Exercise Affects Protein Utilization in Healthy Children\textsuperscript{1,2}

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ABSTRACT Although health initiatives promote increased physical activity in children, the physiologic outcomes have not been well characterized. This investigation examined the effects of programmed aerobic exercise on protein metabolism in children ($n = 7$; mean $\pm$ SEM: 9.14 $\pm$ 0.46 y old; weight, 32.1 $\pm$ 1.6 kg; height, 138 $\pm$ 2.5 cm; and body mass index, 16.21 $\pm$ 0.36 kg/m\textsuperscript{2}) using \textsuperscript{15}N-glycine methodology. Boys ($n = 5$) and girls ($n = 2$) walked (5 d/wk, 3.2–6.4 km/d) for 6 wk. Criterion measures taken at baseline (Pre) and after the exercise program (Post) included anthropometric data, dietary assessment, nitrogen balance, nitrogen flux (Q), protein synthesis (PS), protein breakdown (PB) and net protein balance [(Net) = PS – PB]. After the walking program, there were no significant changes in body weight, fat-free mass or percentage of body fat, whereas height increased ($P < 0.01$). Energy and protein intakes were constant throughout the study. Nitrogen balance was significantly more positive Post than Pre ($P < 0.05$). There was a significant decrease in Q ($P < 0.0001$) with corresponding decreases in PS ($P < 0.001$) and PB ($P < 0.01$). These data provide the first evidence that programmed aerobic exercise alters whole-body protein utilization in healthy, nonobese children. Longitudinal studies are required to further examine changes in protein metabolism associated with increased physical activity in this population. In addition, findings suggest a need to evaluate nutrient requirements for healthy, physically active boys and girls. J. Nutr. 131: 2659–2663, 2001.

KEY WORDS: \textbullet metabolism \textbullet protein turnover \textbullet physical activity \textbullet nitrogen balance \textbullet humans

Current public health initiatives (\textit{Healthy People 2010}) specifically target children for lifestyle modifications that will ultimately improve health. Today, health professionals are challenged to encourage children to increase physical activity in hopes of preventing the increasing incidence of obesity associated with a sedentary lifestyle in this population. Indeed, childhood is a critical period for establishing the framework for a lifestyle that will enable children to become healthy adults.

Although the positive aspects of increased physical activity are apparent in adults, the physiologic implications of routine exercise on growth, development and metabolism in preadolescent children are less well defined. Because optimal deposition of lean body mass is essential in growing children, it is vital to consider the potential consequences of exercise on protein utilization in this population. Six weeks of programmed walking has been shown to influence protein metabolism in healthy obese children during hypocaloric therapy \textsuperscript{(1)} and provides the rationale for execution of a similar protocol in nonobese children. Therefore, the purpose of this research investigation was to employ \textsuperscript{15}N-glycine methodology to examine the effects of programmed aerobic exercise on whole-body protein utilization in healthy, boys and girls aged 8–10 y.

**SUBJECTS AND METHODS**

After approval by the Institutional Review Board, seven healthy children (5 boys and 2 girls) aged 8–10 y were recruited to participate in an 8-wk study, which included testing before and after partaking in a 6-wk walking program. This age range was chosen because younger children may have had difficulty keeping accurate food \textsuperscript{(2)} and activity records and because changes related to puberty would potentially confound interpretation of results. Additionally, there is essentially no research on protein turnover or nitrogen balance in response to exercise in this population.

Criterion measures were assessed during an initial 2-wk baseline period (Pre)\textsuperscript{4} and at the end of the 6-wk exercise intervention period (Post). Measurements included anthropometry, resting energy expenditure (REE), diet assessment, nitrogen balance, nitrogen flux (Q), protein synthesis (PS), protein breakdown (PB) and net protein balance [(Net) = PS – PB]. Each child served as his or her own control, and power calculations utilizing data from similar studies in our laboratory \textsuperscript{(1)} indicated that five subjects would provide power of at least 0.80 ($\beta = 0.20$) to determine the effects of the exercise intervention on criterion measures.


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Exercise intervention

Subjects began the walking program after an initial 2-wk baseline period. The children were instructed to adhere to a formal walking program 5 d/wk. Each exercise session included ~45–60 min of walking with intermittent rest periods to maintain compliance. Emphasis was placed on frequency and duration rather than intensity. However, children were encouraged to cover specific, previously measured distances ranging from 3.2 to 6.4 km. One of the sessions each week was supervised by a graduate student, whereas the remaining sessions were completed with the parents.

