Influence of Growth Rate on Nitrogen Balance in Adolescent Sprint Athletes

Dirk Aerenhouts, Jelle Van Cauwenberg, Jacques Remi Poortmans, Ronald Hauspie, and Peter Clarys

This study aimed to estimate nitrogen balance and protein requirements in adolescent sprint athletes as a function of growth rate and physical development. Sixty adolescent sprint athletes were followed up biannually over a 2-yr period. Individual growth curves and age at peak height velocity were determined. Skeletal muscle mass (SMM) was estimated based on anthropometric measurements and fat mass was estimated by underwater densitometry. Seven-day diet and physical activity diaries were completed to estimate energy balance and protein intake. Nitrogen analysis of 24-hr urine samples collected on 1 weekday and 1 weekend day allowed calculation of nitrogen balance. Body height, weight, and SMM increased throughout the study period in both genders. Mean protein intakes were between 1.4 and 1.6 g kg\(^{-1}\) day\(^{-1}\) in both genders. A protein intake of 1.46 g kg\(^{-1}\) day\(^{-1}\) in girls and 1.35 g kg\(^{-1}\) day\(^{-1}\) in boys was needed to yield a positive nitrogen balance. This did not differ between participants during and after their growth spurt. None of the growth parameters was significantly related to nitrogen balance. It can be concluded that a mean protein intake around 1.5 g kg\(^{-1}\) day\(^{-1}\) was sufficient to stay in a positive nitrogen balance, even during periods of peak growth. Therefore, protein intake should not be enhanced in peak periods of linear or muscular growth.

Keywords: athletic performance, development, nutrition assessment

Pubertal growth and development increase the demand for energy and nutrients (Torun, 2005). During the adolescent growth spurt, 15–25% of the adult height is achieved, 45% of the skeletal growth is acquired (Rees & Christine, 1989), and around 26% of bone mineral is accrued (Bailey et al., 1999). For this lean tissue growth and tissue remodeling, adolescents require sufficient dietary protein. However, few studies cover the nutritional needs of young athletes during that period (Meyer et al., 2007).

Beckett et al. (1997) suggested that ingested protein is more efficiently metabolized during the growth spurt, which can be attributed to hormonal influences. According to Forbes (1981), during adolescence there is a daily nitrogen increment of 160 mg and 320 mg in nonathletic females and males, respectively. At peak height velocity, these values increase up to 360 mg and 610 mg in females and males, respectively. Using the conversion factor of 6.25 (FAO/OMS/UNU, 1986), the corresponding amounts of protein accrual become respectively 1.0 g day\(^{-1}\) and 2.0 g day\(^{-1}\), reaching 2.3 g day\(^{-1}\) and 3.8 g day\(^{-1}\) during the peak of growth spurt. In Belgium, a protein intake of 0.8–0.9 g kg\(^{-1}\) day\(^{-1}\) is recommended for adolescents (Health Council, 2006).

Recommended protein intakes for adult athletes involved in resistance training range between 1.2–1.7 g kg\(^{-1}\) day\(^{-1}\) (Philips et al., 2007; Tipton & Witard, 2007). No specific recommendations exist for strength-trained adolescents and only a limited number of studies have been conducted on the determination of protein needs for adolescent athletes. During the growth spurt, one could expect that adolescent sprint athletes require additional dietary protein to support both their intensive physical activities and the rapid physical changes occurring during this period.

Boisseau et al. (2007) studied 14-year-old male soccer players, playing 10–12 hr per week. These soccer players needed a daily protein intake of 1.04 g kg\(^{-1}\) body weight to be in positive nitrogen balance. The suggested mean protein requirement for these athletes was 1.2 g kg\(^{-1}\) day\(^{-1}\). This study indicated that male adolescent soccer players have higher protein needs than the current guidelines for their nonathletic peers.

To our knowledge, no data exist on the protein requirements in elite male or female adolescent sprint athletes in function of growth rate. Flemish adolescent sprint athletes studied by Aerenhouts et al. (2008) had a mean protein intake of 1.5 g kg\(^{-1}\) day\(^{-1}\). However, in these previous studies on protein requirements for adolescent athletes growth rate and maturational status were not taken into account.

