

Hypocalciuria as a Predictor of Reduced Intestinal Calcium Absorption

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Impaired intestinal calcium absorption contributes to osteoporosis, but its measurement is limited to research settings. We hypothesized that 24-hour urine calcium (24HUC) values could diagnose low fractional calcium absorption (FCA). We performed a *post hoc* analysis of clinical trial data to determine whether 24HUC predicted low FCA compared with the gold standard dual calcium isotope method. Two hundred thirty postmenopausal women <75 years old without osteoporosis underwent 445 FCA measurements using calcium isotopes (8 mg of oral ⁴⁴Ca, 3 mg of intravenous ⁴²Ca) and a 24-hour inpatient urine collection at 0 and 12 months. We determined subjects' total calcium intake via review of food diaries and supplements. Net calcium absorption (NCA) was total calcium intake × FCA. NCA and 24HUC values demonstrated a positive correlation ($r = 0.34$; 95% confidence interval, 0.25 to 0.42; $P < 0.001$). We calculated sensitivity, specificity, positive (PPV) and negative predictive value (NPV) for the ability of 24HUC thresholds to predict calcium malabsorption. When low calcium absorption was defined as <120 mg/d, a 24HUC value <150 mg demonstrated 65% sensitivity, 67% specificity, 31% PPV, and 89% NPV. When calcium malabsorption was defined as <100 mg/d, a 24HUC value <150 mg demonstrated 72% sensitivity, 65% specificity, 22% PPV, and 94% NPV. A 24HUC value <150 mg demonstrated a high NPV for calcium malabsorption. We suggest that 24HUC levels can exclude calcium malabsorption in postmenopausal women.

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Adequate intestinal calcium absorption is crucial for bone mineralization and calcium homeostasis. Calcium absorption is influenced by numerous factors including age, calcium intake, estrogen status, vitamin D stores, gastrointestinal disorders, and genetic factors [1, 2]. Low calcium absorption is a risk factor for osteoporosis and hip fracture [3]. Calcium absorption can be assessed by measuring fractional calcium absorption (FCA) using the dual calcium isotope method, which is the gold standard. However, the cost and limited access to stable calcium isotopes prohibit measurement of FCA in clinical practice. In contrast, clinicians often order inexpensive 24-hour urine calcium (24HUC) levels in the initial evaluation of patients with osteoporosis. Calcium is absorbed across intestinal epithelium cells into the bloodstream, filtered through the kidneys, and excreted in urine. We therefore hypothesized that low 24HUC levels would accurately detect low calcium absorption. To our knowledge, the diagnostic performance of a 24HUC level in detecting inadequate calcium absorption has not been published. To test our hypothesis, we performed a *post hoc* analysis of data from the study “Treatment of Vitamin D Insufficiency in Postmenopausal Women: A Randomized Clinical Trial” (clinicaltrials.gov NCT00933244) [4].

Abbreviations: 1,25(OH)₂D, calcitriol; 24HUC, 24-hour urine calcium; 25(OH)D, calcifediol; CI, confidence interval; FCA, fractional calcium absorption; GFR, glomerular filtration rate; HPLC, high-performance liquid chromatography; NCA, net calcium absorption; NPV, negative predictive value; PPV, positive predictive value; SUCCR, spot urine calcium-to-creatinine ratio.

1. Materials and Methods

We obtained grant support from the National Institutes of Health (R01 AG028739), institutional review board approval from the University of Wisconsin Health Sciences Committee, and written consent from all participants.

We recruited postmenopausal women through local advertisements. Eligible subjects had a serum calcifediol [25(OH)D] level between 14 and 27 ng/mL with high-performance liquid chromatography (HPLC) and were ≥ 5 years past menopause or oophorectomy or were ≥ 60 years if they had undergone hysterectomy without oophorectomy. We excluded women > 75 years old because increasing age is associated with intestinal resistance to vitamin D and decreased calcium absorption [5, 6]. We further excluded women with hypercalcemia, nephrolithiasis, cancer within 5 years (excluding skin cancer), diabetes mellitus, osteoporosis, inflammatory bowel disease, malabsorption, celiac sprue, chronic diarrhea, or a glomerular filtration rate (GFR) < 45 mL/min based on the Modification of Diet in Renal Disease Equation [7]. We also excluded women with adult fragility fracture of the hip, spine, or wrist; use of bone-active medications within the past 6 months, including bisphosphonates, estrogens, calcitonin, teriparatide, oral corticosteroids, or anticonvulsants; or use of > 400 IU of cholecalciferol per day. Subjects' baseline serum calcium, parathyroid hormone, and creatinine and bone mineral density levels were measured.

