Spring 2019

Influence of post-exercise nutrient intake on recovery and subsequent exercise performance in youth cyclists

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Influence of Post-Exercise Nutrient Intake on Recovery and Subsequent Exercise Performance in Youth Cyclists

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A Thesis submitted to the Graduate Faculty of

JAMES MADISON UNIVERSITY

In

Partial Fulfillment of the Requirements

for the degree of

Master of Science

Department of Kinesiology

May 2019

FACULTY COMMITTEE:

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Acknowledgements

I would like to thank Dr. Saunders for being the chair of my thesis and all the help he provided.

I would also like to thank the rest of my committee, Dr. Luden and Dr. Kurti for their valuable input and support.

I would also like to thank the Miller School of Albermarle Endurance Team and their coaches for being excellent subjects.

Finally, I would like to thank Miranda for putting up with this.

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Abstract

Purpose: The purpose of this study was to determine if chocolate milk (CM) consumption after high-intensity cycling exercise affects post-exercise recovery and subsequent exercise performance in youth cyclists, compared to a carbohydrate-only (CHO) and a placebo (PL) beverage. **Methods:** Eight youth cyclists (15-18 y, VO_{2peak} = 61.8 ± 7.7 $mL \cdot kg^{-1} \cdot min^{-1}$) performed an exercise/recovery protocol consisting of 2 bouts of exercise, on 3 separate occasions, in a randomly counterbalanced crossover design. The first exercise bout (EX1) consisted of 30 min of constant-load cycling (40-60% W_{max}), and 60 min of high-intensity intervals (alternating 2 min at 70-90% W_{max}, 2 min at 50% W_{max}). Subjects consumed a recovery beverage (PL, CHO or CM) immediately following EX1 and again 2 h after EX1. EX2 consisted of 30 min of constant-load cycling (60% W_{max}) followed by a simulated 30 km time trial (TT). Ratings of muscle soreness and mental and physical energy/fatigue were obtained prior to EX1, 4 h post-EX1, and pre-EX2. **Results:** Changes in muscle soreness ratings over time were not significantly different between treatments. However, within the PL trial, soreness was significantly elevated from pre-EX1 to 4 h post-EX1 and pre-EX2 (pre-EX1, 4h-post, pre-EX2 = 44.1 ± 23.1, 67.4 ± 22.2, 68.3 ± 19.6 mm, respectively. Physical fatigue ratings increased significantly from pre-EX1 to pre-EX2 in PL. In addition, changes in physical fatigue 4 h following EX1 were greater in CHO than CM, with no other significant within- or between-treatment effects in energy/fatigue ratings. Average power during the TT was not significantly different between treatment trials (PL: 181 ± 27, CHO: 197 ± 39, CM: 195 ± 38 W) (p = 0.23 CHO vs. PL; 0.19 CM vs. PL). **Conclusion:** CM ingestion after exercise may confer some recovery benefits in youth cyclists, as demonstrated by the absence of elevated post-exercise muscle soreness and energy/fatigue ratings in the CM trials. CM ingestion did not significantly improve subsequent cycling performance when compared to CHO or PL beverages. Subsequent research should utilize larger sample sizes to provide more conclusive evidence to enhance the knowledge regarding the impact of CM as a recovery method for youth cyclists.

Chapter 1

Introduction

Introduction:

In competitive athletes, recovery is a critical component of both training and competition. Appropriate post-exercise nutrition is critical to maintain high levels of performance from one exercise session to the next, maximizing training adaptation. In this regard, the potential efficacy of chocolate milk (CM) as a recovery beverage has received considerable attention in recent years. CM contains carbohydrate, protein (casein and whey), and electrolytes (notably, calcium and potassium) in amounts that may influence intra-muscular glycogen levels, hydration status, and muscular function in a manner which may positively influence recovery[1-13]. The effects of nutrient intake, and CM in particular, on each of these components of recovery is discussed below.

Muscle Glycogen Resynthesis

Muscle glycogen resynthesis is a critical component of post-exercise recovery. Long duration exercise or high intensity interval exercise can both result in significant muscle glycogen depletion [14-19]. Muscle glycogen levels are associated with time to fatigue during sustained exercise, so it is important to restore muscle glycogen levels prior to subsequent exercise [3,20]. There is evidence that consumption of carbohydrate (CHO) enhances muscle glycogen resynthesis rates following exercise [14,16,-17]. In addition, because exercise enhances insulin sensitivity and muscle glucose uptake, consumption of CHO in close proximity to exercise cessation may be a useful strategy to maximize glycogen synthesis under conditions where recovery time between exercise sessions is limited [20]. The quantity of ingested CHO

also influences glycogen synthesis post-exercise. Evidence suggests that 1.0 – 1.2 g/kg/hr of CHO is sufficient to maximize glycogen resynthesis rates within a 2-6-hour period following exercise [15,21].

Recent literature has suggested that co-ingestion of carbohydrate and protein (CHO+PRO) may provide further benefits for recovery in endurance athletes, versus CHO alone [2,5,10,11]. This may be due to an enhanced glycogen restoration response after CHO+PRO consumption compared to CHO-only. This could potentially be due to an elevated insulin response that occurs when glucose is paired with certain amino acids such as leucine[3,19]. Van Loon et al. found that a recovery beverage containing of 0.2 - 0.4 g/kg of protein consumed post-glycogen depleting exercise elicited a greater insulin response compared to a CHO-only beverage[41]. Zawadzki *et al.* also found that a CHO+PRO recovery beverage improved muscle glycogen resynthesis versus CHO alone[22]. However, although CHO+PRO ingestion may improve glycogen resynthesis rates versus isocaloric CHO beverages when ingestion rates are < 1 g/kg/hr, most evidence suggests that peak glycogen resynthesis rates with high CHO doses (> 1g/kg/h) are not elevated further with CHO+PRO ingestion[14]. The optimal dose of carbohydrate/protein can fluctuate based on the timing and type of carbohydrate consumed. The amount of exercise-induced glycogen depletion also plays a critical role in determining the optimal dose. In general, protein doses > 0.3g/kg/hr are recommended (in combination with > 0.8 gCHO/kg/hr), in order to increase the likelihood of an augmented insulin response, and optimal glycogen resynthesis[14].

Chocolate milk has been reported to serve as a high-quality CHO+PRO recovery beverage[1,3,4,6,8,9,11-13]. White milk itself is a good source of carbohydrates (lactose), fats, and protein, and the addition of chocolate flavoring adds additional sugars that may be effective to enhance glycogen resynthesis[2,12]. Chocolate milk contains sufficient amounts of CHO and PRO to promote glycogen resynthesis post-exercise[2]. Ferguson-Stegall *et al.* found that post-exercise CM consumption from a drink containing 11.48 g/kg CHO resulted in similar levels of glycogen resynthesis compared to an isocaloric CHO recovery drink containing 15.15 g/kg of carbohydrate [12].

Muscle Damage and Function

Prolonged, intense exercise can cause muscle damage, resulting in reduced force production in damaged muscles[23-24]. During periods of intensified training, or when approaching competition, minimizing muscle damage is critical for maintaining high levels of training quality and maximizing performance. Endurance activities such as running and cycling can result in sufficient muscle damage to negatively affect exercise performance[2,23,24]. This is potentially related to protein turnover, which is the degree to which protein is being broken down (catabolism) versus synthesized (anabolism). A goal of athletes during recovery should be to increase their fractional synthetic rate (FSR), by promoting protein synthesis and minimizing protein break down[24,25]. This will not completely prevent the damaging impact of exercise on muscle, but it can mitigate the effects and promote faster recovery post-exercise[24,25].

CHO+PRO recovery beverages have been found to improve protein balance in athletes, promoting higher protein synthesis rates during recovery[1,3,4,6,8,10,20,28]. This has the benefit of potentially increasing an athlete's ability to recover from a bout of exercise or competitive event more rapidly. This could potentially enhance muscle repair post exercise and reduce feelings of soreness. A number of studies have reported that post-exercise ingestion of CHO+Pro is associated with reduced markers of muscle disruption (plasma CK, LDH) and muscle soreness following exercise compared to CHO-only interventions[1,40]. Additionally, Lunn *et al.* examined the effects of CHO+PRO beverage on protein turnover following treadmill running. One of the primary findings was that Caspase-3, a protein responsible for breaking down muscular proteins, was reduced in athletes who ingested CHO+PRO[6]. Likewise, a concurrent increase in protein synthesis and decreased protein breakdown was reported. The authors theorized that an elevated insulin response following CHO+PRO ingestion potentially maintained higher levels of PI3K pathway activity, which is involved in attenuating protein breakdown[6].

Chocolate Milk (20% whey and 80% casein) contains useful proteins and amino acids that are associated with enhanced protein synthesis post-exercise. The majority of the proteins in CM are casein which require a longer period of time (> 3 h) versus whey, to increase amino acid levels after consumption[3,6,37]. Consumption of these protein sources can elevate protein synthesis and decrease protein breakdown for an extended duration post-consumption[3,6]. This outcome could potentially lead to a prolonged period of muscle repair and enhanced muscle recovery. There is also evidence that CM is more effective than CHO at stimulating intracellular pathways associated with protein synthesis. This could potentially result in enhanced muscle recovery speed and decreased muscle breakdown post-exercise[12]. Greer et al. found that branched chain amino acid (BCAA) supplementation post aerobic exercise attenuated markers of muscle damage, including plasma CK, LDH, and muscle soreness[40]. Similarly, Gilson et al. found that CM consumption post-exercise significantly reduced serum CK in soccer players compared to an isocaloric CHO beverage. However, these findings have not been ubiquitous as other studies have found no effect of CM on muscle recovery versus carbohydrate alone[13,38,].

Rehydration

Proper hydration following exercise is a critical component of recovery. Sweat rates during exercise in the heat can reach 2-3 L/h, resulting in large losses of water and sodium [7,9]. As a result, fluid loss during exercise commonly results in a 2 - 3% loss in body mass [7]. This can result in reduced force production, and impaired performance in long-duration endurance exercise[9]. Wilk *et al.* found that a 1% reduction in body mass from fluid loss resulted in a 15% decrease in TTF at 90% VO_{2max} in adolescent males[29]. Recovery nutrition to ameliorate performance decrements as a result of dehydration should compensate for both water and electrolyte losses [7,9].

The protein and electrolyte composition of CM makes it a potentially useful beverage for rehydration. Chocolate milk contains relatively high calcium and potassium levels, which are critical for proper fluid balance[9]. A study by Volterman *et al.* found that subjects who drank CM maintained a positive fluid balance up to 4 h post exercise, compared to water and water+sodium trials, which resulted in negative fluid balances. This enhanced fluid retention may be due to a prolonged absorption period of CM that slows gastric emptying and helps to maintain fluid balance[39].

Subsequent Exercise Performance

The performance enhancing effect of CHO consumption on subsequent exercise has been well documented. Post-exercise CHO ingestion can increase exercise capacity in subsequent bouts compared to when no post-exercise nutrients are ingested[14,42]. This is theorized to be a result of the enhanced glycogen resynthesis associated with post-exercise CHO consumption, as pre-exercise glycogen levels are strongly correlated with performance[14,21,42]. CHO + PRO recovery beverages have been reported to improve repeat bout exercise performance, and endurance trials assessed via time-to-fatigue (TTF) and time-trials[-13,43]. Saunders et al. found that post-exercise CHO+PRO ingestion increased time to exhaustion (TTE) in a subsequent bout of cycling exercise, in comparison to a CHO beverage matched for carbohydrate content[20]. Similarly, Berardi et al. reported that CHO+Pro consumption improved subsequent performance in a cycling time-trial, versus an isocaloric CHO beverage[43]. Multiple studies have found that post-exercise CM intake improved subsequent bout aerobic performance compared to CHO-only beverages [6,11,12,13,14,20]. Both Ferguson-Stegall *et al.* and Upshaw *et al.* reported that time trial performance in subsequent exercise was significantly improved in subjects who consumed CM during recovery from glycogen-depleting exercise[11,12]. Likewise, Lunn et al. found that post-exercise CM improved TTE in subsequent treadmill running exercise by 23% compared to CHO-only[6]. However, these findings have not been ubiquitous throughout the literature[14].

