Effects of post-exercise milk consumption on whole body protein balance in youth

Kimberly A. Volterman\textsuperscript{1}, Joyce Obeid\textsuperscript{1}, Boguslaw Wilk\textsuperscript{1}, Brian W. Timmons\textsuperscript{1}.

\textsuperscript{1}Child Health & Exercise Medicine Program, Department of Pediatrics, McMaster University, Hamilton, Ontario, Canada.

B.W. and B.W.T conceptualized and designed the research project; K.A.V. acquired the data with assistance from J.O.; K.A.V. and B.W.T. conducted the statistical analysis; K.A.V wrote the final manuscript with manuscript revisions from J.O., B.W., and B.W.T. All authors reviewed and agreed upon the final manuscript.

\textbf{Running Head:} Milk in youth following exercise

\textbf{Contact Information:}

Brian W. Timmons, PhD
Child Health & Exercise Medicine Program
1280 Main Street West, HSC 3N27G
Hamilton, ON, Canada, L8S 4K1
Tel: 905-521-2100, ext 77615
Fax: 905-521-1703
Email: timmonbw@mcmaster.ca

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ABSTRACT

In adults, adding protein to a post-exercise beverage increases muscle protein turnover and replenishes amino acid stores. Recent focus has shifted towards the use of bovine-based milk and milk products as potential post-exercise beverages; however, little is known about how this research translates to the pediatric population. Twenty-eight (15 females) pre- to early-pubertal (PEP, 7-11yrs) and mid- to late-pubertal (MLP, 14-17yrs) children consumed an oral dose of $[^{15}\text{N}]$glycine prior to performing 2 x 20-min cycling bouts at 60% VO$_{2\text{peak}}$ in a warm environment (34.5°C, 47.3% relative humidity). Following exercise, participants consumed either water (W), a carbohydrate-electrolyte solution (CES), or skim milk (SM) in randomized, cross-over fashion in a volume equal to 100% of their body mass loss during exercise. Whole body nitrogen turnover (Q), protein synthesis (S), protein breakdown (B), and whole body protein balance (WBPB) were measured over 16h. Protein intake from SM was 0.40 ± 0.10 g/kg. Over 16h, Q and S were significantly greater (p < 0.01) with SM than W and CES. B demonstrated a trend for a main effect for beverage (p = 0.063). WBPB was more negative (p<0.01) with W and CES than with SM. In the SM trial, WBPB was positive in PEP while it remained negative in MLP. Boys exhibited significantly more negative WBPB than girls (p<0.05). Post-exercise milk consumption enhances WBPB compared to W and CES; however, additional protein intake may be required to sustain a net anabolic environment over 16h.

Key words: children, physical activity, protein metabolism, recovery
Introduction

One of the main goals of a post-exercise beverage, in addition to rehydration (replacing fluid and electrolytes), is to restore muscle glycogen stores that have been utilized during the preceding exercise. The addition of protein to a post-exercise beverage also increases muscle protein turnover and replenishes amino acid stores (14). Therefore, aside from the beneficial effects on rehydration and fluid balance (12), a post-exercise beverage rich in proteins could also contribute to improved recovery from exercise and exercise performance, while providing the nutrients necessary to enhance lean tissue remodeling and increase lean body mass (27).

In adults, much of the focus in recent years has shifted towards the use of bovine-based milk and milk products as potential post-exercise beverages (12; 17; 18; 25); however, very little is known about how this research translates to the pediatric population. While the combined effects of milk (more specifically, calcium) and exercise have been recognized in the promotion of optimal bone development in children (6; 20), the protein needs of this population are not well understood as they remain relatively understudied. This is an important topic when one considers the potential for milk-based products to enhance the anabolic effects of exercise, while facilitating the remodeling and rebuilding process in active, growing children.