Throughout the trial, a single research laboratory measured subjects' serum 25(OH)D level using a HPLC method [8]. Between-run assay coefficients of variation for the assay ranged from 2.6% to 4.9% for 25(OH)D₃ and 3.2% to 12.6% for 25(OH)D₂. Upon addition of known 25(OH)D₃ and 25(OH)D₂ aliquots to serum samples, investigators recovered 95% to 102% of each analyte. The assay's lower detectable limit is 3 ng/mL, and the assay is linear from 5 to 100 ng/mL. Finally, results for samples tested by both HPLC and liquid chromatography/mass spectrometry/mass spectrometry were nearly identical.

Eligible subjects completed 4- to 7-day food diaries within 1 month of the FCA studies, using scales and household measuring tools to record intake. Food diaries were analyzed using Food Processor software (ESHA Research, Salem, OR) to calculate individuals' intake of nutrients. Women who consumed < 600 or > 1400 mg of calcium per day were counseled to modify calcium intake to between 600 and 1400 mg daily.

We measured baseline FCA using the gold standard, the dual stable calcium isotope method [9]. Eastell *et al.* [9] validated this approach by comparing the FCA obtained using balance methods with the FCA obtained using dual stable calcium isotopes; values were 0.26 ± 0.13 and 0.26 ± 0.09 , respectively ($r = 0.71$; $P < 0.01$). Subjects fasted from midnight and arrived at the University of Wisconsin Clinical Research Unit around 7 AM. Participants consumed a breakfast containing a 300-mg calcium load, along with ≤ 50 mL of calcium-fortified orange juice that contained approximately 8 mg of ⁴⁴Ca. At the same time, nurses infused approximately 3 mg of ⁴²Ca intravenously over 5 minutes. Nurses recorded ⁴²Ca and ⁴⁴Ca doses given to each subject. Participants remained on the ward during the 24-hour urine collection. The nutritionist designed each subject's 24-hour inpatient diet to replicate a typical outpatient consumption of nutrients.

We purchased calcium isotopes from Trace Sciences International (Wilmington, DE), reconstituted isotopes into solution, tested solutions for sterility and pyrogenicity [10, 11], and stored solutions at the University of Wisconsin Pharmaceutical Research Center before use. Wisconsin State Laboratory of Hygiene personnel determined concentrations and ratios of calcium isotopes in 24-hour urine specimens by high-resolution inductively coupled plasma mass spectrometry [12, 13]. We measured 24HUC levels and concentrations of calcium isotopes in urine relative to internal and external standards. We calculated FCA as the dose-corrected ratio of the two calcium isotopes in a 24-hour urine collection using the Eastell equation [9]:

$$\text{FCA} = \frac{\Delta\% \text{ excess urinary } ^{44}\text{Ca}}{\Delta\% \text{ excess urinary } ^{42}\text{Ca}} \times \frac{\text{natural ratio } ^{44}\text{Ca}/^{43}\text{Ca}}{\text{natural ratio } ^{42}\text{Ca}/^{43}\text{Ca}} \times \frac{\text{dose } ^{42}\text{Ca}}{\text{dose } ^{44}\text{Ca}}$$

After baseline FCA measures were taken, participants were randomly assigned to receive high-dose cholecalciferol, low-dose cholecalciferol, or placebo for 1 year.

For the current study, we used data from baseline and 1 year later. We graphed data to determine distribution and outliers. We summarized normal data using the mean and standard deviation and skewed data using the median and interquartile range. Previous studies reported an inverse relationship between FCA and calcium intake [3, 14, 15]; therefore, low calcium absorption can occur as a result of low FCA and/or low calcium intake. We therefore calculated net calcium absorption (NCA) as $FCA \times \text{total calcium intake}$, where total calcium intake was the sum of dietary and supplemental calcium intake. We used Spearman correlation coefficients to compare FCA, NCA, and 24HUC levels.

