Long-term protein intake and dietary potential renal acid load are associated with bone modeling and remodeling at the proximal radius in healthy children

Ute Alexy, Thomas Remer, Friedrich Manz, Christina M Neu, and Eckhard Schoenau

ABSTRACT

Background: Protein and alkalizing minerals are increasingly described as playing a major role in influencing bone status, not only in the elderly but also in children and adolescents.

Objective: We examined whether the long-term dietary protein intake and diet net acid load are associated with bone status in children.

Design: In a prospective study design in 229 healthy children and adolescents aged 6–18 y, long-term dietary intakes were calculated from 3-d weighed dietary records that were collected yearly over the 4-y period before a one-time bone analysis. Dietary acid load was characterized as potential renal acid load (PRAL) by using an algorithm including dietary protein, phosphorus, magnesium, and potassium. Proximal forearm bone variables were measured by peripheral quantitative computed tomography.

Results: After adjustment for age, sex, and energy intake and control for forearm muscularity, BMI, growth velocity, and pubertal development, we observed that long-term dietary protein intake was significantly positively associated with periosteal circumference ($P < 0.01$), which reflected bone modeling, and with cortical area ($P < 0.01$), bone mineral content ($P < 0.01$), and polar strength strain ($P < 0.01$). We examined the association of long-term protein intake and dietary potential renal acid load (PRAL) with diaphyseal radial bone in a sample of healthy children and adolescents with the use of peripheral quantitative computed tomography (pQCT).

KEY WORDS Children, bone health, modeling, remodeling, peripheral quantitative computed tomography, dietary protein, potential renal acid load

INTRODUCTION

Apart from genetics and hormonal influences, factors associated with lifestyle—such as muscularity (1), obesity (2), and diet (3)—also affect variables of bone mass and bone dimension. In children, the assessment of the effects of dietary factors on bone accretion has primarily focused on the quantity of calcium required for optimal bone accrual because the skeleton matures at a relatively early age (4). In females, for example, about 90% of total bone mineral content is attained by age 17 y (5). However, the calcium and mineral contents of the skeleton appear to be markedly influenced by nutrients other than calcium, specifically protein (6–8) and alkalizing minerals (9, 10), which are increasingly described as playing a major role.

Clinical studies have provided convincing evidence that protein supplements can have substantial positive effects on bone health in the elderly (11, 12). However, the findings of larger epidemiologic studies are less clear. Evidence for both a negative and a positive effect of protein on bone health exists. An overview of this topic was given by Ginty (8).

We examined the association of long-term protein intake and dietary potential renal acid load (PRAL) with diaphyseal radial bone in a sample of healthy children and adolescents with the use of peripheral quantitative computed tomography (pQCT).

SUBJECTS AND METHODS

Subjects and study design

The study population comprised a subgroup of white children and adolescents participating in the DONALD (Dortmund Nutritional and Anthropometric Longitudinally Designed) Study, a long-term (open cohort) study that collects detailed data on diet and growth in healthy subjects from infancy to adulthood. The subjects were medically examined at regular yearly intervals with concomitant collection of anthropometric data and 3-d weighed dietary records (13).

As a spinoff project, a single pQCT analysis of the forearm was undertaken in 1998–1999 in 371 DONALD participants aged 6–18 y (14, 15). For the present study, we selected 229 (115 boys, 114 girls) of these participants who had ≥4 of the possible 5 three-day weighed dietary records (4 records: $n = 63$ subjects; 5 records: $n = 166$ subjects) and valid reported energy intakes during the 4 y preceding bone analysis.

Ethical permission was obtained from the institutional review board of the Research Institute of Child Nutrition in Dortmund, Germany.

the ethics committee of the medical faculty of the University of Cologne, and the Federal Office for Radiation Protection (Salzgitter, Germany). Parental informed consent and child’s assent were obtained before entry into the study.

