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ORIGINAL ARTICLE

Protein requirements in male adolescent soccer players

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Abstract Few investigations have studied protein metabolism in children and adolescent athletes which makes difficult the assessment of daily recommended dietary protein allowances in this population. The problematic in paediatric competitors is the determination of additional protein needs resulting from intensive physical training. The aim of this investigation was to determine protein requirement in 14-year-old male adolescent soccer players. Healthy male adolescent soccer players ($N = 11$, 13.8 ± 0.1 year) participated in a short term repeated nitrogen balance study. Diets were designed to provide proteins at three levels: 1.4, 1.2 and 1.0 g protein per kg body weight (BW). Nutrient and energy intakes were assessed from 4 day food records corresponding to 4 day training periods during 3 weeks. Urine was collected during four consecutive days and analysed for nitrogen. The nitrogen balances were calculated from mean daily protein intake, mean urinary nitrogen excretion and estimated faecal and integumental nitrogen losses. Nitrogen balance increased with both protein intake and energy balance. At energy equilibrium, the daily protein intake needed to balance nitrogen losses was 1.04 g kg^{-1} day^{-1}. This corresponds to an estimated average requirement (EAR) for protein of 1.20 g kg^{-1} day^{-1} and a recommended daily allowance (RDA) of 1.40 g kg^{-1} day^{-1} assuming a daily nitrogen deposition of 11 mg kg^{-1}. The results of the present study suggest that the protein requirements of 14-year-old male athletes are above the RDA for non-active male adolescents.

Keywords Protein allowances · Adolescent athletes · Growth · Nutrition

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Introduction

During childhood and puberty, the rapid growth and development increase the requirements for energy and nutrients (Torun et al. 1996) and stimulate protein metabolism for lean tissue growth and tissue remodelling (National Research Council Food and Nutrition Board 1989). It has been reported that daily protein intake should amount to about 0.8–1.0 g kg^{-1} body weight (BW) per day in adolescents (Beaufrère et al. 2001, FAO/OMS/UNU 1986, National Research Council Food and Nutrition Board 1989). However, this recommendation is based on "classic" adolescents rather than on those involved in regular intensive physical training. Thus, no daily protein requirements and protein allowances are available for children or adolescents with high level of physical activity (at least 50% higher than the current population).

In adults, acute exercise and regular training induced greater protein oxidation (Poortmans 2004). Literature concerning protein metabolism in children and adolescent athletes is scarce. In 9-year old children, a 6-week walking program altered whole-body protein utilization (Bolster et al. 2001) and a period of 6-week resistance training led to a down-regulation in protein metabolism (Pikosky et al. 2002). However, in both studies, the energy intake was not increased during the training program despite greater energy expenditure. Using the end product method with [^{15}N] glycine, Boisseau et al. (2005) indicated that intense physical activity did not exert a significant effect on protein turnover in young female gymnasts (7–10 year old, 8–10 h of training per week) as compared with untrained age-matched girls. The only study dealing with protein recommendations in adolescent athletes has been performed using a short-term nitrogen balance (7 days) in adolescent soccer players (Boisseau et al. 2002). However, the methodology used was not appropriate. Indeed, in the investigation the authors did not establish protein requirements using different protein levels in the same subjects but they determined a mean protein requirement from free diets in adolescent athletes, and protein intakes were calculated with food composition data drawn from tables only. Thus, no reliable protein requirements and protein allowances are at present available in adolescents with high level of physical activity.

The purpose of the present study was to determine protein requirements in adolescent soccer players using nitrogen balance measurements with four balanced diets including different protein levels (1.4, 1.2, 1.0 and 0.8 g protein per kg BW).

Methods

Subjects

Eleven male adolescents aged [mean (SEM)] 13.8 ± 0.1 years volunteered for this investigation. They were recruited from a secondary school in sport-specialized classes for soccer players and housed in a training centre. All the teenagers performed approximately 10–12 h of soccer per week (at least 2 h of training per day and a match during the week-end). Before the study began, the purpose and objectives were carefully explained to each subject and his parents. A written formal consent signed by the parents and the child was obtained from all the participating subjects. The University of Auvergne Review Board for human subject research approved the study.

