Factors Associated with Calcium Absorption in Postmenopausal Women: A Post Hoc Analysis of Dual-Isotope Studies

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ABSTRACT

Reduced calcium absorption is a risk factor for osteoporosis. This study examined factors associated with fractional calcium absorption (FCA) and net calcium absorption in postmenopausal women in a post hoc analysis of three completed dual-isotope studies. Data were analyzed from 50 postmenopausal women undergoing 121 inpatient research visits in three studies evaluating changes in FCA related to correction of vitamin D insufficiency (n=19), use of proton pump inhibitors (n=21), and use of aromatase inhibitors to treat breast cancer (n=10). Net calcium absorption was the product of FCA and total calcium intake in milligrams per day. Variables included subjects' age, race, body mass index, serum calcium, creatinine, parathyroid hormone, 1,25-dihydroxyvitamin D, 25-hydroxyvitamin D, and habitual intake of kilocalories, protein, fat, carbohydrate, fiber, calcium, iron, magnesium, oxalate, phosphorus, potassium, and vitamin D based on outpatient diet diaries. In multivariate models, subjects' age, dietary intake of kilocalories, carbohydrates, fat, fiber, calcium, and potassium were significant predictors of FCA. In multiple variable models predicting net calcium absorption, dietary intake of kilocalories, fat, fiber, calcium, potassium, and serum 1,25-dihydroxyvitamin D were significant. The square of the correlation between actual and predicted values (an approximation of R^2) was 0.748 for FCA and 0.726 for net calcium absorption. Similar to other studies, this study found that age, 1,25-dihydroxyvitamin D, and dietary calcium and fat were associated with calcium absorption. Dietary intake of kilocalories, carbohydrates, and potassium were new factors that were significantly associated with FCA and net calcium absorption. In summary, the study suggests that several dietary habits play a role in calcium absorption, beyond vitamin D and calcium.
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CALCIUM ABSORPTION EFFICIENCY INFLUENCES calcium balance and, therefore, the likelihood of osteoporosis and subsequent fracture. In the Study of Osteoporotic Fractures, 5,452 nonblack women, 69 years of age or older, underwent measurement of calcium absorption using a single radioisotope level.[1] The age-adjusted relative risk of hip fracture was 1.24 (95% CI 1.05 to 1.48) for each standard deviation (7.7%) decrease in calcium absorption.

Calcium absorption decreases with age,[2-4] with an additional decrease at the time of menopause,[5] which is reversible with estrogen therapy.[6] Cross-sectional studies[2,3,7-12] reported positive associations between calcium absorption and serum 1,25-dihydroxyvitamin D (1,25$[OH]_2$D),[2,3,8,9,11,13,14] estradiol,[14] calcium,[10] dietary fat,[8,14] and obesity.[14] Studies also found negative associations between calcium absorption and increasing age,[3] dietary fiber,[8] alcohol,[8] smoking,[9] and intestinal villus width.[10]

Most calcium absorption studies[2-4,8-11,13] measured calcium absorption using a single isotope. However, the technique can overestimate calcium absorption because intestinal calcium excretion and renal calcium recycling contribute to peak plasma tracer levels. In addition, intestinal transit time,[15,16] volume of distribution,[15,17] and the balance between calcium absorption and clearance[15] can affect the time post dosing that peak plasma tracer levels occur. Some conclude that peak plasma isotope levels are not as reliable as dual-isotope levels when measuring calcium absorption.[16,18] The dual-isotope method is the optimal technique to measure calcium absorption, as it accounts for endogenous fecal calcium excretion and renal calcium recycling.[15,19]

Knowledge of factors affecting fractional calcium absorption (FCA) might allow clinicians to target these factors when caring for postmenopausal women with osteoporosis. Most calcium-absorption studies used a single isotope and/or focused on a limited number of factors affecting calcium absorption, potentially limiting knowledge of factors affecting calcium absorption. A post hoc analysis of three dual-isotope studies in postmenopausal women was performed, to evaluate associations between

22 demographic, dietary, and laboratory characteristics and calcium absorption.

METHODS

Postmenopausal women were recruited for studies evaluating changes in FCA related to treatment of vitamin D insufficiency[20] or therapy with a proton pump inhibitor[21] or aromatase inhibitor.[22] Eligibility was similar across studies. Subjects were 5 or more years past menopause, without stage 4 to 5 chronic kidney disease, malabsorption, achlorhydria, or use of anticonvulsant or systemic glucocorticoid therapy. Subjects in the vitamin D study had serum 25-hydroxyvitamin D (25[OH]D) levels between 16 and 24 ng/mL (40 and 60 nmol/L) and no clinical or densitometric evidence of osteoporosis; calcium absorption was measured at baseline and after correction of vitamin D insufficiency with high-dose ergocalciferol (19 subjects, 38 observations).[20] Subjects in the aromatase inhibitor study had early-stage breast cancer and were beginning adjuvant aromatase inhibitor therapy after lumpectomy and/or radiation therapy; calcium absorption was measured at baseline and after taking anastrazole daily for 6 weeks or more (10 subjects, 20 observations).[22] Subjects in the proton pump inhibitor study underwent two baseline calcium absorption studies 1 month apart, and a third study after taking 40 mg omeprazole daily for approximately 30 days (21 subjects, 63 observations).[21] The University of Wisconsin Human Subjects Committee approved each study, and participants provided written informed consent before study procedures. Each study was registered as a clinical trial (ClinicalTrials.gov NCT00581828, NCT00582972, NCT00766532).