In calcium balance studies performed in 73 adult women and 82 adult men by Hunt and Johnson [16], neutral calcium balance was achieved at a calcium intake of 741 mg/d; mean calcium absorption was $24.9\% \pm 12.4\%$ and increased linearly with calcium intake (Fig. 2). However, calcium absorption decreases with age, with an additional decline at menopause [1, 17, 18]. In postmenopausal women, calcium absorption is approximately 20% of calcium intake [2]. Thus, a healthy postmenopausal woman who consumes 750 mg/d of calcium absorbs approximately 150 mg of calcium per day. Because the normal reference range for NCA is not well established, we used three different thresholds to define low NCA (*i.e.*, $NCA < 150$ mg/d, < 120 mg/d, and < 100 mg/d). We calculated sensitivity, specificity, positive predictive value, and negative predictive value (NPV) using 24HUC cutoff points of 150, 125, and 100 mg/d.

2. Results

Two hundred and thirty postmenopausal women completed the initial FCA measurement and were randomly assigned to receive 1 year of placebo, low-dose vitamin D (800 IU daily), or high-dose vitamin D (50,000 IU daily for 15 days then every 15th day for 1 year); 221 women completed the 1-year trial. Personnel did not record the calcium isotope dose for two FCA visits, a urine sample was lost in another subject, 24HUC values were missing for two subjects, and one subject's calculated FCA was negative, presumably because of laboratory error. Thus, 445 FCA measures were available for the current analysis, including baseline FCA data for 228 subjects.

We summarized baseline characteristics of the 228 subjects in Table 1. Subjects' mean age was 60 ± 6 years; most were Caucasian (90%). Subjects' total calcium intake was 970 ± 465 mg/d. Baseline serum 25(OH)D level was 21 ± 5 ng/mL, FCA was 0.20 ± 0.08 , and NCA level was 184 ± 110 mg/d. At 1 year, subjects' mean 25(OH)D level was 29 ± 13 ng/mL with a range of 4 to 66 ng/mL.

NCA and 24HUC values demonstrated a positive correlation [Spearman correlation coefficient: $r = 0.34$; 95% confidence interval (CI), 0.25 to 0.42; $P < 0.0001$; (Fig. 1)]. We also found positive correlations between NCA and FCA ($r = 0.68$; 95% CI, 0.63 to 0.73; $P < 0.0001$) and between FCA and 24HUC [$r = 0.21$; 95% CI, 0.12 to 0.30; $P < 0.0001$; (Fig. 2)]. We found a negative correlation between FCA and total calcium intake [$r = -0.12$; 95% CI, -0.22 to -0.03 ; $P = 0.009$; (Fig. 3)] consistent with previous studies [4, 14, 15], which demonstrated that greater calcium intake reduced the percentage absorbed. Total calcium intake was positively associated with NCA ($r = 0.59$; 95% CI, 0.52 to 0.65; $P < 0.001$). Age was inversely associated with FCA ($r = -0.17$; 95% CI, -0.26 to -0.07 ; $P = 0.0004$) but not with NCA ($r = -0.03$; $P = 0.51$).

Table 2 summarizes the ability of 24HUC values to diagnose low calcium absorption using various thresholds to define malabsorption. When calcium malabsorption was defined as $NCA < 100$ mg/d, a 24HUC value < 150 mg optimized sensitivity (72%), specificity (65%), and NPV (94%) to diagnose low calcium absorption.

We compared participants with $NCA < 150$ mg/d with those with $NCA \geq 150$ mg/d (Table 1). Baseline calcium, parathyroid hormone, 25(OH)D, calcitriol [$1,25(\text{OH})_2\text{D}$], estradiol, creatinine, and GFR levels were not different between the two groups. Baseline intake of most nutrients, including calcium, vitamin D, carbohydrates, protein, fat, fiber,

