Animal protein intake, serum insulin-like growth factor I, and growth in healthy 2.5-y-old Danish children

Camilla Hoppe, Tina Rovenna Udam, Lotte Lauritzen, Christian Mølgaard, Anders Juul, and Kim Fleischer Michaelsen

ABSTRACT

Background: Studies from developing countries indicate that intake of animal protein, especially of milk, is associated with greater velocity of linear growth in childhood. Whether the same association exists in industrialized countries, where protein intake is high, is not clear.

Objective: Our objective was to examine associations between protein intake, serum insulin-like growth factor I (sIGF-I) concentrations, and height in healthy children.

Design: We analyzed the associations between protein intake, sIGF-I concentrations, and height in 2.5-y-old children. Diet (7-d record) and sIGF-I (radioimmunoassay) data were available from 90 children (54 boys).

Results: The 10th, 50th, and 90th percentiles of protein intake were 2.4, 2.9, and 4.0 g·kg⁻¹·d⁻¹, respectively; 63% was animal protein. In multiple linear regressions with adjustment for sex and weight, height (cm) was positively associated with intakes of animal protein (g/d) [0.10 ± 0.038 (b ± SE); P = 0.01] and milk (0.0047 ± 0.002; P = 0.007), but not with those of vegetable protein or meat. The sIGF-I concentration was significantly associated with intakes of animal protein (1.4 ± 0.53; P = 0.01) and milk (0.049 ± 0.024; P = 0.045), but not with those of vegetable protein or meat. sIGF-I concentrations were positively associated with height (0.019 ± 0.008; P = 0.02).

Conclusion: Milk intake was positively associated with sIGF-I concentrations and height. An increase in milk intake from 200 to 600 mL/d corresponded to a 30% increase in circulating IGF-I. This suggests that milk compounds have a stimulating effect on sIGF-I and, thereby, on growth.


KEY WORDS Dietary protein, animal protein, vegetable protein, children, insulin-like growth factor I, milk, meat, growth

INTRODUCTION

Studies from developing countries indicate that the intake of animal protein, especially that of milk, is associated with a greater velocity of linear growth in childhood (1). Whether the same association exists in industrialized countries, where intakes of both total and animal protein typically are high, is not clear.

A protein intake below physiologic needs results in less growth. Some studies suggest that protein intake and protein quality also have a regulatory effect on growth at intakes above the requirements. Formula-fed infants have greater growth velocities than do infants who are being breastfed (2–4). It has been suggested that this could be due to the higher protein content in formula than in breast milk (3). However, there are many differences between breastfed and formula-fed infants besides protein intake that could explain the differences in growth. Insulin-like growth factor I (IGF-I) plays a central role in the regulation of growth. This fact is supported by many studies showing a strong association between IGF-I and body size during infancy and childhood (5), and IGF-I may mediate the positive association between protein intake and growth. Dietary depletion has a marked, negative effect on IGF-I concentrations in adults (6–8). In malnourished children with low concentrations of IGF-I, there is a quick and marked increase in IGF-I during rehabilitation (9). Evidence suggests that protein restriction results in low IGF-I concentrations in healthy children (10). Data on the relation between protein intake, protein quality and circulating IGF-I in healthy, well-nourished children are lacking. However, a short-term intervention study with prepubertal children suggested that milk intake stimulated IGF-I secretion (11). The aim of this cross-sectional study was to examine the association between protein intake, serum IGF-I (sIGF-I), and height in healthy 2.5-y-old Danish children.

SUBJECTS AND METHODS

Subjects

This cross-sectional study consists of data from a follow-up examination of 2.5-y-old children from an intervention study comparing the effect of fish oil or olive oil supplementation of lactating Danish mothers with a habitual fish intake below the 50th percentile. The selection of the participants has been described elsewhere (12). In brief, participants were selected among women recruited for the Danish National Birth Cohort (13). Nine hundred nineteen pregnant women with a fish intake below the 50th percentile or above the 74th percentile in their 8th

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month of gestation were invited to participate in the trial. Two hundred seventy-three women agreed to participate, and 211 met the other inclusion criteria: the women were required to have an uncomplicated pregnancy, prepregnancy body mass index (BMI; in kg/m²) < 30, no metabolic disorders, and an intention to breastfeed for ≥4 mo. Furthermore, to participate in the study, the newborns had to be healthy (no admission to a neonatal department), term, singleton infants with normal weight for gestation and an Apgar score > 7 at 5 min after delivery. One hundred fifty families completed the initial 4-mo period of the intervention study.