All subjects were in healthy condition without acute or chronic diseases or gastrointestinal disorders, and none of them were taking medications known to alter protein and energy metabolism. They had been training for at least the last 2 years. All the data were collected between January and March to avoid important loss of sweat during training. Height was measured to the nearest 0.5 cm with an anthropometric plane. Body weight (BW) was measured to the nearest 0.1 kg with a calibrated portal digital scale before the study began and at the end of each control period. Percentage of body fat was estimated from skinfolds using a Harpenden Skinfold Caliper (Durnin and Rahaman 1967) just before the study began.

Diets

Four balanced diets consisting of usual foods were composed by the dietician of the training centre according to the French Recommended Nutrient Allowances (Martin 2001). Diets were designed to meet the mean estimated energy requirement of 56 kg adolescent male soccer players (Vermorel and Pérès 2004) and to provide proteins at 4 levels: 1.4, 1.2, 1.0 and 0.8 g protein per kg BW. Theoretically, they were planned to be fed in this descending order during four successive 12-day periods (a 8-day adaptation period and a 4-day balance period). In practice, the last diet including 0.8 g protein per kg BW could not be performed due to both the lack of motivation of the adolescents after 2 months of investigation and the difficulty to prepare appetizing meals with very small amounts of dairy products, bread, fish and meat.

Because of great differences in BW between subjects and since energy requirement is not directly proportional to BW (Vermorel 2004), energy supply was adjusted individually to the so called "Equivalent BW, EBW"[1] to avoid insufficient or excess energy intake in the light and heavy subjects, respectively.

Each food offered and leftovers were weighed individually using an accurate balance (Adventurer Pro, France; 4,000 ± 0.1 g). The leftovers were collected, pooled during each 4-day-period and stored at −18°C.

[1] Calculation of the so-called "equivalent body weight, EBW: if BWm is the mean BW of subjects and BWsi is BW of subject i, his EBW equals: BWm x $BWsi^{0.75}/BWmi^{0.75}$. The EBW of a 37.1 kg subject is 41.1 kg if BWm = 56 kg.

Determination of food intake

Energy and protein intakes were initially intended to be determined using the duplicate meal method. Therefore, representative samples of each food offered were collected, weighed and pooled during each 4-day balance period, and stored at −18°C until analysis. However, leftovers varied greatly depending on food and subject. Therefore, additional food such as cheese, bread, apples, sweet biscuits, and fruit paste were offered to those subjects to meet their energy requirements and prevent uncontrolled snacking. Consequently, the duplicate meal method could not be used thoroughly. Nevertheless, the duplicate meals were homogenized, freeze-dried and analyzed in triplicate for nitrogen using the macro-Kjeldahl method. The measured nitrogen supplies were compared with the protein supplies estimated using the Nutrilog software (Nutrilog SAS, France). The differences were −3.86, +0.78 and +4.80% in diets 1, 2 and 3, respectively. Therefore, protein intake of subjects was assessed individually from individual food intakes using the Nutrilog software and the appropriate corrections. Energy intake was also assessed using the Nutrilog software, which gave similar estimates as the Prodiet software (Proform, France) used in a previous study (Boisseau et al. 2005; Ollier et al. 2006).

Energy requirements and energy expenditure

Energy requirements of subjects during each 4-day balance period were assessed using the factorial method previously used to assess recommended energy allowances for adolescent elite athletes (Vermorel 2004). Energy requirement is the sum of daily energy expenditure (DEE) and energy gain in the body corresponding to protein and lipid accretion. DEE is the sum of energy expenditures (EE) corresponding to the various daily activities (from sleep to competition). EE during an activity depends on basal metabolic rate (BMR, kJ min^{-1}), physical activity ratio [PAR = EE (kJ min^{-1})/BMR (kJ min^{-1})] and the duration (min day^{-1}) of the activity.

Subjects recorded all their daily activities during each 4-day-balance period, indicating the times of the beginning and the end, the nature and the intensity of each activity. BMR was estimated using the FAO/OMS/UNU prediction equation for boys aged 10–18 years (FAO/OMS/UNU 1986). BMR was corrected for body composition (85 kJ kg^{-1} difference in fat free mass)(Ribeyre et al. 2000) since the fat free mass content of athletes is higher than that of untrained subjects. The PAR values corresponding to sleep and 30 sedentary (school, TV, video games, meals, transport, etc.) or physical activities (walking, training, competition, etc.) at three intensity levels were drawn from previous studies and from literature data (Vermorel 2004).