Therefore, the purpose of this study was to determine nitrogen balance and protein requirements in elite adolescent female and male sprint athletes as a function of their growth rate and physical development. It was...
hypothesized that a higher protein intake and nitrogen balance can be observed when adolescent linear and muscular growth peak.

Methods

Participants

One hundred and twenty elite athletes between 12 and 18 years old were selected and invited for participation in this longitudinal study. All athletes were ranked in the top 10 of at least one sprint discipline (60- to 400-m flat and hurdles) of the Flemish Athletics League. They were competing in their discipline for at least 2 years. Due to logistical limitations, only 60 (29 girls and 31 boys aged 14.7 ± 1.6 and 14.8 ± 1.7 years, respectively) of the 76 responders could be retained. All athletes were involved in a sprint-training program of three or more training bouts per week. Participating athletes and their parents were given detailed information about the study. They were asked to give their written informed consent, in accordance with the university’s ethical committee. The study was approved by the university’s medical ethical committee.

Anthropometrics, Energy Expenditure, Energy, and Protein Intake

From autumn 2006 until spring 2008, biannual dietary assessment and anthropometrics of participating athletes were taken. Autumn measurements were carried out during the months November and December, and spring measurements were carried out during the months of May and June, respecting as close as possible the six months interval for every subject. Anthropometrics were carried out following guidelines of the International Society for the Advancement of Kinanthropometry (ISAK; Eston & Reilly, 2009), and body fat was estimated by underwater densitometry. Energy expenditure, physical activity level, and intake of energy and protein were assessed using 7-day activity and diet diaries, respectively. A detailed description of the assessment procedures of anthropometric data collection, body fat estimation, energy expenditure, and dietary intake can be found elsewhere (Aerenhouts et al., 2011b). In addition to the basic anthropometric data collection, upper arm, thigh, and calf circumferences as well as six skinfolds (triceps, biceps, subscapular, supraspinal, thigh, and calf) were measured following standardized procedures (Eston & Reilly, 2009). These measurements allowed the estimation of total skeletal muscle mass (SMM) using the following formula (Poortmans, Boisseau, Moraine, Moreno-Reyes, & Goldman, 2005):

\[
SMM \text{ (kg)} = \text{Ht} \times \left[(0.0064 \times \text{CAG}^2) + (0.0032 \times \text{CTG}^2) + (0.0015 \times \text{CCG}^2)\right] + (2.56 \times \text{gender}) - (0.136 \times \text{age}).
\]

Note. Ht = height (m); gender = 0 for female, 1 for male; age is measured in years; CAG = corrected circumference for upper arm; CTG = corrected circumference for thigh; CCG = corrected circumference for calf.

In addition, height-for-age data from birth until the start of the study, which was collected by governmental childcare organizations, were obtained via the parents. In Belgium, these data are collected with an interval of 1–2 months during the first 2 years after birth, and thereafter about once every year. To obtain the growth curve and age at peak height velocity (PHV), the Jolicoeur-Pontier-Abidi-2 method was applied using the Analysis of Growth Curves software program (Abidi et al., 1996). The increase in growth rate of the smoothed growth curve was used to detect the onset of the growth spurt. The highest value for growth rate was defined as PHV, and the value for growth rate after PHV equal to the one at onset of the growth spurt was used to define the end of the growth spurt. Years from PHV was calculated on every measurement by subtracting age at PHV from calendar age. The individual growth curves allowed the discrimination between athletes measured during their growth spurt (DGS) versus after their growth spurt (AGS).