Table 1. Characteristics of the Participants

| Characteristic | All Participants (n = 228) | Participants With Net Calcium Absorption <150 mg/d (n = 79) | Participants With Net Calcium Absorption ≥150 mg/d (n = 149) | P Value |
|--------------------------------|-------------------------------|---|--|---------|
| Age, mean (SD), y | 61 (6) | 61 (6) | 60 (6) | 0.32 |
| Race, No. (%) | | | | 0.93 |
| White | 205 (90) | 72 (91) | 133 (89) | |
| Black | 14 (6) | 5 (6) | 9 (6) | |
| Asian | 5 (2) | 1 (1) | 4 (3) | |
| American Indian/Alaskan | 2 (1) | 0 (0) | 2 (1) | |
| Hispanic | 2 (1) | 1 (1) | 1 (0.7) | |
| Serum laboratory measures | | | | |
| Calcium, mg/dL | 9.1 (0.4) | 9.0 (0.4) | 9.1 (0.5) | 0.14 |
| Albumin, g/dL | 3.9 (0.3) | 4 (0.3) | 3.9 (0.3) | 0.94 |
| Creatinine, mg/dL | 0.8 (0.2) | 0.8 (0.2) | 0.8 (0.2) | 0.39 |
| GFR, mL/min | 78 (21) | 78 (17) | 78 (20) | 0.47 |
| PTH, pg/mL | 48 (23) | 49 (22) | 48 (23) | 0.84 |
| 25(OH)D, ng/mL | 21 (5) | 20 (5) | 22 (5) | 0.30 |
| 1,25(OH) ₂ D, pg/mL | 41 (23) | 40 (21) | 42 (24) | 0.31 |
| Estradiol, pg/mL | 48 (16) | 50 (12) | 47 (16) | 0.85 |
| Dietary habits | | | | |
| Caloric intake, kcal/d | 1842 (662) | 1672 (432) | 1947 (621) | <0.01 |
| Carbohydrates, g/d | 223 (92) | 192 (91) | 236 (63) | <0.01 |
| Protein, g/d | 76 (19) | 65 (16) | 81 (18) | <0.01 |
| Fat, g/d | 72 (31) | 66 (22) | 77 (34) | <0.01 |
| Fiber, g/d | 19 (11) | 17 (11) | 20 (12) | 0.01 |
| Dietary calcium, mg/d | 910 (397) | 677 (344) | 986 (348) | <0.01 |
| Total calcium intake, mg/d | 970 (471) | 741 (331) | 1066 (425) | <0.01 |
| Vitamin D, IU/d | 196 (152) | 129 (140) | 207 (143) | <0.01 |
| Iron, mg/d | 13 (6) | 12 (5) | 14 (6) | <0.01 |
| Magnesium, mg/d | 306 (123) | 258 (116) | 318 (113) | <0.01 |
| Oxalate, servings per day | 0.9 (1.4) | 0.8 (1.4) | 1.0 (1.4) | 0.22 |

Normal reference ranges for serum laboratory tests are calcium, 8.4–10.2 mg/dL; albumin, 3.5–5.0 g/dL; creatinine, 0.55–1.02 mg/dL; GFR, 60–120 mL/min/1.73 m²; PTH, 23–97 pg/mL; 25(OH)D, 30–80 ng/mL; and 1,25(OH)₂D, 19.9–79.3 pg/mL. Estradiol was measured using an in-house assay with a lower detectable limit of 3 pg/mL and inter- and intra-assay coefficients of variation of 5.0% and 4.3%, respectively. Serum laboratory measures and dietary habits values are given as mean (SD).

Abbreviation: SD, standard deviation; PTH, parathyroid hormone.

iron, magnesium, and kilocalories was significantly higher in participants with NCA ≥150 mg/d.

3. Discussion

Low calcium absorption is a known risk factor for osteoporosis and hip fracture [3]. The gold standard fractional calcium absorption measurement requires administration of two calcium tracers and subsequent 24-hour urine collection. The method is costly and time-consuming, making it impractical for clinical use. We determined whether inexpensive 24HUC levels could be used to detect low calcium absorption. We observed that 24HUC levels positively correlated with FCA and NCA. Compared with 24HUC thresholds of 125 and 100 mg/d, a 24HUC threshold of 150 mg/d optimized sensitivity and specificity for low calcium absorption without compromising NPV.

