The case against ergocalciferol (vitamin D₂) as a vitamin supplement¹,²

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ABSTRACT
Supplemental vitamin D is available in 2 distinct forms: ergocalciferol (vitamin D₂) and cholecalciferol (vitamin D₃). 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 D₃ 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 D₃, the form of vitamin D used in major preparations of prescriptions in North America is vitamin D₂. The case that vitamin D₂ should no longer be considered equivalent to vitamin D₃ is based on differences in their efficacy at raising serum 25-hydroxyvitamin D, diminished binding of vitamin D₂ metabolites to vitamin D binding protein in plasma, and a nonphysiologic metabolism and shorter shelf life of vitamin D₂. Vitamin D₂, or ergocalciferol, should not be regarded as a nutrient suitable for supplementation or fortification.

KEY WORDS Vitamin D₂, vitamin D₃, ergocalciferol, cholecalciferol, vitamin supplement

INTRODUCTION
Vitamin D is available in 2 distinct forms, ergocalciferol (vitamin D₂) and cholecalciferol (vitamin D₃). These are officially regarded as equivalent and interchangeable (1–3). Although sunshine exposure and fish consumption provide vitamin D in the form of D₃, a different bioactive, plant-derived form of vitamin D, named vitamin D₂, 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 D₂ 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 D₂, not vitamin D₃. Multivitamins may contain either vitamin D₂ or vitamin D₃, but most companies are now reformulating their products to contain vitamin D in the D₃ form. Here, we present the case that vitamin D₂ should no longer be considered equivalent to vitamin D₃ and that vitamin D₂, or ergocalciferol, should not be regarded as a nutrient suitable for supplementation or fortification.

INCORRECT PRESUMPTION OF VITAMIN D₂ AND D₃ EQUIVALENCE
Assumptions about the equivalency of the 2 forms of vitamin D were questioned shortly after the discovery of vitamin D₂. As early as 1930, Hess et al (5) suggested that the activity of cod liver oil (vitamin D₃) and Viosterol (vitamin D₂) 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 D₂) may be regarded as being equal to the vitamin D of cod liver oil (vitamin D₃)” (6). As a result, the World Health Organization recommended in 1949 that 1 IU vitamin D be equivalent to 25 ng crystalline vitamin D₃, and no distinction was made between vitamin D₂ and vitamin D₃ (7). Shortly thereafter in Germany in the 1950s, formulations of vitamin D₁ were found to be ~4 times as potent per unit mass as were formulations of vitamin D₂. 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-
mion D₂ and vitamin D₃ were defined to be 65 pmol, such that 1
IU vitamin D₁ (molecular weight: 384) and vitamin D₂ (molecu-
lar weight: 396) would be equivalent to 25 ng and 25.78 ng,
respectively (9). Nevertheless, almost a half century later, British
and American pharmacopoeias 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-
ences in the dose equivalence of vitamin D₂ and D₃, it is likely
that vitamin D₂ 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-
response 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- to 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
D₂ 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)₂D₂ 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 D₁ on raising 25(OH)D concentrations, evalua-
tion of potency was inconclusive due to the effects of confound-
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 D₂ and vitamin D₃ increased serum 25(OH)D con-
centrations, yet the increase in 25(OH)D 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 D/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,
sodium 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 D₁. Clearly,
vitamin D₂ would show efficacy in the treatment of severe vita-
in 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 D₂ to maintain higher 25(OH)D concentrations over
time. Supplementation of vitamin D₂ 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 D₂
than for vitamin D₃ (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-
lism in the kidney to its hormonally active form, 1,25(OH)₂D₃.
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
predisces and converts vitamin D₂ to 25(OH)D₃, 5 times as fast as
it does vitamin D₁ to form 25(OH)D₃ (23). The human micro-
somal fraction also was shown to hydroxylate vitamin D₁ 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, CYP2R1) in hu-
mans that appears able to 25-hydroxylate both vitamin D₂ and
D₃, whereas the mitochondrial enzyme (CYP27A1) only 25-
hydroxylates vitamin D₃ (24, 25).

DIFFERENT 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).

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After 25-hydroxylation, 25(OH)D and 1,25(OH)D undergo additional 24-hydroxylation in the kidney to form 24,25(OH)2D and 1,24,25(OH)3D, respectively. The formation of 1,24,25(OH)3D₂ leads to deactivation of the vitamin D₂ molecule, whereas the analogous vitamin D₃ metabolite, 1,24,25(OH)₃D₃, must undergo additional side-chain oxidation to be biologically deactivated (27). In fact, 1,24,25(OH)₃D₃ has the ability to bind to the vitamin D receptor [VDR; ≈40% more than 1,25(OH)₂D₃] 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 D₃, it does not appear to be a prerequisite for vitamin D₂; evidence (28–30) suggests that 24-hydroxylation of the vitamin D₂ side chain can also occur in the liver, resulting in a significant (20–50%) formation of 24(OH)D₂ (29). Consequently, 1,24(OH)₂D₂, formed in the kidney from 24(OH)D₂, has less affinity for VDR than do 1,25(OH)₂D₃ and 1,24,25(OH)₃D₃ (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 D₃ are conceivably due to the higher affinities of vitamin D₃ and its metabolites than vitamin D₂ for hepatic 25-hydroxylase, DBP, and VDR and because vitamin D₁ is not directly metabolized to 24(OH)D as is vitamin D₂.

**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 D₂ 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 D₃ instead of vitamin D₂ 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 D₂ metabolism.

An older clinical trial in which vitamin D₂ 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 continual application of vitamin D₂ in oil is lacking. The poorer stability of and greater impurities in vitamin D₂ powders may also lead to a higher risk of toxicity than that associated with the vitamin D₃ metabolites. However, it is more likely that the weaker affinity of vitamin D₂ metabolites to DBP produces higher and more biologically available proportions of free 25(OH)D₂ and 1,25-(OH)₂D₃ and may thus be responsible for the greater risk of D₂ toxicity (45).

**Assessment of vitamin D status after D₂ supplementation: challenges to assay methodology**

The production of 25(OH)D₂ as a result of vitamin D₂ supplementation may additionally hinder the assessment of total circulating 25(OH)D, because common assay systems used for clinical purposes have either a diminished capacity or do not detect 25(OH)D₂ with the same efficiency as 25(OH)D₃ (46). Thus, clinical assays used to monitor vitamin D₂ treatment may lead to an erroneous underestimation of vitamin D status. This occurrence may result in additional supplementation with potential adverse consequences, such as hypervitaminosis D.

**CONCLUSION**

Vitamin D₂, if given in high enough doses, prevents infantile rickets and is capable of healing adult osteomalacia. However, the inefficiency of vitamin D₂ compared with vitamin D₃ 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 D₂ 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 D₂ does not fit this current nutritional notion. This is not to suggest that vitamin D₂ 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 D₂ metabolites to DBP in plasma, continual application of vitamin D₂ 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 D₂ or vitamin D₃. The scientific community is aware that these molecules are not equivalent. Therefore, vitamin D₂ should no longer be regarded as a nutrient appropriate for supplementation or fortification of foods.

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