Protein intake at 9 mo of age is associated with body size but not with body fat in 10-y-old Danish children

Camilla Hoppe, Christian Mølgaard, Birthe Lykke Thomsen, Anders Juul, and Kim Fleischer Michaelsen

ABSTRACT

Background: During the complementary feeding period, infants shift from a daily protein intake (PI) of ≈ 1 g/kg body wt to an intake 3–4 times as high. A high PI probably has both endocrine and physiologic effects and may increase the risk of obesity.

Objective: We examined the associations between PI in infancy and body size and composition in late childhood.

Design: We conducted an observational cohort study of 142 Danish healthy term infants (63 boys) born during 1987–1988. At 9 mo of age, diet, weight, length, skinfold thicknesses, insulin-like growth factor I, and serum urea nitrogen were determined. At 10 y of age, 105 children (51 boys) participated in a follow-up study. Diet, weight, height, skinfold thicknesses, percentage of body fat (dual-energy X-ray absorptiometry), insulin-like growth factor I, and serum urea nitrogen were determined.

Results: At 9 mo of age, PI (in g/d and percentage of energy) was strongly correlated with body size (length and weight) but not with measures of adiposity. PI at 9 mo of age was positively associated with height and weight but not with percentage of body fat at 10 y of age. Inclusion of parental body size in the models did not change the associations, but the significant associations were attenuated when body size at 9 mo of age was included.

Conclusions: PI in infancy seems to stimulate early growth. This may explain part of the association between early PI and body size at 10 y of age, but a continuous effect of protein on growth during childhood cannot be excluded. PI in infancy was not associated with any measure of body fat at 10 y of age.


KEY WORDS Protein-adiposity hypothesis, infancy, childhood, breastfeeding, growth, obesity, insulin-like growth factor I, protein intake

INTRODUCTION

Protein intake increases dramatically during the period of complementary feeding. Most healthy infants shift from a protein intake of ≈ 1 g/kg body wt to an intake that is, on average, 3–4 times as high. If an infant eats typical family foods containing 15% of energy from protein, the daily protein intake will be ≈ 3.4 g/kg body wt. There is, however, a wide variation, and some infants have an intake of 5–6 g/kg body wt (1).

Formula-fed infants grow at a faster rate than do infants fed breast milk (2–4). This difference could be caused by a higher protein intake in the formula-fed infants because of the higher protein content in formula than in breast milk (4). There are many other differences between breastfed and formula-fed infants that could explain the difference, but a study comparing the growth of 4–6-mo-old infants fed formulas with different protein contents found that a high protein intake was associated with high growth velocity in infancy (5). In addition, in a study of 5–10-mo-old infants who had protein intakes that exceeded their requirements, a positive association was found between protein intake and weight gain (6).

A high protein intake is likely to have both endocrine and physiologic effects, and it has been suggested that a high protein intake early in life could increase the risk of adiposity later in life (7). The proposed mechanism is that a high protein intake stimulates secretion of insulin-like growth factor I (IGF-I) and thereby triggers precocious cell multiplication and accelerates maturation (8, 9). The increased IGF-I concentrations may then accelerate growth and increase adipose tissue and muscle mass, thereby inducing an early adiposity rebound, which is the point at which the body mass index (BMI; in kg/m2) increases after its nadir; on average, adiposity rebound occurs at the age of 6 y. An early adiposity rebound is associated with an elevated risk of obesity later in childhood and possibly also in adulthood (10–12).

The protein-adiposity hypothesis has been examined in a few observational studies. A study in France (7) and a study in Italy (13) found that a high protein intake early in life is associated with elevated BMI at 8 and 5 y, respectively. A study in the United Kingdom (14) did not find an effect of early protein intake on the timing of adiposity rebound. In the study in Italy (13), the dietary protein content was remarkably high (≈ 20% of energy).

Many studies have suggested that breastfeeding protects against overweight and obesity later in life (15). One of the...
proposed mechanisms for this protection is the lower protein intake in breastfed infants than in formula-fed infants.

The aim of the present study was to examine the associations between protein intake in infancy and body size and body composition in late childhood in a random sample of healthy Danish children. Our emphasis was on testing the protein-adiposity hypothesis.

