The case against ergocalciferol (vitamin D_2) as a vitamin supplement[1,2]

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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. *Am J Clin Nutr* 2006;84:694–7.

KEY WORDS Vitamin D_2, vitamin D_3, ergocalciferol, cholecalciferol, vitamin supplement

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_3 were found to be ≈4 times as potent per unit mass as were formulations of vitamin D_2. Vigantol oil (Merck KGaA, Darmstadt, Germany),

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the most popular vitamin D supplement in Europe, was reformulated to replace its vitamin D_2 content with vitamin D_3 (8).

Sustained advancement in the characterization and metabolism of vitamin D and its metabolites led to the proposed recommendation in 1972 that 1 IU vitamin D be defined in moles or molecules rather than in weight terms. Subsequently, both vitamin D_2 and vitamin D_3 were defined to be 65 pmol, such that 1 IU vitamin D_3 (molecular weight: 384) and vitamin D_2 (molecular 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 nutritional forms of vitamin D with the simple conversion of gram quantity, where 1 IU of either vitamin D_2 or vitamin D_3 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 D_2 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 D_2 or vitamin D_3 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 differences in the dose equivalence of vitamin D_2 and D_3, it is likely that vitamin D_2 is currently provided at a high enough dose per kg infant body weight to maintain adequate bone mineral metabolism. 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 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 D_2 to be only one-tenth as effective as vitamin D_3 at increasing 25(OH)D (11). Likewise, in monkeys, the concentrations of serum 25(OH)D maintained after intake of vitamin D_3 were $\approx$2- to 3-fold those maintained with comparable amounts of vitamin D_2 (12). In rats, however, vitamin D_2 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 D_2 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)D_2 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 D_2 molecule (14).

In humans, vitamin D_3 is more effective than vitamin D_2 at raising serum 25(OH)D concentrations. Although previous studies that compared the 2 versions of vitamin D indicated a greater effect of vitamin D_3 on raising 25(OH)D concentrations, evaluation 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 D_2 or D_3 ($\approx$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 D_2 and vitamin D_3 increased serum 25(OH)D concentrations, yet the increase in 25(OH)D was found to be 70% greater (1.70 times) with vitamin D_3 than the increase obtained with vitamin D_2. When adjusted for concomitant changes in an untreated group, the difference between the 2 groups was $\approx$2-fold. To further complement these findings, a 3-mo supplementation study by Mastaglia et al (20) found that a dose of 250 μg vitamin D_2/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_3/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 D_2 or vitamin D_3 (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 D_2–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 D_3–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 D_3. Clearly, vitamin D_2 would show efficacy in the treatment of severe vitamin D deficiency; however, the authors note that 2000 μg (50 000 IU) vitamin D_2 should be considered equivalent to $\leq$375 μg (15000 IU) vitamin D_3, and likely closer to 125 μg (5000 IU) vitamin D_3 (21).

Several mechanisms could contribute to the greater capacity of vitamin D_3 to maintain higher 25(OH)D concentrations over time. Supplementation of vitamin D_2 produces appreciable amounts of serum 25(OH)D_2 (22), which, as previously mentioned, has a lower affinity for DBP and results in a shorter circulating half-life than that of 25(OH)D_3. Others have suggested a higher affinity of hepatic 25-hydroxylase for vitamin D_3 than for vitamin D_2 (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 metabolism in the kidney to its hormonally active form, 1,25(OH)$_2$D. 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 D_3 to 25(OH)D_3 5 times as fast as it does vitamin D_2 to form 25(OH)D_2 (23). The human microsomal fraction also was shown to hydroxylate vitamin D_3 to some degree, but no detectable vitamin D 25-hydroxylation of vitamin D_2 was observed (23). However, studies have identified a key microsomal liver enzyme (cytochrome P450, CYP2RI) in humans that appears able to 25-hydroxylate both vitamin D_2 and D_3, whereas the mitochondrial enzyme (CYP27A1) only 25-hydroxylates vitamin D_3 (24, 25).

DIFFERENT METABOLIC FATES OF VITAMINS D_2 AND D_3

It was initially thought that both vitamin D_2 and vitamin D_3 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 $1,25(OH)_2D$ undergo additional 24-hydroxylation in the kidney to form $24,25(OH)_2D$ and $1,24,25(OH)_3D$, respectively. The formation of $1,24,25(OH)_3D_2$ leads to deactivation of the vitamin D_2 molecule, whereas the analogous vitamin D_3 metabolite, $1,24,25(OH)_3D_3$, must undergo additional side-chain oxidation to be biologically deactivated (27). In fact, $1,24,25(OH)_3D_3$ has the ability to bind to the vitamin D receptor [VDR; ≈40% more than $1,25(OH)_2D_3$] 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, $1,24(OH)_2D_2$, formed in the kidney from $24(OH)D_2$, has less affinity for VDR than do $1,25(OH)_2D_3$ and $1,24(OH)_2D_3$ (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_3 are conceivably due to the higher affinities of vitamin D_3 and its metabolites than vitamin D_2 for hepatic 25-hydroxylase, DBP, and VDR and because vitamin D_3 is not directly metabolized to 24(OH)D as is vitamin D_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_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_3 have consistently shown preservation of bone density in older adults who adhered to a protocol of daily doses of >10 μg (400 IU) (39, 40). In fact, all successful fracture trials have used vitamin D_3 at doses of ≈20 μg (800 IU)/d (41).

Stability of vitamin D_2 preparations

Synthetic production of vitamin D_3 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_3 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_3 and D_2 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 $1,25\text{-}(OH)_2D_2$ 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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