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 renally excreted citrate 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 acidity 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,

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Abbreviations: BMC, bone mineral content; BMD, bone mineral density; CA, cortical area; DONALD, Dortmund Nutritional and Anthropometric Longitudinally Designed; NAE, net acid excretion; pQCT, peripheral quantitative computed tomography; RAEC, renal acid excretion capacity; SSI, strength strain index; uN, urinary nitrogen; uPRAL, urinary potential renal acid load.

6, 7). Studies on urinary potential renal acid load (uPRAL), a biomarker reflecting diet-dependent acid base 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 health 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 metabolization 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, porassium, 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 $NaHCO_3$) 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 acidotic condition.

Therefore, the aim of the present study was to use and potentially establish urinary citrate excretion as a new and, compared with uPRAL, 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.

Subjects and Methods

Study population

For the present analyses, all 371 healthy participants (aged 6–18 y) of the DONALD Study were eligible (21), 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 2300 to 5000 g and 1 showed a particularly high uPRAL of 50 mEq/d (exceeding 3 SD of the remaining sample), the herein analyzed subcohort included 231 children and adolescents (113 boys). For subsequent analysis 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 on 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

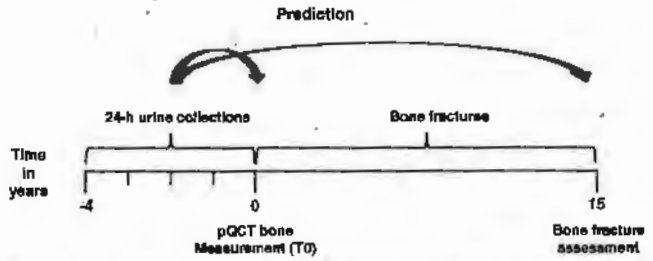

Figure 1. Chronology of urine sample collection, bone measurement, and bone fracture assessment. A total of 231 participants (113 boys, 6–18 y) of the DONALD Study with a peripheral quantitative computed tomography (pQCT) bone measurement at T0 and at least 2 out of 5 possible 24-hour urine collections during the preceding 4-year period. A total of 159 (73 boys) DONALD participants with available questionnaire on bone fractures during the 15-year period from T0 onward and at least 2 out of 5 possible 24-hour urine collections before T0.

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 prepubescence and a pubescence 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 Moellering and Gruber (25).

Urinary nitrogen (uN) was measured by the Kjeldahl technique (Buechi 430 Digestor and Buechi Distillation unit B-324; Büchi Labortechnik) 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 Lüthy et al (26). uPRAL reflecting urinary NAE without its organic acid component (27) was calculated as follows:

(chloride (mmol/d) + sulfate (mmol/d) × 2 + phosphate (mmol/d) × 1.8) − (sodium (mmol/d) + potassium (mmol/d) + magnesium (mmol/d) × 2 + calcium (mmol/d) × 2) (28, 29). Multiplication by the respective ionic valence 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 AS4A 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, an 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, ie, 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-y 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 means and SD values (mean = 0; SD = 1). For each subject, the arithmetic mean of 2–5 individual 24-hour urinary analyte z scores (or the mean 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 uPRAL and sex or developmental group. Because both kinds of interaction (ie, urinary variable-by-sex and urinary variable-by-developmental 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 (ie, urinary citrate excretion or uPRAL) 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 (ie, if changes of the β-coefficients of the predictor variables [citrate or uPRAL] 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 cofounders 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. Aualyses 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 (uPRAL), 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 uPRAL.

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 uPRAL, which was inversely associated only with BMC and CA. After inclusion of uPRAL 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

