Association between long-term consumption of soft drinks and variables of bone modeling and remodeling in a sample of healthy German children and adolescents $^{1,3}$

Lars Libuda, Ute Alexy, Thomas Remer, Peter Stehle, Eckhard Schoenau, and Mathilde Kersting

ABSTRACT

Background: Soft drinks are thought to displace milk in diets of children and adolescents and therefore might affect variables of bone modeling and remodeling.

Objective: We assessed the association between long-term consumption of several types of soft drinks and bone variables in children and adolescents.

Design: Long-term dietary data from 3-d weighed dietary records collected by 228 healthy children and adolescents enrolled in the DONALD Study in 4 y of study participation were used for data analysis. Variables of bone modeling and remodeling of the radius were assessed by using peripheral quantitative computed tomography.

Results: After adjustment for age, sex, total energy intake, muscle area, BMI SD scores, and growth velocity, long-term consumption of all soft drinks and uncaffeinated soft drinks was negatively associated with bone mineral content ($P < 0.05$), cortical area ($P < 0.05$), and polar strength strain index ($P < 0.05$), all of which reflect a combination of bone modeling and remodeling. Long-term consumption of caffeinated soft drinks was negatively associated with polar strength strain index ($P < 0.01$) and periosteal circumference ($P < 0.05$), which reflect bone modeling. Milk intake was positively associated with polar strength strain index ($P < 0.05$). Consumption of all soft drinks was negatively associated with total protein and milk intake, but was not associated with potential renal acid load.

Conclusions: Long-term consumption of caffeinated and uncaffeinated soft drinks appears to have bone catabolic effects in boys and girls. This effect is mainly mediated by the negative association with total protein intake and is not primarily based on milk displacement.

SUBJECTS AND METHODS

Subjects and study design

The study sample consisted of a subgroup of healthy children and adolescents participating in the DONALD Study, an ongoing open cohort study started in 1985 that investigates the relation between long-term consumption of different types of soft drinks and variables of bone modeling and remodeling in children and adolescents from the Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD) Study. Long-term dietary data collected over 4 y of study participation were considered for data analysis. Bone variables of the forearm were measured by peripheral quantitative computed tomography (pQCT). In a second step, we adjusted for several supposed pathway variables, which might influence bone metabolism and therefore might mediate the effect of soft drinks on children’s bone health.

INTRODUCTION

Because of the increase in life expectancy and, consequently, in the proportion of elderly people in industrialized countries, the incidence of bone fractures due to osteoporosis is increasing (1, 2). Since, for example, $\approx 90\%$ of total bone mineral content (BMC) is generated before the age of 17 y (3) in females, adolescence is the most important period of bone mass accretion in a lifetime (4). Therefore, prevention of osteoporosis should already begin in childhood (5–7). Because influencing those genetic and hormonal factors that effect bone mass accretion is difficult, an appropriate lifestyle with an adequate diet beginning from childhood onward is essential for osteoporosis prevention (6). One dietary factor in industrialized countries that is discussed to impair BMC is the consumption of soft drinks. However, the findings of previous studies are conflicting. Some studies indicate that consumption of soft drinks might affect bone mineral density (BMD) and the risk of bone fractures only in girls (8, 9). Others suggest that this relation might be restricted to cola beverages only and does not apply to uncaffeinated soft drinks (10). Additionally, the pathway of a possible bone catabolic effect of soft drinks is not clear. Currently, the discussed mechanisms include the displacement of milk in children’s diets (11, 12), the low pH values of these beverages (13), and the phosphoric acid and caffeine contents of cola drinks (10, 14). However, soft drink consumption might also be an indicator of an unhealthy, sedentary lifestyle (15) and poor diet quality (16, 17).

We therefore analyzed, in a first step, the relation between long-term consumption of different types of soft drinks and variables of bone modeling and remodeling in children and adolescents from the DONALD Nutritional and Anthropometric Longitudinally Designed (DONALD) Study. Long-term dietary data collected over 4 y of study participation were considered for data analysis. Bone variables of the forearm were measured by peripheral quantitative computed tomography (pQCT). In a second step, we adjusted for several supposed pathway variables, which might influence bone metabolism and therefore might mediate the effect of soft drinks on children’s bone health.
between nutrition, development, and metabolism in subjects be-
tween infancy and early adulthood. About 40 subjects are en-
rolled in the DONALD Study each year (18). The regular, non-
invasive assessments that take place in intervals of 1 y include 3-d
weighed dietary records, anthropometric measures, urine sam-
ping, and interviews on lifestyle and medical assessments.

