Section: Original Research

Article Title: The Effect of Post-Exercise Milk Protein Intake on Rehydration of Children

Authors: Kimberly A. Volterman[1], Daniel R. Moore[2], Joyce Obeid[1], Elizabeth A. Offord[3], and Brian W. Timmons[1]

Affiliations: [1]Department of Pediatrics, McMaster University, Hamilton, Ontario, Canada. [2]Department of Kinesiology and Physical Education, University of Toronto, Toronto, Ontario, Canada. 3Nestle Research Centre, Lausanne, Switzerland.

Running Head: Protein intake and rehydration in children

Journal: *Pediatric Exercise Science*

Acceptance Date: August 20, 2015

DOI: http://dx.doi.org/10.1123/pes.2015-0078

ABSTRACT

Purpose: In adults, rehydration after exercise in the heat can be enhanced with a protein-containing beverage; however, whether this applies to children remains unknown. This study examined the effect of milk protein intake on post-exercise rehydration in children. **Method:** Fifteen children (10-12 years) performed three exercise trials in the heat (34.4 ± 0.2 °C, 47.9 ± 1.1% relative humidity). In a randomized, counterbalanced crossover design, participants consumed iso-caloric and electrolyte-matched beverages containing 0-g (CONT), 0.76-g (Lo-PRO) or 1.5-g (Hi-PRO) of milk protein/100mL in a volume equal to 150% of their body mass (BM) loss during exercise. BM was then assessed over 4 h of recovery. **Results:** Fluid balance demonstrated a significant condition × time interaction ($p = 0.012$) throughout recovery; Hi-PRO was less negative than CONT at 2 h ($p = 0.01$) and tended to be less negative at 3 h ($p = 0.07$). Compared to CONT, beverage retention was enhanced by Hi-PRO at 2 h ($p < 0.05$). **Conclusion:** A post-exercise beverage containing milk protein can favourably affect fluid retention in children. Further research is needed to determine the optimal volume and composition of a rehydration beverage for complete restoration of fluid balance.

KEY WORDS: dehydration, adolescents, fluid balance, heat

INTRODUCTION

Proper hydration is important in maintaining normal physiological and mental functions, particularly during exercise in the heat. When fluid replacement does not sufficiently match the exercise- and heat-induced sweat losses, dehydration during competitions (3) or practices (30) can occur. This result is an increase in cardiovascular and thermoregulatory strain, increase in perceived effort, and decrease in physical performance (9). In fact, previous research reports that dehydration as little as 1% of BM loss can impair cycling performance by 15% in young boys (10-12 years of age), whereas 2% dehydration leads to a 20% reduction (44). Other sport-specific skilled performance in young athletes (12- to 15-years) can also be compromised by similar degrees of dehydration (10).

Children typically do not consume sufficient fluid *ad libitum* (even with access to fluids) to avoid dehydration (4, 31). Traditionally, the optimal rehydration program (if time and opportunity permits) is to consume a sufficient volume of fluid in conjunction with normal meals and snacks (2). As the nature of youth sports is often characterized by performing periods of exercise or competition close together (i.e., tournament format, sport camps, 2-a-days), return to activity could occur within hours of the previous bout. Given the aforementioned negative effects of dehydration, ensuring ample hydration and complete restoration of fluid balance, even from as little as 1-2% body fluid loss, prior to a subsequent bout of exercise is of paramount importance for children. Moreover, active children commonly present to both practices and competitions with a mild level of chronic dehydration (i.e., urine specific gravity (U_{sg}) > 1.020) (18) suggesting adequate rehydration does not occur during the recovery intervals of repeated physical activity. Together, these findings suggest that youth are at a high risk of experiencing negative carryover effects from previous-day and/or same-day physical activity (7) and highlights the importance of optimizing post-exercise rehydration. Although pediatric hydration guidelines have been published outlining recommendations for fluid consumption during activity (1, 26, 33), there is much less information pertaining to fluid recommendations during the acute post-exercise period. As such, it is often advised that young athletes adhere to similar recommendations as those given to adult athletes (26).

Despite the importance of the acute post-exercise rehydration period, there still remains a large gap in our understanding of how best to rehydrate children after exercise-induced fluid loss, which is a pre-requisite to providing evidence-based recommendations for youth. For optimal rehydration, it is important to consider both the volume and composition of the beverage. Research in adults suggests that in addition to the electrolyte content, the macronutrient profile of a beverage may also influence rehydration (19, 43). For example, fluid retention after exercise in the heat can be enhanced with a protein-containing beverage in adults (14, 35, 41). In adults, consumption of skim milk after an exercise-induced dehydration of ~2% improved fluid and electrolyte balance to a greater extent after 3-4 h of recovery compared to an iso-volume of water or a carbohydrate-electrolyte solution (CES) (41). Recently, it was demonstrated that compared to an iso-volume (i.e. 100% of fluid loss) of water and CES, children also experience an increase in fluid balance and fluid retention following post-exercise consumption of milk (42). However, due to the vast compositional differences (electrolyte composition, energy density, and macronutrient composition) between beverages, it is unclear which components of the skim milk resulted in the reported increase in fluid retention. Recent research in adults suggests a potential independent role for protein (specifically, milk protein) to enhance rehydration (14, 35); to date, the importance of protein content *per se* of a rehydration beverage has not been investigated in children. Thus, a better understanding of the role of milk protein in replacing body fluid losses after exercise in the heat in active children is warranted.

The purpose of the present study was to examine the effect of varying levels of milk protein intake on rehydration when consumed as a post-exercise beverage following exercise-induced fluid loss (with the goal of achieving body fluid losses up to ~2% of BM) in children. This knowledge will be important for the advancement of pediatric-specific guidelines, as well as nutritional recommendations for children, their parents, and coaches. We hypothesized that rehydration would follow a protein dose-dependent response, with higher milk protein content resulting in more effective rehydration.

METHODS

Participants. Fifteen (8 females, 7 males) volunteers participated in this study approved by the Research Ethics Board. Each participant provided written informed assent and written informed consent was obtained from each parent prior to enrolment in the study. Inclusion criteria required participants to be healthy and physically active (as determined by medical and activity questionnaire), between 10-12 years of age, and to achieve a minimum aerobic fitness based on maximal aerobic power (VO_{2max}) of 35 $ml \cdot kg^{-1} \cdot min^{-1}$. Participants were defined as physically active by participation in organized sport outside of school requirements more than twice per week. Exclusion criteria included participants currently taking any medication, food allergy to milk proteins (e.g. whey or casein), or participants > ~2 years from age of peak height velocity (YPHV).

