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Naturally Occurring Stable Calcium Isotope Ratios in Body Compartments Provide a Novel Biomarker of Bone Mineral Balance in Children and Young Adults

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ABSTRACT

Serum calcium (Ca), bone biomarkers, and radiological imaging do not allow accurate evaluation of bone mineral balance (BMB), a key determinant of bone mineral density (BMD) and fracture risk. We studied naturally occurring stable (non-radioactive) Ca isotopes in different body pools as a potential biomarker of BMB. ^{42}Ca and ^{44}Ca are absorbed from our diet and sequestered into different body compartments following kinetic principles of isotope fractionation; isotopically light ^{42}Ca is preferentially incorporated into bone, whereas heavier ^{44}Ca preferentially remains in blood and is excreted in urine and feces. Their ratio ($\delta^{44/42}Ca$) in serum and urine increases during bone formation and decreases with bone resorption. In 117 healthy participants, we measured Ca isotopes, biomarkers, and BMD by dual-energy X-ray absorptiometry (DXA) and tibial peripheral quantitative CT (pQCT). ^{44}Ca and ^{42}Ca were measured by multi-collector ionization-coupled plasma mass-spectrometry in serum, urine, and feces. The relationship between bone Ca gain and loss was calculated using a compartment model. $\delta^{44/42}Ca_{serum}$ and $\delta^{44/42}Ca_{urine}$ were higher in children ($n = 66$, median age 13 years) compared with adults ($n = 51$, median age 28 years; $p < 0.0001$ and $p = 0.008$, respectively). $\delta^{44/42}Ca_{serum}$ increased with height in boys ($p < 0.001$, $R^2 = 0.65$) and was greatest at Tanner stage 4. $\delta^{44/42}Ca_{serum}$ correlated positively with biomarkers of bone formation (25-hydroxyvitaminD [$p < 0.0001$, $R^2 = 0.37$] and alkaline phosphatase [$p = 0.009$, $R^2 = 0.18$]) and negatively with bone resorption marker parathyroid hormone (PTH; $p = 0.03$, $R^2 = 0.13$). $\delta^{44/42}Ca_{serum}$ strongly positively correlated with tibial cortical BMD *Z*-score ($n = 62$; $p < 0.001$, $R^2 = 0.39$) but not DXA. Independent predictors of tibial cortical BMD *Z*-score were $\delta^{44/42}Ca_{serum}$ ($p = 0.004$, $\beta = 0.37$), 25-hydroxyvitaminD ($p = 0.04$, $\beta = 0.19$) and PTH ($p = 0.03$, $\beta = -0.13$), together predicting 76% of variability. In conclusion, naturally occurring Ca isotope ratios in different body compartments may provide a novel, non-invasive method of assessing bone mineralization. Defining an accurate biomarker of BMB could form the basis of future studies investigating Ca dynamics in disease states and the impact of treatments that affect bone homeostasis. © 2020 The Authors. *Journal of Bone and Mineral Research* published by Wiley Periodicals LLC on behalf of American Society for Bone and Mineral Research (ASBMR).

KEY WORDS: BONE MINERAL BALANCE; BONE MINERAL DENSITY; CALCIUM; ISOTOPES; PERIPHERAL QUANTITATIVE CT SCAN

Introduction

Calcium (Ca) is essential for skeletal growth and mineralization, and the skeleton holds >99% of the body's total Ca.[1,2] Phases of rapid growth in childhood and adolescence are critical periods for bone mass accrual.[3] Thereafter, Ca accumulation in the skeleton continues at a slower pace until peak bone mass is reached[4,5] when bone formation and resorption

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are equal. In older adults, bone resorption predominates. This homeostatic balance between bone formation and resorption at different ages results in changes in the bone mineral balance (BMB) throughout life.[2]

In current clinical practice, serum Ca levels are our only tool for estimating BMB. However, serum Ca accounts for <0.1% of total body Ca, and due to tight negative feedback control, it cannot reflect the total body Ca.[1] Biomarkers lack specificity,[6] bone biopsies are highly invasive,[7] and radiological studies can take months or even years to show changes in bone mineral density (BMD).[8] Traditional Ca balance studies using radioisotopes or stable Ca isotopes have been performed but are highly demanding of participants and mainly limited to Ca absorption studies.[1,9–12] There is a need for markers of BMB that directly reflect changes in bone turnover, are easy to measure, safe, and reproducible. One such potential biomarker is natural Ca isotope fractions in serum and urine.

There are six naturally occurring stable (ie, non-radioactive) Ca isotopes (^{40}Ca, ^{42}Ca, ^{43}Ca, ^{44}Ca, ^{46}Ca, and ^{48}Ca)[13–15] that are present in our diet, taken up in all parts of the body, and excreted in urine and feces.[16] Ca isotopes are sequestered in different body compartments at different rates depending on their atomic mass, following distinct rules of kinetic isotope fractionation;[17] fractionation refers to the physicochemical separation of heavier and lighter isotopes, which in turn results in the enrichment of one isotope relative to the other in at least two separate compartments.[15] Isotopically light Ca is preferentially enriched during chemical transport reactions (for example, incorporation into bone), whereas the heavy isotope is preferentially excreted in urine and feces.[16,18–21] The ratio of Ca isotopes (for example, when studying ^{42}Ca and ^{44}Ca, the ratio would be expressed as $\delta^{44/42}$Ca) gives a direct function of the state of bone turnover. Thus, when bone formation exceeds bone resorption and the net BMB is positive, the $\delta^{44/42}Ca_{serum}$ isotope values are higher compared with $\delta^{44/42}Ca_{serum}$ values measured under conditions when bone resorption is the predominant process. Ca isotope ratios in urine and feces reflect serum isotope values and are similarly high during bone formation and low during bone resorption.[16,22] The relationship between Ca isotope fractions in serum and urine is described mathematically in a compartment model[16,18,19,21,23] that in turn is used to quantitatively determine net bone gain or loss of Ca.

As a proof-of-principle study, our group applied the natural Ca isotope fractionation technique in a healthy 4-year-old boy and a 60-year-old woman with osteoporosis.[18] The boy had a higher isotope ratio, indicating accumulation of isotopically light Ca in bones, whereas the older postmenopausal woman had a lower ratio, reflecting bone demineralization.[18] In a study in older women, those with osteoporosis confirmed by dual-energy X-ray absorptiometry (DXA) scan had significantly lower $\delta^{44/42}Ca_{serum}$ and $\delta^{44/42}Ca_{Urine}$ values compared with age-matched controls without osteoporosis.[19] The sensitivity of natural Ca isotope fractionation measures has been further studied in animal models[14,17,24] and healthy adults,[16,20,22,23,25–28] where $\delta^{44/42}$Ca in serum and urine closely reflected the interventions to alter bone homeostasis.

We hypothesize that the natural Ca isotope fractionation method is a sensitive measure of BMB. We correlated $\delta^{44/42}Ca_{serum}$ and $\delta^{44/42}Ca_{urine}$ with currently used imaging measures of BMD as well as biomarkers of bone formation and resorption to evaluate $\delta^{44/42}Ca_{serum}$ and $\delta^{44/42}Ca_{urine}$ as novel biomarkers of BMB. Identifying a reliable biomarker of BMB will form the basis of future studies investigating Ca dynamics in disease states and in response to treatments such as steroids and antiresorptive therapy that can alter bone homeostasis.

Materials and Methods

This is a prospective cross-sectional study performed at Great Ormond Street Hospital and Institute of Child Health, London, UK, and the GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel, Germany. Healthy children and adults younger than 40 years of age fulfilling all inclusion and exclusion criteria were recruited.

Inclusion and exclusion criteria

Inclusion criteria were children and young adults from birth to 40 years of age with normal renal function (estimated glomerular filtration rate above 90 mL/min/1.73 m^2 [calculated by the Schwartz formula[29]] in children >1 year of age and serum creatinine <35 μMol/L in children <1 year) and weight, height, and body mass index (BMI) within 2 standard deviations (SDs) of normal using World Health Organization (WHO) growth charts.

