INTRODUCTION

The use of vitamin D has never been approached in the same way that we would expect to see for any modern drug. Unlike other nutrients, we have never had dietary intakes of vitamin D as a reasonable reference point for deciding on how much of this nutrient/drug that people should be consuming. The ambiguity between “nutrient” and “drug” is reasonable when it comes to vitamin D, because there were no meaningful amounts of vitamin D in the kinds of foods that Paleolithic humans would likely have been consuming.

Our biology was designed by evolution for life in equatorial Africa. Therefore, consumption of those rare foods that do contain a meaningful amount of vitamin D, like ocean fish, could not have played a role in determining human vitamin D requirements. Requirements for vitamin D were satisfied by the life of the naked ape that became the species, *homo sapien*, in its native, tropical environment. Since our culture and environment no longer match the conditions that defined our biology, we modern humans might benefit if we could compensate for the biological consequences of modern life. One such consequence may be an endemic lack of vitamin D that can be corrected by appropriate supplementation.

My perspective is the North American one, where vitamin D is primarily regarded as a nutrient. However, in Europe and the rest of the world, use of even small doses of vitamin D usually falls into the realm of a prescription drug. That perspective has the advantage of imposing a higher expectation on our understanding of the use of vitamin D. Before approving the clinical use of any new drug, government regulators expect to see the answers to some relatively standard questions. Pharmaceutical firms need to anticipate these issues as they plan the research necessary for implementation of new products. These questions include, but are not limited to, the following:

1a. What is the disease indication for the drug?
1b. What kind of clinical or health effects should we be looking for, based on preclinical animal and laboratory research?
2a. What are the most useful approaches to delivering the drug to people: the vehicle?
2b. What is the appropriate dosage, route of administration, and interval between doses?
3. What is the desirable target for the plasma concentration, what dose would be needed to attain or ensure this?
4. What, if any, are the biological markers to monitor toxicity, and what are our criteria for determining therapeutic effectiveness? What is the "therapeutic index" the ratio between toxic vs beneficial dose levels?

When it comes to plain and simple, nutritional vitamin D, the answer to each of these questions is that we have just started to address it in the past decade. Any opinion about vitamin D here is controversial. In an effort to provide some answers to the preceding questions, I will present perspectives about the vitamin D system that relate to pharmacological aspects of vitamin D in the adult context.
The only officially recognized indications for use of vitamin D in the adult are the prevention of bone loss and fractures. Figure 1 summarizes randomized control trials looking at whether vitamin D, with or without calcium affects risk of non-vertebral fracture. A recent, thorough review of vitamin D, 1,25(OH)2D and its analogs is also available, addressing the issue of osteoporosis prevention and treatment (1). The purpose of Figure 1 is to show that there has been no evidence that doses of vitamin D less than 800 IU/day are effective in preventing osteoporotic fractures.

Figure 1. Summary of randomized-control clinical trials of fracture-prevention using vitamin D, with or without calcium. None of the studies using doses of vitamin D3 providing less than 20 mcg/day was effective in reducing fracture risk (80;124;124). However, all the studies in which there was a reduction in fracture risk used approximately 20 mcg/day of vitamin D3 (2;5-7)(122;122;124). This dose includes the background intake; for the work by Dawson-Hughes background intake, this was 5 mcg/day (6)).

