Is bone equally responsive to calcium and vitamin D intake from food vs. supplements? Use of $^{41}$Ca tracer kinetic model

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ABSTRACT

Background: Few interventions directly compare equivalent calcium and vitamin D from dairy vs. supplements on the same bone outcomes. The radioisotope calcium-$^{41}$ (41Ca) holds promise as a tracer method to directly measure changes in bone resorption with differing dietary interventions.

Objective: Using $^{41}$Ca tracer methodology, determine if 4 servings/day of dairy foods results in greater $^{41}$Ca retention than an equivalent amount of calcium and vitamin D from supplements. Secondary objective was to evaluate the time course for the change in $^{41}$Ca retention.

Methods: In this crossover trial, postmenopausal women ($n = 12$) were dosed orally with 100 nCi of $^{41}$Ca and after a 180 day equilibration period received dairy (4 servings/day of milk or yogurt; ~1300 mg calcium, 400 IU cholecalciferol (vitamin D$_3$/day)) or supplement treatments (1200 mg calcium carbonate/day and 400 IU vitamin D$_3$/day) in random order. Treatments lasted 6 weeks separated by a 6 week washout (WO). Calcium was extracted from weekly 24 h urine collections; accelerator mass spectrometry (AMS) was used to determine the $^{41}/^{40}$Ca ratio. Primary outcome was change in $^{41}$Ca excretion. Secondary outcome was the time course for change in $^{41}$Ca excretion during intervention and WO periods.

Results: The $^{41}/^{40}$Ca ratio decreased significantly over time during both treatments; there was no difference between treatments. Both treatments demonstrated a significant retention of $^{41}$Ca within 1–2 weeks (p = 0.0007 and p < 0.001 for dairy and supplements, respectively). WO demonstrated a significant decrease (p = 0.0024) in $^{41}$Ca retention within 1–2 weeks, back to pre-intervention levels.

Conclusion: These data demonstrate that urinary $^{41}$Ca retention is increased with an increase in calcium and vitamin D intake regardless of the source of calcium, and the increased retention occurs within 1–2 weeks.

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1. Introduction

Currently an estimated 10.2 million adults age 50 and older in the United States have osteoporosis, while an estimated 43.5 million have low bone mass (Wright et al., 2014). Health care costs related to osteoporotic fractures are projected to reach $25.3 billion in the U.S. by 2025 (Cosman et al., 2014). Calcium and vitamin D are widely considered the most critical nutrients for osteoporosis prevention and treatment (Caroli et al., 2011). Calcium absorption in the small intestine declines with age, and women lose approximately 200 mg of calcium per day in the first 3–4 years of menopause, followed by approximately 45 mg per day in the next 5–10 years (Tella and Gallagher, 2014). Calcium can be lost in the feces, urine, skin, hair, nails, and digestive secretions (Bauer, 2013; Heaney, 2000).

Many population groups in the West fall short of the recommended dietary calcium intake, particularly postmenopausal women (Peters and Martini, 2010). The Institute of Medicine now recommends 1200 mg of calcium per day for women 51 years and older (Institute of Medicine (US), 2011), but average intakes for American women are estimated to be 750–850 mg of calcium per day (Bauer, 2013). Calcium supplement use is common: nearly 60% of women in the US take a calcium supplement (Moyer and Vitamin, 2013). Evidence to support the use of calcium and/or vitamin D supplements for fracture prevention...
is the subject of debate (Moyer and Vitamin, 2013; Jackson and Mysiw, 2014). In light of the numerous concerns related to calcium supplements, nutrition experts frequently encourage patients to consume calcium via food, especially dairy products (Moyer and Vitamin, 2013). The 2015–2020 Dietary Guidelines for Americans recommend three cups of low fat or fat free milk (or equivalent dairy products) per day for bone health in persons nine years old and above (US Department of Health and Human Services and US Department of Agriculture). Some observational studies have shown no positive effects of dairy intake on bone in older adults (Feskanich et al., 2003; WADOWLOWSKA et al., 2013; Michaelsson et al., 2014). In contrast, other observational studies support the benefits of dairy products on bone health (Key et al., 2007; Sahni et al., 2014).

Randomized controlled trials (RCT) using dairy foods have reported a beneficial impact on markers of bone turnover (Heaney et al., 2002; Bonjour et al., 2008) as well as on bone mineral density (BMD) (Chee et al., 2003; Moschonis et al., 2010) in postmenopausal women, but very limited work has compared equivalent amounts of calcium from dairy foods vs. supplements on the same bone outcome variables (Recker and Heaney, 1985; Prince et al., 1995; Manios et al., 2007).

