Urinary Citrate, an Index of Acid-Base Status, Predicts Bone Strength in Youths and Fracture Risk in Adult Females

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Context: Diet can impact on bone strength via metabolic shifts in acid-base status. In contrast to the strongly diet-dependent biomarker urinary potential renal acid load (uPRAL), the amount of citrate excreted renally integrates nutritional and systemic influences on acid-base homeostasis, with high citrate indicating prevailing alkalization.

Objective: To examine the association between urinary citrate excretion and bone strength as well as long-term fracture risk.

Design and Participants: Prospective cross-sectional analysis; 231 healthy children (6–18 y) of the Dortmund Nutritional and Anthropometric Longitudinally Designed Study were included, with at least 2 urine collections available during the 4 years preceding peripheral quantitative computed tomography (pQCT) of the nondominant proximal forearm. uPRAL, urinary citrate, and urinary nitrogen excretion were quantified in 857 24-hour urine samples. Data on overall fracture incidence were collected within a 15-year follow-up after pQCT measurement.

Main Outcome Measures: Parameters of bone quality and geometry (pQCT) as well as long-term fracture incidence.

Results: After controlling for confounders, especially forearm length, muscle area, and urinary nitrogen (biomarker of protein intake), urinary citrate excretion was positively associated with various parameters of bone quality and geometry (P < .05). Fracture risk in adult females, but not in males, was inversely associated with urinary citrate and positively with uPRAL (P < .05).

Conclusions: Although urinary citrate has to be confirmed as an integrated noninvasive biomarker of systemic acid-base status in further studies, our results substantiate dietary and metabolic acid as potentially adverse for bone health in the long run from childhood onward. (J Clin Endocrinol Metab 101: 4914–4921, 2016)

Changes in acid base status towards a clinically relevant lower blood pH, eg, due to renal tubular or metabolically induced acidosis, can lead to elevated bone resorption and bone loss (1–3). Also, in healthy individuals, in whom systemic acid base status is predominantly influenced by dietary acid load and usual endogenous metabolic acid production, small shifts to a more acidic status can affect bone status in the long run. A typical Western diet, eg, characterized by a high protein and comparatively low fruit and vegetable intake, already causes a mild acid overload also referred to as subclinical or low-grade metabolic acidosis with blood pH and blood bicarbonate levels still in the normal range (4, 5). This mild acidification is discussed to adversely affect bone quality over time (4).
Subjects and Methods

Study population
For the present analyses, all 371 healthy participants (aged 6-18 y) of the DONALD Study were eligible (7), who underwent a 1-time peripheral quantitative computed tomography (pQCT) bone measurement (8, 9). Of those, all subjects were included, with at least 2 (out of 5 possible) 24-hour urine samples collected during the 4-year observation period before bone analysis (234 children). Because 2 subjects with appropriate urine collections lay outside the accepted birth weight range from 2,300 to 5,000 g and 1 showed a particularly high uPRA of 50 mEq/d (exceeding 3 SD of the remaining sample), the herein analyzed subcohort included 231 children and adolescents (113 boys). For subsequent analyses of long-term fracture risk prediction (15 y after bone measurement), 159 DONALD participants were eligible with both available: 24-hour urine samples and a filled in questionnaire on bone fracture. An overview of the time schedule of the study is given in Figure 1.

The DONALD Study, conducted according to the guidelines laid down in the Declaration of Helsinki, was approved by the ethics committee of the University of Bonn and the additional pQCT measurement was approved by the ethics committee of the medical faculty of the University of Cologne and the German Federal Office for Radiation Protection (Salzgitter, Germany). Written parental consent and (in older children) the child's assent were obtained both before entry into the DONALD Study and before participation in pQCT measurement.

Measurements
pQCT analysis of the nondominant forearm was conducted as described in detail previously (9, 22, 23) using an XCT-2000 device (Stratec, Inc) equipped with a low-energy (38 keV) x-ray tube. In short, in each participant the scanner with an effective radiation of approximately 0.1 µSv was placed at a distance from the ulnar styloid process of 65% of the forearm length. A 2-mm-thick single tomographic slice was sampled at a voxel size of 0.4 mm, along with the cross-sectional forearm muscle area at 65% of the ulnar length (for further specifications, see Refs. 22, 23).