SUBJECTS AND METHODS

The Copenhagen Cohort Study on Infant Nutrition and Growth

This was an observational study in which a random sample of Danish infants was followed from birth to 12 mo of age. Two hundred fifty-one infants born at Hvidovre Hospital, Copenhagen, from 1987 to 1988 fulfilled the following inclusion criteria: parents of Danish origin, singleton births, gestational age between 37 and 42 wk, birth-weight-for-gestational-age between the 10th and 90th percentiles, no neonatal disease resulting in admission to the neonatal department, no severe malformations, and mother and infant admitted to the maternity ward for ≥ 3 d. A more detailed description of the selection and characteristics of the study group was published previously (16). One hundred forty-two infants completed the study.

Follow-up

All 142 subjects from the original study were invited to participate in the follow-up study (October 1997 to February 1998) when they were 10 y old. The study was approved by the Ethics Committee for Copenhagen and Frederiksberg (KF 01-226/97). One hundred fifty (74%) subjects agreed to participate.

Examination at 9 mo of age

Anthropometry

The infants’ weights were measured while the infants were naked and lying on an electronic balance, and length was measured with the use of a wooden measuring board. Triceps and subscapular skinfold thicknesses were measured by using a Harpenden Skinfold Caliper (Chasmors Ltd, London) according to standard procedures. Anthropometric variables for all the infants were measured by the same person.

Breastfeeding

Duration of breastfeeding was registered at 9 mo, and exclusive breastfeeding was distinguished from partial breastfeeding. Exclusive breastfeeding allowed supplements of water or chamomile tea with no sugar or milk added (commonly used for infants in Denmark) and vitamins. Infants were classified as partially breastfed as long as they were breastfed at least once a day.

Food records

When the infants were 9 mo of age, their parents used electronic balances to complete 5-d weighed food records. The 5-d record was scheduled to include 3 weekdays and a weekend. Intake of nutrients was calculated by using the DANKOST computer program, which was developed by the Danish National Food Agency (DANKOST 2.0; Dansk Catering Service, Herlev, Denmark).

Blood analyses

Serum urea nitrogen (SUN) was analyzed on a SMA 12 analyzer (Technicon, Tarrytown, NY). IGF-I concentrations were measured by radioimmunoassay (17). Briefly, serum was extracted by acid-ethanol and cryoprecipitated before analysis to remove interfering IGF-binding proteins. Interassay and intraassay CVs were < 9% and 6%, respectively. Details regarding measurement of IGF-I concentrations were published previously (18).

Follow-up at 10 y of age

Body-composition assessment

The percentage of body fat (%BF) in the whole body was determined by using dual-energy X-ray absorptiometry on a Hologic 1000/W (Hologic Inc, Waltham, MA). For analysis, software version 5.61 was used. The subjects wore only underpants and a cotton T-shirt during the scan. The entrance radiation dose level was 15 μSv, with an effective dose ≤ 10 μSv, which is equal to ≈ 1 d of background radiation in Denmark.

Puberty and anthropometry

Puberty stages were assessed according to the method of Tanner (19). Breast development in girls and pubic hair development in boys were used to assess pubertal stages. Height and weight were measured before dual-energy X-ray absorptiometry scanning. Height was measured to the nearest 1 mm by using a wall-mounted digital stadiometer. Weight was measured to the nearest 0.1 kg by using an electronic digital scale. The subjects wore only underpants and a cotton T-shirt while being weighed. Triceps and subscapular skinfold thicknesses were measured by using the Harpenden Skinfold Caliper according to standard procedures (20). Anthropometric variables for all the children were measured by the same person.

Food intake

With help from their parents, the participants kept a 7-d food record, which was described previously (21).