Milk has distinct compositional differences compared with beverages typically consumed following exercise, for example water and sports drinks (23). One important characteristic of bovine-milk is the presence of protein and amino acids, which contribute to the maintenance of muscle protein synthesis (MPS) and enhancement of protein balance following exercise (17). Milk protein contains ~20% whey protein and 80% casein protein. Whey and casein protein have distinct structural differences that affect their speed of absorption and catabolic properties; they are referred to as “fast” and “slow” proteins, respectively (3). Upon digestion of whey protein,
there is a rapid and transient increase in the appearance of amino acids in the plasma, leading to an acute stimulation of protein synthesis (3; 27). Casein protein, on the other hand, results in a delayed and prolonged rise in plasma amino acids, allowing for the release of insulin and down regulation of muscle protein breakdown (3; 8). The composition of milk protein seemingly produces a beneficial response with respect to MPS and muscle accretion (8). Indeed, adult studies demonstrate that milk enhances MPS to a greater extent than a carbohydrate-electrolyte solution (CES) (27).

The extent to which the beneficial effects of post-exercise milk consumption apply to the pediatric population remains unknown. Given its protein content, milk has the potential to enhance protein balance following exercise. Understanding the role of milk in protein balance is especially important in the pediatric years so as to allow for the promotion of an active lifestyle, while maintaining optimal growth and development. Therefore, the aim of this study was to examine whether milk, a protein-containing beverage, could favourably impact whole body protein balance (WBPB) following exercise in healthy children. Our hypothesis was that due to its protein content, milk would maintain a more positive WBPB following exercise when compared with water and a CES. Additionally, the secondary objective of this study was to assess the effects of puberty and sex, as well as their interaction, on milk’s ability to maintain WBPB.

METHODS

Participants. Twenty-eight pre- to early-pubertal (PEP, 7-11yrs) and mid- to late-pubertal (MLP, 14-17yrs) children participated in this study, approved by the Hamilton Health Sciences/Faculty of Health Sciences Research Ethics Board and conducted in compliance with
the standards set by the Declaration of Helsinki. All participants and their parents were informed of the study protocol and provided written informed assent and consent, respectively, prior to study enrollment. Participants were recruited from the local community through schools and sporting clubs. General medical and activity questionnaires were used to ensure all participants were healthy and habitually physically active. Participant characteristics are summarized in Table 1.

**General Overview.** The present data are secondary outcomes of a study evaluating the effect of milk on rehydration after exercise-induced fluid loss in the heat. Using a randomized, repeated measures cross-over design, participants reported to the laboratory on 4 separate occasions, separated by 4-10 days. The first session was a preliminary screening visit where we obtained basic anthropometrics and aerobic fitness measurements. For an estimate of habitual dietary intake, participants were asked to complete a 3-d dietary record, which was analyzed with The Food Processor SQL (ESHA, Salem, Oregon) software for energy and macronutrient intakes. The following 3 sessions, which took place two weeks after the initial visit, were performed in a counterbalanced manner and consisted of an identical experimental protocol with the exception of the post-exercise beverage consumed. During each of the 3 experimental sessions, participants consumed one of three experimental beverages following exercise: 1) plain water (W); 2) a commercially-available carbohydrate/electrolyte solution (CES), designed for the post-exercise period (Powerade, Coca Cola Ltd, Toronto, Canada); 3) skim milk (SM) (0.1% Skim Milk; Beatrice, Parmalat, Toronto, Canada). The volume consumed was equal to 100% of the body fluid lost during the previous exercise, as previously described (24). The non-invasive oral $^{15}$N\text{glycine} technique, with samples collected over a 16h period (the time in the laboratory for each experimental session plus the subsequent overnight period), was used to determine the
effect of beverage consumption on whole body nitrogen turnover (Q), whole body protein
synthesis (S), whole body protein breakdown (B) and WBPB.