Exclusion criteria were subjects with any chronic illnesses, pre-existing bone disease (inherited or acquired), fractures in the preceding 6 months, any acute illness in the preceding 2 weeks (when the person is unable to maintain their usual diet or has bed rest) and those on glucocorticoid therapy (by any route) in the preceding year, or a lifetime cumulative steroid exposure of ≥6 months.

Recruitment

Pediatric participants were recruited from the siblings and friends of patients at Great Ormond Street Hospital and the children of staff members. In addition, otherwise healthy children who were undergoing minor surgical procedures for dermatology, ear/nose/throat, or plastic surgery were also included after careful screening for all inclusion and exclusion criteria. Young adults were recruited from the staff and their relatives and friends at University College London (n = 21) and women between 18 and 40 years who were part of the GeoOsteo-2016 study from Kiel (n = 30). Demographic details of the study population, their anthropometry, and biomarker profile is described in Supplemental Table S1. All participants and/or their caregivers provided informed written consent and assent as appropriate for their age. The study was approved by the local research ethics committees at both sites.

Study measures

Participants were asked to record a food-frequency questionnaire before their study visit and bring in a first morning urine sample and fecal sample on the day of the study visit. The study visit included anthropometric measures, blood sampling, and radiological tests.

Dietary Ca intake

Dietary Ca intake was assessed in a subgroup of 57 participants (30 young adults and 27 children and their parents/caregivers) who provided complete food diaries/questionnaires for assessment. There was no difference between the subjects in whom dietary Ca intake was assessed versus those in whom it was not based on their age, sex, race, or anthropometric measures. Children completed a prospective 3-day food diary as previously described,[30]

and Ca content was assessed with a software program (CompEat Pro Nutrition Systems; www.compeat.co.uk). A semiquantitative, self-administered food-frequency questionnaire was used to assess the dietary calcium intake of the adults.[31]

Anthropometry and Tanner staging

Height and weight were measured and expressed as absolute values as well as standard deviation score (SDS) for age and sex in participants under 18 years of age.[32] Pubertal staging was determined by the children or their caregivers with a self-reported Tanner staging questionnaire.[33]

Serum biomarkers

Ca isotopes, routine serum biomarkers, and markers of osteoblastic and osteoclastic activity were measured. Samples were frozen at −80°C, thawed only at the time of measurement, and all biomarkers were assayed in the same batch.

Ca isotope measurements

Approximately 250 μL serum, 1000 μL urine, and 100 mg feces were used for analysis. Full details of the Ca isotope measurements have been previously described[19] and are summarized in the Supplemental Materials and Methods.

Routine serum biomarkers

Total calcium, ionized calcium, phosphate, magnesium, bicarbonate, alkaline phosphatase (ALK), intact parathyroid hormone (PTH; Immulite 2000, Siemens Healthcare Diagnostics, Camberley, UK) and 25-hydroxyvitamin D ([25OHD]; isotope-dilution liquid chromatography–tandem mass spectrometry, Waters Xevo TQ-S, Waters, UK) were measured in the Chemical Pathology lab at Great Ormond Street Hospital.

Bone biomarkers

Bone biomarkers of osteoblastic (bone-specific alkaline phosphatase [BAP] and N-terminal propeptide of type I collagen [P1NP]) and osteoclastic (C-terminal telopeptide of type I collagen [CTX]) activity were measured by commercially available ELISAs per manufacturers' instructions (details in Supplemental Materials and Methods). Because smaller volumes of serum were available for some children, a variable number of the different biomarkers were measured; details are provided in the respective Figures as well as in Supplemental Table S1.

Radiological assessment

Radiological assessment was performed in a subgroup of children older than 5 years based on parental consent and ability to stay still for the procedure and 20/21 adults studied at University College London. DXA and peripheral quantitative computed tomography (pQCT) were performed in 20 and 42 children, respectively. None of the participants in the GeoOsteo-2016 study from Kiel had any radiological imaging. There was no difference between the subjects who underwent radiological imaging and those who did not in their age, sex, race, or anthropometric measures.

Dual-energy X-ray absorptiometry

DXA of hip and lumbar spine were performed on the Lunar Prodigy Advance (GE Healthcare, Madison, WI, USA) by trained radiographers according to the manufacturer's protocol and International Society for Clinical Densitometry guidelines.[34] Lumbar spine areal BMD (aBMD) measurements obtained in g/cm^2 were corrected for growth and converted to bone mineral apparent density (BMAD) *Z*-scores for participants under 20 years old, providing age-, sex-, and race-specific *Z*-scores.[35] For the purposes of this analysis, lumbar spine BMAD *Z*-scores for young adults were calculated assuming a maximum age of 20 years. There was no significant difference between reported aBMD *Z*-scores and BMAD *Z*-scores for the young adults.

Peripheral quantitative computed tomography

pQCT scan of the nondominant tibia was performed using the XCT2000 (Stratec Medizintechnik GmbH, Pforzheim, Germany) scanner as previously described.[36,37] Images were acquired at the 3% metaphyseal and 38% diaphyseal site for trabecular and cortical volumetric BMD (g/cm^3), and expressed as age-, height-, sex-, and race-adjusted *Z*-scores (cf. Denburg and colleagues[38]). Tibial cortical BMD measured by pQCT was shown to predict fracture risk in children and young adults[39] and was used as the "gold standard" for assessing bone mineralization in this study.

Calculations for dietary Ca isotope content: The $\delta^{44/42}Ca_{diet}$ values were calculated from the average Ca intake (in grams per day from the food diary or food-frequency questionnaire that the subjects reported), the relative contribution of dairy and non-dairy products, and published values of the Ca isotope fractions in different foods.[40,41] Dairy products show the highest Ca concentrations (~500 mg_{Ca}/100 g), hence are the main source for Ca in a regular European diet followed by vegetables (~100 mg_{Ca}/100 g), fruits (~20 mg_{Ca}/100 g), crop products (~35 mg_{Ca}/100 g), meat (~10 mg_{Ca}/100 g), fat (~7 mg_{Ca}/100 g), and water (~5 mg_{Ca}/100 g). The Ca isotopic composition ($\delta^{44/42}Ca$) of dairy and non-dairy products in the European diet were previously estimated to be approximately −0.58‰ and −0.31‰, respectively.[40,41] The $\delta^{44/42}Ca_{diet}$ values were calculated from the weighted average of the daily consumption of dairy and non-dairy products as follows:

$$\delta^{44/42}{}_{Diet} = X \cdot \delta^{44/42}Ca_{Dairy} + (1 - X) \cdot \delta^{44/42}Ca_{Non-dairy}$$

(where X is the relative amount of dairy products in the daily diet; (1 – X) is the daily amount of the non-dairy products).

Based on this equation, the median $\delta^{44/42}Ca_{diet}$ values were −0.46 (−0.49 to −0.41)‰ in children and −0.40 (−0.45 to −0.39)‰ in adults (Supplemental Table S1), with higher values in adults reflecting their proportionately lower dairy intake. Within our cohort, the Ca isotopic composition of the British and German adult subjects was comparable ($p = 0.082$), pointing to the low variations in the Ca isotope composition of the diet. None of the participants were on any Ca supplements.

Statistical analysis: All variables were assessed for normality. Continuous variables are presented as median and interquartile range (IQR) and categorical variables as frequency and percentage. Comparisons of continuous variables between groups were performed using Mann–Whitney or Kruskal-Wallis tests as appropriate. Independent *t* testing was used for between-group analyses. Categorical variables were compared using chi-square or Fisher's exact *t* test. The relationships between continuous variables were assessed by linear regression. A stepwise multivariable linear regression model was constructed with tibial cortical BMD *Z*-score on pQCT as the dependent variable, and covariates were included if $p < 0.15$ on univariable analyses. A p value of <0.05 (two-sided) was considered statistically

significant. Statistics were performed using SPSS 24.0 (IBM Corp., Armonk, NY, USA) and graphs constructed using GraphPad (La Jolla, CA, USA) Prism (version 8.3).

