Fruit and vegetable intakes are an independent predictor of bone size in early pubertal children

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ABSTRACT

Background: Adequate intakes of fruit and vegetables are recommended for optimum health in children.

Objective: The objective of this study was to determine whether consuming fruit and vegetables \( \geq 3 \) times per day is beneficial to bone mass in children.

Design: Fifty-six white females (Tanner stage 2) recorded dietary intake on 3 independent days. The numbers of servings of fruit and vegetables were recorded for each day and tallied, and the subjects were divided into 2 consumption groups for analysis (low consumption: \(< 3\) servings/d, \( n = 22 \); high consumption: \( \geq 3\) servings/d, \( n = 34 \)). Bone area and the bone mineral content of the whole body and radius were assessed by using dual-energy X-ray absorptiometry. Radioimmunoassays measured serum parathyroid hormone and 25-hydroxyvitamin D. Twenty-four–hour urine samples were assessed for calcium, sodium, and creatinine.

Results: After adjustment for age, body mass index, and physical activity, those children who reported consuming \( \geq 3\) servings fruit and vegetables/d had more bone area of the whole body (6.0%; \( P = 0.03 \)) and radius (8.3%; \( P = 0.03 \)), lower urinary calcium excretion (2.6 \( \pm \) 0.2 compared with 1.8 \( \pm \) 0.3 mg/kg; \( P = 0.04 \)), and lower parathyroid hormone (19.6 \( \pm \) 1.9 compared with 25.0 \( \pm \) 1.6 pg/mL; \( P = 0.01 \)) than did those children who reported consuming \(< 3\) servings fruit and vegetables/d.

Conclusions: High fruit and vegetable intakes have beneficial effects on the bone area of the radius and whole body in early pubertal girls. The lower urinary calcium output associated with higher fruit and vegetable intakes may be a modulating factor.


KEY WORDS Dual-energy X-ray absorptiometry, children, bone mass, urinary calcium excretion

INTRODUCTION

The assessment of dietary factors on bone accretion in children has focused on the quantity of calcium required for optimal bone accrual. In childhood, bone accrual reflects the genetic and environmental factors that contribute to bone metabolism. Urinary calcium excretion is one component of calcium balance that may provide insight into the effects of dietary intake on calcium and bone metabolism. Welch et al (1) showed that 92% of urinary calcium excretion reflects bone metabolism rather than dietary calcium intake. Understanding the factors that contribute to urinary calcium excretion may be crucial to the design of dietary interventions that maximize bone accrual.

The studies relating dietary factors to calcium excretion in children focused on the effect of dietary calcium, protein, phosphorus, and sodium consumption (2, 3). Of these factors, only sodium intake was shown to have modest effects on calcium excretion (2). In theory, each of these nutrients can be related to the excretion of calcium in the urine, but it is the net metabolic effect of the total diet that dictates how much calcium is excreted in the urine.

Wachman and Bernstein (4) proposed that dietary intake is related to the development of osteoporosis through the regulation of the acid-base balance. Bone tissue is a rich source of bases (calcium, carbonate, citrate, magnesium, and potassium) that can buffer acute acid loads (5) and chronic metabolic acidosis (5, 6). Dietary intake is made up of foods that contribute to the metabolic acid load (protein-rich foods, grains, and cereals) and foods that provide base products to neutralize the acid load (fruit and vegetables). In the absence of sufficient base products in the diet, the bone-fluid barrier contributes to the buffering of the acid to maintain pH within narrow limits (5). Thus it follows that high consumption of fruit and vegetables should counterbalance a similar consumption of foods that produce more acid and thus should spare the skeleton.

In adults, evidence has mounted suggesting that a diet with higher consumption of fruit and vegetables is beneficial to bone mineral density (BMD; 7–12). Feeding studies showed that increased consumption of dietary fruit and vegetables in combination with controlled calcium intake reduces urinary calcium excretion (13, 14) and the pH of urine (14). Intestinal absorption of calcium was unchanged, which suggests increased calcium retention (14). Jones et al (15) showed that urinary potassium excretion, a potential marker for fruit and vegetable intakes, correlates with BMD of the total body, femoral neck, and lumbar spine in calcium-replete children. But there are, to our knowledge, no data that tie fruit and vegetable intakes to urinary calcium and bone mass in children.