Nitrogen Balance

Since none of the athletes were vegetarian and their main protein sources were from animal origin (Aerenhouts et al., 2011a), dietary intake of nitrogen was calculated using the nitrogen to protein conversion factor of 6.25 (FAO/OMS/UNU, 1986). During the week of diary completion, participants collected a 24-hr urine sample on 1 weekday and 1 weekend day. To control for completeness, participants ingested 80 mg para-aminobenzoic acid (PABA; Bingham & Cummings, 1983) at breakfast, lunch, and dinner. Volumes of the urine collections were measured and a sample was frozen. Urine samples containing less than 85% of the PABA ingested were excluded for analysis. Urinary nitrogen was determined using the micro-Kjeldahl method (Büchi nitrogen determination System, Switzerland). For practical reasons, stools and sweat losses could not be collected. Generally, daily nitrogen sweat and fecal losses amount to 3 and 12 mg kg⁻¹ body weight respectively (FAO/OMS/UNU, 1986). These values were used to correct for the differences obtained between dietary nitrogen intake and urinary nitrogen loss.

\[
\text{Nitrogen balance} = (0.16^a \times \text{dietary protein (g day}^{-1}))- (\text{urinary nitrogen excretion (g day}^{-1}) + (15 \times \text{body weight (kg)/1,000})^b
\]

\(^a\text{protein to nitrogen conversion factor; }^b\text{estimated nitrogen sweat and fecal losses (FAO/OMS/UNU, 1986)}

Statistical Analysis

Statistical analyses were performed in SAS (version 9.3, SAS Institute, Inc). Taking into account the longitudinal study design, multilevel models (measurements clustered within subjects) were developed using the PROC Mixed procedure (Verbeke & Molenberghs, 2000). First, the
evolutions of the nutritional and anthropometric measurements throughout the study period were analyzed. Time was treated as a continuous variable, as this yielded significant better model fits compared with an unstructured mean structure. Secondly, a model was constructed to investigate the relationship between protein intake and nitrogen balance adjusting for time, body height, skeletal muscle mass, and energy balance.

Models with different mean and (co)variance structures were fitted and compared using Likelihood Ratio tests. When exploratory statistics suggested a curvilinear relationship, a quadratic term was added to the model. To facilitate interpretation, results of models including a quadratic term are presented graphically. Models with different mean structures were fitted by Maximum Likelihood Estimation and then compared using a Likelihood Ratio test. All other models were fitted by Restricted Maximum Likelihood Estimation. Denominator degrees of freedom were estimated by the Kenward and Roger approximation. Level of significance was determined at .05.

Results

Sample Characteristics

Of the 60 included athletes, 7 athletes were excluded for analysis because of unacceptable dietary or nitrogen data on all 4 measurement occasions. Analyses were performed on the 53 remaining athletes, of which 33 delivered unacceptable dietary or nitrogen data on one or more measurement occasions. In 58% of the cases the reason for exclusion was an incomplete urine sample (less than 85% of PABA recovered). The remainder was excluded due to dietary underreporting.

Girls reached their mean PHV (9.3 ± 3.7 cm yr⁻¹) at the age of 11.6 ± 1.5 years. In boys, PHV (10.1 ± 2.0 cm yr⁻¹) occurred at the age of 13.0 ± 1.0 years, which was significantly later than in girls (p < .001). One girl had not reached age at PHV (negative value for calendar age minus age at PHV) on occasion one and two. On occasion one and two, there were four boys who had not reached age at PHV. Three of them reached age at PHV before the third occasion and the last one reached age at PHV before the final occasion.

Evolutions of Anthropometric and Nutritional Variables

Evolutions in anthropometric and nutritional data of girls and boys with accepted diet diaries and urine collections over the four occasions are presented in Table 1 and Table 2, respectively.

In girls, body height and body weight significantly increased in a linear way throughout the study period. Energy balance remained constant and negative while the physical activity level (PAL) and protein intake decreased significantly in a linear way throughout the study period. Figure 1 illustrates the curvilinear relationships (quadratic time effect) for the variables FFM, SMM, body fat percentage, and nitrogen balance. Increases in FFM and SMM stagnated during the study period. Body fat percentage remained stable between the first two occasions, where after it increased. For nitrogen balance, a trend for a linear (p = .050) and quadratic (p = .052) time effect was observed with lower values on occasions 2 and 3.