Nutritional Recovery for Children/Adolescent Athletes

As discussed above, the nutritional composition of CM (CHO, PRO and micronutrients) appear to make it well-suited for post-exercise recovery. In addition, CM contains nutrients such as calcium, vitamin D, and protein which are commonly deficient in the diets of youth athletes[3,6,9]. Furthermore, CM Is a whole food that is commonly consumed in American diets, which has ethical advantages versus promoting nutritional "supplements" to young athletes. These attributes, combined with the relatively low cost and palatability of CM, makes it a strong candidate as a potential recovery beverage for children. However, although there is a fair degree of literature regarding CM's efficacy as a recovery beverage in adult athletes, there is little information regarding its impact on youth athletes. Adolescent athletes (13 – 17 y) have different metabolic demands and recovery needs compared to adults, but guidelines for children are typically just scaled down versions of adult guidelines which may be inappropriate[30-33]. There is evidence that children recover from HII exercise more rapidly than adults, and while this mechanism is unclear, it may be due to differences in glycolytic activity, amount of muscle used during exercise, or substrate utilization[51-53]. In order to maintain high levels of training quality or competition intensity, proper recovery nutrition for children must compensate for the energy expended during training, while also providing the energy necessary to sustain adequate growth and development[30-35]. This is particularly difficult due to the variable rates at which adolescents develop and reach maturity. Adolescents also tend to be less energy efficient than adults [32,34,35]. Frost *et al.* determined that greater co-contraction of antagonist muscles during treadmill in youth aged 7-16 could be a potential factor in reduced metabolic efficiency[32]. Adolescents also tend to be worse than adults at rehydrating properly after exercise when allowed to drink *ad libitum*[7,31].

There is some recent evidence that milk ingestion in children is associated with enhanced protein turnover and rehydration versus CHO only. Volterman et al. performed a study on 28 children (aged 7-17 y) and found that skim milk significantly increased protein synthesis, with a small concurrent

increase in protein degradation compared to a CHO-only intervention[8]. However, no studies to date have specifically examined the effects of CM ingestion on athletic recovery and subsequent exercise performance in youth athletes[1-13]. The purpose of this study is to determine the impact of CM ingestion following a bout of glycogen depleting exercise on subsequent exercise performance (30km time trial). Our primary hypothesis is that CM will improve performance in subsequent exercise in comparison to a carbohydrate only beverage and a placebo.

Chapter 2

Methodology

Methods

Subjects:

10 competitive cyclists (7 males, 3 females) aged 14-18, were recruited from the Miller School of Albermarle (MSA) Endurance Team (n = 8) and Shen Rock (n = 2) youth cycling teams. All subjects were well-trained cyclists, having competed a minimum of 5 h/wk of cycling training over the preceding 3 months and a VO_{2peak} >50 ml/kg/min. Subjects completed a preliminary exercise test, a familiarization trial, and three treatment trials. Parental consent and youth assent were acquired before testing began. All protocols were approved by the IRB of James Madison University.

Protocol:

Preliminary Exercise Testing: Subjects performed a graded exercise cycling test on a magnetically braked cycle ergometer (Velotron; Quarq, Chicago, IL) to assess VO_{2peak} and peak aerobic power (W_{max}). Subjects began with a short warm up (~150 W) followed by progressively increasing resistance (25 W every 2 min) until voluntary fatigue. VO_{2peak} was recorded as the highest 30 second average VO_2 value during the test. W_{max} was used to determine exercise intensities for the experimental conditions, and was determined using the following calculation:

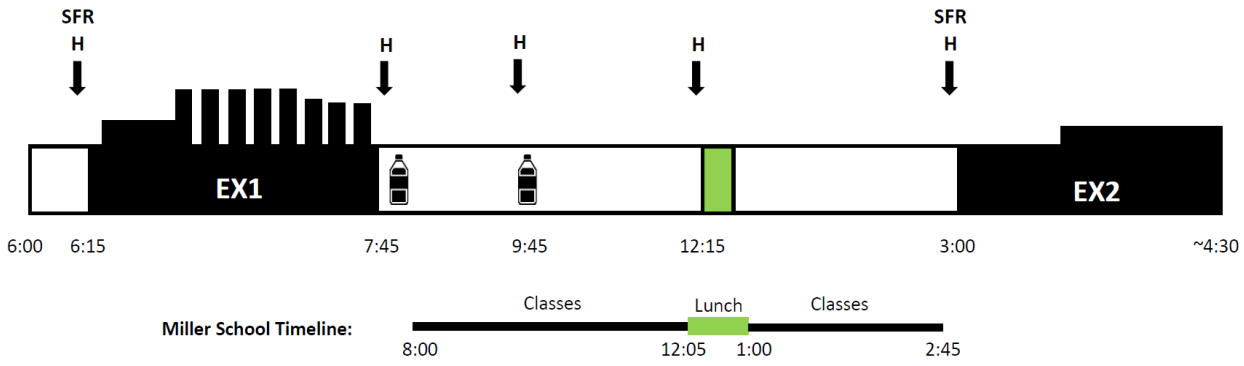
$$W_{max}[(W_{HCS}) + (W_{FS} * \%FSC)]$$

[W_{HCS}: Wattage in highest completed stage; W_{FS}: Wattage is final (incomplete) stage; %FCS: Percent of final stage completed]

Familiarization Trial:A familiarization trial was performed the week prior to the initiation of testing. Subjects performed a scaled-down version of the experimental trials. This included a portion of the continuous training to warm-up, 5 HII's, and a 10k time trial. During this time subjects were familiarized with the instruments and measures being utilized as well as how to operate the cycle ergometers.

Experimental Protocol: An overview of the experimental protocols during treatment trials is shown in Figure 1. Subjects reported to the lab at ~5:45 am, having consumed a standardized snack the night before the trial and another in the morning before arrival (see *Exercise and Diet Controls)*. Subjects completed baseline questionnaires to assess energy/fatigue levels and muscle soreness. After subjects voided their bladders, a body weight was assessed in minimal clothing (in a separate room for their privacy). Subjects were then provided 250 mL's of water and began the preliminary exercise protocol.

Figure 1. Timeline of Protocols during each Experimental Trail



Preliminary Exercise: The initial exercise bout (EX1), was ~ 90 min of total cycling. The first 25 mins was a constant load protocol consisting of a 5 min warm-up at 40% W_{max} followed by 20 mins at 60% W_{max}. The constant-load phase was followed by 60 mins of high-intensity intervals (HII) and a 5 min cooldown period at 40% W_{max}. During the HII segment, subjects completed 15 intervals alternating between 2 mins at 90% W_{max} and 2 mins at 50% W_{max}. Subjects were asked to maintain a cadence above 60rpm during the HII. When participants were unable to maintain the required cadence, workload was reduced

such that subsequent intervals were completed at 80% W_{max}. When the required cadence could not be maintained in subsequent intervals, the load was further reduced to 70% W_{max}. The intervals ended when subjects were incapable of maintaining 60 rpm at 70% W_{max} or when they completed all 15 HII. Subjects were provided with 250 mL of water every 15 mins after initiation of exercise, which they drank ad libitum. The amount of fluid consumed was recorded by the researchers.

Subsequent Exercise: The secondary bout of exercise (EX2) took place ~7 hours after the preliminary exercise bout. It consisted of a constant-load period, including 5 min at 40% W_{max} and 30 mins at 60% W_{max}. After the constant-load phase, subjects were given ~2 mins in which to void their bladder prior to completing a simulated 30km time trial. Subjects were instructed to provide maximal exertion as if the time trial were a race. Subjects were provided with 250 mL water every 15 mins during the constant-load phase, and also every 8km during the time-trial.

Nutritional Intervention: Subjects were provided with recovery beverages immediately after EX1, and 2 hours following EX1. Subjects completed the experimental trials on three occasions, with a different treatment in each trial. Treatments were provided in a randomly-counterbalanced order. Treatments were provided in opaque bottles to ensure both researchers and participants were blinded to the intervention

Chocolate Milk (CM): 11.8 ml/kgBW of low-fat CM (contains approximately 1.2 g CHO, 0.4 g Pro, 0.11 g fat, 9 mg sodium, 21 mg potassium per kgBW).

- For 60 kg subject = 708 ml beverage, 72 g CHO, 24 g Pro, 6.6 g fat & 443 kcal.

Carbohydrate-electrolyte beverage (CHO): 1.72 g/kgBW of chocolate flavored Clif Shot gels (contains 1.2 g CHO, 0 g Pro, 0.08 g fat, 3.1 mg sodium, 4 mg potassium per kgBW), mixed in water to provide 11.8 ml/kgBW of beverage [i.e. matched with CM for fluid & CHO content]

- For 60 kg subject = 708 ml beverage, 72 g CHO, 0 g Pro, 5 g fat & 333 kcal.

Placebo (PL): 11.8 ml/kgBW of an artificially-flavored water beverage [i.e. matched fluid volume versus other treatments]

For each trial, subjects consumed a standardized lunch at 12:15, ~4.75 hours after EX1. For MSA athletes, the meal was provided by the MSA campus cafeteria. Subjects were provided a check sheet with a predetermined list of allowable food items. Subjects circled the items and portion sizes of the items they consumed, and then replicated these foods/amounts during subsequent trials. For non-MSA athletes, food choices for lunch were discussed before the first trial, and an intended lunch menu was agreed upon. The specific foods and amounts consumed in the lunch meal were replicated across all trials.

Dependent Measures:

Subsequent time trial performance: Time to complete the trial, and average power were recorded for each simulated 30km time trial to measure performance differences between conditions.

Physiological Variables during Constant-Load Exercise: All physiological variables were recorded in a 5-min time-frame after 10 mins of cycling at 60% W_{max} for EX1 and EX2. Oxygen consumption (VO_2), ventilation (VE), respiratory exchange ratio (RER) were all measured using the Oxycon Mobile Portable Metabolic Cart (Viasys, Yorba Linda, CA). Gas was collected for 5 mins, the last 3 mins of which were used for data. Heart rate was measured using a Polar heart rate monitor (Lake Success, NY). RPE was measured using a Borg (6-20) RPE scale that the subject could point to. Lactate (Lactate Pro; Arkray, Minneapolis ,MN) and glucose (Cardiocheck; PTS Diagnostics, Indianapolis, IN) was used to measure lactate and glucose levels from whole blood gathered from fingers-sticks after VO_2 was measured.

Muscle Soreness Rating: Muscle Soreness was measured before EX1 and EX2 and 4 h post EX1. Subjects were required to determine their soreness levels using a 100mm visual analog scale. On the scale, a 0 represented an absolute lack of any muscle soreness, and a 100 represented the subject felt their movement was significantly impaired due to soreness.

Mental and Physical Energy/Fatigue Rating: Energy and fatigue scores were gathered at the same time as the muscle soreness data using Part 2 of Mental and Physical State and Trait Energy and Fatigue Scales (MPSTEFS; P.J. O'Connor, personal communication). Subjects were instructed on how to properly utilize the scale prior to reporting.

Hydration Status: Changes in body weight were used to measure changes in hydration status. Body weight was measured before and after EX1, 2 and 4 h post EX1, and immediately prior to EX2.

Exercise and Diet Controls: Subjects were asked to refrain from heavy exercise for 24 h prior to each trial, and followed standardized diet and exercise practices for the 24 h before each experimental trial. Diet and exercise were recorded for 24 h prior to EX1. Participants ate their last meal ≥ 12 h before the initiation of EX1. Subjects were provided with standardized snacks to be consumed at 8:00 pm the night before EX1 (a chewy granola bar (Great Value™ choco chunk; 18 g CHO, 1 g protein, 2 g fat), 30 min prior to EX1 [a chewy granola bar (Great Value™ choco chunk; 18 g CHO, 1 g protein, 2 g fat) and 354 ml of Gatorade (21 g CHO)], and 1 h prior to EX2 bar (Great Value™ choco chunk; 18 g CHO, 1 g protein, 2 g fat) . Subjects were instructed to consume only the recovery beverages and the standardized lunch between EX1 and EX2, and consume no other foods/beverages (including water).

Statistics:

Statistical analyses for the study were performed using IBM SPSS Statistics 25. Mean values and standard deviations were calculated and reported for all dependent measures discussed above. Treatment differences in these variables were assessed using repeated measures ANOVA's, with individual treatment comparisons performed with Fisher's least significant difference test (i.e. no correction for multiple comparisons).