Anthropometric measurements were performed by trained and regularly monitored nurses according to standard procedures. For this, subjects were barefoot and dressed in underwear. Height was measured with a digital stadiometer to the nearest 0.1 cm.

Figure 1. Chronology of urine sample collection, bone measurement, and bone fracture assessment. A total of 231 participants (113 boys, 6-18 y) of the DONALD Study with a peripheral quantitative computed tomography (pQCT) bone measurement at TO and at least 2 out of 5 possible 24-hour urine collections during the preceding 4-year period. A total of 159 (73 boys) DONALD participants with available questionnaire on bone fracture during the 15-year period from TO onward and at least 2 out of 5 possible 24-hour urine collections before TO.

6, 7). Studies on urinary potential renal acid load (uPRA), a biomarker reflecting diet-dependent acid balance, have prospectively confirmed inverse associations of nutritional acidity with diaphyseal bone parameters in healthy children (8, 9). In line with this, neutralization of a Western diet-induced mild acidotic state by subtle elevations in blood bicarbonate levels exerts beneficial effects on bone (7, 10, 11). Numerous other, but not all (12), studies have also found positive associations between an alkaline diet and bone health (13), indicating that a more alkaline milieu is beneficial for bone. However, to our knowledge, studies examining the association of bone heath with a relatively easily measurable biomarker of overall systemic acid base status have not been performed yet.

In the present study, we examined urinary citrate excretion as a new integrative biomarker for the diet- and metabolism-dependent systemic acid-base status. So far, predominantly known as an important inhibiting factor of kidney stone formation (14), urinary citrate excretion also enables a noninvasive and indirect view on renal acid load and metabolism (15). As known for decades, due to its rapid metabolism after absorption in the gut, urinary citrate excretion is independent of dietary citric acid ingestion but not independent of an alkalizing salt (eg, citric acid salt) intake (16). Sodium, potassium, magnesium, and calcium salts of citric acid are alkalizing, whereas citric acid itself has no effect on acid base balance.

Because circulating citrate is freely filtered in the glomerulus and citrate secretion is negligible, alterations in urinary citrate excretion are almost solely caused by changes in renal citrate reabsorption, which is strongly influenced by systemic acid-base status and urinary pH (15, 17, 18). For example, an ingestion of an alkali load of 120 mEq/d (ie, 10-g NaHCO₃) results in an increase of urinary citrate excretion of about 70% (16). Lower intracellular or luminal pH values in the kidney result in higher renal citrate reabsorption and hence in lower citrate excretion either reflecting a prevailing acidosis (17, 19, 20) or a shift to a more acidic condition.

Therefore, the aim of the present study was to use and potentially establish urinary citrate excretion as a new and, compared with uPRA, more integrated (not primarily diet-dependent) biomarker of systemic acid-base status. For this, we examined in healthy participants of the Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD) Study the relationships of urinary citrate: 1) with other urinary indicators of acid base status, 2) with parameters of bone geometry and quality, and 3) with long-term fracture risk.
Cm, and weight was measured with an electronic scale to the nearest 0.1 kg. Tanner stages were determined by a study pediatrician. Based on pubic hair stage as a clinical marker of the beginning of adrenal androgen secretion, the subjects were assigned to a pubescent and a prepubescent group. The 24-hour urine collections, scheduled in yearly intervals in the DONALD Study, were performed at home under standardized conditions (24), and samples were stored at −20°C or below until analyzed. Citrate (mmol/L) was measured with an enzymatic method based on the conversion of citrate via oxaloacetate to L-malate and L-lactate by a citrate kit from Boehringer Mannheim according to the principle described by Moellerling and Gruber (25).