Ca isotope model: We interpret and discuss our data with reference to a Ca isotope balance model[19] that is based on the simple and universal principles that all isotopes follow: immutability of isotopes in natural systems, mass-dependent isotope fractionation, and the conservation of mass during chemical processes.[42,43] Isotope ratios only change as a function of variations in the balance between elemental input and output from a compartment. In the Ca balance model (Fig. 5, inset), the parameters are: (i) F_{Diet} and $\delta^{44/42}Ca_{Diet}$, Ca flux and isotope composition of dietary Ca entering the body; (ii) $F_{Bone\text{-}Gain}$ and $\delta^{44/42}Ca_{Bone\text{-}Gain}$, Ca flux from the blood to the bone and its Ca isotope composition; (iii) $F_{Bone\text{-}Loss}$ and $\delta^{44/42}Ca_{Bone\text{-}Loss}$, Ca flux out of the bone and its isotope composition; (iv) F_{ex} and $\delta^{44/42}Ca_{ex}$, Ca flux and isotope composition of the excreted Ca from the organism; and (v) Δ_{Bone}, which reflects the Ca isotope fractionation during Ca deposition in the bone during mineralization. Δ_{Bone} has been shown to be fairly constant in the order of about −0.3‰ in several different vertebrate species, including chickens, horses, "Göttingen" mini-pigs, and humans, and is independent of the absolute Ca isotope values in blood and bone.[14,21,24,44] The model (Fig. 5) shows a nonlinear relationship of the $\delta^{44/42}Ca_{Blood}$ value to the Ca balance between bone absorption and resorption expressed by the ratio of skeletal Ca loss flux relative to the skeletal Ca gain flux ($F_{Bone\text{-}Loss}/F_{Bone\text{-}Gain}$). Importantly, the equilibrium value for bone Ca absorption and resorption ($F_{Bone\text{-}Loss}/F_{Bone\text{-}Gain} = 1$) can be estimated from basic mass balance principles to correspond to the sum of the Ca isotope value of the diet ($\delta^{44/42}Ca_{Diet}$) and the isotope fractionation value between blood and bone (Δ_{Bone}): $\delta^{44/42}Ca_{Diet} + \Delta_{Bone}$.

Results

Baseline characteristics

A total of 66 children (ages 0.7 to 17.8 years, median 13 years) and 51 adults (ages 18 to 40 years, median 28 years) participated in the study. Supplemental Table S1 describes their demographics and anthropometry. None of the participants reported bone pain or fractures. Three adults used vitamin D supplements (all had normal 25OHD levels) and none of the participants took calcium supplements.

Dietary Ca intake

The median daily Ca intake and the relative contribution from dairy products was calculated (Supplemental Table S1). Estimation of $\delta^{44/42}Ca_{diet}$ is based on previously measured values of Ca isotope fractions in dairy and non-dairy products in the Western European diet.[18,40,41] The median $\delta^{44/42}Ca_{diet}$ values were −0.46‰ in children ($n = 27$) and −0.40‰ in adults ($n = 30$; $p = 0.013$; Fig. 1), with higher values in adults reflecting their proportionately lower dairy intake. From the known Ca isotope composition, the Ca isotope equilibrium value between Ca absorption and resorption can then be calculated to be: $\delta^{44/42}Ca_{Diet} + \Delta_{Bone} = -0.46‰ + -0.3‰ = \sim -0.76$ ‰ based on the $\delta^{44/42}Ca_{Diet}$ of children or to be about −0.70‰ based on the adult $\delta^{44/42}Ca_{Diet}$ value. Eleven participants who were vitamin D deficient (25OHD concentration below 25nMol/L) had the lowest $\delta^{44/42}Ca_{serum}$ levels irrespective of their Ca intake. There was no correlation between the Ca intake from dairy and serum or urine $\delta^{44/42}Ca$ levels.

Ca isotope fractions in different body compartments

The $\delta^{44/42}Ca$ levels measured in serum ($n = 66$ children and 51 adults), urine ($n = 62$ children and 51 adults), and feces ($n = 11$ children and 20 adults) are shown in Fig. 1*A* and Supplemental Table S1, along with the calculated values for $\delta^{44/42}Ca_{diet}$. Children had significantly higher $\delta^{44/42}Ca$ levels in both serum and urine compared with adults ($p < 0.0001$ and $p = 0.008$, respectively). There was no difference in $\delta^{44/42}Ca_{feces}$ between children and adults ($p = 0.9$, Fig. 1*A*). There was a strong correlation between $\delta^{44/42}Ca$ in serum and urine in all patients ($p < 0.0001$, $R^2 = 0.68$; Fig. 1*B*), suggesting that Ca isotope levels in serum and urine are closely interdependent. A significant but weaker correlation was found between $\delta^{44/42}Ca$ in serum and feces ($p = 0.006$, $R^2 = 0.24$; Fig. 1*B*).

Ca isotope fractions and growth

To test our hypothesis that $\delta^{44/42}Ca$ levels in serum and urine are a sensitive measure of BMB, we explored the associations between $\delta^{44/42}Ca$ levels and age, height, and Tanner stage as surrogate measures of bone growth in the pediatric population. In the overall cohort, there was a strong inverse correlation between age and $\delta^{44/42}Ca_{serum}$ ($p < 0.0001$, $R^2 = 0.43$; Fig. 2*A*), which was more marked in females ($p < 0.0001$, $R^2 = 0.53$) compared with males ($p = 0.02$, $R^2 = 0.13$). Similar findings were found between $\delta^{44/42}Ca_{urine}$ and age (Supplemental Fig. S1).

When the $\delta^{44/42}Ca_{serum}$ levels were examined in children and adults separately, in children there was no association between age and $\delta^{44/42}Ca_{serum}$ ($p = 0.43$), whereas an inverse correlation was found in adults ($p = 0.0015$; Supplemental Fig. S2*A*, *B*). However, in children, a strong positive correlation was found between height *Z*-score and $\delta^{44/42}Ca_{serum}$ in boys under 18 years ($p < 0.001$, $R^2 = 0.65$), but there was a non-significant trend in girls ($p = 0.07$, $R^2 = 0.10$; Fig. 2*B*). Given that children were of variable ages at the time of study and that peak height velocity is reached at different ages, we explored the association between Tanner stage and $\delta^{44/42}Ca_{serum}$. Both boys and girls showed significantly higher $\delta^{44/42}Ca_{serum}$ at Tanner stage 4 compared with stage 5 ($p = 0.01$ and $p = 0.03$, respectively; Fig. 2*C*). There was no significant correlation between $\delta^{44/42}Ca_{urine}$ and height or Tanner stage.

Ca isotope fractions and biomarkers of bone formation and resorption

There was no correlation between total serum Ca or ionized Ca levels and $\delta^{44/42}Ca_{serum}$ ($p = 0.61$ and $p = 0.26$, respectively). In contrast, serum 25OHD concentrations showed a strong positive correlation with $\delta^{44/42}Ca_{serum}$ ($p < 0.0001$, $R^2 = 0.37$; Fig. 3*A*). An inverse correlation was found between $\delta^{44/42}Ca_{serum}$ and PTH, $p = 0.03$, $R^2 = 0.13$; Fig. 3*B*). Biomarkers of bone formation (ALK [$p = 0.009$, $R^2 = 0.06$] and BAP [measured in $n = 89$; $p < 0.0007$, $R^2 = 0.18$]) positively correlated with $\delta^{44/42}Ca_{serum}$ (Fig. 3*C*, *D*). Similar correlations were found between $\delta^{44/42}Ca_{urine}$ and ALK and PTH (Supplemental Fig. S3). There was no significant correlation between $\delta^{44/42}Ca_{serum}$ and CTX or P1NP levels (measured in $n = 89$ each; $p = 0.8$ and $p = 0.67$, respectively) or the CTX/P1NP ratio ($p = 0.08$).