**Preliminary visit.** Children attended an initial screening visit, during which we obtained
basic anthropometric and aerobic fitness measurements including stature (Harpenden wall-
mounted Stadiometer), body mass (Tanita BWB-800S digital scale, Tanita Corp., Japan), and
body composition (InBody520 bioelectrical impedance analyzer; Biospace Co., California,
USA). Maturational status was self-assessed according to Tanner criteria (21) using pubic hair
development for boys and breast development for girls. To measure aerobic fitness, we
determined peak oxygen uptake (VO$_{2\text{peak}}$) using the McMaster All-Out Progressive Continuous
Cycling Test. The VO$_{2\text{peak}}$ test was performed in a thermoneutral environment (22°C, 54%
relative humidity (RH)). The highest 30-second VO$_2$ was considered the VO$_{2\text{peak}}$. The test was
terminated when the child could no longer maintain the pre-set cadence of 60 revolutions per
minute (rpm), despite strong verbal encouragement by the investigator. Participants performed
each of their sessions on the same mechanically or electromagnetically braked cycle ergometer
(Fleisch-Metabo, Geneva, Switzerland or Lode Corival, The Netherlands, respectively). Expired
gases were examined throughout the exercise over 30-second intervals in the mixing chamber
setting on a calibrated metabolic cart (Vmax 29, SensorMedics, Yorba Linda, CA, U.S.A), with
appropriately-sized pediatric mouthpieces.

**Experimental protocol.** Children reported to the laboratory at ~3:30 pm for each of their
experimental sessions. On the day of the first experimental session, parents were given a log
book to record everything the child ate and drank throughout the day, before arrival to the
laboratory. Participants were then asked to replicate this diet as closely as possible prior to each
of the subsequent experimental sessions. Participants were also asked to avoid eating at least 1h
before arriving to the laboratory, to avoid any strenuous physical activity on the days of
experimental testing, and to avoid caffeine for 12h prior to each visit. Upon arrival, each child
was asked to empty his/her bladder and provide a spot urine to measure background $[^{15}\text{N}]$
enrichment of urinary ammonia. Participants then consumed 2 mg/kg body mass of $[^{15}\text{N}]$glycine
dissolved in 5 ml/kg body mass of tap water, along with a pre-exercise standardized meal. This
was followed by 1h of rest before entering a climate chamber set to 35°C and 48% relative
humidity to perform 2 × 20-min bouts of cycling at 60% of their previously determined VO$_{2peak}$.

Upon completion of the exercise, participants exited the climate chamber and rested in a
thermoneutral room. At 0, 15 and 30 min following the completion of exercise, participants
consumed three equal aliquots of the experimental beverage in a volume equal to 100% of the
body fluid lost during exercise, as previously described (24). Participants were then asked to rest
in the laboratory for 2h before ingesting their post-exercise standardized meal.

**Urine collection.** All urine produced while in the laboratory, following ingestion of the
[^{15}\text{N}]glycine, was collected at scheduled time points, pooled, and stored at 4°C until the
following day. Upon leaving the laboratory, participants were provided with a urine collection
container and were instructed to collect all urine produced during the evening until the first
urination the following morning (inclusive). Participants were instructed to store the container at
4°C. All urine from the laboratory and home were then pooled and the total volume measured to
the nearest ml. Two 3-ml aliquots representing the 16h measurement period were stored at -20°C
until subsequent analysis.

**Diet.** Each participant was provided with a pre- and post-exercise meal so as to
standardize nutrition throughout the 16h urine collection. These meals, consumed in the
laboratory, consisted of a piece of toast with raspberry jam, an apple, a Nutrigrain bar and a
Boost meal replacement drink. All food was weighed so that each participant received the same amount of food relative to his/her body mass (i.e., g of food or fluid per kg body mass). The total nutrition over the 16h measurement period also included the experimental beverages; thus, due to the nature of the trial, protein intake during the SM trial was higher than during the W and CES trials.