Study Interventions

The dual-isotope method[19] was used to measure FCA. Stable calcium isotopes (^{42}Ca and ^{44}Ca) were purchased as calcium carbonate powder, reconstituted into solution,[20] and tested for sterility and pyrogenicity before human use. The dose-corrected ratio of two calcium isotopes in 24-hour urine collection was used to calculate FCA.[19] Women were admitted to the University of Wisconsin Clinical Research Unit for FCA studies at baseline and after the intervention. Women fasted from midnight until 7:00 AM on the day of admission. The 24-hour urine collection began after each woman voided on the research ward. Nurses drew blood for measurement of 25(OH)D, 1,25$(OH)_2$D, intact parathyroid hormone (PTH), calcium, and creatinine. With breakfast, subjects consumed a glass of milk or calcium-fortified orange juice (50±7 mL) containing 8 to 15 mg of ^{44}Ca. Simultaneously, nurses infused approximately 2 to 3 mg ^{42}Ca intravenously. Nurses weighed the full and empty calcium isotope syringes to record the administered doses of ^{42}Ca and ^{44}Ca. The breakfast meal provided 300 to 305 mg calcium, including the content of the vehicle used to administer the oral isotope.

For all studies, subjects completed outpatient food records lasting 4[22] or 7 consecutive days,[20,21] using a scale to weigh portions. Food records were analyzed using Food Processor Nutrition Analysis Software from ESHA Research to calculate subjects' mean habitual outpatient intake of energy, macronutrient, fiber, calcium, iron, magnesium, sodium, vitamin D, phosphorous, potassium, and oxalate. Data on habitual intake were used to design meals during calcium absorption study visits; food during each 24-hour inpatient study replicated each subject's outpatient diet based on analysis of her diet diary.

Laboratory Analysis

Procedures for measuring serum chemistries, PTH, 25(OH)D and 1,25$(OH)_2$D were similar across studies. Serum 25(OH)D was measured in a University of Wisconsin research laboratory using a semi-automated solid-phase extraction reverse-phase high-performance liquid chromatography assay.[23] Between-run precision coefficients of variation for the assay ranged from 2.6% to 4.9% for 25$(OH)D_3$ and from 3.2% to 12.6% for 25$(OH)D_2$. Serum 1,25$(OH)_2$D was measured using a radioimmunoassay kit with an intra- and interassay coefficient of variation of 8% to 12% and 9% to 15%, respectively. Personnel measured PTH using an electrochemiluminescence immunoassay kit with reported intra- and interassay coefficients of variation of 1% to 4% and 2% to 7%, respectively. Serum calcium and creatinine were measured in a regional laboratory (Meriter Medical Laboratories or University of Wisconsin, Madison) using a Roche Integra autoanalyzer.

Wisconsin State Laboratory of Hygiene personnel measured calcium concentrations and isotope ratios of urine samples using high-resolution inductively coupled plasma mass spectrometry (Finnigan Element 2, Thermo Instruments) as described previously.[24] The laboratory analyzed each subject's urine sample on at least two occasions, and means of data were used to calculate FCA. The Pearson correlation coefficient for values obtained by duplicate analyses of urine specimens was r=0.98 (P<0.0001) for ^{42}Ca/^{43}Ca and r=0.97 (P<0.0001) for ^{44}Ca/^{43}Ca.

Statistical Analysis

All three studies had a similar study design, including use of dual calcium isotopes, inclusion and exclusion criteria, laboratory studies, and careful assessment of habitual intake of nutrients using 4- to 7-day food diaries, with replication of each subject's typical nutrient intake during her inpatient research studies. Only one[20] of three studies detected a significant pair-wise change in subjects' FCA with the study intervention. Nevertheless, the slopes and intercepts of relationships between baseline and subsequent FCA (or net calcium absorption) and each candidate variable (n=22) were carefully examined to determine whether the studies could be combined.

In the proton pump inhibitor study,[21] subjects underwent two baseline absorption studies and a third study after taking omeprazole 40 mg daily for 30 days; FCA was not altered by omeprazole. In the vitamin D study,[20] high-dose ergocalciferol significantly increased FCA, leading to significantly different slopes and intercepts for plots of FCA vs potential covariates for data from the second study visit. Compared with subjects in the other two studies, subjects with breast cancer[22] had significantly lower FCA (Table 1) and significantly different slopes and intercepts when modeling relationships between FCA and candidate variables (n=22). Therefore, for analysis of potential factors associated with FCA in the current study, all proton pump inhibitor study data (up to three measurements per subject) were used, along with baseline data from the vitamin D study.