Relative effectiveness of oral 25-hydroxyvitamin D₃ and vitamin D₃ in raising wintertime serum 25-hydroxyvitamin D in older adults¹–⁴

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ABSTRACT
Background: The relative potency of 25-hydroxyvitamin D₃ to vitamin D₃ needs to be better defined so that food-composition tables can better reflect the true vitamin D nutritive value of certain foods.

Objective: We performed a randomized, controlled intervention study in apparently healthy, free-living adults to investigate whether the intake of 25-hydroxyvitamin D₃ is 5 times more potent in raising serum 25-hydroxyvitamin D [25(OH)D] during winter compared with an equivalent amount of vitamin D₃.

Design: A randomized, placebo-controlled, double-blind intervention study was conducted in adults aged ≥50 y (n = 56) who consumed a placebo, 20 μg vitamin D₃, or 7 or 20 μg 25-hydroxyvitamin D₃ daily throughout 10 wk of winter. Serum 25(OH)D was measured by using an enzyme-linked immunoassay, and serum albumin–corrected calcium (S-Ca) was assessed colorimetrically at the baseline, midpoint, and endpoint of the study.

Results: The mean (±SD) increases (per microgram of vitamin D compound) in serum 25(OH)D concentrations over baseline after 10 wk of supplementation were 0.96 ± 0.62, 4.02 ± 1.27, and 4.77 ± 1.04 nmol·L⁻¹·μg intake⁻¹ for the 20-μg vitamin D₃/d and 7- and 20-μg 25-hydroxyvitamin D₃/d groups, respectively. A comparison of the 7- and 20-μg 25-hydroxyvitamin D₃/d groups with the 20-μg vitamin D₃/d group yielded conversion factors of 4.2 and 5, respectively. There was no effect of treatment on S-Ca concentrations and no incidence of hypercalcemia (S-Ca >2.6 nmol/L).

Conclusions: Each microgram of orally consumed 25-hydroxyvitamin D₃ was about 5 times more effective in raising serum 25(OH)D in older adults in winter than an equivalent amount of vitamin D₃. This conversion factor could be used in food-compositional tables for relevant foods. This study was registered at clinicaltrials.gov as NCT01398202.

INTRODUCTION
For individuals aged ≥1 y, an Estimated Average Requirement and Recommended Dietary Allowance of 400 and 600 IU vitamin D/d (800 IU vitamin D/d for individuals >70 y of age), respectively, has been established in North America (1). The mean daily intake (MDI)⁵ in the range of 40–428 IU vitamin D/d has been reported for children, teenagers, and adults in North America (1, 2) and Europe (3). Clearly, there is a need for sustainable food-based strategies to bridge the gap between current and recommended intakes of vitamin D to minimize the prevalence of vitamin D deficiency, but the development of such strategies requires, in the first instance, the ability to accurately assess vitamin D intakes in the population, including the main food contributors to the MDI of vitamin D.

In countries where the fortification of food with vitamin D is not common, it has been suggested that meat could be a significant source of vitamin D (4). Data on vitamin D intakes from 2 successive national nutrition surveys in Ireland over the past 14 y suggested that 30%, 12–14%, and 9% of the MDI of vitamin D in 18–64 y-olds comes from meat and meat products, fish and fish products, and egg and egg-dish food groupings, respectively (4, 5). Likewise, these 3 food groupings collectively make a significant contribution (55.5%) to the MDI of vitamin D in adults in the United Kingdom (6). Even in countries where fortification with vitamin D is common, the consumption of meat and related products may be important in certain ethnic groups. For example, Van Horn et al (7) recently showed that, in US adolescent girls in the National Heart, Lung and Blood Institute Growth and Health Study, the meat and bean food group contributed ~4% and 26% to the MDI of vitamin D in white and African American girls, respectively. Some of the contributions of these food groups to vitamin D nutriture are due to the fact that 25-hydroxyvitamin D₃, which is one of the major metabolites of vitamin D, is present in meat, fish, and eggs (8–11). Moreover, it is assumed in the United Kingdom (and Danish and Swiss) food-composition databases that the 25-hydroxyvitamin D₃ present in these foods has 5 times the activity of vitamin D₃ (12–14), which is accounted for when their total vitamin D content is estimated. However, Ovesen et al (15) pointed out that there is, as yet, no consensus on the conversion factor that should be used for 25-hydroxyvitamin D₃

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⁵ Abbreviations used: MDI, mean daily intake; PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D.