The above factorial method was validated using the results of two studies performed in 374 15-year-old athlete and non-athlete adolescents in Sweden using the doubly labeled water (DLW) method and activity records (Bratteby et al. 1997, 1998). This method and the same PARs were used to assess EE in fifteen 14-year-old soccer players and the mean estimated energy requirement agreed with the mean energy intake over a 7-day-control period (Ollier et al. 2006).

Determination of protein requirements and protein allowances

Most of the estimates of protein requirement and allowances are based on nitrogen balance studies (FAO/OMS/UNU 1986; Martin 2001; National Research Council Food and Nutrition Board 1989) with at least three levels of protein intake in the same subjects. In adults, protein requirement has been defined as the minimum amount of dietary protein that will balance nitrogen losses from the body of subjects at zero energy balance (FAO/OMS/UNU 1986). The estimated average requirement (EAR) of adolescents aged 14–18 years is based on the adult estimates of maintenance requirements from nitrogen balance studies (Rand et al. 2003) plus an additional amount to meet the needs for growth. Daily protein deposition in 13–15 year male adolescents was reported to correspond to 11 mg N kg^{-1} per day (Dewey et al. 1996).

In the present study, nitrogen balance was calculated as the difference between nitrogen intake drawn from dietary protein intake determinations (see "Determination of food intake") and nitrogen excretion during the last 4 days of the three 12-day experimental periods corresponding to diet 1, 2 and 3, respectively. During this period, urine was collected daily and weighed using an accurate balance. Daily aliquot samples of urine were taken in acidified flasks stored at −18°C. Urinary nitrogen was determined using the micro-Kjeldhal method (Büchi nitrogen determination System, Switzerland). Stools could not be collected for practical reasons. Faecal nitrogen excretion was calculated from nitrogen apparent digestibility of similar diets. Data from literature (Castiglia-Delavaud et al. 1998; Consolazio et al. 1963, 1975; Gattas et al. 1992; Howat et al. 1975; Sinaud et al. 2002; Vermorel et al. 2004) indicate that there is a relationship between nitrogen apparent digestibility and nitrogen intake: nitrogen apparent digestibility (%) = 12.965 × protein intake (g kg^{-1} day^{-1}) + 72.05;

$r = 0.77$; RSD = 2.7. This formula was used to estimate faecal nitrogen excretion for each subject with each diet as: faecal nitrogen excretion (mg kg^{-1} day^{-1}) = nitrogen intake (mg kg^{-1} day^{-1}) × (1 − nitrogen apparent digestibility/100). Integumental and miscellaneous nitrogen losses were taken at 8 mg kg^{-1} day^{-1} (Dewey et al. 1996).

In an attempt to provide recommended dietary allowances (RDA), an estimation of EAR variance was made using the coefficients of variation (CV) for maintenance and protein deposition for adolescents (DRI 2005). Consequently, the RDA for adolescents as indicated at the 97.5 percentile, was estimated as follows:

RDA = EAR + 2[$\sqrt{}$(0.12 × 0.656 g protein/kg/day)^2 + (0.43 × 2.13 × Y g protein/kg/day)^2], where Y = 0.011 × 6.25 = 0.069 g protein kg^{-1} day^{-1}, in this study (DRI 2005).

Statistical analyses

All data are presented as mean ± standard errors of the mean (SEM). The comparison of body weight, energy intake, energy expenditure, energy balance, protein intake, nitrogen excretion and nitrogen balance during the 3 weeks studied was tested using a one-way ANOVA with repeated measures and paired t tests. The relationship between nitrogen balance, nitrogen intake and energy balance was calculated by multiple regression (STATISTICA software, version StatSoft France 1984–2000). Statistical significance was set at $P \leq 0.05$.

Results

Subject's characteristics

Age and anthropometric characteristics of the adolescents are described in Table 1. Interindividual variability was high for height (from 1.53 to 1.77 m), for BW (from 36.8 to 72.1 kg) and percentage of FM (from 7.9 to 18.1%). The mean percentage of FM (11.8%) was lower than the average value (14%) observed in 14-year-old male adolescents of the French population. BW did not vary significantly during the 6 week experimental period being successively 54.2 ± 3.5, 54.5 ± 3.4 and 54.4 ± 3.5 kg with the three diets.