Statistical analyses

All statistical analyses were performed with SPSS (version 11.0; SPSS Inc, Chicago). All descriptive results are expressed as medians (10th–90th percentiles). Differences in the variables between the boys and the girls were tested by the Mann-Whitney test. Pairwise partial correlations with adjustment for sex between diet, anthropometric, and blood variables in infancy and in childhood were calculated. The dependence of the anthropometric measurements at 10 y of age (%BF, BMI, weight, and height) on each protein intake variable at 9 mo of age (SUN, protein intake per kg body wt in g · kg⁻¹ · d⁻¹, and total daily protein intake in g/d) was analyzed by multiple linear regressions after sex was controlled for. The protein intake variables at 9 mo
of age were evaluated by using different parameterizations, either as linear variables or as 3 dummy variables, and the change from one quartile group to the next was measured to evaluate whether any associations were significant only with high intakes of protein. However, because the results were similar with both parameterizations, we present only the results from the models with the linear variables. For each combination of anthropometric measurement at 10 y of age and protein intake variable at 9 mo of age, we evaluated the association in 4 different models. The first model was adjusted for sex. The second model was adjusted for sex and parental size as determined by measurements of the mother’s and father’s weight and height. The third model was additionally adjusted for body size at 9 mo of age (weight and length). In the fourth model, we also included duration of breastfeeding. There was no interaction between sex and the protein intake variables at 9 mo of age in any of the models.

RESULTS

Data at 9 mo of age

Descriptive statistics for anthropometric variables are shown in Table 1. At 9 mo of age, the boys were significantly larger (higher weight, length, and BMI) than were the girls (Table 1), which was in accordance with the boys’ significantly higher total energy intake (Table 2). There were no significant differences between the boys and the girls in duration of breastfeeding or intakes of protein, fat, and carbohydrates. The median energy intake was 3088 kJ/d, which was equivalent to 349 kJ/kg body wt. The median daily protein intake was 2.7 g/kg body wt (13.3% of energy), the range was 0.9–4.2 g/kg body wt (6.5–20% of energy), and the 90th percentile was 3.8 g/kg body wt (17.4% of energy).

SUN and IGF-I concentrations in the infants are shown in Table 3. There were no significant differences between the boys and the girls. As shown in Table 4, SUN was significantly correlated with reported protein intake—expressed either as g/d or as percentage of energy—at 9 mo of age (both P ≤ 0.0001). IGF-I was positively correlated with total daily protein intake and SUN (both P ≤ 0.045) and to a lesser degree with percentage of

TABLE 1

Descriptive statistics for anthropometric variables at 9 mo and 10 y of age$^1$

<table>
<thead>
<tr>
<th></th>
<th>Boys (n = 51)</th>
<th>Girls (n = 53)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 mo of age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (mo)</td>
<td>9.1 (8.9–9.7)</td>
<td>9.1 (8.9–9.6)</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>72.7 (70.1–76.0)</td>
<td>70.2 (67.3–73.8)</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>9320 (8338–10952)</td>
<td>8530 (7350–9582)</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>17.8 (16.0–19.9)</td>
<td>17.0 (15.4–19.0)$^2$</td>
</tr>
<tr>
<td>TSF (mm)</td>
<td>9.0 (6.6–11.9)</td>
<td>8.6 (6.6–11.5)</td>
</tr>
<tr>
<td>SSF (mm)</td>
<td>7.1 (5.1–9.9)</td>
<td>6.7 (5.2–8.9)</td>
</tr>
<tr>
<td>10 y of age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>9.94 (9.87–10.05)</td>
<td>9.97 (9.87–10.10)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>143.2 (134.6–149.9)</td>
<td>141.8 (133.6–148.8)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>34.1 (29.1–41.5)</td>
<td>32.8 (27.9–39.8)</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>16.7 (15.4–19.7)</td>
<td>16.7 (14.5–19.5)</td>
</tr>
<tr>
<td>%BF (%)</td>
<td>18.6 (14.1–25.0)</td>
<td>22.2 (17.9–28.3)$^2$</td>
</tr>
<tr>
<td>TSF (mm)</td>
<td>8.95 (6.32–17.4)</td>
<td>11.7 (7.9–17.9)$^4$</td>
</tr>
<tr>
<td>SSF (mm)</td>
<td>7.2 (4.8–13.9)</td>
<td>9.3 (5.8–15.8)$^4$</td>
</tr>
<tr>
<td>WHR</td>
<td>0.86 (0.81–0.89)</td>
<td>0.83 (0.79–0.90)$^2$</td>
</tr>
</tbody>
</table>

$^1$ Median; 10th–90th percentiles in parentheses. %BF, percentage of body fat measured by dual-energy X-ray absorptiometry; TSF, triceps skinfold thickness; SSF, subscapular skinfold thickness; WHR, waist-to-hip ratio.

