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.

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 D2 were found to be ~4 times as potent per unit mass as were formulations of 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.
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 D3 (molecular weight: 384) and vitamin D2 (mole-
cular 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-
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-
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)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 studies
that compared the 2 versions of vitamin D indicated a greater
effect of vitamin D3 on raising 25(OH)D concentrations, evalua-
tion of potency was inconclusive due to the effects of confounding
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 D2 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 28-
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 D2 would show efficacy in the treatment of severe vita-
m 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 D3 (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 metab-
olism in the kidney to its hormonally active form, 1,25(OH)2D.
In rats, vitamin D2 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 D2 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).
After 25-hydroxylation, 25(OH)D and 1,25(OH)_{2}D undergo additional 24-hydroxylation in the kidney to form 24,25(OH)_{2}D and 1,24,25(OH)_{3}D, respectively. The formation of 1,24,25(OH)_{3}D_{2} leads to deactivation of the vitamin D_{2} molecule, whereas the analogous vitamin D_{3} metabolite, 1,24,25(OH)_{3}D_{3}, must undergo additional side-chain oxidation to be biologically deactivated (27). In fact, 1,24,25(OH)_{3}D_{2} has the ability to bind to the vitamin D receptor [VDR; \approx 40\% more than 1,25(OH)_{2}D_{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_{3}, it does not appear to be a prerequisite for vitamin D_{2}; evidence (28–30) suggests that 24-hydroxylation of the vitamin D_{2} side chain can also occur in the liver, resulting in a significant (20–50\%) formation of 24(OH)D_{2} (29). Consequently, 1,24(OH)_{2}D_{2}, formed in the kidney from 24(OH)D_{2}, has less affinity for VDR than do 1,25(OH)_{2}D_{3} and 1,24(OH)_{2}D_{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_{2} are conceivably due to the higher affinities of vitamin D_{2} and its metabolites than vitamin D_{3} for hepatic 25-hydroxylase, DBP, and VDR and because vitamin D_{2} is not directly metabolized to 24(OH)D as is vitamin D_{3}.

**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_{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_{3} instead of vitamin D_{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_{2} metabolism.

An older clinical trial in which vitamin D_{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_{3} (36), supplementation with vitamin D_{2} caused a reduction in the rate of falls (37); however, another trial using vitamin D_{2} showed no effect (38). Studies using vitamin D_{2} have consistently shown preservation of bone density in older adults who adhered to a protocol of daily doses of \geq 10 \mu g (400 IU) (39, 40). In fact, all successful fracture trials have used vitamin D_{3} at doses of \approx 20 \mu g (800 IU)/d (41).

**Stability of vitamin D_{2} preparations**

Synthetic production of vitamin D_{2} is manufactured in a similar manner to that which occurs naturally in human and animal skin, via the production of 7-dehydrocholesterol from cholesterol and subsequent irradiation to its active D_{2} form. Conversely, vitamin D_{2} is synthetically produced from irradiation of ergosterol derived from the mold ergot (42). In addition to its lower bioactivity, the poor stability of vitamin D_{2} is worrisome, particularly upon exposure of crystalline D_{2} powder to varying temperatures, humidity, or even storage containers (43, 44). In contrast, vitamin D_{3} powder is not as labile. As a result, the vitamin D content by various manufacturers has been found to differ substantially from that of the labeled claim (17). It must be noted that comparative published data on the stability of vitamin D_{2} and D_{3} in oil is lacking. The poorer stability of and greater impurities in vitamin D_{2} powders may also lead to a higher risk of toxicity than that associated with the vitamin D_{3} metabolites. However, it is more likely that the weaker affinity of vitamin D_{2} metabolites to DBP produces higher and more biologically available proportions of free 25(OH)D_{2} and 1,25-(OH)_{2}D_{2} and may thus be responsible for the greater risk of D_{2} toxicity (45).

**Assessment of vitamin D status after D_{2} supplementation: challenges to assay methodology**

The production of 25(OH)D_{2} as a result of vitamin D_{2} 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)D_{2} with the same efficiency as 25(OH)D_{3} (46). Thus, clinical assays used to monitor vitamin D_{2} 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 D_{2}, if given in high enough doses, prevents infantile rickets and is capable of healing adult osteomalacia. However, the inefficiency of vitamin D_{2} compared with vitamin D_{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_{2} prevents fractures (19–21, 47). Given the assumption that the intake of any nutrient will deliver defined effects [ie, supplementation with vitamin D will lead to an increase in 25(OH)D or fracture prevention], it is clear that vitamin D_{2} does not fit this current nutritional notion. This is not to suggest that vitamin D_{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_{2} metabolites to DBP in plasma, continual application of vitamin D_{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_{2} or vitamin D_{3}. The scientific community is aware that these molecules are not equivalent. Therefore, vitamin D_{2} should no longer be regarded as a nutrient appropriate for supplementation or fortification of foods.

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**REFERENCES**

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