Protein and energy intakes

The expected protein intakes were 1.4, 1.2 and 1.0 g protein per kg BW in period 1, 2 and 3 (diet 1, diet 2 and diet 3), respectively. However, since each subject had various leftovers (mainly vegetables and fruit) and as additional foods were offered to limit the energy deficit, protein intake averaged 1.59, 1.50 and 1.25 g protein per kg BW. Energy intake averaged 11.17, 11.88 and 11.82 MJ day^{-1} in period 1, 2 and 3, respectively (Table 2).

Table 1 Initial characteristics of adolescent soccer players (means ± SEM)

Subjects	Age (year)	Height (cm)	Initial body weight (kg)	Initial fat mass (%)
CR	13.9	161.0	46.6	12.9
DJA	13.1	153.0	36.8	7.9
DPA	14.1	168.5	59.6	11.9
DRA	13.8	158.0	46.6	9.3
FC	13.7	161.5	54.1	13.3
FCl	14.1	167.5	55.5	11.8
GC	14.0	176.5	69.6	13.1
HA	14.0	169.0	66.2	12.5
HB	13.5	155.0	39.9	9.5
MJ	13.7	170.0	72.1	18.1
SA	13.5	165.0	49.4	9.6
Mean	13.8	164. 1	54. 2	11.8
SEM	0.1	2. 1	3. 5	0.8

Energy expenditure and energy balance

The subjects spent 113 ± 9 min day^{-1} at actual physical activities including training activities (95 ± 7 min day^{-1}), on average, during the balance periods. Estimated energy expenditure averaged 12.12, 11.88 and 11.82 MJ day^{-1} in period 1, 2 and 3, respectively (Table 2). Consequently, energy balance (energy intake − energy expenditure) was negative in many subjects and averaged −0.94, −1.03 and −1.03 MJ day^{-1} in period 1, 2 and 3, respectively (Table 2). Furthermore it was highly variable between subjects, even when expressed per kg EBW, ranging from −14.49 to 4.47 kJ kg EBW^{-1} per day.

Nitrogen balance

Nitrogen balance and its components are shown in Table 3. Protein intake was significantly lower with diet 3 than with diet 2 and 1 but the difference between diets 1 and 2 was not significant ($P = 0.08$). The between subject differences were significant ($P = 0.03$). Nitrogen balance was significantly positive with diet 1 ($P = 0.03$) and tended to be higher than zero with diet 2 ($P = 0.07$). With diet 3, it was lower than with diets 2 ($P = 0.03$) and 1 ($P = 0.01$) and negative but not significantly lower than zero ($P = 0.21$). Nitrogen balance was significantly ($P < 0.0001$) correlated with nitrogen (or protein) intake ($r = 0.66$) and with energy balance ($r = 0.71$).

Table 2 Mean energy intake (EI; MJ day^{-1}), total energy expenditure (TEE; MJ day^{-1}) and energy balance (EB; MJ day^{-1}) during 4 day measurement periods in adolescent soccer players at the three levels of protein intake

Subjects	Diet 1[a]			Diet 2[b]			Diet 3[c]		
	EI	TEE	EB	EI	TEE	EB	EI	TEE	EB
CR	9.68	11.58	−1.90	9.34	11.40	−2.06	10.63	12.37	−1.74
DJA	10.26	10.68	−0.42	8.89	10.80	−1.91	8.44	9.87	−1.43
DPA	10.81	13.39	−2.58	10.58	12.71	−2.13	–	–	–
DRA	11.06	11.13	−0.07	11.62	11.25	0.37	–	–	–
FC	12.86	13.00	−0.14	11.50	12.27	−0.77	11.89	12.95	−1.06
FCl	11.30	12.49	−1.19	11.24	11.08	0.16	10.60	10.59	0.02
GC	12.77	13.97	−1.19	13.70	13.69	0.00	13.22	13.90	−0.68
HA	12.97	11.55	1.42	11.44	11.69	−0.25	–	–	–
HB	11.40	10.60	0.80	11.28	10.36	0.92	11.43	10.70	0.73
MJ	11.54	13.58	−2.04	11.09	13.53	−2.44	–	–	–
SA	8.27	11.33	−3.06	8.71	11.90	−3.19	9.30	12.36	−3.06
Mean	11.17	12.12	−0.94*	10.85	11.88	−1.03*	10.79	11.82	−1.03 (*)
SEM	0.43	0.37	0.42	0.43	0.33	0.41	0.60	0.55	0.47