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to calculate vitamin D activity, and estimates of the factor that arose from studies in vitamin D–deficient rats, which were performed >35–45 y ago, varied from 1.5 to 5 (16–19). More recently, Jakobsen et al (20) showed that plasma 25-hydroxyvitamin D [25(OH)D] concentrations were similar in pigs fed an equal amount of vitamin D3 and the metabolite for 11 wk.

Priority needs to be given to data from human studies that have experimentally examined the relative potency of 25-hydroxyvitamin D3 to vitamin D3; however, such data have been scant. Conversion-factor estimates of ≈1.4 and 10 arose from 2 intervention studies in patients who required vitamin D treatment (21, 22); however, caution is needed because both studies had design characteristics that may have affected these estimates. In contrast, Bischoff-Ferrari et al (23) very recently showed that healthy postmenopausal women (n = 10 women per group) who were supplemented with 20 μg 25-hydroxyvitamin D3/d had an ≈3-fold greater increase in plasma 25(OH)D after 16 wk than did women supplemented with 20 μg vitamin D3/d. Clearly, additional research is needed to better define the conversion factor so that food-composition tables can better reflect the true vitamin D nutritive value of meats, fish, and eggs.

The aim of this study was to perform a randomized, controlled intervention study in apparently healthy, free-living adults (aged ≥50 y) to investigate whether the intake of 25-hydroxyvitamin D3 is 5 times more potent in raising serum 25(OH)D concentrations during winter than an equivalent amount of vitamin D3.

SUBJECTS AND METHODS

Subjects

A total of 58 apparently healthy, free-living adults aged ≥50 y were recruited in this 10-wk vitamin D3 and 25-hydroxyvitamin D3 intervention trial (www.clinicaltrials.gov; NCT01398202). Subjects were recruited in the Cork area through the use of advertisements placed around University College Cork as well as across the location. Because we predicted that it would be more difficult to recruit men than women, we aimed to recruit a ratio of ≈55:45 women to men. 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 8 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 10-wk intervention) to a location at which 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 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 in accordance with the Helsinki declaration.

Design and conduct of study

This was a double-blind, placebo-controlled trial in which older adults were randomly assigned to receive a capsule that contained a placebo, 20 μg vitamin D3, or 7 or 20 μg 25-hydroxyvitamin D3 daily for 10 wk. Data from Rossini et al (22) and Jetter et al (24), which was a preliminary publication of the Bischoff-Ferrari et al (23) study, that were available at the time the current study was being planned suggested that the relative potency of 25-hydroxyvitamin D3 and vitamin D3 in raising serum 25(OH)D was ≈1 and ≈3, respectively. Therefore, the doses of 20 and 7 μg 25-hydroxyvitamin D3 and 20 μg vitamin D3 were included in the current study to test the possibility of a conversion factor of 1 and 3, respectively. Random assignment of subjects was centralized and computer generated and accounted for sex. The manufactured crystalline 25-hydroxyvitamin D3 and vitamin D3, both of which were identical spray-dried powders stabilized with DL-α-tocopherol, were supplied by DSM Nutritional Products Ltd, and vitamin D3 and 25-hydroxyvitamin D3 capsules and matching placebo capsules were produced by Fisher Clinical Services GmbH. The placebo capsules and active (20 μg vitamin D3 and 7 or 20 μg 25-hydroxyvitamin D3) capsules were identical in appearance and taste. The vitamin D3 or 25-hydroxyvitamin D3 content of the capsules was confirmed by in-house laboratory analysis within DSM Nutritional Products Ltd. Compliance was assessed by capsule counting. An a priori decision was made to include only those subjects who exceeded 80% compliance. The allocation remained concealed until the final analyses, and all outcomes were reported by people who were masked to the allocation scheme.

The study was carried out in Cork, Republic of Ireland (latitude 51° N). All subjects were recruited and commenced the intervention study between 17 and 28 January 2011 and finished 10 wk later between 21 March and 8 April 2011, during which period of time vitamin D status would be expected to decline to a nadir (25). During the intervention phase, each participant was met by researchers on 3 occasions at the study center, once each at the baseline (week 0), midpoint (week 5), and endpoint (week 10) 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 venepuncture 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 (26). The habitual intake of calcium and vitamin D was estimated by using a validated food-frequency questionnaire (27, 28) that was administered by a research nutritionist, and 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 completion of the study protocol.

Laboratory analysis

Serum 25(OH)D

The 25(OH)D concentrations were measured at University College Cork in all serum samples by using an ELISA (OCTEIA 25-Hydroxy Vitamin D; Immuno Diagnostic Systems Ltd). The intraassay and interassay CVs for the ELISA method were 5.9% and 6.6%, respectively. This ELISA assay is used for the quantitative determination of serum and plasma 25(OH)D, additional details of which have been described previously (29). The quality and accuracy of serum 25(OH)D analysis in our
laboratory are assured on an ongoing basis by participation in the Vitamin D External Quality Assessment Scheme (Charing Cross Hospital). A comparison of the performance of our ELISA assay with that of our liquid chromatography–mass spectroscopy method in relation to frozen Vitamin D External Quality Assessment Scheme samples from the first 3 quarters of 2011 (n = 15) showed a high correlation (ELISA = 1.0301 × liquid chromatography–mass spectroscopy + 0.1224; r = 0.992) and a very good agreement by using a Bland-Altman difference plot.

**Serum intact parathyroid hormone**

Serum parathyroid hormone (PTH) concentrations were measured at University College Cork in all serum samples by using an ELISA (intact PTH; MD Biosciences Inc). The 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.

**Statistical analysis**

As mentioned previously, recent estimates of the conversion factor between vitamin D3 and 25-hydroxyvitamin D3 in humans in terms of elevating serum 25(OH)D range from 1.4 to 3.2 (22, 24). Therefore, we used a 40% increase as the lowest potential increment that we would need to be able to detect between groups treated with 20 µg vitamin D3/d and 20 µg 25-hydroxyvitamin D3/d. On the basis of the distribution of wintertime serum 25(OH)D data from older adults in our previous study (30), a study design, whereby 12 volunteers per group were recruited (which included 20% to cover potential dropouts), had 90% power to detect this minimum of a 40% increase in serum 25(OH)D between groups at α = 0.5.

Statistical analysis of the data were conducted with SPSS for Windows (version 16.0; SPSS Inc). Distributions of all variables were tested with Kolmogorov-Smirnov tests. Descriptive statistics (means ± SDs or medians and IQRs, when appropriate) were determined for all variables. Dietary vitamin D and serum PTH were not normally distributed and, thus, were log transformed to achieve near-normal distributions. Serum concentrations of 25(OH)D, albumin-corrected calcium, age, weight, height, or BMI at baseline in the 4 treatment groups (data not shown). There was no difference (P > 0.8) in the mean age, weight, height, or BMI at baseline in the 4 treatment groups (data not shown). Similarly, there was no significant difference in the proportion of men to women in the mean habitual dietary vitamin D or calcium intake or in mean preintervention serum 25(OH)D, PTH, or albumin-corrected calcium concentrations in treatment groups (Table 2).

There were no adverse events reported during the study. Of the 2 dropouts, one subject was from the 20-µg vitamin D3/d group and one from the 20-µg 25-hydroxyvitamin D3/d group. The dropouts during the intervention phase was for a reason of illness that was unrelated to the intervention or loss of interest, and in no instance was the dropout related to the intervention. One subject failed to exceed our minimum 80% compliance, and this subject was excluded from the main analysis. In the remaining subjects, there was good supplement adherence on the basis of the pill count [the overall median (IQR) compliance was 97.0% (92.0–100%), and compliance was similar in the 4 treatment groups; P = 0.9].

With the use of a compliance-based analysis (with the omission of one subject with compliance below our a priori defined 80%), there was a significant (P ≤ 0.0001) time × treatment interaction effect on the mean serum 25(OH)D concentration over the 10-wk intervention period. Within-group repeated-measures ANOVA showed that, in all 4 treatment groups, there was a significant change in serum 25(OH)D over time, with the vitamin D3 and 25-hydroxyvitamin D3 treatment groups exhibiting variable increases over time and the placebo group exhibiting only minor fluctuations (Table 2). Serum 25(OH)D concentrations in the vitamin D3 group increased significantly (by 29%) by midpoint (week 5) but did not significantly increase any further by week 10 (Table 2). Serum 25(OH)D concentrations in both 25-hydroxyvitamin D3 groups had increased significantly by week 5 but were further elevated by week 10 (Table 2). ANCOVA by intervention groups [adjusted for sex, age, dietary vitamin D, and baseline 25(OH)D in the case of week-5 and -10 analyses] showed that there was no significant difference in mean serum 25(OH)D concentrations between the 4 groups at baseline, but compared with the placebo group, the concentrations were significantly higher in all 3 active treatment
groups by weeks 5 and 10 (Table 2). At weeks 5 and 10, relative to the placebo group, the increment in serum 25(OH)D achieved with 20 μg 25-hydroxyvitamin D₃/d was significantly higher than that with either the 20-μg vitamin D₃ or 7-μg 25-hydroxyvitamin D₃/d groups, and the latter 2 groups had similar responses of serum 25(OH)D (Table 2).

There was no significant interaction between treatment and sex (P > 0.8) in the response of serum 25(OH)D to the intervention (data not shown). Similarly, there was no significant interaction with BMI (in kg/m²) < =30 (P > 0.9) or baseline 25(OH)D < =40 nmol/L (P > 0.3).

There was no significant (P > 0.9) time × treatment interaction effect or treatment effect (P > 0.8) on mean serum albumin–corrected calcium concentrations over the 10-wk intervention period. There was a significant time effect (P < 0.0001) with a minor (2.9%) increase in serum albumin–corrected calcium concentrations at midpoint (week 5) compared with at baseline and that had returned to baseline concentrations again by the endpoint (week 10) (Table 2). None of the subjects exhibited hypercalcemia (serum albumin–corrected calcium concentrations >2.6 nmol/L).

There was a significant (P ≤ 0.001) time × treatment interaction effect on the mean serum PTH concentration over the 10-wk intervention period. ANCOVA by intervention groups (adjusted for sex, age, dietary calcium, and baseline PTH in the case of week-5 and -10 analyses) showed that, although there was no significant difference in the mean serum PTH concentration between the 4 groups at baseline, there were significant differences between groups at weeks 5 and 10 (Table 2). Compared with the placebo group, the 7- and 20-μg/d 25-hydroxyvitamin D₃/d groups, but not the 20-μg vitamin D₃/d group, had significantly lower serum PTH concentrations at weeks 5 and 10. There was no significant difference between the 20-μg vitamin D₃/d group and the two 25-hydroxyvitamin D₃/d groups at weeks 5 and 10. Within-group repeated-measures ANOVA showed that, although there were no significant differences in serum PTH concentrations in the placebo and 20-μg vitamin D₃/d groups over the 10-wk period, there were significant differences in the two 25-hydroxyvitamin D₃ groups (Table 2).

In both the 7- and 20-μg 25-hydroxyvitamin D₃/d groups, serum PTH was significantly reduced at weeks 5 and 10 compared with baseline concentrations.

Inclusion of the one noncompliant subject in the 20-μg 25-hydroxyvitamin D₃/d group in an intention-to-treat analysis did not alter the statistical findings for serum 25(OH)D, PTH, or albumin-adjusted calcium (data not shown).

Relative effectiveness of vitamin D₃ and 25-hydroxyvitamin D₃ in raising serum 25(OH)D expressed per microgram of compound

The mean (±SD) increases (expressed per microgram of vitamin D compound) in serum 25(OH)D concentration over baseline after 10 wk of supplementation were 0.96 ± 0.62, 4.02 ± 1.27, and 4.77 ± 1.04 nmol · L⁻¹ · μg intake⁻¹ for the 20-μg vitamin D₃/d and 7- and 20-μg 25-hydroxyvitamin D₃/d groups, respectively. Thus, a comparison of the 20-μg 25-hydroxyvitamin D₃/d group with the 20-μg vitamin D₃/d group yielded a conversion factor of 5 (4.2 if the 7-μg...
TABLE 2
Habitual dietary intakes of vitamin D and calcium and serum 25(OH)D concentrations in treatment groups at baseline, midpoint, and endpoint of a 10-wk intervention in apparently healthy older adults

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Placebo</th>
<th>20 µg vitamin D&lt;sub&gt;D3/d&lt;/sub&gt;</th>
<th>7 µg 25-hydroxyvitamin D&lt;sub&gt;D3/d&lt;/sub&gt;</th>
<th>20 µg 25-hydroxyvitamin D&lt;sub&gt;D3/d&lt;/sub&gt;</th>
<th>ANCOVA by intervention group (P value)&lt;sup&gt;7&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>16</td>
<td>13</td>
<td>14</td>
<td>12</td>
<td>—</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>6:10</td>
<td>5:8</td>
<td>7:7</td>
<td>7:5</td>
<td>0.309</td>
</tr>
<tr>
<td>Dietary vitamin D (µg/d)</td>
<td>6.5 (2.9–7.9)&lt;sup&gt;6&lt;/sup&gt;</td>
<td>7.6 (2.9–5.4)</td>
<td>5.1 (2.8–6.6)</td>
<td>4.4 (3.7–6.1)</td>
<td>0.161</td>
</tr>
<tr>
<td>Dietary calcium (mg/d)</td>
<td>970 ± 503&lt;sup&gt;6&lt;/sup&gt;</td>
<td>1114 ± 494</td>
<td>1008 ± 415</td>
<td>794 ± 309</td>
<td>0.378</td>
</tr>
<tr>
<td>Serum 25(OH)D (mmol/L)&lt;sup&gt;6&lt;/sup&gt;</td>
<td>42.7 ± 12.6&lt;sup&gt;6&lt;/sup&gt;d</td>
<td>49.7 ± 16.2&lt;sup&gt;6&lt;/sup&gt;d</td>
<td>42.5 ± 8.9&lt;sup&gt;6&lt;/sup&gt;d</td>
<td>38.2 ± 9.9&lt;sup&gt;6&lt;/sup&gt;d</td>
<td>0.471</td>
</tr>
<tr>
<td>Before intervention</td>
<td>42.7 ± 11.1&lt;sup&gt;6&lt;/sup&gt;d</td>
<td>64.1 ± 9.5&lt;sup&gt;6&lt;/sup&gt;</td>
<td>60.8 ± 8.1&lt;sup&gt;6&lt;/sup&gt;e</td>
<td>98.1 ± 20.5&lt;sup&gt;6&lt;/sup&gt;e</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>After intervention</td>
<td>41.2 ± 11.1&lt;sup&gt;6&lt;/sup&gt;d</td>
<td>69.0 ± 8.7&lt;sup&gt;6&lt;/sup&gt;e</td>
<td>70.7 ± 9.9&lt;sup&gt;6&lt;/sup&gt;f</td>
<td>134.6 ± 26.0&lt;sup&gt;6&lt;/sup&gt;f</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ANOVA within group (P)&lt;sup&gt;7&lt;/sup&gt;</td>
<td>0.01</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>—</td>
</tr>
<tr>
<td>Serum calcium (mmol/L)&lt;sup&gt;6&lt;/sup&gt;</td>
<td>8.4 ± 0.2</td>
<td>8.3 ± 0.3</td>
<td>8.4 ± 0.2</td>
<td>8.4 ± 0.3</td>
<td>—</td>
</tr>
<tr>
<td>Before intervention</td>
<td>8.4 ± 0.2</td>
<td>8.2 ± 0.7</td>
<td>8.7 ± 0.2</td>
<td>8.7 ± 0.3</td>
<td>—</td>
</tr>
<tr>
<td>After intervention</td>
<td>8.5 ± 0.3</td>
<td>8.5 ± 0.2</td>
<td>8.5 ± 0.1</td>
<td>8.5 ± 0.3</td>
<td>—</td>
</tr>
<tr>
<td>Serum PTH (ng/mL)&lt;sup&gt;6&lt;/sup&gt;</td>
<td>65.6 (47.4–70.2)</td>
<td>47.3 (41.5–57.5)</td>
<td>58.6 (52.8–69.9)&lt;sup&gt;6&lt;/sup&gt;d</td>
<td>57.9 (42.5–73.5)&lt;sup&gt;6&lt;/sup&gt;d</td>
<td>0.339</td>
</tr>
<tr>
<td>Before intervention</td>
<td>66.4 (42.6–85.0)&lt;sup&gt;6&lt;/sup&gt;a</td>
<td>43.3 (39.8–53.3)&lt;sup&gt;6&lt;/sup&gt;b</td>
<td>49.0 (44.5–63.7)&lt;sup&gt;6&lt;/sup&gt;e</td>
<td>48.2 (39.1–66.6)&lt;sup&gt;6&lt;/sup&gt;e</td>
<td>0.013</td>
</tr>
<tr>
<td>After intervention</td>
<td>65.8 (54.5–87.8)&lt;sup&gt;6&lt;/sup&gt;a</td>
<td>44.2 (40.1–52.7)&lt;sup&gt;6&lt;/sup&gt;b</td>
<td>52.7 (41.1–62.7)&lt;sup&gt;6&lt;/sup&gt;e</td>
<td>40.5 (34.6–61.6)&lt;sup&gt;6&lt;/sup&gt;e</td>
<td>0.001</td>
</tr>
<tr>
<td>ANOVA within group (P)&lt;sup&gt;7&lt;/sup&gt;</td>
<td>0.099</td>
<td>0.578</td>
<td>0.010</td>
<td>0.0004</td>
<td>—</td>
</tr>
</tbody>
</table>

1 PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D.
2 Between-group differences explored (with appropriate adjustment for covariates such as age, sex, vitamin D or calcium intake, baseline levels of marker being tested). Different superscript letters represent significant differences in group means, P < 0.05 (Bonferroni-adjusted t test). ANOVA was used in the cases of dietary calcium and vitamin D, and the chi-square test was used in the case of sex.
3 Median (IQR) of nonnormally distributed variable (all such values).
4 Mean ± SD (all such values).
5 Repeated-measures ANOVA was used to test the treatment × time interaction [P < 0.0001 and P < 0.0001 for serum 25(OH)D and serum PTH, respectively]. For serum calcium, there was no significant treatment × time interaction (P > 0.9) or treatment effect (P > 0.8) but a significant (P < 0.0001) time effect, with mean values for week 5 that were significantly higher than for weeks 0 and 10.
6 All baseline blood samples were taken between 17 and 28 January 2011 and all endpoint blood samples were taken between 21 March and 8 April 2011.
7 Significant differences within each intervention group with time were investigated by ANOVA for repeat measures. Different superscript letters represent significant differences in group means, P < 0.05 (Bonferroni-adjusted t test).
8 Albumin corrected.

25-hydroxyvitamin D<sub>D3/d</sub> group was used for the comparison with the vitamin D<sub>3</sub> group). Inclusion of the one noncompliant subject in the 20-µg 25-hydroxyvitamin D<sub>3</sub> group in the analysis yielded a conversion factor of 4.9 compared with the 20-µg vitamin D<sub>3/d</sub> group.

DISCUSSION
Because there is ongoing uncertainty in the data that arose from experimental animal models and very limited human data in relation to the relative effectiveness of dietary 25-hydroxyvitamin D<sub>3</sub> and vitamin D<sub>3</sub>, we compared the effects of daily supplementation with 25-hydroxyvitamin D<sub>3</sub> and vitamin D<sub>3</sub> in raising serum 25(OH)D in late winter after a double-blind, randomized, controlled intervention study over 10 wk in 58 apparently healthy white subjects aged ≥50 y who were living at 51°N. We showed that, when expressed per microgram of vitamin D compound, daily supplementation with 25-hydroxyvitamin D<sub>3</sub> was between 4.2 and 5 times as effective as vitamin D<sub>3</sub> in raising wintertime serum 25(OH)D in older adults. These data largely support the choice of some food-composition databases (12–14) to apply a conversion factor of 5 for 25-hydroxyvitamin D<sub>3</sub> in meat and poultry and also may provide the basis for accounting for the contribution of 25-hydroxyvitamin D<sub>3</sub> in the total vitamin D activity of food in those food-composition databases for which future versions will include the content of the metabolite.

Because of variable study-design characteristics, a comparison of the conversion estimates of the current study with those of previous studies needs to be done cautiously. Our conversion estimates in older adult men and women were higher than those reported previously in older women (22, 24); however, there was no evidence of a sex effect on the response of serum 25(OH)D concentrations to treatment. In the study by Rossini et al (22), which was in osteopenic and osteoporotic women who were complicated by hypovitaminosis D, 25-hydroxyvitamin D<sub>3</sub> was administered weekly as drops (with a daily calcium supplementation), and vitamin D<sub>3</sub> in combination with calcium was administered daily over 12 mo, and the compliance of the women with these 2 treatments was assessed. From the reported serum 25(OH)D concentrations, we were able to estimate that 25-hydroxyvitamin D<sub>3</sub> is ~1.4-fold more potent than vitamin D<sub>3</sub>, but compliance with the daily supplementation regimen was poor because of intolerance to the calcium supplement. Barger-Lux et al (31) performed an open-label trial in which healthy young adult men (with a mean age of 28 y and a relatively good
baseline serum 25(OH)D of 67 nmol/L) received 1 of 3 doses of vitamin D₃, 25-hydroxyvitamin D₃, or 1,25-dihydroxyvitamin D₃ over 8, 4, and 2 wk, respectively, during the wintertime. Although the doses of 25-hydroxyvitamin D₃ (10, 20, 50 μg/d) and vitamin D₃ (25, 250, 1250 μg/d) were not comparable, the former treatment was provided for 4 wk and the latter treatment was provided for 8 wk, and the baseline serum 25(OH)D concentrations were higher than those in the current study, the expression of the reported mean changes (endpoint less baseline) in serum 25(OH)D per microgram of vitamin D compound for the 25-μg vitamin D₃/d of and 20-μg 25-hydroxyvitamin D₃/d groups suggested a conversion factor of 4.2. Data from the recent study by Bischoff-Ferrari et al (23), which was most comparable in design to that of the current study, showed that healthy postmenopausal women (aged 50–70 y) who were supplemented daily with 20 μg (or once weekly with 140 μg/d) 25-hydroxyvitamin D₃ (and for whom there was no significant difference, and thus, the groups were merged to yield an n = 10) had a 3.4-fold greater increase in plasma 25(OH)D after 16 wk than did subjects who were supplemented with an equivalent of 20 μg vitamin D₃/d (or once weekly with 140 μg vitamin D₃/d) over the same time frame. The study was conducted between March and July (E Stöcklin, personal communication, December 2011). The current study was conducted in wintertime to avoid the confounding influence of dermal synthesis of vitamin D₃ on exposure to summer UVB sunlight. The lack of an increase in serum 25(OH)D in the placebo group by week 10 provided assurance in this regard.

In the current study, serum 25(OH)D concentrations appeared to have reached, or had begun to reach, a plateau by week 5 in the group of subjects who received 20 μg vitamin D₃/d. This result is in line with findings that, after the initiation of vitamin D supplementation, serum 25(OH)D concentrations reached equilibrium after 6–8 wk in adult and elderly subjects (32, 33). In contrast, both groups who received 25-hydroxyvitamin D₃ had additional elevated serum 25(OH)D concentrations from weeks 5 to 10. Although it is possible that serum 25(OH)D concentrations may have been further elevated beyond week 10, which would have increased the estimate of the relative effectiveness of 25-hydroxyvitamin D₃ to vitamin D₃ even beyond 4.2–5, the plasma 25(OH)D concentration in the group who received 20 μg metabolite/d in the study by Bischoff-Ferrari et al (23) appeared to plateau by week 11. It is also possible that the timing of the plateau in serum 25(OH)D in the current study may have been different between the 2 groups who received 25-hydroxyvitamin D₃, which might be one explanation for the lower estimate of conversion factor (4.2) that arose from the 7-μg metabolite/d group compared with that (5) in the 20-μg metabolite/d group.

The US 2010 Dietary Guidelines for Americans identified the following 4 nutrients of public concern: vitamin D, dietary fiber, calcium, and potassium (34). Recent data on the source of usual nutrient intakes in the United States show that, in individuals >2 y of age, the median intake of vitamin D from naturally occurring food sources is only 1.7 μg/d (35). However, the US food-composition database does not currently include 25-hydroxyvitamin D₃ data, and thus, vitamin D intakes from meat, fish, and poultry may be underestimated. Data on 25-hydroxyvitamin D₃ will be included in future releases of the US Nutrient Database for Standard Reference (36), and thus, the use of a conversion factor for 25-hydroxyvitamin D₃ in such foods is important in the derivation of the total vitamin D activity. Fulgoni et al (35) have also highlighted the importance of vitamin D fortification or supplementation to usual intakes in the US population. The percentage of the population who did not reach the Estimated Average Requirement fell from 100% to 93.3% and 69.5% when vitamin D from naturally occurring food sources, plus enriched/fortified foods, and plus dietary supplements were accounted for, respectively. Flynn et al (3) has shown that fortified foods, and even nutritional supplements in many cases, do not significantly contribute to high intakes for vitamin D in several European countries. Bischoff-Ferrari et al (23) reported that women supplemented with the metabolite had a 2.8-fold increased odds of maintained or improved lower extremity function, a 5.7-mm Hg decrease in systolic blood pressure, and a more pronounced decrease in several markers of innate immunity, although the trial was small and intended to provide pilot data. Additional research is needed to investigate the potential of 25-hydroxyvitamin D₃ as a vitamin D supplement or a potential fortificant for health purposes in the healthy general public.

Despite significant increases in serum 25(OH)D concentrations in older adults in the current study and in the study of Bischoff-Ferrari et al (23) on supplementation with vitamin D₃ or 25-hydroxyvitamin D₃, there was no case of hypercalcaemia. Most notable was the lack of an alteration in serum calcium concentrations in the group who received 20 μg 25-hydroxyvitamin D₃/d, in whom a mean serum 25(OH)D concentration of 134.6 nmol/L was achieved (and 92% of subjects had serum 25(OH)D concentrations >100 nmol/L at the endpoint of the study).

In conclusion, each microgram of orally consumed 25-hydroxyvitamin D₃ was 4.2 to 5 times more effective in raising serum 25(OH)D in older adults in winter than was an equivalent amount of vitamin D₃. Additional research is needed to verify that the bioavailability of 25-hydroxyvitamin D₃ from meat and other foods is comparable to that from supplements. However, while we await such data, a conversion factor of 5 could be used in conjunction with expected new food-composition data in relation to 25-hydroxyvitamin D₃ to better account for the total vitamin D activity of certain food groups.

The authors’ responsibilities were as follows—MK and KDC: were involved in the conception of the study; TRH, ES, PW, and KDC: contributed to the study design; TRH, AYL, KS, MK, and KDC: contributed to the execution of the study; ES: was the study coordinator within DSM Nutritional Products Ltd and organized the manufacture and analysis of the capsules; TRH and AYL: contributed to sample analyses; and all authors: contributed to the execution of the study. DSM Nutritional Products Ltd and organized the manufacture and analysis of the capsules; TRH and AYL: contributed to sample analyses; and all authors: contributed to the data analysis and writing of the manuscript. DSM Nutritional Products Ltd is a supplier of vitamins, carotenoids, and other fine chemicals to the feed, food, pharmaceutical, and personal care industries. None of the authors had a conflict of interest.

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