In boys, body height, body weight, and FFM increased significantly in a linear way throughout the study period, while body fat percentage remained stable.

Table 1  Evolution of Anthropometric and Nutritional Data of Female Athletes

<table>
<thead>
<tr>
<th></th>
<th>Intercept</th>
<th>B</th>
<th>SE</th>
<th>p</th>
<th>B</th>
<th>SE</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body height (cm)</td>
<td>167.50</td>
<td>0.61</td>
<td>0.17</td>
<td>&lt;.001*</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>53.67</td>
<td>0.82</td>
<td>0.18</td>
<td>&lt;.001*</td>
<td>–</td>
<td>/</td>
<td>–</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>44.62</td>
<td>1.22</td>
<td>0.39</td>
<td>&lt;.001*</td>
<td>–0.33</td>
<td>0.09</td>
<td>.001*</td>
</tr>
<tr>
<td>SMM (kg)</td>
<td>19.09</td>
<td>0.64</td>
<td>0.17</td>
<td>&lt;.001*</td>
<td>–0.10</td>
<td>0.05</td>
<td>.043*</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>16.41</td>
<td>–0.63</td>
<td>0.59</td>
<td>.285</td>
<td>0.48</td>
<td>0.14</td>
<td>.002*</td>
</tr>
<tr>
<td>PAL</td>
<td>1.88</td>
<td>–0.03</td>
<td>0.01</td>
<td>.026*</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Energy balance (MJ)</td>
<td>–2.49</td>
<td>0.08</td>
<td>0.13</td>
<td>.532</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Protein intake (g/kg)</td>
<td>1.48</td>
<td>–0.04</td>
<td>0.02</td>
<td>.020*</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Nitrogen balance (g)</td>
<td>0.86</td>
<td>–1.45</td>
<td>0.73</td>
<td>.050</td>
<td>0.46</td>
<td>0.23</td>
<td>.052</td>
</tr>
</tbody>
</table>

Note. B-values can be interpreted as changes in the anthropometric and nutritional variables between two consecutive measurements (6 months). FFM = fat-free mass; SMM = skeletal muscle mass; PAL = physical activity level.

*aValue of the anthropometric and nutritional variables at begin of the study

*bQuadratic term in the model explaining a curvilinear relationship; values only presented when significant.

*p < .05
Table 2  Evolution of Anthropometric and Nutritional Data of Male Athletes

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intercept</th>
<th>B</th>
<th>SE</th>
<th>p</th>
<th>Time²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body height (cm)</td>
<td>172.98</td>
<td>1.40</td>
<td>0.33</td>
<td>&lt;.001*</td>
<td>–</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>59.43</td>
<td>1.99</td>
<td>0.26</td>
<td>&lt;.001*</td>
<td>–</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>53.89</td>
<td>1.92</td>
<td>0.27</td>
<td>&lt;.001*</td>
<td>–</td>
</tr>
<tr>
<td>SMM (kg)</td>
<td>24.27</td>
<td>1.47</td>
<td>0.20</td>
<td>&lt;.001*</td>
<td>–0.16</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>9.26</td>
<td>–0.22</td>
<td>0.18</td>
<td>.216</td>
<td>–</td>
</tr>
<tr>
<td>PAL</td>
<td>1.89</td>
<td>–0.01</td>
<td>0.01</td>
<td>.360</td>
<td>–</td>
</tr>
<tr>
<td>Energy balance (MJ)</td>
<td>–2.09</td>
<td>–0.11</td>
<td>0.12</td>
<td>.346</td>
<td>–</td>
</tr>
<tr>
<td>Protein intake (g/kg)</td>
<td>1.55</td>
<td>–0.03</td>
<td>0.02</td>
<td>.167</td>
<td>–</td>
</tr>
<tr>
<td>Nitrogen balance (g)</td>
<td>1.80</td>
<td>–2.09</td>
<td>0.73</td>
<td>.006*</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Note. B-values can be interpreted as changes in the anthropometric and nutritional variables between two consecutive measurements (6 months).
FFM = fat-free mass; SMM = skeletal muscle mass; PAL = physical activity level.