Values are means ± SEM on 4-day diet period. Significantly different from zero * $P < 0.05$; (*) $P = 0.069$

[a] 1.59 g protein/kg per day

[b] 1.50 g protein/kg per day

[c] diet 3, 1.25 g protein/kg per day

Table 3 Protein intake (g kg^{-1} day^{-1}), total nitrogen excretion (mg N kg^{-1} day^{-1}) and nitrogen balance (mg N kg^{-1} day^{-1}), in adolescent soccer players during the three levels of protein intake

Subjects	Diet 1			Diet 2			Diet 3		
	Protein intake	N excretion	N balance	Protein intake	N excretion	N balance	Protein intake	N excretion	N balance
CR	1.25	218	−17.53	1.51	264	−22.69	1.10	182	−5.62
DJA	2.01	284	38.48	1.83	275	17.68	1.82	289	1.62
DPA	1.49	236	1.90	1.43	253	−25.15	–	–	–
DRA	1.66	225	40.27	1.56	222	27.79	–	–	–
FC	1.66	180	85.15	1.33	183	29.47	1.11	224	−45.66
FCl	1.67	233	33.73	1.51	184	56.78	1.17	158	29.90
GC	1.50	218	21.64	1.53	211	33.83	1.04	183	−16.54
HA	1.61	249	7.96	1.38	216	4.44	–	–	–
HB	1.92	211	95.83	1.84	223	71.67	1.43	208	20.24
MJ	1.36	202	16.03	1.25	206	−6.60	–	–	–
SA	1.37	263	−44.13	1.34	230	−15.73	1.05	224	−56.21
Mean	1.59	229	25.39	1.50	225	15.90	1.25	210	−10.32
SEM	0.07	0.01	12.30	0.06	9.00	9.65	0.09	16	9.62

Values are means ± SEM on 4-day diet period (1 g of nitrogen is equivalent to 6.25 g protein)

Both protein intakes and energy balance were important determinants of nitrogen balance. A linear multiple regression between nitrogen balance (mg N/kg BW) as dependent variable and protein intake (g/kg BW) and energy balance (kJ/EBW) was calculated.

Nitrogen balance = 69.87(±15.70)**[2] Protein intake + 0.88(±0.17)** Energy balance − 72.77 (±25.44)*[3], $r^2 = 0.78$.

All the subjects who were present but ill or without training sessions have been removed for the calculation—in period 1, one subject (HA); in period 2, two subjects (FCl and HA); in period 3, one subject (FCl). All the coefficients were highly significant. The two variables explained 78% of nitrogen balance variation.

From this relationship it could be calculated that the mean protein intake needed for nitrogen and energy equilibrium was 1.04 g kg^{-1} day^{-1}. As the protein deposition in 13–15 year boys may be considered at 11 mg N kg^{-1} per day (Dewey et al. 1996), the equation gave a mean protein intake of: 1.20 g kg^{-1} day^{-1} that can be considered as protein requirement.

For an estimated average requirement (EAR) of 1.20 g kg^{-1} day^{-1}, the recommended daily allow-

[2] ** $P < 0.001$

[3] * $P < 0.01$

ances (RDA) became (DRI 2005): RDA = EAR + 2[$\sqrt{}$(0.12 × 0.656 g protein/kg/day)^2 + (0.43 × 2.13 × 0.069 g protein/kg/day)^2] = 1.40 g kg^{-1} day^{-1}.

Discussion

The results of the present study showed that the traditional dietary protein allowances (0.8–1.0 g kg^{-1} day^{-1}) were not sufficient to meet protein requirements corresponding to growth and a high level of physical activity in adolescent athletes. They suggest that the recommended protein allowances should be greater for male adolescent soccer players than the presently recommended allowances for non-active male adolescents. However such a recommendation is lower than protein intakes currently observed in France: 2.07 g kg^{-1} per day in 11–14 year boys (Volatier et al. 2000).