**Analysis of samples.** To estimate urinary nitrogen excretion, the sum of the major nitrogen-containing metabolites urea and creatinine were determined by colorimetric analysis using commercially available kits (Quantichrom, Bioassay Systems, USA). The enrichments (i.e. ratio of tracer:trace, t:Tr) of urinary $[^{15}\text{N}]$ammonia (in baseline and 16h samples) were determined in duplicate by isotope ratio mass spectrometry by Metabolic Solutions Incorporated (Nashua, NH, USA). $Q$, determined by the $[^{15}\text{N}]$ammonia end-product method, was then calculated as:

$$Q \text{ (g N/kg)} = \frac{d}{\text{corrected t:Tr}} \frac{\text{BM}}{\text{BM}}$$

where $d$ is the dose of oral $[^{15}\text{N}]$glycine, corrected t:Tr is the baseline corrected $[^{15}\text{N}]$ enrichment of urinary ammonia, and BM is the participant’s body mass. $S$ was calculated as:

$$S \text{ (g protein/kg)} = [Q-(E/\text{BM})] \times 6.25 \text{ g protein/g N}$$

where $E$ is nitrogen excretion expressed as the sum of both measured and estimated nitrogen excretion. Measured nitrogen excretion was calculated as the sum of urinary urea and creatinine nitrogen excretion over the 16h period. Estimated nitrogen excretion was calculated using estimated average sweat nitrogen and amino acid concentrations (1) with an average ~15% nitrogen content of amino acids (13), multiplied by fluid loss estimated by change in body mass for each participant. In agreement with previously published values in children consuming a 1.2
g protein/kg/d diet, fecal nitrogen excretion was estimated to be 0.9 mg/kg/h (10). B was calculated as:

\[ B (\text{g protein/kg}) = \left[ \frac{Q - (I)/BM} \right] \times 6.25 \text{ g protein/g N} \]

where I is nitrogen intake determined by analysis of the standardized meals provided along with the experimental beverage. Finally, WBPB was determined as:

\[ \text{WBPB (g protein/kg)} = S - B \]

**Statistical analysis.** All data were analyzed using Statistica version 5.0. To determine differences in protein intake in the SM trial, a 2-way (puberty × sex) ANOVA was performed. To assess the effects of beverage, a separate 1-way repeated measures ANOVA was used for Q, S, B and WBPB (total of 4 ANOVAs). To assess the effects of puberty and sex on milk’s ability to maintain protein balance, Q, S, B and WBPB from the SM trial were analyzed using separate 2-way (puberty × sex) ANOVAs (total of 4 ANOVAs). When main effects or interactions were significant, the source of statistically significant differences was determined using Tukey’s *post hoc* test. The significance level for all tests was set at \( p < 0.05 \). All data are presented as mean ± SD, as well as 95% confidence intervals where appropriate. Effect sizes for primary outcome variables were calculated using eta squared (\( \eta^2 \)) and interpreted according to Cohen’s guidelines (5).

**RESULTS**

Thirty-eight participants were initially recruited to participate in the study. Six participants were excluded due to failure to provide an overnight urine sample, two participants were excluded due to missing data, and two participants excluded due to values that were greater
than 2 SD from the mean value for their puberty and gender for each of the following variables: Q, S, B and WBPB. As such, our sample size was reduced to 28 participants.

**Experimental diet.** The experimental beverages in both the W and CES trials provided an absolute and relative protein intake of 0 ± 0 g and 0 ± 0 g/kg, respectively. The absolute protein intake from the SM beverage was 18.1 ± 7.0 g, with PEP children consuming a smaller absolute amount of protein than MLP children (12.2 ± 3.8 g and 24.0 ± 3.7 g, respectively; p < 0.001), by virtue of lower sweating rates during the previous exercise. However, when expressed relative to body mass, protein intake from the SM beverage (0.40 ± 0.10 g/kg) did not differ between pubertal groups or between sexes. Macronutrient intake is summarized in Table 2. As a result of the differences in beverage composition (24), macronutrient intake over the 16h observation period (which included the pre- and post-exercise standardized meals, and the experimental beverages) differed between experimental trials for energy (p < 0.05), carbohydrate (p < 0.001), fat (p < 0.05) and protein (p < 0.001) intakes.

