Postexercise protein ingestion increases whole body net protein balance in healthy children

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1Physical Performance and Mobility Group, Nestle Research Centre, Lausanne, Switzerland; 2Faculty of Kinesiology and Physical Education, University of Toronto, Toronto, Canada; and 3Child Health and Exercise Medicine Program, Department of Pediatrics, McMaster University, Hamilton, Canada

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Moore DR, Volterman KA, Obeid J, Offord EA, Timmons BW. Postexercise protein ingestion increases whole body net protein balance in healthy children. J Appl Physiol 117: 1493–1501, 2014. First published October 23, 2014; doi:10.1152/japplphysiol.00224.2014.—Postexercise protein ingestion increases whole body and muscle protein anabolism in adults. No study has specifically investigated the combined effects of exercise and protein ingestion on protein metabolism in healthy, physically active children. Under 24-h dietary control, 13 (seven males, six females) active children (∼11 yr old; 39.3 ± 5.9 kg) consumed an oral dose of [15N]lysine prior to performing a bout of exercise. Immediately after exercise, participants consumed isooenergetic mixed macronutrient beverages containing a variable amount of protein (0, 0.75, and 1.5 g/100 ml for control (CON), low protein (LP), and high protein (HP), respectively) according to fluid losses. Whole body nitrogen turnover (Q), protein synthesis (S), protein breakdown (B), and protein balance (WBPB) were measured throughout exercise and the early acute recovery period (9 h combined) as well as over 24 h. Postexercise protein intake from the beverage was ~0.18 and ~0.32 g/kg body mass for LP and HP, respectively. Q, S, and B were significantly greater (main effect time, all P < 0.001) over 9 h compared with 24 h with no differences between conditions. WBPB was also greater over 9 h compared with 24 h in all conditions (main effect time, P < 0.001). Over 9 h, WBPB was greater in HP (P < 0.05) than LP and CON with a trend (P = 0.075) toward LP being greater than CON. WBPB was positive over 9 h for all conditions but only over 24 h for HP. Postexercise protein ingestion acutely increases net protein balance in healthy children early in recovery in a dose-dependent manner with larger protein intakes (~0.32 g/kg) required to sustain a net anabolic environment over an entire 24 h period.

protein synthesis; lean body mass; youth; physical activity; protein requirements

PHYSICAL ACTIVITY is an essential component for the optimal growth and development of the musculoskeletal system in children. Greater levels of physical activity, especially those which are weight bearing and of higher intensity, have been associated with higher bone mineral density, lean mass, and strength in children (5, 18, 43). The net gain in lean mass with physical activity would require alterations in whole body protein turnover that would favor the net synthesis of body proteins, or, in other words, protein synthesis would be chronically elevated above protein breakdown. Despite the important role physical activity plays in remodeling lean tissues and enhancing lean body mass, few studies have determined the impact of specific episodes of exercise on protein metabolism in children. Whole body protein metabolism is characterized by the continuous turnover and renewal of body proteins through the simultaneous processes of protein synthesis and protein breakdown. Interestingly, previous research by the Rodriguez laboratory (8, 33) demonstrated that 6 wk of structured exercise (either resistance or aerobic) resulted in a downregulation of overnight fasted whole body protein metabolism in healthy children. Despite this potentially counterintuitive suppression of protein metabolism, 24 h net nitrogen balance, a period that would include both fasted and fed states, was elevated with a concomitant increase in lean body mass and height over the 6-wk exercise intervention that would be consistent with the normal growth velocity of the children (8, 33). The discrepancy between overnight fasted and daily net protein balance in these studies (8, 33) could be the result of a potentially increased exercise-induced anabolic sensitivity during the daily fed state, an effect that has been observed previously in exercising adults (12). Therefore, the measurement of protein metabolism in the fasted state may underestimate the anabolic effects of exercise in children.

Provided energy and micronutrient needs are met, dietary protein plays a central role in somatic growth as it provides the substrates necessary to build muscle and other body proteins (21). It is well-established in adults that the postexercise ingestion of protein is essential to maximize muscle protein synthesis (24, 25, 30) and increase whole body protein balance during the acute recovery period (23, 25). The increase in muscle and whole body protein anabolism during this early (e.g., over 3 h) postexercise period facilitates the recovery process and can be sustained over 24 h (36, 42). This synergy between exercise and nutrition for protein anabolism ultimately provides the basis for training adaptations such as lean mass growth (11). Despite the synergies between exercise and protein ingestion in facilitating muscle and whole body protein remodeling in adults, there are a lack of studies that have specifically addressed the combined effects of exercise and protein ingestion on protein metabolism in children. Therefore, the present study evaluated, for the first time in children, the effect of postexercise protein ingestion on the ability to modify whole body protein metabolism during the exercise and early (i.e., over 9 h) and late (i.e., over 24 h) recovery periods after physical activity. Given that postexercise protein ingestion improves muscle protein balance in a dose-dependent manner (9, 30), and that this enhances 24 h net protein balance in adults (36, 42), we hypothesized that whole body protein balance in the present study would demonstrate a similar ingested protein dose response after activity in children. We present here novel

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data as secondary outcomes from a larger study that yield important, hypothesis-generating information on the importance of postexercise protein ingestion to increase whole body net protein balance in healthy children.