pQCT of forearm bone and forearm muscle area

An XCT-2000 device (Stratec Inc, Pforzheim, Germany) equipped with a low-energy (38 keV) X-ray tube was used to conduct the pQCT analysis (14–16) on the nondominant forearm. The effective radiation was ≈0.1 μSv from a radiation source of 45 kV at 15 μA. The scanner was placed on the forearm, where the distance from the ulnar styloid process was 65% of the forearm length. A 2-mm thick single tomographic slice was sampled at a voxel size of 0.4 mm. The speed of the translational scan movement was 15 mm/s. The resulting time for a measurement run was ≈2–3 min in the younger and 4–5 min in the older subjects, depending on the cross-sectional size of the forearm. Image processing and calculation of numerical values were performed by using the manufacturer’s software package (version 5.40; Statex Inc, Paris, France). The cross-sectional area of cortical bone was determined by detecting the outer and inner cortical bone contour at a threshold of 710 mg/cm³. Periosteal circumference was determined under the assumption that the bone is cylindrical, whereby the outer bone radius was calculated as follows:

\[
\text{Outer bone radius} = \left( \frac{\text{total area}}{\pi} \right)^{0.5}
\]

Volumetric cortical density and bone mineral content were also determined at a threshold of 710 mg/cm³. Cortical density represents the mass of mineral (in mg) per unit volume (in cm³) of the radial cortex slice, and bone mineral content was defined as the mass of mineral per unit of axial bone length (in mm). To assess the bone strength strain index, a threshold of 480 mg/cm³ was used. This lower threshold accounted for the fact that, in the analysis of strength strain index, the individual density reading of each voxel was used for calculation (16). The strength strain index was calculated as the product of section modulus and cortical density normalized to the maximal physiologic cortical density of human bones and is an indication of bone stability (16).

These measurements allowed the evaluation of bone modeling and remodeling, which are 2 mechanisms used to construct and reconstruct the skeleton (17). Modeling characterizes the expansion process of the bone’s cross-section assessed by determination of the periosteal circumference. In a cross-sectional study design, higher periosteal circumference values indicate that more modeling, ie, more skeletal construction has taken place in the respective subjects. Remodeling indicates the changes in cortical density or cortical porosity, ie, reconstruction. Bone mineral content, cortical area, and polar strength strain index reflect a combination of modeling and remodeling. Cross-sectional forearm muscle area was also determined with the XCT-2000 device at 65% of the ulnar length as previously described (15, 16, 18).

Anthropometric measurements and Tanner stages

Body weight was measured with an electronic scale to the nearest 0.1 kg and standing height to the nearest 0.1 cm with a digital telescopic wall-mounted stadiometer. From these measurements, body mass index [BMI; weight (kg)/height² (m)] was calculated and converted into SD scores of BMI (SDS-BMI) by using Cole’s LMS method (19), which allows the assessment of individual BMI in relation to a reference population. The recent data derived from 17 regional German surveys published by Kromeyer-Hauschild et al (20) were used as a reference.

Growth velocity (GV) was calculated in 2 ways: 1) as the 4-y GV taken from height measurements over the 4 y before pQCT and 2) as the mean GV at pQCT measurement, averaged from GV 1 y before and 1 y after pQCT.

Tanner stage 1-5 of the study participants were determined by a pediatrician. On the basis of pubic hair as a clinical marker of the beginning of adrenal androgen secretion (18), the subjects were assigned to 2 groups: without (prepubescent) and with (pubescent) pubic hair. The age of menarche in girls and of voice change in boys were reported by the children via standardized questioning.

Dietary survey

The parents of the children or of the older subjects themselves weighed and recorded all foods and fluids consumed, ingredients of home-prepared meals, as well as leftovers using electronic food scales (± 1 g) on 3 consecutive days. Semiquantitative recording (eg, number of spoons, scoops) was allowed if weighing was not possible. However, in 75% of the completed records, >90% of the food items were weighed (21).

Energy and nutrient intakes, including food fortification and nutrition supplements, were calculated as individual means on the recorded days by using our nutrient database LEBTAB (22).

To check for the validity of the dietary measurements, we used the reported energy intake as a surrogate measure of the general quality of the dietary data (23). For this, according to Goldberg et al (24), the ratio of reported energy intake and predicted basal metabolic rate were used. Basal metabolic rate was calculated by using the equations of Schofield (25), which included the measured height and weight of the individuals. For identification of implausible records, which were excluded from further analysis, sex- and age-specific cutoffs were used, considering CVs for energy intake and physical activity levels of light physical activity (26).