General overview. A repeated measures crossover design was incorporated such that each participant consumed each of the experimental beverages in a randomized, double-blind fashion. Participants reported to the laboratory on 5 separate occasions: a preliminary screening visit, a familiarization visit, and three identical experimental trials. The experimental trials were separated by a 4-10 d washout. A schematic representation of the experimental trials is provided in **Figure 1**.

Preliminary visit. A preliminary session was completed to determine each participant's height (Harpenden wall-mounted Stadiometer), BM (BWB-800, Tanita Corp., Japan), percent body fat (InBody520 bioelectrical impedance analyzer; Biospace Co., California, USA), body mass index (BMI), chronological age and maturity offset, the latter of which was estimated as YPHV (27). Mean ± SD for these variables were: 150.4 ± 7.0 cm, 39.3 ± 5.8 kg, 15.7 ± 5.7 %, 17.5 ± 1.8 $kg{\cdot}m^{-2}$, 11.6 ± 0.6 years, -1.3 ± 0.9 YPHV, respectively. Since menstrual cycle does not appear to have an impact on fluid replacement after exercise-induced dehydration (20), neither age at menarche nor phase of menstrual cycle were considered when scheduling participants. An assessment of VO_{2max} was then conducted using an All-Out Progressive Cycling Test on a cycle ergometer (Fleisch-Metabo, Geneva, Switzerland), as previously described (5). Mean ± SD for VO_{2max} was 45.0 ± 7.0 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$.

Familiarization visit. At the beginning of the familiarization visit, each participant performed a blind taste testing of each of the 3 experimental beverages, in a randomized order, to assess whether the addition of milk protein affected the taste and palatability of the beverage. Participants were asked to assess beverage taste, overall beverage preference, drink sweetness, drink saltiness, and drink sourness (by analog and category scales, as previously described (25)). Following beverage tasting, the fastest running speed on a treadmill was determined as described previously (28). The remainder of the familiarization session was based on the same format as the experimental sessions (see below, excluding blood samples), with a shortened post-exercise recovery period. Prior to leaving the laboratory, participants were given a log book to record food and beverage consumption for the 24 h prior to their first experimental visit, and were then asked to replicate this diet and approximate eating times prior to the subsequent experimental trials. Participants were asked to avoid any caffeine and strenuous physical activity for 12 and 24 h, respectively, before each of the experimental trials.

Experimental Trials. Participants arrived to the laboratory between 07:30 h and 09:30 h following an overnight (minimum 10 h) fast and provided a baseline spot-urine sample to estimate pre-exercise hydration status. The U_{sg} was determined using a handheld clinical refractometer (ATAGO, Model SUR-NE). A baseline blood sample (10 mL) was collected via individual venipuncture into a chilled EDTA-containing vacutainer and placed on ice. Within 20 min of sampling, tubes were centrifuged (2,000×g) for 20 min. Plasma was stored at -20°C until further analysis. Participants then consumed a small standardized breakfast (a Boost meal replacement drink; Boost Meal Replacement, Nestle Canada Inc., North York, Ont., Canada) and, if extra calories were required, a calculated volume of a Chubby carbonated beverage (Chubby, S.M. Jaleel & Co. Ltd., Florida, USA) providing ~12 and ~15% of their predicted daily caloric and protein intake from food (i.e. not including the experimental beverages), respectively, along with 5 $ml \cdot kg^{-1}$ BM of water. Additional details of the standardized diet have been described elsewhere (28). If participants were determined to be in a hypohydrated state upon arrival to the laboratory (baseline spot-urine $U_{sg} > 1.020$) (8), an additional 2 $ml \cdot kg^{-1}$ BM of water was provided with breakfast. Participants then rested for 60 min in a thermoneutral room. After 30 min of rest, a second urine sample was collected, following which participants' nude BM (representing the pre-exercise BM) and mass of their exercise clothes (including shoes) were taken. Participants were asked to wear the same exercise clothes for each experimental session, with boys exercising shirtless wearing light shorts and girls wearing light shorts and a bikini top/sports bra.

To achieve a dehydration target of up to 2%, defined as an acute reduction in pre-exercise to post-exercise BM, each participant performed 45 min of alternating running and cycling exercise (Figure 1) in a climate chamber (34.4 ± 0.2 °C, 47.9 ± 1.1% relative humidity) with restricted fluid intake. A privacy screen was provided for the collection of in-chamber urine samples, as necessary, and scheduled BM measurements (Figure 1).

Immediately upon exiting the chamber, participants were asked to dry any surface sweat from their bodies and void their bladder prior to the measurement of their post-exercise nude BM. The mass of the post-exercise dry clothes was subsequently recorded. Participants then sat comfortably in a thermoneutral room for the remainder of their visit. Starting 15 min post-exercise, participants orally consumed the experimental beverage in 3 equal aliquots provided every 15 min for a total volume of 150% of the body fluids lost during exercise (measured as a change in BM, with 100g = 100mL of fluid). Because there are no pediatric-specific rehydration guidelines, our fluid replacement scheme was based on the current recommendations of the American College of Sports Medicine (ACSM) consensus statement for rapid (i.e. within 4-6 h) rehydration in adults (2).

Each experimental trial was identical except for the post-exercise beverage consumed. Beverages were provided by Nestec Ltd. (Lausanne, Switzerland) in blinded packages in powder form and were reconstituted in 450mL of deionized water. Beverage characteristics are provided in **Table 1.** Briefly, the experimental beverages consisted of a blend of carbohydrates (sucrose) and milk protein (both whey and casein protein fractions in a ratio of ~1:4) concentrates (skim or full fat for Hi-PRO and Lo-PRO, respectively) at a concentration of 0 g/100 mL (CONT), 0.76

g/100 mL (Lo-PRO) or 1.5 g/100 mL (Hi-PRO). At the time of study conception, previous research had shown that fluid retention and rehydration after exercise was enhanced with beverages containing milk protein at 1.5-2.5% (weight:volume) in adults (14, 35). Therefore, to determine the potential minimum protein content required to similarly enhance fluid retention in children, the present study selected the previously determined minimum protein content in adults (1.5%) as the protein content for Hi-PRO and half that content for Lo-PRO so as to construct a relative dose-response. Beverages were iso-caloric (30 kcal/100 mL (CONT), 28.4 kcal/100 mL (Lo-PRO) or 28.1 kcal/100 mL (Hi-PRO)). Given the relatively greater importance of Na^+ than K^+ in enhancing fluid retention in adults (21, 39), a small amount of electrolytes in the form of Gatorlytes (18.6 mg/100 mL; Gatorade electrolyte mix, Series PRO 02 Perform) that contain Na^+ and K^+ in a ratio of ~2:1 (9.5 mg and 4.9 mg per 100 mL of the beverage, respectively) were added to each beverage. All beverages were served chilled to 8° to 10°C in opaque bottles, and it was visually verified that the beverage volume was fully consumed.