Current methods of assessing bone health have limitations. Dual energy X-ray absorptiometry (DXA) is used clinically to monitor BMD, but is limited by low sensitivity as well as the inability to capture all aspects of bone strength. Image quality and spatial resolution of the scans are poor, and six months to two years are required to detect significant changes in BMD after an intervention. Quantitative computed tomography (qCT) scans of bone, which measure volumetric BMD, are more sensitive and precise than DXA, but expose subjects to a greater radiation dose (Beck, 2003). Biomarkers of bone turnover can be measured to assess bone remodeling rates. Although biomarkers of bone turnover require less time than imaging techniques to see the effects of an intervention, they are limited by high inter- and intra-individual variation (Civitelli et al., 2009).

The radioisotope 41Ca, in conjunction with accelerator mass spectrometry (AMS), holds promise as a technique to measure small changes in calcium retention in a short period of time. In brief, calcium kinetic studies can be done to monitor appearance and disappearance of the tracer in a variety of biological specimens (Lin et al., 2004). These techniques reveal changes in bone accretion and resorption in response to experimental interventions. Because bone turnover is slow the 41Ca isotope can be used in vivo to label the skeleton. The 41Ca isotope has a half-life of 1.03×10⁶ years, negligible radiological risk to human and can be used to assess short term changes in 41Ca retention with a variety of interventions following a single small dose (CIVITELLI et al., 2009; LIN et al., 2004). Following an oral dose, the 41Ca tracer is taken up into a short-term pool where it awaits incorporation into the skeleton. The tracer not incorporated into the skeleton is gradually excreted from the short-term pool into urine; this takes approximately 6 months (Dekn et al., 2006). After the 6 month equilibration period any tracer in the body resides in mineralized bone, and the appearance of 41Ca in urine is a direct result of bone turnover (41Ca being lost from the skeleton). The amount of 41Ca lost from the skeleton is evaluated by comparing the tracer excretion curve extrapolated from equilibration data prior to intervention to the tracer excretion curve during intervention (Dekn et al., 2007; FitzGerald et al., 2005). In addition, subjects can also serve as their own controls in crossover studies, so smaller sample sizes can be used (Dekn et al., 2007). To date, this method has not been employed in a nutritional intervention with dairy foods.

2. Aims

The aims of this study were to 1) determine if 4 servings/day of dairy foods increase 41Ca retention more than an equivalent amount of calcium & vitamin D from supplements. Secondary objective was to evaluate the time course for change in 41Ca retention in healthy postmenopausal women.

3. Materials and methods

3.1. Subjects

A total of 12 healthy women at least two years post-menopause completed the study protocol. Subjects ranged in age from 50 to 65 years, were weight stable (±2.3 kg in the past three months), and were classified as low dairy consumers (≤2.5 serves/day). Exclusion criteria included the use of oral hormone therapy in the past year, BMD T scores > 0 or < −1.5, use of calcium or vitamin D supplements, autoimmune or inflammatory disorders, history of non-traumatic bone fracture, and lactose intolerance. Subjects were recruited from the community via flyers, newspaper ads, and email list serves as well as informational booths at local farmers’ markets. The study was conducted at the USDA, Western Human Nutrition Research Center (WHNRC) on the University of California, Davis campus and was approved by the Institutional Review Boards of the University of California, Davis (#22919-7) and Lawrence Livermore National Laboratory (LLNL) (#11-008). A CONSORT diagram of enrollment and follow up of study subjects is shown in Fig. 1. All study participants gave informed consent prior to starting the study protocol. The study was registered at clinicaltrials.gov as NCT01394484.

3.2. Study design

The study design is summarized in Fig. 2. After enrollment, subjects received an oral labeling dose of 100 nCi 41Ca and began a 180 day equilibration period during which the isotope excretion stabilized to a steady rate of natural loss. Subjects were instructed to continue their normal low dairy diet and lifestyle during this time. Subjects provided 24 h urine collections prior to the 41Ca dose (day 0), and during the equilibration period at days 90, 120, 150, and 180.

Following the 180 day equilibration period subjects were randomized to one of two 42 day interventions followed by a 42 day washout (WO) period. After completion of the initial intervention and WO period women continued the study on the second intervention. The dairy intervention included 20 (1cup/237 mL) servings of milk (1% fat, 400 mg calcium and 100 International Units (IU) cholecalciferol (vitamin D3)/serving) and 8 (8 oz./227 g) servings of yogurt (low fat vanilla, 200 mg calcium and 100 IU vitamin D3 per serving) per week. Women were instructed to consume the dairy foods throughout the day. Supplement intervention included a calcium supplement tablet (600 mg calcium per tablet; Caltrate) twice daily and a vitamin D supplement tablet (400 IU vitamin D3 per tablet) once daily. No other supplements were permitted.

Dairy provided ~1300 mg calcium and 400 IU vitamin D3/day, and supplements provided 1200 mg calcium and 400 IU vitamin D3/day. Subjects were instructed by the study diettian how to adjust their food intake to account for the energy associated with the dairy servings (~350 kcal per day) and where dairy foods could be included in their diets, e.g. coffee latte for 1 serving of milk. Subjects were instructed to follow their usual (low dairy diet) during the supplement and WO periods.

Subjects completed 24 h urine collections for the measurement of the excreted 41Ca, as well as other urinary minerals. Urine collections were made at the beginning and at weekly intervals for each intervention and WO phase; for a total of 24 (24 h) urine collections. Blood was drawn at ~0800, following an overnight fast of 10 h, at the beginning and end of each intervention period.
3.3. Compliance

During the intervention periods, subjects reported to the WHNRC weekly to pick up the dairy products or supplement tablets. Compliance to the interventions was measured via empty milk and yogurt containers that were returned and by pill count on returned supplement foil-blister packs. Additionally, diet records were kept and reviewed for compliance by the study dietitian weekly when women returned to the WHNRC to pick dairy or supplement supplies.

3.4. Dietary assessment

Women kept 3-day food logs each week for each intervention and the WO period. The first day of the first diet record was randomly

Fig. 1. Enrollment and follow up of participants in the randomized crossover trial.

Fig. 2. Study design. After enrollment, women received a minute labeling dose of $^{41}$ calcium, which was incorporated into the skeleton over a period of 180 days. Women were then randomly assigned to either the dairy or supplement interventions for 42 days. After a 42 day WO period, subjects completed the second intervention for 42 days.
nium hydroxide (NH$_4$OH) was used to adjust the samples to pH 10 and calcium from the 14 urine samples was converted to calcium oxalate
operator to decrease the variance in the measurement data.

DXA instrument during the course of the study was 0.457% for the lumbar
100 °C. The use of the 40Ca in the 41Ca/40Ca standards is important
ions using cation exchange chromatography. Concentrated
S100; Ayrton Corp, Prior Lake, MN). Body mass index (BMI) was calcu-
0.1 cm with a wall-mounted stadiometer (Ayrton stadiometer, model
and dried overnight in a muf.

Anthropometric measurements were taken for each woman by a trained research assistant. Body weight was measured to the nearest
0.1 kg using an electronic scale (Circuits and Systems Inc., E. Rockaway,
NY). Standing height, without shoes, was measured to the nearest
0.1 cm with a wall-mounted stadiometer (Ayrton stadiometer, model
S100; Ayrton Corp, Prior Lake, MN). Body mass index (BMI) was calculat-
based on weight and standing height measurements and expressed as kg/m$^2$.

3.6. Body composition and bone mineral density

Body composition (lean mass and fat mass of the total body) as well as bone mineral content (BMC), and areal BMD of the lumbar spine and hip were measured with a Delphi-W QDR DXA bone densitometer (Hologic Inc., Bedford, MA). Calibration procedures were carried out daily according to
manufacturer instructions. The coefficient of variation (CV) for the
DXA instrument during the course of the study was 0.457% for the lumbar
spine BMD calibration phantom. All DXA scans were analyzed by a single
operator to decrease the variance in the measurement data.

3.7. Accelerator mass spectrometry: measurement of $^{41}$Ca

Calcium was extracted from each of the 24 h urines collected and
used to determine the $^{41}$Ca/$^{40}$Ca ratio. The calcium extraction for the
$^{41}$Ca tracer has been previously described (Lin et al., 2004). Briefly, sam-
ple were converted to acid solution with HCl to pH < 1.9. Acid soluble
calcium from the 14 urine samples was converted to calcium oxalate
by adding saturated ammonium oxalate solution. Concentrated ammo-
nium hydroxide (NH$_4$OH) was used to adjust the samples to pH 10 and
thus promote the release of less soluble metal oxalates. The oxalate
pellet was re-suspended in acid, and calcium was isolated from other cat-
ions using cation exchange chromatography. Concentrated hydrofluoric acid (28.9 molarity) was added to yield calcium fluoride, which was then pelletized by centrifugation and washed with de-ionic-
ized water. Samples were dried at 100 °C for 20 h in a muffle furnace.
Samples were shipped from the WHNRC to the LLNL (Livermore, CA).
A small amount of niobium powder (1 part Nb:4 parts CaF$_2$ by mass)
was added to increase thermal and electrical conductivity for ion
beam stability in the AMS source. Primary isotopic standards, secondary
standards, and backgrounds were prepared at LLNL following previously
described methods (Lin et al., 2004). Acidic solutions with known
$^{41}$Ca/$^{40}$Ca ratios were used to precipitate calcium fluoride by the addi-
tion of concentrated hydrofluoric acid, followed by centrifugation, rins-
ing with de-ionized water, and drying overnight in a muffle furnace at
100 °C. The use of the $^{40}$Ca in the $^{41}$Ca/$^{40}$Ca standards is important
because it represents urinary calcium excretion from all sources, e.g. di-
etary intake, compared to the $^{41}$Ca whose only source, after equilibra-
tion, is from the skeleton. All samples and standards were analyzed on the
HVEE-FN-class AMS system at LLNL operated as described
(Fitzgerald et al., 2005). >88% of the normalized primary standards
were within 5% of the published value (Nishizumi et al., 2000). Average
repeatability of the secondary standards was generally 1–7% for
$^{41}$Ca/$^{40}$Ca ranging 9 × 10$^{-9}$ to 9 × 10$^{-12}$.

3.8. Statistical analysis

Sample size calculation for the 12 subject study was based on the
assumption that the change in the $^{41}$Ca/$^{40}$Ca ratio is not correlated within
subjects and that the standard deviation of the change in the ratio is
10 percentage points and resulted in an 80% power to detect a 12.6% change in urinary $^{41}$Ca/$^{40}$Ca over time.

The change in the $^{41}$Ca/$^{40}$Ca ratio was the primary outcome variable for
this study. Evaluation of the calcium loss was based on the
$^{41}$Ca/$^{40}$Ca urinary excretion from the weekly 24-h urine collections over the
6 weeks of each intervention and the WO. Therefore, each period (intervention and WO) had 6 urinary data points, one per week for the duration of the 6-week intervention. Data were coded for order effect (dairy first or supplements first) and analyzed by analysis of variance (ANOVA) to determine if an order effect existed. ANOVA was also
used to determine if significant differences existed between dairy and supplement interventions in the urinary $^{41}$Ca/$^{40}$Ca excretion over the 6-
week intervention (treatment by time interaction).

Continuous variables were assessed for normality using Shapiro-
Wilk and D’Agostino-Pearson normality tests and were transformed as
appropriate. Nonparametric tests were used on data that were not con-
ductive to transformation. Descriptive statistics were performed on
$^{41}$Ca dose baseline characteristics. The secondary outcome of time to
change in $^{41}$Ca was evaluated by ANOVA using the difference between
weekly $^{41}$Ca/$^{40}$Ca values.

Additionally, to test whether the $^{41}$Ca/$^{40}$Ca excretion ratio was different
from what would be expected in the case of no intervention the kinetic
model developed by Denk et al. (Denk et al., 2006) was used to establish
the predicted curve of $^{41}$Ca loss from the $^{41}$Ca excretion data during the
180 equilibration period plus the values during the WO. The actual
values during the two interventions were compared to the predicted
values with a mixed model analysis that included type of measurement
(actual vs. predicted), intervention (dairy vs. supplement), week of in-
tervention (1 through 6), and period of intervention (first vs. second)
and all of their estimable interactions as main effects, and subject as a
random effect. SAS for Windows Release 9.4 (SAS Institute, Inc., Cary,
NC) and GraphPad Prism 6.0c (GraphPad Software, Inc., La Jolla, CA)
were used for statistical analyses.

4. Results

Subject characteristics at baseline (prior to the $^{41}$Ca dose on day 0)
are shown in Table 1. All subjects were healthy postmenopausal
women with hip and spine bone densities at the low end of the normal
range. Compliance to treatment regimens was 100% for both phases.

The ratio of urinary $^{41}$Ca/$^{40}$Ca excretion over time for each treatment is shown in Fig. 3. There was a significant decline in the
$^{41}$Ca/$^{40}$Ca excretion during Intervention I compared to the predicted value with no interven-
ion. Conversely, there was a significant increase in the $^{41}$Ca/$^{40}$Ca excretion
during WO returning to pre-intervention or untreated levels. During In-
tervention II the $^{41}$Ca/$^{40}$Ca excretion declined significantly a second time.
The response to either Intervention or WO was observed within 1–2
weeks and continued to decline throughout the 6 weeks with the
maximum reduction occurring by week 6 (Table 2; Fig. 3). The urinary
$^{41}$Ca excretion decreased during both interventions, confirming a fast-
exchangeable pool suggested in kinetic models (Denk et al., 2007;
Wastney et al., 1996). During WO the excretion increased also within
However, the change in $^{41}$Ca was not entirely explained by the interventions and only a small portion of the increased calcium intake. This translates to a 20.7% increase in urinary calcium during 59 mg vs. 150 ± 60 mg) compared to the value just before the intervention or WO (Fig. 4).

Given that both dairy and supplement interventions demonstrated no significant differences between these treatments were not significant. The investigators concluded that the milk powder and calcium carbonate were essentially equivalent in preventing bone loss (Prince et al., 1995). Later, Manios et al. (Manios et al., 2007) reported a 12 month RCT of 101 postmenopausal Greek women and compared three servings of low fat dairy products versus a control group. They found that the dairy group had a significantly greater increase in bone mineral density (BMD) compared to the control group.

Table 1

Subject characteristics at baseline (n = 12 females).

| Parameter             | Mean ± Standard deviation |
|-----------------------|---------------------------|
| Age (years)           | 55.4 ± 2.5                |
| Height (cm)           | 163.0 ± 4.9               |
| Weight (kg)           | 63.9 ± 8.5                |
| BMI (kg/m²)           | 24.2 ± 3.7                |
| Body fat (%)          | 38.0 ± 7.4                |
| Fat-free mass (kg)    | 37.2 ± 3.2                |
| Spine BMC (g)         | 45.6 ± 4.1                |
| Spine BMD (g/cm²)     | 1.1 ± 0.1                 |
| Spine T-Score         | −0.82 ± 0.62              |
| Hip BMC (g)           | 27.0 ± 2.4                |
| Hip BMD (g/cm²)       | 0.9 ± 0.1                 |
| Hip T-Score           | −0.88 ± 0.06              |
| Systolic blood pressure (mm Hg) | 117 ± 21               |
| Diastolic blood pressure (mm Hg) | 75 ± 14                 |
| PTH (pmol/L)          | 59 ± 19                   |

Note: Average of right and left hip values.

By measuring the change in $^{41}/^{40}$Ca in urine by AMS we have demonstrated that interventions using calcium and vitamin D from either dairy foods or supplements exert equal effects on calcium metabolism. Furthermore, our study is the first to show that the rapid turn-over pool suggested in calcium kinetic model literature (Wastney et al., 1996) is responsive to a calcium and vitamin D intervention, whether from food or supplements, as quickly as one week.

The $^{41}$Ca-AMS method has been used to assess the effect of bisphosphonates in postmenopausal women (Denk et al., 2007), in a comparison of healthy vs. end stage renal disease patients (Fitzgerald et al., 2005) as well as in a comparison of different isoflavone sources in postmenopausal women (Wastney et al., 1996). The present study is the first to utilize the $^{41}$Ca isotope with AMS quantification in an intervention with dairy foods vs. supplements. Denk and colleagues (Denk et al., 2007), in a study using bisphosphonates with postmenopausal women, present a 3-compartment kinetic model similar to that of Wastney et al. (Wastney et al., 1996) in adolescent girls and young women. In both studies the authors indicate that compartment 2, the fast exchange compartment, serves as a transfer station to deposit into or remove calcium from a slower turnover pool – presumably the skeleton. Wastney and coauthors provide kinetic details, after oral and intravenous administration of calcium isotopes, in serum, urine and feces in adolescent girls compared to young women and demonstrated higher rates of absorption, lower rates of excretion, higher turnover of bone and higher calcium retention in the girls vs. the young women. They did not conduct an intervention to determine responsiveness in the rapidly exchanging compartment 2 to calcium and vitamin D use.

5. Discussion

Schild et al. (Schild et al., 2015) also using $^{41}$Ca labeling method found that urinary $^{41}$Ca retention was increased with increasing levels of daily vitamin D supplementation taken daily for 3 months by healthy postmenopausal women. Supplementation was positively associated with a downward shift in the urinary $^{41}/^{40}$Ca ratio compared to the predicted change without intervention. Schild et al. also demonstrated that increasing levels of vitamin D affected the transfer rate from the central compartment to the fast exchange compartment. They hypothesized that the fast exchange compartment represented an exchange from the extracellular space to the surface of the bone. Our results also demonstrate the rapidity with which the fast exchangeable pool responds to increases or decreases in calcium and vitamin D intake.