The main objective of this research project was to evaluate the influence of fruit and vegetable intakes on urinary calcium excretion.
excretion and bone mass in a group of early pubertal girls. A secondary aim was to evaluate whether parathyroid hormone (PTH), 25-hydroxyvitamin D [25(OH)D], and markers of bone formation and resorption were related to fruit and vegetable intakes.

SUBJECTS AND METHODS

Subjects

Fifty-six white females were recruited to participate in a randomized calcium supplementation trial through direct mailing and advertisements in the local media. The girls were between the ages of 8 and 13 y, and they presented at Tanner stage 2 of sexual development. Girls were excluded from entering the study if they reported chronic use of medicines known to affect calcium metabolism. Each subject and her legal guardian provided written informed consent in accordance with the human investigation and review boards at the University of Tennessee.

Study measurements

Subjects completed height, weight, and bone measurements and provided a 24-h urine sample, a blood sample, and a 1-d food record before being randomly assigned to receive placebo or 1000 mg calcium carbonate/d. Two additional 1-d food records were collected after randomization. Tanner stage was self-reported.

Anthropometry

Body weight was measured on a balance-beam scale to the nearest 0.1 kg. Height was measured twice to the nearest 0.1 cm by using a Harpenden stadiometer (Holtain, Crymych, United Kingdom). If the 2 height measurements differed by >0.5 cm, a third measurement was performed. The average of the 2 measurements within 0.5 cm was used for these analyses. Body mass index (BMI) was calculated as weight in kg divided by height in meters squared.

Dual-energy X-ray absorptiometry of the whole body and wrist

Dual-energy X-ray absorptiometry (DXA) was performed by using a Hologic Quantitative Digital Radiography bone densitometer (model 2000; Hologic Inc, Bedford, MA) to measure bone area, bone mineral content (BMC), and BMD of the whole body and nondominant wrist. Whole-body measurements were assessed by using the array mode and analyzed by using enhanced whole-body software (version 5.73a; Hologic Inc). The nondominant wrist was scanned in the pencil-beam mode and analyzed by using the same software. The DXA quality assurance manual for the study was used to standardize subject positioning and scan analysis. Two scans at the baseline visit were obtained with repositioning. The average of the 2 scans are reported as the bone indexes. In addition, we applied the method developed by Brismar et al (16) for calculating the whole-body bone mineral apparent density [in g/cm² = areal BMD of whole body/(whole body area − head area/(body height − 18 cm))].

Laboratory assessments

All subjects collected a 24-h urine sample for measurement of calcium, creatinine, and sodium. Subjects were given detailed verbal as well as written instructions on how to collect a complete 24-h urine sample and given a 2.5-L plastic bottle to take with them. Time and volume of collections were recorded. Samples were considered to be complete if the volume exceeded 24 mL urine/kg body wt and if creatinine was within the 2 SDs of creatinine values for the subject’s height (17). Urinary calcium was measured with the use of o-cresolphthalein. Urinary sodium was measured by using an ion-selective electrode. Urinary creatinine was measured by using the Jaffe reaction (picric acid). The intra-assay CVs for these assays were 0.3–2.3% for calcium, 0.3–1.6% for sodium, and 0.6–1.5% for creatinine. Urinary deoxypyridoline was measured by using a competitive enzyme immunoassay (Pyrilinks D; Metra Biosystems Inc, Mountain Home, CA). The inter-assay CVs for these assays were 0.9–1.2% for calcium, 1.1–2.7% for sodium, 2.5–3.2% for creatinine, and 5% for deoxypyridoline.