Participants remained in the laboratory for 4 h following beverage consumption to allow for monitoring during recovery period. Blood samples (10 mL each) were collected via individual venipuncture immediately after the experimental beverage was consumed in its entirety (i.e., 0 h of recovery), and after 4 h of recovery. Urine samples and clothed BM measurements were recorded at regular intervals (i.e., 1 h, 2 h, 3 h, and 4 h) (**Figure 1**). In cases where the participants needed to urinate before the scheduled urine collection, the sample was collected, the time recorded, and the sample was then added to the hourly sample.

Primary outcome*:* The change in BM was used as an estimate of fluid balance (with the assumption that 1 mL is equal to 1 g) and was our primary outcome. Fluid balance over the experimental visit was determined by the following equation:

$$\text{Fluid balance (mL)} = BM_t - BM_i$$

Where BM_i is the pre-exercise BM in grams and BM_t is the BM at a given time in grams.

Secondary outcomes. Secondary outcomes included: fraction of beverage retained, urine production, plasma osmolality and plasma albumin concentration. The rehydration potential of each beverage was determined by examining the fraction of beverage retained in the body, calculated by the following equation:

$$\text{Fraction retained (\%)} = (\text{Beverage volume (mL)} - \text{Urine volume (mL)}) / \text{Beverage volume (mL)} \times 100$$

The potential for each beverage to influence urine production was determined by examining urine output after each beverage had been consumed in its entirety. The cumulative urine output (in L) was calculated by the sum of the urine samples collected (measured to the nearest mL using a volumetric cylinder) throughout the recovery phase. To understand factors involved in rehydration, we also measured plasma osmolality (P_{osm}) and plasma albumin concentrations. P_{osm} was assessed by freezing point depression using an Advanced Instruments Micro-Osmometer Model 3320 (Advanced Instruments, Norwood, MA). Plasma albumin levels were assessed using the QuantiChrom™ BCG Albumin Assay Kit (DIAG-250) for cuvettes.

Sample size. The primary outcome in this study was body fluid balance and, thus, sample size was based on this variable. As a conservative estimate, our study was powered on the observed difference of 326 ± 354 mL (mean ± SD; p value = 0.051) in favour of skim milk over a carbohydrate control at 3 h of recovery in a previous study involving adults (43). With t = 2.43 from a t-distribution with df = 7-1 = 6 degrees of freedom, the within subject SD was calculated as $sd_w = \Delta / t * \text{sqrt}(n) = 355$ mL (43). Therefore, with the main *a priori* comparisons of Hi-PRO vs. CONT and Lo-PRO vs. CONT, we estimated that 18 participants were needed to detect a difference of 326 mL between conditions with alpha-level = 0.05/2 and power = 0.9.

Statistical analysis. Based on the expected difference in fluid balance that is typically observed in adults (14, 41, 43), we planned *a priori* comparisons between Hi-PRO vs. CONT and Lo-PRO vs. CONT at each hour of recovery using paired t-tests with $\alpha = 0.05$, or a non-parametric alternative if necessary (i.e., Wilcoxon). However, due to the exploratory nature of this trial in the absence of any previous pediatric studies, and the uncertainty about the extent of similarities between children and adults, all data were also submitted to a 2-way repeated-measures analyses of variance (ANOVAs; condition × time). This allowed us to obtain comparisons between all three experimental beverages that were not examined in the *a priori* analyses and, thus, establish a further understanding of the dose-response relationship. Beverage volumes, fluid loss, spot-urine U_{sg} and cumulative urine production were compared using a one-way ANOVA (condition). When main effects of within-subject interactions were significant, the source of statistically significant differences was determined using the Bonferroni post hoc test. Statistical significance for all comparisons was set at $p < 0.05$. Statistical analyses were performed using the statistical software STATISTICA 10.0 (StatSoft Inc., Tulsa, OK). Data are presented as mean ± SD unless stated otherwise.

RESULTS

In order to meet the required n=18, 20 participants were initially recruited for participation in the study. Data from one participant was excluded due to failure to consume the required volume (i.e., participant consumed ~30% of experimental beverage volume) and 4 other participants dropped out due to personal reasons related to the study, which included: i) refusal to complete the exercise protocol (n=1); ii) refusal to provide all blood samples (n=1), and; iii) refusal to drink the experimental beverage due to taste (n=2). Therefore, data presented herein represent the participants (n=15) who completed the study.

Throughout the dehydration protocol, participants lost a mean of 0.62 ± 0.14 kg (0.64 ± 0.16 kg, CONT; 0.65 ± 0.15 kg, Lo-PRO; and 0.59 ± 0.13 kg, Hi-PRO). This corresponded to a dehydration of 1.60 ± 0.35 % (CONT), 1.63 ± 0.24 % (Lo-PRO) and 1.49 ± 0.29 % (Hi-PRO) of initial BM. There was no difference in the degree of dehydration (mean 1.57 ± 0.31 % of initial BM) attained between the three experimental trials ($p = 0.45$). As a result there were no differences between trials in the volume of beverage ingested ($p = 0.52$); participants consumed 958 ± 249 mL (CONT), 971 ± 230 mL (Lo-PRO), and 836 ± 222 mL (Hi-PRO) of fluid. Analysis of the beverages revealed that participants ingested 0, 7.0 ± 2.1, and 12.5 ± 3.7 g (or 0, 0.18 ± 0.03, and 0.32 ± 0.07 $g \cdot kg^{-1}$) of protein after exercise in the CONT, Lo-PRO, and Hi-PRO conditions, respectively. Results of the blind taste testing revealed that there were no significant differences in beverage taste ($p = 0.59$), overall beverage preference ($p = 0.57$), drink sweetness ($p = 0.11$), drink saltiness ($p = 0.12$), and drink sourness ($p = 0.65$) between the CONT, Lo-PRO and Hi-PRO beverages.