Our findings of no difference in calcium retention between food vs. supplemental intake of calcium and vitamin D is in contrast with those of Recker and Heaney (Recker and Heaney, 1985) who reported that both a low-fat milk and calcium carbonate supplement improved calcium balance in 30 healthy postmenopausal women, but calcium carbonate suppressed bone remodeling to a greater extent than milk. However, this conclusion was based on data from two separate studies and was, therefore, not a direct comparison.

More consistent with the present findings were those of Prince and colleagues (Prince et al., 1995), who conducted a two-year study of 168 postmenopausal women and evaluated the effects of three different calcium treatments or placebo on BMD. Calcium lactate gluconate tablets and skim milk powder significantly attenuated bone loss at certain sites (inter-trochanteric hip, ultradistal tibia) compared to placebo, but differences between these treatments were not significant. The investigators concluded that the milk powder and calcium carbonate were essentially equivalent in preventing bone loss (Prince et al., 1995).
to calcium and vitamin D supplementation and a control (usual) diet. Of the three groups, dairy consumption led to the greatest attenuation of bone resorption (a 23% decrease in CTx). Unlike our study, the dairy group had significantly greater BMD at the pelvis, total spine and total body after 12 months compared to the supplement and control groups, suggesting an advantage of dairy treatment on multiple parameters of bone health (Manios et al., 2007).

Neither the present study nor the study by Prince and colleagues (Prince et al., 1995) showed robust differences in the anti-resorptive effects of the calcium supplement versus dairy foods, but advantages of dairy become evident when dietary intake data are examined. In the present study, subjects consumed significantly greater amounts of protein, carbohydrate, vitamin A, zinc and potassium during the dairy treatment than during either the supplement intervention or WO (Supplemental Table 1). The dairy intervention also led to significant increases in dietary intake of folate, phosphorus, and magnesium compared to the subjects’ typical intake (WO). These nutrients are well recognized as bone-enhancing nutrients (Caroli et al., 2011; Peters and Martini, 2010; Weaver, 2009) and, over a longer duration, may result in a shift toward a significant difference between food and supplement treatments.

It is possible that the six week time frame of the present study was too brief to observe mineralization changes represented by bone formation markers such as bone alkaline phosphatase (BAP) (Supplemental Table 2). The lack of change in BAP is consistent with a study of postmenopausal women by Bonjour and colleagues (Bonjour et al., 2008). This 16-week crossover trial compared treatment with 1200 mg/day calcium from semi skimmed milk vs. no milk supplement and found significant changes in bone formation markers amino-terminal propeptide of type 1 procollagen (P1NP) and osteocalcin, but not in BAP. Likewise,
no significant PTH change during either treatment is consistent with previous reports that calcium intake prevents increases in PTH over time (Chee et al., 2003; Manios et al., 2007). Calcium tablets have been shown to significantly decrease PTH after 6 months (Prince et al., 1995), but the six week treatment duration in the present study may have been insufficient to observe this suppressive effect. The sample size of the present study is comparable to other 41Ca-AMS studies by Weaver and colleagues (Weaver, 2009) (n = 11) and Denk and colleagues (Denk et al., 2007) (n = 6). The crossover design allowed each woman to serve as her own control, thereby limiting inter-individual variability and allowing for a smaller sample size.

A limitation of the present study is the racial homogeneity in this sample of Caucasian women. Racial differences in bone density and bone structure are well known (Heaney, 2000), so results of the present study cannot be extrapolated to other women of other races. An added limitation of the present study is that the vitamin D3 dose of 400 IU/day given during both treatments does not reflect the most current RDA value of 600 IU per day for women 51–70 years old. The RDA changed from 400 IU to 600 IU while the present study was underway (Institute of Medicine (US), 2011), so we proceeded with the original study protocol. Future studies using daily doses of vitamin D3 = 400 IU are needed (Moyer and Vitamin, 2013).

The present study is the first to use the 41Ca-AMS method in a dietary intervention study with dairy products and directly compares calcium and vitamin D intake from dairy foods vs. supplements on the same bone variables. The highly sensitive 41Ca-AMS technique represents an um and vitamin D intake from dairy foods vs. supplements on the same no signi

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