Commentary

The case against ergocalciferol (vitamin D$_2$) as a vitamin supplement

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ABSTRACT

Supplemental vitamin D is available in 2 distinct forms: ergocalciferol (vitamin D$_2$) and cholecalciferol (vitamin D$_3$). Pharmacopoeias have officially regarded these 2 forms as equivalent and interchangeable, yet this presumption of equivalence is based on studies of rickets prevention in infants conducted 70 y ago. The emergence of 25-hydroxyvitamin D as a measure of vitamin D status provides an objective, quantitative measure of the biological response to vitamin D administration. As a result, vitamin D$_3$ has proven to be the more potent form of vitamin D in all primate species, including humans. Despite an emerging body of evidence suggesting several plausible explanations for the greater bioefficacy of vitamin D$_3$, the form of vitamin D used in major preparations of prescriptions in North America is vitamin D$_2$. The case that vitamin D$_2$ should no longer be considered equivalent to vitamin D$_3$ is based on differences in their efficacy at raising serum 25-hydroxyvitamin D, diminished binding of vitamin D$_2$ metabolites to vitamin D binding protein in plasma, and a nonphysiologic metabolism and shorter shelf life of vitamin D$_2$. Vitamin D$_2$, or ergocalciferol, should not be regarded as a nutrient suitable for supplementation or fortification.

INTRODUCTION

Vitamin D is available in 2 distinct forms, ergocalciferol (vitamin D$_2$) and cholecalciferol (vitamin D$_3$). These are officially regarded as equivalent and interchangeable (1–3). Although sunshine exposure and fish consumption provide vitamin D in the form of D$_3$, a different bioactive, plant-derived form of vitamin D, named vitamin D$_2$, was produced in the early 1920s through ultraviolet exposure of foods. This process was patented and licensed to pharmaceutical companies, which led to the development of a medicinal preparation of vitamin D$_2$ called Viosterol (4). Because antirachitic bioassays were used to establish “rat units” for vitamin D (ie, the amount of vitamin D required for recalcification of the epiphyseal end of tibiae in rats), early workers found it extremely difficult to distinguish between the specific biological value of the 2 forms.

To this day, the major preparations of vitamin D for prescription use in North America are in the form of vitamin D$_2$, not vitamin D$_3$. Multivitamins may contain either vitamin D$_2$ or vitamin D$_3$, but most companies are now reformulating their products to contain vitamin D in the D$_3$ form. Here, we present the case that vitamin D$_2$ should no longer be considered equivalent to vitamin D$_3$ and that vitamin D$_2$, or ergocalciferol, should not be regarded as a nutrient suitable for supplementation or fortification.

INCORRECT PRESUMPTION OF VITAMIN D$_2$ AND D$_3$ EQUIVALENCE

Assumptions about the equivalency of the 2 forms of vitamin D were questioned shortly after the discovery of vitamin D$_2$. As early as 1930, Hess et al (5) suggested that the activity of cod liver oil (vitamin D$_3$) and Viosterol (vitamin D$_2$) used in the treatment of rickets may have different biologic values. They found that one unit of cod liver oil could be as effective in preventing rickets as 4 units of Viosterol. Over the next 10 y, more than 40 studies were conducted to determine whether the 2 distinct forms of vitamin D were equally effective. The results from these studies were confusing, and, in 1940, Park (6) noted that the work done was of poor quality, making a comparison of the 2 forms exceedingly difficult. Despite these misgivings, Park stated that any effect due to differences between the 2 forms would be minimal and concluded that, “For practical purposes, the vitamin D in Viosterol (vitamin D$_2$) may be regarded as being equal to the vitamin D of cod liver oil (vitamin D$_3$)” (6). As a result, the World Health Organization recommended in 1949 that 1 IU vitamin D be equivalent to 25 ng crystalline vitamin D$_3$, and no distinction was made between vitamin D$_2$ and vitamin D$_3$ (7). Shortly thereafter in Germany in the 1950s, formulations of vitamin D$_2$ were found to be 4 times as potent per unit mass as were formulations of vitamin D$_3$. Vigantol oil (Merck KGaA, Darmstadt, Germany),...
the most popular vitamin D supplement in Europe, was reformu-
lated to replace its vitamin D2 content with vitamin D3 (8).

