Effects of postexercise milk consumption on whole body protein balance in youth

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Volterman KA, Obeid J, Wilk B, Timmons BW. Effects of postexercise milk consumption on whole body protein balance in youth. J Appl Physiol 117: 1165–1169, 2014. First published September 25, 2014; doi:10.1152/japplphysiol.01227.2013.—In adults, adding protein to a postexercise beverage increases muscle protein turnover and replenishes amino acid stores. Recent focus has shifted toward the use of bovine-based milk and milk products as potential postexercise beverages; however, little is known about how this research translates to the pediatric population. Twenty-eight (15 girls) pre- to early pubertal (PEP, 7–11 yr) and mid- to late-pubertal (MLP, 14–17 yr) children consumed an oral dose of [15N]glycine prior to performing 2 × 20-min cycling bouts at 60% V̇O₂peak 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 a 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), and protein breakdown (B), and whole body protein balance (WBPB) were measured over 16 h. Protein intake from SM was 0.40 ± 0.10 g/kg. Over 16 h, 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, although it remained negative in MLP. Boys exhibited significantly more negative WBPB than girls (P < 0.05). Postexercise milk consumption enhances WBPB compared with W and CES; however, additional protein intake may be required to sustain a net anabolic environment over 16 h.

In adults, much of the focus in recent years has shifted toward the use of bovine-based milk and milk products as potential postexercise beverages (12, 17, 18, 25), however, very little is known about how this research translates to the pediatric population. Although 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 because 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 downregulation 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 postexercise 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 favorably affect 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 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).

Participants. Twenty-eight pre- to early pubertal (PEP, 7–11 yr) and mid- to late-pubertal (MLP, 14–17 yr) 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.

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1165
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.

Table 1. Participant characteristics

<table>
<thead>
<tr>
<th></th>
<th>PEP</th>
<th>MLP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Boys</td>
<td>Girls</td>
</tr>
<tr>
<td>$n$</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Age, y</td>
<td>9.4 ± 1.0</td>
<td>9.7 ± 0.8</td>
</tr>
<tr>
<td>Stature, cm</td>
<td>137 ± 8</td>
<td>136 ± 9</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>34.2 ± 7.7</td>
<td>29.6 ± 5.7</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>14.5 ± 8.4</td>
<td>14.3 ± 6.1</td>
</tr>
<tr>
<td>Tanner stage</td>
<td>2 (1)</td>
<td>1 (0)</td>
</tr>
</tbody>
</table>

PEP, pre- to early pubertal; MLP, mid- to late-pubertal. *Significant difference from prepubertal, $P < 0.001$. †Significant difference between sexes, $P < 0.05$. *Determined using bioelectrical impedance analysis as described in (20). Data are presented as mean ± SD or median (interquartile range).

**Experimental protocol.** Children reported to the laboratory at ~3:30 P.M. 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 1 h before arriving to the laboratory, to avoid any strenuous physical activity on the days of experimental testing, and to avoid caffeine for 12 h prior to each visit. Upon arrival, each child was asked to empty his or her bladder and provide a spot urine to measure background [15N] enrichment of urinary ammonia. Participants then consumed 2 mg/kg body mass of [15N]glycine dissolved in 5 mL/kg body mass of tap water, along with a preexercise standardized meal. This was followed by 1 h of rest before entering a climate chamber set to 35°C and 48% relative humidity to perform $2 \times 20$-min bouts of cycling at 60% of their previously determined VO$_2$ peak.

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 2 h before ingesting their postexercise standardized meal.

**Urine collection.** All urine produced while in the laboratory following ingestion of the [15N]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 milliliter. Two 3-ml aliquots representing the 16-h measurement period were stored at −20°C until subsequent analysis.