Dietary PRAL: estimation method and background

The dietary component (PRAL) of net endogenous acid production was estimated according to Remer et al (27, 28):

\[
\text{PRAL} = 0.4888 \times \text{protein (g/d)} + 0.0366 \times \text{phosphorus (mg/d)} - 0.0205 \times \text{potassium (mg/d)} - 0.0263 \times \text{magnesium (mg/d)}
\]

The PRAL model has been validated in dietary experiments (29, 30) and proved to be highly significantly correlated with analyzed urinary net acid excretion (NAE) (28). PRAL does not include an estimate of organic acid (OA) anion excretion, which, according to our own results (30) and those of others (31, 32), is largely independent of dietary acid load or macronutrient composition (33). However, an effect of base-forming foods on OA excretion is under discussion (34, 35). The findings of Kleinman and Lemann (36) suggest that diet alkali load may increase OA anion excretion. Because it has been shown that the measured urinary OA excretion of children, adolescents (28), and adults (30) can be reasonably estimated from body surface area, we calculated the PRAL and not an estimate of the NAE.
The variable residual is uncorrelated with total energy intake or energy intakes or age. For nutrient intakes, this was done for each bone variables) of the individuals were regressed on their total residuals.

Calcium (mg/d), and PRAL (mEq/d) were adjusted for age, sex, eggs, and grain.

Statistical analysis

For final analysis of long-term dietary intakes on measures of pQCT, stepwise multiple regression was used. Apart from dietary protein, calcium, and PRAL significantly correlated with each other (P < 0.0001, Pearson’s correlation coefficient)—even after adjustment for age, sex, and energy intake—collinearity diagnostics according to Belsley, Kuh, and Welsch (38) were performed. No evidence of collinearity between any of the dietary factors (expressed as residuals) could be detected. Therefore, the residuals were included in subsequent multivariate analysis. Each of these residuals was the arithmetic mean of the 4 or 5 individual residuals obtained from the yearly diet records, thus reflecting long-term dietary intakes.

Preliminary analysis of covariance (ANCOVA) was used to test for interactions between diet and sex or developmental group. Because diet-by-sex and diet-by—developmental group interactions exclusively were nonsignificant (P > 0.1) for all bone variables studied, the subsequent analyses were performed with the total sample of 229 subjects.

For final analysis of long-term dietary intakes on measures of pQCT, stepwise multiple regression was used. Apart from dietary protein, PRAL, and calcium, muscle area, BMI, GV, menarche (voice change), and Tanner stages (calculated as dummy variables) were also included in the model as potential confounders. Regression analyses were run separately with GV at pQCT stages).

RESULTS

Mean (±SD) values for bone measures and for all continuous independent variables and potential confounders used in these analyses are presented in Tables 1 and 2. The study sample was almost equally divided into subgroups of males and females and likewise for the developmental stages of prepubescence and pubescence.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Prepubescent</th>
<th>Pubescent</th>
<th>P²</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Boys (n = 67)</td>
<td>Girls (n = 57)</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>8.8 ± 2.0</td>
<td>8.3 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>Age at voice change or menarche (y)</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>31.7 ± 10.2</td>
<td>29.1 ± 8.2</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>136 ± 13</td>
<td>132 ± 11</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>16.7 ± 2.7</td>
<td>16.4 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>Growth velocity at pQCT (cm/y)</td>
<td>5.9 ± 0.9⁹</td>
<td>6.0 ± 0.7⁷</td>
<td>5.9 ± 2.6⁹</td>
</tr>
<tr>
<td>Muscle area (mm³)</td>
<td>1973 ± 37⁹</td>
<td>1760 ± 32⁶</td>
<td>3150 ± 71⁸</td>
</tr>
<tr>
<td>Periosteal circumference (mm)</td>
<td>32.6 ± 3.4⁴</td>
<td>31.8 ± 2.9⁹</td>
<td>38.6 ± 2.9⁸</td>
</tr>
<tr>
<td>Cortical density (mg/cm³)</td>
<td>1008 ± 45⁴</td>
<td>986 ± 57⁷</td>
<td>1034 ± 48⁶</td>
</tr>
<tr>
<td>Cortical area (mm²)</td>
<td>45.2 ± 9.8</td>
<td>39.6 ± 11.4</td>
<td>68.2 ± 16.8</td>
</tr>
<tr>
<td>Bone mineral content (mg/mm)</td>
<td>45.8 ± 11.1</td>
<td>39.6 ± 12.9</td>
<td>71.1 ± 20.1</td>
</tr>
<tr>
<td>Polar bone strength strain index (mm²)</td>
<td>147 ± 48</td>
<td>125 ± 41</td>
<td>253 ± 65</td>
</tr>
</tbody>
</table>

¹ All values are ± SD. pQCT, peripheral quantitative computed tomography. Values in the same row with different superscript letters are significantly different, P < 0.05.