Criterion measures

Anthropometry. Body mass and height were determined using a balance beam scale equipped with a measuring rod (Health-o-meter, Bridgeview, IL). Body composition was assessed using bioelectric impedance (BIA-101Q, RJL Systems, Detroit, MI) and skinfold calipers (Harpenden, British Indicators, West Sussex, UK) measurements. Fat mass (FM), fat-free mass (body weight – FM), and relative percentage of body fat (FM/body weight) were determined using the regression equation of Goran et al. (3). Independent variables in the regression equation included a resistance index obtained from bioelectric impedance (height²/R), triceps and subscapular skinfolds, body mass and gender.

Diet analysis. Food intake was documented with 7-d food records kept during alternate weeks. In addition, a researcher conducted biweekly 24-h recalls to complement food records. These methods were used to estimate and monitor nutrient intake of each child throughout the study. Children and parents were instructed on appropriate procedures for recording dietary intake. Food models were used when instructing children and parents on estimation of food portion sizes. Food intake data were analyzed using the Nutritionist IV software program (N² Computing, Salem, OR). Children consumed diets analogous to baseline diets with regard to composition and macronutrient composition were derived from 7-d food records, probing when necessary and monetary incentive when efforts of the children were successful.

Resting metabolic rate. REE was determined by indirect calorimetry using a metabolic cart (Medical Graphics Corporation, St. Paul, MN). Parents drove the children to the testing site after a 12-h overnight fast, with minimal physical activity before testing. REE was assessed for 15–20 min with the subject lying supine in a quiet, temperature-controlled room using a “canopy” system to collect expired gases.

Physical activity. Physical activity was assessed using physical activity logs and Tritrac R3D accelerometers (Hemokinetics, Madison, WI). The Tritrac unit was fastened at the waist in a neoprene pouch. Each child was instructed to wear the monitor for seven consecutive days during alternate weeks from the food records. Monitors were worn throughout the baseline and exercise intervention period, except when bathing, swimming and during the night. Parents tracked the time intervals when the Tritrac was not worn and provided written descriptions of the activities performed during these periods. Data obtained from the Tritrac monitors were recorded at 1-min intervals and subsequently downloaded to a personal computer for analysis and interpretation. Software supplied by the manufacturer was utilized to approximate physical activity–related energy expenditure at baseline compared with that estimated during the exercise intervention. For weeks in which the Tritrac monitors were not worn, physical activity logs were kept to document physical activity and sports-related exercise.

Nitrogen balance. Nitrogen balance (24-h) was measured as a single pooled sample for each child during the baseline data collection (Pre) and after the exercise intervention (Post). Urine was collected in provided bottles, which contained 15 mL of 30% hydrochloric acid to preserve urinary ammonia. Total nitrogen content of the urine was determined in duplicate using a micro-Kjeldahl technique (Tecator Kjeltec System, Höganäs, Sweden). Adjustments in nitrogen excretion (E) were made to account for fecal and intestinal nitrogen losses (4,5). Nitrogen intake (I), as determined by food recalls for the 24 h coinciding with the urine collection period, and urinary nitrogen excretion (E) were utilized to calculate apparent nitrogen balance (Nitrogen Balance = I – E).

Protein turnover. Whole-body protein utilization was assessed using the single-pulse method of 15N-glycine and collecting total urine output (6). Protein turnover studies were conducted at night to improve accuracy and compliance, while minimizing the effects of physical activity on protein utilization. This method was convenient, noninvasive and posed no risk to the subjects. A single oral dose of 15N-glycine (2mg/kg body; 98% atom % enrichment; Cambridge Isotope Laboratories, Andover, MA) was mixed with fruit juice and delivered to the child’s home for administration at bedtime by a member of the research team. Subjects provided a baseline “spot” urine sample for determination of background 15N-ammonia and then emptied their bladders. Urine was then collected 10 h after the dose and subjects refrained from consumption of food and beverages. The 15N enrichment of urinary ammonia (i.e., ratio of tracer to tracee, t/t) was determined using isotope ratio mass spectroscopy (Metabolic Solutions, Merriam, MA). The t/t ratio for the cumulative sample was corrected for background 15N-ammonia enrichment. Nitrogen intake (I) during the evening meal was determined on the basis of analysis of food records and recalls performed during the 15N administration. Nitrogen flux (Q), protein synthesis (PS), protein breakdown (PB) and net protein balance (Net) were calculated using the following equations, where D denotes the oral dose of 15N (D = g glycine · 0.1972).