Sustained advancement in the characterization and metabo-
lism of vitamin D and its metabolites led to the proposed recom-
mendation in 1972 that 1 IU vitamin D be defined in moles or
molecules rather than in weight terms. Subsequently, both vita-
min D2 and vitamin D3 were defined to be 65 pmol, such that 1
IU vitamin D2 (molecular weight: 384) and vitamin D3 (molec-
ular weight: 396) would be equivalent to 25 ng and 25.78 ng,
respectively (9). Nevertheless, almost a half century later, British
and American pharmacopeias continue to generalize the 2 nu-
tritional forms of vitamin D with the simple conversion of gram
quantity, where 1 IU of either vitamin D2 or vitamin D3 equals
25 ng (1, 3).

Despite early evidence of differences in potency between the
2 vitamin D forms on a per weight basis, it must be highlighted
that the widely practiced addition of vitamin D2 to milk in the
United States and Europe in the 1930s served to successfully
eradicate rickets as a significant health problem. Additionally,
fortification of milk with either vitamin D2 or vitamin D3 to this
day has proven effective in the elimination of infantile rickets in
North America. To prevent infantile rickets, a minimal intake of
2.5 μg (100 IU) vitamin D/d in infants with little sun exposure
was shown to be efficacious (10). Thus, despite potential differ-
ces in the dose equivalence of vitamin D2 and D3, it is likely
that vitamin D3 is currently provided at a high enough dose per kg
infant body weight to maintain adequate bone mineral metabo-
lism. However, compared with the use of cruder markers (ie,
rickets or “units equivalence” of the bioassays shown by the older
rat data), the use of serum 25-hydroxyvitamin D [25(OH)D] as an
objective and quantitative marker of nutritional adequacy has
consistently shown specific differences in the biological re-
sponse of the 2 nutritional vitamin D forms.

The use of 25(OH)D as a biomarker in nonhuman species such
as birds showed vitamin D2 to be only one-tenth as effective as
vitamin D3 at increasing 25(OH)D (11). Likewise, in monkeys,
the concentrations of serum 25(OH)D maintained after intake of
vitamin D2 were 2–3-fold those maintained with comparable
amounts of vitamin D3 (12). In rats, however, vitamin D2 was
found to be more effective (13). These differences have been
largely explained on the basis of the relative binding affinity of
vitamin D and its metabolites to the plasma vitamin D binding
protein (DBP) (14, 15). The weaker binding affinity of vitamin
D3 metabolites to DBP would lead to a shorter circulating half-
life and an increased rate of clearance from circulation. Thus,
in the case of birds and monkeys, the 25(OH)D2 metabolite is likely
less able to compete for binding sites on DBP. This difference in
the binding ability is potentially explained by the presence of a
methyl group at carbon 24 on the D2 molecule (14).

In humans, vitamin D3 is more effective than vitamin D2 at
raising serum 25(OH)D concentrations. Although previous stud-
ies that compared the 2 versions of vitamin D indicated a greater
effect of vitamin D3 on raising 25(OH)D concentrations, evalu-
ation of potency was inconclusive due to the effects of confound-
ing variables (eg, seasonal solar exposure), insufficient sample
size, or both (16–18). In an effort to resolve the uncertainties of
earlier work, Trang et al (19) compared the ability of an equal
molar dose of vitamin D2 or D3 (∼100 μg or 4000 IU) to elevate
serum 25(OH)D over 2 wk between February and early May,
when vitamin D concentrations and solar exposure are minimal.

Both vitamin D3 and vitamin D1 increased serum 25(OH)D con-
centrations, yet the increase in 25(OH)D2 was found to be 70%
greater (1.70 times) with vitamin D3 than the increase obtained
with vitamin D2. When adjusted for concomitant changes in an
untreated group, the difference between the 2 groups was ∼2-
fold. To further complement these findings, a 3-mo supplemen-
tation study by Mastaglia et al (20) found that a dose of 250 μg
vitamin D2/d (2.5-fold) was needed to achieve similar serum
25(OH)D concentrations to those of the later study using a dose of
100 μg vitamin D3/d.

A comparison of the time course of serum 25(OH)D over a period
of 28 d after a single dose of either vitamin D2 or vitamin
D3 (2000 μg, or 50 000 IU, for both) was conducted by Armas et
al (21). Both forms of vitamin D produced similar rises in serum
25(OH)D concentration over the first 3 d, suggesting comparable
absorption of the 2 forms. In the vitamin D2–treated subjects,
serum 25(OH)D concentrations fell rapidly, reaching baseline
values by day 14. Interestingly, 25(OH)D concentrations then
continued to decline in this group and fell below baseline values
by day 28. In the D3–treated subjects, 25(OH)D continued to rise,
peaking by day 14 and remaining above baseline until at least day
28. A comparison of the areas under the curve (concentration
versus time) showed a >3-fold potency with vitamin D3. Clearly,
vitamin D3 would show efficacy in the treatment of severe vita-
min D deficiency; however, the authors note that 2000 μg
(50 000 IU) vitamin D2 should be considered equivalent to ≤375
μg (15 000 IU) vitamin D3, and likely closer to 125 μg (5000 IU)
vitamin D1 (21).

Several mechanisms could contribute to the greater capacity of
vitamin D3 to maintain higher 25(OH)D concentrations over
time. Supplementation of vitamin D3 produces appreciable
amounts of serum 25(OH)D2 (22), which, as previously men-
tioned, has a lower affinity for DBP and results in a shorter
circulating half-life than that of 25(OH)D3. Others have sug-
gested a higher affinity of hepatic 25-hydroxylase for vitamin
D3 than for vitamin D2 (23). In the liver, hepatic enzyme 25-
hydroxylase places a hydroxyl group in the 25 position of the
molecule, resulting in the formation of 25(OH)D. This reaction
is the initial step in the activation of vitamin D before its metabo-
lim in the kidney to its hormonally active form, 1,25(OH)2D.
In rats, vitamin D 25-hydroxylase has been shown to exist in liver
mitochondrial and microsomal fractions. In humans, previous
work has shown that mitochondrial vitamin D 25-hydroxylase
presides and converts vitamin D3 to 25(OH)D3, 5 times as fast as
it does vitamin D2 to form 25(OH)D2 (23). The human micro-
somal fraction also was shown to hydroxylate vitamin D3 to some
degree, but no detectable vitamin D 25-hydroxylation of vitamin
D2 was observed (23). However, studies have identified a key
microsomal liver enzyme (cytochrome P450, CYP2RI) in hu-
mans that appears able to 25-hydroxylate both vitamin D3 and
D2, whereas the mitochondrial enzyme (CYP27A1) only 25-
hydroxylates vitamin D3 (24, 25).

DIFERENT METABOLIC FATES OF VITAMINS D2
AND D3

It was initially thought that both vitamin D2 and vitamin D3
follow the same metabolic pathway. However, minor differences
in the chemistry of side chains between the 2 forms of vitamin D
result in differences in the site of hydroxylation and leads to the
production of unique biologically active metabolites (26).
After 25-hydroxylation, 25(OH)D and 1,25(OH)\textsubscript{2}D undergo additional 24-hydroxylation in the kidney to form 24,25(OH)\textsubscript{2}D and 1,24,25(OH)\textsubscript{3}D, respectively. The formation of 1,24,25(OH)\textsubscript{3}D\textsubscript{2} leads to deactivation of the vitamin D\textsubscript{2} molecule, whereas the analogous vitamin D\textsubscript{3} metabolite, 1,24,25(OH)\textsubscript{3}D, must undergo additional side-chain oxidation to be biologically deactivated (27). In fact, 1,24,25(OH)\textsubscript{3}D\textsubscript{2} has the ability to bind to the vitamin D receptor [VDR; \(\approx40\%\) more than 1,25(OH)\textsubscript{2}D\textsubscript{3}] and, thus, is able to potentially generate significant biological activity. It was suggested that this 24-hydroxylation of the side chain could occur only after 25-hydroxylation (15). Although this may be the case for vitamin D\textsubscript{3}, it does not appear to be a prerequisite for vitamin D\textsubscript{2}; evidence (28–30) suggests that 24-hydroxylation of the vitamin D\textsubscript{2} side chain can also occur in the liver, resulting in a significant (20–50\%) formation of 24(OH)\textsubscript{2}D\textsubscript{2} (29). Consequently, 1,24(OH)\textsubscript{2}D\textsubscript{2}, formed in the kidney from 24(OH)\textsubscript{2}D\textsubscript{2}, has less affinity for VDR than do 1,25(OH)\textsubscript{2}D\textsubscript{3} and 1,24(OH)\textsubscript{2}D\textsubscript{3} (31). Binding to VDR represents a molecular event important to the biological action of the vitamin D metabolites. Taken together, the most plausible explanations for the greater bioefficacy of vitamin D\textsubscript{3} are conceivably due to the higher affinities of vitamin D\textsubscript{3} and its metabolites than vitamin D\textsubscript{2} for hepatic 25-hydroxylase, DBP, and VDR and because vitamin D\textsubscript{3} is not directly metabolized to 24(OH)D as is vitamin D\textsubscript{2}.