Between July 1998 and June 1999, a single pQCT of the
forearm was conducted as part of a subproject in 371 DONALD
participants aged 6–18 y. We considered 228 of these partici-
pants who had ≥4 of 5 possible plausible 3-d dietary records in
the 4 y before bone analysis. Implausible dietary records were
excluded by using age- and sex-specific cutoff values for the ratio
of reported total energy intake and predicted basal metabolic rate
(19). Basal metabolic rate was calculated by equations using
measured height and weight (20).

The DONALD Study was approved by the ethical com-
mittee of the Rheinische Friedrich-Wilhelms-Universita¨t Bonn
and with respect to bone analysis by the Federal Office for Radia-
tion Protection (Salzgitter, Germany). All examinations and as-
sessments are performed initially with parental consent and later with
the children’s written consent.

Dietary survey

For dietary records, all foods and beverages before consump-
tion and leftovers had to be weighed with electronic food scales
and recorded by the parents of the children, or by the older
subjects themselves, on 3 consecutive days. Semiquantitative
recording (eg, numbers of glasses and cups) was allowed if
weighing was not possible. Intake of macro- and micronutrients
was calculated by using our in-house nutrient database LEBTAB
(21), which contains detailed data on the energy and nutrient
contents of all recorded food items and is continuously up-
dated. For LEBTAB, the nutrient content of basic food items
was taken from standard nutrient tables, and the nutrient con-
tent of commercial food items was derived either from the
product labels or from simulating recipes from the ingredients
listed on the labels (21).

Individual daily consumption of dietary variables and bever-
age groups in each year was calculated as the mean value of each
3-d record. Subsequently, the individual long-term consumption
in the 4 y before pQCT was calculated as mean values of these 3-d
averages. For this examination, we considered the following
beverage groups:

1) all soft drinks: including caffeinated and uncaffeinated,
carbonated and uncarbonated, sugar- or artificially sweet-
ened beverages, eg, lemonades, iced tea, and fruit drinks
diluted and sweetened fruit juices), sports drinks, and energy
drinks;
2) caffeinated soft drinks: sugar- or artificially sweetened soft
drinks containing caffeine and phosphoric acid, such as
cola, iced tea, and energy drinks;
3) uncaffeinated soft drinks: sugar- or artificially sweetened
soft drinks without caffeine and with citric acid as an acidifi-
cier, such as lemonades and fruit drinks; and
4) milk: including only beverage milk (eg chocolate milk),
but not milk as a component of foods (eg, together with
cereals).

We further calculated 3-d means and long-term values of en-
ergy intake (TEI), protein intake, calcium, and potential renal
acid load (PRAL) in the 4 y before pQCT. Dietary PRAL was
calculated according to Remer et al (22) using the following
equation:

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

This model of calculation considers the different absorption rates
of minerals and of sulfur-containing proteins. Consideration of
the sulfur-containing amino acid intake would result in a more
precise estimation of dietary PRAL. However, in our food and
nutrient database LEBTAB, as well as in other common data-
bases, the methionine and cysteine contents of specific foods are
not available. Therefore, we used average values for sulfur-
containing amino acids, which have already proved to be a nearly
accurate estimator of measured urinary sulfur excretion in
lactovegetarian, moderate-protein diets as well as in high-
protein omnivorous diets (23). This model of PRAL calcula-
tion was recently validated not only in adults (23), but also in
children and adolescents, because a strong correlation was
observed between calculated PRAL values and measured uri-
nary net acid excretion (22).

Anthropometric measurements

Anthropometric measurements and assessments of pubertal
status were conducted at the time of the dietary records by trained
and regularly monitored nurses according to standard proce-
dures. Body weight was measured to the nearest 0.1 kg with an
electronic scale (Seca 753 E; Seca GmbH & Co KG, Hamburg,
Germany). Height was measured while the subjects were in a
standing position to the nearest 0.1 cm with a digital telescopic
stadiometer (Harpenden; Holtain Ltd, Crymych, United King-
dom). Body mass index (BMI) was calculated as body weight
(kg) divided by height\(^2\) (m). Sex- and age-independent BMI SD
scores (BMI-SDS) were calculated by using the German national
reference data (24). Growth velocity was calculated as the dif-
ference between height at pQCT and height in the year before
pQCT divided by the time interval in years between the 2 mea-
surements. Puberty stage at pQCT (prepubescent or pubescent)
was assessed by using Tanner stage 1–5, which was determined
by a study pediatrician. Subjects with Tanner stage 1 were clas-
sified as prepubescent, and subjects with Tanner stages ≥2 were
classified as pubescent.

Peripheral quantitative computed tomography

Measurement of bone and muscle variables of the nondomi-
nant forearm with pQCT was performed by using an XCT-2000
device (Stratec Inc, Pforzheim, Germany), which is equipped
with a low-energy X-ray tube (38 keV). The measurement was
conducted at the maximum circumference of the forearm, ie, at a
distance to the ulnar styloid process of 65% of the forearm length
proximal to the radial endplate (25). The effective radiation was
\(\equiv 0.1 \mu \text{sV} \) from a radiation source of 45 kV at 15 \(\mu \text{A} \). A 2-mm
thick single tomographic slice was sampled at a voxel size of 0.4
\(\times 0.4 \times 2 \text{ mm} \). The speed of the translational scan movement
was 15 mm/s, which resulted in a measurement time of 2–3 min in
the younger and of 4–5 min in the older subjects, depending on the
cross-sectional size of the forearm. Image processing and the
calculation of numerical values were performed with the use of
the manufacturer’s software package (software version 5.40).
Cortical area (CA), ie, the cross-sectional area of cortical bone, was determined by detecting the outer and inner cortical bone contour at a threshold of 710 mg/cm². BMC, defined as the mass of mineral (in mg) per unit of axial bone length (in mm), was also identified at a threshold of 710 mg/cm². Periosteal circumference (PC) was determined under the assumption of a cylindrical bone shape, whereby the outer bone radius was calculated by using the following equation: outer bone radius = (total area/π)¹⁄². Polar strength strain index (SSI), an indicator of bone stability, was calculated as the product of section modulus and cortical density normalized to the maximal physiologic cortical density of human bones (26).

Individual bone architecture is determined by processes of modeling and remodeling. PC served as an indicator for bone modeling, which reflects the expansion process of bone cross section due to aggregation of bone matrix on the periosteal surface. Remodeling indicates the change in cortical density or cortical porosity (27). BMC, CA, and SSI indicated a combination of modeling and remodeling. Cross-sectional forearm muscle area was also determined by using the XCT-2000 device at 65% of the ulnar length. Muscle area was separated from bone and fat tissue by using a built-in software algorithm (software version 5.40).

### Statistical analysis

All statistical tests were performed by using the SAS procedures (version 8.02, 2001; Statistical Analysis Systems, Cary, NC). Descriptive data are given as means ± SDs, unless indicated otherwise. Main effects of sex and puberty level on anthropometric and dietary variables were tested with a 2-factor analysis of variance.

First, a repeated-measures regression analysis model (PROC Mixed) regarding individual 3-d means of dietary variables was used to estimate the longitudinal effect of the change in soft drink consumption on the concurrent change in possible confounding dietary variables. Separate analyses included 3-d mean beverage milk consumption (g/d), calcium intake (mg/d), PRAL (mEq/d), or protein intake (% of TEE/d) as the dependent variable. The models included time in years (0 for the first assessment), age, baseline consumption of soft drinks, the interaction between baseline consumption of soft drinks with time, and the change in consumption in the 4-y study period. The change in soft drink consumption was calculated by subtracting baseline consumption from the consumption at each year of assessment. In this way, the regression coefficient of the change in soft drink consumption represents the slope of the change in the dietary variable (ie, milk consumption, calcium intake, PRAL, and protein intake) on the concurrent change in soft drink consumption (last assessment minus first assessment).

The association between long-term beverage consumption and markers of bone modeling and remodeling was then evaluated in a cross-sectional analysis using a multivariate linear regression model (PROC GLM). Individual long-term consumption of different beverage groups in the 4 y before pQCT was chosen as the respective independent variable. Separate analyses included BMC, CA, PC, or SSI as the dependent variable. Model 1 was adjusted for age, sex, TEE, BMI-SDS, growth velocity, and muscle area of the forearm. In further models, we separately included various variables that are discussed as potential mediators of the bone catabolic effect of soft drink consumption. Because soft drinks are thought to displace milk beverages, which might have an impact on bone mineralization, we included long-term consumption of milk as a confounder in model 2. We further included either long-term calcium intake (model 3), long-term PRAL (model 4), or long-term protein intake as a percentage of TEE (model 5) as potential pathway variables. Both PRAL and protein intake were recently shown to have an impact on bone metabolism (28). Because we found no significant sex interaction, we did not analyze boys and girls separately. In all statistical tests, a P value <0.05 was considered significant.