Some clinicians use the spot urine calcium-to-creatinine ratio (SUCCR) to diagnose hypercalciuria on the basis of studies showing a correlation between the SUCCR and 24HUC levels [19, 20]. However, others found robust correlations only in subjects with hypercalciuria

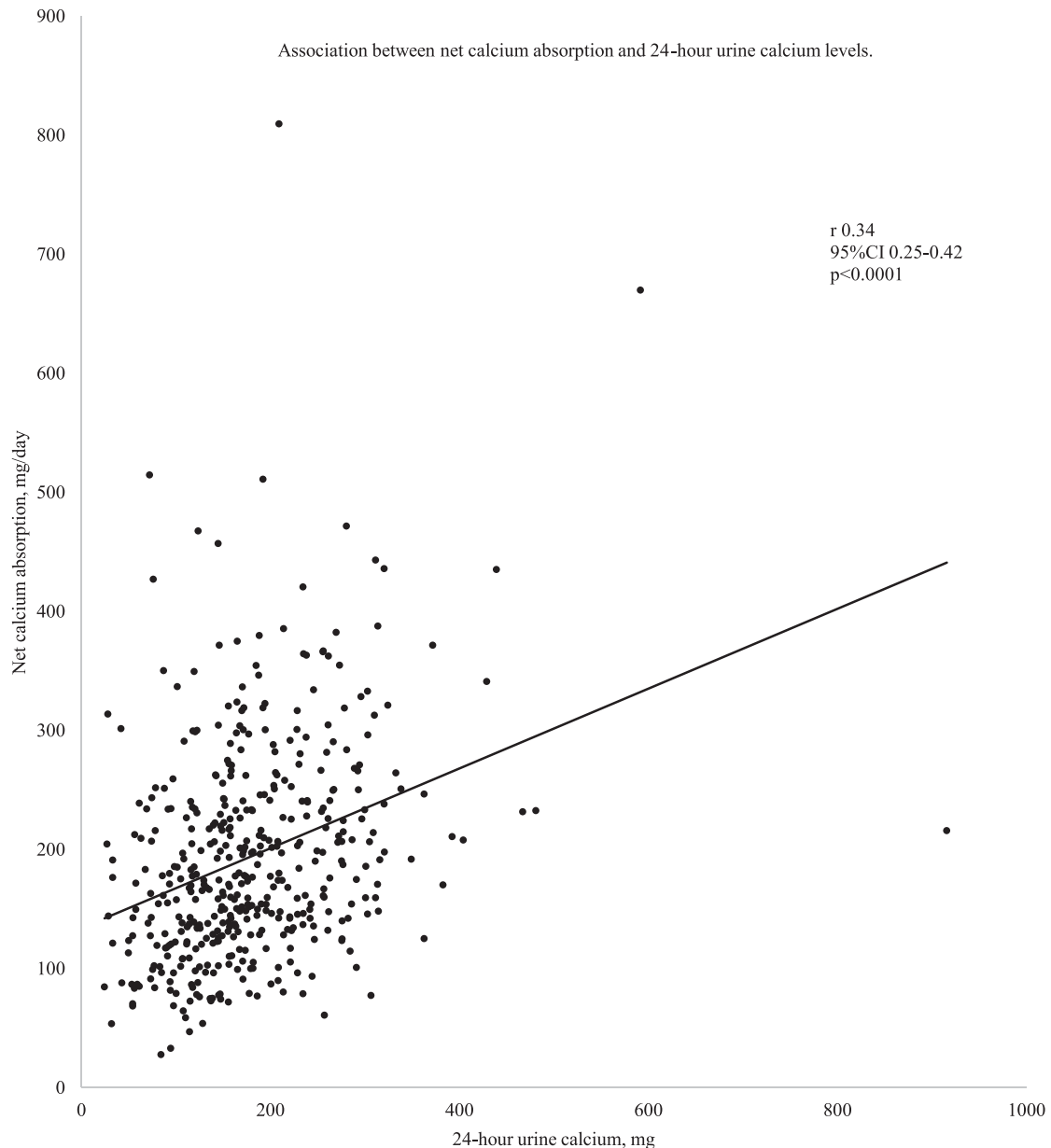


Figure 1. Association between net calcium absorption and 24-hour urine calcium levels. Net calcium absorption and 24-hour urine calcium values demonstrated a positive correlation ($r = 0.34$; 95% CI, 0.25 to 0.42; $P < 0.0001$).