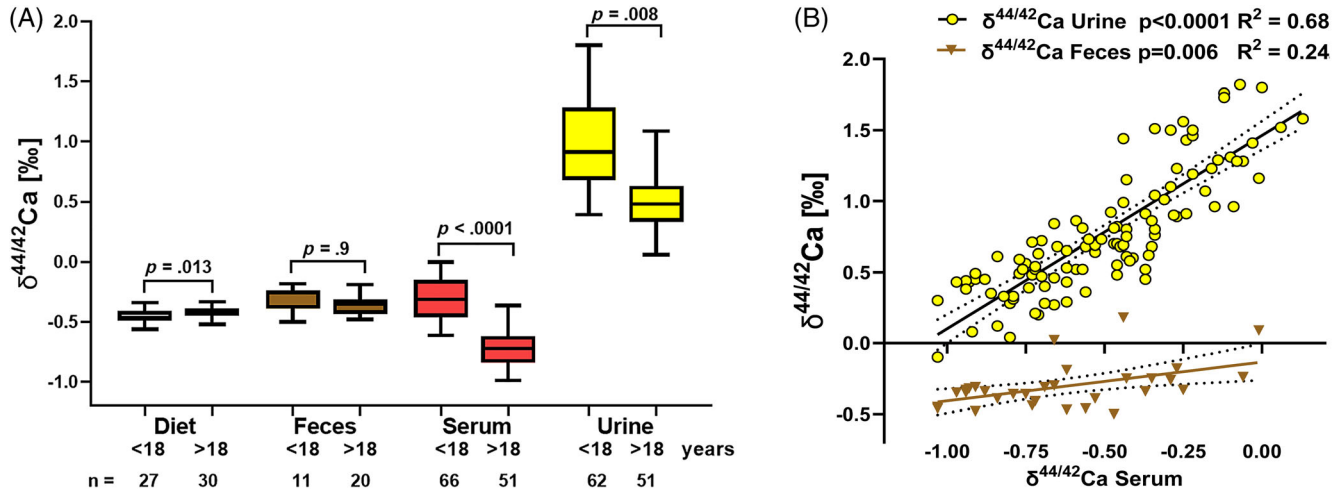

Fig 1. (*A*) Ca isotope ratios expressed as $\delta^{44/42}$Ca (‰) in diet (calculated values) and feces, serum, and urine (measured values) in children (age range 5 to 18 years) and young adults (18 to 40 years of age). Boxes mark the 25th and 75th quartiles, the horizontal line marks the median, and whiskers mark the 1% and 99% limits of the data. Unpaired *t* tests compare between-group differences, and significance values are shown on the graph. $\delta^{44/42}$Ca of serum and urine values of the <18 group is significantly higher than for the >18 group. (*B*) $\delta^{44/42}Ca_{serum}$ (‰) shows a strong correlation with $\delta^{44/42}Ca_{urine}$ and $\delta^{44/42}Ca_{feces}$, indicating the close interdependency based on the Ca balance model (Fig. 5, inset). A similar but less pronounced correlation was found between $\delta^{44/42}Ca_{serum}$ and $\delta^{44/42}Ca_{feces}$. Dotted lines show 95% confidence intervals.

Ca isotope fractions and radiological measures

In children, DXA hip *Z*-score positively correlated with serum $\delta^{44/42}$Ca levels ($n = 20$; $p = 0.02$, $R^2 = 0.18$), but no significant correlation was found between $\delta^{44/42}Ca_{serum}$ and any DXA measures in adults ($n = 20$; $p = 0.23$ and $p = 0.07$, respectively).

In children ($n = 42$) and adults ($n = 20$), both the tibial cortical and trabecular BMD *Z*-scores measured by pQCT showed a positive correlation with $\delta^{44/42}Ca_{serum}$ ($p < 0.001$, $R^2 = 0.39$ and $p = 0.003$, $R^2 = 0.16$, respectively; Fig. 4*A*, *B*), indicating increasing bone mineral content. This was further confirmed by a positive correlation between the total bone mineral content *Z*-score and $\delta^{44/42}Ca_{serum}$ ($p = 0.005$, $R^2 = 0.29$; Fig. 4*C*), implying increasing BMD as bone Ca content increases. When these data were analyzed separately for children and adults, the tibial cortical BMD *Z*-scores and total bone mineral content *Z*-score remained significantly positively correlated with $\delta^{44/42}Ca_{serum}$ in children and adults, but the trabecular BMD *Z*-scores showed only a weak positive correlation in adults (Supplemental Table S2). $\delta^{44/42}Ca_{urine}$ levels showed a similar correlation with the trabecular BMD and total bone mineral content *Z*-scores (Supplemental Fig. S4).

Multivariable linear regression analysis showed that significant and independent predictors of tibial cortical BMD *Z*-score were $\delta^{44/42}Ca_{serum}$ ($p = 0.004$, $\beta = 0.37$), 25-hydroxyvitamin D ($p = 0.04$, $\beta = 0.19$), and PTH ($p = 0.03$, $\beta = -0.13$). These variables together predicted 76% of the variability in tibial cortical BMD *Z*-score. There were no significant predictors of DXA hip *Z*-score on multivariable analysis.

Discussion

In this study, we have shown that the naturally occurring Ca isotope ratio in serum is a significant and independent predictor of

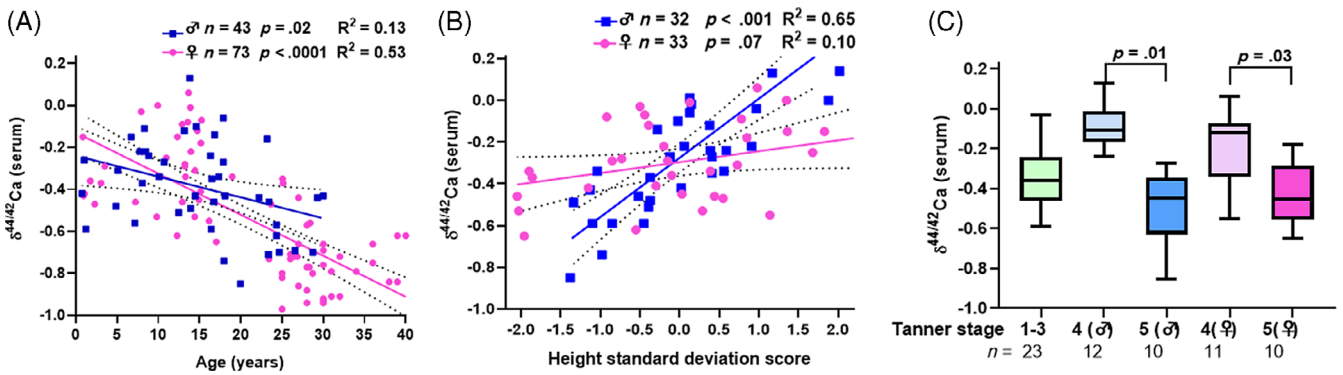

Fig 2. (*A*) In the overall cohort, there was an inverse association between age and $\delta^{44/42}Ca_{serum}$ in both males and females. (*B*) In children, a positive correlation was found between $\delta^{44/42}Ca_{serum}$ and height *Z*-score that reached statistical significance in males only. (*C*) In both boys and girls, $\delta^{44/42}Ca_{serum}$ was highest at Tanner stage 4 compared with stage 5.