Urinary nitrogen (un) was measured by the Kjeldahl technique (Buechi 430 Digestor and Buechi Distillation unit B-324; Büchi Labotechnik) and creatinine by the kinetic Jaffé procedure on a creatinine analyzer (Beckmann-2; Beckman Instruments, Inc) in a total of 857 24-hour urine samples. Urinary net acid excretion (NAE) was calculated as titratable acid + ammonium minus bicarbonate, which were quantified in freshly, thawed aliquots of the 24-hour urine samples according to the titration method of Luthy et al (26). uPRAL reflecting urinary NAE without its organic acid component (27) was calculated as follows:

\[(\text{chloride (mmol/d)} + \text{sulfate (mmol/d)} \times 2 + \text{phosphate (mmol/d)} \times 1.3) - (\text{sodium (mmol/d)} + \text{potassium (mmol/d)} + \text{magnesium (mmol/d)} \times 2 + \text{calcium (mmol/d)} \times 2) \times 28, 29).\]

Multiplication by the respective ionic valences converts the ion excretions in mmol/d to charge quantities in milliequivalents per day (mEq/d).

The cations sodium, potassium, magnesium, and calcium were measured by flame atomic absorption spectrometry (PerkinElmer 1100 Spectrometer; PerkinElmer GmbH), and the anions chloride, phosphate, and sulfate were measured by Dionex 2000i/SP ion chromatography with an ion Pac AS9A column (Dionex GmbH).

Fracture incidence after the pQCT measurement was assessed by a questionnaire, which included information on each fracture that occurred, its localization, and particular accident circumstances.

Renal acid excretion capacity (RAEC), which is the amount of net acid per day, individual excretes or is able to excrete at a given pH, was defined as the residual of a linear regression of 24-hour renal NAE on 24-hour urine pH (30). Here, residuals denote the proportion of variance that is not explained by the independent variable, i.e., a positive residual indicates that a higher than average amount of net acid is excreted at a given pH.

Statistical analysis

SAS procedures (version 9.1; SAS Institute, Inc) were used for data analysis. Sex- and age-standardized data were derived for urinary parameters by Z-transformation. For this, mean and SD were calculated for separate age groups (covering 2-9 age intervals [5–6 y, 7–8 y, etc]). Individual Z scores for all available measurements of the respective urine analyte were then derived against these age group-specific mean and SD values (mean = 0; SD = 1). For each subject, the arithmetic mean of 2–5 individual 24-hour urinary analyte Z scores (the means of nontransformed 24-h excretion rates) were calculated. Descriptive data are presented as median (1st and 4th quartile).

Main effects of sex and puberty status on anthropometric, bone, and urinary variables were tested with two-way ANOVA.

All nonnormally distributed outcome variables [bone mineral content (BMC), bone mineral density (BMD), cortical area (CA), strength strain index (SSI), periosteal circumference, and endosteal circumference] were log10 transformed for subsequent analyses. An initial analysis of covariance was performed to test for interactions between urinary citrate or uPRL and sex or developmental group. Because both kinds of interaction (i.e., urinary variable-by-sex and urinary variable-by-development group) were nonsignificant (P value each >.1) for all bone variables studied, no correspondingly stratified analysis was done and both sexes were combined.

Partial correlation was used to first investigate roughly the association between urinary citrate excretion and its main predictors. The final analyses of the relationship between biomarker of acid-base status as predictors (i.e., urinary citrate excretion or uPRL) and parameters of bone status as outcome were done by multiple linear regressions (Proc GLM). Each biologically plausible covariate or confounder was initially considered stepwise and included, if the predictor-outcome association was substantially modified (i.e., if changes of the β-coefficients of the predictor variables [citrate or uPRL] were >10%) and/or if the covariate had its own significant fixed effect (P <.05). The following potential confounders were considered, but not included by default in the model: age, sex, pubertal stage, growth velocity, fat mass index, forearm muscle area, forearm length, and urine volume as well as the excretions of urinary sodium, calcium, nitrogen, and creatinine.