INDICATIONS AND CLINICAL USE: POTENTIAL HEALTH EFFECTS OF VITAMIN D

While all of the effects in Table 1 are statistically significant, most of the evidence for a role of vitamin D is circumstantial. Epidemiological studies show that higher serum 25(OH)D, and/or environmental ultraviolet exposure is associated with lower rates of breast, ovarian, prostate, and colorectal cancers (21-28). More recent statistical analyses also show significant relationships including non-Hodgkin's lymphoma, and cancer of the bladder, esophagus, kidney, lung, pancreas, rectum, stomach and corpus uteri (29). Multiple sclerosis is more prevalent in populations having lower levels of vitamin D nutrition or ultraviolet exposure (26;30-32), and it has been proposed that vitamin D intake, ranging from...
1,300 to 3,800 units per day, helps prevent the disease (32). Established osteoarthritis progresses more slowly (is less severe) in adults with higher vitamin D nutritional status, with serum 25(OH)D that exceeds 75 nmol/L (33;34). The prevalence of hypertension increases with population distance, north or south, from the equator (35). Blood pressure is lowered in mildly hypertensive patients whose 25(OH)D levels are raised to over 100 nmol/L by tanning (36). One randomized intervention study showing that vitamin D supplementation at 20 µg/d (800 IU/d) lowers blood pressure in elderly women (37). Vitamin D deficiency impairs immune function in animals (38), and in children there is a strong association between pneumonia and nutritional rickets (39). The concept that there is a connection between vitamin D nutrition and immune function is further supported by the apparent protective effect of improved vitamin D nutrition during infancy and childhood against type I diabetes mellitus (40). If any of these non-traditional effects of vitamin D were taken into account, they would result in a substantial upward revision of the AI (RDA) for vitamin D.

The level of evidence needed to make a health claim that can be sanctioned officially involves more than the circumstantial evidence of laboratory experiments and epidemiology. It requires direct intervention, the controlled administration of the agent to many healthy people, and showing an effect that stands up to statistical analysis. We need randomized intervention trials to take this field beyond pre-clinical basic research and epidemiological evidence. There are ongoing randomized trials involving “vitamin D” that relate to cancer, multiple sclerosis, and osteoporosis, but for the most part, they deal with analogs of 1,25(OH)2D, not the nutrient.

The nutrient has been very much overlooked for all purposes except rickets, osteomalacia and osteoporosis. There are three reasons for this. First, the financial incentive lies with the proprietary analogs, driven by private funding that diverts the focus of investigators who are able to do such studies. Second, an optimized dose of vitamin D has never been established for adults. Therefore, “plain” vitamin D sometimes compares poorly with 1,25(OH)2D and its analogs whose dose is more thoroughly optimized (12), and whose dose is usually designed to be very close to the point where it could cause hypercalcemia. As I will discuss later, optimal doses of vitamin D probably vary, depending on the indication, so that one dose may not always be optimal. Third, the official mis-representation that vitamin D2 and vitamin D3 are equal has resulted in efficacy studies at higher doses usually involving vitamin D2 because high-dose commercial preparations of vitamin D are comprised of this. One example of this is work looking at whether vitamin D2 supplementation might prevent bone loss in steroid-treated patients (41;42); the effects of “vitamin D” were marginal, but since plain and simple vitamin D3 was never part of the experimental protocol, the issue remains unresolved. Another example of the unfortunate focus on vitamin D2 instead of the D3 form is the recent Australian study using vitamin D2 at a decent dose, 10,000 IU (250 mcg) weekly, producing no significant effect on bone density preservation, but showing essentially no effect on serum 25(OH)D either (43).

Administration of vitamin D (cholecalciferol) is unusual in pharmacology or in endocrinology, because this molecule is two metabolic steps away from the biologically active agent, 1,25(OH)2D. Furthermore, the laboratory test to monitor dose is the concentration of a metabolite, 25(OH)D. Figure 2 illustrates the metabolite “compartments” occupied by vitamin D after ingestion or exposure to sunshine. Less than the 25 percent of vitamin D that enters the body actually becomes 25(OH)D. More than 75 percent of vitamin D entering the circulation bypasses what we recognize as the vitamin D endocrine system. Instead, most vitamin D entering the circulation is excreted and/or metabolized by other routes not shown here, and most likely, excreted into the bile.

Figure 2 consists of two panels to illustrate the metabolic adaptations that exist so that the vitamin D endocrine system can accommodate to a wide range in the substrate concentration. The vitamin D system is optimized to maintain plasma 1,25(OH)2D levels according to the requirements of calcium homeostasis. The earliest compromise to progressive restriction in vitamin D supply is a diminished capacity of non-renal tissues to produce 1,25(OH)2D. This compromise at non-renal tissues is illustrated in Figure 2 by the greater height of one of the valves representing the 1-hydroxylase on the “pail” that represents the 25(OH)D compartment.