Chapter 3

Manuscript

Influence of Post-Exercise Nutrient Intake on Recovery and Subsequent Exercise Performance in Youth Cyclists

Andrew Sterling Law

A Thesis submitted to the Graduate Faculty of

JAMES MADISON UNIVERSITY

In

Partial Fulfillment of the Requirements

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Exercise Science

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FACULTY COMMITTEE:

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Purpose: The purpose of this study was to determine if chocolate milk (CM) consumption after high-intensity cycling exercise affects markers of post-exercise recovery and subsequent exercise performance in youth cyclists, compared to a carbohydrate-only (CHO) and a placebo (PL) beverage. **Methods:** Eight youth cyclists (15-18 y, VO_{2peak} = 61.8 ± 7.7 $mL \cdot kg^{-1} \cdot min^{-1}$) performed an exercise/recovery protocol consisting of 2 bouts of exercise, on 3 separate occasions, in a randomly counterbalanced crossover design. The first exercise bout (EX1) consisted of 30 min of constant-load cycling (40-60% W_{max}), and 60 min of high-intensity intervals (alternating 2 min at 70-90% W_{max}, 2 min at 50% W_{max}). Subjects consumed a recovery beverage (PL, CHO or CM) immediately following EX1 and again 2 h after EX1. A standardized lunch was consumed approximately 4 h post-EX1, after which a second exercise session (EX2) was completed ~ 7 h after EX1. EX2 consisted of 30 min of constant-load cycling (60% W_{max}) followed by a simulated 30 km time trial (TT). Body weight measurements (to assess fluid retention) were obtained after ingestion of the first beverage, and again 2 hours later. Ratings of muscle soreness and mental and physical energy/fatigue were obtained prior to EX1, 4 h post-EX1, and pre-EX2. Average power (W) during the 30 km TT was used to assess subsequent exercise performance. **Results:** Changes in body weight in the 2 h following beverage ingestion were not significantly different between treatments (PL: - 0.6 ± 0.6, CHO: -0.7 ± 0.7, CM: -0.6 ± 1.0 lbs). Changes in muscle soreness ratings over time were not significantly different between treatments. However, within the PL trial, soreness was significantly elevated from pre-EX1 to 4 h post-EX1 and pre-EX2 (pre-EX1, 4h-post, pre-EX2 = 44.1 ± 23.1, 67.4 ± 22.2, 68.3 ± 19.6 mm, respectively). Within CHO, soreness tended to be elevated (p = 0.051) at 4 h post-exercise (37.4 ± 25.7, 51.6 ± 27.6, 48.1 ± 24.4 mm); and was not elevated at any post-exercise time-point in CM (41.0 ± 15.6, 46.3 ± 23.0, 47.0 ± 27.4 mm). Physical fatigue ratings increased significantly from pre-EX1 to pre-EX2 in PL. In addition, changes in physical fatigue 4 h following EX1 were greater in CHO than CM, with no other significant within- or between-treatment effects in energy/fatigue ratings. Average power during the TT was not significantly different between treatment trials (PL: 181 ± 27, CHO: 197 ± 39, CM: 195 ± 38 W) (p = 0.23 CHO vs. PL; p = 0.19 CM vs. PL). **Conclusion:** CM ingestion after exercise may confer some recovery benefits in youth cyclists, as demonstrated by the absence of elevated post-exercise muscle soreness and energy/fatigue ratings in the CM trials. However, a lack of consistent treatment*time effects on these ratings minimizes the impact of these findings. CM ingestion did not significantly improve subsequent cycling performance when compared to CHO or PL beverages. Subsequent research should utilize larger sample sizes to provide more conclusive evidence to enhance the knowledge regarding the impact of CM as a recovery method for youth cyclists.

Introduction

Recovery from heavy exercise is critical for both training and performance for competitive athletes. Consequently, nutritional approaches to optimize recovery have been of critical importance. In recent years, chocolate milk (CM) has emerged as a potential beverage to enhance recovery and there is a growing body of evidence that CM ingestion after heavy aerobic exercise can enhance performance in subsequent exercise[6,11,12,13,14,20]. For example, both Ferguson-Stegall et al. and Upshaw et al. reported that time trial performance in subsequent exercise was significantly improved in subjects who consumed CM during recovery from glycogen-depleting cycling exercise[11,12]. Additionally Lunn et al. demonstrated a 23% increase in time to exhaustion (TTE) in runners that consumed CM compared to those that consumed CHO[6].

The mechanisms by which CM influences subsequent performance are not clearly elucidated. However, CM contains carbohydrates (CHO), protein (PRO; casein and whey), and electrolytes (notably, calcium and potassium) in amounts that could benefit recovery[1-13]. For instance, post-exercise CHO + PRO ingestion has been reported to enhance muscle glycogen replenishment rates compared to CHO, in some studies[1,3,12]. CM contains sufficient amounts of CHO and PRO to promote glycogen resynthesis post-exercise, potentially at a faster rate than CHO alone[12,14]. It is generally believed that CHO + PRO ingestion augments resynthesis rates over isocaloric amounts of CHO when consumed at carbohydrate ingestion doses of $\leq$ 1g/kg/hr, and this may be a result of an elevated insulin response due to the additional protein[14]. Prolonged intense exercise also results in muscle damage, leading to reduced force production and muscle soreness[23-24]. Although findings are somewhat mixed, numerous studies have reported that post-exercise ingestion of CHO + PRO (and/or CM) is associated with reduced markers of muscle disruption (i.e. plasma CK) and muscle soreness following exercise compared to CHO-only interventions[1,3,4,6,8,14,40,44]. Furthermore, CM may also influence post-exercise recovery via enhanced

rehydration. Milk/CM has been demonstrated to increase fluid retention during recovery, potentially due to high calcium/potassium content combined with reduced rate of gastric emptying[7,9].

Investigations into the influence of CM on exercise recovery have been exclusively performed with adults. While guidelines for children are typically scaled-down versions of adult guidelines, this may be inappropriate[30-33], as youth athletes (13 – 17 y) have different metabolic demands and recovery needs compared to adults. There is also evidence that children recover from HII exercise more rapidly than adults, and while this mechanism is unclear, it may be due to differences in glycolytic activity, amount of muscle used during exercise, or substrate utilization[51-53]. Additionally, in order to maintain high levels of training quality or competition intensity, proper recovery nutrition for young athletes must compensate for the energy expended during training, while also providing the energy necessary to sustain adequate growth and development[30-35]. There is some evidence that CM can improve post-exercise rehydration and protein synthesis in children, but no studies have examined the impact of CM on subsequent performance in youth athletes[8,9]. The purpose of this study is to determine the impact of CM ingestion following high-intensity cycling intervals on markers of recovery, and subsequent exercise performance (30-km time trial). Our primary hypothesis is that CM will improve performance in subsequent exercise compared to a CHO beverage and a placebo.

Methods

Subjects:

10 competitive cyclists (7 males, 3 females) aged 14-18, were recruited from the Miller School of Albermarle (MSA) Endurance Team (n = 8) and Shen Rock (n = 2) youth cycling teams, of which 8 completed all testing protocols. All subjects were well-trained cyclists, having competed a minimum of 7 h/wk of cycling training over the preceding 3 months and a VO_{2peak} > 50 ml/kg/min. Subjects completed a preliminary exercise test, a familiarization trial, and three treatment trials. Parental consent and youth assent were acquired before testing began. All protocols were approved by the IRB of James Madison University.

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[W_{HCS}: Wattage in highest completed stage; W_{FS}: Wattage is final (incomplete) stage; %FCS: Percent of final stage completed]

Familiarization Trial: A familiarization trial was performed the week prior to the initiation of testing. Subjects performed a scaled-down version of the experimental trials. This included a portion of the continuous training to warm-up, 5 high intensity intervals (HII), and a 10-km time trial. During this time

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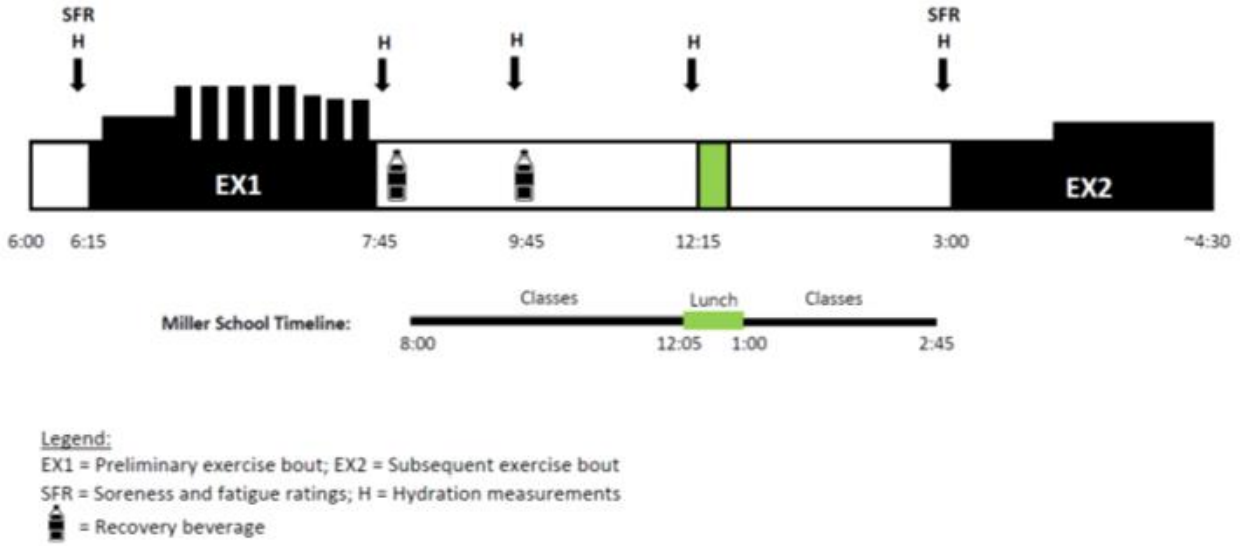
Figure 1. Timeline of Protocols during each Experimental Trail



Legend:
EX1 = Preliminary exercise bout; EX2 = Subsequent exercise bout
SFR = Soreness and fatigue ratings; H = Hydration measurements
= Recovery beverage

Preliminary Exercise: The initial exercise bout (EX1) included 90 min of total cycling. The first 25 min were constant-load consisting of a 5 min warm-up at 40% W_{max} followed by 20 min at 60% W_{max}. The constant-load phase was followed by 60 min of high-intensity intervals (HII) and a 5 min cooldown period at 40% W_{max}. During the HII segment, subjects completed 15 intervals alternating between 2 min at 90% W_{max} and 2 min at 50% W_{max}. Subjects were asked to maintain a cadence above 60 rpm during

the HII. When participants were unable to maintain the required cadence, workload was reduced such that subsequent intervals were completed at 80% W_{max}. When the required cadence could not be maintained in subsequent intervals, the load was further reduced to 70% W_{max}. The intervals ended when subjects were incapable of maintaining 60 rpm at 70% W_{max} or when they completed all 15 HII. Subjects were provided with 250 mL of water every 15 mins after initiation of exercise, which they drank ad libitum. The amount of fluid consumed was recorded by the researchers.

Subsequent Exercise: The secondary bout of exercise (EX2) took place ~ 7 h after the preliminary exercise bout. It consisted of a constant-load period, including 5 min at 40% W_{max} and 30 min at 60% W_{max}. After the constant-load phase, subjects were given ~ 2 min in which to void their bladder prior to completing a simulated 30 km time trial. Subjects were instructed to provide maximal exertion as if the time trial were a race. Subjects were provided with 250 mL water every 15 min during the constant-load phase, and also every 8 km during the time-trial.

Nutritional Intervention: Subjects were provided with recovery beverages immediately after EX1, and 2 h following EX1. Subjects completed the experimental trials on three occasions, with a different treatment in each trial. Treatments were provided in a randomly-counterbalanced order. Treatments were provided in opaque bottles to ensure both researchers and participants were blinded to the intervention

Chocolate Milk (CM): 11.8 ml/kgBW of low-fat CM (contains approximately 1.2 g CHO, 0.4 g Pro, 0.11 g fat, 9 mg sodium, 21 mg potassium per kgBW).

- For 60 kg subject = 708 ml beverage, 72 g CHO, 24 g Pro, 6.6 g fat & 443 kcal.

Carbohydrate-electrolyte beverage (CHO): 1.72 g/kgBW of chocolate flavored Clif Shot gels (contains 1.2 g CHO, 0 g Pro, 0.08 g fat, 3.1 mg sodium, 4 mg potassium per kgBW), mixed in water to provide 11.8 ml/kgBW of beverage [i.e. matched with CM for fluid & CHO content]

- For 60 kg subject = 708 ml beverage, 72 g CHO, 0 g Pro, 5 g fat & 333 kcal.

Placebo (PL): 11.8 ml/kgBW of an artificially-flavored water beverage [i.e. matched fluid volume versus other treatments]

For each trial, subjects consumed a standardized lunch at 12:15, ~4.75 h after EX1. For MSA athletes, the meal was provided by the MSA campus cafeteria. Subjects were provided a check sheet with a predetermined list of allowable food items. Subjects circled the items and portion sizes of the items they consumed, and then replicated these foods/amounts during subsequent trials. For non-MSA athletes, food choices for lunch were discussed before the first trial, and an intended lunch menu was agreed upon. The specific foods and amounts consumed in the lunch meal were replicated across all trials.

Dependent Measures:

Subsequent time trial performance: Time to complete the trial, and average power were recorded for each simulated 30 km time trial to measure performance differences between conditions.

Physiological Variables during Constant-Load Exercise: All physiological variables were recorded in a 5 min time-frame after 10 min of cycling at 60% W_{max} for EX1 and EX2. Oxygen consumption (VO_2), ventilation (VE), respiratory exchange ratio (RER) were all measured using the Oxycon Mobile Portable Metabolic Cart (Viasys, Yorba Linda, CA). Gas was collected for 5 min, the last 3 min of which were used for data. Heart rate was measured using a Polar heart rate monitor (Lake Success, NY). RPE was measured using a Borg (6-20) RPE scale that the subject could point to immediately prior to placing the mask on the subject to collect VO_2. Lactate (Lactate Pro; Arkray, Minneapolis ,MN) and glucose

(Cardiocheck; PTS Diagnostics, Indianapolis, IN) was used to measure lactate and glucose levels from whole blood gathered from fingers-sticks after VO_2 was measured.