Logistic regression (Proc Logistic) was used to examine the influence of urinary citrate on long-term fracture risk. Important potential confounders were tested and adjusted for, if they significantly modified regression coefficients in the basic model by more than 10%, had their own significant and independent effect on the outcome variable, or led to an improvement of the Akaike Information Criterion. Analyses were performed sex-stratified as girls had substantially less fractures than boys. P < .05 was considered significant in all analyses.

Results

A general description of the study sample with characteristics at bone analysis, average dietary intakes and 24-hour urine excretion data during the 4-year time period before bone measurement is given in Table 1. Gross correlations between citrate excretion in 24-hour urine and nutritional (uPRL), functional (RAEC), and metabolic (urinary pH) parameters of acid base status are shown in Table 2. Adjusted for 1 important confounder, to eliminate the influence of a second parameter, which is related to acid-base status, urinary citrate showed positive correlations with the kidney's function to eliminate protons (RAEC) and the 24-hour urine pH, and an inverse correlation with uPRL.

Long-term urinary citrate excretion was significantly positively associated with BMC, SSI, CA, and periosteal circumference but not with BMD (Table 3). Less clear associations were seen for the biomarker of dietary acidity uPRL, which was inversely associated only with BMC and CA. After inclusion of uPRL or NAE together with
Table 1. Anthropometric and Diaphyseal Characteristics of the Study Population of 231 Children and Adolescents at the Time of pQCT Bone Analysis and Average 24-Hour Urine Excretion Data and Dietary Intake Data During the 4 Years Before pQCT Measurement

<table>
<thead>
<tr>
<th>Characteristics at bone analysis</th>
<th>Boys (n = 113)</th>
<th>Girls (n = 118)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>11.1 (8.2, 13.5)</td>
<td>11.0 (8.0, 13.5)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>43.1 (28.9, 55.8)</td>
<td>38.6 (27.2, 55.6)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>152 (135, 167)</td>
<td>151 (130, 161)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>18.0 (15.9, 19.7)</td>
<td>18.1 (15.4, 20.6)</td>
</tr>
<tr>
<td>Fat mass index (kg/m²)</td>
<td>2.4 (1.9, 3.3)</td>
<td>3.1 (2.3, 4.2)</td>
</tr>
<tr>
<td>Body surface area (m²)</td>
<td>1.4 (1.1, 1.6)</td>
<td>1.4 (1.0, 1.6)</td>
</tr>
<tr>
<td>BMD (mg/mm)</td>
<td>54.5 (44.5, 64.3)</td>
<td>54.3 (38.6, 70.3)</td>
</tr>
<tr>
<td>BMC (mg/cm³)</td>
<td>1019 (993, 1053)</td>
<td>1037 (986, 1083)</td>
</tr>
<tr>
<td>Polar bone SSI (mm³)</td>
<td>188 (137, 241)</td>
<td>166 (118, 225)</td>
</tr>
<tr>
<td>Endosteal circumference (mm)</td>
<td>23.6 (20.9, 26.6)</td>
<td>21.9 (19.6, 24.9)</td>
</tr>
<tr>
<td>Periosteal circumference (mm)</td>
<td>35.5 (31.8, 38.2)</td>
<td>33.8 (31.3, 37.0)</td>
</tr>
<tr>
<td>CA (mm²)</td>
<td>53.6 (43.8, 62.4)</td>
<td>52.2 (39.0, 65.3)</td>
</tr>
<tr>
<td>Muscle area (mm²)</td>
<td>2320 (1947, 2918)</td>
<td>2206 (1674, 2668)</td>
</tr>
<tr>
<td>Forearm length (cm)</td>
<td>23.9 (20.8, 26.1)</td>
<td>23.5 (19.8, 24.9)</td>
</tr>
<tr>
<td>Dietary variables (daily intakes)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake (kcal)</td>
<td>1757 (1515, 2013)</td>
<td>1551 (1323, 1817)</td>
</tr>
<tr>
<td>Protein intake (g/d)</td>
<td>56.0 (46.2, 65.5)</td>
<td>48.2 (40.5, 57.6)</td>
</tr>
<tr>
<td>Protein intake (g/d · kg⁻¹ body weight)</td>
<td>1.82 (1.50, 2.08)</td>
<td>1.65 (1.38, 1.99)</td>
</tr>
<tr>
<td>Calcium intake (mg/d)</td>
<td>824 (690, 1024)</td>
<td>712 (601, 881)</td>
</tr>
<tr>
<td>Calcium intake (mg/d · kg⁻¹ body weight)</td>
<td>27.5 (21.9, 33.9)</td>
<td>24.8 (19.7, 30.9)</td>
</tr>
<tr>
<td>24-Hour urine excretion data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine volume (ml)</td>
<td>679 (553, 873)</td>
<td>629 (510, 860)</td>
</tr>
<tr>
<td>Creatinine (mmol/d)</td>
<td>5.5 (3.7, 6.9)</td>
<td>4.9 (3.2, 6.7)</td>
</tr>
<tr>
<td>Nitrogen (mmol/d)</td>
<td>522 (421, 633)</td>
<td>434 (367, 573)</td>
</tr>
<tr>
<td>Nitrogen (mmol · d⁻¹ · 1.73 m²)</td>
<td>624 (722, 932)</td>
<td>715 (617, 823)</td>
</tr>
<tr>
<td>Calcium (mmol/d)</td>
<td>1.1 (0.8, 2.0)</td>
<td>1.2 (0.8, 2.1)</td>
</tr>
<tr>
<td>Citrate (mmol/d)</td>
<td>1.5 (1.1, 2.2)</td>
<td>1.5 (1.1, 2.3)</td>
</tr>
<tr>
<td>uPRAL (mEq/d)</td>
<td>5.0 (-0.3, 9.6)</td>
<td>1.3 (-4.3, 6.1)</td>
</tr>
<tr>
<td>uPRAL (mEq · d⁻¹ · 1.73 m²)</td>
<td>7.6 (-0.5, 16.4)</td>
<td>2.0 (-7.0, 9.5)</td>
</tr>
<tr>
<td>NAE (mEq/d)</td>
<td>38.2 (29.1, 47.8)</td>
<td>29.8 (23.3, 39.2)</td>
</tr>
<tr>
<td>NAE (mEq · d⁻¹ · 1.73 m²)</td>
<td>60.4 (48.2, 69.1)</td>
<td>49.7 (42.4, 55.5)</td>
</tr>
</tbody>
</table>