Serum was drawn between 1600 and 1900. Serum PTH was measured by using a 2-site immunoradiometric assay and 2 affinity-purified antibodies. One antibody raised against the mid- and C-terminal (PTH 39–84) is immobilized on plastic beads, and the other antibody raised against the N-terminal (PTH 1–34) is labeled with 125I (DiaSorin, Stillwater, MN). The CV for this assay is <7%, and the range for normal children is 13–50 pg/mL. We evaluated the effect of consuming foods and beverages within 4 h of the blood draw on PTH concentrations between the 2 fruit and vegetable consumption groups. There were no differences between the 2 groups with regard to food consumption. Serum 25(OH)D was measured by using a radioimmunoassay (DiaSorin). The intra-assay and inter-assay CVs are <10%. The reference range for 25(OH)D was 15–60 ng/L. We used a competitive enzyme immunoassay and a monoclonal antibody to measure both intact osteocalcin and its large N-terminal midfragment (Nichols Inc, San Juan Capistrano, CA). The inter-assay CV for osteocalcin was <4.9%.

Dietary intake

Food records

Each subject and her parent or guardian were provided detailed instructions on keeping a 1-d food record by using a standardized collection form. Food models and photographs of serving sizes of foods were used to estimate the quantity of all foods and beverages consumed. A technician was trained and certified by a registered dietitian to provide instructions to the subjects for recording all food and beverages and to review the completed records with each girl and her parent or guardian. Ninety-six percent of the subjects provided food records for 3 independent days within 12 mo of beginning the study; 2% did so by 15 mo, and 2% did so by 24 mo.

Food group consumption

A trained technician assigned each food consumed to 1 of 6 food categories: meat and meat alternatives, fats and sweets, milk and milk products, grain products, fruit, and vegetables. Each time a food in one of the major food categories was consumed, its food category was credited with one point. The
method used to assign food items to food groups is described in detail by Krebs-Smith et al (18). Ten percent of the records were coded by another research technician and checked for accuracy. A third researcher rectified differences between the 2 coders in assigning a food to a food group. For statistical analyses, data from 3 d were summarized for each subject. Subjects were grouped into consumption groups; a low intake was <3 servings/d, and a high intake was ≥3 servings/d.

**Nutrient intake**

The food records used to estimate food group consumption were analyzed for nutrient content by using NUTRIBASE 2001 CLINICAL software (version 3.03; CyberSoft Inc, Phoenix, AZ). Nutrient intake reflects the average of the independent food records for 3 d. For each nutrient, the actual intake was compared with the estimated average requirement or adequate intake set by the Institute of Medicine (19–22).

**Physical activity**

Subjects completed a 1-d physical activity checklist on the same days that diet records were collected. The physical activity log used for this study was developed and validated by Sallis et al (23). All physical activities for a 24-h period, including activities of daily living, were recorded. Energy expenditure was calculated on the basis of the subject’s weight and the frequency and duration of each activity recorded (24, 25).

**Statistical analysis**

Data were analyzed by using SAS software (26). Means and SEs were used to describe all continuous measures according to the consumption groups: urinary measures, serum measures, and physical characteristics of the participants. Student’s t tests were used to compare differences between the fruit and vegetable consumption groups in age, height, weight, BMI, percentage body fat, household size, and parent education. A chi-square test was used to determine whether there were differences between the fruit and vegetable consumption groups with respect to family income and the percentage of subjects who consumed the estimated average requirement of the adequate intake of the respective nutrient. The energy intake differed significantly between the 2 consumption groups (low consumption: 2059 ± 98 kcal; high consumption: 2394 ± 94 kcal; P = 0.02). Nutrient intakes of the 2 food consumption groups were compared after adjustment for differences in energy intake. Comparisons of bone indexes, urinary sodium and calcium, serum PTH and 25(OH)D, and markers of bone turnover over the 2 consumption groups were evaluated after adjustment for age, BMI, and physical activity. A P value of <0.05 was considered significant when the 2 consumption groups were compared. The adjusted means are presented for nutrient intake and bone assessments.

**RESULTS**

The characteristics of the study population according to fruit and vegetable intake groups are shown in Table 1. Sixty-one percent of the girls reported consuming ≥3 servings fruit or vegetables/d. The consumption groups did not differ significantly with respect to age, height, weight, BMI, percentage body fat, household size, parent education, physical activity expenditure, or family income. The sample represents a fairly affluent group: 75% of the participants’ families reported an income of >$50 000/y.