METHODS

Participants. Thirteen (six females, seven males) volunteers participated in this study, which conformed to the standards set by the Declaration of Helsinki and carried with it approval from the Faculty of Health Sciences/Hamilton Health Sciences Research Ethics Board. All participants and their parents were informed of the purpose, procedures, and potential risks of the study both verbally and with a written copy of the information sheet. Each participant provided written informed assent and written informed consent was obtained from each parent prior to enrolment in the study. All participants were healthy and physically active as determined by medical and activity questionnaire.

General overview. As indicated previously, participants were a subset of a larger study evaluating the effect of protein in a beverage on rehydration after exercise-induced fluid loss, and, therefore, the present data are secondary outcomes from that study. Participants reported to the laboratory on five separate occasions: a preliminary visit, a familiarization visit, and three intervention visits, the latter of which were separated by a 4- to 10-d washout period (for further details, see below). The intervention visits were conducted in a randomized double-blind crossover fashion (Fig. 1). Following exercise on the intervention visits, participants ingested a mixed macronutrient beverage containing a variable amount of protein at a volume equivalent to 150% of their exercise-induced fluid loss. For each intervention visit, participants were provided with energy and macronutrient-matched controlled diets (see below for details). Whole body protein turnover was measured during the exercise and early (i.e., 9 h) acute recovery period as well as the complete 24-h period by oral [15N]glycine to determine the effect of postexercise protein ingestion on whole body protein synthesis, breakdown, and net protein balance.

Preliminary visit. Participants completed a preliminary session during which height (Harpenden wall-mounted Stadiometer), body mass (BWB-800, Tanita, Japan), percent body fat (InBody520 bioelectrical impedance analyzer, Biospace), body mass index (BMI), chronological age, and maturity offset were determined. Maturity offset was determined as the estimated years from age at peak height velocity (28). Maximal aerobic power (VO₂ max) was then assessed on a cycle ergometer (Fleisch-Metabo, Geneva, Switzerland), as previously described (41). Physical characteristics of the participants are presented in Table 1.

Familiarization visit. The purpose of this visit was to 1) familiarize the participants with the exercise equipment, 2) confirm appropriate exercise intensities, 3) ensure participants could successfully complete the exercise, and 4) determine individual estimates of fluid loss during exercise to be expected during subsequent trials. During this visit, the fastest running speed on a treadmill was determined based on a modified version of Bundle et al. (10). Briefly, prior to beginning the test, participants ran at a low to moderate speed (between 3.0 to 4.0 mph) for 30 s to allow them to become comfortable with running on a treadmill. Participants then rested for 2 min. The test then began between 4.0 to 4.5 mph and increased at 0.1 mph every 3 s until the participant 1) strongly stated verbally that they could not continue, 2) grabbed onto the handrails to stop, 3) drifted significantly backwards, or 4) pressed the stop button. The test lasted between 48 and 141 s with the fastest running speed of the participants averaging 7.4 ± 1.1 mph. Following the determination of fastest running speed, participants rested for 7 min prior to beginning the exercise protocol [using

<table>
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<th>Table 1. Participant characteristics</th>
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<tr>
<td><strong>Females (n = 6)</strong></td>
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<tr>
<td>Age, yr</td>
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<td>Height, cm</td>
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<td>Height, percentile</td>
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<td>Body mass, kg</td>
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<td>Lean body mass, kg</td>
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<td>Maturity offset</td>
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<td>Tanner stage</td>
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<td>Maximal aerobic capacity, ml·kg⁻¹·min⁻¹</td>
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Tanner staging was self-assessed based on breast development for girls and pubic hair development for boys. Percentile rankings relative to 2000 CDC growth charts for the United States (22). *Maturity offset defined as the difference between chronological age and estimated age of peak height velocity, the latter of which was calculated as previously described (28). †Different between sexes, P < 0.05. With the exception of Tanner stage (presented as n per stage), data are presented as means (SD).

![Fig. 1. A: schematic representation of the trial day with a solid time line representing time spent in the laboratory and a hashed time line representing time spent in a free-living setting with a controlled diet. The total postexercise macronutrient intake was consumed in three equal volumes every 15 min after exercise. The meals represent food that was consumed within the laboratory. B: schematic representation of the exercise protocol. PMP, peak mechanical power.](image-url)
the same protocol as the intervention visit (see below) with a shortened postexercise recovery period; this familiarization session permitted the estimation of the exercise-induced fluid loss in order to design the controlled diet for each subsequent trial (see below for details).