$^2$–$^4$Significantly different from boys: $^2$P < 0.001, $^3$P < 0.05, $^4$P < 0.01.

TABLE 2

Intakes of energy and macronutrients at 9 mo of age as assessed by 5-d food records, duration of breastfeeding at 9 mo of age, and intakes of energy and macronutrients at 10 y of age as assessed by 7-d food records

<table>
<thead>
<tr>
<th></th>
<th>Boys (n = 47)</th>
<th>Girls (n = 53)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 mo of age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(kJ/d)</td>
<td>3193 (2465–4191)$^4$</td>
<td>3025 (2464–3555)$^2$</td>
</tr>
<tr>
<td>(kJ · kg body wt$^{-1}$ · d$^{-1}$)</td>
<td>330 (261–427)</td>
<td>355 (276–417)</td>
</tr>
<tr>
<td>Protein intake</td>
<td>(g/d)</td>
<td>(g · kg body wt$^{-1}$ · d$^{-1}$)</td>
</tr>
<tr>
<td>(g/d)</td>
<td>25 (14–38)</td>
<td>23 (14–31)</td>
</tr>
<tr>
<td>(% of energy)</td>
<td>2.7 (1.6–3.9)</td>
<td>2.8 (1.8–3.6)</td>
</tr>
<tr>
<td>Fat intake</td>
<td>(g/d)</td>
<td>(g · kg body wt$^{-1}$ · d$^{-1}$)</td>
</tr>
<tr>
<td>(g/d)</td>
<td>27 (16–37)</td>
<td>25 (18–33)</td>
</tr>
<tr>
<td>(% of energy)</td>
<td>31 (22–39)</td>
<td>31 (24–41)</td>
</tr>
<tr>
<td>Carbohydrate intake</td>
<td>(g/d)</td>
<td>(g · kg body wt$^{-1}$ · d$^{-1}$)</td>
</tr>
<tr>
<td>(g/d)</td>
<td>103 (83–136)</td>
<td>99 (76–122)</td>
</tr>
<tr>
<td>(% of energy)</td>
<td>55 (45–68)</td>
<td>55 (47–64)</td>
</tr>
<tr>
<td>Breastfeeding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exclusive (mo)</td>
<td>3.0 (0.0–5.0)</td>
<td>3.0 (0.0–5.0)</td>
</tr>
<tr>
<td>Total (mo)</td>
<td>6.0 (0.8–9.0)</td>
<td>6.5 (1.0–9.0)</td>
</tr>
<tr>
<td>Partially breastfed at 9 mo of age (%)</td>
<td>29</td>
<td>33</td>
</tr>
<tr>
<td>10 y of age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(kJ/d)</td>
<td>10 295 (7829–12 228)</td>
<td>8945 (7501–11 076)$^2$</td>
</tr>
<tr>
<td>(kJ · kg body wt$^{-1}$ · d$^{-1}$)</td>
<td>298 (233–356)</td>
<td>272 (206–337)$^2$</td>
</tr>
<tr>
<td>Protein intake</td>
<td>(g/d)</td>
<td>(g · kg body wt$^{-1}$ · d$^{-1}$)</td>
</tr>
<tr>
<td>(g/d)</td>
<td>80 (60–99)</td>
<td>72 (57–91)$^2$</td>
</tr>
<tr>
<td>(% of energy)</td>
<td>2.3 (1.8–3.0)</td>
<td>2.2 (1.6–2.9)</td>
</tr>
<tr>
<td>Fat intake</td>
<td>(g/d)</td>
<td>(g · kg body wt$^{-1}$ · d$^{-1}$)</td>
</tr>
<tr>
<td>(g/d)</td>
<td>97 (69–122)</td>
<td>81 (67–109)$^4$</td>
</tr>
<tr>
<td>(% of energy)</td>
<td>36 (32–41)</td>
<td>36 (31–40)</td>
</tr>
<tr>
<td>Carbohydrate intake</td>
<td>(g/d)</td>
<td>(g · kg body wt$^{-1}$ · d$^{-1}$)</td>
</tr>
<tr>
<td>(g/d)</td>
<td>299 (238–363)</td>
<td>273 (218–332)$^4$</td>
</tr>
<tr>
<td>(% of energy)</td>
<td>50 (45–54)</td>
<td>50 (46–57)</td>
</tr>
</tbody>
</table>

$^1$ Median; 10th–90th percentiles in parentheses.