\[
Q \left[ \text{g N/(kg ⋅ d)} \right] = \left[ D/(corrected \ t/t) \right]/10 \ h \cdot 24 \ h/\text{body weight} \\
PS \left[ \text{g/kg ⋅ d} \right] = [Q - (E/10 \ h \cdot 24 \ h/\text{body weight})] \cdot 6.25 \ g \text{protein/g N} \\
PB \left[ \text{g/kg ⋅ d} \right] = [Q - (E/10 \ h \cdot 24 \ h/\text{body weight})] \cdot 6.25 \ g \text{protein/g N} \\
\text{Net} \left[ \text{g/kg ⋅ d} \right] = PS - PB
\]

Statistics

Pre and Post means (± SEM) were compared using Student’s t test. Statistical analyses were conducted using Microsoft Excel (Microsoft Office, 1997; Microsoft Corporation, Redmond, WA).

RESULTS

Description of subjects. Body weight, fat-free mass, fat mass, percentage of body fat (Table 1) and waist-to-hip ratio (not shown) did not change during the study, whereas heights increased significantly (P < 0.01).

Macronutrient intake. Descriptive data for energy intake and macronutrient composition were derived from 7-d food records and 24-h dietary recalls obtained throughout the study. Energy and protein intakes during the exercise intervention remained constant. Energy consumption averaged 1844 ± 926 kcal.

TABLE 1

Characteristics of the children studied before (Pre) and after (Post) the 6-wk walking program

<table>
<thead>
<tr>
<th></th>
<th>Height</th>
<th>Weight</th>
<th>Body fat</th>
<th>Fat-free mass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cm</td>
<td>kg</td>
<td>g/100 g</td>
<td>kg</td>
</tr>
<tr>
<td>Pre</td>
<td>138 ± 2.5</td>
<td>32.1 ± 1.6</td>
<td>16.7 ± 1.1</td>
<td>25.1 ± 1.4</td>
</tr>
<tr>
<td>Post</td>
<td>140 ± 2.5*</td>
<td>32.2 ± 1.3</td>
<td>15.6 ± 1.3</td>
<td>25.5 ± 1.2</td>
</tr>
</tbody>
</table>

* Values are means ± SEM, n = 7; * significant change from Pre, P ≤ 0.05.
kcal/d (7685 ± 384 kJ/d), and protein consumption averaged 70 ± 0.1 g/d. Total energy intake was comprised of ~56% carbohydrate, 29% fat and 15% protein. All subjects consumed more than adequate protein to satisfy recommendations with protein intake averaging >2 g/kg body · d. Dietary data collected via 24-h recalls did not differ from that provided by 7-d food records.

**Energy expenditure.** Tritrac accelerometry data showed an ~20% increase in physical activity–related energy expenditure above baseline levels (P < 0.02) after the walking program. Additionally, accelerometry data indicated that non-exercise activity or spontaneous activity after programmed walking was not reduced compared with baseline values (Pre). It stayed the same or in some cases, increased. REE tended to increase Post vs. Pre (1327 ± 44 vs. 1279 ± 32 kcal/d, respectively, P = 0.13) or (5530 ± 183 vs. 5330 ± 133 kJ/d).

**Protein utilization.** Nitrogen balance among subjects (n = 6) remained positive during the study and was significantly greater Post (5.9 ± 1.2 g/d) compared with Pre (2.87 ± 1.3 g/d) (P < 0.05). Nitrogen intake during the 24-h nitrogen balance studies averaged 12.5 ± 2.1 and 10.8 ± 1.1 g for Post and Pre, respectively, and nitrogen excretion averaged 8.8 ± 0.7 and 7.9 ± 0.7 g for Post and Pre, respectively. Nitrogen balance data were excluded from one subject because urine was inappropriately collected. Protein utilization is depicted in Figure 1 for Q (a measure of amino acid cycling between protein and free amino acid pools), PS, PB and Net. There was a significant decrease in Q after the walking program (P < 0.0001). Corresponding decreases in PS (P < 0.001) and PB (P < 0.01) were also observed. Net protein balance tended to decrease (P = 0.36) after the walking program.

**DISCUSSION**

This research provides the first evidence that changes in whole-body protein utilization are elicited in response to programmed aerobic exercise in healthy, preadolescent children. The only other investigation that has evaluated whole-body protein turnover in children in response to exercise was in obese children undergoing hypocaloric therapy (7). The present investigation was conducted to assess the implications of consistent aerobic activity on protein metabolism in healthy, nonobese preadolescent children, given the findings of Ebbeling and Rodriguez (7).