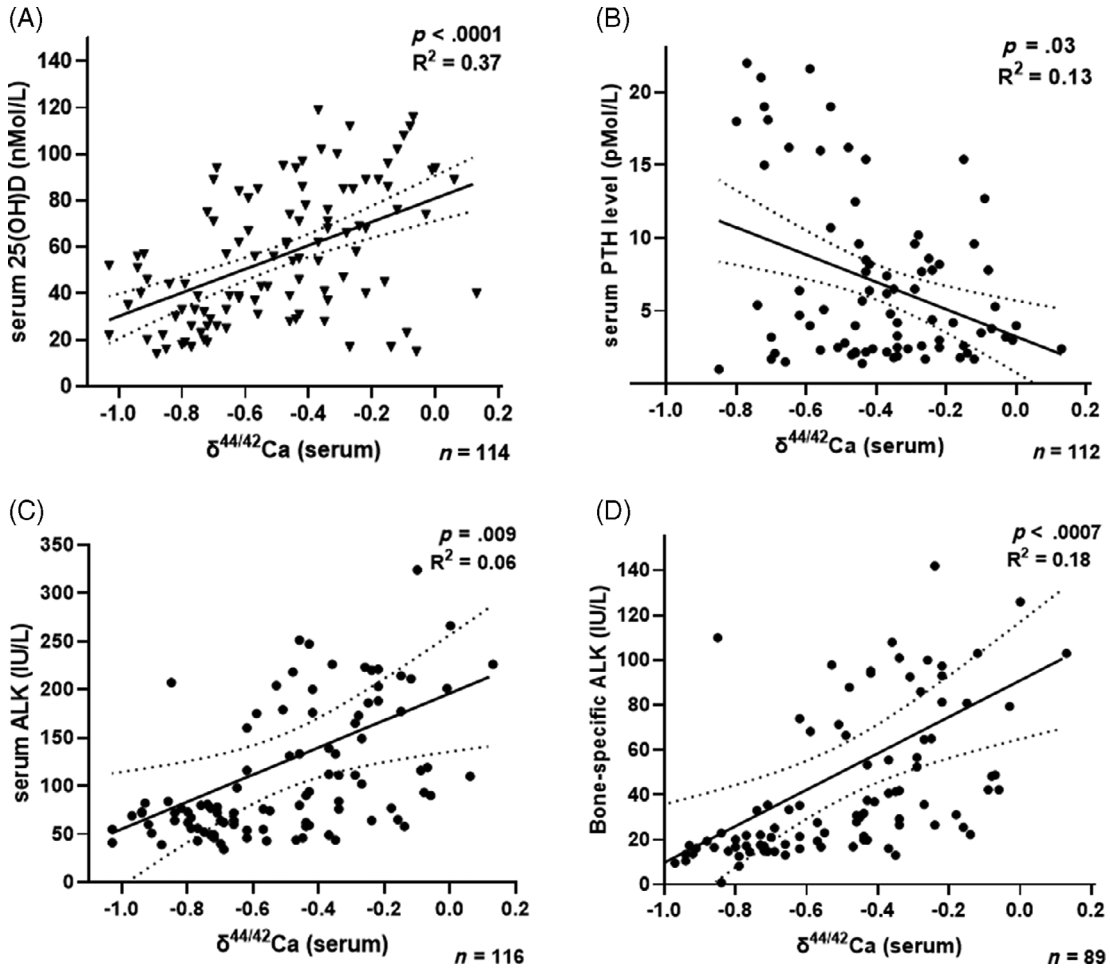

Fig 3. Correlation of $\delta^{44/42}Ca_{serum}$ (‰) with biomarkers of bone formation (*A*) 25-hydroxyvitamin D [25(OH)D], (*C*) alkaline phosphatase (ALK), and (*D*) bone-specific alkaline phosphatase (BAP), and bone resorption (*B*) parathyroid hormone (PTH).

BMB and may be a novel biomarker of bone mineralization in children and young adults. These data need to be evaluated in larger groups of children and adults with bone-related diseases to determine the utility of Ca isotopic ratios as potential tools for the diagnosis, therapeutic monitoring, and prognosis of bone mineralization disorders.

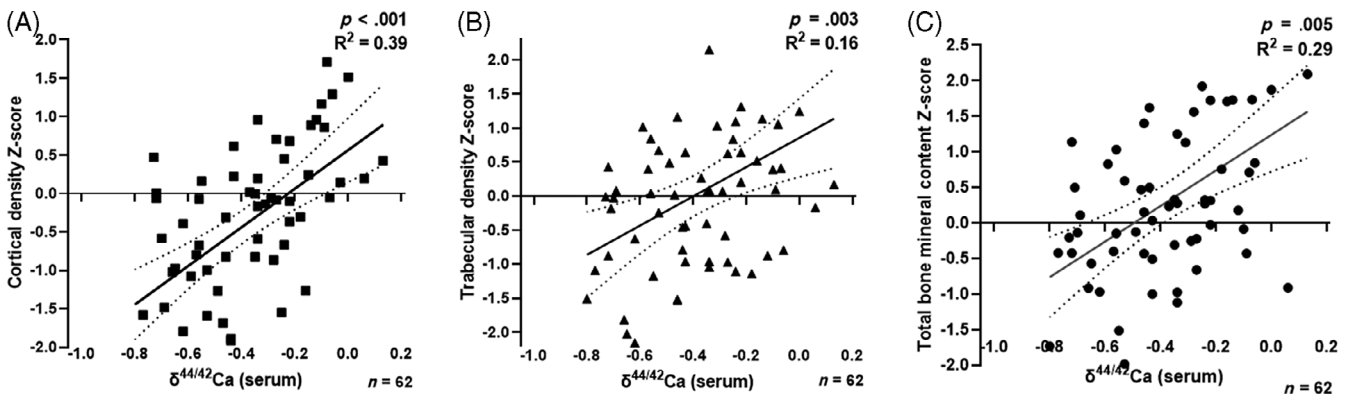

Fig 4. Correlation of $\delta^{44/42}Ca_{serum}$ (‰) with bone densitometry markers on peripheral quantitative computed tomography scan of the tibia in 62 participants (42 children older than 5 years and 20 adults). Tibial cortical density *Z*-score (*A*), trabecular density *Z*-score (*B*), and total bone mineral content *Z*-score (*C*) showed significant correlations with $\delta^{44/42}Ca_{serum}$, confirming an increase in bone mineral density as bone Ca content increases.

Ca balance in the human body is primarily controlled by the exchange of Ca between blood and bone and to a lesser extent by Ca isotope fractionation during filtration and reabsorption by the kidneys. Bone is a dynamic organ and continuously replaced through remodeling, resulting from the coupled actions of bone-forming cells (osteoblasts) and bone-resorbing cells (osteoclasts). There are two critical Ca fluxes, one from the blood to the bone ($F_{BoneGain}$) and the other from bone to the blood ($F_{BoneLoss}$; Fig. 5, inset). The precipitation of bone mineral is an incomplete kinetic reaction involving a preferential uptake of the lighter Ca isotope into bone, depleting blood of light Ca isotope. Bone resorption releases this isotopically light Ca back into the blood. Thus, when BMB is positive (ie, $F_{BoneGain} > F_{BoneLoss}$) $\delta^{44/42}Ca_{serum}$ is relatively high, and when BMB is negative ($F_{Bone\text{-}Loss} > F_{Bone\text{-}Gain}$), $\delta^{44/42}Ca_{serum}$ is relatively low. The Ca isotope composition of blood and bone always differs by about −0.3‰ across different animal species, so that $\delta^{44/42}Ca_{bone}$ is reflected by $\delta^{44/42}Ca_{serum}$, just offset by −0.3‰; this value is similar throughout a number of vertebrate species and is a basic characteristic of bone mineral precipitation.[45]

The variations in Ca isotope fractions in the blood are reflected in urine as both are strongly interdependent. The Ca isotopic composition of urine reflects the separation (ie, fractionation) of light (^{42}Ca) and heavy (^{44}Ca) Ca isotopes during the process of filtration and reabsorption in the renal tubules. The Ca isotope fractionation occurs as the primary urine forms, is reabsorbed by tubules, and then again filtered, with fraction between the reabsorbed and excreted fraction (also called secondary urine). In contrast, the Ca isotopic composition of the feces is influenced by the fractionation of Ca isotopes from the diet, with the lighter isotope (^{42}Ca) absorbed into the blood and the heavier isotope (^{44}Ca) excreted in the feces. In addition, the Ca content of digestive fluids, of yet unknown Ca isotope composition, which also go through cycles of secretion and reabsorption, may affect the Ca isotopic composition of the feces. Also, the Ca isotopic composition of the diet does not have a significant influence on the serum $\delta^{44/42}Ca_{serum}$ because only a small fraction (~20% to 30%) of dietary Ca is absorbed from the gastrointestinal tract, and this contributes only about 5% to the daily Ca turnover. Based on these very different processes influencing the Ca isotopic composition of urine and feces, no correlation between dietary, fecal, and urinary Ca isotopic compositions can be expected.