**Whole body protein metabolism.** Rates of Q, S, B and WBPB over 16h are summarized in Table 3. A main effect for beverage was observed for Q (p < 0.001), S (p < 0.01), and WBPB (p = 0.01), while B demonstrated a trend for a main effect for beverage (p = 0.063). Rates of Q, S, B, and WBPB according to puberty and sex in the SM trial are summarized in Table 4. There were no main effects for puberty or sex, nor were statistically significant puberty × sex interactions observed for Q, S or B. WBPB demonstrated a main effect for both puberty (p < 0.001) and sex (p < 0.05), however, no puberty × sex interaction was found.

**DISCUSSION**

The potential benefits of adding protein to a post-exercise beverage to enhance lean tissue
remodeling and increase lean body mass in growing children cannot be overlooked. In this study, we demonstrate that SM, a protein-rich beverage, stimulates protein synthesis to a greater extent than W and a CES, and as a result creates a less catabolic environment over 16h following exercise in a warm environment. Despite the improvement in WBPB, it is apparent that children require a higher protein intake than that of the current study to achieve a net anabolic state during an overnight period. Furthermore, it is important to consider age- and sex-specific recommendations; we demonstrated that PEP and MLP children, as well as boys and girls showed differences in post-exercise WBPB following the consumption of SM. For example, although all children received the same relative dose of protein following exercise, MLP children experienced a more negative WBPB than PEP children. Furthermore, only the PEP girls were able to attain a positive WBPB over the 16h post-exercise recovery period.

Over a 16h recovery period, the post-exercise consumption of SM significantly increased the rate of S, and had a tendency to increase B. While exercise training has known effects on protein metabolism in children, including a decrease in protein turnover and increase in nitrogen balance (4; 16), we are not aware of studies examining the acute protein response to specific episodes of exercise. Our results suggest that the post-exercise ingestion of SM had a greater effect on the stimulation of protein synthesis than of protein breakdown. Although we lack the ability to determine the extent to which the metabolism within the skeletal muscle of the children influenced changes in WBPB, the observation that post-exercise protein synthesis was stimulated by post-exercise protein ingestion is consistent with previous adult studies (14; 28). Since changes in protein synthesis are a large contributing factor to changes in protein balance (15), it is not surprising that children had a significantly more negative WBPB following the ingestion of protein-free beverages, such as W and CES.
An important consideration with regards to growth in active children is the attainment of a positive net protein balance – whereby the anabolic pathways are activated to a greater extent than the catabolic pathways. However, a large proportion of children in the present study, regardless of experimental condition (25 of 28 in W, 24 of 28 in CES, 19 of 28 in SM), experienced a negative net WBPB over the 16h recovery period. This observation was made despite the fact that all children in the SM trial consumed a significantly greater amount of protein than the relative dose of dietary protein shown to maximally stimulate post-exercise muscle protein synthesis in young adults (~0.40 g/kg vs. ~0.25 g/kg, respectively) (14). Although it is possible that children require a larger relative protein dose due to higher rates of tissue remodeling, the negative WBPB observed is more likely a result of the observation period used.

In our study, children spent a large portion of the recovery period in the post-prandial and overnight fasted states. Despite the elevated rate of protein turnover as a result of the SM beverage, it is possible that the lack of additional feeding periods resulted in an insufficient stimulation of protein synthesis to offset the fasted losses that were experienced. It is unclear whether the children in the present study would have reached a positive WBPB over a 24h observation period that takes into account additional feedings. These findings emphasize the need for future studies to investigate the impact of post-exercise milk consumption over an entire 24h period to further our understanding of optimal energy and protein intake in active children.