[21]. Jones *et al.* [22] found that a fasting SUCCR underestimated 24HUC values by a mean of 83 mg. Isaacson and Jackson [21] reported large intraindividual variations in SUCCR test results over a 24-hour interval. In another paper, Jones *et al.* [23] found that neither fasting nor postprandial SUCCR level could reliably replace 24HUC values. Therefore, the SUCCR is an inadequate substitute for 24HUC levels.

We also analyzed factors associated with low calcium absorption in postmenopausal women. We found that dietary intake of kilocalories, calcium, vitamin D, carbohydrates, protein, fat, fiber, iron, and magnesium was significantly higher in participants with an NCA ≥ 150 mg/d. Previous studies found that age, calcium intake, $1,25(\text{OH})_2\text{D}$ level, dietary intake of kilocalories, carbohydrates, and fat were significantly associated with calcium absorption, whereas serum $25(\text{OH})\text{D}$ level was not associated with calcium absorption [2, 3, 5, 17, 18, 24, 25]. In our study, which excluded subjects with vitamin D deficiency, $25(\text{OH})\text{D}$ and

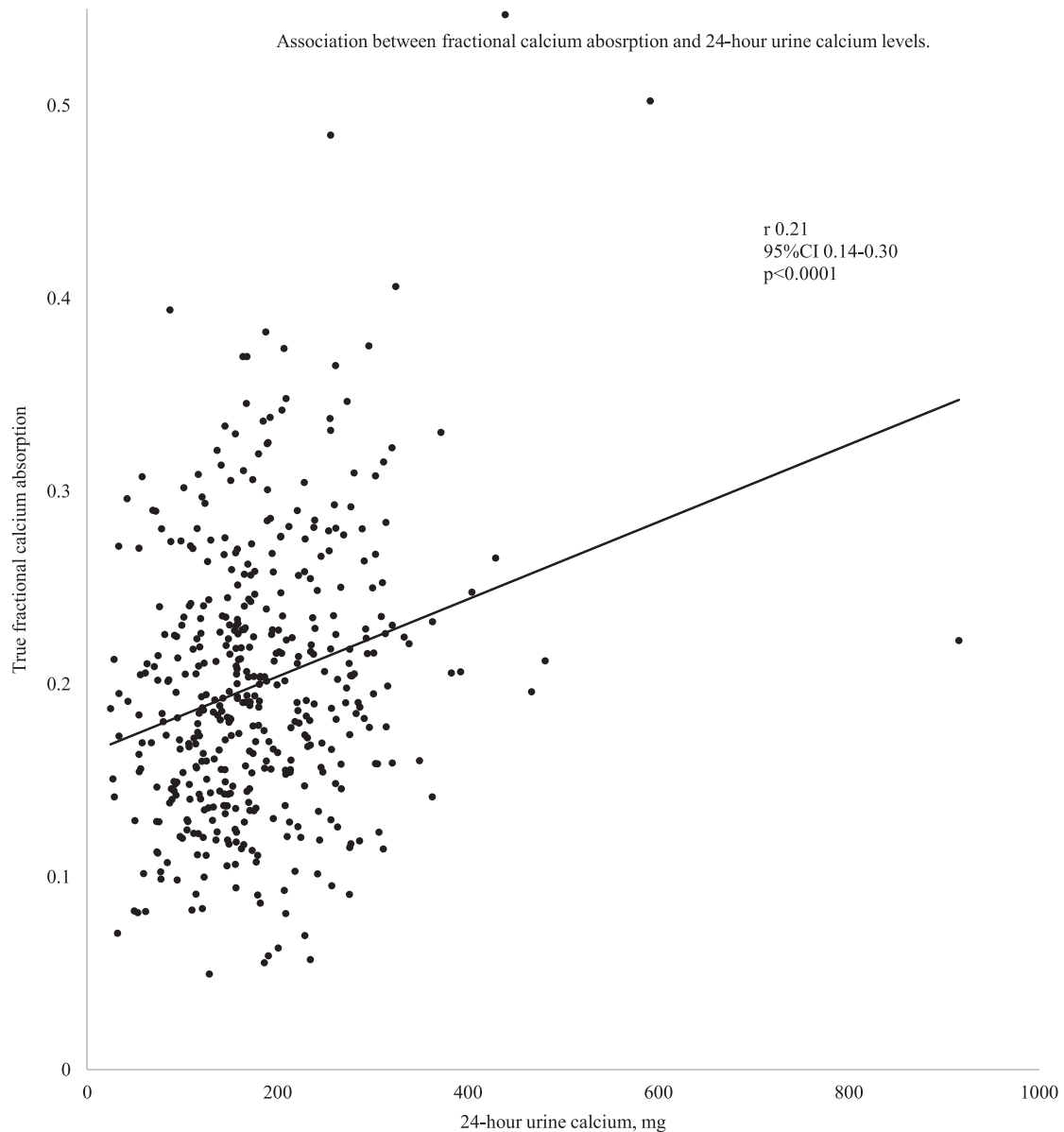


Figure 2. Association between fractional calcium absorption and 24-hour urine calcium levels. Fractional calcium absorption and 24-hour urine calcium values demonstrated a positive correlation ($r = 0.21$; 95% CI, 0.14 to 0.30; $P < 0.0001$).

1,25(OH)₂D levels were similar in participants with NCA <150 mg/d and in those with NCA ≥150 mg/d [26].

Our study had several strengths. We used the gold standard dual isotope method to measure FCA, and participants remained on the Clinical Research Unit, permitting a complete 24-hour urine collection. We replicated typical outpatient dietary habits during FCA measurement. We studied postmenopausal women, a group most likely to benefit from a diagnosis of impaired calcium absorption because of their higher prevalence of osteoporotic fractures. Our study encompassed a large spectrum of 25(OH)D levels because we measured FCA at baseline and again 1 year later, when subjects' vitamin D levels ranged from 4 to 66 ng/mL.

We also note some study limitations. Subjects collected urine during an inpatient research study with assistance from research nurses. Thus, the reliability of outpatient urine collections may be lower. We excluded individuals with GFR <45 mL/min and therefore cannot