Fluid balance (primary outcome). According to the protocol, in case of outliers, the analysis was performed by a Wilcoxon test at 1 h, 2 h, 3 h, and 4 h of recovery, separately, to determine the difference between Hi-PRO and CONT and then the difference between Lo-PRO and CONT. Treatment differences between Hi-PRO and CONT and Lo-PRO and CONT are provided in Table 2. We found no difference between Hi-PRO and CONT at 1 h ($T = 34.0$, $Z = 0.80$, $p = 0.42$). However, there was a difference between Hi-PRO and CONT at 2 h ($T = 8.5$, $Z = 2.93$, $p = 0.003$) and 3 h ($T = 14.0$, $Z = 2.42$, $p = 0.02$), with a strong trend at 4 h ($T = 21.5$, $Z = 1.94$, $p = 0.051$). For the comparison between Lo-PRO and CONT, there was no difference at 1 h (T = 45.5, Z = 0.82, $p = 0.41$), 2 h (T = 50.0, Z = 0.57, $p = 0.57$), 3 h (T = 48.5, Z = 0.57, $p = 0.57$), or 4 h (T = 59.0, Z = 0.057, $p = 0.95$). Exploratory analysis of the primary outcome using a 2-way repeated measures ANOVA (**Figure 2**) found no main effect for condition ($p = 0.31$), but indicated a significant condition × time interaction ($p = 0.01$) throughout recovery. Post-hoc analyses of the condition × time interaction revealed that fluid balance was less negative in Hi-PRO compared with CONT at 2 h of recovery ($p < 0.05$), and tended to be less negative at 3 h ($p = 0.06$). Fluid balance in Lo-PRO was not different than CONT or Hi-PRO at any time during recovery.

Fraction of beverage retained (secondary outcome). The fraction of beverage retained decreased throughout the recovery period (main effect time, $p < 0.001$). The fraction of beverage retained also demonstrated a significant condition × time interaction ($p < 0.01$), where participants had retained significantly more of the beverage in Hi-PRO compared with CONT at 2 h (p value < 0.05; **Figure 3**). Lo-PRO was not different than CONT or Hi-PRO at any time during recovery.

Urine output (secondary outcome). The spot-urine U_{sg} values (1.020 ± 0.007, 1.021 ± 0.008, and 1.023 ± 0.005 in the CONT, Lo-PRO and Hi-PRO conditions, respectively) were not significantly different between trials (main effect condition, $p = 0.43$), giving an average spot-urine U_{sg} value of 1.021 ± 0.007. Eight participants had an average spot U_{sg} between 1.021 and 1.031, suggesting that more than half the participants arrived to the laboratory in a hypohydrated state. More specifically, all 15 participants had at least one spot $U_{sg} \geq 1.021$; three participants had one, nine participants had two, and three participants had all spot $U_{sg} \geq 1.021$. Hourly urine output ($L \cdot h^{-1}$) did not differ between experimental trials (main effect condition, $p = 0.12$, **Figure 4**). The volume of urine produced each hour was greatest in the first two hours following beverage consumption, and decreased by 3 h (main effect time, $p < 0.001$). Furthermore, a condition × time interaction was found for hourly urine production throughout the recovery period ($p < 0.001$); hourly urine volume at 2 h was significantly greater in CONT vs. Hi-PRO ($p < 0.001$) and CONT vs. Lo-PRO (all p values < 0.05). There were no differences in cumulative urine production throughout the recovery period between trials (main effect condition, $p = 0.14$; 543 ± 154 mL, CONT; 503 ± 120 mL, Lo-PRO; 455 ± 159 mL, Hi-PRO).

Blood parameters (secondary outcomes). At rest, P_{osm} averaged 296 ± 5 mOsm. There were no main effects for condition ($p = 0.35$), time ($p = 0.77$), or a condition × time interaction ($p = 0.97$). At rest, plasma albumin concentration averaged 0.348 ± 0.059 $g \cdot dL^{-1}$. Plasma albumin concentration demonstrated a trend towards a main effect for condition ($p = 0.074$; 0.363 ± 0.081 $g \cdot dL^{-1}$, CONT; 0.364 ± 0.084 $g \cdot dL^{-1}$, Lo-PRO; 0.337 ± 0.060 $g \cdot dL^{-1}$, Hi-PRO), but no significance for time ($p = 0.49$) or condition × time ($p = 0.33$). For plasma albumin content, there was a trend for a main effect for condition ($p = 0.076$; 1.87 ± 0.43 $g \cdot kg^{-1}$, CONT; 1.93 ± 0.33 $g \cdot kg^{-1}$, Lo-PRO; 1.74 ± 0.32 $g \cdot kg^{-1}$, Hi-PRO). There was also a main effect for time ($p < 0.01$) and a condition × time interaction ($p = 0.01$). For the estimated change in plasma volume (data not shown), there was no main effect of condition ($p = 0.83$) or time ($p = 0.94$), nor was there an interaction effect ($p = 0.36$)

DISCUSSION

Hydration status is an important consideration for optimal health and performance in physically active youth (1, 6). Despite the fact that active children commonly present to sporting events with a level of chronic dehydration (18) and have been reported to voluntarily dehydrate during physical activity (4, 31, 32), there remains a paucity of information in the literature regarding the effects of different types of rehydration beverages in children. We compared, for the first time in children, beverages that were matched for energy and electrolyte content and show that the presence of milk protein results in less negative indices of rehydration after exercise when children consumed a volume of 150% of fluid losses. Using body fluid balance as our primary outcome to be consistent with previous adult literature (14–16, 43), we found a less negative fluid balance at 2 h and 3 h of recovery after consumption of Hi-PRO vs. CONT, but there were no differences between Lo-PRO and CONT. This finding is in contrast to our hypothesis that protein ingestion would exhibit a dose-dependent response. Instead, when matched for energy and electrolyte content, a potential threshold for the positive effects of milk protein on post-exercise fluid balance may exist, although the addition of milk protein at levels below this level does not impair rehydration.

The factors contributing to rapid and complete rehydration in adults have been extensively studied, with recommendations focusing on the synergistic roles of the volume and electrolyte (specifically Na^+) content of the ingested fluids (for review, see: Maughan *et al.* (22)). Only recently has the potential for bovine milk and protein-containing beverages been investigated for enhanced rehydration after endurance exercise in adults (14, 41, 43). Both Shirreffs *et al.* (41) and Watson *et al*. (43) demonstrated that when low-fat milk was consumed in a volume equivalent to 150% of sweat losses following an exercise-induced dehydration of ~2%, fluid retention and net fluid balance over 3-4 h of recovery was greater compared to when a CES was consumed. In children, it was recently determined that post-exercise consumption of low-fat milk also improved fluid balance and fluid retention over 2 h of recovery compared to a CES or water, when consumed at a volume equivalent to 100 % of BM loss to replace a similar ~2% fluid loss (42). While these studies demonstrate the applied benefit of consuming milk post-activity for improved rehydration (41–43), the specific mechanism underlying this enhanced fluid retention is confounded by the myriad of compositional differences between the beverages including protein, energy and electrolyte contents.

The specific role of protein on rehydration after exercise has been investigated in the adult literature. For example, Seifert *et al*. (35) first demonstrated that a carbohydrate beverage containing 1.5 % protein increased fluid retention over 3 h of recovery compared to a carbohydrate-only solution and water. While carbohydrate content was similar between beverages, the energy content was slightly greater with the protein-containing beverage. James *et al*. (14) later demonstrated that the addition of milk protein (2.5% w/v) to a carbohydrate-electrolyte beverage enhanced fluid retention and induced a less negative whole body net fluid balance than an energy and electrolyte-matched carbohydrate beverage. The present study attempted to elucidate, for the first time in children, the specific role of milk protein in a post-exercise beverage when matched for energy and electrolyte content. We found similar results to James *et al.* (14) in that that presence of milk protein in a rehydration beverage helped to maintain a less negative body fluid balance, retain a greater fraction of the beverage, and decrease the volume of urine produced 2 h post-exercise in children. While collectively these results would support a role for protein-containing beverages to enhance rehydration, the enhanced fluid retention in the present study occurred earlier in recovery (i.e. 2 h) compared to the adult literature (i.e. 3-4 h), albeit after ingestion of a 2.5 % protein-containing beverage (14). Moreover, the benefit of protein-containing beverages for rehydration is not universal as 3 subsequent studies in adults (13, 16, 17) reported no effect of protein (1.5 – 2.0 % whey protein) on urine output or beverage retention during the post-exercise period when given in a volume equivalent to 150 % of sweat losses. Whether the lack of effect of a beverage containing ≤ 2 % protein on rehydration when matched for energy and electrolyte content in adults is due to the protein dose itself or the fact that whey protein isolate was used instead of milk protein remains unclear. As bovine milk protein contains both casein and whey protein fractions, differences in the gastric-emptying properties of the milk and whey protein (12) may have contributed to the differences in findings between studies. Nevertheless, the present study found that, in children, a beverage containing 1.5 % milk protein was effective at enhancing post-exercise fluid balance, whereas beverages containing half that content (0.75 % milk protein) appeared to have no additional effect compared with a protein-free beverage. Therefore, it is possible that 1) children may benefit from a relatively lower beverage protein content than what has been observed in the adult literature when consuming 150% of fluid loss (14, 16, 43), and 2) rather than a dose-dependent response, there may be a potential threshold for the positive effects of protein on post-exercise fluid balance. Whether protein contents above those used in this study would have additional benefits on rehydration remain unknown. Regardless, both our results and those from the adult literature (13, 14, 16, 17, 43) suggest that strategies to enhance rehydration after exercise are not "one size fits all" and that population specific research is required.

The mechanisms by which protein can enhance rehydration have not been fully elucidated. One of the proposed mechanisms by which protein might exert its effect is by decreasing the rate at which the beverage moves through the gastrointestinal system. It is possible that the slower digestion of milk protein (which is ~80% casein) may impact the rate of gastric emptying compared to the ingestion of simple carbohydrates alone (12, 14), which would subsequently reduce its rate of absorption into the circulation (23). This lower rate of fluid absorption can attenuate the decline in P_{osm} and subsequent secondary fluid loss (due to an enhanced urine production observed with the rapid ingestion of large volumes of dilute fluid) (11). However, in the present study, P_{osm} values at the time points measured remained stable throughout each session, regardless of the beverage consumed. This is not altogether surprising, as other indices of rehydration (body fluid balance, fraction of beverage retained, and urine output) did not different between conditions at 4 h of recovery when P_{osm} was assessed. On a similar note, recovery of plasma volume did not differ between conditions and remained stable at the 2 post-exercise time periods. While we cannot disregard the possibility that differences in P_{osm} or plasma volume may have been present at times we did not measure (i.e. at 1 h, 2 h and 3 h of recovery when there were differences in urine production and fluid retention), our data do not allow us to address this possibility.

Another proposed mechanism by which protein might exert its effect on rehydration is through alterations in oncotic pressure. In adults, post-exercise protein ingestion has been shown to enhance plasma albumin protein concentration, which, due to its contribution to oncotic pressure, is known to enhance plasma volume expansion during the post-exercise recovery period (29). Despite the possibility that enhanced albumin production could bind water within the intravascular space and theoretically enhance fluid retention we did not observe changes in plasma albumin concentration that would support this potential mechanism. Although the present study, as well as previous work in adults (14, 41, 43), demonstrate the benefit of protein in a rehydration beverage, further research is needed to determine the specific mechanism(s) by which protein can enhance fluid retention during the post-exercise recovery period.

Although we reported a greater fluid retention with the protein-containing beverages, we found that regardless of condition participants did not return to a euhydrated state (i.e. pre-exercise BM) by 4 h of recovery. For adults to achieve complete rehydration there is general consensus that the volume of fluid consumed must be greater than the volume of fluid lost, in order to account for obligatory urine losses (19, 40); this has led to the recommendation that a volume equal to 150% of BM losses is optimal (34, 38). Due to a lack of pediatric-specific guidelines, this 150% fluid volume was used as the basis for the rehydration volume in the present study. Despite consuming this volume our participants did not achieve euhydration, highlighting the importance of additional studies to determine the optimal rehydration volume in children. Another possible reason for the unsuccessful return to a euhydrated state after beverage consumption in each trial may be related, in part, to the relatively low sodium content (18.6 mg/100 ml) of the rehydration beverages in comparison to the quantity found in commercially available sports drink or beverages commonly used in the post-exercise period (36, 37, 41). However, given the growth-related differences that exist in sweat sodium concentrations (24) (and, therefore, the amount of sodium that a post-exercise beverage would need to replace), whether increasing the sodium content would have had an impact on the rehydration potential of the beverages remains unknown. Collectively, the discrepancies between the present study and those performed previously in adults underscore the importance of performing pediatric-specific research to generate population-specific guidelines.

CONCLUSIONS

In conclusion, we demonstrate that in healthy active children, adding 1.5 g/100 mL of milk protein to a rehydration beverage resulted in a less negative body fluid balance during the early (2 h) post-exercise recovery period compared to a protein-free carbohydrate beverage. Therefore, when the period between exercise sessions is short (i.e. 2 h), children may benefit from rehydration with protein-containing beverages. At the end of the 4 h rehydration period, children remained in a net negative fluid balance despite ingesting a volume of fluid recommended for adults. Further research is needed to determine the optimal rehydration volume, beverage composition, and/or ingestion pattern for children.

ACKNOWLEDGEMENTS

The authors would like to thank the participants and their families for their time and dedication to this study. BW Timmons is supported by a Salary Award from the Canadian Institutes of Health Research.

CONFLICT OF INTEREST

This study was funded by Nestec Ltd., which is a subsidiary of Nestlé Ltd. and provides professional assistance, research, and consulting services for food, dietary, dietetic, and pharmaceutical products of interest to Nestlé Ltd. The authors have no conflicts of interest to declare.

REFERENCES

1. American Academy of Pediatrics. American Academy of Pediatrics Committee on Sports Medicine: Climatic heat stress and the exercising child. Pediatrics. 1982;69(6):808–9.

2. American College of Sports Medicine, Sawka MN, Burke LM, et al. American College of Sports Medicine position stand. Exercise and fluid replacement. Med Sci Sports Exerc. 2007;39(2):377–90.

3. Aragón-Vargas LF, Wilk B, Timmons BW, Bar-Or O. Body weight changes in child and adolescent athletes during a triathlon competition. Eur J Appl Physiol. 2013;113(1):233–9.

4. Bar-Or O, Dotan R, Inbar O, Rotshtein A, Zonder H. Voluntary hypohydration in 10- to 12-year-old boys. J Appl Physiol. 1980;48(1):104–8.

5. Bar-Or O, Rowland TW. Pediatric Exercise Medicine. From Physiologic Principles to Health Care Application. Champaign, IL: Human Kinetics, 2004.

6. Bergeron MF. Youth sports in the heat: recovery and scheduling considerations for tournament play. Sports Med. 2009;39(7):513–22.

7. Bergeron MF, Laird MD, Marinik EL, Brenner JS, Waller JL. Repeated-bout exercise in the heat in young athletes: physiological strain and perceptual responses. J Appl Physiol. 2009;106(2):476–85.

8. Casa DJ, Armstrong LE, Hillman SK, et al. National athletic trainers' association position statement: fluid replacement for athletes. J Athl Train. 2000;35(2):212–24.

9. Cheuvront SN, Kenefick RW, Montain SJ, Sawka MN. Mechanisms of aerobic performance impairment with heat stress and dehydration. J Appl Physiol. 2010;109(6):1989–95.

10. Dougherty KA, Baker LB, Chow M, Kenney WL. Two percent dehydration impairs and six percent carbohydrate drink improves boys basketball skills. Med Sci Sports Exerc. 2006;38(9):1650–8.

11. Evans GH, Shirreffs SM, Maughan RJ. Postexercise rehydration in man: the effects of osmolality and carbohydrate content of ingested drinks. Nutrition. 2009;25(9):905–13.

12. Hall WL, Millward DJ, Long SJ, Morgan LM. Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite. Br J Nutr. 2003;89(2):239–48.

13. Hobson R, James L. The addition of whey protein to a carbohydrate-electrolyte drink does not influence post-exercise rehydration. J Sports Sci. 2015;33(1)77-84.

14. James LJ, Clayton D, Evans GH. Effect of milk protein addition to a carbohydrate-electrolyte rehydration solution ingested after exercise in the heat. Br J Nutr. 2011;105(3):393–9.

15. James LJ, Evans GH, Madin J, et al. Effect of varying the concentrations of carbohydrate and milk protein in rehydration solutions ingested after exercise in the heat. Br J Nutr. 2013;110(7):1285–91.

16. James LJ, Gingell R, Evans GH. Whey protein addition to a carbohydrate-electrolyte rehydration solution ingested after exercise in the heat. J Athl Train. 2012;47(1):61–6.

17. James LJ, Mattin L, Aldiss P, Adebishi R, Hobson RM. Effect of whey protein isolate on rehydration after exercise. Amino Acids. 2014;46(5):1217–24.

18. Kavouras SA, Arnaoutis G, Makrillos M, et al. Educational intervention on water intake improves hydration status and enhances exercise performance in athletic youth. Scand J Med Sci Sports. 2012;22(5):684–9.

19. Maughan RJ, Leiper JB, Shirreffs SM. Restoration of fluid balance after exercise-induced dehydration: effects of food and fluid intake. Eur J Appl Physiol. 1996;73(3-4):317–25.

20. Maughan RJ, McArthur M, Shirreffs SM. Influence of menstrual status on fluid replacement after exercise induced dehydration in healthy young women. Br J Sports Med. 1996;30(1):41–7.

21. Maughan RJ, Owen JH, Shirreffs SM, Leiper JB. Post-exercise rehydration in man: effects of electrolyte addition to ingested fluids. Eur J Appl Physiol. 1994;69(3):209–15.

22. Maughan RJ, Shirreffs SM, Watson P. Exercise, heat, hydration and the brain. J Am Coll Nutr. 2007;26(5 Suppl):S604–12.

23. McHugh PR, Moran TH. Calories and gastric emptying: a regulatory capacity with implications for feeding. Am J Physiol. 1979;236(5):R254–60.

24. Meyer F, Bar-Or O, MacDougall D, Heigenhauser GJ. Sweat electrolyte loss during exercise in the heat: effects of gender and maturation. Med Sci Sports Exerc. 1992;24(7):776–81.

25. Meyer F, Bar-Or O, Salsberg A, Passe D. Hypohydration during exercise in children: effect on thirst, drink preferences, and rehydration. Int J Sport Nutr. 1994;4(1):22–35.

26. Meyer F, O'Connor H, Shirreffs SM, International Association of Athletics Federations. Nutrition for the young athlete. J Sports Sci. 2007;25(1 Suppl):S73–82.

27. Mirwald RL, Baxter-Jones ADG, Bailey DA, Beunen GP. An assessment of maturity from anthropometric measurements. Med Sci Sports Exerc. 2002;34(4):689–94.

28. Moore DR, Volterman KA, Obeid J, Offord EA, Timmons BW. Post-exercise protein ingestion increases whole body net protein balance in healthy children. J Appl Physiol. 2014;117(12)1493-501.

29. Okazaki K, Hayase H, Ichinose T, Mitono H, Doi T, Nose H. Protein and carbohydrate supplementation after exercise increases plasma volume and albumin content in older and young men. J Appl Physiol. 2009;107(3):770–9.

30. Palmer MS, Spriet LL. Sweat rate, salt loss, and fluid intake during an intense on-ice practice in elite Canadian male junior hockey players. Appl Physiol Nutr Metab. 2008;33(2):263–71.