$^2$–$^4$Significantly different from boys: $^2$P < 0.05, $^3$P < 0.001, $^4$P < 0.01.

TABLE 3

Blood variables at 9 mo and 10 y of age$^2$

<table>
<thead>
<tr>
<th></th>
<th>Boys (n = 36)</th>
<th>Girls (n = 42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 mo of age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SUN (mmol/L)</td>
<td>4.1 (1.6–6.8)</td>
<td>4.0 (2.1–6.0)</td>
</tr>
<tr>
<td>IGF-I (ng/mL)</td>
<td>72 (47–110)</td>
<td>75 (37–130)</td>
</tr>
<tr>
<td>10 y of age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SUN (mmol/L)</td>
<td>4.4 (3.5–5.7)</td>
<td>4.2 (2.8–5.6)</td>
</tr>
<tr>
<td>IGF-I (ng/mL)</td>
<td>271 (192–370)</td>
<td>311 (227–487)$^2$</td>
</tr>
</tbody>
</table>

$^1$ Median; 10th–90th percentiles in parentheses. SUN, serum urea nitrogen; IGF-I, insulin-like growth factor I.

$^2$ Significantly different from boys, P < 0.05.
TABLE 4
Partial correlations between energy intake (EI), protein intake (PI), serum urea nitrogen (SUN), insulin-like growth factor I (IGF-I), and anthropometric variables at 9 mo of age after sex was controlled for

<table>
<thead>
<tr>
<th></th>
<th>EI (kJ/d)</th>
<th>PI (g/d)</th>
<th>PI (% of energy)</th>
<th>SUN (mmol/L)</th>
<th>IGF-I (ng/mL)</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>SN (mm)</th>
</tr>
</thead>
</table>
| PI (g/d) | 0.729
| PI (% of energy) | 0.299
| SUN (mmol/L) | 0.343
| IGF-I (ng/mL) | 0.12
| Weight (kg) | 0.258
| Height (cm) | 0.214
| TSF (mm) | -0.17
| SSF (mm) | -0.06

1 n = 43–105. TSF, triceps skinfold thickness; SSF, subscapular skinfold thickness.
2 P < 0.001.
3 P < 0.010.
4 P < 0.050.

Data at 10 y of age

At 10 y of age, there were no significant differences between the boys and the girls in age, height, weight, or BMI (Table 1). However, as expected, the girls had significantly higher %BF and triceps and subscapular skinfold thicknesses and significantly lower waist-to-hip ratios than did the boys. According to international cutoffs (22), overweight (adult BMI ≥ 25) corresponds to a BMI of 19.84 and 19.86 in 10-y-old boys and girls, respectively, and obesity (adult BMI ≥ 30) corresponds to a BMI of 24.00 and 24.11 in 10-y-old boys and girls, respectively. In this group of children, 8 children (4 boys) were overweight, and none were obese. The percentage of overweight children was 7.8% in the boys and 7.5% in the girls. Most of the children in this study (49 boys and 34 girls) had no sign of pubertal development (Tanner stage 1), 2 boys and 18 girls were in Tanner stage 2, and no boys and 2 girls were in Tanner stage 3.

Dietary intakes of energy and macronutrients are shown in Table 2. The boys had significantly higher absolute intakes of all macronutrients than did the girls, in accordance with the boys’ higher energy intake (P < 0.001). When macronutrient intakes were expressed as percentages of energy, there were no significant differences between the boys and the girls (all P ≥ 0.37). IGF-I concentrations were significantly higher in the girls than in the boys (Table 3).

IGF-I was strongly positively correlated with height, weight, and BMI (Table 5). Height was positively correlated with absolute energy intake, total daily protein intake, and percentage of energy from protein. SUN was not correlated with body size (height and weight) but was positively correlated with percentage of energy from protein; however, the correlation was not as strong as at 9 mo of age.