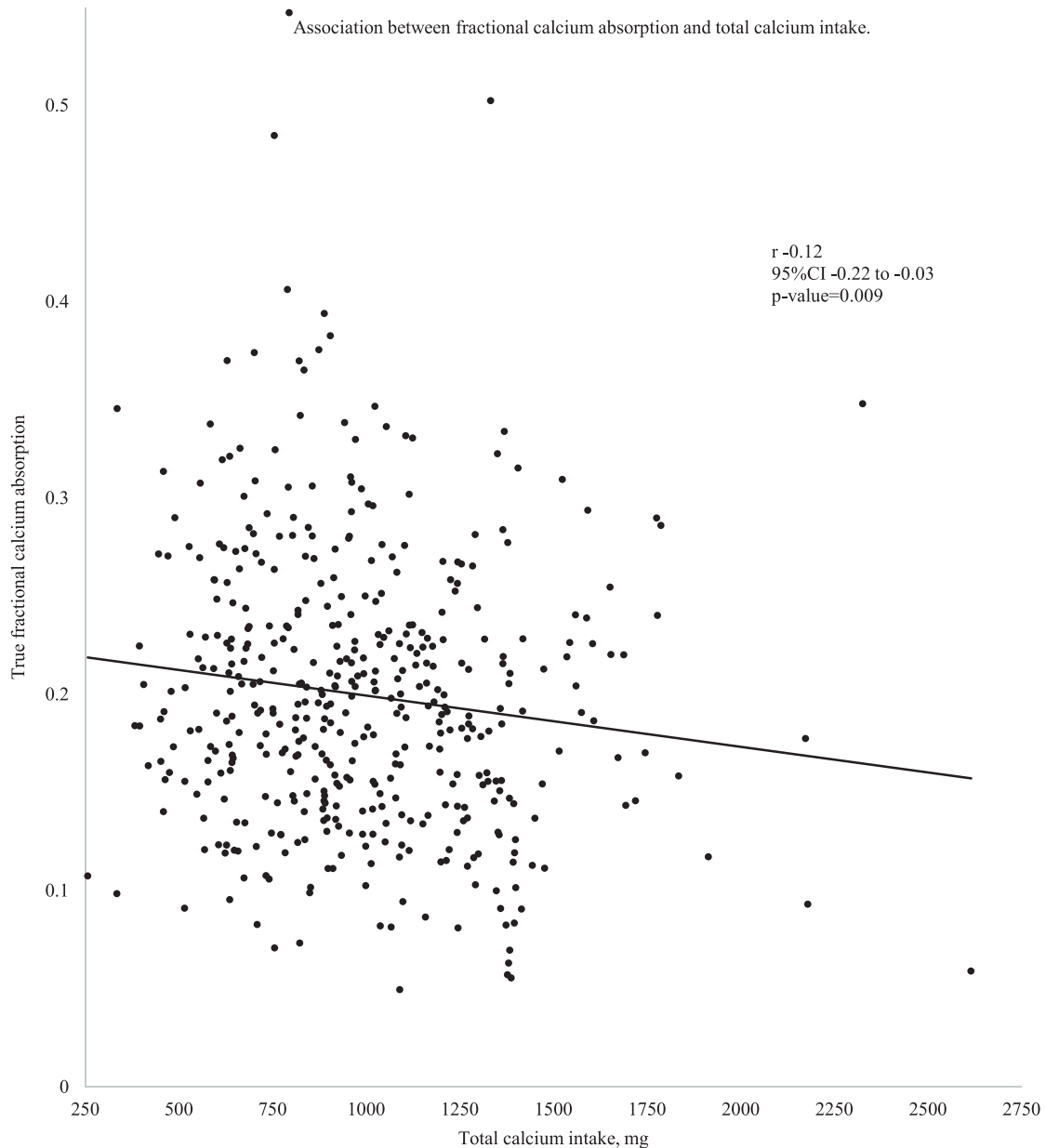


Figure 3. Association between fractional calcium absorption and total calcium intake. Fractional calcium absorption and total calcium intake demonstrated an inverse correlation ($r = -0.12$; 95% CI, -0.22 to -0.03 ; $P = 0.009$).

determine whether 24HUC values can be used to exclude impaired FCA in this group. Most of our participants were Caucasian women; therefore, we cannot state whether our results apply to other populations. Clearly, it is imperative to validate these findings in additional studies, particularly in other patient populations.

In conclusion, adequate calcium intake and absorption is a crucial physiologic process that maintains bone health and when impaired contributes to osteoporosis. Measuring calcium absorption could allow clinicians to optimize nutritional habits for patients with osteoporosis. We found that 24HUC levels correlated with gold standard FCA and NCA values. A 24HUC level <150 mg/d demonstrated high NPV, excluding calcium malabsorption. We suggest that clinicians consider 24HUC levels as a way to screen for calcium malabsorption and potentially guide dietary recommendations for optimal bone health.

Table 2. Ability of 24-Hour Urine Calcium Levels to Diagnose Low Calcium Absorption

| 24-Hour Urine Calcium Threshold | Low Calcium Absorption Defined as: | Sensitivity | Specificity | Positive Predictive Value | Negative Predictive Value |
|---------------------------------|------------------------------------|-------------|-------------|---------------------------|---------------------------|
| 150 mg/d | NCA <150 mg/d | 0.55 | 0.70 | 0.53 | 0.72 |
| | NCA <120 mg/d | 0.65 | 0.67 | 0.31 | 0.89 |
| | NCA <100 mg/d | 0.72 | 0.65 | 0.22 | 0.94 |
| 125 mg/d | NCA <150 mg/d | 0.39 | 0.79 | 0.53 | 0.68 |
| | NCA <120 mg/d | 0.50 | 0.78 | 0.34 | 0.87 |
| | NCA <100 mg/d | 0.54 | 0.76 | 0.24 | 0.92 |
| 100 mg/d | NCA <150 mg/d | 0.23 | 0.89 | 0.57 | 0.66 |
| | NCA <120 mg/d | 0.32 | 0.88 | 0.39 | 0.85 |
| | NCA <100 mg/d | 0.35 | 0.87 | 0.28 | 0.91 |

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