Published in final edited form as:

J Acad Nutr Diet. 2014 May ; 114(5): 761–767. doi:10.1016/j.jand.2013.07.041.

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

Karishma Ramsubeik, MD,

Rheumatology Fellow, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA; Phone 608-263-3457, FAX 608-263-7353; KRamsubeik@uwhealth.org

Nicholas S Keuler, MS,

Department of Statistics, College of Letters and Science, University of Wisconsin, Madison, WI, USA; Phone 608-265-9219, keuler@stat.wisc.edu

Lisa A Davis, RD, MS, and

University of Wisconsin Institute for Clinical and Translational Research, Madison, WI, USA, Phone 608-263-8244, FAX 608-265-9225, LDavis5@uwhealth.org

Karen E Hansen, MD, MS

Associate Professor of Medicine, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA, phone 608-265-8162, FAX 608-263-7353, keh@medicine.wisc.edu

Abstract

Reduced calcium absorption is a risk factor for osteoporosis. This study examined factors associated with fractional calcium absorption (FCA) and net calcium absorption (NCA) in postmenopausal women in a post-hoc analysis of three completed dual isotope studies. Data were analyzed from fifty 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). NCA was the product of FCA and total calcium intake in mg/day. Variables included subjects' age, race, body mass index, serum calcium, creatinine, parathyroid hormone, 1,25(OH)₂D, 25(OH)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 NCA, dietary intake of kilocalories, fat, fiber, calcium, potassium and serum 1,25(OH)₂D were significant. The square of the correlation between actual and predicted values (an approximation of R²) was 0.748 for FCA and 0.726 for NCA. Similar to other studies, this study found that age, 1,25(OH)₂D and dietary calcium and fat were associated with calcium absorption. Dietary intake of kilocalories, carbohydrates and potassium were new factors that significantly associated with

© 2013 Academy of Nutrition and Dietetics. Published by Elsevier Inc. All rights reserved.

Author responsible for correspondence and reprint requests: Karen E. Hansen.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

FCA and NCA. In summary, the study suggests that several dietary habits play a role in calcium absorption, beyond vitamin D and calcium.

Keywords

calcium absorption; calories; fat; fiber; postmenopausal women

Introduction

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 underwent measurement of calcium absorption using a single radioisotope level.⁽¹⁾ The age-adjusted relative risk of hip fracture was 1.24 (95% confidence interval, 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⁾ that is reversible with estrogen therapy.⁽⁶⁾ Cross-sectional studies^(2, 3, 7–12) reported positive associations between calcium absorption and serum 1,25(OH)₂D,^(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^(2–4, 8–11, 13) calcium absorption studies 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. Additionally, 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. Thus, 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 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 years past

menopause, without stage 4–5 chronic kidney disease, malabsorption, achlorhydria, or use of anticonvulsant or systemic glucocorticoid therapy. Subjects in the vitamin D study had serum 25(OH)D levels between 16 and 24 ng/mL 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 anastrozole daily for 6 weeks (10 subjects, 20 observations).⁽²²⁾ Subjects in the omeprazole study underwent two baseline calcium absorption studies one month apart, and a third study after taking 40 mg omeprazole daily for ~30 days (21 subjects, 63 observations).⁽²¹⁾ The University of Wisconsin (UW) Human Subjects Committee approved each study, and participants provided written informed consent prior to 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 (⁴²Ca and ⁴⁴Ca) were purchased as calcium carbonate powder, reconstituted into solution⁽²⁰⁾ and tested for sterility and pyrogenicity prior to 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 UW Clinical Research Unit for FCA studies at baseline and after the intervention. Women fasted from midnight until 0700 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)₂D, intact parathyroid hormone (PTH), calcium and creatinine. With breakfast, subjects consumed a glass of milk or calcium-fortified orange juice (50 ± 7 mL) containing 8–15 mg of ⁴⁴Ca. Simultaneously, nurses infused ~2–3 mg of ⁴²Ca intravenously. Nurses weighed the full and empty calcium isotope syringes to record the administered doses of ⁴²Ca and ⁴⁴Ca. The breakfast meal provided 300–305 mg of calcium, including the content of the vehicle used to administer the oral isotope.