*aValue of the anthropometric and nutritional variables at begin of the study.

¹Quadratic term in the model explaining a curvilinear relationship; values only presented when significant.

*p < .05

Figure 1 — Quadratic time effects for FFM, SMM, body fat %, and nitrogen balance in female athletes. Dotted lines are 95% confidence intervals.
PAL, energy balance and protein intake remained stable. Quadratic time effects were observed for SMM and nitrogen balance (Figure 2). For SMM, the increases between occasions became smaller toward the end of the study. For nitrogen balance, a significant linear and quadratic time effect was observed with lower values on occasions 2 and 3.

**Final Model for the Relationship Between Protein Intake and Nitrogen Balance**

Table 3 presents the results of the final model for the relationship between protein intake and nitrogen balance. The inclusion of the dichotomous growth spurt variable (during vs. after growth spurt) and its interaction effect with protein intake did not significantly improve the model fit, and their parameter estimates were not significant. This shows that the dietary protein intake needed to obtain a positive nitrogen balance is similar for athletes during and after their growth spurt. On the other hand, a significant interaction effect was found for gender; the significant positive relationship between protein intake and nitrogen was stronger in boys compared with girls. The inclusion of a quadratic term for protein intake shows that nitrogen balance does not increase linearly with increasing protein intake. High levels of protein intake do not further increase the nitrogen balance.

**Figure 2** — Quadratic time effects for SMM and nitrogen balance in male athletes. Dotted lines are 95% confidence intervals.
Figure 3a shows the predicted nitrogen balance by protein intake for girls with an average body height, muscle mass and energy balance. To obtain a nitrogen balance that is significantly higher than 0, girls needed a protein intake of 1.46 g kg\(^{-1}\) day\(^{-1}\). Protein intakes higher than 1.70 g kg\(^{-1}\) day\(^{-1}\) did not lead to further increases in nitrogen balance.

Predicted nitrogen balance by protein intake for boys with an average body height, muscle mass and energy balance is displayed in Figure 3b. A protein intake of 1.35 g kg\(^{-1}\) day\(^{-1}\) was needed to result in a nitrogen balance that was significantly higher than 0. Protein intakes higher than 2.20 g kg\(^{-1}\) day\(^{-1}\) did not lead to further increases in nitrogen balance.

**Discussion**

Mean protein intake as studied in these adolescent sprint athletes ranged between 1.4 and 1.6 g kg\(^{-1}\) day\(^{-1}\) in both genders. A positive nitrogen balance was reached from a protein intake of 1.46 g kg\(^{-1}\) day\(^{-1}\) in girls and 1.35 g kg\(^{-1}\) day\(^{-1}\) in boys. Although significant increases in
Nonetheless, a certain degree of dietary underreporting, possibly in combination with overreporting of physical activities, may have occurred. Both dietary underreporting and activity overreporting are commonly observed, also in highly motivated athletes (Burke, 2001; Irwin et al., 2001). Indeed, diary-based estimations revealed consistent negative energy balances in these sprint athletes. The available data did not allow a reliable adjustment of energy and protein intake at the individual level and was therefore not performed. Instead, to detect and exclude underreporters from analysis, the cut-off value of Goldberg et al. (1991) was applied. Self-measured body weight at start and the day after the recording weeks were stable. This indicates that habitual dietary and physical activity habits were maintained during the recording weeks. Moreover, the changes in height and weight were consistent according to the Flemish reference data for height and weight (Roelants et al., 2009), while SMM increased and body fat percentage remained stable or increased in case of the girls in this study. This indicates that physical activities, recovery, and nutrition were not misbalanced during the study period.