Tracking of diet and anthropometric and blood variables

Percentage of energy from protein at 9 mo of age was positively correlated with percentage of energy from protein at 10 y of age (r = 0.28, P = 0.01), which indicated tracking of dietary

TABLE 5
Partial correlations between energy intake (EI), protein intake (PI), serum urea nitrogen (SUN), insulin-like growth factor I (IGF-I), and anthropometric variables at 10 y of age after sex was controlled for

<table>
<thead>
<tr>
<th></th>
<th>EI (kJ/d)</th>
<th>PI (g/d)</th>
<th>PI (% of energy)</th>
<th>SUN (mmol/L)</th>
<th>IGF-I (ng/mL)</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>SSF (mm)</th>
<th>BMI (kg/m²)</th>
</tr>
</thead>
</table>
| PI (g/d) | 0.768
| PI (% of energy) | -0.04
| SUN (mmol/L) | -0.02
| IGF-I (ng/mL) | 0.11
| Weight (kg) | 0.17
| Height (cm) | 0.314
| TSF (mm) | 0.003
| SSF (mm) | -0.09
| BMI (kg/m²) | 0.02
| %BF (%) | -0.07

1 n = 92–97. TSF, triceps skinfold thickness; SSF, subscapular skinfold thickness; %BF, percentage of body fat measured by dual-energy X-ray absorptiometry.
2 P < 0.001.
3 P < 0.050.
4 P < 0.010.
habits. In addition, between 9 mo and 10 y of age, there were strong positive correlations for weight \((r = 0.35, P < 0.0001)\) and length or height \((r = 0.53, P < 0.0001)\), as expected; there was also a positive correlation for IGFI-1 \((r = 0.27, P = 0.03)\) but not for SUN \((r = -0.17, P = 0.19)\) or triceps \((r = 0.13, P = 0.34)\) or subscapular \((r = 0.07, P = 0.60)\) skinfold thickness.

**Relation between diet in infancy and anthropometric variables in childhood**

We studied the associations of protein intake variables (SUN, percentage of energy from protein in the diet, protein intake per kilogram body weight, and total daily protein intake) at 9 mo of age with body size (weight and height) and body composition (%BF and BMI) at 10 y of age. All analyses were performed with adjustment for sex.

None of the protein variables at 9 mo of age was significantly associated with %BF at 10 y of age (Table 6). This result did not change when parental body size or infant body size at 9 mo of age was included in the models (data not shown).

As shown in Table 6, the only predictor at 9 mo of age for BMI at 10 y of age was SUN \((P = 0.001)\). Inclusion of parental body size in the models did not change the association \((P = 0.008)\), but the association was attenuated when infant body size at 9 mo of age was included in the model \((P = 0.06)\).

An increase of 1% of energy from protein at 9 mo of age was associated with an increased weight at 10 y of age of \(\approx 0.44\) kg \((P = 0.007)\) (Table 6), and weight at 10 y of age was also significantly associated with SUN \((P = 0.006)\) and total daily protein intake \((P = 0.012)\) at 9 mo of age. Inclusion of parental body size in the models slightly attenuated the associations (SUN, \(P = 0.02\); percentage of energy from protein, \(P = 0.02\); total daily protein intake, \(P = 0.03\)), but they disappeared when infant body size at 9 mo of age was included in the model (SUN, \(P = 0.10\); percentage of energy from protein, \(P = 0.32\); total daily protein intake, \(P = 0.42\)).

An increase of 1% of energy from protein at 9 mo of age was associated with an increased height at 10 y of age of \(\approx 0.51\) cm \((P = 0.009)\) (Table 6), and height at 10 y of age was also significantly associated with SUN \((P = 0.006)\) and total daily protein intake \((P = 0.012)\) at 9 mo of age \((P = 0.003)\). The significant associations were not affected by the inclusion of parental body size in the models (percentage of energy from protein, \(P = 0.005\); total daily protein intake, \(P = 0.007)\), but they disappeared when infant body size at 9 mo of age was included in the models (SUN, \(P = 0.25\); total daily protein intake, \(P = 0.42\)).

**DISCUSSION**

Our main finding was a positive association between protein intake at 9 mo of age and body size at 10 y of age. This is remarkable in a group of healthy Danish children who had no indication of malnutrition and whose protein intake was severalfold that required to satisfy physiologic needs. We did not find an association between protein intake at 9 mo of age and body fatness at 10 y of age expressed as %BF or BMI. Thus, our study did not support the protein-adiposity hypothesis.