If one looks at the system of vitamin D metabolism in Figure 2 from the perspective of a system designed to control something, it becomes clear that this is a system better designed to cope with an abundance of supply, not a lack of it. The flow of vitamin D toward 25(OH)D is remarkably inefficient, with most bypassing it. Furthermore, there is no way to correct for deficiency of vitamin D, other than to redirect utilization of 25(OH)D toward 1,25(OH)2D production, which is the pathway most acutely important for life. That is, when supplies of vitamin D are severely restricted, its metabolism is directed only toward the maintenance of calcium homeostasis. To expand on the point that the system of vitamin D metabolism is effectively a designed for adjusting for higher inputs, not lower inputs, I offer the example of an air-conditioner system. Air conditioners are designed to compensate for excessive heat, but they are a useless way to compensate for a cold environment. The environment under which human vitamin D metabolism was effectively designed was for people without clothing, living at latitudes where UVB intensity was always enough to produce a relative abundance of vitamin D. In contrast, most modern humans cover close to 95 percent of skin surface and avoid sunshine. The vitamin D endocrine/paracrine system is not designed to cope with the lack of vitamin D created by our modern culture of clothing and sun-avoidance. Inadequate supplies of vitamin D limit the paracrine control that many tissues need so they can function properly. As a result, it is possible that what we regard as a modern “normal” prevalence of some of the diseases listed in Table 1 could be reduced substantially if we were to increase our intakes of vitamin D (44-47).

Control of metabolism in the vitamin D endocrine system is very different from the way other steroid hormones are regulated. For conventional steroid hormones, the concentration of substrate (cholesterol) is far higher than the substrate in the vitamin D system.

Figure 3 illustrates the effective in-vivo Km of 1-hydroxylase, in relation to the physiological concentration

OVERVIEW OF THE SYSTEM OF VITAMIN D METABOLISM, AND ITS REGULATION.
range of its substrate. Our circulating cholesterol concentration is in the order of 5 million nmol/liter; in contrast, 25(OH)D typically circulates at less than 200 nmol/liter. Cholesterol concentration is not a rate-limiting aspect of the body’s capacity to generate steroid hormones; however, 25(OH)D concentration is absolutely rate-limiting for 1,25(OH)2D production.

In the acute situation, before adjustments can be made to 24-hydroxylase and catabolic pathways (before the “valves” in Figure 2 can be adjusted), the in vivo production of 1,25(OH)2D is directly proportional to circulating 25(OH)D concentration. In rats, the acute injection of 25(OH)D into the circulation produces a rapid increase in 1,25(OH)2D, directly proportional to the percentage increase in 25(OH)D (48;49). Since in vivo concentrations of 25(OH)D change slowly, over many days and weeks, this first-order relationship between 25(OH)D and 1,25(OH)2D is not normally seen in adults (18). However, in situations where 1-hydroxylase is tonically stimulated, either because of primary hyperparathyroidism (50) or in granulomatous disease (51;52), modest increases in vitamin D supply will raise plasma 1,25(OH)2D concentration and aggravate hypercalcemia.

Differences between steroid hormones and the vitamin D system are amplified further by the large differences in concentrations of their respective plasma carrier proteins. Sex-steroid binding globulin, and glucocorticoid binding globulin circulate in concentrations of about 200 nmol/L, in the same order of magnitude as their ligands (53); in contrast, the concentration of vitamin D binding protein is 4700 nmol/L (54); this represents a 50-fold excess over its vitamin D-derived ligands.

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**Figure 2A. Metabolism of vitamin D under conditions of low vitamin D supply.** The vessels represent metabolic compartments, stages in the metabolism of vitamin D. The height of the shaded portion of each vessel represents the relative concentration of each metabolite indicated in the figure. This figure illustrates the concept that vitamin D metabolism in vivo functions below its Km, i.e. the system behaves according to the first-order reaction kinetics. Just as the flow of water through a hole in a pail reflects the height of water in that pail, the rates of metabolism in the vitamin D system reflect the concentration of precursor at each step. Open passages represent steps in metabolism in which the pertinent enzymes are relatively unregulated. Valves represent steps in metabolism in which there is regulation of flow at the enzyme (this regulation is usually through changes in amount of enzyme protein in specific tissues, and not allosteric). When vitamin D supplies are low, flow of 25(OH)D through other potential pathways is compromised to maintain the circulating concentration of 1,25(OH)2D at the level determined by the priority requirements of bone and mineral metabolism.