The above principles of Ca isotope fractionation are confirmed in our cohort of healthy children and young adults. We show that the growing skeleton of children avidly absorbs calcium with the highest $\delta^{44/42}Ca$ serum and urine values in children under 18 years (Fig. 5). Phases of rapid growth in childhood and adolescence are critical periods for bone mineral accrual: In healthy individuals, the Ca content of the skeleton increases from ~25 g at birth to ~1000 to 1200 g in adults.[4,11] However, bone mineral accretion continues into the 30s, when peak bone mass (the amount of bone acquired at the end of skeletal development) is reached.[4] Thus, as shown in Fig. 5, the Ca isotope ratios show a significant inverse correlation with age, reflecting the relative predominance of bone mineralization or demineralization at different ages. Based on compartment model calculations,[19] the relatively high Ca isotope values in young people are

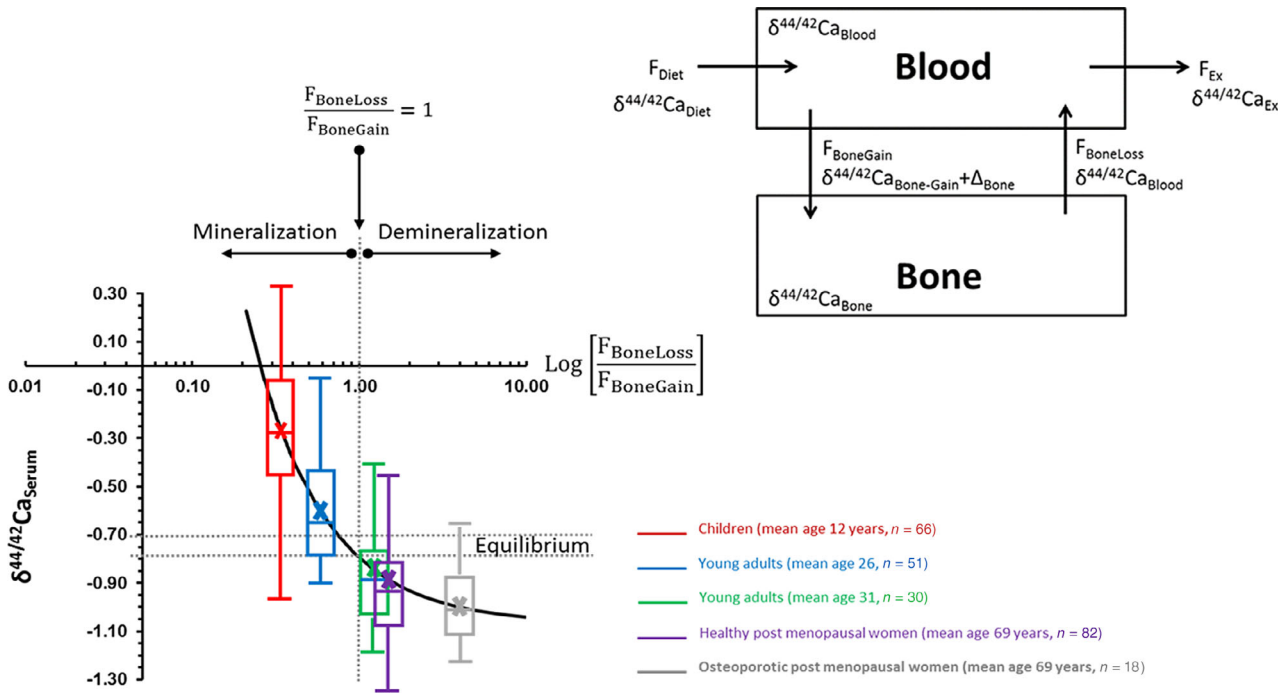

Fig 5. In healthy individuals, the serum Ca isotope composition ($\delta^{44/42}Ca_{Blood}$) depends on age from childhood (mean age 12 years), young adulthood (mean ages 26 and 31 years in two cohorts), as well as healthy and postmenopausal women (mean ages 69 years for both). Modeling indicates an equilibrium value ($F_{BoneLoss}/F_{BoneGain}$ ratio) between 0.7 and 0.76‰ (gray dotted lines). The more positive values of younger subjects are above the equilibrium value, indicating a positive Ca balance ($F_{BoneLoss}/F_{BoneGain}$ ratio > 1) and increasing skeletal mass. In contrast, $\delta^{44/42}Ca_{Blood}$ values below the equilibrium values indicate a negative Ca balance ($F_{BoneLoss}/F_{BoneGain}$ ratio < 1) and skeletal demineralization with a net loss of bone mass.

reflected by a $F_{BoneLoss}/F_{BoneGain}$ ratio of ~0.4, which means that 2.5 times more Ca is accumulated in the bones than lost by bone resorption. In contrast, as shown in our earlier work,[19] osteoporotic women show a $F_{BoneLoss}/F_{BoneGain}$ ratio of ~3, indicating that the Ca loss from bone is three times higher than the Ca uptake from the blood. Furthermore, Fig. 5 shows that at the age of about 30 years, the skeletal gain and loss of Ca are in equilibrium ($F_{BoneLoss}/F_{BoneGain} \sim 1$), at a $\delta^{44/42}Ca_{serum}$ value of about −0.8‰. This value, within statistical uncertainties, is in agreement with the estimated value of about 0.7 to 0.77‰ based on the theoretical principles of isotope mass balance and simplified model assumptions described in Materials and Methods. From Fig. 5, it is shown that for the cohort of young adults, the $\delta^{44/42}Ca_{serum}$ value is in the range of −0.72 to −0.8‰, falling within the equilibrium range. This reflects the average age when peak bone mass is achieved[4] and there is no further net gain or loss of Ca from the skeleton. Lower values for $\delta^{44/42}Ca_{serum}$, as found in healthy and osteoporotic postmenopausal women, reflect gradual age-related bone resorption, leading to a net loss of Ca. In contrast, the higher values in children indicate net accumulation of Ca in the growing skeleton.

The rate of bone Ca accrual varies with age, pubertal status, linear growth, weight gain, and change in lean body mass. Our data show that children have a uniformly high $\delta^{44/42}Ca$ serum and urine levels irrespective of age. Given that children in this cross-sectional study were of different pubertal stages and would have varying growth velocity,[46] we were able to show a stronger correlation of $\delta^{44/42}Ca_{serum}$ with height, an indicator of skeletal size, as well as Tanner stage, rather than age. DXA studies have shown that the highest bone mineral accretion takes place in Tanner stages 4 or 5 when peak height velocity is reached[47]: ~25% of total skeletal mass is laid down during the 2-year interval of attaining peak height velocity.[4] Given that the Ca isotope method is far more sensitive than X-ray densitometry,[8,19] we infer that during periods of very rapid bone growth, ie, in Tanner stage 5, there may be a lag between osteoid deposition and its mineralization.

Our study was designed to compare Ca isotope values against a wide range of currently available measures of bone mineral status, including bone densitometry and biomarkers in clinical and research practice. Of note, serum Ca and ionized Ca that are routinely measured and used to assess Ca status and adjust medication dosage did not show any correlation with $\delta^{44/42}Ca_{serum}$, implying that they do not reflect the bone turnover status. A poor correlation between DXA measures and $\delta^{44/42}Ca_{serum}$ is explained by their different methodological approaches. DXA captures only a single area of the skeleton, cannot distinguish between cortical and trabecular bone and increased bone density due to abnormal bone remodeling,[48] and radiological changes can take months to years to manifest a change.[8,49] pQCT provides a more sensitive measure of BMD—it provides volumetric density data in g/cm^3 and can distinguish between trabecular and cortical bone compartments independent of size of the subject.[49,50] pQCT is shown to predict fracture risk even in children.[38] We found that $\delta^{44/42}Ca_{serum}$ was a significant and independent predictor of tibial cortical BMD *Z*-score in children and young adults. However, pQCT is a research tool only—it requires expensive equipment that is not widely available, measurements are highly observer dependent, reference data are heterogenous,[49] and as with DXA, it provides information on only a single area of the skeleton. Biomarkers of osteoblastic and osteoclastic activity are easy to measure and relatively inexpensive but can be affected by sex, age, body weight, circadian rhythm, food intake, exercise, renal or liver function, and recent fractures.[51] Although these preliminary data show that Ca isotope ratios have a statistically significant correlation with biochemical markers, they have a minimal predictive value, perhaps because biomarkers are strongly influenced by pubertal stage. Thus, the predictive value of Ca isotope ratios at different stages of puberty needs to be studied in a larger cohort of healthy children. Importantly, bone mineralization is a dynamic process, but radiological and histological measures cannot provide information on short-term changes in Ca status, such as with nutritional or pharmacological interventions. Ca isotope ratios give a "real-time" picture of the mineralization—demineralization fluxes of the whole skeleton, and serial measures allow monitoring changes in response to a disease process or therapy.