	Boys (n = 113)	Girls (n = 118)
Characteristics at bone analysis		
Age (y)	11.1 (8.2, 13.5)	11.0 (8.0, 13.5)
Weight (kg)	43.1 (28.9, 55.8)	38.6 (27.2, 55.6)
Height (cm)	152 (135, 167)	151 (130, 161)
Body mass index (kg/m^2)	18.0 (15.9, 19.7)	18.1 (15.4, 20.6)
Fat mass index (kg/m^2)	2.4 (1.9, 3.3)	3.1 (2.3, 4.2)
Body surface area (m^2)	1.4 (1.1, 1.6)	1.4 (1.0, 1.6)
BMD (mg/mm)	54.5 (44.5, 64.3)	54.3 (38.6, 70.3)
BMC (mg/cm^3)	1019 (993, 1053)	1037 (986, 1083)
Polar bone SSI (mm^3)	188 (137, 241)	166 (118, 225)
Endosteal circumference (mm)	23.6 (20.9, 26.6)	21.9 (19.6, 24.9)
Periosteal circumference (mm)	35.5 (31.8, 38.2)	33.8 (31.3, 37.0)
CA (mm^2)	53.6 (43.8, 62.4	52.2 (39.0, 65.3)
Muscle area (mm^2)	2320 (1947, 2918)	2206 (1674, 2668)
Forearm length (cm)	23.9 (20.8, 26.1)	23.5 (19.8, 24.9)
Dietary variables (daily intakes)		
Energy intake (kcal)	1757 (1515, 2013)	1551 (1323, 1817)
Protein intake (g/d)	56.0 (46.2, 65.5)	48.2 (40.5, 57.6)
Protein intake (g/d · kg^{-1} body weight)	1.82 (1.50, 2.08)	1.65 (1.38, 1.99)
Calcium intake (mg/d)	824 (690, 1024)	712 (601, 881)
Calcium intake (mg/d · kg^{-1} body weight)	27.5 (21.9, 33.9)	24.8 (19.7, 30.9)
24-Hour urine excretion data		
Urine volume (mL)	679 (553, 873)	629 (510, 860)
Creatinine (mmol/d)	5.5 (3.7, 6.9)	4.9 (3.2, 6.7)
Nitrogen (mmol/d)	522 (421, 633)	434 (367, 573)
Nitrogen (mmol × d^{-1} × 1.73 m^2)	824 (722, 932)	715 (617, 823)
Calcium (mmol/d)	1.1 (0.8, 2.0)	1.2 (0.8, 2.1)
Citrate (mmol/d)	1.5 (1.1, 2.2)	1.5 (1.1, 2.3)
uPRAL (mEq/d)	5.0 (-0.3, 9.6)	1.3 (−4.3, 6.1)
uPRAL (mEq × d^{-1} × 1.73 m^2)	7.6 (-0.5, 16.4)	2.0 (−7.0, 9.5)
NAE (mEq/d)	38.2 (29.1, 47.8)	29.8 (23.3, 39.2)
NAE (mEq × d^{-1} × 1.73 m^2)	60.4 (48.2, 69.1)	49.7 (42.4, 55.5)

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

urinary citrate in the regression models, the association between urinary citrate and bone status was substantially weakened. Only the association with SSI remained significant, whereas BMC, CA, and periosteal circumference were no longer significant ($P \geq .1$) (data not shown), confirming that dietary acidity as reflected in the forms of uPRAL or NAE also results in increased renal citrate reabsorption and consequently in a down-regulation of citrate excretion.

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

	Citrate Excretion (mmol/d)	
	r	*P*
uPRAL[a]	−0.37	.0001
RAEC[b]	0.63	.0001
urinary pH[c]	0.33	.0001

[a] uPRAL was adjusted for RAEC to exclude grossly the influence of kidney's acid excretion function on citrate excretion.

[b] RAEC was adjusted for uPRAL to exclude specifically the influence of diet-dependent acid load on citrate excretion.

[c] Urinary pH was adjusted for NAE to examine particularly the influence of urinary free protons on citrate excretion independently of total daily acid load.

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, eg, 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 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

	Bone Quality									Bone Geometry								
	Bone Mineral Content (mg/mm) [log 10]			Bone Mineral Density (mg/cm³) [log 10]			Strength Strain Index (mm³) [log 10]			Cortical Area (mm²) [log 10]			Endosteal Circumference (mm) [log 10]			Periosteal Circumference (mm) [log 10]		
	β	SE	P	β	SE	P	β	SE	P	β	SE	P	β	SE	P	β	SE	P
Citrate	0.02	0.007	.02	0.002	0.002	.2	0.02	0.006	.006	0.01	0.006	.02	0.005	0.006	.4	0.005	0.003	.04
uPRAL	−0.02	0.01	.043	−0.003	0.002	.13	−0.01	0.01	.2	−0.02	0.008	.046	0.007	0.009	.4	0.0005	0.003	.9

Results are from multiple linear regression analyses (adjusted for age, sex, pubertal stage, forearm muscle area, forearm length, urinary calcium excretion, and uN).

marker of systemic acid base status and could show for the first time significant positive relationships between urinary citrate excretion and different parameters of bone quality and bone geometry in healthy children. So far in children, such associations have only been demonstrated for the biomarker uPRAL, which reflects almost exclusively diet-dependent influences on acid-base status (9). Urinary citrate, however, can be used as a window on renal metabolism and reflects both, diet dependent and independent (partly genetically determined), systemic influences on acid-base status (15). Generally, renal citrate excretion is elevated under alkaline and decreased under acidic conditions (15, 20). Using exclusively noninvasive urine analytics, our results indirectly confirm this dependence of urinary citrate on systemic acid-base status 1) for the kidney's functional parameter "RAEC", 2) for the biomarker of dietary acid load "uPRAL", and 3) for the kidney's overall free proton activity "24-hour urine pH" (Table 2).