**Figure 2B. Metabolism of vitamin D under conditions of adequate vitamin D supply.** When vitamin D supplies are adequate, flow of 25(OH)D through other potential pathways, including its utilization by peripheral tissues for paracrine regulation, is no longer compromised. Higher 25(OH)D concentration makes available routes of metabolism other than the one path needed for bone and mineral metabolism. Furthermore, a higher supply of vitamin D leads to an upregulation of 24-hydroxylase and the catabolic pathways associated with it, this accelerates rate of metabolic clearance and metabolite turnover in each compartment.
Recent new knowledge of the megalin/cubulin system has shed light on the mechanisms of DBP-tissue interactions, and tissue-specific uptake of DBP (chapters 8-10 of this book) (57). Megalin and cubulin are cell-surface, endocytic receptors, members of the low-density lipoprotein receptor gene family. These help to regulate the concentration of ligands in the extracellular fluids and deliver metabolites to cells in need of these metabolites (58). Differences in tissue distribution of these cell-surface proteins will affect the accessibility of different tissues to circulating 25(OH)D.

**DOSAGE CONSIDERATIONS**

**Infants**

Cholecalciferol, or vitamin D3, given in the form of cod liver oil, has been a folk remedy in northern Europe since the 1700’s. Empirically, a small teaspoon-full daily was thought to help infants thrive. This arbitrary dose of cod-liver oil has turned out to be a good guess, so far as infants are concerned. The 375 IU (9 mcg) of vitamin D3 contained in that teaspoon (59) was confirmed relatively recently as being appropriate for infants (60;61). A French study utilizing vitamin D2 concluded that neonates might need somewhat more, 1000 IU (62). If safety of vitamin D during infancy is a concern, it should be kept in mind, that until the late 1960’s, the recommended amount of vitamin D for infants in Finland was 2000 IU/day (50 mcg/day). A large epidemiologic study suggests that this higher dose lowered risk of juvenile diabetes before age 30 years, by 85% compared to people not receiving vitamin D as infants (40).

Compared to the adult, vitamin D nutrition in the infant and child has been well characterized, and it is the focus of Chapter 65 (Pettifor). There is also an excellent review of the field available, by Chesney (63). The present chapter focuses on the pharmacology of vitamin D in the adult.

**Adults**

Until it became clear that vitamin D was important to the health of adults, there was very little thought directed at how much vitamin D adults might need to consume. Until recently, there was been no consensus about what the objective criteria should be for appropriate vitamin D nutrition. In England, an adult recommendation of 2.5 mcg (100 IU)/d was set simply because 7 women with severe nutritional osteomalacia showed a response to this amount (64).

Interestingly, the oils of different fish contain different amounts of vitamin D. For example, a teaspoon full of halibut liver oil contains twice as much vitamin D3 as does cod liver oil. If it had been halibut liver oil used in the past, recommendations for vitamin D supplementation could well have been double what they have been through most of the last century.

Into the 1960s, the absence of overt rickets or osteomalacia was the only criterion that vitamin D nutrition was adequate (65). By the same criterion, anthropologists also consider vitamin D nutrition to have been a relatively minor problem for ancient populations. This is now explained by the new concept that the lack of vitamin D resulted in a natural selection for white skin colour as a way to prevent rickets and osteomalacia within defined environments (66). Women with osteomalacia would have produced few offspring, while those able to produce enough vitamin D to prevent rickets and
osteomalacia would have been the vast majority in any region - survival depended on adequacy of vitamin D nutrition, and natural selection of skin color helped to ensure adequacy.

In the 1960s, an expert committee on vitamin D could provide only anecdotal support for “the hypothesis of a small requirement” for vitamin D in adults and recommended one-half the infant dose, to ensure that adults obtain some from the diet (65). Despite the new knowledge uncovered since that time, dietary vitamin D recommendations for adults have remained very conservative, and still derive from amounts established for neonates. In contrast to the way decisions are made about the dose of any new drug, recommendations for vitamin D have been arbitrary, because there was no firm evidence on which to base decisions. However, even though the evidence about the effects of vitamin D dosages on adult health have become characterized scientifically, those with the final say in setting official nutrient guidelines (not the experts they consulted) continued to focus on lower doses of vitamin D than had been shown effective in the fracture prevention trials discussed above. The revised recommendations were referred to as the “adequate intake” (AI), because there was no published evidence of efficacy for them (67,68).