**Intervention visit.** Participants recorded their habitual dietary intake for the 24 h prior to reporting to the laboratory in an overnight fasted condition and were asked to match as closely as possible this diet prior to all subsequent trials. After providing a morning spot urine for baseline [15N]ammonia and urea enrichment (see below), participants consumed 2 mg/kg of [15N]glycine dissolved in water and a light breakfast providing ~12 and ~15% of each participant’s total daily energy and protein intake (details for controlled diet provided below), respectively, from food (i.e., not including the experimental beverage intake). After resting quietly for 1 h, participants then performed three blocks of 15 min of exercise for a total of 45 min (Fig. 1B). Each exercise block comprised 5 min of running at 50% of their previously determined maximal running speed and 10 min of cycling at 50% of their maximal cycling power interspersed with 10-s sprints at 100% of their maximal cycling power.

Following the laboratory period, the 24-h controlled diet comprised a breakfast providing aliquots of a beverage that contained 0 g [control (CON)], 0.76 g [low], 1.5 g [moderate (M)], and 2.24 g [high (HP)] protein/100 ml, which subsequently contained both whey and casein protein fractions in a ratio ~1:4. Beverages were also isoenergetic (28 kcal/100 ml) and provided a variable amount of carbohydrate (7.0, 5.3, and 5.3 g/100 ml) and fat (0.46, and 0.1 g/100 ml) for CON, LP, and HP, respectively. The beverages were provided in a volume equal to 150% of the participant’s measured fluid loss (based on the change in body mass with 100 g = 100 ml of fluid) during the previous bout of exercise, which, because of interindividual differences in sweat rates, resulted in a variable amount of protein being ingested between each participant. However, because fluid loss and subsequent beverage intake were similar between crossover trials, this resulted in a graded amount of protein ingested within each participant. Participants then rested comfortably in the lab for 4 h before consuming lunch, which provided ~46 and ~45% of each participant’s total daily energy and protein intake, respectively, from food (i.e., not including the experimental beverage intake).

All urine produced within the lab and after the initial morning spot urine was collected, pooled, and stored at 4°C until the following day (see below). Participants were then provided with the remainder of the controlled diet and allowed to leave the laboratory. Participants were provided with two urine containers and were instructed to collect all urine produced up to their dinner meal in one container and all urine produced in the evening and overnight fasted period, including the first urination the following morning, in the second container and store at 4°C before returning them to the laboratory the following day. All urine produced prior to the dinner meal (i.e., first urine container) was pooled with the urine collected within the laboratory, and its volume was measured to the nearest ml, after which an aliquot was taken and stored at ~80°C prior to analysis; this sample represented the 9-h exercise and early recovery period. Following this, the evening and overnight fasted urine was then pooled with the remaining 9-h sample to generate an aggregate 24-h urine sample, which was measured to the nearest ml prior to taking a second aliquot for storage at ~80°C and subsequent analysis; this sample represented the complete 24-h period.

**Controlled diet.** Participants were provided with a controlled diet for the 24-h period during which protein metabolism measures were performed. Resting energy requirements were estimated using standard equations (21a) and were corrected with an activity factor of 1.5. Based on the exercise-induced fluid loss measured during the familiarization session and assuming a similar fluid loss during all experimental trials, it was estimated that the rehydration beverages (which were all isoenergetic) would provide ~14% of each participant’s daily energy. The energy content of the beverages were subsequently added to the food portion of each participant’s controlled diet in order to calculate 24-h energy intake. In addition, the protein intake during the 24-h controlled diet was targeted to be ~1.4 g kg⁻¹d⁻¹ when participants were ingesting the estimated fluid intake (i.e., 1.5 × fluid loss) of the LP beverage (0.76 g/100 ml); hence, due to the double-blind nature of the trial, protein intakes during the controlled diet were subsequently lower and higher than 1.4 g kg⁻¹d⁻¹ during the CON and HP trials, respectively. However, all trials were targeted to provide adequate protein (i.e., ≥0.95 g kg⁻¹d⁻¹) according to current recommendations (21b). Aside from the energy and macronutrient profiles of the test beverages, the 24-h controlled diets were supplied as isoenergetic breakfast and lunch meals (consumed within the laboratory, providing ~11 and 40% of 24-h energy intake, respectively) and dinner meals (consumed outside the laboratory, providing ~35% of 24-h energy intake; Fig. 1B), with the remaining ~14% of energy coming from the test beverages. The breakfast, lunch, and dinner meals were also isoprotein and provided ~15, 45, and 40% of the 24-h food protein intake, respectively, with the test beverages providing a variable amount of protein in addition to the meal protein intake. Participants were instructed not to ingest the dinner meal away from the laboratory until the final 9-h urine sample was collected. To estimate habitual dietary intakes, participants completed a 3-d dietary record that was analyzed with The Food Processor SQL (ESHA, Salem, Oregon) software for energy and macronutrient intakes.