For all studies, subjects completed outpatient food records lasting four⁽²²⁾ or seven consecutive days,^(20, 21) using a scale to weigh portions. Food records were analyzed using Food Processor Nutrition Analysis Software from ESHA Research (Salem, Oregon) to calculate subjects' average 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)₂D were similar across studies. Serum 25(OH)D was measured in a UW research laboratory using a semi-automated solid phase extraction reverse phase HPLC assay.⁽²³⁾ Between-run precision coefficients of variation (CVs) for the assay ranged from 2.6% to 4.9% for 25(OH)D₃ and 3.2% to 12.6% for 25(OH)D₂. Serum 1,25(OH)₂D was measured using a radioimmunoassay

kit with an intra- and interassay CV of 8% to 12% and 9% to 15%, respectively. Personnel measured PTH using an electrochemiluminescence immunoassay kit with reported intra- and interassay CVs 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, WI, USA) using a Roche Integra autoanalyzer.

Wisconsin State Lab of Hygiene laboratory personnel measured calcium concentrations and isotope ratios of urine samples using high-resolution inductively coupled plasma mass spectrometry (Finnigan Element 2, Thermo Instruments, Bremen, Germany) as previously described.⁽²⁴⁾ The laboratory analyzed each subject's urine sample on at least two occasions, and averages of data were used to calculate FCA. The Pearson correlation coefficient for values obtained by duplicate analyses of urine specimens was $r=0.98$ ($p<.0001$) for $^{42}\text{Ca}/^{43}\text{Ca}$ and $r=0.97$ ($p<.0001$) for $^{44}\text{Ca}/^{43}\text{Ca}$.

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 four to seven 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 NCA) and each candidate variable ($n=22$) were carefully examined to determine if the studies could be combined.

In the proton pump 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 versus 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.

Net calcium absorption (NCA, milligrams of calcium absorbed per day) was the product of total calcium intake (dietary and supplemental calcium consumed in mg/day) times FCA. When FCA data was transformed into NCA data, the slopes and intercepts of candidate variables impacting NCA across the three studies (seven visits) were not significantly different. Therefore data from all studies were combined, to evaluate factors associated with NCA.

Data was graphed to ensure relationships were linear, if they existed, and to detect the presence of outliers. Mean FCA was 0.209 ± 0.084 while NCA was 239 ± 102 mg/day, with two outliers in the FCA dataset (one subject with 3.5% and the other with 46.8% FCA values) and three outliers in the NCA dataset (subjects who absorbed 39 mg, 533 mg and

739 mg of calcium per day). Outliers were excluded in univariate and multivariate analyses. Linear mixed models were used to assess relationships between calcium absorption (FCA and NCA) and subjects' demographic, laboratory and nutritional variables (n=22), with subject ID as a random effect to account for potential correlation among the multiple measurements on a single subject. Demographic variables included subjects' age, race and body mass index. Laboratory variables included subjects' serum levels of calcium, creatinine, PTH, 1,25(OH)₂D and 25(OH)D. Glomerular filtration rate was calculated from the Modification of Diet in Renal Disease formula.⁽²⁵⁾ Nutritional variables included subjects' habitual dietary intake of kilocalories, protein, carbohydrate, fat, fiber, calcium, iron, magnesium, oxalate, phosphorus, potassium, and vitamin D based on outpatient food records. Dietary sodium data was skewed; therefore sodium was excluded in all analyses.

After fitting the single-variable linear mixed models, a stepwise (simultaneous backward and forward) procedure was used to determine the subset of variables providing the best fit for the response variable, FCA or NCA, using the Akaike information criterion as the fit criterion. Since R^2 is not well-defined for mixed models, and the Akaike information criterion is only a relative measure of fit, two other quantities were used to assess absolute model fit. The square of the correlation between the predicted and observed calcium absorption values (equivalent to R^2 for a linear model without mixed effects) and the root mean square prediction error (RMSPE, indicating accuracy of the model to predict FCA or NCA for a new subject) were calculated. Data were analyzed using R software (version 2.13.1 The R Project for Statistical Computing, <http://www.r-project.org>) and p values < 0.05 were considered significant.