![Figure 1](https://example.com/figure1.png)

**FIGURE 1** Protein utilization assessed as nitrogen flux (Q), protein synthesis (PS), protein breakdown (PB) and net protein balance (Net) before (Pre) and after (Post) the 6-wk walking program in boys and girls. Values are means ± SEM, n = 7. Letters indicate significant change from Pre: *P < 0.0001; †P < 0.001; ‡P < 0.01.

The goal of this project with regard to exercise was to systematically increase physical activity in children through a routine walking program. Our data suggest that spontaneous activity in response to the increased programmed exercise was not decreased in a compensatory manner, but rather that an increased energy expenditure was achieved after the exercise intervention. The frequency and duration of walking was given priority over the intensity of exercise to maintain subject compliance. Moreover, it has been suggested that the total time spent on physical activity is more important than the combined energy cost of a number of activities. That is, prolonged bouts of exercise may be more beneficial in promoting an active lifestyle and reducing the risk for developing obesity (8). Therefore, we did not attempt to document changes in fitness levels of subjects. Rather, the intent was to increase habitual physical activity via the walking program.

Physically active children must receive proper nutrition to support optimal growth and development as well as to meet energy demands associated with increased physical activity. The question remains concerning whether programmed exercise in children has long-term effects on overall health by acutely affecting nutrient, specifically protein, utilization. The present investigation demonstrated an apparent change in protein metabolism, with the possibility that the modulation of protein turnover may be energy based. That is, in contrast to the findings of Ebbeling and Rodriguez (7) with obese children, the 6 wk of programmed walking in this study did not increase net protein turnover in healthy, nonobese children. Rather, the changes noted in protein utilization (i.e., decreased Q, PS and PB) were analogous to those observed during an additional 6 wk of negative energy balance (7).

Subjects in the Ebbeling and Rodriguez study (7) were in an established state of negative energy balance before beginning the walking program (7). Walking appeared to improve protein utilization in obese children undergoing a weight loss regimen by increasing, or upregulating, protein turnover. This response occurred in the presence of no change in energy balance or physical activity–related energy expenditure. However, the fact that a downregulation of protein turnover was noted with 6 wk of hypocaloric therapy (i.e., induction of negative energy balance) provides insight into a possible energy-related mechanism for the decreases observed in protein metabolism variables in the present study. That is, one factor common to both studies is the coexistence of a negative energy balance. Although Ebbeling and Rodriguez (7) established negative energy balance via dietary intervention, negative energy balance may have occurred in the present study due to an increase in energy expenditure without a concomitant increase in energy intake. It is also possible that the slight, although nonsignificant, increase in REE may be of physiologic importance in the present study when coupled with the additional energy expended during the walking program.

Energy intake did not change throughout the study. By simple difference, a state of negative energy balance could have been imparted during the walking protocol. Furthermore, the 10th edition of the Recommended Dietary Allowances (9) recommends 70 kcal/kg · d [292 kJ/(kg · d)] for young children aged 8–10 y. On average, children in this study were consuming 1844 kcal/d (7685 kJ/d) or ~56 kcal/kg (233 kJ/kg). This represents a deficit of ~400 kcal/d (1667 kJ/d) with respect to energy intake or ~160 kcal/d (~670 kJ/d), considering the overall recommendation of 2000 kcal/d (8336 kJ/d) for this population.

Potential limitations in our measurements must be considered regarding our energy balance data. Ambler et al. (10) investigated the effect of 5 wk of endurance training on energy...
intake in adolescent males and females (15–17 y). They compared the energy intake with doubly labeled measurement of total energy expenditure (TEE). The authors hypothesized that endurance type training (i.e., running, dance, basketball) would lead to increases in food intake to balance the increased energy expenditure that accompanies physical activity. They found that males and females had significantly higher TEE than total energy intake during the training period. Energy expenditure exceeded self-reported energy intake, but despite the seemingly negative energy balance, the subjects in the exercise group experienced no weight loss. The authors speculated that the subjects might have subconsciously underestimated total energy intake. Therefore, our results may be limited by the accuracy of our dietary records as well as our inability to detect meaningful changes in energy balance.

We took efforts to ensure collection of accurate dietary data from study participants and their families. A researcher reviewed the records with the children and their parents on a routine basis. When records were submitted, probing took place to complete dietary data and reminder phone calls were placed to children throughout the study. In addition, 24-h dietary recalls were conducted biweekly throughout the study to validate subject record keeping. As a result of these efforts, we have confidence in the dietary data and believe that protein metabolism may have been affected by the coexistence of an energy deficit in the children participating in the walking program in the present study. Given that protein intake was more than adequate, these observations would support a need to provide additional energy to young, consistently active children, to support optimal protein utilization.