### RESULTS

Mean (± SD) anthropometric and long-term dietary data including all dependent and independent variables and confounders are presented in Table 1 and Table 2, respectively. All anthropometric and bone variables were significantly greater in the pubescent group than in the prepubescent group, except for growth velocity. For example, the mean BMC was ≈1.6 (1.7) greater in boys (girls) in the pubescent group than in boys (girls) in the prepubescent group. Additionally, we observed significant sex differences in all bone variables and in the muscle area of the

#### Table 1

Anthropometric and bone characteristic variables in a sample of 228 German children and adolescents at the time of peripheral quantitative computed tomography (pQCT)¹

<table>
<thead>
<tr>
<th></th>
<th>Prepubescent Boys (n = 67)</th>
<th>Girls (n = 56)</th>
<th>Pubescent Boys (n = 48)</th>
<th>Girls (n = 57)</th>
<th>P for differences² Sex</th>
<th>Puberty</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>8.8 ± 2.0</td>
<td>8.2 ± 1.6</td>
<td>13.8 ± 2.2</td>
<td>13.3 ± 2.3</td>
<td>0.0786</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>31.7 ± 10.2</td>
<td>28.6 ± 7.1</td>
<td>55.9 ± 9.7</td>
<td>52.5 ± 11.7</td>
<td>0.0123</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>136.1 ± 12.6</td>
<td>131.5 ± 10.8</td>
<td>167.5 ± 11.2</td>
<td>160.7 ± 10.5</td>
<td>0.0003</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>16.7 ± 2.7</td>
<td>16.3 ± 2.0</td>
<td>19.8 ± 9.7</td>
<td>20.1 ± 3.2</td>
<td>0.7055</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI-SD score</td>
<td>−0.06 ± 0.97</td>
<td>−0.09 ± 0.90</td>
<td>0.19 ± 0.74</td>
<td>0.23 ± 0.99</td>
<td>0.9699</td>
<td>0.0220</td>
</tr>
<tr>
<td>Growth velocity at pQCT (cm/y)</td>
<td>6.0 ± 1.0</td>
<td>5.9 ± 0.9</td>
<td>5.8 ± 2.7</td>
<td>4.6 ± 3.0</td>
<td>0.0273</td>
<td>0.0071</td>
</tr>
<tr>
<td>Bone mineral content (mg/mm)</td>
<td>45.8 ± 11.1</td>
<td>39.5 ± 13.0</td>
<td>71.1 ± 16.8</td>
<td>68.2 ± 17.3</td>
<td>0.0216</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cortical area (mm²)</td>
<td>45.2 ± 9.8</td>
<td>39.6 ± 11.5</td>
<td>68.2 ± 16.8</td>
<td>63.2 ± 14.0</td>
<td>0.0032</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Periosteal circumference (mm)</td>
<td>32.6 ± 3.4</td>
<td>31.7 ± 2.9</td>
<td>38.6 ± 2.9</td>
<td>35.9 ± 3.2</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Polar bone strength strain index (mm³)</td>
<td>147.4 ± 47.7</td>
<td>124.3 ± 41.4</td>
<td>253.5 ± 64.7</td>
<td>216.6 ± 59.6</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Muscle area (mm²)</td>
<td>1973.1 ± 379.1</td>
<td>1749.3 ± 318.1</td>
<td>3150.2 ± 718.0</td>
<td>2605.5 ± 401.2</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

¹ All values are means ± SDs.
² Main effects of sex and puberty were tested by 2-factor ANOVA.
Long-term values for caffeinated and uncaffeinated soft drink consumption, TEI, PRAL, and absolute intake of protein, calcium, and phosphorus were higher in the pubescent group; no differences in the densities (per MJ) of protein, calcium, and phosphorus and PRAL between the prepubescent and pubescent group were observed (Table 2). In contrast with the consumption of all soft drinks, milk consumption was not higher in the pubescent group. Mean values for most dietary variables were higher in boys than in girls without any differences in BMI or BMI-SDS.

Results of the repeated-measured regression analysis on the association between the change in consumption of all soft drinks and the concurrent change in dietary variables are shown in Table 3. The change in consumption of all soft drinks was negatively associated with a concurrent change in milk consumption and calcium intake. The change in consumption of all soft drinks was not associated with a concurrent change in PRAL. Protein intake decreased with increasing consumption of all soft drinks.

Results of the linear regression analysis on the association between long-term beverage consumption and bone status are shown in Table 4. Long-term consumption of all soft drinks was negatively associated with all bone variables in model 1, except for PC. Whereas similar results were observed for uncaffeinated soft drinks, caffeinated soft drinks were negatively associated with PC and SSI only. Long-term consumption of milk was positively associated with SSI, but not with the remaining bone variables. Adjustment for long-term milk consumption in model 2 resulted in moderate attenuation of significance levels in the analyses of all soft drinks and in those of both subgroups of soft drinks. However, long-term consumption of all soft drinks was still negatively associated with CA and SSI and consumption of caffeinated soft drinks was still significantly associated with PC and SSI. Adjustment for long-term calcium intake in model 3 did not result in major modifications of the effect of soft drinks on bone variables, except for SSI, for which the association with all types of soft drinks was attenuated after adjustment for calcium intake. Inclusion of long-term PRAL values in model 4 did not substantially change significance levels for any beverage group or bone variable. After adjustment for long-term protein intake (% of total energy intake) in model 5, only the association for PC. Whereas similar results were observed for uncaffeinated soft drinks, caffeinated soft drinks were negatively associated with PC and SSI only. Long-term consumption of milk was positively associated with SSI, but not with the remaining bone variables. Adjustment for long-term milk consumption in model 2 resulted in moderate attenuation of significance levels in the analyses of all soft drinks and in those of both subgroups of soft drinks. However, long-term consumption of all soft drinks was still negatively associated with CA and SSI and consumption of caffeinated soft drinks was still significantly associated with PC and SSI. Adjustment for long-term calcium intake in model 3 did not result in major modifications of the effect of soft drinks on bone variables, except for SSI, for which the association with all types of soft drinks was attenuated after adjustment for calcium intake. Inclusion of long-term PRAL values in model 4 did not substantially change significance levels for any beverage group or bone variable. After adjustment for long-term protein intake (% of total energy intake) in model 5, only the association

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Pubescent (n = 48)</th>
<th>Girls (n = 57)</th>
<th>P for differences&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Boys (n = 67)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All soft drinks (g/d)</td>
<td>136.8 ± 137.3</td>
<td>119.8 ± 129.2</td>
<td>0.01085 0.0001</td>
</tr>
<tr>
<td>Caffeinated soft drinks (g/d)</td>
<td>19.0 ± 47.4</td>
<td>10.1 ± 20.5</td>
<td>0.0145 &lt;0.0001</td>
</tr>
<tr>
<td>Uncaffeinated soft drinks (g/d)</td>
<td>117.8 ± 112.3</td>
<td>109.7 ± 126.6</td>
<td>0.3259 0.0027</td>
</tr>
<tr>
<td>Milk (g/d)</td>
<td>207.1 ± 124.5</td>
<td>185.5 ± 107.7</td>
<td>&lt;0.0001 0.6894</td>
</tr>
<tr>
<td>TEI (MJ/d)</td>
<td>6.4 ± 1.1</td>
<td>5.6 ± 0.7</td>
<td>&lt;0.0001 0.0001</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>48.2 ± 10.2</td>
<td>42.5 ± 7.3</td>
<td>0.0001 0.0001</td>
</tr>
<tr>
<td>Protein (% of TEI)</td>
<td>12.5 ± 1.4</td>
<td>12.7 ± 1.6</td>
<td>0.5754 0.1159</td>
</tr>
<tr>
<td>Calcium (mg/d)</td>
<td>744.4 ± 180.3</td>
<td>664.0 ± 157.9</td>
<td>&lt;0.0001 &lt;0.0001</td>
</tr>
<tr>
<td>Calcium (mg/MJ)</td>
<td>116.6 ± 21.8</td>
<td>119.2 ± 24.6</td>
<td>0.2702 0.4623</td>
</tr>
<tr>
<td>Calcium (mg/kg body wt)</td>
<td>31.2 ± 7.1</td>
<td>31.1 ± 7.4</td>
<td>0.0181 &lt;0.0001</td>
</tr>
<tr>
<td>PRAL (mEq/d)</td>
<td>8.5 ± 6.6</td>
<td>7.6 ± 5.5</td>
<td>0.0096 0.0004</td>
</tr>
<tr>
<td>PRAL (mEq/MJ)</td>
<td>1.3 ± 0.9</td>
<td>1.3 ± 0.9</td>
<td>0.3409 0.3722</td>
</tr>
<tr>
<td>Phosphorus (mg/d)</td>
<td>950.6 ± 202.8</td>
<td>838.5 ± 147.7</td>
<td>&lt;0.0001 &lt;0.0001</td>
</tr>
<tr>
<td>Phosphorus (mg/MJ)</td>
<td>148.2 ± 20.7</td>
<td>149.5 ± 20.2</td>
<td>0.3313 0.3933</td>
</tr>
</tbody>
</table>

<sup>1</sup> All values are means ± SDs. PRAL, potential renal acid load; TEI, total energy intake.

<sup>2</sup> Main effects of sex and puberty were tested by 2-factor ANOVA.
between consumption of caffeinated soft drinks and PC and SSI remained significant. Long-term protein intake itself was positively associated with all bone variables in model 5 (BMC: $\beta = 1.02$, $P = 0.03$; CA: $\beta = 0.97$, $P = 0.01$; PC: $\beta = 0.28$, $P = 0.02$; SSI: $\beta = 5.23$, $P < 0.01$).

DISCUSSION
The main finding of the present analysis was the negative association between consumption of different types of soft drinks and variables of bone modeling and remodeling in German children and adolescents. This association might have been due to the negative relation between consumption of soft drinks and intake of protein, which had anabolic effects on bone. However, in contrast with uncaffeinated soft drinks, the impact of caffeinated soft drink consumption on bone status was not fully mediated by protein intake.

Our findings are in line with results from various studies that observed a displacement of milk in the diets of children and adolescents by soft drinks (8, 30–32). In the present examination, the low long-term milk consumption of subjects in the puberty group in particular might be of concern because milk is known to be the most important source of calcium intake in children (33, 34). The displacement of milk and the concurrent decrease in calcium intake is often thought to be one of the main mediators of the negative effect of soft drinks on bone status (11, 12, 35, 36). Interestingly, the effect size of soft drink consumption and milk consumption per 100 g on the various bone variables was comparable in the present examination. However, milk consumption was only significantly associated with SSI before adjustment for long-term protein intake, but not with the remaining bone variables. Additionally, adjustment for milk consumption weakened the relation between soft drink consumption and bone variables only to a minor degree. Furthermore, adjustment for long-term calcium intake had no major effect on significance levels. These results support the findings of McGartland et al (9), who observed that the association between consumption of soft drinks and BMD in girls was weakened but still significant after adjustment for beverage milk consumption. Therefore, the displacement of milk in the diet of children and adolescents seems to be only one factor that mediates the bone catabolic effect of soft drink consumption. Long-term soft drink consumption in our study sample, which ranged from 120 to 250 g/d, especially the consumption of caffeinated soft drinks, was relatively low. In comparison, mean daily soft drink consumption (including fruit drinks) of children and adolescents $< 19$ y of age in the United States was $\approx 425$ g/d (37). Therefore, it is possible that, in our

### Table 4

<table>
<thead>
<tr>
<th>Independent variable (g/d)</th>
<th>Bone mineral content (mg/mm)</th>
<th>Cortical area (mm²)</th>
<th>Periosteal circumference (mm)</th>
<th>Polar strength strain index (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$</td>
<td>SE</td>
<td>$P$</td>
<td>$\beta$</td>
</tr>
<tr>
<td><strong>All soft drinks</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1 $^c$</td>
<td>$-0.09$</td>
<td>0.004</td>
<td>0.036</td>
<td>$-0.09$</td>
</tr>
<tr>
<td>Model 2 $^d$</td>
<td>$-0.08$</td>
<td>0.004</td>
<td>0.053</td>
<td>$-0.08$</td>
</tr>
<tr>
<td>Model 3 $^e$</td>
<td>$-0.11$</td>
<td>0.005</td>
<td>0.020</td>
<td>$-0.10$</td>
</tr>
<tr>
<td>Model 4 $^f$</td>
<td>$-0.09$</td>
<td>0.004</td>
<td>0.034</td>
<td>$-0.09$</td>
</tr>
<tr>
<td>Model 5 $^g$</td>
<td>$-0.05$</td>
<td>0.004</td>
<td>0.248</td>
<td>$-0.05$</td>
</tr>
<tr>
<td><strong>Caffeinated soft drinks</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1 $^c$</td>
<td>$-0.01$</td>
<td>0.014</td>
<td>0.243</td>
<td>$-0.02$</td>
</tr>
<tr>
<td>Model 2 $^d$</td>
<td>$-0.01$</td>
<td>0.014</td>
<td>0.282</td>
<td>$-0.01$</td>
</tr>
<tr>
<td>Model 3 $^e$</td>
<td>$-0.01$</td>
<td>0.015</td>
<td>0.225</td>
<td>$-0.02$</td>
</tr>
<tr>
<td>Model 4 $^f$</td>
<td>$-0.01$</td>
<td>0.014</td>
<td>0.241</td>
<td>$-0.02$</td>
</tr>
<tr>
<td>Model 5 $^g$</td>
<td>$-0.09$</td>
<td>0.014</td>
<td>0.522</td>
<td>$-0.01$</td>
</tr>
<tr>
<td><strong>Uncaffeinated soft drinks</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0.004</td>
<td>0.050</td>
<td>$-0.09$</td>
</tr>
<tr>
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<td>$-0.10$</td>
</tr>
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<td>0.005</td>
<td>0.047</td>
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</tr>
<tr>
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<td>$-0.05$</td>
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<tr>
<td><strong>Milk</strong></td>
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<tr>
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<td>0.005</td>
<td>0.240</td>
<td>0.005</td>
</tr>
<tr>
<td>Model 2 $^d$</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Model 3 $^e$</td>
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<td>0.006</td>
<td>0.141</td>
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<tr>
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<td>0.002</td>
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<td>0.738</td>
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</table>

$^1$ Results of the linear regression analysis.
$^2$ Each beverage group was included in a separate analysis.
$^3$ Adjusted for age, sex, total energy intake, muscle area, BMI-SD score, and growth velocity.
$^4$ Adjusted as for model 1 plus long-term milk intake (g/d).
$^5$ Adjusted as for model 1 plus long-term calcium intake (g/d).
$^6$ Adjusted as for model 1 plus long-term potential renal acid load (mEq/d).
$^7$ Adjusted as for model 1 plus for long-term protein intake (% of total energy intake).
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study sample, soft drinks might not have displaced milk to the same extent as in the American children.

Another mechanism thought to explain the bone catabolic effect of soft drinks is the acid load, because it is assumed to be associated with consumption of these beverages (13). Dietary acid load is known to be positively associated with bone resorption due to osteoclast stimulation (38, 39) and negatively associated with BMD (28, 39). We observed positive long-term PRAL values representing an excess of dietary acid load in our study sample. The calculated PRAL value of 0.21 mEq for each 100-g consumption of a common cola beverage was, however, moderate. Because long-term consumption of all soft drinks in this analysis was between 120 and 250 g/d, soft drinks would be responsible for only 3% to 4.5% of mean long-term PRAL values. Additionally, we found no association between the change in consumption of all soft drinks and the change in PRAL. Because the bone catabolic effect of soft drink consumption remained significant after long-term PRAL was controlled for, the presumed impact of soft drinks on PRAL does not seem to mediate the effect on bone status in children and adolescents.

Our findings confirm the negative relation of soft drink consumption with protein intake that was observed in various other studies (31, 35, 40–42). Protein intake was found to have anabolic effects on bone in children, adolescents, and young adults when the intake of calcium and alkalizing minerals is adequate (28, 43). Accordingly, most of the significant associations between soft drink consumption and the bone variables were no longer significant after adjustment for long-term protein intake. Therefore, the negative association with long-term protein intake appears to be the main mediator of the catabolic effect of soft drinks on bone. However, the effects of caffeinated soft drinks remained significant even after adjustment for long-term protein intake. Additionally, consumption of caffeinated soft drinks affected PC, which was not the case for the consumption of uncaffeinated soft drinks.