Follow-up study

When the children were 2.5 y old, all 150 families were invited to participate in the follow-up study, which was carried out from November 2001 to September 2002 at the Department of Human Nutrition, Frederiksberg, Denmark. Of the 150 families who completed the initial 4-mo intervention study, 11 could not be located, 6 had moved far away, 15 did not wish to participate in the follow-up for various personal reasons, and 13 did not give any reason for the lack of participation. One hundred five families agreed to participate in the follow-up study. Four of these 105 families did not complete the study because the children were uncooperative during the examination; thus, 101 children participated in this study, from 90 of whom blood samples were available. At the time of the follow-up study, the children were healthy: ie, they were not taking any medication continuously, and they did not have any chronic disease.

The protocols for the intervention trial and follow-up study were approved by the Scientific and Ethical Committee of Copenhagen and Frederiksberg (KF 01–300/98 and KF 01–183/01). Both parents of all participating children gave written informed consent to their children’s participation.

Diet

The description of the recording of the dietary intakes of the children was provided elsewhere (12). Briefly, the diet of the children was recorded by the parents for 7 consecutive days with the use of a coded dietary questionnaire, which we adapted from the questionnaire used in the Danish National Food Surveys (14). The amount of food was given in household measures or standard portion sizes estimated from a collection of pictures. All data from the questionnaires were entered into a computer program that contained standard recipes for all coded dishes and standard serving sizes for 296 food types that were used to convert household measures into grams. The average daily nutrient intakes of each child were calculated from the resulting diet with the use of a computerized nutrient database, GIES (version 0.9, Danish Veterinary and Food Administration, Søborg, Denmark).

Blood

Blood samples were drawn by venipuncture after an average fast of 3 h 23 min (SD: 1 h 9 min; range: 1 h 15 min–7 h 30 min). The sIGF-I concentration was ascertained with the use of a radioimmunoassay as previously described (15). Briefly, serum was extracted by using acid-ethanol and was cryoprecipitated before analysis to remove interfering IGF binding proteins. Interassay and intraassay variations were <9% and <6%, respectively. Details regarding measurement of the IGF-I concentration were presented previously (16).

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Girls (n = 36)</th>
<th>Boys (n = 54)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (MJ/d)</td>
<td>5.98 ± 1.36</td>
<td>6.14 ± 1.10</td>
</tr>
<tr>
<td>Protein (% of energy)</td>
<td>12.4 ± 1.43</td>
<td>11.6 ± 1.48</td>
</tr>
<tr>
<td>Protein (g·kg⁻¹·d⁻¹)</td>
<td>3.07 ± 0.66</td>
<td>3.06 ± 0.68</td>
</tr>
<tr>
<td>Total protein (g/d)</td>
<td>43.5 ± 10.8</td>
<td>41.8 ± 8.34</td>
</tr>
<tr>
<td>Animal protein (g/d)</td>
<td>28.4 ± 7.72</td>
<td>26.15 ± 6.36</td>
</tr>
<tr>
<td>Vegetable protein (g/d)</td>
<td>13.9 ± 4.13</td>
<td>14.54 ± 4.15</td>
</tr>
<tr>
<td>Milk (g/d)</td>
<td>410 ± 179</td>
<td>369 ± 130.2</td>
</tr>
<tr>
<td>Meat (g/d)</td>
<td>33.7 ± 16.3</td>
<td>39.4 ± 25.9</td>
</tr>
</tbody>
</table>

1 All values are $\bar{x} \pm$ SD.
2 Significantly different from girls, $P = 0.035$ (two-tailed Student’s $t$ test).

### Anthropometric measurements

Height was measured in barefoot children to 0.1 cm accuracy with the use of a stadiometer (model 28. P.4, CMS Weighing Equipment Ltd, London). Body weight was measured to 0.1 kg accuracy with the use of a digital scale (Lindeltronic 8000; Samhall Lavi AB, Kristianstad, Sweden). Most of the measurements were made by one observer, and the rest were made by 1 of 2 well-trained stand-ins.

### Statistical analysis

Statistical analyses were performed with the use of SPSS software (version 11.0; SPSS Inc, Chicago). All descriptive results are expressed as means ± SDs. Differences in the variables between boys and girls were ascertained by two-tailed Student’s $t$ tests. To examine the bivariate relations between the variables, Pearson’s correlation coefficients were calculated. The dependence of IGF-I and height on intakes of animal protein, vegetable protein, milk, and meat and the dependence of height on the IGF-I concentration were analyzed by multiple linear regressions after control for sex and body weight. There was no interaction between sex and the independent variables in any of the models.

### RESULTS

The mean energy intake was 6.08 MJ/d (range: 3.8–9.8 MJ; Table 1). The 10th, 50th, and 90th percentiles of protein intake were 2.4, 2.9, and 4.0 g·kg⁻¹·d⁻¹, respectively; 63% of the protein was animal protein. The percentage of energy as protein in the diet was higher in girls than in boys ($P = 0.035$). There were no other significant differences between the boys and girls regarding dietary variables. Anthropometric variables of the children, as well as serum IGF-I concentrations, are shown in Table 2. There were no significant anthropometric differences between boys and girls. Mean values for sIGF-I were 86 and 132 ng/mL for boys and girls, respectively ($P < 0.0001$).