The strength of our study was the design, in which we examined a relatively large group of children at very narrow age ranges with the use of a detailed dietary record covering 5–7 d and dual-energy X-ray absorptiometry scanning, which is regarded as the gold standard for assessing body fat content in epidemiologic studies. Furthermore, we measured IGFI-I concentrations at both 9 mo and 10 y of age, and IGFI-I is likely to be a mediator between nutrition and growth (23, 24). However, there were also limitations to the study. Only 8 of the children were overweight, and none were obese. Furthermore, the protein content of the diet (8; 13% of energy; 90th percentile: 17% of energy) was not as high as in some of the other studies examining the protein-adiposity hypothesis, in which average intakes were 16.3% (7) and 20% (13) of energy. Thus, our study had only a limited power to show an association between protein intake in infancy and fatness at 10 y of age if the association is mainly caused by a tendency among those with a very high protein intake to become obese.

**Protein intake and adiposity**

Although there was no association between protein intake in infancy and fatness in late childhood, SUN at 9 mo of age was associated with BMI at 10 y of age. BMI was used as a measure of adiposity in some studies testing the protein-adiposity hypothesis, but BMI is not a good measure of fatness in a population of children with a normal distribution of weight, especially a population of boys (25). BMI also reflects growth and pubertal development because BMI increases with age in prepubertal chil-

**TABLE 6**

Effect estimates for multiple linear regressions between protein intake variables [serum urea nitrogen (SUN), percentage of energy from protein in the diet, protein intake per kilogram body weight, and total daily protein intake] at 9 mo of age and body size (weight and height) at 10 y of age after adjustment for sex

<table>
<thead>
<tr>
<th>Variable at 9 mo of age</th>
<th>Variable at 10 y of age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%BF (%)</td>
</tr>
<tr>
<td>SUN (mmol/L)</td>
<td>0.44 (−0.093, 0.98)</td>
</tr>
<tr>
<td>Protein (% of energy)</td>
<td>0.13 (−0.13, 0.39)</td>
</tr>
<tr>
<td>Protein intake</td>
<td></td>
</tr>
<tr>
<td>(g · kg body wt⁻¹ · d⁻¹)</td>
<td>0.22 (−0.79, 1.2)</td>
</tr>
<tr>
<td>(g/d)</td>
<td>0.045 (−0.056, 0.15)</td>
</tr>
</tbody>
</table>

¹ ²P < 0.005.
² ²P < 0.01.
³ ²P < 0.001.
⁴ ²P = 0.012.
children. Thus, the finding of Scaglioni et al (13) that children with a BMI above the 90th percentile at 5 y of age have a significantly higher protein intake at 1 y of age than do children with lower BMI values at 5 y of age could in part be explained by an effect of early protein intake on growth.

SUN was associated with reported protein intake at both 9 mo and 10 y of age, which was expected because SUN is regarded as a valuable biomarker of recent protein intake (26, 27). The association was much stronger at the age of 9 mo, when almost 60% of the variation in protein intake could be explained by SUN, and this result may be due to the larger range in protein intake in infancy. Whether SUN is a better proxy for true protein intake than is percentage of energy from protein calculated from food records is not known.

Protein intake and insulin-like growth factor I

Rolland-Cachera et al (7) have suggested that the association that they found between a high protein intake early in life and adiposity later in childhood is mediated through an increased IGF-I concentration. There is some evidence suggesting that protein restriction results in decreased IGF-I concentrations (28), and in malnourished children with low IGF-I concentrations, IGF-I concentrations increase quickly and markedly during re-habilitation (29). Animal studies suggest that an excessive protein intake could result in increased IGF-I concentrations (30, 31), but data on children and infants are lacking. Some studies suggest that consumption of milk stimulates circulating IGF-I concentrations. Cadogan et al (32) found that supplementation with 1 pt (0.47 L) of milk for 18 mo stimulated IGF-I concentrations in 12-y-old girls. Two observational studies in adults showed a significant positive association between milk intake and IGF-I concentrations (33, 34).

In our study, IGF-I concentrations were associated with protein intake and SUN concentrations at 9 mo of age. Thus, our results suggest that a high protein intake increases IGF-I concentrations in healthy infants who have no signs of malnutrition and whose protein intakes far exceed requirements. At 10 y of age, IGF-I was associated only with SUN concentrations, but not with protein intakes. In the present study, there was no association between milk intake and IGF-I (data not shown).