In the literature, only three studies dealt with protein metabolism in active children (7–10-year-old). They indicate that training had either no effect (Boisseau et al. 2005) or decreased protein turnover (Bolster et al. 2001; Pikosky et al. 2002).

From these data, one could expect that daily protein requirement should not be different from those specified in non-active children or adolescents. However, during puberty (from 13–17 year), the increase in muscle mass, especially in male adolescent athletes, could stimulate protein metabolism. The only study performed using a short term nitrogen balance (7 days) in adolescent soccer players aged 15 year (Boisseau et al. 2002) was not appropriate to determine protein requirement that needs accurate nitrogen balances using different protein levels in the same subjects (Scrimshaw 1996).

In the present study, protein intakes and nitrogen excretion were accurately determined but other difficulties have been encountered. The main difficulties were related to compliance of the young adolescent volunteers and kitcheners with the dietary experimental design. Both the length of the study and the unusual composition of the diet designed for 0.8 g of protein kg^{-1} per day led to suppress this diet. Furthermore leftovers and substitutions resulted in a 10% energy deficiency and alterations in protein level intakes. However as every event was recorded, this could be quantified but the usual method to assess protein requirements could not be used.

During the 4-day balance periods subjects spent, on average, 6 and 14 min day^{-1} less at actual sport activities than during two previous studies (Ollier et al. 2006; Vermorel 2004). After correction for differences in height and body weight between subjects of the three studies, daily energy expenditure was 0.51 and 0.58 MJ (4.1 and 4.6%) lower than in the two previous studies . Nevertheless energy balance was negative (−0.94, −1.03 and −1.03 MJ day^{-1}) during the 4-day periods 1, 2 and 3 because of leftovers. Subjects kept BW constant throughout the experimentation probably because they compensated for the energy deficit during the last three days of the balance weeks spent at home.

Due to the negative energy balance (Scrimshaw 1996) and its high variability, the classical method based on the determination of individual requirements using individual response curve to the three diets to calculate the mean protein requirement from these individual estimations, could not be used. Using the multiple regression method, it has been shown that protein intake and energy balance significantly altered nitrogen balance ($P < 0.001$). Through this methodology using the 3 diets on healthy and active subjects, our study indicated an estimated average requirement (EAR) of 1.20 g of protein per kg day^{-1} and a recommended daily allowance (RDA) of 1.40 g kg^{-1} day^{-1}.

The difference between the present result in protein EAR (1.20 g of protein kg^{-1} per day) and current data (0.73 g of protein kg^{-1} per day) is rather high. However a comparison with a control group studied in similar condition would be necessary to demonstrate thoroughly that protein requirements were higher in trained adolescents than in the current population. Nevertheless the fact that dietary energy and protein efficiencies drawn from the coefficients of the multiple regression are in keeping with data from literature suggests that this model is rather sound. Indeed, increasing protein intake by 1.0 g kg^{-1} day^{-1} improved nitrogen balance by 69.87 mg N kg^{-1} day^{-1} (i.e. 0.44 g protein kg^{-1} day^{-1}) per g of dietary protein kg^{-1} day^{-1}. Such an efficiency (0.44) is lower than 0.59 reported in 12.7-year-old children (Gattas et al. 1992) but similar to 0.47 reported in adults (DRI 2005) and to the value (0.40) calculated in endurance trained young men (Gaine et al. 2006). With the increase in energy balance there was an increase of 0.88 mg N per 1 kJ kg EBW^{-1} day^{-1} (corresponding to 0.092 kJ if we assume that it is body proteins). This is higher than the increase (0.51 mg. N per added kJ) reported in trained young men (Butterfield et al. 1984) probably because our subjects were younger and because they were mostly in negative energy balance.

Conclusion

Soccer is a high-intensity, intermittent activity which requires both strength and endurance over a period of 90 min (Lemon 1994). Consequently, an adequate protein intake must be provided in adolescent soccer players to sustain growth and the increased amino acid oxidation that may occur during training and competition. The results of this study suggest greater protein needs in male adolescent athletes. Additional investigations are necessary to confirm these results and establish protein recommended allowances in young athletes with respect to age, sex, sport, and level of practise.

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