The consumption of milk and milk products, meat and meat alternatives, cereals and grains, and fats and sweets did not differ significantly between the low and high fruit and vegetable consumption groups, with respect to the reported intake (Table 2). The energy-adjusted nutrient intakes for the high and low fruit and vegetable consumption groups are shown in Table 3. The report of higher fruit and vegetable intakes reflects higher nutrient intakes of vitamin A, vitamin C, potassium, and magnesium. There were no significant differences between the 2 groups in calcium, phosphorus, vitamin D, or protein intakes, nor were there significant differences in sodium contained in the 24-h urine sample. When the percentage of subjects in the low and high fruit and vegetable consumption groups who met the estimated average requirement or the adequate intake for the respective nutrients were compared, the low-consumption group had significantly fewer persons reporting adequate intake of vitamin C and calcium, but no differences for the other nutrients. Overall, both fruit and vegetable consumption groups met the estimated average requirement for the macronutrients but fell short for calcium, phosphorus, magnesium, and vitamin A.

**Bone assessments**

Compared with the low-consumption group, the high fruit and vegetable consumption group had a 6% and 8.3% larger bone area of the whole body (P = 0.03) and of the wrist (P = 0.03), respectively. The BMC of the whole body was 7.4% larger (P = 0.07) and that of the wrist was 7.0% larger (P = 0.09) in the high-consumption group than in the low-consumption group (Figure 1). BMD of the whole body did not differ significantly between the low- and high-consumption groups (0.712 ± 0.005 and 0.719 ± 0.006 mg/cm 2 , respectively; P > 0.05). Similar results were obtained for BMD of the wrist (low-consumption group: 0.389 ± 0.005 mg/cm 2; high-

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**Table 1**

<table>
<thead>
<tr>
<th>Consumption groups</th>
<th>Low (n = 22)</th>
<th>High (n = 34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>10.3 ± 0.3</td>
<td>9.9 ± 0.2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>139.7 ± 2.1</td>
<td>141.0 ± 1.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>38.2 ± 1.5</td>
<td>37.7 ± 1.5</td>
</tr>
<tr>
<td>BMI (kg/m 2)</td>
<td>19.5 ± 0.6</td>
<td>18.8 ± 0.6</td>
</tr>
<tr>
<td>Percentage body fat (%)</td>
<td>29.9 ± 1.9</td>
<td>28.9 ± 1.5</td>
</tr>
<tr>
<td>Residents in household (n)</td>
<td>4.3 ± 0.3</td>
<td>4.7 ± 0.2</td>
</tr>
<tr>
<td>Education of parent (y)</td>
<td>14.4 ± 0.7</td>
<td>16.0 ± 0.6</td>
</tr>
<tr>
<td>Physical activity (kcal)</td>
<td>559 ± 60</td>
<td>598 ± 48</td>
</tr>
<tr>
<td>Family income (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;$30 000</td>
<td>5.4</td>
<td>1.8</td>
</tr>
<tr>
<td>$30 000–49 999</td>
<td>3.6</td>
<td>8.9</td>
</tr>
<tr>
<td>$50 000–69 999</td>
<td>14.3</td>
<td>14.3</td>
</tr>
<tr>
<td>$70 000–49 999</td>
<td>14.3</td>
<td>32.1</td>
</tr>
</tbody>
</table>

7 There were no significant differences between consumption groups. Low consumption, <3 servings/d; high consumption, ≥3 servings/d.

2 SE.
URINARY CALCIUM

Grain products

The difference in 24-h urinary calcium concentrations between the 2 consumption groups after adjustment for age, physical activity, and BMI is graphically depicted in Figure 2. There were no significant differences in urinary calcium/kg body wt (P < 0.05). Differences between the 2 groups were also found when urinary calcium was corrected for creatinine (low-consumption group: 0.37 ± 0.04 mmol/L; high-consumption group: 0.27 ± 0.03 mmol/L; P < 0.02).

Parathyroid hormone and 25-hydroxyvitamin D

PTH concentrations were higher in the low fruit and vegetable consumption group than in the high-consumption group (Figure 2). There were no significant differences in 25(OH)D stores between the 2 groups (low-consumption group: 26.1 ± 1.5 ng/mL; high-consumption group: 29.4 ± 1.5 ng/mL). Adjustment for age, BMI, and physical activity did not change the results.