² Two-way ANOVA was used to examine the main effects of developmental group and sex and the interaction between developmental group and sex. If the interaction term was P < 0.1, a subgroup analysis was done by using Tukey’s Studentized range (honestly significant difference) test.

³ In 26 boys and 28 girls, pQCT was performed after attainment of voice change and menarche, respectively.
The mean age at the time of the pQCT measurement was 11 y; 8 y in the prepubescent group and 13 y in the pubescent group. All anthropometric and bone characteristics were significantly different between the prepubescent and the pubescent groups (Table 1). Muscle area was 1.6-fold higher in pubescent than in prepubescent boys; the respective difference for girls was 1.5 fold. The bone variables increased from the prepubescent to the pubescent stage, showing a negative association. Of all the examined independent variables, only mean GV at pQCT showed a significant negative association with cortical density ($r^2 = 0.04$, $P = 0.0015$). However, GV at pQCT was not associated with any of the other bone variables, whereas 4-y GV showed significant positive associations with cortical area ($r^2 = 0.03$, $P = 0.0016$) and bone mineral content ($r^2 = 0.02$, $P = 0.0091$) (data not shown). The associations of the bone variables with protein intake and dietary PRAL remained unchanged when the regression analyses were run with 4-y GV instead of GV at pQCT. Calcium did not enter the model for any bone variable. Only sporadic associations with bone variables were seen for BMI and menarche or voice change (Table 3). Additionally, Tanner stages (especially Tanner stage 5) positively predict the already age-adjusted cortical area and bone mineral content.

Overall, protein and PRAL accounted for 3–6% and 2%, respectively, of the variation in bone indexes; muscle area accounted for 24–36% (Table 3). For each bone variable, the standardized regression coefficients were highest for muscle area (Table 3).

The associations between age-adjusted bone variables and long-term dietary protein intake (calculated as a percentage of energy intake) and PRAL (adjusted for protein intake, age, and sex) are shown in Figures 1 and 2. Subjects with a higher or a

### TABLE 2  
Long-term dietary intakes of the study population

<table>
<thead>
<tr>
<th></th>
<th>Prepubescent Boys ($n = 67$)</th>
<th>Prepubescent Girls ($n = 57$)</th>
<th>Pubescent Boys ($n = 48$)</th>
<th>Pubescent Girls ($n = 57$)</th>
<th>Developmental group Interaction</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (MJ/d)</td>
<td>6.5 ± 1.1$^{a}$</td>
<td>5.7 ± 0.7$^{b}$</td>
<td>8.8 ± 1.4$^{b}$</td>
<td>7.4 ± 1.0$^{b}$</td>
<td>&lt;0.0001</td>
<td>0.0442</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>48.5 ± 10.3$^{a}$</td>
<td>43.0 ± 7.5$^{b}$</td>
<td>68.8 ± 12.5$^{c}$</td>
<td>56.5 ± 10.7$^{d}$</td>
<td>&lt;0.0001</td>
<td>0.0133</td>
</tr>
<tr>
<td>(g/MJ)$^{d}$</td>
<td>7.5 ± 0.8</td>
<td>7.6 ± 0.9</td>
<td>7.9 ± 0.7</td>
<td>7.6 ± 1.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(g/kg)$^{d}$</td>
<td>2.0 ± 0.3$^{a}$</td>
<td>2.0 ± 0.4$^{a}$</td>
<td>1.6 ± 0.3$^{b}$</td>
<td>1.4 ± 0.3$^{b}$</td>
<td>&lt;0.0001</td>
<td>0.0372</td>
</tr>
<tr>
<td>Calcium (mg/d)</td>
<td>745 ± 182$^{a,c}$</td>
<td>667 ± 159$^{a}$</td>
<td>1056 ± 278$^{b}$</td>
<td>823 ± 239$^{b}$</td>
<td>&lt;0.0001</td>
<td>0.0075</td>
</tr>
<tr>
<td>(mg/MJ)$^{d}$</td>
<td>116 ± 22</td>
<td>119 ± 25</td>
<td>121 ± 26</td>
<td>111 ± 25</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Calcium/protein (mg/g)</td>
<td>15.5 ± 2.4</td>
<td>15.6 ± 2.5</td>
<td>15.5 ± 3.0</td>
<td>14.5 ± 2.6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Potassium (mg/d)</td>
<td>2102 ± 431$^{a}$</td>
<td>1848 ± 292$^{b}$</td>
<td>2884 ± 579$^{a}$</td>
<td>2419 ± 500$^{d}$</td>
<td>&lt;0.0001</td>
<td>0.0841</td>
</tr>
<tr>
<td>(mg/MJ)$^{d}$</td>
<td>328 ± 47</td>
<td>330 ± 46</td>
<td>331 ± 51</td>
<td>328 ± 49</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Magnesium (mg/d)</td>
<td>217 ± 46$^{a}$</td>
<td>197 ± 38$^{a}$</td>
<td>320 ± 72$^{b}$</td>
<td>267 ± 58$^{c}$</td>
<td>&lt;0.0001</td>
<td>0.028</td>
</tr>
<tr>
<td>(mg/MJ)$^{d}$</td>
<td>33.8 ± 5.2</td>
<td>35.0 ± 5.8</td>
<td>36.6 ± 2.7</td>
<td>36.2 ± 6.0</td>
<td>0.0082</td>
<td>—</td>
</tr>
<tr>
<td>Phosphorus (mg/d)</td>
<td>953 ± 206$^{a}$</td>
<td>847 ± 155$^{b}$</td>
<td>1362 ± 267$^{c}$</td>
<td>1099 ± 237$^{d}$</td>
<td>&lt;0.0001</td>
<td>0.0070</td>
</tr>
<tr>
<td>(mg/MJ)$^{d}$</td>
<td>148 ± 21</td>
<td>150 ± 20</td>
<td>156 ± 19</td>
<td>149 ± 24</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PRAL (mEq/L)</td>
<td>8.8 ± 6.7$^{a}$</td>
<td>8.1 ± 5.7$^{b}$</td>
<td>14.5 ± 8.1$^{b}$</td>
<td>10.0 ± 7.9$^{a}$</td>
<td>&lt;0.0001</td>
<td>0.0454</td>
</tr>
<tr>
<td>(mEq/L/MJ)$^{d}$</td>
<td>1.3 ± 0.9</td>
<td>1.4 ± 0.9</td>
<td>1.6 ± 0.9</td>
<td>1.3 ± 1.1</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

1 All values are $\bar{x}$ ± SD from individual means of 4–5 yearly 3-d dietary records. PRAL, potential renal acid load. Values in the same row with different superscript letters are significantly different, $P < 0.05$.

2 Two-way ANOVA was used to examine the main effects of developmental group and sex and the interaction between developmental group and sex. If the interaction term was $P < 0.1$, a subgroup analysis was done by using Tukey’s Studentized range (honestly significant difference) test.

3 The daily nutrient intake was energy-corrected by dividing by the daily energy (MJ) intake.

4 The daily nutrient intake was body weight–corrected by dividing by weight (kg).
Predictors of proximal radial diaphyseal bone in 229 children and adolescents

<table>
<thead>
<tr>
<th>Predictor</th>
<th>β</th>
<th>βstand</th>
<th>r²</th>
<th>P</th>
<th>β</th>
<th>βstand</th>
<th>r²</th>
<th>P</th>
<th>β</th>
<th>βstand</th>
<th>r²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle area (mm²)</td>
<td>0.003</td>
<td>0.40</td>
<td>0.24</td>
<td>&lt;0.0001</td>
<td>0.012</td>
<td>0.44</td>
<td>0.33</td>
<td>&lt;0.0001</td>
<td>0.013</td>
<td>0.41</td>
<td>0.28</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>0.072</td>
<td>0.17</td>
<td>0.03</td>
<td>0.0014</td>
<td>0.420</td>
<td>0.27</td>
<td>0.04</td>
<td>0.00001</td>
<td>0.461</td>
<td>0.26</td>
<td>0.03</td>
<td>0.0011</td>
</tr>
<tr>
<td>PRAL (mEq/d)</td>
<td>−0.252</td>
<td>−0.17</td>
<td>0.02</td>
<td>0.0075</td>
<td>−0.345</td>
<td>−0.20</td>
<td>0.02</td>
<td>0.0055</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.402</td>
<td>0.13</td>
<td>0.01</td>
<td>0.0421</td>
<td></td>
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<td></td>
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1. Results of stepwise multiple regression. Because only significant variables entering the final model are presented, adjusted calcium intake, mean growth velocity at the time of peripheral quantitative computed tomography, and sex are not shown. Tanner stages were entered into the model for cortical area (Tanner stage 5; \( P = 0.0410 \)) and bone mineral content (Tanner stages 3 and 5; \( P < 0.05 \) each). Menarche and voice change (0 = not yet attained; 1 = attained) were entered into the model for bone mineral content (\( P < 0.05 \). PRAL, potential renal acid load; \( \beta \), parameter estimate; \( \beta_{\text{stand}} \), standardized parameter estimate; \( r^2 \), partial \( R^2 \).

2. Adjusted for age and sex.

3. Adjusted for age, sex, and energy intake.

lower proportion of sulfur-containing amino acids in their dietary protein did not show any consistent bias.

DISCUSSION

Although an adequate dietary intake of protein is essential for growth, it is not known whether variations in protein intake and quality contribute to variations in bone size and mineral content (40). Therefore, in a prospective study, we evaluated the relation of long-term dietary protein intake and dietary acid load with specific bone variables analyzed by pQCT in children and adolescents.

In general, our results agreed with findings in elderly patients who had bone anabolic effects after protein supplementation of their initially low-protein diets (11, 12). Our study provides evidence of a consistent positive association of dietary protein with periosteal circumference, cortical area, bone mineral content, and polar strength strain index at the proximal diaphyseal radius in children and youth. This potential protein anabolism was found for habitual Western diets with higher protein intakes and explained 3–4% of the examined variability in bone variables. This is clearly less than what is explained by muscularity (Table 3) and adrenarchal hormones (\( r^2 \leq 0.1 \)) (18).

In line with recent findings in a juvenile longitudinal cohort (41), which reported a very poor correlation of GV with diaphyseal bone, we also found a negative association of GV measured by pQCT only with cortical density. Whether the observed positive association of long-term growth (4-year GV) with cortical area and bone mineral content might reflect a common anabolic cause deserves further research.

Interestingly, variations in protein intake did not associate with cortical density, in line with the explanation that the metabolic activity in cortical bone (remodeling) is influenced more by estrogens than by androgens (14) and that muscularity, which interacts with the growth hormone–insulin-like growth factor (IGF) system, has almost no or only a modest effect on cortical density (18). However, the extent of protein intake seems to stimulate modeling, i.e., the main process for increasing bone strength during childhood and adolescence (1).

A protein-induced increase in IGF-I is strongly assumed to be the most likely explanation for an osteotrophic effect of protein (8). IGF-I is a major determinant of bone growth and mineral content (42). Until now, associations between dietary protein intake and IGF-I were primarily studied in elderly or malnourished children. However, in a recent study, Hoppe et al (43) showed a significant positive association between protein intake, growth, and circulating IGF-I concentrations in healthy young children. This may underline the in vivo potential of protein for tissue anabolism via IGF-I. Similarly, though more bone-related, Cadogan et al (44) found that supplementation in 12-y-old girls with \( \approx 570 \) mL milk/d for 18 mo was associated with an increase in plasma IGF-I and bone mineral status compared with control subjects. As discussed by the authors (44) and summarized by Ginty (8), the higher protein content of milk could have mediated an increase in plasma IGF-I that, in turn, may have been stimulatory for osteoblast activity or promoted bone mineralization. Additionally, the daily amount of protein ingested may also influence calcium homeostasis together with parathyroid hormone secretion and 1,25-dihydroxy vitamin D status (45). However, in a recent study, parathyroid hormone and vitamin D status remained unaffected by corresponding changes in protein intake (46).