Our data and inferences complete and extend earlier observations that the Ca isotope ratio closely reflects the BMB. Heuser and colleagues induced pharmacological osteoporosis with glucocorticoids and vitamin D–deficient diets in Göttingen mini-pigs and showed that $\delta^{44/40}Ca_{urine}$ closely reflected the interventions to change bone homeostasis.[24] In an effort to mimic bone demineralization in an anti-gravity environment, groups from NASA have studied healthy adult volunteers subjected to complete bed rest and showed a significant drop in $\delta^{44/42}Ca$ in serum and urine starting by 10 days of initiating bed rest, correlating with markers of bone resorption.[21,22,28] Also, sensitivity of the Ca isotope fractionation technique in experiments comparing bed rest versus osteoclast inhibition with bisphosphonates has been shown.[16] Recently our group has shown that the Ca isotope composition of blood and urine of osteoporotic postmenopausal women is significantly lighter when compared with healthy postmenopausal women and correlated with DXA measures.[19] Finally, in a group of healthy young men, Rangarajan and colleagues showed an increase in Ca isotope ratios after 3 weeks of vitamin D supplementation.[27] Importantly, these interventional studies have shown that Ca isotope ratios in blood and urine change within days in response to bone demineralization and therefore provide a sensitive tool to monitor disease processes or treatments that affect bone homeostasis.

Some limitations of our study must be acknowledged. Because this is a cross-sectional study, we were unable to monitor height velocity and changes in Ca isotope ratios at different stages of growth. The numbers of subjects at each Tanner stage were very small, and self-reporting may have led to inaccuracies, particularly when distinguishing between Tanner stages 4 and 5. In addition, as bone turnover is closely related to sex hormones during puberty, further studies must include measures of sex hormones as part of the analysis. Different techniques for dietary Ca assessment were used for adults from Kiel as they formed part of a different study protocol; food-frequency questionnaire being retrospective in nature may underestimate or overestimate Ca intake.[30,52] We could not determine Ca absorption, which is strongly influenced by an individual's vitamin D status.[53] Indeed, we found that participants who were 25OHD deficient had the lowest $\delta^{44/42}Ca_{serum}$ levels irrespective of Ca intake (confirming findings by Rangarajan and colleagues[27]); this may of course reflect 25OHD effects on bone, too,[54] and requires further study. The $\delta^{44/42}Ca_{urine}$ showed a significant albeit lower degree of correlation than with $\delta^{44/40}Ca_{serum}$ with most measures, likely due to individual changes in Ca isotope fractionation occurring in the kidneys as previously described.[18,22,55,56] Importantly, our data suggest that $\delta^{44/42}Ca_{serum}$ is a more sensitive measure of BMB and must be measured in preference to

$\delta^{44/42}Ca_{urine}$, at least in children and young adults. Given that Ca isotope measures reflect real-time changes in bone homeostasis, repeated measurements have the potential to greatly improve monitoring the effects of medications on bone health compared with currently used X-ray densitometry or biomarkers; these studies are ongoing.

In summary, our data suggest that the naturally occurring stable Ca isotope ratio in serum is a significant and independent predictor of BMB in children and young adults and are more accurate than currently used DXA or bone biomarkers in determining BMD. Further studies are required to test the clinical utility of Ca isotopes as a novel biomarker in children and adults with diseases or treatments that affect bone health, such as renal failure, chronic childhood diseases affecting nutrition and growth, inherited bone diseases like osteogenesis imperfecta, physiological changes with age as with osteoporosis, effects of steroids, chemotherapy, and antiresorptive treatments, and prognostication of fracture risk in all of these conditions.

Disclosures

AE and AK part own Osteolabs company and receive an honorarium from Osteolabs. AE receives patent royalties from Geomar.

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References

1. Bushinsky DA. Contribution of intestine, bone, kidney, and dialysis to extracellular fluid calcium content. Clin J Am Soc Nephrol. 2010;5 (Suppl 1):S12–22.
2. Peacock M. Calcium metabolism in health and disease. Clin J Am Soc Nephrol. 2010;5(Suppl 1):S23–30.
3. Bailey DA, Martin AD, McKay HA, Whiting S, Mirwald R. Calcium accretion in girls and boys during puberty: a longitudinal analysis. J Bone Miner Res. 2000;15(11):2245–50.
4. Baxter-Jones AD, Faulkner RA, Forwood MR, Mirwald RL, Bailey DA. Bone mineral accrual from 8 to 30 years of age: an estimation of peak bone mass. J Bone Miner Res. 2011;26(8):1729–39.
5. Teegarden D, Proulx WR, Martin BR, et al. Peak bone mass in young women. J Bone Miner Res. 1995;10(5):711–5.
6. Fischer DC, Mischek A, Wolf S, et al. Paediatric reference values for the C-terminal fragment of fibroblast-growth factor-23, sclerostin, bone-specific alkaline phosphatase and isoform 5b of tartrate-resistant acid phosphatase. Ann Clin Biochem. 2012;49(Pt 6):546–53.
7. Bacchetta J, Harambat J, Cochat P, Salusky IB, Wesseling-Perry K. The consequences of chronic kidney disease on bone metabolism and growth in children. Nephrol Dial Transplant. 2012;27(8):3063–71.
8. Watts NB. Fundamentals and pitfalls of bone densitometry using dual-energy X-ray absorptiometry (DXA). Osteoporos Int. 2004;15 (11):847–54.
9. Abrams SA. Using stable isotopes to assess mineral absorption and utilization by children. Am J Clin Nutr. 1999;70(6):955–64.
10. Abrams SA. Calcium absorption in infants and small children: methods of determination and recent findings. Nutrients. 2010;2(4): 474–80.
11. Matkovic V, Heaney RP. Calcium balance during human growth: evidence for threshold behavior. Am J Clin Nutr. 1992;55(5):992–6.
12. O'Brien KO, Abrams SA. Using stable isotope tracers to study bone metabolism in children. J Physiol. 2019;597(5):1311–9.
13. DePaolo DJ. Calcium isotopic variations produced by biological, kinetic, radiogenic and nucleosynthetic processes. Rev Mineral Geochem. 2004;55(1):255–88.
14. Skulan J, DePaolo DJ. Calcium isotope fractionation between soft and mineralized tissues as a monitor of calcium use in vertebrates. Proc Natl Acad Sci U S A. 1999;96(24):13709–13.
15. Nielson LC, Yang W, Brown ST, DePaolo DJ. Calcium isotopes as tracers of biogeochemical processes. In Baskaran M, ed. Handbook of environmental isotope geochemistry. Berlin: Springer; 2011 pp 105–24.
16. Skulan J, Bullen T, Anbar AD, et al. Natural calcium isotopic composition of urine as a marker of bone mineral balance. Clin Chem. 2007;53 (6):1155–8.
17. Reynard LM, Henderson GM, Hedges REM. Calcium isotope ratios in animal and human bone. Geochim Cosmochim Acta. 2010;74(13): 3735–50.
18. Heuser A, Eisenhauer A. A pilot study on the use of natural calcium isotope ((44)ca/ca-40) fractionation in urine as a proxy for the human body calcium balance. Bone. 2010;46(4):889–96.
19. Eisenhauer A, Muller M, Heuser A, et al. Calcium isotope ratios in blood and urine: a new biomarker for the diagnosis of osteoporosis. Bone Rep. 2019;10:100200.
20. Morgan JLL, Skulan JL, Gordon GE, Romaniello SJ, Smith SM, Anbar AD. Using natural stable calcium isotopes to rapidly assess changes in bone mineral balance using a bed rest model to induce bone loss. FASEB J. 2012;26:244.1.
21. Morgan JLL, Skulan JL, Gordon GW, Romaniello SJ, Smith SM, Anbar AD. Rapidly assessing changes in bone mineral balance using natural stable calcium isotopes. Proc Natl Acad Sci USA. 2012;109(25): 9989–94.
22. Channon MB, Gordon GW, Morgan JLL, Skulan JL, Smith SM, Anbar AD. Using natural, stable calcium isotopes of human blood to detect and monitor changes in bone mineral balance. Bone. 2015;77:69–74.
23. Morgan JL, Zwart SR, Heer M, Ploutz-Snyder R, Ericson K, Smith SM. Bone metabolism and nutritional status during 30-day head-down-tilt bed rest. J Appl Physiol. 2012;113(10):1519–29.
24. Heuser A, Eisenhauer A, Scholz-Ahrens KE, Schrezenmeir J. Biological fractionation of stable ca isotopes in Gottingen minipigs as a physiological model for ca homeostasis in humans. Isotopes Environ Health Stud. 2016;52(6):633–48.
25. Shackelford LC, LeBlanc AD, Driscoll TB, et al. Resistance exercise as a countermeasure to disuse-induced bone loss. J Appl Physiol. 1985;97 (1):119–29.