Data are presented as median (1st, 4th quartile).

In the present study, we used citrate measurements in repeatedly collected 24-hour urine samples as a new bio-


t are no longer significant (P > .1) (data not shown), confirming that dietary acidity as reflected in the forms of uPRAL or NAE also results in increased renal citrate re-absorption and consequently in a down-regulation of citrate excretion.

Logistic regressions revealed that in girls, but not in boys, 24-hour urinary citrate excretion during childhood and adolescence was prospectively and inversely associated with the fracture risk during the subsequent 15-year period after pQCT bone analysis (Table 4). A comparable, but not inverse association, emerged for uPRAL. Additional inclusion of potential confounders, e.g., of total uN (as biomarker of protein intake), did neither improve the models, nor changed the β-value of the predictor or showed significant own effects.

**Discussion**

In the present study, we used citrate measurements in repeatedly collected 24-hour urine samples as a new bio-

**Table 2. Gross Correlations Between Raw 24-Hour Urinary Citrate Excretion and Its Main Determining Factors, Each Relationship Adjusted for 1 Important Confounder**

<table>
<thead>
<tr>
<th>Citrate Excretion (mmol/d)</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>uPRAL*</td>
<td>-0.37</td>
<td>.0001</td>
</tr>
<tr>
<td>RAEC*</td>
<td>0.63</td>
<td>.0001</td>
</tr>
<tr>
<td>urinary pH*</td>
<td>0.33</td>
<td>.0001</td>
</tr>
</tbody>
</table>