Biomarkers of bone turnover

Deoxypyridoline did not differ significantly between the low (20.2 ± 1.3 nmol/mL) and high (21.7 ± 0.9 nmol/mL) fruit and vegetable consumption groups (P > 0.05). Nor did osteocalcin differ significantly between the low (34 ± 2.4 ng/mL) and high (24.8 ± 1.9 ng/mL) fruit and vegetable consumption groups (P > 0.05).

DISCUSSION

Early pubertal girls who reported consuming fruit and vegetables ≥3 times a day had, when compared with similar girls who reported consuming fruit and vegetables <3 times a day, lower urinary calcium excretion, lower PTH concentrations, and larger bone size as indicated by bone area of the whole body and of the nondominant wrist after control for age, BMI, and physical activity. The girls with high fruit and vegetable intakes reported higher concentrations of potassium, magnesium, vitamin A, and vitamin C than did those who had low fruit and vegetable intakes, and there was no difference between the 2 groups in protein, phosphorus, and calcium intakes after adjustment for differences in caloric intake. These results suggest that a diet high in fruit and vegetable intakes may be important to the developing skeleton, especially as related to bone size.

Diet, metabolic acidosis, and buffering

Diet in Westernized countries are characterized as rich in meat products and lower in fruit and vegetables than is recommended for optimal health. The net metabolic acid load of these diets is considered to produce larger quantities of acid, as indicated by the bicarbonate and pH of the blood (27). In humans with normal kidney function, the acid-base balance is dependent on the ability of the kidney to excrete excess acid and the availability of a base for buffering (28). Fruit and vegetables provide a natural source of base to buffer the acid produced by other dietary components. In the acute phase, potassium and sodium contained in the bone-fluid barrier are the first line of defense for buffering metabolic acidosis, and thus they spare the bone tissue (28). In a chronic state of metabolic acidosis, bone crystals are dissolved to provide calcium, carbonate, and citrate for buffering (28). Much of the work to support these observations has been done in adults or animal models (29, 30). In contrast, different assumptions may be required about the role of bone and nutrient intake in modulating the acid-base balance in children.

Skeletal effects

In the growing skeleton, increases in BMC should parallel increases in bone area, with modest changes in BMD (31). Within 3–6 y after cessation of longitudinal growth, the secondary consolidation of bone mineral should be complete (32). It is only at this point or when peak bone mass is reached that differences in bone area, BMC, and BMD due to dietary influences such as high fruit and vegetable consumption can be realized. Factors that can affect the skeletal size at any point in time during puberty include Tanner stage of sexual maturation.
genetics, and environmental influences (33). To control for nondifferential error attributed to these influences, we included chronologic age, physical activity, and BMI in all of the analyses.

Our cross-sectional analyses imply that high fruit and vegetable consumption may also affect BMC. Because the hydration of bone tissue is proportional to the age of the bone and is a primary determinant of the chemical reactivity of bone (29, 30), there may be an increased capacity in children to buffer acid loads without affecting the density of bone tissue, but that capacity may limit the accrual of BMC and ultimately BMD at peak bone mass. This would be in accord with adult studies linking diets with higher fruit and vegetable intakes to greater bone density (7–12). In contrast with our study, Jones et al (15) linked urinary potassium as a surrogate for fruit and vegetable intakes to greater bone density of the hip, lumbar spine, and whole body in prepubertal children, but they did not report on bone size or BMC. Differences that may have contributed to the disparate findings include a larger sample size of boys and girls residing in Tasmania, pubertal status, calcium-replete status, and the use of urinary potassium excretion as a surrogate for fruit and vegetable intakes.

**Urinary excretion**

One consequence of greater endogenous acid production is an increase in calcium excreted in the urine (6, 13, 14, 27). However, sodium excretion has been reported to be a significant determinant of calcium excretion in children (2, 3, 34). In both our study and that of Jones et al (15), there were no differences in 24-h urinary sodium concentrations between the groups according to fruit and vegetable consumption groups (ie, high or low consumption) or urinary potassium excretion.