Muscle Soreness Rating: Muscle soreness was measured before EX1 and EX2 and 4 h post EX1. Subjects were required to determine their soreness levels using a 100 mm visual analog scale. On the scale, a 0 represented an absolute lack of any muscle soreness, and a 100 represented the subject felt their movement was significantly impaired due to soreness, as described previously[1].

Mental and Physical Energy/Fatigue Rating: Energy and fatigue scores were gathered at the same time as the muscle soreness data using Part 2 of Mental and Physical State and Trait Energy and Fatigue Scales (MPSTEFS; P.J. O'Connor, personal communication). Subjects were instructed on how to properly utilize the scale prior to reporting.

Hydration Status: Changes in body weight were used to measure changes in hydration status. Body weight was measured before and after EX1, 2 and 4 h post EX1, and immediately prior to EX2.

Exercise and Diet Controls: Subjects were asked to refrain from heavy exercise for 24 h prior to each trial, and followed standardized diet and exercise practices for the 24 h before each experimental trial. Diet and exercise were recorded for 24 h prior to EX1. Participants ate their last meal ≥ 12 h before the initiation of EX1. Subjects were provided with standardized snacks to be consumed at 8:00 pm the night before EX1 (a chewy granola bar (Great Value™ choco chunk; 18 g CHO, 1 g protein, 2 g fat), 30 min prior to EX1 [a chewy granola bar (Great Value™ choco chunk; 18 g CHO, 1 g protein, 2 g fat) and 354 ml of Gatorade (21 g CHO)], and 1 h prior to EX2 bar (Great Value™ choco chunk; 18 g CHO, 1 g protein, 2 g fat) . Subjects were instructed to consume only the recovery beverages and the standardized lunch between EX1 and EX2, and consume no other foods/beverages (including water).

Statistics:

Statistical analyses for the study were performed using IBM SPSS Statistics 25. Mean values and standard deviations were calculated and reported for all dependent measures discussed above. Treatment differences in these variables were assessed using repeated measures ANOVA's, with individual treatment comparisons performed with Fisher's least significant difference test (i.e. no correction for multiple comparisons).

Results:

Ten subjects volunteered for the study (7 male, 3 female). One subject (male) withdrew from the study prior to completing any of the treatment trials. Another male subject was unable to complete consistent work intensities between trials during EX1, and was removed from data analysis. Analyses were performed on the remaining 8 subjects; demographic data for these subjects are reported in Table 1. One of these subjects experienced stomach issues during the PL trial (prior to starting EX2), and did not complete this trial. For ease of visual interpretation, this subject is not included in the mean values reporting within- and between-treatment effects (Tables 2-7, Figures 2-3). However, statistical analyses from trials that did not include the PL trial included data from this subject.

Table 1. Subject Demographic Data

	Age (y)	Height (cm)	Weight (kg)	Cycling (h/wk)	W_{max} (W)	VO_{2max} (ml/kg/min)
Total (8)	16.1 ± 1.1	174 ± 10	62.9 ± 5.2	9.2 ± 2.1	317 ± 50	61.8 ± 7.7
Females (3)	15.7 ± 0.9	166 ± 6	59.3 ± 5.3	9.7 ± 1.7	256 ± 5	53.3 ± 2.4
Males (5)	16.4 ± 1.0	177 ± 5	65.0 ± 3.7	8.9 ± 2.2	353 ± 20	66.9 ± 4.5

Muscle soreness ratings obtained Pre-EX1, Post-4h, and Pre-EX2 are reported in Table 2. There was a significant increase in muscle soreness in PL from Pre-EX1 to both Post-4h and Pre-EX2 ($p < .05$). There was a trend for a difference in the CHO trial between Pre-EX1 and Post-4h ($p = .051$). There were no significant differences between treatments.

Table 2. Muscle Soreness Ratings Before and After Cycling

Treat	Pre-EX1	4 h Post-EX1	Pre-EX2
PL	44.1 ± 23.1	67.4 ± 22.2*	68.3 ± 19.6*
CHO	37.4 ± 25.7	51.6 ± 27.6#	48.1 ± 24.4
CM	41.0 ± 15.6	46.3 ± 23.0	47.0 ± 27.4

Table excludes 1 subject due to missing data in the placebo trial;
*Within-trial effects: *indicates a significant difference between Pre-EX1; # indicates a trend towards difference compared to Pre-EX1 (p =.051).*

Physical and mental energy and fatigue ratings are reported in Table 3 and 4 respectively. Changes in physical fatigue from Pre-EX1 to Post-4h were lower in the CM trial compared to the CHO trial ($p < .05$). No other significant treatment differences in these variables were observed.

Table 3. Effects of Recovery Beverages on Ratings of Physical Fatigue and Energy

	Physical Fatigue			Physical Energy		
Treatment	**Pre-Ex1**	**Post-4h**	**Pre-Ex2**	**Pre-Ex1**	**Post-4h**	**Pre-Ex2**
Placebo	48 ± 0	61 ± 14	67 ± 10*	51 ± 21	50 ± 17	42 ± 14
CHO	52 ± 14	63 ± 19	55 ± 11	42 ± 19	38 ± 17	43 ± 18
CM	58 ± 9	58 ± 17#	53 ± 16	45 ± 12	37 ± 17	51 ± 18

Table excludes 1 subject due to missing data in the placebo trial.
*Within-trial effects: *indicates a significant difference from Pre-EX1 (p<.05)*
Treatment*time: # indicates a significant interaction of the change in muscle soreness from Pre-Ex1 to Post-4 between CM and CHO

Table 4. Effects of Recovery Beverages on Ratings of Mental Fatigue and Energy

	Mental Fatigue			Mental Energy		
Treatment	**Pre-Ex1**	**Post-4h**	**Pre-Ex2**	**Pre-Ex1**	**Post-4h**	**Pre-Ex2**
Placebo	48 ± 28	49 ± 27	65 ± 11	42 ± 25	46 ± 26	34 ± 16
CHO	52 ± 21	54 ± 16	57 ± 18	31 ± 22	34 ± 16	30 ± 12
CM	60 ± 11	61 ± 18	58 ± 26	43 ± 19	36 ± 17	38 ± 24

Table excludes 1 subject due to missing data in the placebo trial.

Body weight assessed immediately after consumption of the first recovery beverage and prior to consumption of the second recovery beverage and changes in body weight (Post-Bev2 **–** Post-Bev1) are presented in table 5. No significant treatment effects were observed in these variables.

Table 5. Body Weight Changes Following Beverage Consumption

Treatment	**Post-Bev1 (lbs)**	**Pre-Bev2 (lbs)**	**Δ Pre-Bev2 – Post-Bev1 (lbs)**
Placebo	139.7 ± 17.4	139.1 ±17.8	- 0.6 ± 0.6
CHO	139.7 ± 14.0	139.0 ± 4.6	-0.7 ± 0.7
CM	140.4 ± 13.6	139.8 ± 14.2	-0.6 ± 1.0

Physiological responses to exercise (HR, RER, VO_2, glucose, and lactate) during EX1 and EX2 are presented in Table 6. There were no significant differences between treatments during EX1. During EX2 the PL group had a significantly higher plasma glucose compared to the CHO group ($p < .05$).

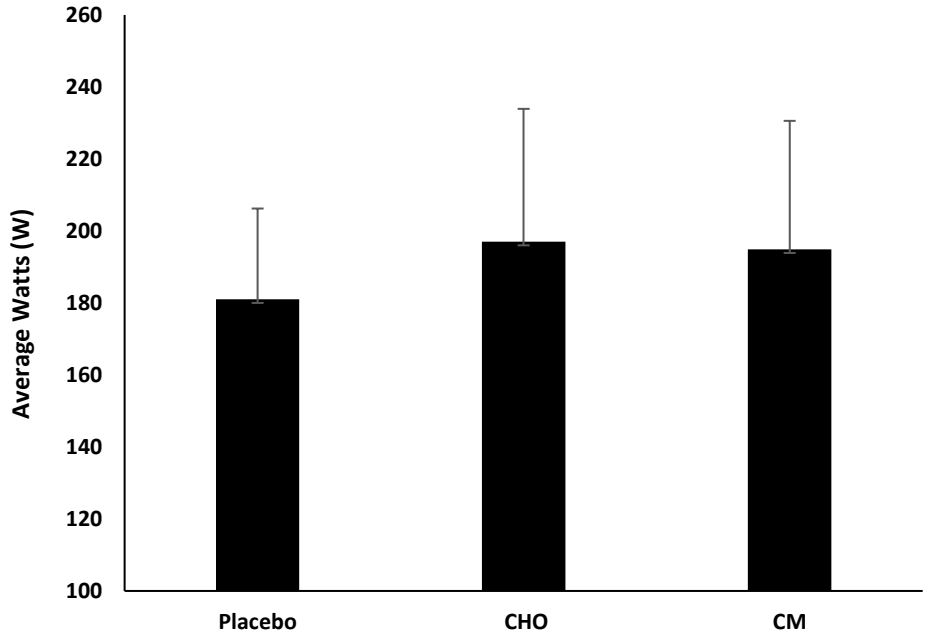
Table 6. Physiological Responses during Constant-Load Exercise (179±43W for all trials)

Ex Bout	Trial	HIIT (kj)	VO_2 (ml /min)	Ve (L/min)	RER	HR (bpm)	RPE	Glucose (mmol/L)	Lactate (mmol/L)
Ex1	PL	625 ±177	2427 ±654	65.6 ±14.4	.95 ±.03	159 ±10	14.0 ±1.7	71.0 ±11.9	1.6 ±0.8
	CHO	625 ±177	2670 ±639	71.3 ±14.1	.96 ±.07	158 ±7	13.0 ±2.3	80.3 ±15.5	1.9 ±0.6
	CM	625 ±177	2547 ±492	69.32 ±11.2	.97 ±.02	160 ±15	13.13 ±2.6	73.9 ±18.3	1.7 ±0.7
Ex2	PL	-	2265 ±499	60.62 ±9.7	.96 ±.04	152 ±8	13.00 ±1.0	71.3 ±8.4*	1.3 ±0.3
	CHO	-	2388 ±521	65.14 ±12.9	.99 ±.03	147 ±9	11.75 ±1.7	68.71 ±10.1	1.1 ±0.3
	CM	-	2430 ±556	68.37 ±13.8	.99 ±.01	153 ±12	12.13 ±2.3	61.43 ±11.3	1.3 ±0.3

** indicates a significant difference (p<.05) of PL compared to CHO*

Performance in EX2 (average watts for 30km TT) for PL, CHO, and CM groups are shown in Figure 2. No significant treatment differences were observed between trials. Similarly, time to complete the 30 km were not different between PL (57.13 ± 3.23 min), CHO (55.36 ± 4.49 min) and CM (55.45 ± 4.26 min) trials.

Figure 2. Effects of Recovery Beverages on Power Output during a 30 km Time-trial



Discussion

The purpose of this study was to examine the effects of varied post-exercise nutrient intake (CM, CHO and PL) on markers of short-term recovery (ratings of perceived soreness, energy, and fatigue) and subsequent exercise performance in youth cyclists. The primary findings were that the CM did not significantly improve subsequent exercise performance in a 30 km TT, compared to the CHO and PL trials. However, some markers of recovery appeared to be improved with CM, as demonstrated by the absence of elevated post-exercise muscle soreness and energy/fatigue ratings in the CM trials. By contrast, changes in some of these variables were present in the PL and CHO trials.

One of the primary objectives of this study was to elucidate the impact of a CM recovery beverage on 30 km time trial performance compared to CHO and PL. During the CM trial subjects averaged 195 ± 38 W which was not significantly different from CHO (197 ± 39 W) or PL (181 ± 27 W) (Figure 2). These findings differ from the results of Ferguson-Stegall et al. who reported that those who consumed CM after heavy cycling exercise (90 min at 70% VO_{2max} and 10 min of HII) maintained

significantly higher average power outputs during a 40 km TT performed 4 h later[12]. Lunn et al. observed similar outcomes utilizing a TTE protocol, in which subjects who consumed CM during recovery ran 23% longer before fatigue in comparison to when they received CHO-only during recovery[6]. However, not all studies have observed an increase in performance resulting from CM consumption as opposed to CHO consumption. For example, Gilson et al. reported no differences in subsequent performance in soccer players who received post-exercise CM during 4 days of heavy training, in comparison to when they received CHO after exercise[1]. The lack of an effect of CM on subsequent performance in the present study may been a result of a longer recovery period between exercise bouts, as most studies have utilized a recovery period of 4 h as opposed to our 7 h which may have provided enough recovery time to minimize differences between trials. Additionally, we provided a standardized meal 3 h prior to EX2 which contained normalized amounts of protein across all trials, which may have blunted any potential treatment effects of additional protein resulting from the presence of protein in the CM treatment. While our approach may have reduced our ability to detect significant treatment effects, this protocol is more applicable to what athletes typically experience between workouts. This hypothesis is supported by the aforementioned study from Gilson et al., who reported no effects of post-exercise CM when performance measurements were assessed the day following beverage ingestion[1].