31. Rivera-Brown AM, Gutiérrez R, Gutiérrez JC, Frontera WR, Bar-Or O. Drink composition, voluntary drinking, and fluid balance in exercising, trained, heat-acclimatized boys. J Appl Physiol. 1999;86(1):78–84.

32. Rivera-Brown AM, Ramírez-Marrero FA, Wilk B, Bar-Or O. Voluntary drinking and hydration in trained, heat-acclimatized girls exercising in a hot and humid climate. Eur J Appl Physiol. 2008;103(1):109–16.

33. Rowland T. Fluid replacement requirements for child athletes. Sports Med. 2011;41(4):279–88.

34. Roy BD. Milk: the new sports drink? A Review. J Int Soc Sports Nutr. 2008;5:15.

35. Seifert J, Harmon J, DeClercq P. Protein added to a sports drink improves fluid retention. Int J Sport Nutr Exerc Metab. 2006;16(4):420–9.

36. Sharp RL. Role of sodium in fluid homeostasis with exercise. J. Am. Coll. Nutr. 2006;25(3 Suppl):S231–9.

37. Shirreffs SM, Aragon-Vargas LF, Keil M, Love TD, Phillips S. Rehydration after exercise in the heat: a comparison of 4 commonly used drinks. Int J Sport Nutr Exerc Metab. 2007;17(3):244–58.

38. Shirreffs SM, Armstrong LE, Cheuvront SN. Fluid and electrolyte needs for preparation and recovery from training and competition. J Sports Sci. 2004;22(1):57–63.

39. Shirreffs SM, Maughan RJ. Volume repletion after exercise-induced volume depletion in humans: replacement of water and sodium losses. Am J Physiol. 1998;274(5 Pt 2):F868–75.

40. Shirreffs SM, Taylor AJ, Leiper JB, Maughan RJ. Post-exercise rehydration in man: effects of volume consumed and drink sodium content. Med Sci Sports Exerc. 1996;28(10):1260–71.

41. Shirreffs SM, Watson P, Maughan RJ. Milk as an effective post-exercise rehydration drink. Br J Nutr. 2007;98(1):173–80.

42. Volterman KA, Obeid J, Wilk B, Timmons BW. Effect of milk consumption on rehydration in youth following exercise in the heat. Appl Physiol Nutr Metab. 2014;39(11):1257–64.

43. Watson P, Love TD, Maughan RJ, Shirreffs SM. A comparison of the effects of milk and a carbohydrate-electrolyte drink on the restoration of fluid balance and exercise capacity in a hot, humid environment. Eur J Appl Physiol. 2008;104(4):633–42.

44. Wilk B, Meyer F, Bar-Or O, Timmons BW. Mild to moderate hypohydration reduces boys' high-intensity cycling performance in the heat. Eur J Appl Physiol. 2014;114(4):707–13.

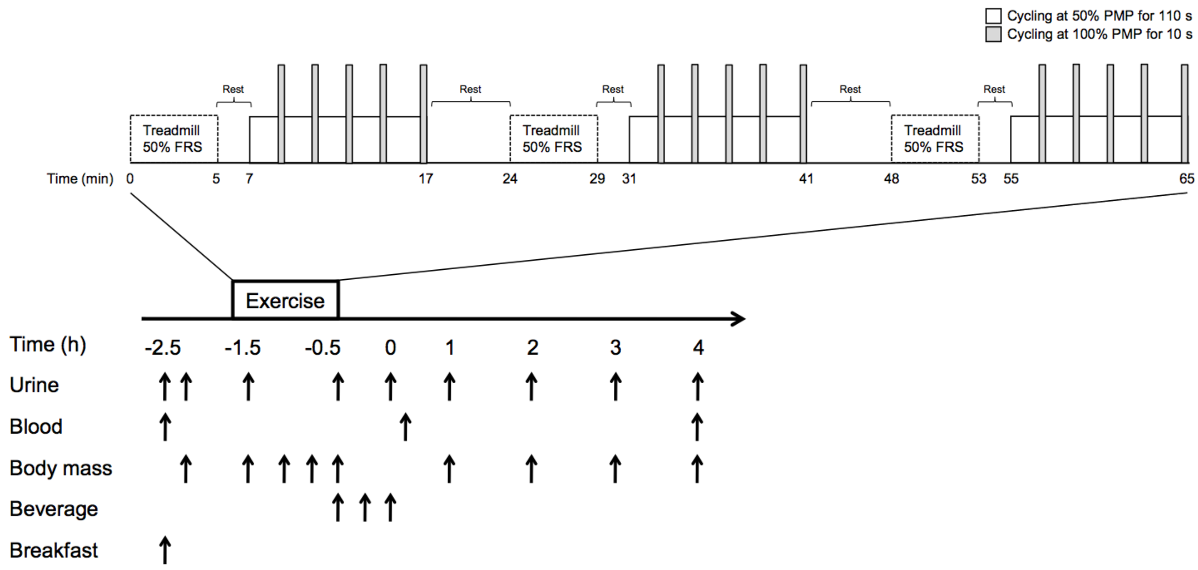

Figure 1. Schematic representation of trial day. PMP, peak mechanical power; FRS, fastest running speed; Beverage, experimental beverage (CONT, Lo-PRO or Hi-PRO) corresponding to respective experimental trial.

