Dietary calcium does not interact with vitamin D$_3$ in terms of determining the response and catabolism of serum 25-hydroxyvitamin D during winter in older adults$^{1-3}$


ABSTRACT

Background: Interactions between calcium and vitamin D may have implications for the regulation of serum 25-hydroxyvitamin D [25(OH)D] and its catabolism and, consequently, the vitamin D dietary requirement.

Objective: We investigated whether different calcium intakes influenced serum 25(OH)D and indexes of vitamin D activation and catabolism during winter and in the context of both adequate and inadequate vitamin D intakes.

Design: A 15-wk winter-based, randomized, placebo-controlled, double-blind vitamin D$_3$ intervention (20 mg/d) study was carried out in free-living men and women aged ≥70 y ($n$ = 125) who were stratified according to calcium intakes (moderate-low [<700 mg/d] or high [≥1000 mg/d] intake). The serum 25(OH)D concentration was the primary outcome, and serum calcium, parathyroid hormone (PTH), 1,25-dihydroxyvitamin D [1,25(OH)$_2$D], 24,25-dihydroxyvitamin D [24,25(OH)$_2$D], the ratio of 24,25(OH)$_2$D:25(OH)D, vitamin D-binding protein, and free 25(OH)D were exploratory outcomes.

Results: A repeated-measures ANOVA showed there was no significant ($P = 0.2$) time × vitamin D treatment × calcium intake grouping interaction effect on the mean serum 25(OH)D concentration over the 15-wk intervention period. Serum 25(OH)D concentrations increased ($P = 0.005$) and decreased ($P = 0.002$) in vitamin D$_3$ and placebo groups, respectively, and were of similar magnitudes in subjects with calcium intakes <700 mg/d (and even <550 mg/d) compared with >1000 mg/d. The response of serum PTH, 1,25(OH)$_2$D, 24,25(OH)$_2$D, the ratio of 24,25(OH)$_2$D:25(OH)D, and free 25(OH)D significantly differed in vitamin D$_3$ and placebo groups but not by calcium intake grouping.

Conclusions: We found no evidence of a vitamin D sparing effect of high calcium intake, which has been referred to by some authors as “vitamin D economy.” Thus, recent dietary vitamin D requirement estimates will cover the vitamin D needs of even those individuals who have inadequate calcium intakes. This trial was registered at clinicaltrials.gov as NCT01990872.

INTRODUCTION

Calcium and vitamin D are metabolically interrelated to serve endocrine functions. Vitamin D, as the active metabolite (1,25-dihydroxyvitamin D [1,25(OH)$_2$D])$^4$, forms part of the endocrine system that maintains serum calcium concentrations, which are required for bone mineralization (1) and several nonskeletal processes (2). Interactions between vitamin D and calcium may have implications for the regulation of serum 25-hydroxyvitamin D [25(OH)D] concentrations and its catabolism and, consequently, the dietary vitamin D requirement. For example, if dietary calcium intake is low, and serum calcium concentrations decrease, the compensatory metabolic response is an accelerated conversion of 25(OH)D to 1,25(OH)$_2$D [via parathyroid hormone (PTH)], which normalizes serum calcium concentrations. There have been animal data that suggested that the plasma half-life of 25(OH)D will be reduced by low calcium intake accompanied by an increased hepatic conversion of 25(OH)D to metabolites, which are excreted in bile (3).

Mechanistically, the increased synthesis of 1,25(OH)$_2$D during times of low calcium intakes induces the synthesis of the 24-hydroxylase enzyme, which converts serum 25(OH)D to 24,25-dihydroxyvitamin D [24,25(OH)$_2$D]. In vitro experiments with purified 24-hydroxylase suggested that 24-hydroxylation is just the first step in a 5-step, vitamin D–inducible pathway to water-soluble truncated degradation products (4). However, older animal data suggested that 24,25(OH)$_2$D [and its metabolite 1,24,25-trihydroxyvitamin D] may stimulate intestinal calcium absorption and bone calcium mobilization (5, 6), suggesting a biological activity in addition to a role in the degradation of vitamin D. The use of intravenously administered radiolabeled 25(OH)D in patients has shown that its elimination half-time in plasma was significantly shortened by oral 1,25(OH)$_2$D treatment (7). There has also been supportive evidence from children with
Subjects and Methods

Subjects

A total of 125 apparently healthy, free-living adults aged ≥ 50 y were recruited in this 15-wk vitamin D$_3$ intervention trial. Subjects were recruited in the Cork area through the use of advertisements placed around University College Cork and across the location as well as on radio. We aimed to recruit a ratio of ~2.5:1 women to men because this is the ratio of women to men (aged ≥50 y) with moderate-low (<700 mg/d; see Design and conduct of study) calcium intakes as reported in the National Adult Nutrition Survey in Ireland (21) and the National Diet and Nutrition Survey in the United Kingdom (22).

White men and women aged ≥50 y who provided consent were included in the study. Volunteers were excluded if they were unwilling to discontinue the consumption of vitamin D–containing supplements 4 wk before the initiation of the study and throughout the study. Volunteers were also excluded if they planned to take a winter vacation (during the course of the 15-wk intervention) to a location where either the altitude or the latitude was predicted to result in significant cutaneous vitamin D synthesis from solar radiation (eg, a winter-sun coastal resort or a mountain ski resort) or if they used tanning facilities of any type. A severe medical illness, hypercalcemia, known intestinal malabsorption syndrome, excessive alcohol use, and the use of medications known to interfere with vitamin D metabolism were also reasons for exclusion. The study was approved by the Clinical Research Ethics Committee of the Cork Teaching Hospitals, University College Cork. All participants gave their written consent according to the Helsinki Declaration. This trial was registered at clinicaltrials.gov as NCT01990872.

Design and conduct of study

The study was a double-blind, placebo-controlled trial in which older adults, who were stratified according to calcium intakes (moderate-low or high intake), were randomly assigned to receive a capsule that contained a placebo or 20 µg vitamin D$_3$ for 15 wk throughout the winter. During the recruitment phase, subjects were initially screened for their habitual intake of calcium (and vitamin D) via a validated food-frequency questionnaire (23, 24) that was administered by a research nutritionist. For the purposes of this study, moderate-low calcium intake was defined as <700 mg/d, whereas high calcium intake was defined as >1000 mg/d. The reference nutrient intake and population reference intake value for calcium in the United Kingdom and European Union, respectively, is700 mg/d (25, 26), and ~49% and ~69% of UK men and women (aged 50–64 y), respectively, have calcium intakes below this value (22). The European Union estimated average requirement (EAR) for calcium is 550 mg/d (26) and ~5 and ~15% of men and women, respectively, in the United Kingdom have intakes below this value (22). In relation to high calcium intake, the North American Institute of Medicine (IOM) has suggested that EAR and Recommended Dietary Allowance (RDA) values for calcium in women aged >50 y and men aged >70 y are 1000 and 1200 mg/d, respectively (9). For men aged 50–70 y, the RDA is 1000 mg Ca/d (9). In addition, for the purposes of informing the design of the current study, we revisited our recent meta-analysis of the relation of vitamin D intake to serum 25(OH)D, which used data from RCTs performed at latitudes >50° (27), and included data on average habitual calcium intake from each of the studies. Stratification of the 11 RCTs by the median (~1000 mg/d) of reported average calcium intakes in these studies showed that there was a significant (P < 0.05) interaction between calcium intake (>1000 or <1000 mg/d) and vitamin D intake such that subjects with lower calcium intakes had a less-steep slope [response of serum 25(OH)D] than did subjects with higher calcium intakes (the 2 slopes were significantly different; 0.030 compared with 0.044, respectively; P = 0.023; unpublished data). Therefore, the achievement of a serum 25(OH)D concentration of 50 nmol/L required lower vitamin D intake in subjects with a higher calcium intakes (>1000 mg/d) than in subjects with lower intake (<1000 mg Ca/d).

To maximize the subject recruitment in the study, any subject (n = 38) whose habitual calcium intake was >700 mg/d but did not reach the >1000-mg/d target was asked during recruitment if they would take additional calcium. Subjects were given a choice as to whether they wished to consume the additional calcium in the form of a supplement [one Calcichew tablet that contained 500 mg Ca alone (Shire Pharmaceuticals Ireland Ltd); n = 36 opted for this route] or as additional milk in their diet (n = 2 opted for this route), so that their calcium intakes would be >1000 mg/d for the duration of the intervention phase. These
additional calcium intakes were started 4 wk before the intervention phase commenced to allow for normalization of calcitropic hormone concentrations (28).

The IOM has suggested that the RDA-like serum 25(OH)D concentration required to meet the needs of 97.5% of the population aged ≥1 y is 50 nmol/L (9). On the basis of this serum concentration of 25(OH)D, the IOM has established the RDA for vitamin D at 15 and 20 μg vitamin D3/d for individuals aged 1–70 and >70 y, respectively, in North America (9). We have previously reported that a vitamin D RDA estimate of 24.7 μg/d was needed to maintain a wintertime serum 25(OH)D concentration >50 nmol/L in 97.5% of adults aged ≥64 y residing in Ireland (51–55°N) (29) and this estimate was confirmed again recently in our RCT with vitamin D compared with 25(OH)D in subjects aged ≥50 y (30). Therefore, we chose to use 20 μg supplemental vitamin D3/d, which, together with a habitual intake of ~5 μg vitamin D/d in Irish adults (21), would provide about ~25 μg/d in the current study.

The random assignment of subjects was centralized and computer generated and accounted for calcium intake grouping (moderate-low or high intakes) and sex. Vitamin D3 and matching placebo capsules were produced by Laboratorium Medisan. Vitamin D3 capsules contained 20 μg vitamin D3 (as Quali-D, a pure crystalline, powder form of vitamin D3; DSM Nutritional Products), and placebo and vitamin D3 capsules contained filling additives (potato starch and talcum). Placebo and vitamin D3 capsules were identical in appearance and taste. The vitamin D3 content of capsules was confirmed by an in-house laboratory HPLC analysis within the Vitamin D Research Group at University College Cork. Compliance was assessed by capsule counting. An a priori decision was made to include only subjects who exceeded 80% compliance. The allocation remained concealed until final analyses, and all outcomes were reported by people masked to the allocation scheme.

The study was carried out in Cork, Republic of Ireland (latitude: 51°N). All subjects were screened, recruited, and commenced the intervention study between 5 and 23 November 2012 and finished the study 15 wk later between 18 February and 14 March 2013, during which the vitamin D status would be expected to decline to a nadir (31). During the intervention phase, each participant was met by researchers on 3 occasions at the study center, once each at baseline (week 0), week 8 (which was close to the midpoint), and the endpoint (week 15) of the study. At each visit, an overnight fasting blood sample was taken from each participant between 0830 and 1030 by a trained phlebotomist. Blood was collected by venipuncture into an evacuated tube with no additive and processed to serum, which was immediately stored at −80°C until required for analysis. Anthropometric measures, including height and weight, were taken as described previously (32). A health and lifestyle questionnaire, which assessed physical activity, general health, smoking status, and alcohol consumption, was completed at baseline. Participants were contacted regularly by phone or correspondence to promote compliance and encourage the completion of the study protocol.

Laboratory analysis

**Serum 25(OH)D**

Concentrations of total 25(OH)D [ie, 25(OH)D2 plus 25(OH)D3] in all serum samples were measured by the Vitamin D Research Group at University College Cork by using a liquid chromatography–tandem mass spectrometry (LC–tandem MS) method that has been described in detail elsewhere (33). The quality and accuracy of the serum 25(OH)D analysis by using the LC–tandem MS in our laboratory is monitored on an ongoing basis by participation in the Vitamin D External Quality Assessment Scheme (Charing Cross Hospital). In addition, the Vitamin D Research Group is a participant in the Vitamin D Standardization Program (34) and Vitamin D Standardization Certification Program (35).

**Serum intact PTH**

Serum PTH concentrations were measured at University College Cork in all serum samples by using an ELISA (Intact PTH; MD Biosciences Inc). Intraassay and interassay CVs were 3.4% and 3.8%, respectively.

**Serum total calcium**

Total calcium and albumin concentrations in all serum samples were measured at Cork University Hospital, Cork, Ireland. Serum calcium concentrations were adjusted for albumin concentrations.

**Serum 1,25(OH)2D**

Concentrations of 1,25(OH)2D were measured at University College Cork in baseline and endpoint serum samples by using an ELISA (IDS 1,25-DihydroxyVitamin D EIA; Immuno Diagnostic Systems Ltd). Intraassay and inter assay CVs for the ELISA method were 10.5% and 17.1%, respectively.

**Serum 24,25(OH)2D**

Concentrations of 24,25(OH)2D in baseline and endpoint serum samples were measured by the Vitamin D Research Group at University College Cork by using an in-house LC–tandem MS method that was a modification of the method for measurement of 25(OH)D as described elsewhere (33). See supplemental methodologic information under “Supplemental data” in the online issues for additional details of the LC–tandem MS method for serum 24,25(OH)2D.

**Serum vitamin D–binding protein**

Vitamin D–binding protein (DBP) concentrations were measured at University College Cork in baseline and endpoint serum samples by using an ELISA (Human Vitamin D BP Quantikine ELISA Kit; R&D Systems). Intraassay and inter assay CVs for the ELISA method were 6.2% and 7.4%, respectively.

**Estimation of serum free 25(OH)D concentrations and 24,25(OH)2D:25(OH)D ratio**

It has been suggested that free bioavailable 25(OH)D may provide for a more-meaningful marker of vitamin D function (36). For example, an individual with inadequate serum total 25(OH)D (<50 nmol/L), might nevertheless have adequate concentrations of free 25(OH)D if serum concentrations of DBP are low (36). Therefore, we calculated the concentration of free 25(OH)D by using a previously described equation (37). The ratio of 24,25(OH)2D:25(OH)D has also been suggested as possible sensitive marker of vitamin D status and possesses clinical
utility as a marker for vitamin D catabolism because its production is reduced in the early stages of vitamin D deficiency and may be increased when the vitamin D supply is high as the first step in the catabolic pathway of 25(OH)D (19, 38).

Statistical analysis

Berlin and Bjorkhem (16) reported a mean difference in winter serum 25(OH)D concentrations of 17 nmol/L in Swiss men supplemented compared with unsupplemented with calcium. Stratification of vitamin D RCTs included in our previous meta-analysis of functional markers of vitamin D status by whether vitamin D was administered alone or in conjunction with calcium (39) suggested a difference in the average group mean serum 25(OH)D concentration between groups who received vitamin D alone compared with calcium plus vitamin D of ~13 nmol/L. Therefore, we used a more conservative 10-nmol/L difference as what we would need to be able to detect between calcium intake groups. On the basis of the distribution of winter time serum 25(OH)D data from older adults from our previous study (30), a study design that recruited 30 volunteers/group (which included 20% to cover potential dropouts) would have 90% power to detect a 10-nmol/L difference in serum 25(OH)D between groups at \( \alpha = 0.5 \).

Statistical analyses of the data were conducted with SPSS for Windows software (version 17.0; SPSS Inc). Distributions of all variables were tested by using Kolmogorov-Smirnov tests. Descriptive statistics (means \( \pm \) SDs or medians and IQRs when appropriate) were determined for all variables. Dietary vitamin D, serum PTH, 24,25(OH)2D, free 25(OH)D, and 1,25(OH)2D were not normally distributed and, thus, were either log or square-root transformed to achieve near-normal distributions. Serum concentrations of 25(OH)D, albumin-corrected calcium, DBP, the 24,25(OH)2D:25(OH)D ratio as well as age, weight, height, BMI, and dietary calcium were normally distributed. Baseline characteristics of men and women were compared by using unpaired Student’s t tests. Baseline characteristics of subjects in the different intervention groups were compared by using the chi-square test (for the ratio of men to women) and 1-factor ANOVA. Linear models of the response in a repeated-measures analysis for differences in serum 25(OH)D, albumin-corrected calcium, PTH, 24,25(OH)2D, 1,25(OH)2D, 24,25(OH)2D:25(OH)D, free 25(OH)D, and DBP concentrations were constructed. Linear models included 3- and 2-way interactions between main effects (calcium intake grouping and vitamin D treatment), and these were reported. If no significant calcium intake grouping \( \times \) vitamin D treatment \( \times \) time interaction was observed, then calcium intake grouping \( \times \) time and vitamin D treatment \( \times \) time interactions were explored. Models were also run to include sex as an additional factor. Bonferroni-adjusted \( t \) tests were used for post hoc analysis to the ANOVA. \( P < 0.05 \) was taken as being statistically significant.

RESULTS

Baseline characteristics of subjects

Of 125 subjects recruited onto the study, 121 subjects completed the intervention. The progress of these subjects through the trial is shown in Figure 1. Baseline characteristics of subjects who entered the intervention are shown in Table 1. Although women were, on average, lighter and smaller than men (both \( P < 0.0001 \)) and had lower serum 1,25(OH)2D concentrations (\( P < 0.05 \)), there was no difference (\( P > 0.1 \)) in the mean age, BMI, mean habitual intakes of vitamin D and calcium, serum 25(OH)D, albumin-corrected calcium, PTH, 24,25(OH)2D, DBP, or free 25(OH)D concentrations between men and women (data not shown).

Effects of intervention with vitamin D3 in moderate-low- and high-calcium intake groups

There was no difference (\( P > 0.3 \)) in the mean age, weight, height, or BMI at baseline in the 4 treatment groups (placebo and vitamin D3 with moderate-low-calcium intake groups;
placebo and vitamin D$_3$ with high–calcium intake groups (data not shown). Similarly, there was no significant difference in the proportion of men to women in treatment groups (Table 2). Mean habitual dietary vitamin D was significantly ($P = 0.001$) higher in the group with high calcium intake who were randomly assigned to receive vitamin D supplementation than in the group with moderate–low calcium intake who were randomly assigned to receive the placebo, but otherwise there was no significant difference (Table 2). Mean habitual dietary calcium was similar in the 2 groups with moderate–low calcium intakes and similar in the 2 groups with high calcium intakes, with the latter 2 groups with significantly higher calcium ($P < 0.0001$) than for the former 2 groups (Table 2). Mean total calcium intakes ( habitual plus additional calcium intakes) for the 2 groups with high calcium intakes are shown in Table 2, and these intakes were similar ($P > 0.7$).

There were no adverse events reported during the study. Of 4 dropouts, 2 subjects were in the period between baseline and midpoint samplings (1 subject was from the group with moderate–low calcium intake plus 20 μg vitamin D$_3$/d, 1 subject was from the group with high calcium intake plus the placebo), and 2 subjects (both from the group with high calcium intake plus 20 μg vitamin D$_3$/d) were in the period between midpoint and endpoint samplings. Dropout during the intervention phase was for reasons of unavailability or loss of interest, and in no instance was dropout related to the intervention. There was good supplement adherence on the basis of the pill count (median IQR compliance was 98.6% (94.3%, 100%), compliance was similar in the 4 treatment groups; $P = 0.9$).

Effects of vitamin D treatment in the 2 calcium intake groupings ( moderate–low compared with high intakes) on serum 25(OH)D, albumin–corrected calcium, and PTH at the baseline, midpoint, and endpoint are shown in Table 2. There was no significant difference in preintervention serum 25(OH)D concentrations in treatment groups (Table 2). A repeated–measures ANOVA showed that there was no significant ($P = 0.2$) time × vitamin D treatment × calcium intake grouping interaction effect on mean serum 25(OH)D concentration over the 15–wk intervention period (Table 2). Likewise, there was no significant ($P = 0.8$) time × calcium intake grouping interaction effect, but there was a significant ($P < 0.0001$) time × vitamin D treatment interaction effect on mean serum 25(OH)D concentration over the 15–wk intervention period (Table 2). Because calcium intake grouping had no effect, the mean (±SD) baseline serum 25(OH)D of the 2 groups combined who received 20 μg vitamin D$_3$/d (54.3 ± 23.4 nmol/L; $n = 64$) was similar ($P > 0.6$) to that of the 2 groups combined who received the placebo (56.1 ± 16.7 nmol/L; $n = 61$). Mean (±SD) serum 25(OH)D concentrations in combined groups who received 20 μg vitamin D$_3$/d, irrespective of the calcium intake grouping, increased significantly (to 74.3 ± 15.5 nmol/L; $P < 0.0001$) by approximately midpoint (week 8), and there was a modest but significant ($P = 0.005$) additional increase (to 76.9 ± 17.1 nmol/L) by week 15. Conversely, mean (±SD) serum 25(OH)D concentrations in combined groups who received the placebo, irrespective of the calcium intake grouping, decreased significantly (to 43.8 ± 15.8 nmol/L; $P < 0.0001$) by week 8, and there was a modest but significant ($P = 0.002$) additional decrease (to 41.7 ± 15.5 nmol/L) by week 15.

There was no significant interaction between time × vitamin D treatment × calcium intake grouping (or time × vitamin D treatment or time × calcium intake grouping) and sex (all $P > 0.4$) in the response of serum 25(OH)D to the intervention (data not shown). There was no significant difference in preintervention serum albumin–corrected calcium concentrations in treatment groups (Table 2). There was no significant time × vitamin D treatment × calcium intake grouping interaction effect or time × vitamin D treatment or time × calcium intake grouping interaction effects (all $P > 0.3$) on mean serum albumin–corrected calcium concentrations over the 15–wk intervention period.

A repeated–measures ANOVA showed that there was a significant ($P < 0.05$) difference in preintervention serum PTH concentrations in groups stratified by calcium intakes. The 2 groups with moderate–low calcium combined ($n = 56$) had a modest but significantly ($P = 0.037$) higher median (IQR) baseline serum PTH than did the 2 groups combined ($n = 69$) who had a high calcium intake [50.2 pg/mL (38.2, 67.6 pg/mL) compared with 43.9 pg/mL (34.7, 55.9 pg/mL), respectively]. There was no significant ($P = 0.3$) time × vitamin D treatment × calcium intake grouping interaction effect on the median (IQR) serum PTH concentration over the 15–wk intervention period (Table 2). Likewise, there was no significant ($P = 0.7$) time × calcium intake grouping interaction effect, but there was a significant ($P = 0.013$) time × vitamin D treatment interaction effect on the median (IQR) serum PTH concentration over the 15–wk intervention period (Table 2). Median serum PTH concentrations in the 2 groups who received 20 μg vitamin D$_3$/d, irrespective of the calcium intake grouping, remained unchanged ($P > 0.3$) over the 15 wk, whereas median serum PTH concentrations in the 2 groups who received the placebo, irrespective of the calcium intake grouping, remained unchanged ($P > 0.7$) over the first 8 wk but had increased significantly ($P = 0.034$) by week 15 [44.8 pg/mL (35.2, 56.9 pg/mL), 45.1 pg/mL (40.1, 58.4 pg/mL), and 47.8 pg/mL (49.0, 57.1 pg/mL), respectively].

Effects of vitamin D treatment in the 2 calcium intake groupings ( moderate–low compared with high intakes) on the

### TABLE 1
Baseline characteristics of subjects who entered the intervention study

<table>
<thead>
<tr>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
</tr>
<tr>
<td>M:F</td>
</tr>
<tr>
<td>Age (y)</td>
</tr>
<tr>
<td>Height (m)</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
</tr>
<tr>
<td>Serum calcium (mmol/L)</td>
</tr>
<tr>
<td>Serum PTH (pg/mL)</td>
</tr>
<tr>
<td>Serum PTH (mg/mL)</td>
</tr>
</tbody>
</table>

$\text{DBP}$, vitamin D–binding protein; $\text{PTH}$, parathyroid hormone; 25(OH)D, 25–hydroxyvitamin D; 25(OH)D$\text{D}$, 25–hydroxyvitamin D. $\text{M}$ mean ± SD (all such values)

Median; IQR in parentheses [nonnormally distributed variable (all such values)].
Dietary intakes of vitamin D and calcium and serum 25(OH)D, calcium, and PTH concentrations in treatment groups at baseline, midpoint, and endpoint of the 15-wk intervention in apparently healthy older adults.

<table>
<thead>
<tr>
<th></th>
<th>Moderate-low calcium intake (&lt;700 mg/d)</th>
<th>High calcium intake (&gt;1000 mg/d)</th>
<th>Repeated-measures ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>20 µg vitamin D3/d</td>
<td>Placebo</td>
</tr>
<tr>
<td>n (at endpoint)</td>
<td>60</td>
<td>61</td>
<td>56</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>8:20</td>
<td>8:19</td>
<td>8:24</td>
</tr>
<tr>
<td>Habital dietary vitamin D (µg/d)</td>
<td>3.0 (2.2, 5.5)³</td>
<td>4.0 (2.8, 6.6)³</td>
<td>3.8 (3.2, 6.1)³</td>
</tr>
<tr>
<td>Dietary calcium (mg/d)</td>
<td>15.5 to 76.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Habitat</td>
<td>514 ± 156.4 a</td>
<td>479 ± 169 a</td>
<td>1061 ± 373 b</td>
</tr>
<tr>
<td>Serum 25(OH)D (nmol/L)</td>
<td>58.3 ± 16.7</td>
<td>54.3 ± 25.1</td>
<td>54.3 ± 16.7</td>
</tr>
<tr>
<td>Midpoint</td>
<td>44.3 ± 15.8</td>
<td>76.1 ± 16.4</td>
<td>43.4 ± 16.0</td>
</tr>
<tr>
<td>Postintervention</td>
<td>42.1 ± 14.8</td>
<td>80.4 ± 18.7</td>
<td>41.4 ± 16.3</td>
</tr>
<tr>
<td>Serum calcium (mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preintervention³,⁹,¹⁰</td>
<td>47.0 (35.6, 59.9)</td>
<td>53.7 (38.2, 70.5)</td>
<td>43.1 (35.0, 52.2)</td>
</tr>
<tr>
<td>Midpoint</td>
<td>45.0 (40.5, 65.2)</td>
<td>52.9 (41.4, 64.4)</td>
<td>45.2 (39.2, 55.6)</td>
</tr>
<tr>
<td>Postintervention</td>
<td>50.2 (42.2, 61.8)</td>
<td>47.0 (37.7, 61.4)</td>
<td>46.5 (39.4, 55.9)</td>
</tr>
</tbody>
</table>

¹ Different superscript letters (a and b) represent significant differences in group means, P < 0.05 (Bonferroni-adjusted t test). PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D.
² Repeated-measures ANOVA was used to test the time × vitamin D treatment × calcium intake grouping interaction effect.
³ Repeated-measures ANOVA was used to test the time × calcium intake grouping interaction effect.
⁴ Repeated-measures ANOVA was used to test the time × vitamin D treatment interaction effect.
⁵ Chi-square test was used to test differences for between-group differences in the case of sex (P > 0.9), and ANOVA was used in the case of dietary calcium (both P > 0.0001) and vitamin D (P < 0.01)
⁶ Median; IQR in parentheses [nonnormally distributed variable (all such values)].
⁷ Mean ± SD (all such values).
⁸ There were no significant differences in baseline concentrations across the 4 groups (P > 0.4 in all cases).
⁹ All baseline blood samples were taken between 5 and 23 November 2012, and all endpoint blood samples were taken between 18 February and 14 March 2013.
¹⁰ Mean (±SD) serum 25(OH)D concentrations in the combined groups with intake of 20 µg vitamin D3/d (n = 64), irrespective of calcium intake grouping, increased significantly from baseline by week 8 (P < 0.0001; Bonferroni-adjusted t test) and further by week 15 (P = 0.005) (54.3 ± 23.4 to 74.3 ± 15.5 to 76.9 ± 17.1 nmol/L, respectively). Conversely, mean (±SD) serum 25(OH)D concentrations in the combined placebo groups (n = 61) decreased significantly from baseline by week 8 (P < 0.0001) and further by week 15 (P = 0.002) (56.1 ± 16.7 to 43.8 ± 15.8 to 41.7 ± 15.5 nmol/L, respectively).
¹¹ Albmin corrected.
¹² Median (IQR) baseline serum PTH in the 2 groups with moderate-low calcium intake combined (n = 66) was significantly higher than that in the 2 groups with high calcium intake combined (n = 69) [50.2 pg/mL (38.2, 67.6 pg/mL) compared with 43.9 pg/mL (34.7, 55.9 pg/mL); P = 0.037 (unpaired t test)].

Serum 24,25(OH)₂D, ratio of 24,25(OH)₂D:25(OH)D, 1,25(OH)₂D, free 25(OH)D, or DBP concentrations at the baseline and endpoint are shown in Table 3. There was no significant difference in preintervention concentrations for any of these variables in treatment groups (Table 3). A repeated-measures ANOVA showed that there was no significant (P > 0.1) for all time × vitamin D treatment × calcium intake grouping interaction effect in the serum 24,25(OH)₂D, ratio of 24,25(OH)₂D:25(OH)D, 1,25(OH)₂D, free 25(OH)D, or DBP concentrations over the 15-wk intervention period (Table 3). Likewise, there was no significant (P > 0.1) for all time × calcium intake grouping interaction effect on the mean serum 24,25(OH)₂D, ratio of 24,25(OH)₂D:25(OH)D, 1,25(OH)₂D, free 25(OH)D and DBP concentrations over the 15-wk intervention period (Table 3). Although there was no significant (P = 0.7) time × vitamin D treatment interaction effect on mean serum DBP, there were significant (P ≤ 0.0001 for all) time × vitamin D treatment interaction effects on mean serum 24,25(OH)₂D, the ratio of 24,25(OH)₂D:25(OH)D, 1,25(OH)₂D, and free 25(OH)D concentrations over the 15-wk intervention period (Table 3).

Because the calcium intake grouping had no effect, the mean baseline 24,25(OH)D₃, ratio of 24,25(OH)D₃:25(OH)D, 1,25(OH)₂D₃, and free 25(OH)D₃ of the 2 groups combined who received 20 µg vitamin D₃/d (n = 61) were similar (P = 0.2 for all) to those of the 2 groups combined who received the placebo (n = 60) (data not shown). The 2 placebo groups combined (n = 60) had a significantly (P < 0.0001) lower median (IQR) serum 24,25(OH)D₃ at the endpoint than that of the 2 vitamin D₃-supplemented groups combined (n = 61) [1.14 nmol/L (0.59, 1.85 nmol/L) compared with 3.54 nmol/L (2.87, 4.54 nmol/L), respectively]. The 2 placebo groups combined (n = 60) had a significantly (P = 0.0001) lower mean (±SD) serum 24,25(OH)D₃:25(OH)D ratio at the endpoint than that of the 2 vitamin D₃-supplemented groups combined (n = 61) (0.030 ± 0.014...
TABLE 3
Serum 24,25(OH)2D, 1,25(OH)2D, free 25(OH)D, and DBP concentrations and serum 24,25(OH)2D:25(OH)D ratio in treatment groups at the baseline and endpoint of the 15-wk intervention in apparently healthy older adults.

<table>
<thead>
<tr>
<th></th>
<th>Moderate-low calcium intake (&lt;700 mg/d)</th>
<th>High calcium intake (&gt;1000 mg/d)</th>
<th>Repeated-measures ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo 20 µg Vitamin D3/d</td>
<td>Placebo 20 µg Vitamin D3/d</td>
<td></td>
</tr>
<tr>
<td>n (at endpoint)</td>
<td>28</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Serum 24,25(OH)2D (nmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preintervention</td>
<td>2.3 (1.8, 3.7)</td>
<td>2.4 (1.3, 3.3)</td>
<td></td>
</tr>
<tr>
<td>Postintervention</td>
<td>1.3 (0.7, 1.9)</td>
<td>1.0 (0.5, 1.7)</td>
<td></td>
</tr>
<tr>
<td>Serum 24,25(OH)2D:25(OH)D ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preintervention</td>
<td>0.041 ± 0.017</td>
<td>0.043 ± 0.017</td>
<td></td>
</tr>
<tr>
<td>Postintervention</td>
<td>0.031 ± 0.014</td>
<td>0.030 ± 0.014</td>
<td></td>
</tr>
<tr>
<td>Serum 1,25(OH)2D (pmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preintervention</td>
<td>79.9 (64.4, 103.6)</td>
<td>59.5 (48.8, 82.8)</td>
<td></td>
</tr>
<tr>
<td>Postintervention</td>
<td>69.2 (52.1, 91.5)</td>
<td>64.2 (46.4, 91.4)</td>
<td></td>
</tr>
<tr>
<td>Serum free 25(OH)D (pmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preintervention</td>
<td>20.3 (15.6, 26.0)</td>
<td>16.2 (11.5, 23.1)</td>
<td></td>
</tr>
<tr>
<td>Postintervention</td>
<td>15.1 (10.9, 20.5)</td>
<td>11.5 (8.2, 18.0)</td>
<td></td>
</tr>
<tr>
<td>Serum DBP (mg/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preintervention</td>
<td>211.4 ± 62.5</td>
<td>242.5 ± 75.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>206.9 ± 58.9</td>
<td>234.9 ± 80.3</td>
<td></td>
</tr>
</tbody>
</table>

1 There were no significant differences in preintervention concentrations across the 4 groups (P > 0.1 in all cases). All baseline blood samples were taken between 5 and 23 November 2012, and all endpoint blood samples were taken between 18 February and 14 March 2013. DBP, vitamin D–binding protein; P2, vitamin D treatment interaction effect. 
2 Repeated-measures ANOVA was used to test the time × vitamin D treatment × calcium intake grouping interaction effect. 
3 Repeated-measures ANOVA was used to test the time × calcium intake grouping interaction effect. 
4 Repeated-measures ANOVA was used to test the time × vitamin D treatment interaction effect. 
5 Median; IQR in parentheses [nonnormally distributed variable (all such values)]. 
6 Mean ± SD (all such values).

Effects of intervention with vitamin D3 in groups with low compared with high calcium intakes: secondary analysis of serum 25(OH)D

A secondary analysis of data in which subjects with low calcium intakes [ie, with the cutoff set at <530 mg/d (mean ± SD: 391 ± 136 mg/d); n = 32] were compared with subjects with high calcium intake [ie, with the cutoff set at >1000 mg/d but which was achieved from habitual intake alone and without any additional calcium needed in the study period (mean ± SD: 1376 ± 371 mg/d); n = 31] showed that there was no significant (P > 0.2) time × vitamin D treatment × calcium intake grouping interaction effect on the mean serum 25(OH)D concentration over the 15-wk intervention period.

Effects of intervention with vitamin D3 in groups with moderate-low and high calcium intakes stratified by adequacy of the baseline serum 25(OH)D concentration: secondary analysis of serum 25(OH)D

An additional secondary analysis of data was performed to see whether the adequacy of baseline serum 25(OH)D concentrations influenced the main outcome. All subjects were stratified on the basis of baseline serum 25(OH)D concentrations (<39.9 and ≥40 nmol/L, which were cutoffs suggested by the IOM as EAR-like concentrations). Mean (±SD) baseline serum 25(OH)D of resulting subgroups was 31.1 ± 4.6 nmol/L (n = 30) and 62.8 ± 17.2 nmol/L (n = 92) for <40 and >40 nmol/L, respectively. A repeated-measures ANOVA showed that there was no significant (P > 0.2 for both) time × vitamin D treatment × calcium intake grouping interaction effect on the mean serum 25(OH)D concentration over the 15-wk intervention period in either subgroup (data not shown).

DISCUSSION

Recent dietary requirement estimates for vitamin D from both sides of the Atlantic have prioritized wintertime as a critical...
compared with

centrationsequate vitamin D intake in maintaining serum 25(OH)D concentrations above chosen cutoffs (9, 40, 41). The IOM, while setting the most recent DRI for vitamin D, highlighted the uncertainty and gaps in available data about the influence of calcium intake on the regulation of vitamin D activation and catabolism (9). Thus, although, as per convention, the vitamin D DRI was established by the IOM on the assumption that the requirement for dietary calcium was being met (9), an inadequacy in calcium intake could cause changes in the efficient handling of or physiologic response to vitamin D that might not otherwise be present. A significant portion of adult populations in Europe and North America fail to meet respective dietary calcium requirements (9, 21, 42). The 2010 Dietary Guidelines Advisory Committee identified 4 nutrients of public health concern with calcium being one of them. Thus, a clarification of whether low intakes of calcium increase the dietary requirement for vitamin D is important from a public health perspective and also in devising preventative strategies for vitamin D deficiency. With the use of a 15-wk winter-based vitamin D intervention study in 125 apparently healthy, free-living, white adults aged =50 y at a latitude of 51°N, we examined potential interactions of dietary calcium intake on both the decline of serum 25(OH)D over the winter while having a habitual inadequate vitamin D intake as well as on the response of serum 25(OH)D to an intake aimed at achieving at least the IOM suggested RDA-like 25(OH)D concentration of 50 nmol/L (9). We showed that responses in serum 25(OH)D (bound and free) concentrations throughout winter, as well as indexes of vitamin D activation and catabolism as potential explanatory variables, were similar in older adults irrespective of whether they were had relatively low or high habitual calcium intakes.

These data suggest that recently proposed dietary requirement estimates for vitamin D (9, 40, 41, 43) will ensure the adequacy of serum 25(OH)D concentrations in older adults even when calcium intakes of these adults are in the inadequate range (<700 mg/d, and possibly even <550 mg/d). The winter-based, 2 × 2 factorial design of the current vitamin D intervention study differed from the designs of the limited number of previous RCTs in this area (16–18). The design allowed us to assess the possible interaction effect of 2 different nutritionally and public health–relevant calcium intake bands (ie, <550 and <700 compared with >1000 mg/d) on the natural decline in serum 25(OH)D concentrations that are a result of absent or markedly diminished UVB sunlight as well as on the potential of an adequate vitamin D intake in maintaining serum 25(OH)D concentrations ≥50 nmol/L (6) during winter. Berlin and Bjorkhem (16) reported that the supplementation of healthy young adult men (n = 14/group) with 2000 mg Ca/d in addition to the usual diet for 6–7 wk during late autumn and early winter in Sweden led to a significant increase in serum 25(OH)D concentrations (by 30%) relative to that in a control group who consumed a normal diet. Unfortunately, the habitual dietary intake of calcium was not assessed in the study, but the authors worked on the assumption that it was ≈800 mg/d (16). Although none of the subjects received vitamin D supplementation, serum 25(OH)D concentrations increased in both calcium and control groups over the 6–7-wk study period (increasing from a mean of 67–71 nmol/L in the control group) that was from late October to early December (16). Of note, the authors suggested that their unpublished data on an identical trial that was conducted from February to March still suggested an elevatory effect of additional calcium on serum 25(OH)D [mean decrease in serum 25(OH)D of 21% compared with 9% in control and calcium-supplemented groups, respectively; P < 0.002 (actual data not provided)]. McCullough et al (18) also performed a randomized, 2 × 2 factorial design, pilot intervention study with vitamin D and calcium supplements in US-based (42°N) previous adenomatous colonic polyp patients (aged 30–74 y; 70% of subjects were men) as part of a chemoprevention trial of biomarkers of risk of colorectal adenoma. Four groups of patients, with group mean habitual calcium intakes in the range from 625 to 753 mg/d were randomly assigned to receive daily either a placebo, additional 2000 mg Ca, additional 20 μg vitamin D3, or additional 2000 mg Ca plus 20 μg vitamin D3 for 6 mo (n = 22/23 per group) (18). The additional calcium did not influence the response of serum 25(OH)D to vitamin D supplementation. Baseline blood sampling occurred throughout the year, with only 26% during winter. Consequently, there was no significant reduction in mean serum 25(OH)D concentrations over the intervention period in the 2 groups who were not randomly assigned to receive vitamin D supplements, whereas there were expected significant increases in mean serum 25(OH)D in the 2 vitamin D–supplemented groups. Goussous et al (17) performed an RCT in which healthy older men and women (aged ≥50 y; 74% women on average; mean habitual calcium intake: 577 mg/d) were randomly assigned to receive calcium supplements (1000 mg/d) or a placebo (n = 23/29 per group) for 90 d throughout winter. However, because all participants received vitamin D3 (20 μg/d) over the intervention period, neither group had a decline in mean winter serum 25(OH)D concentrations but, instead, had the expected increase. The response of serum 25(OH)D to vitamin D3 supplementation did not differ between calcium and placebo groups (17).

The IOM used data from winter-based RCTs with vitamin D alone to establish the DRI for vitamin D (9). This method was used because of the concern that altering calcium intake as well as vitamin D intake in RCTs may affect serum 25(OH)D concentrations because of the altered regulation of vitamin D activation and catabolism. All calcium-supplemented subjects in the 3 RCTs previously mentioned (16–18) had increases in their usual calcium intake of the order of an additional 1000–2000 mg Ca/d, which likely brought their total mean calcium intakes into the range from >1500 to >2700 mg/d. This perturbation in calcium intake alone may have altered calcitropic hormone concentrations from the baseline to endpoint. One study showed a significant reduction in serum 1,25(OH)2D (but not PTH) with calcium supplementation (16), whereas 1,25(OH)2D (17, 18) and, surprisingly, PTH (17) were unaffected by calcium supplementation in the other studies. Responses of these hormones to interventions have been variable (17). In the current study, we tried to minimize the perturbation of usual calcium intake and possible knock-on effects on calcitropic hormones by stratifying subjects at recruitment by habitual calcium intake, and only in subjects (n = 38) who fell short of the cutoff for high calcium intake (>1000 mg/d) did we add supplemental calcium to meet this target [started 4 wk before baseline to facilitate normalization of calcitropic hormone concentrations (28)]. In the comparison of subjects with <550 and >1000 mg Ca/d, there was no perturbation because these were all habitual intakes of calcium. Thus, declines and increases in serum 25(OH)D over the winter, which were associated with inadequate and adequate