Results and Discussion

Table 1 summarizes subjects' baseline characteristics including their average intake of nutrients based on the outpatient food diary. All data exhibited a normal or approximately normal distribution and are summarized using the mean $\pm$ standard deviation. Subjects' age was 60 ± 8 years; most were Caucasian with a dietary calcium intake of 940 ± 304 mg/day and supplemental calcium intake of 358 ± 512 mg/day. Participants' baseline serum 25(OH)D level was 28 ± 11 ng/mL; 11 had subjects levels <20 ng/mL. Mean FCA was 0.209 ± 0.084 while NCA was 239 ± 102 mg/day.

FCA was first assessed as a function of race. White women had significantly lower FCA (0.194 ± 0.081) compared to Hispanic (0.308 ± 0.091 , $p=0.0008$) and Black (0.310 ± 0.094 , $p=0.0007$) women. However, because race was a dichotomous variable and there were a small numbers of non-White subjects, race was excluded as a variable in multiple regression models predicting FCA.

In single-variable linear mixed models of FCA, age and intake of calcium and magnesium were negatively associated with FCA, whereas PTH was positively associated with FCA (Table 2). In stepwise models predicting FCA (Table 3), subjects' age and dietary intake of kilocalories, carbohydrates, fat, fiber, calcium and potassium were significant. Addition of serum PTH, 1,25(OH)₂D levels and dietary vitamin D intake improved the model. The square of the correlation between predicted and observed FCA using these ten variables

(approximation of R^2) was 0.748 and the RMSPE was 0.044 absolute units of FCA (a 4.4% error in calcium absorption).

NCA was assessed as a function of race; no significant difference was found across racial groups. Net calcium absorption was 253 ± 113 mg/day in White, 228 ± 89 mg/day in Hispanic ($p=0.565$ vs. White) and 178 ± 55 mg/day in Black ($p=0.086$ vs. White) women. In single-variable linear mixed models of NCA (Table 2), intake of kilocalories, carbohydrates, calcium, magnesium, phosphorus, potassium, vitamin D and serum creatinine, GFR and $1,25(\text{OH})_2\text{D}$ levels were all significant. In stepwise models predicting NCA (Table 3), dietary intake of kilocalories, carbohydrates, fat, fiber, calcium, potassium and serum $1,25(\text{OH})_2\text{D}$ levels were significant; addition of GFR improved the model. The square of the correlation between predicted and observed NCA using these variables (approximation of R^2) was 0.726 and the RMPSE was 52 mg of calcium/day.

In keeping with other studies, age, $1,25(\text{OH})_2\text{D}$ and consumption of calcium and fat were significantly associated with calcium absorption. Subjects' total calcium intake showed a strong and consistent association with both FCA and NCA, whereas the association with $1,25(\text{OH})_2\text{D}$ was weaker, and of borderline or significance in models of FCA. Researchers reported that FCA was inversely correlated with calcium intake in 24 premenopausal⁽²⁶⁾ and postmenopausal women.⁽²⁷⁾ Consistent with previous studies,^(2, 3, 7, 9, 10, 13, 14, 28) this study found no relationship between $25(\text{OH})\text{D}$ levels and calcium absorption, though most subjects had $25(\text{OH})\text{D}$ levels > 20 ng/mL.

Dietary fat was positively associated with FCA and NCA. Wolf et al.⁽⁸⁾ also reported a positive association between dietary fat and FCA in 142 healthy pre- and perimenopausal women. Likewise, Shapses et al.⁽¹⁴⁾ found that dietary fat was a positive predictor of FCA in 229 women ages 54 ± 11 years old, and suggested that fat might indirectly increase FCA by increasing serum estradiol levels.

Dietary fiber was inversely associated with calcium absorption in multiple-variable models of FCA and NCA. Wolf et al. also reported an inverse association between dietary fiber and calcium absorption in women.⁽⁸⁾ However, Abrams et al. reported that a dietary fiber supplement with an inulin type fructan increased calcium absorption in adolescents.⁽²⁹⁾ The type of fiber seems important; wheat bran binds to calcium and reduces its absorption.⁽³⁰⁾

Several findings of this study are unique. In this study, dietary intake of kilocalories, carbohydrates and potassium were significantly associated with FCA and NCA. Weight loss decreases FCA, but prior studies have not directly linked caloric intake to reduced FCA.⁽³¹⁾ A recent study⁽¹⁴⁾ reported a positive relationship between carbohydrate intake and FCA ($r=0.189$, $p<0.05$), providing additional support for the relationship. Increased potassium intake might reflect a low dietary acid load leading to lower renal calcium excretion and bone resorption,⁽³²⁾ but no direct studies assessing dietary potassium intake and calcium absorption were found.

The study has both strengths and limitations. Strengths include the “gold standard” technique to measure FCA⁽¹⁹⁾ and use of food records, permitting the investigation of associations between many dietary habits and absorption. The current report represents a

post-hoc analysis of data from three studies. Slopes and intercepts of data comparing FCA to candidate variables were carefully analyzed to determine whether data across studies could be combined, but study participants had different health problems and medical treatments that might affect calcium absorption in ways that were not considered. A prospective sham-controlled blinded study is the optimal method by which to examine the effect of a nutrient on calcium absorption. Therefore, this cross-sectional study can only identify associations, not prove causation. Since a large number of variables were examined, the potential to detect some associations by chance exists.

Conclusion

Like other studies, this study found that age, calcium intake, 1,25(OH)₂D levels and dietary fat were significantly associated with calcium absorption whereas serum 25(OH)D levels did not associate with calcium absorption.^(2, 3, 7, 9, 10, 28) Several findings of this study are unique. Dietary intake of kilocalories, carbohydrates and potassium were significantly associated with FCA and NCA, suggesting that several factors influence calcium absorption, beyond the traditional focus on calcium and vitamin D. Additional studies are needed to evaluate these new findings.

References

1. Ensrud KE, Duong T, Cauley JA, et al. Low Fractional Calcium Absorption Increases the Risk for Hip Fracture in Women with Low Calcium Intake. *Ann Intern Med.* 2000; 132:345–353. [PubMed: 10691584]
2. Pattanaungkul S, Riggs BL, Yergey AL, et al. Relationship of Intestinal Calcium Absorption to 1,25-Dihydroxyvitamin D [1,25(OH)₂D] Levels in Young Versus Elderly Women: Evidence for Age-Related Intestinal Resistance to 1,25(OH)₂D Action. *JCEM.* 2000; 85:4023–4027. [PubMed: 11095427]
3. Nordin BEC, Need AG, Morris HA, et al. Effect of age on calcium absorption in postmenopausal women. *Am J Clin Nutr.* 2004; 80:998–1002. [PubMed: 15447911]
4. Ebeling PR, Yergey AL, Vieira NE, et al. Influence of age on effects of endogenous 1,25-dihydroxyvitamin D on calcium absorption in normal women. *Calcified tissue international.* 1994; 55:330–334. [PubMed: 7866911]
5. Heaney RP, Recker RR, Stegman MR, et al. Calcium absorption in women: relationships to calcium intake, estrogen status, and age. *J Bone Miner Res.* 1989; 4:469–475. [PubMed: 2816496]
6. Gallagher JC, Riggs BL, DeLuca HF. Effect of estrogen on calcium absorption and serum vitamin D metabolites in postmenopausal osteoporosis. *J Clin Endocrinol Metab.* 1980; 51:1359–1364. [PubMed: 6255005]
7. Tellez M, Arlot ME, Mawer EB, et al. Gastrointestinal calcium absorption and dietary calcium load: relationships with bone remodelling in vertebral osteoporosis. *Osteoporos Int.* 1995; 5:14–22. [PubMed: 7703619]
8. Wolf RL, Cauley JA, Baker CE, et al. Factors associated with calcium absorption efficiency in pre- and perimenopausal women. *Am J Clin Nutr.* 2000; 72:466–471. [PubMed: 10919942]
9. Need AG, Kemp A, Giles N, et al. Relationships between intestinal calcium absorption, serum vitamin D metabolites and smoking in postmenopausal women. *Osteoporosis Int.* 2002; 13:83–88.
10. Barger-Lux MJ, Heaney RP. Effects of above average summer sun exposure on serum 25-hydroxyvitamin D and calcium absorption. *Journal Clin Endocrinol Metab.* 2002; 87:4952–4956. [PubMed: 12414856]
11. Devine A, Wilson SG, Dick IM, et al. Effects of vitamin D metabolites on intestinal calcium absorption and bone turnover in elderly women. *Am J Clin Nutr.* 2002; 75:283–288. [PubMed: 11815319]

12. Cao JJ, Johnson LK, Hunt JR. A Diet High in Meat Protein and Potential Renal Acid Load Increases Fractional Calcium Absorption and Urinary Calcium Excretion without Affecting Markers of Bone Resorption or Formation in Postmenopausal Women. *J Nutr.* 2011; 141:391–397. [PubMed: 21248199]
13. Sai AJ, Walters RW, Fang X, et al. Relationship between Vitamin D, parathyroid hormone, and bone health. *J Clin Endocrinol Metab.* 2011; 96:E436–E446. [PubMed: 21159838]
14. Shapses SA, Sukumar D, Schneider SH, et al. Hormonal and dietary influences on true fractional calcium absorption in women: role of obesity. *Osteoporos Int.* 2012; 23:2607–2614. [PubMed: 22282301]
15. Griffin IJ, Abrams SA. Methodological considerations in measuring human calcium absorption: relevance to study the effects of inulin-type fructans. *Br J Nutr.* 2005; 93(Suppl 1):S105–S110. [PubMed: 15877883]
16. Harrison JE, McNeill KG, Wilson DR, et al. An evaluation of isotopic calcium absorption tests. *Clin Biochem.* 1973; 6:237–245. [PubMed: 4798239]
17. Heaney RP, Recker RR. Estimation of true calcium absorption. *Ann Intern Med.* 1985; 103:516–521. [PubMed: 4037557]
18. Spencer H, Fuller H, Norris C, et al. Effect of magnesium on the intestinal absorption of calcium in man. *J Am Coll Nutr.* 1994; 13:485–492. [PubMed: 7836628]
19. Eastell R, Vieira NE, Yergey AL, et al. One-day test using stable isotopes to measure true fractional calcium absorption. *J Bone Miner Res.* 1989; 4:463–468. [PubMed: 2816495]
20. Hansen KE, Jones AN, Lindstrom MJ, et al. Vitamin D insufficiency: disease or no disease? *J Bone Miner Res.* 2008; 23:1052–1060. [PubMed: 18302509]
21. Hansen KE, Jones AN, Lindstrom MJ, et al. Do proton pump inhibitors decrease calcium absorption. *Journal of Bone and Mineral Research.* 2010; 25:2786–2795. [PubMed: 20578215]
22. Tevaarwerk A, Burkard ME, Wisinski KB, et al. Aromatase inhibitors and calcium absorption in early stage breast cancer. *Breast Cancer Res Treat.* 2012
23. Lensmeyer GL, Wiebe DA, Binkley N. HPLC method for 25-hydroxyvitamin D measurement: comparison with contemporary assays. *Clin Chem.* 2006; 52:1120–1126. [PubMed: 16574756]
24. Field M, Shapses S, Cifuentes M. Precise and accurate determination of calcium isotope ratios in urine using HR-ICP-SFMS. *Journal of Analytical Atomic Spectrometry.* 2003; 18:727–733.
25. Levey AS, Bosch JP, Lewis JB, et al. A More Accurate Method To Estimate Glomerular Filtration Rate from Serum Creatinine: A New Prediction Equation. *Annals of Internal Medicine.* 1999 Mar 16;130:461–470. 1999. [PubMed: 10075613]
26. Heaney RP, Weaver CM, Fitzsimmons M. Influence of Calcium Load on Absorption Fraction. *J Bone Miner Res.* 1990; 5:1135–1138. [PubMed: 2270776]
27. Chen YM, Teucher B, Tang XY, et al. Calcium absorption in postmenopausal Chinese women: a randomized crossover intervention study. *Br J Nutr.* 2007; 97:160–166. [PubMed: 17217572]
28. Ebeling PR, Sandgren ME, DiMaggio EP, et al. Evidence of an age-related decrease in intestinal responsiveness to vitamin D: relationship between serum 1,25-dihydroxyvitamin D3 and intestinal vitamin D receptor concentrations in normal women. *J Clin Endocrinol Metab.* 1992; 75:176–182. [PubMed: 1320048]
29. Abrams SA, Griffin IJ, Hawthorne KM, et al. A combination of prebiotic short- and long-chain inulin-type fructans enhances calcium absorption and bone mineralization in young adolescents. *The American journal of clinical nutrition.* 2005 Aug;82:471–476. [PubMed: 16087995]
30. Weaver CM, Heaney RP, Teegarden D, et al. Wheat bran abolishes the inverse relationship between calcium load size and absorption fraction in women. *The Journal of nutrition.* 1996 Jan; 126:303–307. [PubMed: 8558315]
31. Cifuentes M, Riedt CS, Brolin RE, et al. Weight loss and calcium intake influence calcium absorption in overweight postmenopausal women. *Am J Clin Nutr.* 2004; 80:123–130. [PubMed: 15213038]
32. Lemann J Jr, Bushinsky DA, Hamm LL. Bone buffering of acid and base in humans. *Am J Physiol Renal Physiol.* 2003 Nov;285:F811–F832. [PubMed: 14532161]

Table 1

Demographic, Dietary and Laboratory Characteristics of Subjects

Variable	Aromatase Inhibitor Study ²²	Proton Pump Inhibitor Study ²¹	Vitamin D Study ²⁰	All Studies
Number of Subjects [†]	n=10	n=21	n=19 [†]	n=50
Studies per Subject	2	3	2	
Age, years	66 ± 7	58 ± 7	58 ± 8	60 ± 8
Race	9 White 1 Black	17 White 2 Black 2 Hispanic	17 White 1 Black 1 Indian	43 White 4 Black 2 Hispanic 1 Indian
Body Mass Index, kg/m ²	29 ± 4	29 ± 5	29 ± 5	29 ± 5
Fractional Calcium Absorption	0.155 ± 0.042	0.204 ± 0.097	0.243 ± 0.070	0.209 ± 0.084
Net Calcium Absorption	251 ± 88	257 ± 127	212 ± 74	239 ± 102
Kilocalories, day	1923 ± 418	2195 ± 337	1660 ± 368	1826 ± 409
Protein, g/day	86 ± 20	88 ± 21	73 ± 17	79 ± 20
Fat, g/day	84 ± 31	87 ± 22	66 ± 27	74 ± 26
Carbohydrates, g/day	219 ± 60	265 ± 58	192 ± 77	220 ± 60
Fiber, g/day	20 ± 5	24 ± 11	16 ± 7	20 ± 9
Dietary Calcium, mg/day	994 ± 372	998 ± 330	832 ± 215	940 ± 304
Calcium supplement,	720 ± 530	403 ± 568	85 ± 170	358 ± 512
All Calcium Intake, mg/day	1714 ± 640	1410 ± 648	912 ± 287	1281 ± 612
Iron, mg/day	12 ± 3	16 ± 5	12 ± 3	13 ± 5
Magnesium, mg/day	378 ± 100	364 ± 110	260 ± 66	320 ± 110
Oxalate, servings/day	1.5 ± 1.0	1.2 ± 1.0	0.7 ± 0.6	0.9 ± 0.8
Phosphorus, mg/day	1378 ± 490	1521 ± 409	1252 ± 318	1388 ± 405
Potassium, mg/day	3062 ± 971	3318 ± 829	2613 ± 692	2993 ± 855
Vitamin D, IU/day	217 ± 111	151 ± 96	107 ± 84	146 ± 102
Serum Calcium, mg/dL	9.3 ± 0.5	9.1 ± 0.4	9.3 ± 0.3	9.2 ± 0.4
Serum Creatinine, mg/dL	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
GFR, mL/minute	77 ± 11	86 ± 19	80 ± 17	83 ± 18
PTH, pg/dL	48 ± 25	49 ± 24	53 ± 15	50 ± 21
25(OH)D, ng/mL	40 ± 7	28 ± 13	22 ± 4	28 ± 11
1,25(OH) ₂ D, pg/mL	44 ± 17	47 ± 26	37 ± 12	43 ± 20

The table summarizes the baseline data of all subjects, including habitual intake of nutrients based on analysis of four to seven day food diaries. Data exhibited a normal or approximately normal distribution, and are summarized using the mean and standard deviation for each study and for all studies.

[†] Data include the baseline visit of one subject whose urine sample from one study visit was mishandled; therefore her paired calcium absorption data were not reported in the original study.

Table 2

Single Variable Linear Mixed Models of Calcium Absorption

Variable	Fractional Calcium Absorption (FCA)		Net Calcium Absorption (NCA)	
	Slope	P value	Slope	P value
Demographic Characteristics				
Age, years	−0.00383	0.027	−0.892	0.571
Body Mass Index, kg/m ²	+0.00328	0.227	−2.746	0.291
Nutritional Characteristics				
Kilocalories, day	−0.00004	0.229	+0.058	0.032
Protein, g/day	−0.00115	0.065	+0.879	0.144
Fat, g/day	−0.00046	0.353	+0.389	0.395
Carbohydrates, g/day	−0.00005	0.791	+0.368	0.025
Fiber, g/day	−0.00220	0.083	+2.108	0.101
All Calcium intake, mg/day	−0.00008	<0.001	+0.079	<0.001
Iron, mg/day	−0.00497	0.062	+1.722	0.538
Magnesium, mg/day	−0.00028	0.019	+0.312	0.004
Oxalate, servings/day	−0.02648	0.061	+15.113	0.275
Phosphorus, mg/day	−0.00006	0.063	+0.068	0.025
Potassium, mg/day	−0.00003	0.084	+0.045	0.001
Vitamin D, IU/day	−0.00022	0.095	+0.270	0.019
Laboratory Characteristics				
Serum Calcium, mg/day	−0.01283	0.640	−21.603	0.387
Serum Creatinine, mg/dL	+0.01066	0.891	−182.519	0.022
GFR, mL/minute	+0.00032	0.571	+1.375	0.018
PTH, pg/dL	+0.00135	0.017	−0.359	0.473
25(OH)D, ng/mL	−0.00033	0.608	+0.291	0.440
1,25(OH) ₂ D, pg/mL	+0.00035	0.382	+0.995	0.009

FCA predictors were determined using all three inpatient fractional calcium absorption studies performed on twenty-one subjects taking part in the proton pump inhibitor study, and the baseline visit from the vitamin D insufficiency study, excluding two outliers, yielding 80 observations. NCA predictors were determined using all three calcium absorption studies minus 3 outliers, yielding 118 observations.

Table 3**Multiple-Variable Linear Mixed Models of Calcium Absorption**

	Fractional Calcium Absorption (FCA)		Net Calcium Absorption (NCA)	
Variable	Slope	P value	Slope	P Value
Age, years	−0.0035	0.031	-	-
Kilocalories, day	−0.0004	0.004	−0.199	0.034
Carbohydrates, g/day	+0.0015	0.002	+0.796	0.081
Fat, g/day	+0.0032	0.015	+1.976	0.050
Fiber, g/day	−0.0052	0.008	−4.422	0.027
Calcium, mg/day	−0.0005	0.026	−0.065	<0.001
Potassium, mg/day	+0.0001	0.004	−0.058	0.030
Vitamin D, IU/day	−0.0003	0.066	-	-
GFR, mL/minute	-	-	+0.981	0.061
PTH, pg/dL	+0.0009	0.083	-	-
1,25(OH) ₂ D, pg/mL	+0.0008	0.052	+1.063	0.007
Model Root Mean Square Error	0.044 TFCA		52 mg calcium/day	
Square of Correlation between predicted and actual calcium absorption values	0.748		0.726	
	We analyzed data from the proton pump inhibitor study and the baseline visit from the vitamin D insufficiency study. We excluded two outliers and subjects with no missing data, yielding 74 observations.		We analyzed data from all three calcium absorption studies. We excluded 3 outliers and subjects with missing data, yielding 111 observations.	