* uPRAL was adjusted for RAEC to exclude grossly the influence of kidney's acid excretion function on citrate excretion.
* RAEC was adjusted for uPRAL to exclude specifically the influence of diet-dependent acid load on citrate excretion.
* Urinary pH was adjusted for NAE to examine particularly the influence of urinary free protons on citrate excretion independently of total daily acid load.
Urinary acid-base status substantiates dietary and metabolic influences on bone health in the long run. The fact that the prospective relationships between dietary acidity and bone outcomes (i.e., parameters of bone quality and bone geometry) and the (anabolic and catabolic) biomarkers of acid base status are similar for citrate and uPRAL underlines the importance of nutrition, which is more closely reflected by PRAL. During clinical metabolic acidosis with reduced blood bicarbonate and pH levels below 7.35, systemic endocrine changes, e.g., decreased serum IGF-1 levels (31, 32) and increased glucocorticoid secretion (33, 34), lead to a disadvantageous endocrine-metabolic milieu for bone and promote bone loss (2, 3, 35, 36). In subtle acidosis, inducible already by common diet modifications, these endocrine and metabolic changes are observable as well, however, in an attenuated form with still unfavorable effects for bone health in the long run (11, 37). Nevertheless, the impact on bone of only slight shifts towards a more acidic status is discussed controversially (12). One major reason why a number of studies did not find significant associations between dietary acidity and bone outcomes is, among others, that protein intake and its confounding anabolic effect on bone is usually not considered (6, 38). Protein intake acts as a strong confounder of the acid-base-bone relationship and even as a strong confounder of the glucocorticoid-bone relationship, thereby masking dietary acidity’s (9) as well as cortisol or cortisone’s (37) influence on bone function. Also the other way round, the anabolic protein effect on bone is often not, or only to some extent, identified (39) probably due to the nonconsideration of counterbalancing acid-base action. Thus, with regard to studies of rather mild (but in the long-term important) influences of nutrition or acid base balance on measures of bone densitometry, not only valid dietary record data of good quality but also the allowance of protein intake as a major confounder is required (6, 8, 9). Additionally, to uncover these rather mild influences, important confounders have to be con-

Table 3. Prospective Associations of Acid-Base Status, as Assessed by Repeated Measurements of the Biomarkers 24-Hour Urinary Citrate or uPRAL, With Diaphysal Parameters of Bone Quality and Geometry at the Proximal Radius in Healthy Children, and Adolescents

<table>
<thead>
<tr>
<th>Bone Quality</th>
<th>Bone Mineral Density (mg/cm²) [log 10]</th>
<th>Strength-Strain Index (mm²) [log 10]</th>
<th>Bone Geometry</th>
<th>Endostelial Circumference (mm) [log 10]</th>
<th>Periostael Circumference (mm) [log 10]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone Mineral Content (mg/mm) [log 10]</td>
<td>β</td>
<td>SE</td>
<td>p</td>
<td>β</td>
<td>SE</td>
</tr>
<tr>
<td>Citrate</td>
<td>0.02</td>
<td>0.007</td>
<td>.2</td>
<td>0.002</td>
<td>0.006</td>
</tr>
<tr>
<td>uPRAL</td>
<td>-0.02</td>
<td>0.01</td>
<td>.4</td>
<td>-0.002</td>
<td>0.001</td>
</tr>
<tr>
<td>Results are from multiple linear regression analyses (adjusted for age, sex, pubertal stage, forearm muscle area, forearm length, urinary calcium excretion, and BMI).</td>
<td></td>
<td></td>
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</tr>
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</table>

Furthermore, the present findings on urinary citrate excretion as an integrated noninvasive biomarker of systemic acid-base status substantiates dietary and metabolic acidity as potentially adverse for bone health in the long run from childhood onward.