The 90 children with full follow-up data were similar to the children with no follow-up data with respect to gestational age, weight, and length at birth (data not shown). There were no differences between the original intervention groups (fish oil or olive oil) regarding protein intake, IGF-I concentrations, height, or weight at age 2.5 y.

Bivariate relations are presented as Pearson’s correlation coefficients in Table 3. Total protein intake was positively correlated with IGF-I concentration, height, and weight. Total protein...
was divided into animal and vegetable protein. Height was positively associated with the intakes of animal protein and milk, but not with those of vegetable protein or meat. The sIGF-I concentration was positively associated with the intakes of animal protein and milk, but not with those of vegetable protein or meat. Weight was also positively correlated with total protein intake. However, the association with animal protein was not significant ($P = 0.120$), whereas that with vegetable protein was significant.

We also performed multiple linear regression analyses of the associations between intakes of animal protein, vegetable protein, milk, and meat and between sIGF-I concentrations and height (Table 4). Sex and body weight were included in these models because of the significant difference in IGF-I concentration and protein intake between boys and girls and because height and weight are highly correlated. Because we used the absolute dietary intake in our analysis, body weight was also included in the analysis to control for the influence of body size on dietary intake. The results were not altered considerably by control for the time since the previous meal, body size at birth (weight and length), or mean parental height. Height (cm) as a function of IGF-I (ng/mL) is shown in Figure 1, and height (cm) as a function of milk intake (g/d) is shown in Figure 2.

### Table 3

Pearson’s correlation coefficients between intakes of energy, protein, milk, and meat and between serum insulin-like growth factor I (sIGF-I), height, and weight in 2.5-y-old children

<table>
<thead>
<tr>
<th>Protein intake (%) of energy</th>
<th>sIGF-I (ng/mL)</th>
<th>P</th>
<th>Height (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (%)</td>
<td>−0.129</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (g/d)</td>
<td>0.837</td>
<td>0.427</td>
<td></td>
</tr>
<tr>
<td>Animal (g/d)</td>
<td>0.312</td>
<td>0.630</td>
<td>0.909</td>
</tr>
<tr>
<td>Vegetable (g/d)</td>
<td>0.835</td>
<td>−0.053</td>
<td>0.730</td>
</tr>
<tr>
<td>Milk (g/d)</td>
<td>0.467</td>
<td>0.484</td>
<td>0.695</td>
</tr>
<tr>
<td>Meat (g/d)</td>
<td>0.277</td>
<td>0.049</td>
<td>0.279</td>
</tr>
<tr>
<td>sIGF-I (ng/mL)</td>
<td>0.054</td>
<td>0.281</td>
<td>0.230</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>0.288</td>
<td>0.057</td>
<td>0.297</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.316</td>
<td>−0.087</td>
<td>0.248</td>
</tr>
</tbody>
</table>

1 $n = 90$. 2 $P < 0.0001$. 3 $P < 0.01$. 4 $P < 0.05$. 5 $P < 0.001$. 6 Mean of group.

### Table 4

Effect estimates for multiple linear regressions between dietary variables (animal protein, vegetable protein, milk, and meat) with serum insulin-like growth factor I (sIGF-I) and height

<table>
<thead>
<tr>
<th>Energy intake (MJ/d)</th>
<th>sIGF-I (ng/mL)</th>
<th>P</th>
<th>Height (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein intake (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animal protein (g/d)</td>
<td>1.4 ± 0.53</td>
<td>0.013</td>
<td>0.10 ± 0.038</td>
</tr>
<tr>
<td>Vegetable protein (g/d)</td>
<td>0.12 ± 1.00</td>
<td>0.909</td>
<td>−0.021 ± 0.069</td>
</tr>
<tr>
<td>Milk (g/d)</td>
<td>0.049 ± 0.024</td>
<td>0.045</td>
<td>0.0047 ± 0.002</td>
</tr>
<tr>
<td>Meat (g/d)</td>
<td>0.15 ± 0.17</td>
<td>0.368</td>
<td>−0.012 ± 0.012</td>
</tr>
</tbody>
</table>

1 $n = 90$. All analyses were performed with adjustment for sex and body weight. 2 Values are $b ± SE$. 3 $P < 0.001$. 4 $P < 0.01$. 5 $P < 0.05$. 6 $P < 0.0001$. 7 $P < 0.001$. 8 Mean of group.

### DISCUSSION

In this cross-sectional study of well-nourished, healthy 2.5-y-old children, circulating IGF-I was positively associated with intakes of animal protein and milk, but not with the intake of vegetable protein or meat. We also found that height was associated both with IGF-I and with intake of milk. This may suggest that milk contains some growth-stimulating components, and that these components stimulate IGF-I.