At the beginning of the study, FFM increased on average at 2.44 kg year\(^{-1}\) in girls and 3.84 kg year\(^{-1}\) in boys. Assuming the protein content of FFM to be 19.4% (Brozek et al., 1963), this corresponded with a daily 1.30 g and 2.04 g protein accrual in girls and boys, respectively. In boys measured during their growth spurt, yearly increases of FFM were between 7.5 and 10 kg, which would correspond with a daily protein accrual between 4.0 and 5.3 g. This is somewhat higher than the values for daily accrual of protein in nonathletic adolescents as estimated by Forbes (1981). According to Forbes’ estimations (1981), girls in their growth spurt accrue about 2.3 g and boys about 3.8 g additional protein each day. After the growth spurt these estimations are respectively 1.0 g day\(^{-1}\) and 2.0 g day\(^{-1}\), as was on average observed in the athletes in this study.

The athletes in this study reached a significant positive nitrogen balance from a daily protein intake of 1.46 g kg\(^{-1}\) in girls and 1.35 g kg\(^{-1}\) in boys, irrespective of their growth rate or increase in FFM. Even athletes with the highest increases in FFM, requiring additional protein of 5.3 g day\(^{-1}\), had sufficient protein with the observed daily intake between 1.4 and 1.6 g kg\(^{-1}\). Intakes around these values were observed in athletes during their growth spurt, and equally during the entire evaluated adolescence period as demonstrated in our previous paper on these athletes (Aerenhouts et al., 2011b). Even during the adolescent growth spurt, growth only represents a small period as demonstrated in our previous paper on these athletes (1996; Scrimshaw, 1996). Age at PHV also fell outside the range of 13.8–14.2 years, as estimated on samples of European boys (Malina et al., 2004). However, PHV did fall within the range for European boys. Athletes measured after their growth spurt were not earlier mature than athletes measured during their growth spurt, as indicated by their similar age at PHV and higher chronological age.

When studying nitrogen balance, it is important that caloric intake matches caloric requirement and that normal dietary and activity patterns are maintained (Scrimshaw, 1996). Stable isotope techniques and the doubly labeled water method for the assessment of protein balance and energy expenditure, respectively, are considered the most accurate (Melanson & Freedson, 1996; Scrimshaw, 1996). However due to practical and ethical reasons these methods could not be applied in this longitudinal study conducted on a relative large sample of minors. Instead, energy expenditure was estimated through administration of an activity diary. Objective accelerometry in a subsample of these athletes confirmed the results of energy expenditure as estimated by the activity diary (Aerenhouts et al., 2011c).
balances. However, increases in body weight, FFM, or muscularity did not influence nitrogen balance. Protein intake throughout appeared to meet the requirements for growth, development and physical activities, with a margin for periods of increased FFM gain. The ceiling effect observed in the relationship between protein intake and nitrogen balance is an indication that an excess of protein is not necessarily used by the body. Nonetheless, two individuals (1 girl, 1 boy) with a protein intake near 2 g kg⁻¹ day⁻¹ still had a negative nitrogen balance. Retrospective control of these outliers did not show unrealistic physical changes, energy expenditure, or dietary intakes of energy and nutrients. These outliers indicate interindividual variation in protein needs and, therefore, the mean protein intake necessary to be in a positive nitrogen balance found in this study may not be generalized and should be interpreted with care.

To conclude, the sprint athletes in this longitudinal study over 2 consecutive years reached a positive nitrogen balance from a mean protein intake of 1.46 g kg⁻¹ in girls and 1.35 g kg⁻¹ in boys. Therefore, the estimated daily protein intake around 1.5 g kg⁻¹ in both genders covers the basic needs for protein dependent systems in the body and tissue growth. Such amount of protein seems to be sufficient both during the linear growth spurt as well as thereafter, when athletes enter the peak muscle mass velocity. Therefore, protein intake should not necessarily be enhanced in periods of high linear or muscular growth. The current dietary protein intake around 1.5 g kg⁻¹ day⁻¹ appears to be sufficient throughout adolescence in sprint athletes of both genders, with the exception of some individuals with a higher protein need. These results can only be applied in well-nourished athletes who have access to an abundance and variety of well-balanced foods and training facilities, allowing them to optimize training.

Acknowledgments

The authors thank the athletes for their voluntary participation in this study. The university research council provided funding for this study.

References