The fact that the prospective relationships between bone outcomes (i.e., parameters of bone quality and geometry as well as bone fractures) and the (anabolic and catabolic) biomarkers of acid base status are similar for citrate and uPRAL underlines the importance of nutrition, which is more closely reflected by PRAL. During clinical

Table 4. Twenty-Four-Hour Citraturia and uPRAL as Predictors of Long-Term Fracture Risk in Boys and Girls

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Fractures/No Fractures</th>
<th>Odds Ratio (95% Confidence Interval)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys, n = 73</td>
<td>24-h citraturia</td>
<td>31/42</td>
<td>0.94 (0.52–1.71)</td>
</tr>
<tr>
<td></td>
<td>uPRAL</td>
<td>31/42</td>
<td>1.63 (0.64–4.17)</td>
</tr>
<tr>
<td>Girls, n = 86</td>
<td>24-h citraturia</td>
<td>25/61</td>
<td>0.47 (0.22–0.99)</td>
</tr>
<tr>
<td></td>
<td>uPRAL</td>
<td>25/61</td>
<td>2.53 (1.02–6.28)</td>
</tr>
</tbody>
</table>

Adjusted for renal calcium excretion and maternal birth age.
largely determined sex dimorphism may mask this association for males.

A limitation of our study is the lack of repeated pQCT measurements. The association of acid-base status and bone health could therefore not be examined with regard to changes in the predictor and outcome variables. Another limitation is that due to a comparatively small sample size all types of fractures that occurred during the follow-up were included in the analysis, among them also fractures of small bones (eg, of hands and feet), which are less likely to reflect bone density than long bone and vertebral fractures. Moreover, potential confounders (eg, individual genetic and/or endocrine-metabolic influences) may also impact on 24-hour citraturia and its association with urine pH and citrate excretion.

In summary, the presented data confirm together with the physiological and pathophysiological data from the literature, the strong potential of urinary citrate as an integral and noninvasive biomarker of systemic acid-base status. Additionally, high urinary citrate excretions during childhood or adolescence do not only predict a stronger bone status but also indicate a lower long-term fracture risk in women. This is in line with results from Jehle et al (41), who reported that alkalinization with potassium citrate increased BMD and reduced fracture risk score in healthy older men and women. In our study on healthy young subjects, a certain endocrine-metabolically determined sex dimorphism may mask this association for males.

The fact that in our study a higher citrate excretion associates with a higher RAEC (reflecting the kidney's ability to excrete acid loads via ammonium at a given level of free proton stimulation) confirms that urinary citrate can be expected to decline with diminished kidney function (47). Accordingly, a person with a good RAEC is rather able to excrete a higher acid load at a given level of free renal protons and does not need to activate the above described metabolic processes associated with enhanced ammoniagenesis, gluconeogenesis and citrate reabsorption (30).

In conclusion, the presented data confirm together with the physiological and pathophysiological data from the literature, the strong potential of urinary citrate as an integral and noninvasive biomarker of systemic acid-base status. Additionally, high urinary citrate excretions during childhood or adolescence do not only predict a stronger bone status but also indicate a lower long-term fracture risk in women. This is in line with results from Jehle et al (41), who reported that alkalinization with potassium citrate increased BMD and reduced fracture risk score in healthy older men and women. In our study on healthy young subjects, a certain endocrine-metabolically determined sex dimorphism may mask this association for males.

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follow-up period of 15 years, in which the association of biomarkers of acid-base status with bone fractures was analyzed. For this, urinary citrate, a new, but also simpler and cheaper, index of systemic acid-base status compared with, eg, NAE was used for the first time.

Conclusion

Urinary citrate excretion used as a new noninvasive, integrated biomarker of systemic acid-base status substantiates potential detrimental influences of acid loads still in the physiological range on bone strength and long-term fracture risk. Compared with almost completely diet-dependent uPRAL, urinary citrate appears to be rather a more integrated predictor of long-term acid-base effects on bone.

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Author contributions: J.E. and T.R. contributed to the statistical analyses, the interpretation of the data, and drafted the manuscript. S.J. and T.R. drafted the questionnaire on bone fractures. L.S. and T.R. contributed to the study design and the statistical analyses. E.S. took responsibility for the pQCT biomechanical measurements. T.R. (principal investigator) was responsible for the manuscript critically for important intellectual content and approved the article’s final version.

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References

28. Remer T, Manz F. Estimation of the renal net acid excretion by