However, this study has some limitations. The main limitation is that we cannot establish causality between the observed associations, because the study is cross-sectional. However, in this group of well-nourished and healthy children, the range of dietary intakes and that of circulating IGF-I are wide. We therefore believe that the study can contribute to the exploration of dietary regulation of IGF-I and growth, although the observed milk intake could be a marker of some other factor in the diet or dietary pattern.

Studies in populations with marginal or poor nutritional status have shown associations between milk intake and growth. In 12–36-mo-old children from 5 Latin American countries, milk intake was significantly associated with higher height-for-age $z$ scores after control for potential confounding factors of the child.
mother, and household (1). Interventions with milk have led to
greater velocity of linear growth in schoolchildren from New
Guinea than did consumption of margarine or extra taro and
sweet potato (17, 18), and American teenagers with chronic
nutritive failure had greater velocity of linear growth after 20 mo
of supplementation with whole milk or nonfat milk than after no
supplementation (19, 20). In the 1920s, Scottish schoolchildren
aged 5–14 y were randomly assigned for 1 yr to intervention with
either whole milk, skimmed milk, biscuits with energy content
similar to that of skimmed milk, or no supplement. The 2 milk
groups had a greater increase in height than did the other 2 groups
(21, 22).

In well-nourished populations, observational studies have also
shown positive associations between protein intake and growth.
In 105 healthy Danish children, we saw a significant positive
association between protein intake at age 9 mo and height at age
10 y (23).

Furthermore, it seems that children who do not drink milk are
shorter than those who do. Black et al (24) observed that 50
children aged 3–10 y from New Zealand who avoided drinking
cow milk were shorter than were similar children who drank
milk. In addition, children with lactose intolerance (25) and with
milk allergy (26, 27) are shorter than the population average.

A positive association between milk intake and circulating
IGF-I has been found in adults in some studies (28, 29) but not in
all (30). And in vegan women, the IGF-I concentration was 13%
lower than that in meat-eaters and vegetarians (31). These data
suggest that a plant-based diet without milk is associated with
lower circulating concentrations of total IGF-I.

When they randomly assigned 83 well-nourished 12-y-old
girls to consume 1 pint milk/d or no milk for 18 mo, Cadogan et
al (32) found that milk stimulated IGF-I concentrations. How-
ever, the increase in IGF-I was not accompanied by a significant
increase in height: only 6% in the intervention group and 5% in
the control group. In an intervention study with 8-y-old boys who
consumed the same amount of animal protein for 1 wk—12 boys
consuming low-fat milk and 12 consuming meat—we found that
IGF-I concentrations increased in the milk group but not in the
meat group (11).

The positive association between milk and height suggests a
growth-stimulating effect of milk, which might, at least in part,
involve the stimulation of IGF-I. However, further studies are
needed to explore the mechanisms.

Increase in linear growth is likely to have both positive and
negative effects. Height in infancy and childhood is negatively
associated with risk of coronary heart disease (33) and high
cholesterol (34) but positively associated with cancer risk (35)
and blood pressure (34). However, the role of early diet in these
associations is not known. Several epidemiologic studies found
associations between IGF-I concentrations and some noncom-
municable diseases. In adults, low concentrations of IGF-I may
be associated with increased rates of cardiovascular disease (36),
whereas high concentrations of IGF-I are associated with an
increased risk of hormonal cancers such as breast and prostate
cancer and with colorectal cancer (37–40). The association be-
tween milk intake and cancer also is not simple. In one study, a
high intake of milk was associated with a lower risk of colorectal
cancer but a higher risk of prostate cancer (41).
In summary, milk intake was positively associated with circulating IGF-I in healthy, well-nourished 2.5-y-old children. This association suggests that an increase in milk intake from 200 to 600 mL/d would result in a 30% increase in circulating IGF-I.

These data suggest that milk has a stimulating effect on the sIGF-I concentration, and that it is components in milk rather than the intake of animal protein or of total protein per se that influences IGF-I concentrations. Further research is needed to understand the regulatory effect of nutrition on IGF-I and thereby on growth. The growth-stimulating effect of milk seems to take place also in well-nourished populations, where nutritional deficiencies are not likely.

We are grateful to M Ege and H Mathiesen for valuable assistance in the data collection. LL and KFM were responsible for the intervention study idea and design. TRU was responsible for data collection and management. CH conducted the statistical analyses in collaboration with KFM, CM, and LL and prepared the first draft of the manuscript. AJ was responsible for all IGF-I measurements. All contributors participated in interpreting the results and were involved in preparing the final draft of the manuscript. None of the authors had any financial or personal conflicts of interest.

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