There is also evidence indicating that youth may recover more rapidly from HII exercise compared to adults while also being more fatigue resistant[52,54]. Specifically, Ratel and colleagues reported that over the course of 10 x 10 sec cycling HII's, adult males saw a 28.5% decrease in peak power output (PPO), youth saw an attenuated decrease (18.5%), and young children saw no decrease in PPO when given 30 seconds rest between bouts[54]. The precise mechanism for this age-related effect is somewhat unclear, but it could be to differences in glycolytic activity during exercise, amount of muscle mass utilized, or muscle morphology throughout this age range[52]. Although speculative, these factors could have contributed to subjects in the present study recovering from HII more quickly than adults of

prior investigations (irrespective of beverage treatment) [51-53]. This outcome, particularly in combination with a longer recovery period and additional nutrients from the standardized lunch, could have diminished the likelihood to observe potential treatment effects in our small sample.

One of the secondary outcome measures of this study was ratings of perceived soreness, collected at 3 timepoints throughout the day (pre-EX1, 4h-post, pre-EX2). There were no statistically significant treatment*time interactions for muscle soreness. However, in the PL trial, soreness ratings increased significantly from pre-EX1 (44.1 ± 23.1 mm) to 4h-post (67.4 ± 22.2 mm), and pre-EX2 (68.3 ± 19.6 mm). In the CHO trial, muscle soreness also tended to increase (p = .051) from pre-EX1 (37.4 ± 25.7 mm) to 4h-post (51.6 ± 27.6 mm). By contrast, muscle soreness did not increase at either post-exercise time-point in the CM trial (Table 2). This indicates that the CM intervention may have prevented increases in muscle soreness otherwise induced by EX1. This outcome is similar to results by Papacosta et al. who examined the effects of post-exercise CM ingestion (versus an isovolumetric water beverage) on ratings of muscle soreness over 5 days of intense judo training. The authors found that muscle soreness of the CM group was significantly reduced in comparison to the water group during days 3 and 5 of intensive training. However, the authors assessed muscle soreness over a much longer time frame and did not have a CHO-only group, which limits our ability to directly compare these findings with the present study[45]. In addition, as discussed above, an accelerated recovery response of youth to HII training may have also contributed to the lack of a significant treatment*time effect in the present study[51-53], as it is possible that our subjects recovered from EX1 relatively well independent of any recovery beverages provided.

The potential protective effect of a CM drink on perceived muscle soreness may be an effect of the protein in the beverage. A number of prior studies have reported that the co-ingestion of CHO + PRO following exercise has been associated with attenuated muscle soreness versus carbohydrate alone[46,47]. Luden et al. found that runners experienced reduced markers of muscle damaged and had lower

reported muscle soreness as when given a CHO + PRO + antioxidant beverage as compared to CHO[47]. Additionally, there is some evidence that CHO + PRO can mitigate markers of muscle disruption, such as plasma CK levels, after heavy exercise[1,6,20]. It is unclear if reduced post-exercise muscle soreness is the result of protein reducing muscle damage, initiating faster muscle recovery, or some other mechanism. However, reduced levels of muscle soreness after exercise is presumably a desirable outcome and may be associated with improved post-exercise muscle function with CHO + PRO ingestion[40,49,50].

Our study also examined the impact of the recovery beverages on perceived ratings of mental and physical energy and fatigue. In the PL trial, there was a significant increase in physical fatigue from pre-EX1 (44 ± 20 mm) to pre-EX2 (67 ± 10 mm) (Table 3). By contrast, physical fatigue ratings did not increase significantly in the CM or CHO trials. Additionally, the change in physical fatigue between pre-EX1 and post-4h was significantly less for CM (-4 ± 11 mm) versus CHO (1 ± 33 mm). This indicates that that CM may have conferred some beneficial effects with respect to physical energy and fatigue, though these were not consistently observed for all measurements, or at all time-points. The additional protein of the CM beverage may have mitigated some of the physically fatiguing effects of EX1 similar to that described above for ratings of perceived soreness[44,45]. Similarly, Papacosta et al. found that CM could reduce muscle soreness over a 3 to 5 day high intensity training block of Judo as compared to water alone[45].

There was a significant difference in plasma glucose during the steady state portion of EX2 between PL (71.3 ± 8.4 mmol/L) and CM (61.43 ± 11.3 mmol/L). There were no other significant differences in the other measured physiological variables. This outcome may be an effect of the increased insulinemic response after consuming a CHO + PRO recovery beverage that has been reported by Shearer et al.[3,19]. The increased insulin response may have left the CM group with a lower blood glucose during EX2. However, this seems unlikely in this case, as this measurement occurred ~ 5 h after the consumption of the last treatment beverage (and ~ 3 h after a standardized lunch). It is also possible

that this outcome resulted from slight differences in the timing of the standardized snack that was provided prior to EX2, which may have influenced blood glucose measurements.

One of the limitations of the present study was that the beverages were matched for total carbohydrate content as opposed to total caloric content. This approach allows for the study outcomes to be generalizable to commercially available recovery beverages that include both carbohydrate and protein. By controlling for carbohydrate content, we can determine the effect of the added protein substrate on exercise performance. However, the increased energy content due to the added protein may have also led to differences between trials, so it cannot be determined if any treatment differences between CHO and CM were due to protein per se. Additionally, the standardized lunch consumed in our trials reduced the overall differences in macronutrient content between the treatments, which may have reduced potential treatment effects.

In conclusion, the additional protein in a carbohydrate-controlled CM beverage can potentially lead to some recovery benefits in youth cyclists, as demonstrated by the absence of elevated post-exercise muscle soreness and energy/fatigue ratings in the CM trials. However, the impact of these findings is diminished by the absence of consistent treatment*time effects on these ratings. CM ingestion did not significantly improve subsequent cycling performance when compared to CHO or PL beverages. Subsequent research should utilize larger sample sizes to provide more conclusive evidence to support/refute the trends observed in this study.

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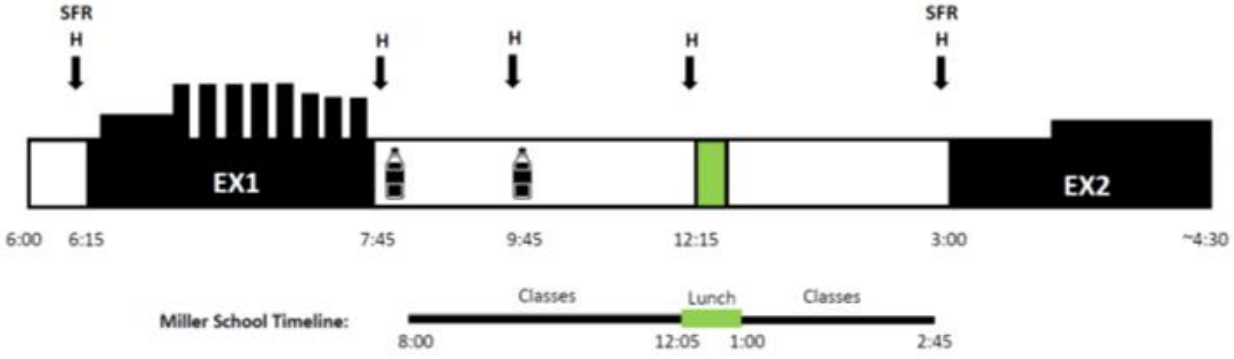
Tables and Figures

Figure 1. Timeline of Protocols during each Experimental Trail



Legend:
EX1 = Preliminary exercise bout; EX2 = Subsequent exercise bout
SFR = Soreness and fatigue ratings; H = Hydration measurements
= Recovery beverage

Table 1. Subject Demographic Data

	Age (y)	Height (cm)	Weight (kg)	Cycling (h/wk)	W_{max} (W)	VO_{2max} (ml/kg/min)
Total (8)	16.1 ± 1.1	174 ± 10	62.9 ± 5.2	9.2 ± 2.1	317 ± 50	61.8 ± 7.7
Females (3)	15.7 ± 0.9	166 ± 6	59.3 ± 5.3	9.7 ± 1.7	256 ± 5	53.3 ± 2.4
Males (5)	16.4 ± 1.0	177 ± 5	65.0 ± 3.7	8.9 ± 2.2	353 ± 20	66.9 ± 4.5

Table 2. Muscle Soreness Ratings Before and After Cycling

Treat	Pre-EX1	4 h Post-EX1	Pre-EX2
PL	44.1 ± 23.1	67.4 ± 22.2*	68.3 ± 19.6*
CHO	37.4 ± 25.7	51.6 ± 27.6#	48.1 ± 24.4
CM	41.0 ± 15.6	46.3 ± 23.0	47.0 ± 27.4

Table excludes 1 subject due to missing data in the placebo trial;
*Within-trial effects: *indicates a significant difference between Pre-EX1; # indicates a trend towards difference compared to Pre-EX1 (p =.051).*

Table 3. Effects of Recovery Beverages on Ratings of Physical Fatigue and Energy

	Physical Fatigue			Physical Energy		
Treatment	**Pre-Ex1**	**Post-4h**	**Pre-Ex2**	**Pre-Ex1**	**Post-4h**	**Pre-Ex2**
Placebo	48 ± 0	61 ± 14	67 ± 10*	51 ± 21	50 ± 17	42 ± 14
CHO	52 ± 14	63 ± 19	55 ± 11	42 ± 19	38 ± 17	43 ± 18
CM	58 ± 9	58 ± 17#	53 ± 16	45 ± 12	37 ± 17	51 ± 18

Table excludes 1 subject due to missing data in the placebo trial.
*Within-trial effects: *indicates a significant difference from Pre-EX1 (p<.05)*
Treatment*time: # indicates a significant interaction of the change in muscle soreness from Pre-Ex1 and Post-4 between CM and CHO

Table 4. Effects of Recovery Beverages on Ratings of Mental Fatigue and Energy

	Mental Fatigue			Mental Energy		
Treatment	**Pre-Ex1**	**Post-4h**	**Pre-Ex2**	**Pre-Ex1**	**Post-4h**	**Pre-Ex2**
Placebo	48 ± 28	49 ± 27	65 ± 11	42 ± 25	46 ± 26	34 ± 16
CHO	52 ± 21	54 ± 16	57 ± 18	31 ± 22	34 ± 16	30 ± 12
CM	60 ± 11	61 ± 18	58 ± 26	43 ± 19	36 ± 17	38 ± 24

Table excludes 1 subject due to missing data in the placebo trial.

Table 6. Physiological Responses during Constant-Load Exercise (179±43W for all trials)

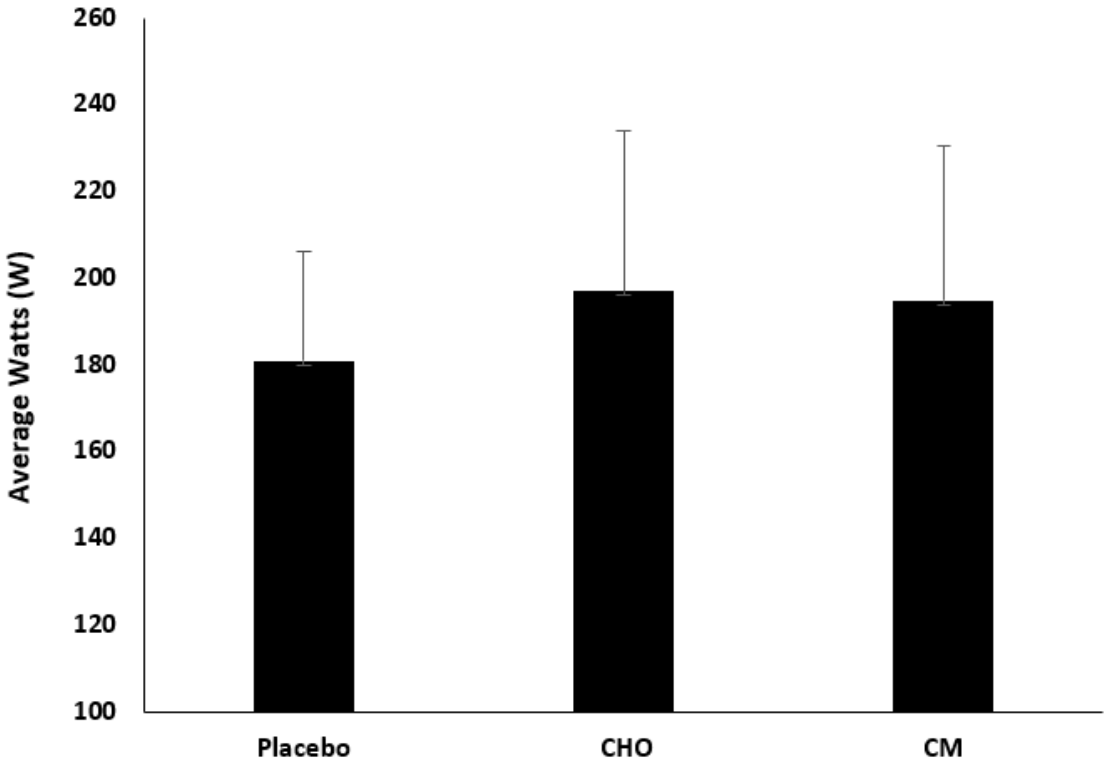
Ex Bout	Trial	HIIT (kj)	VO_2 (ml /min)	Ve (L/min)	RER	HR (bpm)	RPE	Glucose (mmol/L)	Lactate (mmol/L)
Ex1	PL	625 ±177	2427 ±654	65.6 ±14.4	.95 ±.03	159 ±10	14.0 ±1.7	71.0 ±11.9	1.6 ±0.8
	CHO	625 ±177	2670 ±639	71.3 ±14.1	.96 ±.07	158 ±7	13.0 ±2.3	80.3 ±15.5	1.9 ±0.6
	CM	625 ±177	2547 ±492	69.32 ±11.2	.97 ±.02	160 ±15	13.13 ±2.6	73.9 ±18.3	1.7 ±0.7
Ex2	PL	-	2265 ±499	60.62 ±9.7	.96 ±.04	152 ±8	13.00 ±1.0	71.3 ±8.4*	1.3 ±0.3
	CHO	-	2388 ±521	65.14 ±12.9	.99 ±.03	147 ±9	11.75 ±1.7	68.71 ±10.1	1.1 ±0.3
	CM	-	2430 ±556	68.37 ±13.8	.99 ±.01	153 ±12	12.13 ±2.3	61.43 ±11.3	1.3 ±0.3