Our findings indicate that growth took precedence over the short-term or acute energy deficits that might have occurred in response to the programmed exercise. The unique process of nutrient partitioning may explain in part how the body supported growth despite suppressed protein turnover. Nutrient partitioning refers to a physiologic state (i.e., growth) in which nutritional intake is specifically “partitioned” in metabolic organs and tissues to “accommodate successful execution of the dominant productive function” (11). Nutrient partitioning during growth is often marked by increases in nitrogen balance without changes in dietary intake; over an extended period of time, it is accompanied by reductions in lipid accretion (11). In addition, nutrient partitioning can occur in the presence of an apparent negative energy balance. Although a more sensitive measure of body composition in our study may have detected subtle differences, significant changes in the percentage of body fat and fat-free mass are unlikely during an acute phase (6 wk) of moderate exercise. Thus, the apparent negative energy balance and increases in nitrogen balance observed in this study suggest nutrient partitioning as a possible short-term adaptation for supporting the hierarchical function of growth and development despite the increased energy expenditure of physical activity.

Nitrogen balance increased significantly after the exercise program, indicating retention of nitrogen for promoting global anabolic functions. Nitrogen balance was utilized in this study as an adjunct to the stable isotope modeling. Although nitrogen balance methodology has been pervasive in its application, one of its consistent criticisms is that the values represent a “black box” scenario. This scenario infers that although nitrogen balance provides information regarding the net difference in nitrogen utilization, the technique cannot provide specific data regarding the metabolic role of the nitrogen within the body (i.e., protein synthesis, breakdown and oxidation) (12). For instance, a positive balance can be attained by increasing only protein synthesis, by increasing synthesis more than breakdown, by decreasing only breakdown and by decreasing breakdown more than synthesis. The last-mentioned example may apply to this investigation in that protein synthesis and breakdown were both downregulated and as long as synthesis remained higher, a positive nitrogen balance would result.

Although endocrine parameters were not assessed in the present study, previous studies of hormones in exercising children support a potential relationship between energy availability and anabolic functions, which may provide insight into our findings. Growth hormone (GH) and insulin-like growth factor-1 (IGF-1) have been shown to be affected by exercise and diet, with nutritional status as a major factor in the proper functioning of the GH–IGF axis (13). Recent work investigating the effect of increased physical activity in adolescents on GH binding protein (GHBP) and IGF-1, found a significant reduction in circulating GHBP and IGF-1 in the training group as well as a significant increase in IGFBP-2 (14). The reduced GHBP might suggest diminished responsiveness to GH. Similarly, a recent study demonstrated that 5 wk of increased physical activity in adolescent females led to a 14% drop in plasma IGF-1 concentrations (15). Interestingly, the changes noted in IGF-1 and IGFBP-2 in those studies are consistent with energy-deficient states, yet the increased energy expenditure in the trained group did not result in weight loss. Our results suggest a similar paradox and are consistent with increased energy expenditure without a corresponding weight loss. Although our subjects were not “food restricted” per se, it is possible that no change in energy intake during the exercise intervention period affected the multiple energy-requiring processes associated with protein utilization.

From an empirical standpoint, children routinely engage in physical activity and sports while growing and suffer no apparent ill effects. What remains to be defined is whether a unique “window” of time exists during growth during which children may be more susceptible to metabolic alterations when faced with the additional energy demands associated with consistent programmed aerobic exercise. Although there are not enough longitudinal data to support a consensus regarding the long-term effects of physical activity in young children, it appears that consistent exercise might modulate protein utilization when nutritional status, particularly energy intake, is suboptimal. The responses noted in this investigation may be reversed or possibly blunted by a modest increase in energy intake to offset the increased energy demands associated with consistent physical activity.

Changes in protein utilization occurred in the presence of increases in linear height. Protein and energy intakes remained constant throughout the study. Although protein intake was adequate, our data suggest that energy intake may not have balanced expenditure, creating an acute situation of negative energy balance that was physiologically relevant with regard to protein utilization. Although a nonexercise control group was not included in the present study’s design, each subject served as his or her own control. At this time, we do not believe that the apparent modulation in protein turnover after the exercise intervention can be accounted for solely by the accompanying growth of the children, particularly because similar, more pronounced changes were noted in response to a resistance training program in a similar population (16). Without quantifying these findings, further investigation in longitudinal studies incorporating a nonexercise control group and longer periods of exercise training are required to further document our observations and to better characterize the
implications of consistent, programmed aerobic exercise on dietary requirements and protein utilization in young children.

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LITERATURE CITED