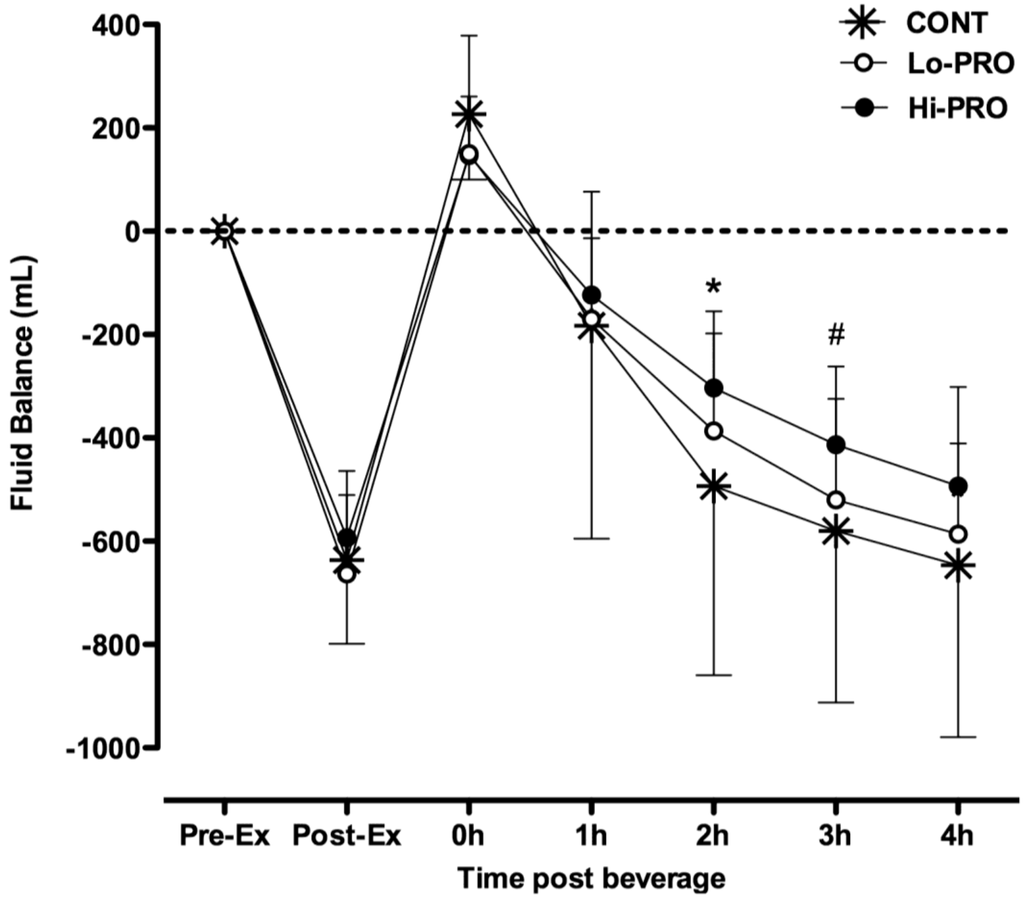

Figure 2. Body fluid balance during CONT, Lo-PRO, and Hi-PRO experimental trials. *, indicates that value in Hi-PRO is significantly different than CONT. #, indicates that value in Hi-PRO tended to be different than CONT (p = 0.06).

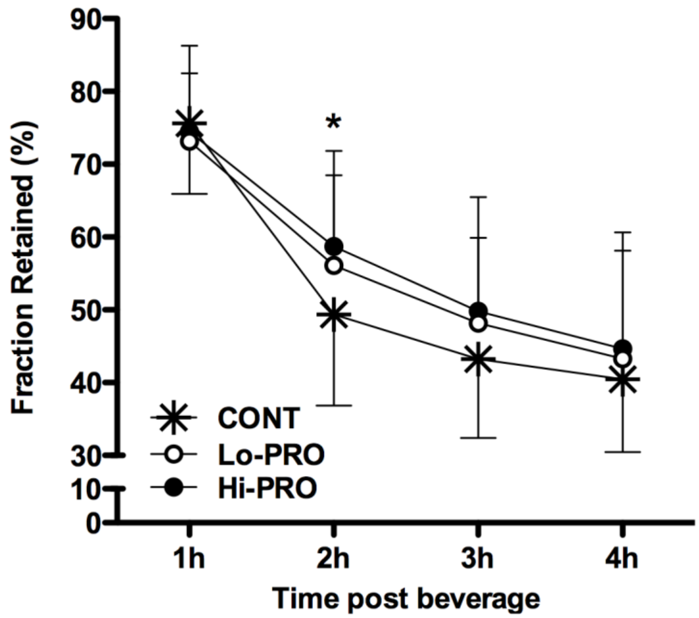

Figure 3. Fraction of beverage retained during recovery after consumption of CONT, Lo-PRO, and Hi-PRO. *, indicates that value in Hi-PRO is higher than CONT.

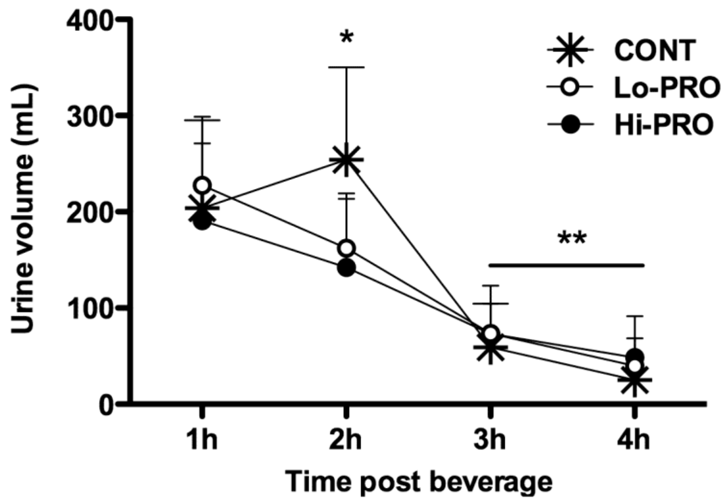

Figure 4. Hourly urine output during recovery after consumption of CONT, Lo-PRO, and Hi-PRO. *, indicates that value in Hi-PRO and Lo-PRO are lower than CONT; **, indicates that values differ significantly from 1 h and 2 h time points.

Table. 1. Energy density, carbohydrate content, protein content, fat content, sodium concentration, potassium concentration and osmolality of the CONT, Lo-PRO and Hi-PRO beverages.

	CONT	Lo-PRO	Hi-PRO
Energy (kcal/100ml)	30	28.4	28.1
CHO (g/100ml)	7	5.3	5.3
PRO (g/100ml)	0	0.76	1.5
Fat (g/100ml)	0	0.46	0.10
Na^+ (mmol/l)	94.9	94.9	94.9
K+ (mmol/l)	48.7	48.7	48.7
Osmolality (mOsm)	222	249	245

CHO, carbohydrates; PRO, protein.

Table. 2. Difference in fluid balance between Hi-PRO and CONT and Lo-PRO and CONT at 1h, 2h, 3h and 4h of the recovery period**.**

	Treatment differences (mL)	
	Hi-PRO - CONT	Lo-PRO - CONT
1h	60 ± 403 [-144, 264]	13 ± 488 [-234, 260]
2h	190 ± 354* [11, 369]	107 ± 409 [-100, 314]
3h	167 ± 302* [14, 320]	60 ± 368 [-126, 246]
4h	153 ± 338 [-18, 324]	60 ± 379 [-132, 252]

CONT; Control condition; Lo-PRO; Low-protein condition; Hi-PRO; High-protein condition. Data are presented as mean ± SD [95% confidence interval]. *significant difference between conditions ($p < 0.05$).