**Analysis.** The concentration of the major nitrogen-containing metabolites urea and creatinine were determined colorometrically by commercially available kits (QuantaChrom, Bioassay Systems) as an estimate of urinary nitrogen excretion. The [15N] enrichments (i.e., ratio of tracer-trace, t/Tr) of urinary ammonia (at baseline, 9 h, and 24 h) and urea (at baseline and 24 h) were determined in duplicate by isotope ratio mass spectrometry by Metabolic Solutions Incorporated (Nashua, NH) to determine whole body nitrogen turnover and protein metabolism (19). Whole body nitrogen turnover (Q) by the [15N]ammonia end product was calculated as Q (g N kg⁻¹h⁻¹) = d/corrected t/Tr·t/Tr·t/diet (17). Whole body protein synthesis (S) was calculated as S (g protein·kg⁻¹·d⁻¹) = (Q – E)/(t/Tr·t/diet) · t/diet (1). Whole body protein breakdown (B) was calculated as B (g protein·kg⁻¹·h⁻¹) = E(t/Tr×t/diet) · t/diet (1). Whole body protein balance (WBPB) was determined as WBPB = S – B.
Table 2. Dietary intakes

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<thead>
<tr>
<th></th>
<th>9-h Intake</th>
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<th>24-h Intake</th>
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<th>24-h Habitual Intake</th>
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<tr>
<td></td>
<td>CON</td>
<td>LP</td>
<td>HP</td>
<td>CON</td>
<td>LP</td>
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<tr>
<td>Energy, kcal/kg</td>
<td>31.5 (4.0)</td>
<td>31.4 (3.2)</td>
<td>31.0 (3.5)</td>
<td>48.7 (5.9)</td>
<td>48.6 (5.1)</td>
</tr>
<tr>
<td>Carbohydrate, g/kg</td>
<td>5.34 (0.81)a</td>
<td>4.91 (0.71)b</td>
<td>4.83 (0.74)b</td>
<td>8.11 (1.35)a*</td>
<td>7.69 (1.18)b*</td>
</tr>
<tr>
<td>Fat, g/kg</td>
<td>0.82 (0.22)a</td>
<td>0.92 (0.24)b</td>
<td>0.84 (0.22)a</td>
<td>1.29 (0.21)a*</td>
<td>1.39 (0.21)b*</td>
</tr>
<tr>
<td>Protein, g/kg</td>
<td>0.69 (0.11)a</td>
<td>0.87 (0.09)b</td>
<td>1.02 (0.09)c</td>
<td>1.16 (0.15)a*</td>
<td>1.35 (0.12)b*</td>
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</table>

Dietary intakes over the 9- and 24-h controlled diets include the contribution from the experimental beverages. Twenty-four-hour habitual intake was determined by a 3-d diet log and was only available for nine subjects. Comparisons to the 24-h habitual intake were only performed for the 24-h controlled diet. Means with different letter are significantly different from each other within the respective time period, P < 0.05. CON, control; LP, low protein; HP, high protein. *Different from 24-h habitual intake, P < 0.05.

Results

Beverage macronutrient intake. Absolute protein intake from the beverages was 0 ± 0, 7.1 ± 1.5, and 12.8 ± 3.6 g for CON, LP, and HP, respectively. This resulted in a relative protein intake of 0 ± 0, 0.18 ± 0.03, and 0.32 ± 0.07 g/kg for CON, LP, and HP, respectively. The drinks provided similar amounts of energy (268 ± 70, 275 ± 65, and 242 ± 62 kcal; CON = LP = HP, respectively; P = 0.38) but differing amounts of carbohydrate (67 ± 17, 51 ± 12, and 46 ± 12 g; CON > LP = HP, respectively; P < 0.01) and fat (0 ± 0, 0.9 ± 0.2, and 4.4 ± 1.1 g; CON < HP < LP; P < 0.01).

Dietary macronutrient intake. When the drinks were included in the diets, energy intake over 9 and 24 h was identical for all conditions (Table 2). However, there were expected differences in protein intake between conditions (HP > LP > CON; P < 0.001) and subtle differences in carbohydrate and fat intakes for the 9 and 24 h periods (Table 2). Twenty-four-hour controlled diets were similar in energy to the participants’ habitual intakes. Moreover, protein intake during the 24-h period for HP was similar to habitual intakes, whereas CON and LP were ~26 and 14% lower, respectively (P < 0.05). Because of a low-protein intake during dinner on the LP trial that was replicated on all other trials, one participant consumed a daily protein intake that was less than the current recommended daily allowance during the CON (~0.81 g·kg⁻¹·d⁻¹) but not LP or HP trials (1.04 and 1.18 g·kg⁻¹·d⁻¹). However, this participant was included in all analysis as their exclusion did not alter the results given that, because of the within-subject design, they had the expected graded protein intake over 9 and 24 h. Relative to the habitual dietary intake, carbohydrate intake was 17–25% higher, whereas fat intake was 13–19% lower for all controlled diets (all P < 0.05).

Whole body protein metabolism by [¹⁵N]ammonia end-product enrichment. Whole body Q was not different (main effect for condition, P = 0.19; interaction, P = 0.26) between conditions over 9 h (CON = 59 ± 21 mg N·kg⁻¹·h⁻¹; LP = 53 ± 14 mg N·kg⁻¹·h⁻¹; HP = 64 ± 23 mg N·kg⁻¹·h⁻¹) and 24 h (CON = 28 ± 8 mg N·kg⁻¹·h⁻¹; LP = 26 ± 7 mg N·kg⁻¹·h⁻¹; HP = 31 ± 9 mg N·kg⁻¹·h⁻¹). However, there was a significant effect of time (main effect, P < 0.001) with rates of Q being ~50% lower after 24 h compared with the early 9-h period. Similarly, there were no differences between conditions for S (main effect for condition, P = 0.15; interaction, P = 0.22) or B (main effect for condition, P = 0.32; interaction, P = 0.36) during the 9- or 24-h periods (Fig. 2). S and B were ~58 and 55% lower, respectively, during the 24-h measurement period (main effect for time, P < 0.001). Rates of Q, S, and B were greater when expressed relative to body mass but revealed similar differences between conditions and across time as when expressed relative to body mass (data not presented).

Fig. 2. Whole body protein synthesis (S) (A) and protein breakdown (B) (B) over 9 and 24 h calculated using urinary [¹⁵N]ammonia end product enrichment. ***Main effect for time, P < 0.01. CON, control; HP, high protein; LP, low protein.
When collapsed across conditions, linear trend analysis revealed no relation between the WBPB over 9 h ($r = 0.26, P = 0.11$) or 24 h ($r = 0.05, P = 0.74$) and energy intake (data not shown). However, there were expected linear relationships between WBPB and protein intake over 9 h ($r = 0.51, P < 0.01$) and 24 h ($r = 0.33, P < 0.05$; Fig. 3, B and C, respectively).

**Whole body protein metabolism by harmonic mean of $[^{15}\text{N}]$ammonia and $[^{15}\text{N}]$urea end product enrichment.** Similar to rates calculated by the $[^{15}\text{N}]$ammonia end product method, there were no differences between conditions for $Q$ ($P = 0.40$), $S$ ($P = 0.27$), $B$ ($P = 0.63$), or WBPB ($P = 0.13$) calculated by the harmonic mean of urinary $[^{15}\text{N}]$ammonia and $[^{15}\text{N}]$urea (Table 3). WBPB was, however, significantly different from zero for HP ($P < 0.05$) but not LP ($P = 0.83$) or CON ($P = 0.74$).

**DISCUSSION**

Adherence to an active lifestyle is associated with greater strength and lean body mass in children (5), which ultimately would necessitate alterations in protein turnover and tissue remodeling that would favor net anabolism. While previous studies have investigated the impact of physical activity on fasting (8, 33) or daily rates (7) of whole body protein metabolism in healthy active children, to the best of our knowledge, we present here the first data to address the cumulative effect of exercise and nutrition. Specifically, we show that postexercise protein ingestion has little effect on whole body protein synthesis and protein breakdown yet induces a dose-dependent increase in WBPB during the early 9-h exercise and recovery period. However, while net protein balance was positive for all conditions during the early 9-h period, ingestion of $\sim 13$ g of protein immediately after exercise was necessary to maintain a net anabolic environment (i.e., positive protein balance) over the entire 24-h period in our healthy children.

During the early acute (i.e., 9 h) and prolonged (i.e., 24 h) recovery period, there was no measurable effect of protein ingestion on whole body protein synthesis or breakdown. This may be related in part to the relatively small differences in protein ingestion between conditions ($\sim 0.3$ g/kg at the most) and/or the relatively short duration of the intervention (i.e., 24 h) in the present study. For example, chronic (i.e., 2 wk) ingestion of diets differing in protein intake by $\sim 0.4–0.9$ g·kg$^{-1}$·d$^{-1}$ has been reported to measurably modify whole body rates of protein turnover as measured by oral $[^{15}\text{N}]$glycine in adults (31). Moreover, lower habitual protein intake (by $\sim 0.31$ g/kg) in free-living young female gymnasts is associated with attenuated rates of whole body protein breakdown relative to their nontraining peers (7). Therefore, given the relatively small differences in protein intake between conditions, it is possible that if our intervention period persisted for a greater duration of time that rates of whole body protein turnover may eventually diverge between conditions. Alternatively, the sensitivity of oral $[^{15}\text{N}]$glycine may have precluded

### Table 3. Twenty-four-hour whole body protein metabolism

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<th>CON</th>
<th>LP</th>
<th>HP</th>
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<tr>
<td>$Q$, g N·kg$^{-1}$·d$^{-1}$</td>
<td>$0.84 \pm 0.22$</td>
<td>$0.80 \pm 0.12$</td>
<td>$0.90 \pm 0.22$</td>
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<tr>
<td>$S$, g·kg$^{-1}$·d$^{-1}$</td>
<td>$4.16 \pm 1.46$</td>
<td>$3.78 \pm 0.62$</td>
<td>$4.45 \pm 1.28$</td>
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<tr>
<td>$B$, g·kg$^{-1}$·d$^{-1}$</td>
<td>$4.13 \pm 1.42$</td>
<td>$3.71 \pm 0.82$</td>
<td>$4.18 \pm 1.37$</td>
</tr>
<tr>
<td>WBPB, g·kg$^{-1}$·d$^{-1}$</td>
<td>$0.03 \pm 0.31$</td>
<td>$0.02 \pm 0.25$</td>
<td>$0.21 \pm 0.28^*$</td>
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Whole body nitrogen turnover ($Q$), protein synthesis ($S$), protein breakdown ($B$), and net protein balance (WBPB) calculated using the harmonic mean of $[^{15}\text{N}]$ammonia and $[^{15}\text{N}]$urea as the end-product. *Significantly different from zero, $P < 0.05$. 

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Fig. 3. A: whole body protein balance (WBPB) over 9 and 24 h calculated using urinary $[^{15}\text{N}]$ammonia end product enrichment. B: WBPB relative to protein intake over 9 h expressed relative to protein intake. Linear correlation (collapsed across all conditions): $r = 0.51, P < 0.01$; line of best fit: $y = 73.7x - 32.3$, $x$-intercept $= 0.44$ g·kg$^{-1}$·9 h$^{-1}$. C: WBPB relative to protein intake over 24 h expressed relative to protein intake. Linear correlation (collapsed across all conditions): $r = 0.33, P < 0.05$; line of best fit: $y = 20.0x - 24.5$, $x$-intercept $= 1.23$ g·kg$^{-1}$·24 h$^{-1}$. *Significantly different from zero, $P < 0.05$. **Significantly different from zero, $P < 0.01$. ***Main effect for time, $P < 0.01$. #Significantly different from CON and LP at 9 h, $P < 0.01$. Main effect for condition (HP > LP = CON), $P < 0.05$. 

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our ability to detect differences in protein turnover with the relatively small acute differences in protein intake between conditions that other methodology, such as intravenous infusions, may have permitted; however, discrepancies between measured rates of whole body protein kinetics by oral \[^{15}\text{N}]\text{glycine and }[^{13}\text{C}]\text{leucine infusion are more pronounced with measurements made in the fasted rather than fed state (14). Nevertheless, in general, whole body protein turnover (i.e., both synthesis and breakdown) is higher in children than adults and can be less sensitive to even large fluctuations in protein intake (19), which opens the possibility that even subtle changes in whole body protein synthesis and/or protein breakdown with exercise or nutrition could translate into physiologically more meaningful differences in WBPB because of these rapid rates of tissue remodeling.

In comparison with previously published whole body protein kinetics in healthy children, our rates of protein synthesis and protein breakdown calculated over 9 h appear to be greater than those previously measured in exercise-trained children over a similar time period but in the overnight fasted state (8, 33). In contrast, 24-h rates of whole body protein turnover in the present study were equivalent to those measured in healthy free-living children utilizing similar methods (7); this is likely due to the incorporation of a fed-state period in the previous (7) and present studies, which would be necessary to elevate rates of protein turnover. In the present study, for example, rates of protein synthesis and protein breakdown as well as WBPB were greatest during the early 9-h exercise and recovery period, which is in agreement with the ability of exercise and protein feeding to increase whole body and muscle protein turnover in adults (11, 14). The overnight fasted period, on the other hand, is characterized by lower rates of turnover and a net catabolism of lean tissue, which likely contributed to the attenuation in protein turnover and the subsequent WBPB when measured over 24 h in the present study. The greater rates of protein synthesis and breakdown consonant with a positive net balance during the early acute recovery period highlights the importance of measuring protein metabolism in the fed state to elucidate the potential synergies between exercise and protein ingestion in children.

Arguably the most important variable for active children who would be experiencing concurrent growth in essentially all lean tissues would be WBPB, which would represent the sum of all anabolic and catabolic pathways. We observed that WBPB was positive during the early acute 9-h recovery period in all conditions, which is not surprising given that at least \(~27\) g (\(\sim 0.7\) g/kg) of protein was consumed within the breakfast and lunch meals independently of the postexercise beverage. As such, the positive protein balance over 9 h would be a reflection of the fed-state anabolism that occurs with protein intake (27). In contrast, 24-h WBPB, which included both the daytime fed and overnight fasted periods, was markedly attenuated such that balance was lower than over 9 h and remained positive only during the HP intake condition. This suggests the fed-state gains in WBPB were generally counterbalanced by the overnight fasted state losses, which is characteristic of the diurnal pattern of protein metabolism (27, 31) and highlights the importance of maximizing fed-state anabolism to sustain a net positive 24-h protein balance.

In the present study there was a dose response of protein ingestion on WBPB during the early 9-h exercise and recovery period. While it is unclear to what extent changes in WBPB reflect the metabolism within skeletal muscle in active, growing children, this dose response during the acute recovery period in the present study is congruent with previous observations in both young and older adults of a graded stimulation of postexercise muscle protein synthesis with ingested protein (30, 47). Interestingly, maintenance of a net positive WBPB over 24 h occurred only in the condition that consumed \(~0.32\) g/kg of protein after exercise, which is greater than the relative dose of protein that is required to maximally stimulate muscle protein synthesis after exercise in young adults (\(~0.25\) g/kg) (30). Given that skeletal muscle is a major storage reservoir for body amino acids and that the acute postexercise muscle anabolism that occurs with exogenous amino acid ingestion is additive to the cumulative 24-h response in adults (42), it is tempting to speculate the sustained positive WBPB over 24 h with HP was related in part to an optimal postexercise stimulation of muscle protein synthesis with the relatively higher protein ingestion. Indeed, a reasonable agreement between \[^{15}\text{N}]\text{glycine-determined WBPB measured over 12 h and the exercise-induced increase in muscle protein synthesis in adults suggests these rates reflect, to some degree, changes in muscle protein metabolism (2, 29). However, the children in the present study would be experiencing normal somatic growth and, therefore, could be expected to have a positive 24-h WBPB with adequate protein and energy intake. As such, we cannot discount the possibility that the positive WBPB during HP was a reflection of the children consuming a protein intake that was similar to their habitual intake (i.e., \(~1.49\) vs. 1.56 g·kg\(^{-1} \cdot d^{-1}\), respectively) and in line with recent revised recommendations for protein intake in children (15). In contrast, the neutral balance during CON (\(\sim 1.16\) g·kg\(^{-1} \cdot d^{-1}\)) and LP (\(\sim 1.35\) g·kg\(^{-1} \cdot d^{-1}\)) may have been related, in part, to a marginally lower (yet still adequate according to the current recommended dietary allowance of 0.95 g·kg\(^{-1} \cdot d^{-1}\)) protein intake relative to their habitual dietary pattern, which could have resulted in an attenuated 24-h anabolic response that can occur during the early adaptation to a lower protein intake (34). Ultimately, substantiation of the potential positive synergies of exercise and protein ingestion on muscle protein metabolism in children and the relative importance of consuming said protein during the immediate postexercise period would need to be determined through longer-term studies, given the current limitations in measuring protein turnover in skeletal muscle of healthy children.

It has been highlighted previously that protein source, and not just protein quantity, should be considered when addressing the needs for optimal growth and development in children (35). In the present study, we provided milk proteins as the source of dietary nitrogen in the postexercise beverages given that they are complete, high-quality proteins that are generally associated with greater muscle remodeling and recovery after exercise in adults (32). For example, studies in adults have revealed that milk and associated dairy-based proteins (especially whey) support greater rates of muscle protein synthesis and net protein balance after exercise compared with plant-based proteins such as soy (38, 46), which can ultimately translate into greater training-induced gains in muscle and lean body mass (20, 44). The greater anabolic effect of dairy compared with soy protein in adults occurs despite these sources having similarly high [albeit artificially truncated (37)]
protein quality ratings according to the protein digestibility-corrected amino acid score (32). Nevertheless, it is unclear whether protein quality may similarly affect protein anabolism in active, concurrently growing children. As such, it may be premature to suggest that similar elevations of postexercise net protein balance observed in the present study could occur with the ingestion of similar quantities of other protein sources that may be of lower quality (e.g., plant-based) given the complete lack of studies evaluating the effect of protein source, let alone protein quantity, on postexercise recovery in children. Therefore, we echo the sentiments of Rodriguez (35) that additional research is required on this important macronutrient to determine the optimal protein amount and/or composition for active children.

Our decision to utilize [15N]glycine methodology to measure whole body protein metabolism, which has a long history of use in humans of all ages (14), was related to the ease with which protein kinetics can be measured over relatively long time frames (e.g., up to 24 h) (19), the relatively low within-subject variability (16), and, because of the oral ingestion and urinary endpoint analysis, its feasible and ethical application in healthy children. In addition, although urinary nitrogen excretion can account for ~80–85% of all nitrogen loss in active adults (39, 40), we also utilized the measured exercise-induced change in body mass with average sweat nitrogen content (1) to estimate sweat nitrogen excretion, which can contribute ~10% of nitrogen loss in adult males (39) and, if not accounted for, would subsequently increase the apparent net protein balance (1); this approach, in contrast to previous studies in active children that only considered urinary nitrogen excretion (7, 8, 33), would result in a conservative estimate of WBPB in our study in the absence of the technically and logistically challenging determination of all potential routes of nitrogen loss (e.g., fecal, integumentary, hair, etc.). As such, our study was able to assess the changes in whole body protein synthesis, breakdown, and net protein balance over periods encompassing both the exercise and early recovery period (i.e., 24 h) period up to, and including, the entire 24-h day to determine how protein ingestion after exercise modulates these variables in a single cohort of healthy children. The potential limitation to oral tracers, such as [15N]glycine, is they represent the net sum of all nitrogen metabolism in the body (e.g., within muscle, splanchnic bed, etc.) and generally have a lower time resolution given the need to adequately collect the metabolic end product, which based on differences in pool size is ~9 h and ~24 h for urinary [15N]ammonia and [15N]urea, respectively (19). In addition, the noninvasive study design precluded our ability to measure other potential biological effectors of protein metabolism such as circulating insulin, which can suppress whole body protein breakdown (Arslanian) and, provided there was a differential response between conditions, may have also contributed to the changes in WBPB observed herein. Nevertheless, other stable isotope methodologies are available to study whole body protein metabolism such as the primed constant [13C]leucine infusion (45), which has been utilized successfully in research in children (13) and is arguably the current gold standard methodology (45). Despite the disadvantage of requiring repeated breath and blood samples that is not always feasible in children, the advantages of [13C]leucine infusion include its preferential metabolism within skeletal muscle, the ability to measure changes in circulating amino acid and insulin concentrations, and the greater time resolution over which changes in protein turnover can be measured. For example, utilization of non-steady-state kinetics can permit the measurement of changes in leucine oxidation, leucine rate of appearance (marker of protein breakdown), and nonoxidative leucine disposal (marker of protein synthesis) over 20–30 min intervals (6), which makes it ideally suited to determine the effect of a single nutrition intervention (e.g., protein-containing beverage similar to that used in the present study) on acute protein metabolism. Therefore, given the need to better understand population-specific protein requirements (35), future studies in active children should endeavor, where possible, to utilize a variety of tracer methodologies to more accurately determine the effect of nutrition on the early (e.g., 0–4 h) and later (e.g., 6–24 h) postexercise recovery periods. Additionally, inclusion of nonexercise days would help advance our understanding of the potential interactive effects of physical activity and nutrition on protein metabolism in children.

In summary, we report here the first study to address the effects of graded postexercise protein ingestion on whole body protein metabolism in active healthy children. Elevated rates of protein turnover during the early exercise and recovery period are congruent with an increased remodeling of lean body tissues that is well-characterized in adult populations (11). However, despite the presence of an ingested protein dose response over the early exercise and 9-h recovery period between conditions, a sustained 24-h net positive WBPB was only observed with HP, suggesting a potential threshold is required to obtain a benefit of postexercise protein ingestion over the entire day in healthy children. Future studies should evaluate whether the acute elevation of a positive WBPB over 24 h observed in the present study would be sustained over more chronic periods of weeks to months, which ultimately would be a prerequisite for the remodeling and growth of lean tissue in active healthy children. Moreover, utilization of alternate stable isotope methodologies (e.g., [13C]leucine infusion) that may have a greater sensitivity and permit the determination of other biological effects of protein metabolism (e.g., blood amino acid and insulin concentrations) would be helpful in confirming the conclusions reached in this preliminary study around the importance of postactivity protein ingestion to enhance WBPB in children. Finally, leveraging the present results and those from future studies with alternative methodologies to pediatric populations whose growth may be impaired by chronic disease represents a fruitful area of further study.

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DISCLOSURES

Elizabeth Offord-Cavin is currently employed by Nestle Research Centre, which funded this study. When the study was completed, Daniel Moore was also employed by Nestle Research Centre. Dr. Moore is now employed by the University of Toronto.

AUTHOR CONTRIBUTIONS

D.R.M., E.A.O., and B.W.T. conception and design of research; D.R.M., K.A.V., J.O., and B.W.T. analyzed data; D.R.M., K.A.V., and B.W.T. inter-
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