**Diet.** Each participant was provided with a pre- and postexercise meal to standardize nutrition throughout the 16-h urine collection. These meals, consumed in the laboratory, consisted of a piece of toast with raspberry jam, an apple, a Nutrigain bar, and a Boost meal replacement drink. All food was weighed so that each participant received the same amount of food relative to his or her body mass (i.e., gram of food or fluid per kilogram of body mass). The total nutrition over the 16 h 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 (QuantiChrome; Bioassay Systems). The enrichments (i.e., ratio of tracer:trace, t:Tr) of urinary [15N]ammonia (in baseline and 16-h samples) were determined in duplicate by isotope ratio mass spectrometry by Metabolic Solutions (Nashua, NH). Q, determined by the [15N]ammonia end-product method, was then calculated as Q (g N/kg) = d/corrected t:Tr/BM, where $d$ is the dose of oral [15N]glycine, corrected t:Tr is the baseline corrected [15N] enrichment of urinary ammonia, and BM is the participant’s body mass. S was calculated as $S$ (g protein/kg) = $[Q - (E/BM)] \times 6.25$ 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 16-h 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$^{-1}$·day$^{-1}$ diet, fecal nitrogen excretion was estimated to be 0.9 mg·kg$^{-1}$·h$^{-1}$ (10). B was calculated as $B$ (g protein/kg) =
Table 2. Dietary intakes

<table>
<thead>
<tr>
<th>Intake, 16 h</th>
<th>W</th>
<th>CES</th>
<th>SM</th>
<th>Habitus Intake, 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kcal/kg</td>
<td>29.14 ± 5.33a</td>
<td>33.49 ± 6.82b</td>
<td>31.95 ± 5.37a</td>
<td>30.51 ± 5.86a</td>
</tr>
<tr>
<td>Carbohydrate, g/kg</td>
<td>5.54 ± 1.00a</td>
<td>6.14 ± 1.24b</td>
<td>5.89 ± 0.98c</td>
<td>6.87 ± 1.15a</td>
</tr>
<tr>
<td>Fat, g/kg</td>
<td>0.44 ± 0.08a</td>
<td>0.44 ± 0.09a</td>
<td>0.42 ± 0.07b</td>
<td>0.65 ± 0.08a</td>
</tr>
<tr>
<td>Protein, g/kg</td>
<td>0.83 ± 0.16a</td>
<td>0.82 ± 0.16a</td>
<td>1.24 ± 0.23b</td>
<td>1.24 ± 0.23b</td>
</tr>
</tbody>
</table>

Intake over 16 h consisted of controlled diet consumed within the laboratory; intake over 24 h consisted of the 16-h in-laboratory diet and an extrapolated analysis of consumption in the 8 h prior to arrival at the laboratory analyzed by dietary logs; habitual intake over 24 h was the average of the 3-day diet log prior to study commencement. W, water; CES, carbohydrate-electrolyte solution; SM, skim milk. Data are reported as mean ± SD. Conditions with different letters are significantly different from each other within the respective measurement time period, P < 0.05.

Statistical analysis. All data were analyzed using Statistica version 5.0. To determine differences in protein intake in the SM trial, a two-way (puberty × sex) ANOVA was performed. To assess the effects of beverage, a separate one-way repeated measures ANOVA was used for Q, S, B, and WBPB. To compare 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 two-way (puberty × sex) ANOVAs (a total of four ANOVAs). When main effects or interactions were significant, the source of differences was assessed by a post hoc test. The significance level for all tests was set at P < 0.05. All statistical tests were performed using Statistica version 5.0.

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 were 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 16-h observation period (which included the pre- and postexercise 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 16 h 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), whereas 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 postexercise 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 16 h 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—boys and girls both—showed differences in postexercise WBPB following the consumption of SM. For example, although all children

Table 3. Whole body protein metabolism over 16 h

<table>
<thead>
<tr>
<th>W</th>
<th>CES</th>
<th>SM</th>
<th>P</th>
<th>ω²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q, g N/kg</td>
<td>0.62 ± 0.11a (0.58, 0.67)</td>
<td>0.61 ± 0.12a (0.57, 0.67)</td>
<td>0.69 ± 0.12b (0.65, 0.74)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>S, g/kg</td>
<td>2.94 ± 0.59a (2.77, 3.22)</td>
<td>2.90 ± 0.72a (2.67, 3.22)</td>
<td>3.33 ± 0.64a (3.10, 3.64)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>B, g/kg</td>
<td>3.30 ± 1.12a (3.06, 3.54)</td>
<td>3.26 ± 0.14a (2.98, 3.54)</td>
<td>3.56 ± 0.15a (3.26, 3.85)</td>
<td>0.06</td>
</tr>
<tr>
<td>WBPB, g/kg</td>
<td>-0.32 ± 0.28a (-0.41, -0.20)</td>
<td>-0.33 ± 0.25a (-0.42, -0.22)</td>
<td>-0.19 ± 0.36a (-0.32, -0.05)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Q, whole body nitrogen turnover; S, protein synthesis; B, protein breakdown; WBPB, whole body net protein balance determined using the [15N]ammonia end-product method. Data are reported as mean ± SD and (95% confidence interval). Conditions with different letters are significantly different from each other within the respective variable group, P < 0.05.
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 16-h postexercise recovery period.