<table>
<thead>
<tr>
<th>Page</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>CASHMAN ET AL</td>
</tr>
</tbody>
</table>

---

**Footnotes:**

1. McCullough et al (18) performed a randomized, 2 × 2 factorial design, pilot intervention study with vitamin D and calcium supplements in US-based (42°N) previous adenomatous colonic polyp patients (aged 30–74 y; 70% of subjects were men) as part of a chemoprevention trial of biomarkers of risk of colorectal adenoma. Four groups of patients, with group mean habitual calcium intakes in the range from 625 to 753 mg/d were randomly assigned to receive daily either a placebo, additional 2000 mg Ca, additional 20 μg vitamin D3, or additional 2000 mg Ca plus 20 μg vitamin D3 for 6 mo (n = 22/23 per group) (18). The additional calcium did not influence the response of serum 25(OH)D to vitamin D supplementation. Baseline blood sampling occurred throughout the year, with only 26% during winter. Consequently, there was no significant reduction in mean serum 25(OH)D concentrations over the intervention period in the 2 groups who were not randomly assigned to receive vitamin D supplements, whereas there were expected significant increases in mean serum 25(OH)D in the 2 vitamin D–supplemented groups. Goussous et al (17) performed an RCT in which healthy older men and women (aged ≥50 y; 74% women on average; mean habitual calcium intake: 577 mg/d) were randomly assigned to receive calcium supplements (1000 mg/d) or a placebo (n = 23/29 per group) for 90 d throughout winter. However, because all participants received vitamin D3 (20 μg/d) over the intervention period, neither group had a decline in mean winter serum 25(OH)D concentrations but, instead, had the expected increase. The response of serum 25(OH)D to vitamin D3 supplementation did not differ between calcium and placebo groups (17).

2. The IOM used data from winter-based RCTs with vitamin D alone to establish the DRI for vitamin D (9). This method was used because of the concern that altering calcium intake as well as vitamin D intake in RCTs may affect serum 25(OH)D concentrations because of the altered regulation of vitamin D activation and catabolism. All calcium-supplemented subjects in the 3 RCTs previously mentioned (16–18) had increases in their usual calcium intake of the order of an additional 1000–2000 mg Ca/d, which likely brought their total mean calcium intakes into the range from >1500 to >2700 mg/d. This perturbation in calcium intake alone may have altered calcitropic hormone concentrations from the baseline to endpoint. One study showed a significant reduction in serum 1,25(OH)2D (but not PTH) with calcium supplementation (16), whereas 1,25(OH)2D (17, 18) and, surprisingly, PTH (17) were unaffected by calcium supplementation in the other studies. Responses of these hormones to interventions have been variable (17). In the current study, we tried to minimize the perturbation of usual calcium intake and possible knock-on effects on calcitropic hormones by stratifying subjects at recruitment by habitual calcium intake, and only in subjects (n = 38) who fell short of the cutoff for high calcium intake (>1000 mg/d) did we add supplemental calcium to meet this target [started 4 wk before baseline to facilitate normalization of calcitropic hormone concentrations (28)]. In the comparison of subjects with <550 and >1000 mg Ca/d, there was no perturbation because these were all habitual intakes of calcium. Thus, declines and increases in serum 25(OH)D over the winter, which were associated with inadequate and adequate
dietary vitamin D, respectively, were compared in subjects with their habitual low calcium intakes and their habitual, or as close as, high calcium intakes.

With the use of the 24,25(OH)2D concentration and ratio of 24,25(OH)2D:25(OH)D as markers for vitamin D catabolism (4, 19, 38), the lack of effect of the concentration of habitual intake of calcium on the response of both variables during the current study was in keeping with the main findings of a lack of interaction between calcium and vitamin D on the response of serum 25(OH)D. In contrast, supplementation with vitamin D3 significantly increased the serum 24,25(OH)2D concentration and ratio of 24,25(OH)2D:25(OH)D as well as serum 25(OH)D, which suggested the induction of the catabolic pathway via increased 24-hydroxylase activity. Serum 1,25(OH)2D has been suggested to be a key regulator of catabolism of 25(OH)D (3, 7), whereas it appears less clear whether PTH itself has an independent effect on the catabolism of 25(OH)D (3). Vitamin D3 supplementation during winter had no effect on serum PTH in the current study but significantly increased serum 1,25(OH)2D concentrations. Although serum 1,25(OH)2D was not increased by vitamin D supplementation in some RCTs (17, 18), it has been increased in other studies (44). The serum 24,25(OH)2D concentration and ratio of 24,25(OH)2D:25(OH)D were significantly reduced during winter in placebo groups, which suggested a possible 24-hydroxylase–mediated sparing effect aimed at maintaining serum 25(OH)D even though concentrations, as expected, decreased during the winter. Although serum PTH was significantly increased over the winter time in placebo groups, serum 1,25(OH)2D was unchanged.

Although the current study did not include an entire group of subjects with extremely low calcium intakes, in whom an effect on serum 25(OH)D catabolism might have occurred and, thus, may have been a potential limitation of the study, 50% of subjects in the moderate-low calcium intake group had intakes <550 mg Ca/d, 25% of subjects had intakes <400 mg Ca/d, and all subjects had intakes <700 mg Ca/d. Within the population, only 3% and 13% of Irish adults have calcium intakes <400 and <550 mg Ca/d, respectively (21).

In conclusion, the findings of the current study suggest that responses in serum 25(OH)D (both bound and free) concentrations throughout winter as well as indexes of vitamin D activation and catabolism were similar in older adults irrespective of whether they had relatively low or high habitual calcium intakes. Thus, recent dietary vitamin D–requirement estimates will cover the vitamin D needs of even those individuals who do not have adequate calcium intakes.

The authors’ responsibilities were as follows—KMS, M Kiely, and KDC: were involved in the conception of the work and are grant holders; KMS and KDC: contributed to the study design; KMS, AH, SMO, and KDC: contributed to the execution of the study; AH, JYZ, M Kinsella, and KG: contributed to the sample analysis; and all authors: contributed to the data analysis and writing of the manuscript. None of the authors had a conflict of interest.

REFERENCES