26. Baecker N, Tomic A, Mika C, et al. Bone resorption is induced on the second day of bed rest: results of a controlled crossover trial. J Appl Physiol. 1985;95(3):977–82.
27. Rangarajan R, Mondal S, Thankachan P, Chakrabarti R, Kurpad AV. Assessing bone mineral changes in response to vitamin D supplementation using natural variability in stable isotopes of calcium in urine. Sci Rep. 2018;8(1):16751.
28. Heuser A, Frings-Meuthen P, Rittweger J, Galer SJG. Calcium isotopes in human urine as a diagnostic tool for bone loss: additional evidence for time delays in bone response to experimental bed rest. Front Physiol. 2019;10:12.
29. Schwartz GJ, Munoz A, Schneider MF, et al. New equations to estimate GFR in children with CKD. J Am Soc Nephrol. 2009;20(3):629–37.
30. McAlister L, Pugh P, Greenbaum L, et al. The dietary management of calcium and phosphate in children with CKD stages 2–5 and on dialysis-clinical practice recommendation from the pediatric renal nutrition taskforce. Pediatr Nephrol. 2020;35(3):501–18.
31. Nothlings U, Hoffmann K, Bergmann MM, Boeing H. Fitting portion sizes in a self-administered food frequency questionnaire. J Nutr. 2007;137(12):2781–6.
32. Cole TJ. The LMS method for constructing normalized growth standards. Eur J Clin Nutr. 1990;44(1):45–60.
33. Feingold D. Pediatric endocrinology. Atlas of pediatric physical diagnosis. 2nd ed. Philadelphia: WB Saunders; 1992 pp 16–9.
34. ISCD. 2019 ISCD Official Positions—Pediatric. Available at: https://www.iscd.org/official-positions/2019-iscd-official-positions-pediatric/. 2019. Accessed 2019 Dec 8.
35. Crabtree NJ, Shaw NJ, Bishop NJ, et al. Amalgamated reference data for size-adjusted bone densitometry measurements in 3598 children and young adults—the ALPHABET study. J Bone Miner Res. 2017;32(1):172–80.
36. Binkley TL, Specker BL. pQCT measurement of bone parameters in young children: validation of technique. J Clin Densitom. 2000;3(1):9–14.
37. Wetzsteon RJ, Kalkwarf HJ, Shults J, et al. Volumetric bone mineral density and bone structure in childhood chronic kidney disease. J Bone Miner Res. 2011;26(9):2235–44.
38. Denburg MR, Tsampalieros AK, de Boer IH, et al. Mineral metabolism and cortical volumetric bone mineral density in childhood chronic kidney disease. J Clin Endocrinol Metab. 2013;98(5):1930–8.
39. Denburg MR, Kumar J, Jemielita T, et al. Fracture burden and risk factors in childhood CKD: results from the CKiD cohort study. J Am Soc Nephrol. 2016;27(2):543–50.
40. Chu N, Henderson G, Hedges R. Ca isotope variations in modern dietary systems and their potential to assess the importance of dairying in past cultures. Geophys Res Abstr. 2005;1:047426.
41. Chu NC, Henderson GM, Belshaw NS, Hedges REM. Establishing the potential of ca isotopes as proxy for consumption of dairy products. Appl Geochem. 2006;21(10):1656–67.
42. Dauphas N, Schauble EA. Mass fractionation laws, mass-independent effects, and isotopic anomalies. Annu Rev Earth Planet Sci. 2016;44(1):709–83.
43. Young ED, Galy A, Nagahara H. Kinetic and equilibrium mass-dependent isotope fractionation laws in nature and their geochemical and cosmochemical significance. Geochim Cosmochim Acta. 2002;66(6):1095–40.
44. Skulan J, DePaolo DJ, Owens TL. Biological control of calcium isotopic abundances in the global calcium cycle. Geochim Cosmochim Acta. 1997;61(12):2505–10.
45. Bullen TD, Eisenhauer A. Metal stable isotopes in low-temperature systems: a primer. Elements. 2009;5(6):349–52.
46. Granados A, Gebremariam A, Lee JM. Relationship between timing of peak height velocity and pubertal staging in boys and girls. J Clin Res Pediatr Endocrinol. 2015;7(3):235–7.
47. Zemel BS, Kalkwarf HJ, Gilsanz V, et al. Revised reference curves for bone mineral content and areal bone mineral density according to age and sex for black and non-black children: results of the bone mineral density in childhood study. J Clin Endocrinol Metab. 2011;96(10):3160–9.
48. Baim S, Wilson CR, Lewiecki EM, Luckey MM, Downs RW Jr, Lentle BC. Precision assessment and radiation safety for dual-energy X-ray absorptiometry: position paper of the International Society for Clinical Densitometry. J Clin Densitom. 2005;8(4):371–8.
49. Lalayiannis AD, Crabtree NJ, Fewtrell M, et al. Assessing bone mineralisation in children with chronic kidney disease: what clinical and research tools are available? Pediatr Nephrol. 2019;35(6):937–57.
50. Crabtree N, Ward K. Bone densitometry: current status and future perspective. Endocr Dev. 2015;28:72–83.
51. Eastell R, Pigott T, Gossiel F, Naylor KE, Walsh JS, Peel NFA. Diagnosis of endocrine disease: bone turnover markers: are they clinically useful? Eur J Endocrinol. 2018;178(1):R19–31.
52. Ortiz-Andrellucchi A, Henriquez-Sanchez P, Sanchez-Villegas A, Pena-Quintana L, Mendez M, Serra-Majem L. Dietary assessment methods for micronutrient intake in infants, children and adolescents: a systematic review. Br J Nutr. 2009;102(Suppl 1):S87–117.
53. Shroff R, Wan M, Nagler EV, et al. Clinical practice recommendations for native vitamin D therapy in children with chronic kidney disease stages 2–5 and on dialysis. Nephrol Dial Transplant. 2017;32(7):1098–113.
54. Shroff R, Knott C, Rees L. The virtues of vitamin D—but how much is too much? Pediatr Nephrol. 2010;25(9):1607–20.
55. Gussone N, Eisenhauer A, Heuser A, et al. Model for kinetic effects on calcium isotope fractionation (delta ca-44) in inorganic aragonite and cultured planktonic foraminifera. Geochim Cosmochim Acta. 2003;67(7):1375–82.
56. Gussone N, Langer G, Thoms S, et al. Cellular calcium pathways and isotope fractionation in Emiliania huxleyi. Geology. 2006;34(8):625–8.