**FORM OF VITAMIN D USED IN CLINICAL STUDIES**

As with all drugs that differ in molecular structure, care should be taken to distinguish the form of vitamin D used in clinical studies. For example, after vitamin D\textsubscript{2} supplementation, Harris et al (32) reported almost double the increase in 25(OH)D concentrations in young men compared with those in older men. This result suggested a general age-related impairment in vitamin D metabolism. However, a follow-up study by the same researchers in which they used supplemental vitamin D\textsubscript{3} instead of vitamin D\textsubscript{2} showed similar increases in plasma concentrations of 25(OH)D between age groups (33). Harris et al (33) then reaffirmed that although there are age-related changes in vitamin D metabolism, impairment with age pertains solely to vitamin D\textsubscript{2} metabolism.

An older clinical trial in which vitamin D\textsubscript{2} was used showed prevention loss of bone density on the basis of radiographs of the hand (34), whereas another trial showed no effect on the basis of dual-energy X-ray absorptiometry (35). One clinical trial showed that, similar to supplementation with vitamin D\textsubscript{3} (36), supplementation with vitamin D\textsubscript{2} caused a reduction in the rate of falls (37); however, another trial using vitamin D\textsubscript{2} showed no effect (38). Studies using vitamin D\textsubscript{3} have consistently shown preservation of bone density in older adults who adhered to a protocol of daily doses of \(>10 \mu g\) (400 IU) (39, 40). In fact, all successful fracture trials have used vitamin D\textsubscript{3} at doses of \(\approx20 \mu g\) (800 IU)/d (41).

**Stability of vitamin D\textsubscript{2} preparations**

Synthetic production of vitamin D\textsubscript{2}, if given in high enough doses, prevents infantile rickets and is capable of healing adult osteomalacia. However, the inefficiency of vitamin D\textsubscript{2} compared with vitamin D\textsubscript{3}, on a per mole basis, at increasing 25(OH)D is now well documented, and no successful clinical trials to date have shown that vitamin D\textsubscript{2} prevents fractures (19–21, 47). Given the assumption that the intake of any nutrient will deliver defined effects [i.e., supplementation with vitamin D will lead to an increase in 25(OH)D or fracture prevention], it is clear that vitamin D\textsubscript{2} does not fit this current nutritional notion. This is not to suggest that vitamin D\textsubscript{2} is not efficacious, but, because the units of the 2 forms is clearly not equivalent, likely due to its distinct metabolic features and diminished binding of vitamin D\textsubscript{2} metabolites to DBP in plasma, continual application of vitamin D\textsubscript{2} in clinical use, including in research trials, only serves to confound our understanding of optimal vitamin D dosing recommendations. Furthermore, the public expects to derive the equivalent effect per unit dose of vitamin D, whether it is vitamin D\textsubscript{2} or vitamin D\textsubscript{3}. The scientific community is aware that these molecules are not equivalent. Therefore, vitamin D\textsubscript{2} should no longer be regarded as a nutrient appropriate for supplementation or fortification of foods.

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