In addition, it is possible that the oral [\textsuperscript{15}N]glycine methodology used was not sensitive enough to detect relatively small, albeit, potentially physiologically relevant, differences in protein turnover between conditions that may have been seen with other methodology (e.g. intravenous infusion). Moreover, a potential limitation of oral tracers, including [\textsuperscript{15}N]glycine, is they represent the net sum of all nitrogen metabolism in the body (e.g. within muscle, splanchnic bed,
etc.), whereas other stable isotope methodologies, like \(^{13}\)C-leucine infusion, are preferentially metabolized within the skeletal muscle. The decision to utilize the \(^{15}\)N-glycine methodology in the present study was based on the following: 1) the relatively low within-subject variability (9); 2) the ease of measuring protein kinetics over relatively long time frames (i.e. 16h) (11); and 3) its feasible application in healthy children (7). Future studies are needed to gain a better understanding of post-exercise protein requirements using alternative tracer methodologies in healthy, active children.

In healthy children, puberty is characterized by a number of metabolic and hormonal changes (19), including an increase in insulin resistance that is highest during mid-puberty (2). Although we did not assess insulin resistance in the present study, it is possible that the MLP group may have been in a state of relative insulin resistance. As a result, the MLP children may have experienced a reduction in sensitivity to both the insulin-induced stimulation of protein synthesis and to amino acid feeding which would explain the resultant negative WBPB over the 16h recovery period that was not experienced by the PEP group. Although the exact mechanisms for the relatively more negative WBPB in MLP is unknown, our findings suggest that higher protein doses (>0.40 g/kg) or the frequency and timing of protein intake may be more important in this group compared with pre- and early-pubertal youth. Future studies are needed to examine the relationship between protein dose and timing of protein intake in pubertal children in order to maximize WBPB.

PEP girls were able to attain a positive WBPB over the 16h recovery period, whereas the PEP boys remained in a net negative WBPB, suggesting that sex-specific differences should also be considered. However, it is important to note that in the present study, we did not control for menstrual cycle nor did we assess hormonal markers, thus, we cannot decipher the mechanism
by which these differences might exist. Indeed, the effect of testosterone and growth hormone on protein metabolism remains controversial (22; 26); however, it is possible that hormonal differences between the girls and the boys contributed to the differences in WBPB between groups. Regardless of the mechanisms, it is apparent that further studies involving a greater sample size are needed to appropriately compare boys and girls by maturity status. Another limitation of this study is that we only examined 1 type of protein, as both protein source and protein quality are important factors to consider in dietary recommendations for growing children (16). Skim milk, the protein source of the present study, is considered to be a high quality, nutrient dense protein source (16) with a number of additional essential micronutrients. Adult studies have shown that in general, proteins of higher quality are better able to support muscle protein accretion and enhance WBPB after exercise (15; 27). To date, there are no studies examining the effects of protein source or protein quality on protein metabolism following exercise in children. Therefore, whether different protein sources (e.g. plant-based) would have similar effects of post-exercise protein metabolism is unknown and should be investigated in future studies.

In conclusion, this is the first study to investigate the effects of post-exercise milk ingestion on protein metabolism in active youth. SM consumption resulted in elevated Q, S and WBPB, and a trend of elevated rates of B compared to W and a CES. Despite the relatively large dose of protein ingested in SM, children were unable to attain a positive WBPB over the 16h recovery period, probably as a result of the timing of our meals and nitrogen assessments. Regardless, our findings suggest that SM is more effective than W or a commercially available sport drink at stimulating protein synthesis and promoting a more favourable environment for the remodeling of lean tissues following exercise in a hot environment. This study highlights the fact
that youth can benefit from consuming a high-quality protein source post-exercise for enhancements of WBPB. Future studies should seek to assess graded levels of protein intake in order to gain a better understanding of the doses required for healthy, active youth.
ACKNOWLEDGEMENTS