** indicates a significant difference (p<.05) of PL compared to CHO*

Table 5. Body Weight Changes Following Beverage Consumption

Treatment	Post-Bev1 (lbs)	Pre-Bev2 (lbs)	Δ Pre-Bev2 – Post-Bev1 (lbs)
Placebo	139.7 ± 17.4	139.1 ±17.8	- 0.6 ± 0.6
CHO	139.7 ± 14.0	139.0 ± 4.6	-0.7 ± 0.7
CM	140.4 ± 13.6	139.8 ± 14.2	-0.6 ± 1.0

Figure 2. Effects of Recovery Beverages on Power Output during a 30 km Time-trial



Appendices

Appendix A – 1 (Consent forms for any subjects who are 18 years of age or older)

Influence of Post-Exercise Nutrient Intake on Recovery in Youth Cyclists

Informed Consent

Identification of Investigators & Purpose of Study

You are being asked to participate in a research study conducted by Dr. Mike Saunders and Dr. Nick Luden from James Madison University (JMU). The purpose of this study is to examine the effects of different beverages/sports drinks on post-exercise recovery in youth cyclists.

Research Procedures

Should you decide to participate in this research study, you will be asked to sign this consent form once all your questions have been answered to your satisfaction.

Participants in the study will complete the following activities, all of which will be completed in an exercise room at the Miller School of Albemarle:

Preliminary Visit (~ 1 h)

You will have your height and body weight measured by a researcher. Then, you will complete a cardiovascular fitness test to determine your cardiovascular fitness ("VO_{2max}"). This test will begin at a comfortable exercise intensity, after which the workload will be increased every two minutes until fatigue is reached, determined by either: 1) you request to stop due to fatigue, or 2) inability to maintain a pedal cadence of ≥ 50 rpm. During this test, you will breathe through a mouthpiece/breathing apparatus which collects expired air (10-15 minutes). Heart rate and perceived exertion ratings will be measured and recorded at the end of each stage of the test.

Familiarization Trial (1.5 h)

To ensure familiarity with operation of the stationary bicycle, you will complete an exercise session consisting of a) 50 min of cycling (a mix of moderate-intensity cycling, and high-intensity intervals), b) 15

min rest, c) 25 min of cycling (a 10 min warm-up followed by a 15 min time-trial). No data will be collected during this trial.

3 Exercise/Recovery Trials (3 h each, not including recovery periods)

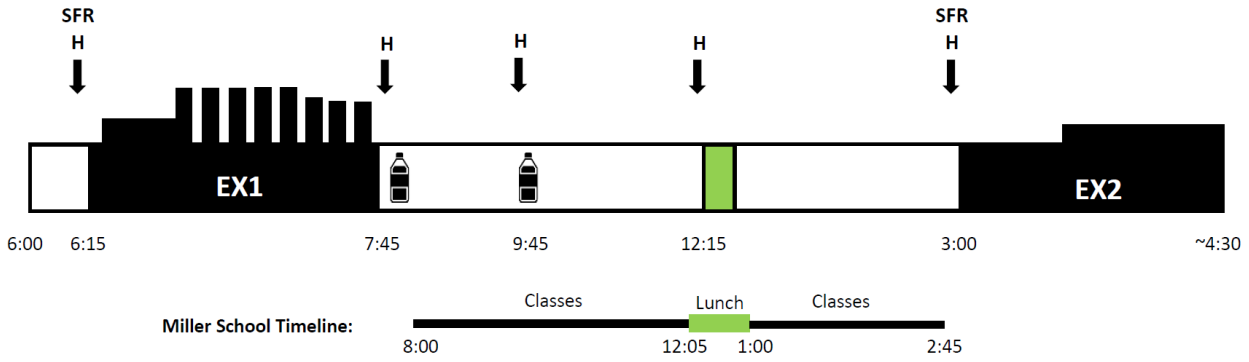
You will complete the exercise/nutrition protocols shown in Figure 1 (below) on three occasions, with a different beverage/sports drink utilized during each trial (~ 1 week between trials).

Briefly, you will complete an initial bout of cycling (EX1), consisting of ~90 min of a mix of moderate-intensity cycling and high-intensity intervals. Following EX1, you will consume a beverage/sports drink immediately following exercise, and 2 h post-exercise (and a standardized lunch 4.5 h post-exercise). A second bout of cycling (EX2), including a simulated 30 km time-trial, will be completed after 7.25 h of recovery, to determine the effectiveness of the beverage/sports drinks on subsequent exercise performance. Testing will be timed such that EX1 occurs before the school day, and EX2 occurs after school (lunch will be consumed at the usual time/place). Further details regarding protocols and measurements are provided below.

Beverages:

A different beverage/sports drink will be provided during each trial. During each trial, you will consume ~700 ml of the respective beverage at the indicated time-points (immediately and 2 h following exercise). Beverages may contain gluten and/or milk products, so you will be provided with a form to alert the researchers to any food allergies to these (or other) items.

Figure 1. Timeline of Protocols during each Experimental Trail



Legend:
EX1 = Preliminary exercise bout; EX2 = Subsequent exercise bout
SFR = Soreness and fatigue ratings; H = Hydration measurements
= Recovery beverage

Measurements:

Exercise performance will be determined from time to complete the 30-km time-trial in EX2.

Measurements such as oxygen consumption, ventilation, etc. will be measured with a breathing device once during EX1 and once during EX2. This will require you to breathe into a mask for 5 minutes on each occasion. In addition, heart rate will be recorded, so you will wear a chest strap with a transmitter during exercise.

Finger stick blood samples will be obtained and assessed for blood glucose and lactate. These measurements will be taken once during EX1 and once during EX2. Each of these samples will be obtained by a fingerstick with a small lancet. A very small amount of blood (~2 drops) will be collected at each time point.

Perception of exertion will be assessed in EX1 and EX2 by having you point to a value on a numbered scale from 6-20.

Ratings of a) muscle soreness and b) mental/physical energy and fatigue will be obtained immediately prior to EX1 and EX2. You will use a pencil to mark their rating of soreness (etc.) on a line that is 100 mm long (i.e. 0 = very low, 100 = very high).

Changes in body weight will be assessed periodically during recovery, to determine changes in hydration following beverage consumption (marked with 'H' on the timeline in Figure 1).

Exercise/Dietary Controls:

You will be instructed to record dietary intake and exercise in written logs for 48 h prior to each trial. To standardize dietary intake participants will be provided with a snack to consume the night before exercise, and another to consume 30 min before exercise. In addition, the foods consumed at lunch from the cafeteria will be held constant during the 3 trials.

Risks

Exercise Tests

According to the American College of Sports Medicine's Guidelines for Exercise Testing and Prescription, people without signs, symptoms, or diagnosis of cardiovascular, metabolic, or renal disease are cleared for vigorous exercise. The conditions that the exercise sessions are to take place are likely safer than the typical exercise environments for competitive cyclists. If you do not meet ACSM criteria of clearance for vigorous exercise (or if you have not received physician's clearance for participation in sport at MSA), you will not be allowed to participate in the study. In the unlikely event of cardiac or other complications during exercise, an emergency plan is in place. This includes immediate access to a phone to call emergency personnel, and an AED during testing. In addition, at least one of the listed investigators will be present during the exercise sessions, and all are CPR certified. You may also experience fatigue and some muscle soreness following each trial, similar to what you experience in their regular cycling training/competition.

Blood Sampling

The risks of blood sampling using finger stick technique include possible mild bruising, and the risk of transfer of blood-borne pathogens, as well as possible risks of infection or skin irritation. These risks are considered to be minimal, and all safety precautions for handing blood samples will be followed according to OSHA protocols, including: investigators will wear nitrile gloves at all times during blood sampling and testing. A sharps container lined with a biohazard bag will be used for all sharp objects involved in the blood sampling; all other materials (i.e. gloves, gauze pads, etc.) used during the sampling will be put in a separate waste disposal unit lined with a biohazard bag. All investigators who will be involved in blood

sampling (and handling of blood) have been trained in these techniques, and completed JMU blood-borne pathogen training. Additional risks include injury to blood vessels, as well as dizziness, fainting, and nausea. A total of <10 ml of blood will be obtained throughout the course of the study, which is roughly 2% of the amount of blood typically obtained during blood donation (1 pint or 473 milliliters).

Benefits

This research will enhance our understanding about the efficacy of nutritional recovery strategies for young athletes, which has inherent potential to provide useful information for youth athletes and their coaches/parents in the future.

Incentives

Upon completion of the graded exercise test, you will be informed of your cardiovascular fitness (VO_{2max}), which is an important measurement fori endurance athletes (and can cost >$100 to obtain privately). In addition, participants who complete the study will receive a free Human Performance Lab t-shirt and $150 in the form of a payment voucher. If you do not complete all testing, this amount will be prorated to $50 for each experimental trial completed.

Confidentiality

The results of this research will be presented in classroom presentations, conferences and research journals. You will be identified in the research records by a code name or number. The researchers retain the right to use and publish non-identifiable data. When the results of this research are published or discussed in conferences, no information will be included that would reveal your identity. All data will be stored in a secure location accessible only to the researchers. All information that matches up your responses with your code number will be stored in a secure location separate from the data.

Participation & Withdrawal

Your participation is entirely voluntary. You are free to choose not to participate. Should you choose to participate, you can withdraw at any time without consequences of any kind.

Questions about the Study

If you have questions or concerns during the time of your participation in this study, or after its completion or you would like to receive a copy of the final aggregate results of this study, please contact Dr. Mike Saunders [saundemj@jmu.edu; (540) 568-8121] or Dr. Nicholas Luden [ludennd@jmu.edu; (540) 568-4069].

For any questions about Your Rights as a Research Subject, please contact Dr. David Cockley:

Chair, Institutional Review Board, James Madison University [cocklede@jmu.edu; (540) 568-2834].

Giving of Consent

I have read this consent form and I understand what is being requested of me as a participant in this study. I freely consent to participate. I have been given satisfactory answers to my questions. The investigator provided me with a copy of this form. I certify that I am at least 18 years of age.

_____

Name of Participant (Printed)

_____ _____

Signature of Participant (Signed) Date

_____ _____

Name of Researcher (Signed) Date

Appendix A – 2 (Consent forms for parents of subjects under 18 years of age)

Influence of Post-Exercise Nutrient Intake on Recovery in Youth Cyclists

Parent/Guardian Informed Consent

Identification of Investigators & Purpose of Study

Your child is being asked to participate in a research study conducted by Dr. Mike Saunders and Dr. Nick Luden from James Madison University (JMU). The purpose of this study is to examine the effects of different beverages/sports drinks on post-exercise recovery in youth cyclists.

Research Procedures

Should you decide to allow your child to participate in this research study, you will be asked to sign this consent form once all your questions have been answered to your satisfaction.

Participants in the study will complete the following activities, all of which will be completed in an exercise room at the Miller School of Albemarle:

Preliminary Visit (~ 1 h)

Your child will have their height and body weight measured by a researcher. Then, they will complete a cycling test to determine their cardiovascular fitness ("VO_{2max}"). This test will begin at a comfortable exercise intensity, after which the workload will be increased every two minutes until fatigue is reached, determined by either: 1) they request to stop due to fatigue, or 2) inability to maintain a pedal cadence of ≥ 50 rpm. During this test, they will breathe through a mouthpiece/breathing apparatus which collects expired air (10-15 minutes). Heart rate and perceived exertion ratings will be measured and recorded at the end of each stage of the test.