One difference between caffeinated and uncaffeinated soft drinks is the type of acidulant added, ie, phosphoric acid in caffeinated soft drinks and citric acid in uncaffeinated soft drinks. Whereas the addition of phosphoric acid increases the PRAL value of foods, addition of dietary organic acids (eg, citric acid), which are metabolically oxidized to bicarbonate and water, will not (22). Because we adjusted for total PRAL, the phosphoric acid content of caffeinated soft drinks does not seem to be the reason for the differences in the effects of caffeinated and uncaffeinated soft drinks on bone variables. This result supports the findings of Heaney and Rafferty (12), who found no effect of the acidulant type (phosphoric acid compared with citric acid) on calciauria in a short-term intervention period of 5 wk in women between 20 and 40 y of age. However, they observed excess calciauria after consumption of caffeinated soft drinks, but not after consumption of uncaffeinated soft drinks.

Differences in the effect of these soft drink subgroups on bone variables were also observed in various studies (10, 14, 44). In contrast with the present analysis, consumption of uncaffeinated soft drinks had no effect on bone variables in these studies. Others found significant effects only for uncaffeinated soft drinks, but not for caffeinated soft drinks (9). Potential differences might also be explained by specific cola ingredients other than phosphoric acid, such as caffeine (14) or cola extract (10). However, results of the impact of caffeine on BMD are conflicting. Two studies in young women found no effect of self-reported caffeine consumption on BMD (45, 46). In a double-blind, placebo-controlled, crossover study in premenopausal women, a caffeine intake of 400 mg/d showed either no or only minor effects on variables of calcium economy (47). The authors suggested, therefore, that moderate caffeine intake is not a risk factor for osteoporosis in women with adequate calcium intakes. Another explanation for the different impact of caffeinated and uncaffeinated soft drinks might be specific lifestyle factors of cola consumers, eg, poor diet quality with inadequate intake of bone protective micronutrients such as vitamin D, magnesium, and zinc or a sedentary lifestyle. Even though we indirectly adjusted for physical activity using muscle area of the forearm as a covariable, the impact of a sedentary lifestyle might not be fully accounted for by our approach. At this time, the reason for the differences in the effect of caffeinated and uncaffeinated soft drinks on bone modeling and remodeling cannot be finally clarified.

In the present analysis we observed no sex differences in the effect of soft drink consumption on bone variables. In contrast, some previous studies found catabolic effects on bone in girls and female adults only (8–10, 44). One hypothesis for this restriction to girls is a suggested threshold effect of soft drink consumption depending on the individual calcium intake level, because girls have lower mean calcium intake levels than boys (11). However, our data do not support the hypothesis of a threshold effect. Therefore, girls might be at special risk for the bone catabolic effects of soft drinks, but an effect on bone variables in boys cannot be excluded.

Some strengths and limitations of the present examination need to be mentioned. First, only one pQCT measurement was carried out in each subject, so we were not able to estimate the effect of soft drink consumption on longitudinal bone development and individual modeling and remodeling. Such longitudinal data are desirable to prove causality between soft drink consumption and bone catabolic effects. Second, dietary data were self-reported and might be subject to underreporting or recording errors. However, the high standard of dietary recording in the DONALD Study was recently shown in an analysis that compared dietary iodine intake from weighed amounts of different food groups with concurrent 24-h urinary iodine excretion, which reflected precisely the iodine content of food groups measured by chemical analysis (48). Additionally, in contrast with other studies, we considered long-term dietary data of each subject instead of a single dietary survey for characterization of individual beverage consumption patterns, which results in a more accurate estimation of dietary behavior. The third limitation of our examination may have been the missing information on physical activity. However, we were able to indirectly adjust for physical activity using pQCT information on muscle area at the analyzed bone site. A strength of our approach is the application of pQCT instead of the dual energy X-ray absorptiometry method. The pQCT method is known to provide more sensitive measurements of bone quality in children and produces valid surrogate measures of fracture risk in children (49, 50).

Conclusion

Consumption of both caffeinated and uncaffeinated soft drinks is negatively associated with variables of bone modeling and remodeling in boys and girls. This relation seems to be
mainly mediated by the negative association with long-term protein intake and not by milk displacement. The negative association of soft drink consumption with BMC and SSI might increase the risk of fractures in childhood and osteoporosis in later life.

We are grateful to the staff of the Research Institute of Child Nutrition for collecting the anthropometric and dietary data.

The authors’ responsibilities were as follows—LL, UA, and TR: conceived the project and performed the initial statistical analyses; LL: conducted further analyses and drafted the manuscript; PS, ES, and MK: provided critical input on the data analyses and on the early versions of the manuscript; and TR, ES, MK, supervised the study. None of the authors had any personal or financial conflicts of interest.

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