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DISCLOSURES

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REFERENCES


### Table 1. Participant characteristics.

<table>
<thead>
<tr>
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<th>PEP</th>
<th>MLP</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Boys</td>
<td>Girls</td>
<td>Boys</td>
<td>Girls</td>
</tr>
<tr>
<td>N</td>
<td>6</td>
<td>8</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Age (y)</td>
<td>9.4 ± 1.0</td>
<td>9.5 ± 0.8</td>
<td>15.6 ± 0.5*</td>
<td>14.8 ± 0.4*†</td>
</tr>
<tr>
<td>Stature (cm)</td>
<td>137 ± 8</td>
<td>136 ± 9</td>
<td>171 ± 8*</td>
<td>169 ± 4*</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>34.2 ± 7.7</td>
<td>29.6 ± 5.7</td>
<td>59.4 ± 9.0*</td>
<td>60.5 ± 8.4*</td>
</tr>
<tr>
<td>Body fat (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.5 ± 8.4</td>
<td>14.3 ± 6.1</td>
<td>15.7 ± 8.4</td>
<td>21.3 ± 5.5</td>
</tr>
<tr>
<td>Tanner stage</td>
<td>2 (1)</td>
<td>1 (0)</td>
<td>4 (1)</td>
<td>4 (1)</td>
</tr>
</tbody>
</table>

PEP, Pre- to early-pubertal; MLP, mid- to late-pubertal. *Significant difference from pre-pubertal, p < 0.001, †Significant difference between sexes, p < 0.05. a determined using bioelectrical impedance analysis as described (20). Data are presented as mean ± SD or median (interquartile range).
Table 2. Dietary intakes.

<table>
<thead>
<tr>
<th></th>
<th>16-h Intake</th>
<th>24-h Intake</th>
<th>24-h Habitual Intake</th>
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<tbody>
<tr>
<td></td>
<td>W</td>
<td>CES</td>
<td>SM</td>
</tr>
<tr>
<td>Energy (kcal/kg)</td>
<td>29.14 ± 5.33 a</td>
<td>33.49 ± 6.82 b</td>
<td>31.95 ± 5.37 a</td>
</tr>
<tr>
<td>Carbohydrate (g/kg)</td>
<td>5.54 ± 1.00 a</td>
<td>6.14 ± 1.24 b</td>
<td>5.89 ± 0.98 c</td>
</tr>
<tr>
<td>Fat (g/kg)</td>
<td>0.44 ± 0.08 a</td>
<td>0.44 ± 0.09 a</td>
<td>0.42 ± 0.07 b</td>
</tr>
<tr>
<td>Protein (g/kg)</td>
<td>0.83 ± 0.16 a</td>
<td>0.82 ± 0.16 a</td>
<td>1.24 ± 0.23 b</td>
</tr>
</tbody>
</table>

16-h intake consisted of controlled diet consumed within the lab; 24-h Intake was comprised of the 16-h in-lab diet, as well as an extrapolated analysis of the 8-h prior to arrival to the lab, analyzed by dietary logs; 24-h Habitual Intake was the average of the 3-day diet log prior to study commencement. Water (W); carbohydrate-electrolyte solution (CES); skim milk (SM). Data reported as means ± SD. Conditions with different letters are significantly different from each other within the respective measurement time period, p < 0.05.
Table 3. 16-h whole body protein metabolism.

<table>
<thead>
<tr>
<th></th>
<th>W</th>
<th>CES</th>
<th>SM</th>
<th>p-value</th>
<th>η²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q (g N/kg)</td>
<td>0.62 ± 0.11&lt;sup&gt;a&lt;/sup&gt; 0.58, 0.67</td>
<td>0.61 ± 0.12&lt;sup&gt;a&lt;/sup&gt; 0.57, 0.67</td>
<td>0.69 ± 0.12&lt;sup&gt;b&lt;/sup&gt; 0.65, 0.74</td>
<td>&lt; 0.001</td>
<td>0.080</td>
</tr>
<tr>
<td>S (g/kg)</td>
<td>2.94 ± 0.59&lt;sup&gt;a&lt;/sup&gt; 2.77, 3.22</td>
<td>2.90 ± 0.72&lt;sup&gt;a&lt;/sup&gt; 2.67, 3.22</td>
<td>3.33 ± 0.64&lt;sup&gt;b&lt;/sup&gt; 3.10, 3.64</td>
<td>&lt; 0.01</td>
<td>0.081</td>
</tr>
<tr>
<td>B (g/kg)</td>
<td>3.30 ± 1.12&lt;sup&gt;a,b&lt;/sup&gt; 3.06, 3.54</td>
<td>3.26 ± 0.14&lt;sup&gt;a&lt;/sup&gt; 2.98, 3.54</td>
<td>3.56 ± 0.15&lt;sup&gt;b&lt;/sup&gt; 3.26, 3.85</td>
<td>0.06</td>
<td>0.034</td>
</tr>
<tr>
<td>WBPB (g/kg)</td>
<td>-0.32 ± 0.28&lt;sup&gt;a&lt;/sup&gt; -0.41, -0.20</td>
<td>-0.33 ± 0.25&lt;sup&gt;a&lt;/sup&gt; -0.42, -0.22</td>
<td>-0.19 ± 0.36&lt;sup&gt;b&lt;/sup&gt; -0.32, -0.05</td>
<td>0.01</td>
<td>0.043</td>
</tr>
</tbody>
</table>