Familiarization Trial (1.5 h)

To ensure familiarity with operation of the stationary bicycle, participants will complete an exercise session consisting of a) 50 min of cycling (a mix of moderate intensity cycling, and high-intensity intervals),

b) 15 min rest, c) 25 min of cycling (a 10 min warm-up followed by a 15 min time-trial). No data will be collected during this trial.

3 Exercise/Recovery Trials (3 h each, not including recovery periods)

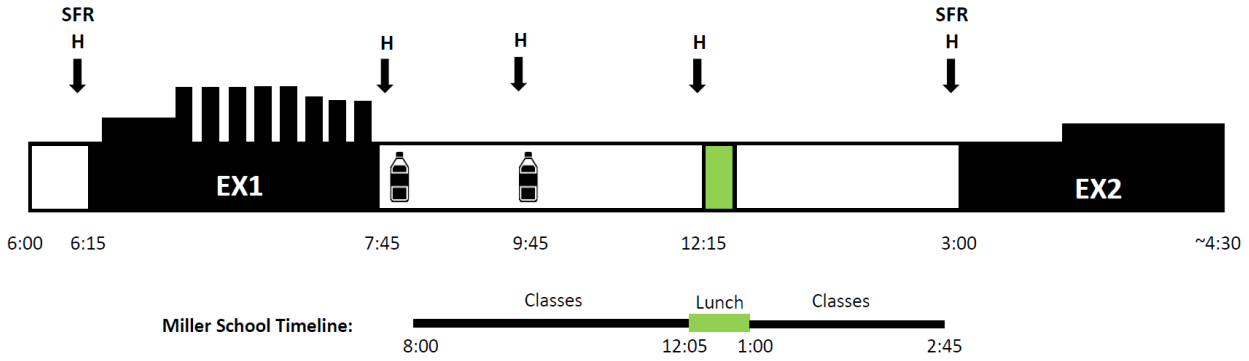
Participants will complete the exercise/nutrition protocols shown in Figure 1 (below) on three occasions, with a different beverage/sports drink utilized during each trial (~ 1 week between trials).

Briefly, participants will complete an initial bout of cycling (EX1), consisting of ~90 min of a mix of moderate-intensity cycling and high-intensity intervals. Following EX1, participants will consume a beverage/sports drink immediately following exercise, and 2 h post-exercise (and a standardized lunch 4.5 h post-exercise). A second bout of cycling (EX2), including a simulated 30 km time-trial, will be completed after 7.25 h of recovery, to determine the effectiveness of the beverage/sports drinks on subsequent exercise performance. Testing will be timed such that EX1 occurs before the school day, and EX2 occurs after school (lunch will be consumed at the usual time/place). Further details regarding protocols and measurements are provided below.

Beverages:

A different beverage/sports drink will be provided during each trial. During each trial, participants will consume ~700 ml of the respective beverage at the indicated time-points (immediately and 2 h following exercise). Beverages may contain gluten and/or milk products, so participants will be provided with a form to alert the researchers to any food allergies to these (or other) items.

Figure 1. Timeline of Protocols during each Experimental Trail



Legend:
EX1 = Preliminary exercise bout; EX2 = Subsequent exercise bout
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= Recovery beverage

Measurements:

Exercise performance will be determined from time to complete the 30-km time-trial in EX2.

Measurements such as oxygen consumption, ventilation, etc. will be measured with a breathing device once during EX1 and once during EX2. This will require the participant to breathe into a mask for 5 minutes on each occasion. In addition, heart rate will be recorded, which will require the participant to wear a chest strap with a transmitter during exercise.

Finger stick blood samples will be obtained and assessed for blood glucose and lactate. These measurements will be taken once during EX1 and once during EX2. Each of these samples will be obtained by a fingerstick with a small lancet. A very small amount of blood (~2 drops) will be collected at each time point.

Ratings of exertion will be assessed in EX1 and EX2 by having the participant point to a value on a numbered scale from 6-20.

Ratings of a) muscle soreness and b) mental/physical energy and fatigue will be obtained immediately prior to EX1 and EX2. Participants will use a pencil to mark their rating of soreness (etc.) on a line that is 100 mm long (i.e. 0 = very low, 100 = very high).

Changes in body weight will be assessed periodically during recovery, to determine changes in hydration following beverage consumption (marked with 'H' on the timeline in Figure 1).

Exercise/Dietary Controls:

Participants will be instructed to record dietary intake and exercise in written logs for 48 h prior to each trial. To standardize dietary intake participants will be provided with a snack to consume the night before exercise, and another to consume 30 min before exercise. In addition, the foods consumed at lunch from the cafeteria will be held constant during the 3 trials.

Risks

Exercise Tests

According to the American College of Sports Medicine's Guidelines for Exercise Testing and Prescription, people without signs, symptoms, or diagnosis of cardiovascular, metabolic, or renal disease are cleared for vigorous exercise. The conditions that the exercise sessions are to take place are likely safer than the typical exercise environments for competitive cyclists. If your child does not meet ACSM criteria of clearance for vigorous exercise (or if they have not received physician's clearance for participation in sport at MSA), they will not be allowed to participate in the study. In the unlikely event of cardiac or other complications during exercise, an emergency plan is in place. This includes immediate access to a phone to call emergency personnel, and an AED during testing. In addition, at least one of the listed investigators will be present during the exercise sessions, and all are CPR certified. Your child may also experience fatigue and some muscle soreness following each trial, similar to what they experience in their regular cycling training/competition.

Blood Sampling

The risks of blood sampling using finger stick technique include possible mild bruising, and the risk of transfer of blood-borne pathogens, as well as possible risks of infection or skin irritation. These risks are considered to be minimal, and all safety precautions for handing blood samples will be followed according to OSHA protocols, including: investigators will wear nitrile gloves at all times during blood sampling and testing. A sharps container lined with a biohazard bag will be used for all sharp objects involved in the blood sampling; all other materials (i.e. gloves, gauze pads, etc.) used during the sampling will be put in a separate waste disposal unit lined with a biohazard bag. All investigators who will be involved in blood sampling (and handling of blood) have been trained in these techniques, and completed JMU blood-borne pathogen training. Additional risks include injury to blood vessels, as well as dizziness, fainting, and

nausea. A total of <10 ml of blood will be obtained throughout the course of the study, which is roughly 2% of the amount of blood typically obtained during blood donation (1 pint or 473 milliliters).

Benefits

This research will enhance our understanding about the efficacy of nutritional recovery strategies for young athletes, which has inherent potential to provide useful information for youth athletes and their coaches/parents in the future.

Incentives

Upon completion of the graded exercise test, participants will be informed of their cardiovascular fitness (VO_{2max}), which is generally of great interest to trained endurance athletes (and can cost >$100 to obtain privately). In addition, participants will receive a free Human Performance Lab t-shirt and $150 in the form of a payment voucher. In the case of those who do not complete all testing, this amount will be prorated to $50 for each experimental trial completed.

Confidentiality

The results of this research will be presented in classroom presentations, conferences and research journals. Your child will be identified in the research records by a code name or number. The researchers retain the right to use and publish non-identifiable data. When the results of this research are published or discussed in conferences, no information will be included that would reveal your child's identity. All data will be stored in a secure location accessible only to the researchers. All information that matches up individual respondents with their code number will be stored in a secure location separate from their data.

Participation & Withdrawal

Your child's participation is entirely voluntary. He/she is free to choose not to participate. Should you and your child choose to participate, he/she can withdraw at any time without consequences of any kind.

Questions about the Study

If you have questions or concerns during the time of your child's participation in this study, or after its completion or you would like to receive a copy of the final aggregate results of this study, please contact Dr. Mike Saunders [saundemj@jmu.edu; (540) 568-8121] or Dr. Nicholas Luden [ludennd@jmu.edu; (540) 568-4069].

For any questions about Your Rights as a Research Subject, please contact Dr. David Cockley:

Chair, Institutional Review Board, James Madison University [cocklede@jmu.edu; (540) 568-2834].

Giving of Consent

I have read this consent form and I understand what is being requested of my child as a participant in this study. I freely consent for my child to participate. I have been given satisfactory answers to my questions. The investigator provided me with a copy of this form. I certify that I am at least 18 years of age.

_____

Name of Child (Printed)

_____

Age of Child (Printed)

_____

Name of Parent/Guardian (Printed)

_____ _____

Name of Parent/Guardian (Signed) Date

_____ _____

Name of Researcher (Signed) Date

Appendix B

YOUTH ASSENT FORM (Ages 14-17)

IRB #

Influence of Post-Exercise Nutrient Intake on Recovery in Youth Cyclists

We are inviting you to participate in this study because you are a competitive cyclist at the Miller School of Albemarle, and we are interested in studying the effects of different beverages/sports drinks on post-exercise in competitive youth cyclists.

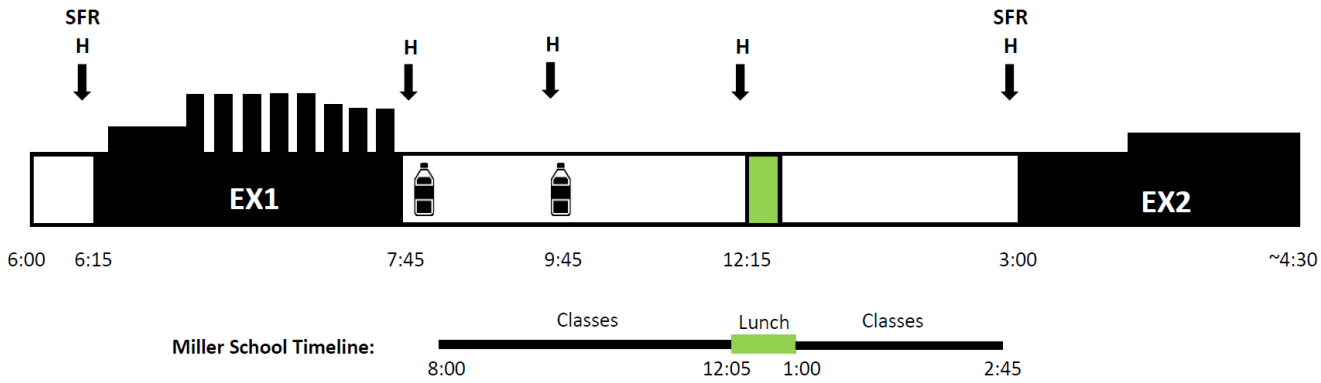
If you participate in this study, you will complete five different exercise/testing days over the course of one month. These testing days will consist of the following:

Trial 1: Preliminary visit (1 hr) – You will have your height and body weight measured by a researcher. Then, you will complete a fitness test on a stationary bike in which the workload will be increased every two minutes until fatigue (or you request to stop). During this test, you will breathe through a mask, and wear a chest-strap to measure heart rate.

Trial 2: Familiarization Trial (1.5 h) – You will complete an exercise session consisting of a) 50 min of cycling (a mix of moderate intensity cycling, and high-intensity intervals), b) 15 min rest, c) 25 min of cycling (a 10 min warm-up followed by a 15 min time-trial). No data will be collected during this trial.

Trials 3-5 Exercise/Recovery Trials (see below) – You will complete the exercise and nutrition protocols shown in Figure 1 (below). This will consist of an exercise session before school (EX1), and an exercise session after school (EX2). EX1 will consist of ~90 min of moderate-intensity cycling and high-intensity intervals. EX2 will include a 30 km time-trial (after a warm-up and some moderate effort riding). You will consume food and fluid intake from the researchers on these days, including beverage/sports drinks consumed after exercise, and a standardized lunch from the cafeteria. A different beverage/sports drink will be provided during each trial.

Figure 1. Timeline of Exercise/Recovery Trials



Legend:
EX1 = Preliminary exercise bout; EX2 = Subsequent exercise bout
SFR = Soreness and fatigue ratings; H = Hydration measurements
= Recovery beverage

Measurements:

Your time to complete the 30-km time-trial in EX2 will be measured. You will be asked to provide a maximal effort during this trial (i.e. treat it like a race).

You will breathe into a mask for 5 minutes during EX1 (and again during EX2) to measure oxygen consumption. In addition, you will wear a chest strap during exercise to measure heart rate.

Blood samples will be obtained once during EX1 and once during EX2. To do so, the researcher will perform a fingerstick with a small lancet to obtain a very small amount of blood (~2 drops).

You will be asked to provide your ratings of a) exertion/effort, b) muscle soreness, and c) energy/fatigue at different times during exercise, or before/after exercise.

Changes in your body weight will be measured periodically during recovery, to determine changes in hydration following beverage consumption.

You will also be asked to record your dietary intake and exercise in written logs for 48 h prior to each trial. You will be provided with a snack to consume the night before each exercise trial, and on the morning of each exercise trial.