Protein intake

The median daily protein intake at 9 mo of age was 2.7 g/kg body wt, which was considerably above the requirement of 1.1 g/kg body wt for infants aged 6–12 mo (35, 36). The 90th percentile value was 3.8 g/kg body wt. Some of the infants included in this analysis had their diet assessed at 12 mo also (16). In those infants, protein intake increased further: the median intake was 5.2 g/kg body wt, and the 90th percentile value was 4.7 g/kg body wt. The very high protein intake of some infants in late infancy is also seen in other countries, and protein intake was especially high in a study of Italian infants, whose mean intake was 5.1 g/kg body wt (1).

Protein intake as a percentage of energy at 9 mo of age was positively correlated with that at 10 y of age; this correlation is referred to as “tracking.” This tracking is most likely caused by the dietary habits of the family; for example, some families may have a habitually high protein intake because of high intakes of meat and milk. However, it could also be caused by children’s food preferences that are persistent throughout childhood. Tracking of nutrient intake between infancy and childhood has also been observed in other studies (37, 38).

Protein intake and growth

Protein intake as a percentage of energy was positively associated with length or height at both 9 mo and 10 y of age. The stronger association was seen at 9 mo of age. Furthermore, we found that the protein intake variables (SUN, percentage of energy from protein, and total daily protein intake) at 9 mo of age were associated with body size (height and weight) at 10 y of age, but this association disappeared after adjustment for body size at 9 mo of age. Thus, it is not possible to determine from our data whether a high protein intake during infancy has a long-term programming effect on growth or whether the association we see between 9 mo and 10 y of age is caused by a stimulatory effect of a persistently high protein intake. If there is an early programming effect, it could be through programming of the IGF-I axis. In a study by Fall et al (39), IGF-I concentrations were, as expected, higher in tall and heavy children than in short and light ones, but when sex and current body size were controlled for, children with a low birth weight had higher IGF-I concentrations than did those with a high birth weight. The authors interpret this as a reprogramming of the IGF-I axis that is related to catch-up growth in low-birth-weight infants.

Some other studies suggest that a high protein intake during infancy stimulates growth. In another study of 339 Danish infants aged 5–10 mo, we found that weight gain was positively associated with protein intake as a percentage of energy (6). There was an overall effect of protein intake, but a post hoc analysis showed that the effect was mainly due to a higher weight gain among the infants with the highest protein intake (≥16% of energy) than in those with lower protein intakes. Axelsson et al (5) suggested that a high protein intake stimulates insulin secretion, which in turn stimulates weight gain. They found higher values of C-peptide, a metabolite of insulin production, in infants with a high protein intake than in those with lower protein intakes and a positive association between C-peptide concentrations and weight gain (5).

Our data suggest that a high protein intake stimulates growth. This is supported by the results of a few other studies, but the data are inconclusive because most of the studies are observational. If there is an effect of protein intake on growth, it cannot be due to protein deficiency, because the 10th percentile of protein intake for the boys and the girls combined was 1.7 g·kg⁻¹·d⁻¹. High intakes of certain amino acids could modulate the hormonal regulation of growth. Some studies have suggested that cow milk stimulates growth and that an effect of protein could be caused by milk. If this is the case, the mechanism could be an effect of the bioactive factors in cow milk rather than of the amount of protein.

The long-term implications of increased growth caused by a high protein intake are unknown. A high growth velocity has traditionally been regarded as beneficial, and some studies have found that high adult stature is associated with low overall mortality (40, 41) and reduced risks of certain noncommunicable diseases (42–44). However, other studies have suggested that a high growth velocity during certain periods of childhood might result in an increased risk of other noncommunicable diseases, especially certain forms of cancer (45–48).
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KFM established the cohort and planned the study. CH conducted the statistical analyses in collaboration with BLT and prepared the first draft of the manuscript in collaboration with CM and KFM. AJ was responsible for statistical analyses in collaboration with BLT and prepared the first draft of the manuscript in collaboration with CM and KFM. AJ was responsible for statistical analyses in collaboration with BLT and prepared the first draft of the manuscript. No author had a financial or personal conflict of interest related to this research or its source of funding.

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