Furthermore, the present findings on urinary citrate excretion as an integrated noninvasive biomarker of systemic acid-base status substantiate 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 (ie, 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 metabolic acidosis with reduced blood bicarbonate and pH levels below 7.35, systemic endocrine changes, eg, 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, hereby 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 4. Twenty-Four-Hour Citraturia and uPRAL as Predictors of Long-Term Fracture Risk in Boys and Girls

	Predictor	Fractures/ No Fractures	Odds Ratio (95% Confidence Interval)	P
Boys, n = 73	24-h citraturia	31/42	0.94 (0.52–1.71)	.84
	uPRAL	31/42	1.63 (0.64–4.17)	.31
Girls, n = 86	24-h citraturia	25/61	0.47 (0.22–0.99)	.048
	uPRAL	25/61	2.53 (1.02–6.28)	.046

Adjusted for renal calcium excretion and maternal birth age.

sidered. For example, to account for the influence of sex hormones as well as individual body size and muscularity on parameters of bone quality and geometry, and because of the wide age range of the children under study, we additionally had to adjust the multiple linear regression analyses for age and tanner stage at bone measurement, forearm length, and forearm muscle area. However, despite the difficulties to detect associations between only slight changes in dietary or systemic acid load and bone outcomes in observational studies, randomized controlled trials have shown that shifts by dietary intervention to a more alkaline status are associated with higher BMD (10, 40, 41).

According to its assumed role as a more holistic biomarker, urinary citrate turned out as a somewhat more distinct predictor of bone status than the mostly diet-dependent biomarker uPRAL. This is especially evident for bone's SSI and periosteal circumference.

Regarding the role of citrate in acidosis, different mechanisms have been shown to contribute on a tissue and cellular level to the sensitivity of the kidney's citrate excretion against small changes in systemic acidity. The main determining factor is acid-base-dependent renal proximal tubular reabsorption of citrate, because citrate secretion by the kidney is negligible (15). Reabsorption can be divided into transport and metabolic processes (17). On the one hand, an intracellular decrease in pH, in consequence of an elevated systemic acid load, promotes an up-regulation of the citrate reabsorption by the sodium dicarboxylate cotransporter-1 in the apical membrane of the proximal tubule cells (42, 43). Also, the luminal pH of the tubular fluid influences reabsorption, because higher concentrations of free protons increase the abundance of the transported divalent citrate anion (17, 42, 43). The significant positive association between 24-hour urine pH and citrate excretion is in line herewith (Table 2).

On the other hand, apart from transport processes, the increased intracellular citrate metabolization in an acidotic state further contributes to an enhanced reabsorption. The intracellular citrate degradation is strongly linked to ammoniagenesis and gluconeogenesis. In case of manifest acidosis, the kidney is supplied with increasing amounts of glutamine from glucocorticoid-mediated muscle degradation (34, 44). Along with the increased glutamine uptake especially in renal proximal tubule cells (19, 45), ammoniagenesis rises strongly. Mitochondrial glutamine degradation is enhanced, eventually leading to a marked NH_3 production thus stimulating acid excretion capacity via NH_4. To keep this process running, intramitochondrial and cytoplasmatic degradation of citrate is stimulated along with correspondingly elevated tubular citrate reabsorption. Altogether, the combination of stimulated citrate cycle and gluconeogenesis (19, 20, 46) stimulates ammoniagenesis and provides the necessary energy for cellular transport, transcription, and enzyme expression processes under acidic conditions in the kidney.

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 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 alkalization with potassium citrate increased BMD and reduced fracture risk score in healtby older men and women. In our study on healthy young subjects, a certain endocrine-metabolically 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, yet to be identified, confounders (eg, individual genetic and/or endocrine-metabolic influences) may also impact on 24-hour citraturia and its association with urine pH and uPRAL. Strength of the present DONALD investigation is an accurate data collection, encompassing repeated 24-hour urine measurements. This allows appropriately adjusting for an obligate confounder namely dietary protein intake, as has been done via the biomarker total uN excretion. Additionally, we used, in contrast to many other studies, the more accurate pQCT measurement instead of dual-energy X-ray absorptiometry to analyze bone status, which allows a more precise assessment especially of bone strength (48). Another strength of the study is the long

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 bone measurements. T.R. (principal investigator) was responsible for conceiving the project and realizing it. All authors were involved in revising the manuscript critically for important intellectual content and approved the article's final version.

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