Risks/Benefits:

There are health risks associated with maximal exercise (i.e. muscle soreness, and in rare cases, heart attacks, etc.), but the risks of exercise in this study are probably less than those during a typical cycling race. In addition, there are small risks of infection or skin irritation from the finger-stick blood draws. However, these risks are minimal, and safety procedures will be followed to minimize any risks to participants.

This research will enhance our understanding about recovery strategies for young athletes, which can provide useful information for youth athletes and their coaches/parents in the future.

Benefits to participants include:

a) Information about your cardiovascular fitness (VO_{2max}), an important training variable for cyclists
b) A free Human Performance Lab t-shirt
c) $150 in the form of a payment voucher (if you are unable to complete the entire study, you will receive $50 for each exercise/recovery trial completed).

Additional Information

Your individual data will be completely confidential. No information will be revealed in presentations or publications of the data that will reveal your identity.

We have asked your parents for their permission for you to do this study. Please talk this over with them before you decide whether or not to participate.

If you have any questions at any time, please ask one of the researchers.

If you check "yes," it means that you have decided to participate and have read everything that is on this form. You and your parents will be given a copy of this form to keep.

_____ Yes, I would like to participate in the study.

_____ _____

Signature of Subject Date

_____ _____

Signature of Investigator Date

If you have any questions or concerns, please contact Dr. Mike Saunders at saundemj@jmu.edu and (540) 568-8121 or Dr. Nicholas Luden at ludennd@jmu.edu and (540) 568-4069.

Appendix C

(Preliminary Recruitment e-mail - content to be included in e-mail from MSA coaches)

Dear MSA Endurance Team Parent,

We would like to make you aware of an opportunity for your child to participate in an interesting study during this academic year. Dr. Mike Saunders, a Professor of Exercise Physiology from James Madison University, will be conducting a study on the *Influence of Post-Exercise Nutrient Intake on Recovery in Youth Cyclists* at the Miller School in 2018-19. This study will be conducted on the MSA campus, and will not interfere with your child's class schedule.

Additional information about the study is provided in the attached forms, and Dr. Saunders will be at the Miller School in late August to provide information about the study to our athletes, and answer any questions. If your child is aged 14-18 and interested in participating, please review the attached documents, and encourage your child to attend the information session with Dr. Saunders. Benefits for participants include: a) a free test of aerobic capacity during cycling (VO_{2max}), which usually costs ~ $100, b) $150 for study completion, c) a free JMU 'Human Performance Lab' t-shirt, and d) training data regarding post-exercise recovery and nutrition. Individuals who wish to participate must complete the attached forms (parental informed consent, and youth assent) before they can be included in the study. Please note that participation in this study is entirely voluntary, and your child is under no pressure to participate.

Appendix D

(Prescreening Questionnaire – To be completed prior to acceptance into the study)

Subject Prescreening Information

Age:_____ years

Height_____ Weight_____

Typical Exercise Habits over the Past 2 Months:

Average number of days of cycling exercise per week:_____

Average number of hours cycling per week:_____

How many cycle rides ≥ 2 hr in past 8 weeks:_____

Average number of days of running exercise per week:_____

Average number of hours running per week:_____

Briefly describe your aerobic exercise habits over the past 2 months:

Briefly describe your resistance training habits over the past 2 months:

Food Allergies:

Allergic to gluten? _____ If yes, provide details:_____

_____

Allergic to milk/milk products:_____ If yes, provide details:_____

_____

Describe any other food allergies:_____

_____

_____

Do you have a muscle or joint injury/condition that may prevent completion of the exercise described in this study? If yes, please explain.

Appendix E

2017 PAR-Q+

FOLLOW-UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

1. Do you have Arthritis, Osteoporosis, or Back Problems?

If the above condition(s) is/are present, answer questions 1a-1c If **NO** ☐ go to question 2

1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES☐ NO☐
1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	YES☐ NO☐
1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	YES☐ NO☐

2. Do you currently have Cancer of any kind?

If the above condition(s) is/are present, answer questions 2a-2b If **NO** ☐ go to question 3

2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and/or neck?	YES☐ NO☐
2b.	Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)?	YES☐ NO☐

3. Do you have a Heart or Cardiovascular Condition? *This includes Coronary Artery Disease, Heart Failure, Diagnosed Abnormality of Heart Rhythm*

If the above condition(s) is/are present, answer questions 3a-3d If **NO** ☐ go to question 4

3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES☐ NO☐
3b.	Do you have an irregular heart beat that requires medical management? (e.g., atrial fibrillation, premature ventricular contraction)	YES☐ NO☐
3c.	Do you have chronic heart failure?	YES☐ NO☐
3d.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	YES☐ NO☐

4. Do you have High Blood Pressure?

If the above condition(s) is/are present, answer questions 4a-4b If **NO** ☐ go to question 5

4a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES☐ NO☐
4b.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer **YES** if you do not know your resting blood pressure)	YES☐ NO☐

5. Do you have any Metabolic Conditions? *This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes*

If the above condition(s) is/are present, answer questions 5a-5e If **NO** ☐ go to question 6

5a.	Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies?	YES☐ NO☐
5b.	Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness.	YES☐ NO☐
5c.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, **OR** the sensation in your toes and feet?	YES☐ NO☐
5d.	Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease, or liver problems)?	YES☐ NO☐
5e.	Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future?	YES☐ NO☐

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6. **Do you have any Mental Health Problems or Learning Difficulties?** *This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome*

If the above condition(s) is/are present, answer questions 6a-6b — If **NO** ☐ go to question 7

6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
6b.	Do you have Down Syndrome **AND** back problems affecting nerves or muscles?	YES ☐ NO ☐

7. **Do you have a Respiratory Disease?** *This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure*

If the above condition(s) is/are present, answer questions 7a-7d — If **NO** ☐ go to question 8

7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
7b.	Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	YES ☐ NO ☐
7c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	YES ☐ NO ☐
7d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	YES ☐ NO ☐

8. **Do you have a Spinal Cord Injury?** *This includes Tetraplegia and Paraplegia*

If the above condition(s) is/are present, answer questions 8a-8c — If **NO** ☐ go to question 9

8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
8b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	YES ☐ NO ☐
8c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	YES ☐ NO ☐

9. **Have you had a Stroke?** *This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event*

If the above condition(s) is/are present, answer questions 9a-9c — If **NO** ☐ go to question 10

9a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
9b.	Do you have any impairment in walking or mobility?	YES ☐ NO ☐
9c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	YES ☐ NO ☐

10. **Do you have any other medical condition not listed above or do you have two or more medical conditions?**

If you have other medical conditions, answer questions 10a-10c — If **NO** ☐ read the Page 4 recommendations

10a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months **OR** have you had a diagnosed concussion within the last 12 months?	YES ☐ NO ☐
10b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	YES ☐ NO ☐
10c.	Do you currently live with two or more medical conditions?	YES ☐ NO ☐

PLEASE LIST YOUR MEDICAL CONDITION(S) AND ANY RELATED MEDICATIONS HERE: _____

_____

GO to Page 4 for recommendations about your current medical condition(s) and sign the PARTICIPANT DECLARATION.

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If you answered NO to all of the follow-up questions about your medical condition, you are ready to become more physically active - sign the PARTICIPANT DECLARATION below:

- It is advised that you consult a qualified exercise professional to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually - 20 to 60 minutes of low to moderate intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate intensity physical activity per week.
- If you are over the age of 45 yr and NOT accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.

If you answered **YES** to **one or more of the follow-up questions** about your medical condition:

You should seek further information before becoming more physically active or engaging in a fitness appraisal. You should complete the specially designed online screening and exercise recommendations program - the **ePARmed-X+** at www.eparmedx.com and/or visit a qualified exercise professional to work through the ePARmed-X+ and for further information.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at www.eparmedx.com before becoming more physically active.
- Your health changes - talk to your doctor or qualified exercise professional before continuing with any physical activity program.

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The authors, the PAR-Q+ Collaboration, partner organizations, and their agents assume no liability for persons who undertake physical activity and/or make use of the PAR-Q+ or ePARmed-X+. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.

PARTICIPANT DECLARATION

- All persons who have completed the PAR-Q+ please read and sign the declaration below.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that a Trustee (such as my employer, community/fitness centre, health care provider, or other designate) may retain a copy of this form for their records. In these instances, the Trustee will be required to adhere to local, national, and international guidelines regarding the storage of personal health information ensuring that the Trustee maintains the privacy of the information and does not misuse or wrongfully disclose such information.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

For more information, please contact
www.eparmedx.com
Email: eparmedx@gmail.com

citation for PAR-Q+
Warburton DER, Jamnik VK, Bredin SSD, and Gledhill N on behalf of the PAR-Q+ Collaboration. The Physical Activity Readiness Questionnaire for Everyone (PAR-Q+) and Electronic Physical Activity Readiness Medical Examination (ePARmed-X+). Health & Fitness Journal of Canada 4(2):3-23, 2011.

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The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+ Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or the BC Ministry of Health Services.

01-01-2017

Appendix F – Muscle Soreness

Muscle Soreness Questionnaire

Subject #_____ Trial # Timepoint:_____Date:_____

Muscle Soreness

➔Please place a mark on the line below corresponding to your level of muscle soreness

0 millimeters (left) = complete absence of muscular soreness

100 millimeters (right) = extremely sore with noticeable pain and stiffness at all times

0 mm _____ 100 mm

Appendix G – MPSTEFS Questionnaire

(note: formatting corrupted by insertion – originals have 100 mm lines separating the ratings)

Fatigue/Energy Scales

Subject #: _____ Trial # :_____ Date: _____

Directions. This part of the questionnaire asks about your current feelings of energy and fatigue. We are interested in how you feel right now, even if it is different than how you usually feel. Therefore, it is important that you focus on how you feel right now at this moment in responding to each item. There are no right or wrong answers. Please be as honest and accurate as possible in your responses. Make a vertical line through each horizontal line below to indicate the intensity of your current feelings. If you have a complete absence of the feeling described then place a vertical mark at the left edge of the horizontal line. If your feelings are the strongest intensity that you have ever experienced then place a vertical mark at the right edge of the horizontal line. If your feelings are between these two extremes, then use the distance from the left edge to represent the intensity of your feelings.

How do you feel right now with regard to your capacity to perform your typical ***PHYSICAL ACTIVITIES***....

29. I feel I have no energy _____ Strongest feelings of energy ever felt

30. I feel no fatigue _____ Strongest feelings of fatigue ever felt

31. I feel I have no vigor _____ Strongest feelings of vigor ever felt

32. I feel no exhaustion _____ Strongest feelings of exhaustion ever felt

33. I feel I have no pep _____ Strongest feelings of pep ever felt

34. I have no feelings of being worn out _____ Strongest feelings of being worn out ever felt

How do you feel right now with regard to your capacity to perform your typical ***MENTAL ACTIVITIES***....

35. I feel I have no energy _____ Strongest feelings of energy ever felt

36. I feel no fatigue _____ Strongest feelings of fatigue ever felt

37. I feel I have no vigor _____ Strongest feelings of vigor ever felt

38. I feel no exhaustion _____ Strongest feelings of exhaustion ever felt

39. I feel I have no pep _____ Strongest feelings of pep ever felt

40. I have no feelings of being worn out _____ Strongest feelings of being worn out ever felt

Note to administrator - horizontal lines must be 10 cm in length, photocopying can change the length

Appendix H

24-HOUR DIET RECORD

*Subject number*_____ *Date*_____ *Day of Week*_____

Time	Food and/or Drink	Method of Preparation	Quantity Consumed	

Adapted From: Lee RD, Nieman DC. *Nutritional Assessment.* 2nd ed. United States of America: Mosby; 1996

Appendix H

Physical Activity Records

Subject #_____ Trial #_____ Date:_____

Date	Type of Exercise Performed	Duration of Exercise (minutes)	Intensity of Exercise (use scale below)

Intensity Scale

6

7 Very, very light

8

9 Very light

10

11 Fairly light

12

13 Somewhat hard

14

15 Hard

16

17 Very hard

18

19 Very, very hard

20

Appendix I – Site Coordinator Letter of Permission

Note: Signatory was out of town at the time of proposal submission. A signed copy of this form from a Miller School official will be submitted prior to IRB evaluation of this proposal

Site Coordinator Letter of Permission

6/7/18

Institutional Review Board

James Madison University

MSC 5738

601 University Boulevard

Harrisonburg, VA 22807

Dear Institutional Review Board,

I hereby agree to allow Dr. Mike Saunders, from James Madison University to conduct his research at The Miller School of Albemarle, in Charlottesville, VA. I understand that the purpose of the study is to investigate the effects of nutrient intake on post-exercise recovery in youth cyclists.

By signing this letter of permission, I am agreeing to the following:

☒ JMU researcher(s) have permission to be on The Miller School of Albemarle premise, to conduct data collection for their research project.

☒ JMU researcher(s) have access to the data collected to perform the data analysis both for presentation to The Miller School of Albemarle and/or for publication purposes.

Sincerely,

Name of Authorized Individual, Title

The Miller School of Albemarle

_____

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