Dietary protein was shown to increase the excretion of calcium in adults (35–38). The primary mechanism is through the deamination of nitrogen during protein metabolism, which yields a higher metabolic acid load. Hydrogen ions are also derived from oxidation of sulfur-containing methionine and

### Table 3

Energy-adjusted nutrient intakes according to fruit and vegetable consumption

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Consumption groups</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Low (n = 22)</td>
</tr>
<tr>
<td></td>
<td>Intake</td>
</tr>
<tr>
<td>Protein (g/kg)</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>300 ± 7.5</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>85.9 ± 2.5</td>
</tr>
<tr>
<td>Vitamin A (µg RAE)</td>
<td>489 ± 79</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>53.6 ± 8.8</td>
</tr>
<tr>
<td>Vitamin D (IU)</td>
<td>109 ± 20</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>778 ± 59</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>732 ± 61</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>1306 ± 125</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>128 ± 11</td>
</tr>
<tr>
<td>Caffeine (mg)</td>
<td>32.6 ± 4.7</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>2562 ± 222</td>
</tr>
</tbody>
</table>

*Fructose-adjusted daily energy intake (kcal/kg); EAR, estimated adequate requirement; AI, adequate intake; DRI, dietary reference intake; RAE, retinol activity equivalent. Low consumption, <3 servings/d; high consumption, ≥3 servings/d.

3 Refers to EAR or AI where appropriate.

4,5 Significantly different from low-consumption group: 4 P < 0.05 (Student’s t test), 6 P < 0.05 (chi-square test).

5 EAR.

6 AI.

7 From a 24-h urine sample.
cysteine. Because our nutrient analysis program did not specify the amino acid content of the diets, we cannot exclude the possibility that there were differences in sulfur-containing amino acids between the 2 consumption groups that contributed to differences in urinary calcium excretion or bone size.

Dietary potassium has been implicated as a calcium-sparing nutrient because of its ability to buffer metabolic acid load through either supplements (39, 40) or diet (14, 36). Whether this effect is due to the potassium content of the diet or is secondary to higher fruit and vegetable intakes cannot be ascertained from our study.

Indicators of bone metabolism

Feeding studies have shown that consuming an alkaline diet inhibits bone resorption (14, 41) in adults, whereas in vitro studies show that osteoclastic activity mobilizes bone calcium to buffer metabolic acid loads (42). The lack of association between bone turnover in our 2 fruit and vegetable consumption groups may be due to a cross-sectional study design, insufficient sample size, or measurement error associated with diet-assessment techniques.

Diet assessment

Adequate representation of the diet is critical to the underlying assumptions of this research. We averaged 3 independent days of intake over a 24-mo period to incorporate seasonal and weekday variations to obtain a better estimate of usual intake. We controlled for this potential bias of underreporting of fruit and vegetable intake in the low-consumption group by adjusting for energy in our statistical analyses of nutrient intakes. Because fruit and vegetable intake were quantified as a unit when reported on the food records, we could not exclude the possibility that serving size may make a difference in the ability to buffer acid load, and our results may have been attenuated. The report of similar intakes of each food group except fruit and vegetables by the 2 consumption groups suggests that differences in the quality of the diet are factors mitigating the lower urinary calcium excretion and larger bone size.

Study limitations

Whereas our study does provide support that consistent intakes of fruit and vegetables may be beneficial to bone mass in early pubertal girls, it does have limitations. This study was cross-sectional, and thus we cannot confer causality between diet and bone mass. The sample was small—limited to 56 white girls from affluent families—and thus our results cannot be generalized to all ethnic or socioeconomic groups. The study included only one 24-h urine sample, which raises the possibility that excretion of urinary calcium and sodium could be underestimated or overestimated (43).

Summary

In summary, high fruit and vegetable intakes have beneficial effects on bone size of the radius and whole body in early-pubertal white girls after control for age, BMI, and physical activity. The lower urinary calcium output and serum PTH associated with higher fruit and vegetable intakes may be a modulating factor. However, cumulative effects on bone mass accrual and peak bone mass remain to be determined.

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