Until now, many studies concerning dietary protein intake on bone health focused on its potential negative effect. The primary assumed mechanism by which bone resorption may be increased in response to higher dietary protein intakes is the metabolic oxidation of the \( S \)-containing amino acids methionine and cysteine to \( H_2SO_4 \) with a consecutive reduction of blood pH (47). However, the acidifying effect of protein cannot be regarded as isolated, because other alkalizing nutrients (e.g., potassium, magnesium) can counterbalance it. So far, there is only predominantly indirect evidence for such an acid-base homeostatic effect on bone: increasing intakes of fruit and vegetables, i.e., alkali-forming foods (48, 49), or alkali-forming diets (50) decrease urinary calcium excretion. Additionally, observational, clinical, and intervention studies found a positive effect of alkali-forming foods, i.e., fruit and vegetables, on bone health (51) in elderly (52, 53) and in early pubertal children (3, 54). Only one recent study associated directly the estimated dietary net acid production with indexes of bone health, finding that lower estimates of net endogenous non–carbonic acid production were correlated with higher bone mass and a tendency to less bone resorption in premenopausal and perimenopausal women (10). Because it has postulated and supported by measurements of serum bicarbonate
and blood pH levels that the capability to excrete protons gradually decreases with age as the glomerular filtration rate drops (55, 56), our findings of a negative association of PRAL with bone variables, even during childhood and adolescence, when renal function should be near its optimum, are all the more remarkable. However, definite biochemical data on the age-dependency of the renal function in eliminating acidity have still to be established.

In line with our findings, Cadogan et al (44), who examined the effects of milk supplementation in 12-y-old girls, also found no association between calcium intake and bone variables. Although intervention trials in children and adolescents have regularly shown positive effects of calcium or dairy supplementation on bone mass acquisition (57–59), observational studies—especially those that have examined long bones (60, 61)—failed to detect associations.

One study with a comparable study design to ours exists; however, the results of the 2 studies are conflicting (62). In this study, neither positive nor negative relations between long-term protein intake and bone mineral densities at different sites were
found. Several reasons may have accounted for this. First, different bone sites may be differently susceptible to metabolic influences (14). Second, the dual-energy X-ray absorptiometry (DXA) method used for the measurements may not have been accurate enough to specifically identify association with protein intake, because it yields only a 2-dimensional projection (areal bone density), which tends to underestimate volumetric density in smaller and overestimate in larger subjects (1, 63). The PQCT method, however, provides a 3-dimensional assessment of the structural and geometric properties of the skeleton and thus allows a more sensitive measurement of bone quality (63, 64). In this context, the periosteal circumference and cortical density determined by pQCT more realistically reflect modeling and remodeling, respectively, than the corresponding variables calculated from DXA. This applies also to those variables reflecting a combination of modeling and remodeling.

The limitations of the current study also warrant mention. First, although weighed dietary records are regarded to be particularly reliable (65), some skepticism against the dietary assessment tool might remain. Second, a more specific analysis using individual data on methionine and cysteine intakes would be desirable, although in our present evaluation we did not see any association between bone variables and the intake of food groups with a higher content of sulfur-containing amino acids. Third, an assessment of serum IGF-I could support the hypothesis that bone anabolism by protein is driven by this hormone.

In conclusion, our data provide evidence of a positive link between long-term dietary protein intake and diaphyseal bone stability in healthy children and adolescents. Hereby, 2 seemingly contradictory mechanisms appear to be effective: an anabolic effect (probably mediated by IGF-I) on periosteal circumference, cortical area, bone mineral content, and strength strain index and a catabolic effect mediated by dietary acid load and characterizable by PRAL. A high PRAL, which indicates an inadequate intake of alkalizing minerals, can at least partly negate an osteotrophic protein effect. Our findings support the hypothesis that bone anabolism by protein is driven by this hormone.

UA and TR were primarily responsible for the data analysis, interpretation of the resultant data, and preparation of the manuscript. ES participated in the conceptualization and interpretation of results. CMN was responsible for the bone measurements. FM was responsible for the implementation of bone analyses as part of the DONALD Study and played a role as a principal investigator in all areas associated with the preparation of this manuscript. None of the authors had any conflict of interest.

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