Whole body nitrogen turnover (Q), protein synthesis (S), protein breakdown (B), and net protein balance (WBPB) determined using the $[^{15}\text{N}]$ammonia end-product method. Water (W); carbohydrate-electrolyte solution (CES); skim milk (SM). Data reported as means ± SD and [95% confidence interval]. Conditions with different letters are significantly different from each other within the respective variable group, p < 0.05.
### Table 4. 16h whole body protein metabolism across pubertal groups and sex.

<table>
<thead>
<tr>
<th></th>
<th>PEP girls</th>
<th>PEP boys</th>
<th>MLP girls</th>
<th>MLP boys</th>
<th>Puberty p-value ($\eta^2$)</th>
<th>Sex p-value ($\eta^2$)</th>
<th>Interaction p-value ($\eta^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q (g N/kg)</td>
<td>0.73 ± 0.13</td>
<td>0.71 ± 0.15</td>
<td>0.62 ± 0.11</td>
<td>0.72 ± 0.11</td>
<td>0.336 (0.036)</td>
<td>0.405 (0.026)</td>
<td>0.234 (0.055)</td>
</tr>
<tr>
<td>S (g/kg)</td>
<td>3.59 ± 0.84 [2.89, 4.29]</td>
<td>3.15 ± 0.65 [2.47, 3.83]</td>
<td>3.08 ± 0.52 [2.60, 3.56]</td>
<td>3.62 ± 0.68 [2.99, 4.24]</td>
<td>0.938 (0.002)</td>
<td>0.852 (0.001)</td>
<td>0.076 (0.125)</td>
</tr>
<tr>
<td>B (g/kg)</td>
<td>3.42 ± 0.79 [2.76, 4.07]</td>
<td>3.26 ± 0.91 [2.30, 4.22]</td>
<td>3.43 ± 0.63 [2.85, 4.02]</td>
<td>4.09 ± 0.61 [3.52, 4.66]</td>
<td>0.144 (0.079)</td>
<td>0.381 (0.027)</td>
<td>0.161 (0.072)</td>
</tr>
<tr>
<td>WBPB (g/kg)</td>
<td>0.17 ± 0.20 a [0.00, 0.34]</td>
<td>-0.11 ± 0.42 a,b [-0.55, 0.33]</td>
<td>-0.35 ± 0.14 b [-0.49, -0.22]</td>
<td>-0.47 ± 0.14 b [-0.60, -0.35]</td>
<td>&lt;0.001 (0.420)</td>
<td>0.040 (0.086)</td>
<td>0.402 (0.013)</td>
</tr>
</tbody>
</table>

Whole body nitrogen turnover (Q), protein synthesis (S), protein breakdown (B), and net protein balance (WBPB) determined using the $^{15}$N ammonia end-product method during the skim milk (SM) trial. Data reported as means ± SD and [95% confidence interval]. Groups with different letters are significantly different from each other within the respective variable group, p < 0.05.