Over a 16-h recovery period, the postexercise consumption of SM significantly increased the rate of S, and had a tendency to increase B. Although 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 postexercise 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 postexercise protein synthesis was stimulated by postexercise protein ingestion is consistent with previous adult studies (14, 28). Because 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, and 19 of 28 in SM), experienced a negative net WBPB over the 16-h recovery period. This observation was made despite all children in the SM trial consuming a significantly greater amount of protein than the relative dose of dietary protein shown to maximally stimulate postexercise 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 postprandial and overnight fasted states. Despite an 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 24 h observation period that takes into account additional feedings. These findings emphasize the need for future studies to investigate the impact of postexercise milk consumption over an entire 24-h period to further our understanding of optimal energy and protein intake in active children. In addition, it is possible that the oral

\[ ^{15}\text{N} \text{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 observed with another methodology (e.g., intravenous infusion). Moreover, a potential limitation of oral tracers, including \( ^{15}\text{N} \text{glycine}, \text{is that they represent the net sum of all nitrogen metabolism in the body (i.e., within muscle, splanchnic bed, etc.), whereas other stable isotope methodologies, such as \( ^{13}\text{C} \text{leucine} \text{, are preferentially metabolized within the skeletal muscle. The decision to utilize the \( ^{15}\text{N} \text{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., 16 h) (11); and 3) its feasible application in healthy children (7). Future studies are needed to gain a better understanding of postexercise 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 midpuberty (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 16-h recovery period that was not experienced by the PEP group. Although the precise 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 to maximize WBPB.

PEP girls were able to attain a positive WBPB over the 16-h 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

Table 4. Whole body protein metabolism across pubertal groups and sex over 16 h

<table>
<thead>
<tr>
<th>Puberty</th>
<th>Sex</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEP Girls</td>
<td>PEP Boys</td>
<td>MLP Girls</td>
</tr>
<tr>
<td>Q, g N/kg</td>
<td>0.73 ± 0.15 (0.550.86)</td>
<td>0.62 ± 0.10 (0.520.72)</td>
</tr>
<tr>
<td>S, g/kg</td>
<td>3.59 ± 0.84 (2.894.29)</td>
<td>3.15 ± 0.65 (2.473.83)</td>
</tr>
<tr>
<td>B, g/kg</td>
<td>3.42 ± 0.79 (2.764.07)</td>
<td>3.26 ± 0.91 (2.304.22)</td>
</tr>
<tr>
<td>WBPB, g/kg</td>
<td>0.17 ± 0.20 (0.000.34)</td>
<td>−0.11 ± 0.42 (−0.550.33)</td>
</tr>
</tbody>
</table>

WBPB, whole body net protein balance was determined using the \( ^{15}\text{N} \text{ammonia end-product method during the skim milk trial. Data are reported as mean ± SD and (95% confidence interval). Groups with different letters are significantly different from each other within the respective variable group, } P < 0.05.}
of this study is that we examined only one type of protein, because both protein source and protein quality are important factors to consider in dietary recommendations for growing children (16). SM, 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 postexercise protein metabolism is unknown and should be investigated in future studies.

In conclusion, this is the first study to investigate the effects of postexercise milk ingestion on protein metabolism in active youth. SM consumption resulted in elevated Q, S, and WBPB, and a trend toward elevated rates of B compared with W and a CES. Despite the relatively large dose of protein ingested in SM, children were unable to attain a positive WBPB over the 16-h 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 favorable 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 postexercise for enhancements of WBPB. Future studies should seek to assess graded levels of protein intake to gain a better understanding of the doses required for healthy, active youth.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES