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Aromatase inhibitors and calcium absorption in early stage breast cancer

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Abstract

To investigate the effect of aromatase inhibitors (AI) on intestinal calcium absorption, measured using the gold-standard dual stable calcium isotope method. In this pilot study, we recruited 10 postmenopausal women with hormone receptor-positive breast cancer who planned to initiate AI

therapy; women receiving chemotherapy were excluded. Women completed two 24 h inpatient calcium absorption study visits, the first prior to AI therapy and the second at least 6 weeks following onset of AI therapy. We calculated total fractional calcium absorption (TFCA) using the dose-corrected fractional recovery of two stable isotopes from 24 h urine collections. Ten postmenopausal women (mean $\pm$ SD age, 66 ± 7 years; $25(\text{OH})\text{D}$ 40 ± 7 ng/mL, and total calcium intake of $1,714 \pm 640$ mg/day) exhibited no change in TFCA related to AI therapy (0.155 ± 0.042 prior to and 0.160 ± 0.064 following AI therapy, $p = 1.0$). Subjects exhibited a surprisingly small decline in serum estradiol levels with AI therapy that was not statistically significant. However, there was a significant correlation between duration of AI therapy and the decline in serum estradiol levels ($r = -0.65$, $p = 0.040$). In this pilot study, AI therapy did not decrease TFCA. Women with early stage breast cancer exhibited an unexpectedly low TFCA, most likely due to their high calcium intake. The null effect of AI therapy on TFCA might relate to the brief duration of AI therapy, the minimal effect of AI therapy on estradiol levels, subjects' high calcium intake or excellent vitamin D status.

Keywords

Aromatase inhibitors; Breast cancer; Calcium absorption; Osteoporosis

Introduction

In postmenopausal women with hormone receptor-positive breast cancer, physicians initiate aromatase inhibitors (AI) either as initial adjuvant therapy or following a course of tamoxifen. Compared with tamoxifen, AIs improve disease-free and overall survival in women with breast cancer [1, 2]. Based on these results, the American Society of Clinical Oncology clinical practice guidelines recommend that clinicians consider AIs for all postmenopausal women with hormone receptor-positive breast cancer [3]. However, unlike tamoxifen which has estrogen-like effects on the skeleton, AI therapy causes bone loss [4]. Postmenopausal women with breast cancer on AI therapy have an increased fracture risk compared to their peers [5–7].

AIs inhibit conversion of androgens to estrogens, reportedly reducing serum estrogen levels by 81–96% within 2–6 weeks of starting therapy [8, 9]. Presumably through low estrogen levels, AI therapy associates with increased bone turnover, a decline in bone mineral density, and an increased risk of fracture [4, 7, 10]. To our knowledge, no studies have investigated whether AI-induced declines in tissue estrogen levels lead to a decline in intestinal calcium absorption.

Menopause and subsequent reduced estrogen levels result in decreased intestinal calcium absorption. Researchers recruited 189 women ages 35–45 years old and studied longitudinal changes in calcium absorption over two decades, reporting a decline in calcium absorption with age ($\sim 0.2\%$ per year), and an additional one time decrease in calcium absorption ($\sim 2.2\%$) at the time of menopause [11]. In another study [12], women entering menopause experienced a sudden decline in calcium absorption that corresponded to a decline in serum estradiol levels, whereas women with ongoing menses experienced stable calcium absorption. In a third study [13], women experienced a significant decline in calcium absorption following oophorectomy, which was reversed with estrogen therapy. In summary, several studies report that menopause results in a decrease in calcium absorption efficiency.

Since AIs decrease serum estrogen levels, and menopause with associated estrogen deficiency results in decreased calcium absorption efficiency, we hypothesized that AIs

might also decrease intestinal calcium absorption. If true, decreased calcium absorption might contribute to AI-associated declines in bone mineral density and a commensurate increase in risk of fracture. We conducted a pilot study to determine the feasibility of testing this hypothesis in breast cancer patients starting AI therapy.

Methods

We recruited postmenopausal women with recently diagnosed hormone receptor-positive breast cancer who planned to initiate AI therapy following definitive local therapy (lumpectomy or mastectomy, and/or local radiation therapy). The primary study outcome was the change in subjects' total fractional calcium absorption (TFCA) [14] following at least 6 weeks of AI therapy. Secondary study outcomes included changes in bone turnover markers, Short-Form 12 survey scores, and the incidence of musculoskeletal signs and symptoms related to AI therapy. The University of Wisconsin Human Subjects Committee approved the study protocol and all participants provided written informed consent prior to study procedures. The study was registered as a clinical trial (ClinicalTrials.gov identifier: NCT00766532).

Statistical analysis and sample size

TFCA is a continuous variable with a between-subject standard deviation of 1% and a within-subject precision error of TFCA of <1%, among postmenopausal women studied in our center [15]. Although researchers have previously reported a ~ 2% decline in TFCA related to menopause [11], such small changes in TFCA have not been linked to relevant health outcomes such as a higher fracture risk. By contrast, in the Study of Osteoporotic Fractures [16], 5,452 women underwent a single measurement of calcium absorption and subsequently, researchers noted that an 8% decrease in fractional calcium absorption corresponded to an increased risk of hip fracture among these women. We therefore chose to calculate sample size for this pilot study based on findings of the Study of Osteoporotic Fractures. Recruitment of 10 women and use of the paired *t* test allowed us to detect an 8% or greater difference in TFCA within subjects after AI therapy with ~ 99% power and a two-sided type 1 error of 0.05. Our ability to detect an 8% or greater difference in TFCA with AI therapy was deemed clinically relevant to the risk of fracture in women initiating aromatase inhibitor therapy.

All data was graphed and analyzed using the Shapiro–Wilk test for normality. Subjects' demographic, laboratory and dietary characteristics were summarized using the mean and standard deviation, with the addition of the median and interquartile range for the few variables without a normal distribution. Within-subject changes in TFCA, laboratory and dietary variables were analyzed using the paired *t* test for normally distributed data, or the Wilcoxon test for data without a normal distribution, with its use noted next to the relevant *p* value. Analyse-It software (Leeds, UK) was used for all analyses.

Participants

We recruited women with recently diagnosed hormone receptor-positive breast cancer who planned to start AI therapy following definitive local therapy (lumpectomy, mastectomy, and/or local radiation therapy). Eligible women were also required to be at least 5 years past the onset of menopause, defined as the date of last menses or bilateral oophorectomy. We excluded women undergoing chemotherapy for breast cancer, due to its potential independent effects on intestinal health, calcium homeostasis, and bone turnover. We excluded women with intestinal conditions associated with malabsorption, including Crohn disease, ulcerative colitis, pernicious anemia, bacterial overgrowth, celiac sprue, chronic diarrhea, or use of antibiotics within the month preceding the screening visit. We also

excluded women with known Stage 4 or 5 chronic kidney disease, defined as an estimated GFR <30 mL/min, as our calcium absorption method is not validated in this population. We excluded women taking medications known to interfere with calcium or vitamin D metabolism, including oral glucocorticoids or anticonvulsants.

Oncologists (AT, MEB, and KW) referred potential participants to our research center shortly after their diagnosis of breast cancer, and prior to onset of AI therapy. Recruitment began in January 2009 and ended in June 2010. Twenty-eight women with breast cancer were interviewed by phone (Fig. 1). We met women who appeared eligible based on questions administered by phone, and obtained written informed consent. Eligible subjects underwent two 24 h inpatient TFCA study visits, the first study serving as a baseline measurement prior to onset of AI therapy. Baseline TFCA measurements permitted us to control for multiple patient-specific variables that influence TFCA [17, 18] including age, race, calcium intake, and serum vitamin D levels. Based on studies showing that estrogen levels decrease by 81–96% from baseline levels approximately 2–6 weeks after onset of AI therapy [8, 9], we required at least 6 weeks of AI therapy before the second TFCA study. We considered the 6-week interval to be sufficient to detect the full effect of estrogen suppression on calcium homeostasis, based on guidance from the Institute of Medicine [19].

Measurement of calcium absorption

We measured TFCA using two stable calcium isotopes, which are calcium compounds of different atomic weights with no known toxicity [20]. The isotopic distribution of calcium in the environment is 96.9% ⁴⁰calcium, 2.1% ⁴⁴calcium, 0.6% ⁴²calcium, with minor amounts of calcium with atomic weights of 43, 46, and 48. We purchased purified ⁴⁴calcium and ⁴²calcium from Trace Sciences International (Wilmington, DE) as calcium carbonate powder. The University of Wisconsin Waisman Clinical Biomanufacturing Facility prepared the calcium isotope solutions for this study. Personnel dissolved the calcium powders in HCl, mixed the resulting solution with NaCl and adjusted the solution pH 6 for intravenous injection or pH 4 for oral administration. Solutions were sterile filtered using a Sterivex GV 0.22 micron sterile filter, aseptically dispensed into sterile vials and tested for sterility and pyrogenicity. We administered one isotope (⁴⁴calcium) orally to trace intestinal TFCA and administered the other isotope (⁴²calcium) intravenously to trace renal recycling and intestinal calcium secretion. The ratio of the two isotopes in a 24 h urine collection provides an accurate estimate of TFCA and is considered the current gold-standard method for measuring calcium absorption [14]. The Wisconsin State Lab of Hygiene-Trace Element Research Group, under the direction of Dr. Martin Shafer, measured the calcium isotope content of urine samples using high-resolution mass spectrometry as described elsewhere [15, 18], with a ratio standard deviation of 0.2–0.5% for each sample.

Subjects fasted from midnight until 0700 when they arrived on the research unit to undergo measurement of calcium absorption. Subjects provided a urine sample upon arrival on the research unit, representing the second morning void; this sample was used to measure urine N-telopeptide levels. A morning blood sample was collected for measurement of calcium, creatinine, 25(OH)D, 1,25(OH)₂D, intact parathyroid hormone, estradiol, bonespecific alkaline phosphatase, and C-reactive protein. Serum estradiol levels were measured by chemiluminescent microparticle immunoassay (Architect assay, Abbott Laboratories) with a lower limit of detection of 7 pg/mL and a between-run coefficient of variation of 4.9% for measurement of levels in the low range, defined as a mean value of 45 pg/mL.

With breakfast, women consumed a glass of calcium-fortified orange juice (50 mL) containing one stable calcium isotope tracer (8 mg of ⁴⁴Ca). At the same time, the nurse injected another stable calcium isotope tracer (3 mg of ⁴²Ca) intravenously, flushing the line with saline and weighing the syringe before and after use to record the dose of ⁴²Ca. Over

the next 24-h period, we collected all urine to calculate TFCA based on the dose-corrected fractional recovery of the two stable calcium isotopes established by Eastell et al. [14] using the formula shown below:

$$\text{TFCA} = \frac{\text{Urinary } ^{44}\text{Ca}/^{43}\text{Ca}}{\text{Urinary } ^{42}\text{Ca}/^{43}\text{Ca}} \times \frac{\text{natural abundance } ^{44}\text{Ca}}{\text{natural abundance } ^{42}\text{Ca}} \times \frac{\text{Dose } ^{42}\text{Ca}}{\text{Dose } ^{44}\text{Ca}}$$

During each TFCA study, the study dietitian (LAD) adjusted the breakfast meal to ensure that subjects consumed 300 mg of dietary calcium from breakfast. Breakfast was consumed while subjects received oral and intravenous stable calcium isotope tracers. Including breakfast, the mean $\pm$ SD dietary calcium intake during the first and second TFCA studies was $1,090 \pm 290$ mg, and $1,070 \pm 260$ mg, respectively. During each TFCA study, women also continued to take their outpatient supplements, including calcium. Subjects consumed three meals and one or two snacks with approximately 17% of caloric intake from protein, 38% from fat, and 45% from carbohydrates. Food provided during the first and second TFCA study was identical.

Subjects completed two 4 day diet diaries, one immediately after the first TFCA study and one just before or after the second TFCA study. The research dietitian taught subjects to complete accurate diet diaries, and each subject received a scale to weigh food portions. Subjects recorded what they ate every other day for 4 days including 1 weekend day, to capture daily variability in nutrient intake. Diet diaries were analyzed using Food Processor[®] Nutrition Analysis Software, ESHA Research (Salem, OR). We used data collected from diet diaries as covariates when determining factors influencing TFCA.

At each TFCA visit, subjects rated their degree of morning stiffness, pain (10-point visual analog scale) and underwent a joint examination by a rheumatologist (KEH) to determine the number of tender and swollen joints among 28 joints. In addition, subjects completed the Short-Form 12 survey version 1 as a means of assessing mental and physical health.

Results

Twelve postmenopausal women with early stage breast cancer provided written informed consent to take part in the study (Fig. 1). Two women subsequently withdrew from the study, one due to inability to establish intravenous access, and the other due to a personal decision to defer AI therapy.

Ten women (nine Caucasian and one African American) with a mean $\pm$ SD age of 66 ± 7 years old completed all study visits. All 10 women underwent lumpectomy and 8 women were also treated with local radiation therapy. Women began AI therapy (anastrozole 1 mg by mouth daily) immediately after completing their first TFCA study and took AI therapy an average of 53 days (range 42–90 days) between their first and second TFCA study visits. Self-reported adherence to anastrozole was excellent; no subject reported interruption of therapy. Unlike two other studies [8, 9], subjects in our study exhibited a surprisingly small decline in serum estradiol levels with AI therapy that was not statistically significant (Table 1). However, there was a significant correlation between duration of AI therapy and the decline in serum estradiol levels ($r = -0.65$, $p = 0.040$).

TFCA did not change as a result of AI therapy. TFCA was 0.155 ± 0.042 at baseline, and 0.160 ± 0.064 following the onset of AI therapy ($p = 1.000$). There was no significant relationship between the absolute change in fractional calcium absorption and duration of AI therapy ($r = -0.04$, $p = 0.907$). We found no significant relationship between serum estradiol

levels and TFCA at baseline ($r = -0.04$, $p = 0.905$) or following AI therapy ($r = -0.49$, $p = 0.146$). Likewise, there was no significant relationship between AI-induced declines in estradiol levels and change in fractional calcium absorption ($r = 0.06$, $p = 0.873$). Finally, we analyzed changes in TFCA as a function of detectable ($n = 5$) or undetectable ($n = 5$) estradiol levels following AI therapy. Among subjects with undetectable estradiol levels, TFCA increased by 2.2%. By contrast, TFCA decreased by 1% in subjects with measurable estradiol levels, a between-group change that was not statistically significant ($p = 0.55$, Wilcoxon test).

We next analyzed changes in milligrams of calcium absorbed per day (TFCA times total calcium intake from diet and supplements). Follow-up measurements for milligrams of calcium absorbed were non-parametric, and therefore all data are presented as the median (interquartile range). Subjects absorbed 259 mg (190–308 mg) of calcium at baseline and 255 mg (118–328 mg) of calcium following AI therapy ($p = 1.000$, Wilcoxon test). There was no significant relationship between the absolute change in milligrams of calcium absorbed and duration of AI therapy ($r = -0.07$, $p = 0.852$). We also found no significant relationship between serum estradiol levels and milligrams of calcium absorbed at baseline ($r = -0.43$, $p = 0.218$) or following AI therapy ($r = -0.15$, $p = 0.679$). Finally, there was no significant relationship between AI-induced declines in estradiol levels and the change in milligrams of calcium absorbed per day ($r = 0.11$, $p = 0.761$).

Table 1 summarizes patients' laboratory results and dietary habits at each inpatient study visit. Six variables demonstrated a non-normal distribution and are also summarized using the median (interquartile range). Of note, baseline serum 25(OH)D levels were >30 ng/mL in 9 of 10 subjects and the remaining subject had a serum 25(OH)D level of 29.7 ng/mL, indicating vitamin D repletion in all subjects [21]. During the study, subjects exhibited no significant changes in parameters of calcium homeostasis including bone turnover markers. Subjects' 4 day food records detected no significant changes in dietary habits between study visits. Most importantly, subjects did not report significant changes in their use of supplemental calcium or their consumption of dietary calcium.

Adverse events from AI therapy

Participants reported mild pain that did not change during the study (pain level 1.2 ± 1.5 before, and 1.4 ± 1.4 following onset of AI therapy, $p = 0.910$). One woman had rheumatoid arthritis; her joint pain, tender joint count, swollen joint count and duration of morning stiffness did not change with AI therapy. Aside from this individual, no other subject reported morning stiffness. No subject experienced new joint pain or joint swelling during AI therapy.

Subjects completed the SF12 survey during each calcium absorption study visit. The physical component summary scale remained unchanged during the study (48.5 ± 10.3 prior to and 48.4 ± 10.0 following AI therapy, $p = 1.000$). Similarly, the mental component summary scale did not change during the study (54.2 ± 5.2 prior to and 53.5 ± 6.7 following AI therapy, $p = 0.508$).

Discussion

Postmenopausal women who receive AI therapy to treat breast cancer can experience a decline in bone mineral density and a commensurate increased risk of fracture [5–7]. We hypothesized that because calcium absorption decreases in the setting of estrogen deficiency, that AI therapy might decrease calcium absorption, thereby contributing to a greater risk of osteoporosis and fracture. In this pilot study, we found no significant decline in calcium absorption resulting from 6 weeks of AI therapy.

We found an unexpectedly low TFCA among subjects in this study, compared to postmenopausal women participating in other studies in our center [15, 18]. We believe the observed low TFCA among subjects in this study relates to subjects' high calcium intake, as several other researchers [11, 14, 17, 22] report an inverse relationship between calcium intake and TFCA.

Possible explanations for the null effect of AI therapy on calcium absorption in this study include the short duration of AI therapy, the null effect of AI therapy on estradiol levels, the high calcium intake among participants, and participants' excellent vitamin D status. Excellent vitamin D status might reduce the effects of AI therapy on calcium absorption and/or bone mineral density. In early stage breast cancer patients randomized to exemestane, researchers [23] found a trend toward greater rates of spine and hip bone loss among subjects with baseline serum 25(OH)D levels <30 ng/mL, compared to subjects with baseline levels >30 ng/mL. In a subsequent review article, Geisler and Lonning [5] emphasized calcium and vitamin D supplements as one strategy to manage bone loss related to AI therapy. It is possible that the excellent vitamin D status of subjects in the current study helped to minimize any negative effect of AI therapy on TFCA or bone turnover.

As joint pain is a common side effect of AI therapy, we systematically monitored joint pain and swelling during the conduct of this pilot study. None of our subjects reported joint pain or were noted to have new synovitis when examined by a rheumatologist. The lack of new synovitis is not surprising, as the median time to onset of initial joint pain in the "Arimidex, Tamoxifen, Alone or In Combination" (ATAC) Trial was 14 months [24]. In addition, our subjects did not have prior chemotherapy, which might be a risk factor for AI-induced joint pain [24].

Our study had several key strengths, including the use of a gold-standard method to measure calcium absorption, and performance of calcium absorption studies on the inpatient unit, permitting complete 24 h urine collections to measure calcium absorption. We used a paired study design in which each subject served as her own control, thereby controlling for patient-specific factors that influence calcium absorption including age, race, habitual calcium intake, and vitamin D status. In terms of potential weaknesses, our sample size was small and therefore we had limited power to detect small changes in fractional calcium absorption. We measured changes in calcium absorption after 6 weeks of AI therapy; it is possible that the true effect of AI therapy on calcium absorption might take longer than 6 weeks, especially since subjects did not demonstrate a significant decline in estradiol levels despite excellent self-reported adherence. The precision of the clinical estradiol assay used in this study might not have captured the true effect of AI therapy on estradiol levels in our subjects. In addition, it is possible that the excellent vitamin D status of our subjects might have prevented AI-induced declines in TFCA. We did not restrict or standardize calcium intake, and therefore cannot determine from this study whether breast cancer itself might cause, or be associated with, low TFCA.

Conclusions

In this pilot study, subjects with early stage breast cancer did not experience a decrease in TFCA following 6 weeks of AI therapy. Women with early stage breast cancer exhibited an unexpectedly low TFCA, most likely due to their high calcium intake. The null effect of AI therapy on TFCA might relate to the brief duration of AI therapy, the null effect of AI therapy on estradiol levels, subjects' high calcium intake or excellent vitamin D status. We suggest that future studies assessing the influence of AI therapy on calcium absorption recruit a larger sample of subjects, standardize subjects' calcium intake and vitamin D

status, and prescribe AI therapy for a longer duration in order to better evaluate whether AI therapy truly decreases TFCA.

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References

1. Howell A, Cuzick J, Baum M, Buzdar A, Dowsett M, Forbes JF, Hocht-Boes G, Houghton J, Locker GY, Tobias JS. Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. *Lancet*. 2005; 365(9453):60–62. [PubMed: 15639680]
2. Colleoni M, Giobbie-Hurder A, Regan MM, Thurlimann B, Mouridsen H, Mauriac L, Forbes JF, Paridaens R, Lang I, Smith I, et al. Analyses adjusting for selective crossover show improved overall survival with adjuvant letrozole compared with tamoxifen in the BIG 1–98 study. *J Clin Oncol*. 2011; 29(9):1117–1124. [PubMed: 21321298]
3. Burstein HJ, Prestrud AA, Seidenfeld J, Anderson H, Buchholz TA, Davidson NE, Gelmon KE, Giordano SH, Hudis CA, Malin J, et al. American Society of Clinical Oncology clinical practice guideline: update on adjuvant endocrine therapy for women with hormone receptor-positive breast cancer. *J Clin Oncol*. 2010; 28(23):3784–3796. [PubMed: 20625130]
4. Gonnelli S, Cadirni A, Caffarelli C, Petrioli R, Montagnani A, Franci MB, Lucani B, Francini G, Nuti R. Changes in bone turnover and in bone mass in women with breast cancer switched from tamoxifen to exemestane. *Bone*. 2007; 40(1):205–210. [PubMed: 16904960]
5. Geisler J, Lonning PE. Impact of aromatase inhibitors on bone health in breast cancer patients. *J Steroid Biochem Mol Biol*. 2010; 118(4–5):294–299. [PubMed: 19833206]
6. Rabaglio M, Sun Z, Price KN, Castiglione-Gertsch M, Hawle H, Thurlimann B, Mouridsen H, Campone M, Forbes JF, Paridaens RJ, et al. Bone fractures among postmenopausal patients with endocrine-responsive early breast cancer treated with 5 years of letrozole or tamoxifen in the BIG 1–98 trial. *Ann Oncol*. 2009; 20(9):1489–1498. [PubMed: 19474112]
7. Eastell R, Adams J, Clack G, Howell A, Cuzick J, Mackey J, Beckmann MW, Coleman RE. Long-term effects of anastrozole on bone mineral density: 7-year results from the ATAC trial. *Ann Oncol*. 2011; 22(4):857–862. [PubMed: 20929964]
8. Geisler J, Haynes B, Anker G, Dowsett M, Lonning PE. Influence of letrozole and anastrozole on total body aromatization and plasma estrogen levels in postmenopausal breast cancer patients evaluated in a randomized, cross-over study. *J Clin Oncol*. 2002; 20(3):751–757. [PubMed: 11821457]
9. Dowsett M, Donaldson K, Tsuboi M, Wong J, Yates R. Effects of the aromatase inhibitor anastrozole on serum oestrogens in Japanese and Caucasian women. *Cancer Chemother Pharmacol*. 2000; 46(1):35–39. [PubMed: 10912575]
10. Francini G, Petrioli R, Montagnani A, Cadirni A, Campagna S, Francini E, Gonnelli S. Exemestane after tamoxifen as adjuvant hormonal therapy in postmenopausal women with breast cancer: effects on body composition and lipids. *Br J Cancer*. 2006; 95(2):153–158. [PubMed: 16835585]
11. Heaney RP, Recker RR, Stegman MR, Moy AJ. Calcium absorption in women: relationships to calcium intake, estrogen status, and age. *J Bone Miner Res*. 1989; 4(4):469–475. [PubMed: 2816496]
12. Wishart JM, Scopacasa F, Horowitz M, Morris HA, Need AG, Clifton PM, Nordin BE. Effect of perimenopause on calcium absorption: a longitudinal study. *Climacteric*. 2000; 3(2):102–108. [PubMed: 11910650]

13. 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(6):1359–1364. [PubMed: 6255005]
14. Eastell R, Vieira NE, Yergey AL, Riggs BL. One-day test using stable isotopes to measure true fractional calcium absorption. *J Bone Miner Res.* 1989; 4(4):463–468. [PubMed: 2816495]
15. Hansen KE, Jones AN, Lindstrom MJ, Davis LA, Ziegler TE, Penniston KL, Alvig AL, Shafer MM. Do proton pump inhibitors decrease calcium absorption? *J Bone Miner Res.* 2010; 25(12): 2786–2795. [PubMed: 20578215]
16. Ensrud KE, Duong T, Cauley JA, Heaney RP, Wolf RL, Harris E, Cummings SR. Low fractional calcium absorption increases the risk for hip fracture in women with low calcium intake. Study of Osteoporotic Fractures Research Group. *Ann Intern Med.* 2000; 132(5):345–353. [PubMed: 10691584]
17. Wolf RL, Cauley JA, Baker CE, Ferrell RE, Charron M, Caggiula AW, Salamone LM, Heaney RP, Kuller LH. Factors associated with calcium absorption efficiency in pre- and perimenopausal women. *Am J Clin Nutr.* 2000; 72(2):466–471. [PubMed: 10919942]
18. Hansen KE, Jones AN, Lindstrom MJ, Davis LA, Engelke JA, Shafer MM. Vitamin D insufficiency: disease or no disease? *J Bone Miner Res.* 2008; 23(7):1052–1060. [PubMed: 18302509]
19. Institute of Medicine FaNB. Dietary reference intakes: calcium, phosphorus, magnesium, vitamin D and fluoride. Washington: National Academy Press; 1999.
20. Abrams SA. Using stable isotopes to assess mineral absorption and utilization by children. *Am J Clin Nutr.* 1999; 70(6):955–964. [PubMed: 10584039]
21. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, Gallagher JC, Gallo RL, Jones G, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metab.* 2011; 96(1):53–58. [PubMed: 21118827]
22. Devine A, Wilson SG, Dick IM, Prince RL. Effects of vitamin D metabolites on intestinal calcium absorption and bone turnover in elderly women. *Am J Clin Nutr.* 2002; 75(2):283–288. [PubMed: 11815319]
23. Geisler J, Lonning PE, Krag LE, Lokkevik E, Risberg T, Hagen AI, Schlichting E, Lien EA, Offord ES, Eide GE, et al. Changes in bone and lipid metabolism in postmenopausal women with early breast cancer after terminating 2-year treatment with exemestane: a randomised, placebo-controlled study. *Eur J Cancer.* 2006; 42(17):2968–2975. [PubMed: 16963261]
24. Coleman RE, Bolten WW, Lansdown M, Dale S, Jackisch C, Merkel D, Maass N, Hadji P. Aromatase inhibitor-induced arthralgia: clinical experience and treatment recommendations. *Cancer Treat Rev.* 2008; 34(3):275–282. [PubMed: 18082328]

Figure 1.
Study recruitment

Table 1

Subjects' demographic, laboratory, and dietary characteristics

Characteristics	Visit 1 Baseline	Visit 2 Follow-up ^a	<i>p</i> value
Demographic characteristics			
Age, years	66 ± 7		
Race	9 Caucasian, 1 Black		
Body mass index, kg/m ²	29 ± 4		
Laboratory characteristics			
Calcium, mg/dL	9.3 ± 0.5	9.4 ± 0.5	0.84
25(OH)D, ng/mL	40 ± 7	40 ± 7	0.63
1,25(OH) ₂ D, pg/dL	44 ± 17	46 ± 13	0.770 ^c
PTH, pg/mL	48 ± 25	42 ± 20	0.275 ^c
Estradiol, pg/mL	17.6 ± 5.8	11.2 ± 13.7	0.884 ^c
Creatinine, mg/dL	0.8 ± 0.1	0.8 ± 0.1	0.823
C-reactive protein, mg/dL	0.5 ± 0.7	1.1 ± 2.5	1.000 ^c
Urine calcium, mg/24 h	184 ± 92	183 ± 86	0.996
Bone-specific alkaline phosphatase, u/L	41 ± 10	44 ± 11	0.382
N-telopeptide, nM BCE	555 ± 380	519 ± 211	0.922 ^c
Dietary habits ^b			
Calcium from diet, mg	994 ± 372	966 ± 322	0.712
Calcium from supplements, mg	720 ± 530	720 ± 530	1.000
Kilocalories	1,848 ± 400	1,724 ± 290	0.234
Carbohydrates, g	227 ± 52	206 ± 43	0.081
Protein, g	77 ± 21	78 ± 15	0.827
Fat, g	72 ± 25	66 ± 20	0.283
Fiber, g	21 ± 8	19 ± 6	0.143
Vitamin D, IU	189 ± 101	192 ± 111	0.913
Sodium, mg	2,552 ± 695	2,652 ± 859	0.41
Magnesium, mg	338 ± 129	331 ± 101	0.782
Iron, mg	13 ± 2	14 ± 3	0.271
Caffeine, mg	119 ± 103	125 ± 102	0.342
Oxalate, servings	0.7 ± 0.6	0.9 ± 0.7	0.338

Continuous data are summarized using the mean ± standard deviation. Six variables with a non-normal distribution are summarized using the median (interquartile range) and include the following. The 1,25(OH)₂D level at visit 1 was 41 (31–48) pg/dL, PTH at visit 1 was 42 (37–45) pg/mL, estradiol levels at visit 2 were 6 (0–16) pg/mL, N-telopeptide levels at visit 2 were 569 (300–699) nM BCE, the C-reactive protein at visit 1 was 0 (0–2) mg/dL and at visit 2 was 0 (0–1.1) mg/dL.

^aThe follow-up visit occurred after subjects took AI therapy for at least 6 weeks

^bDietary habits are reported in units consumed per day, and are based on analysis of subjects' 4 day food records, completed within approximately 1 week of each inpatient study visit

^cIndicates use of the Wilcoxon test instead of the paired *t* test