The objective measure of vitamin D nutritional status is the 25-hydroxyvitamin D (25(OH)D) concentration in serum or plasma (67). The consensus on this point has made it possible for researchers to focus on a measurable target when it comes to vitamin D nutrition. Table 2 summarizes two views of the relationships between long-term vitamin D intakes and the anticipated range of 25(OH)D concentration associated with them.

Figure 4 is a dose-response curve to showing the final average 25(OH)D concentrations attained in studies reported in the literature (44;69). Table 3 summarizes incremental responses to different treatment strategies to raise 25(OH)D to steady-state concentrations. Responsiveness to vitamin D administration, as measured by the nmol per liter increase per mcg consumption per day, increases with: a) lower vitamin D dosage, b) lower initial 25(OH)D concentration; c) longer duration of supplementation, suggesting a long half-life and time to plateau.

The conventional approach to improving vitamin D nutritional status has been to give either vitamin D3 or vitamin D2 (ergocalciferol). Until recently, availability of 25(OH)D was another option (supply of this product has been discontinued by Organon, NJ, USA). The company’s discontinuation of 25(OH)D may have made sense, because the objective of increasing plasma 25(OH)D concentrations can be almost as easily achieved by providing enough vitamin D3. Nonetheless, useful perspectives can be gained from previous experience with 25(OH)D. Barger-Lux and Heaney et al. have shown that as 25(OH)D dosage increases, there is effectively a linear increase in the average 25(OH)D concentration achieved (Table 3). However, when vitamin D3 is used, the increment in 25(OH)D per mcg per day of vitamin D3 decreases as dose increases.
<table>
<thead>
<tr>
<th>Disease</th>
<th>Type of evidence supporting the association</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rickets</td>
<td>Long established, preventive</td>
<td></td>
</tr>
<tr>
<td>Osteomalacia</td>
<td>Long established, preventive</td>
<td></td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>Placebo-controlled, randomized studies that vitamin D prevents loss of bone density, and lessens fracture risk</td>
<td>(2;4-6)</td>
</tr>
<tr>
<td>Poor calcium absorption</td>
<td>Modest increase in Vitamin D nutrition increases this.</td>
<td>Heaney2003dieteticj</td>
</tr>
<tr>
<td>blood-pressure regulation</td>
<td>Epidemiological and randomized interventional data</td>
<td>(8;9;35;36)</td>
</tr>
<tr>
<td>Risk of diabetes</td>
<td>Epidemiological and case-control data</td>
<td>(40;126;127)</td>
</tr>
<tr>
<td>progression osteoarthritis</td>
<td>Epidemiological, cross-sectional studies</td>
<td>(33;34)</td>
</tr>
<tr>
<td>diminished intra-uterine growth</td>
<td>Presumed effect</td>
<td>(128),</td>
</tr>
<tr>
<td>Effects on brain development</td>
<td>Rat experiments</td>
<td>(129)</td>
</tr>
<tr>
<td>resistance to pneumonia</td>
<td>Epidemiological association with rickets</td>
<td>(39)</td>
</tr>
<tr>
<td>Multiple sclerosis, occurrence and progression</td>
<td>Epidemiological data, and lab effects on tissue</td>
<td>(32;130;131)</td>
</tr>
<tr>
<td>prevention of tuberculosis, Prevent Depression or SAD or Improve mood</td>
<td>Epidemiological data, and lab effects on tissue</td>
<td>(132;133)</td>
</tr>
<tr>
<td></td>
<td>Small RCT’s 400 IU/day or 100000 in winter</td>
<td>(134;135)</td>
</tr>
<tr>
<td></td>
<td>No mood effect of 400 IU/day</td>
<td>(136)</td>
</tr>
<tr>
<td>Lessen risk/severity of Fibromyalgia</td>
<td>Cross-sectional study</td>
<td>(137)</td>
</tr>
<tr>
<td>Protection against cancers</td>
<td>Breast</td>
<td>(138;139)</td>
</tr>
<tr>
<td>prostrate</td>
<td>Prostate</td>
<td>(140)</td>
</tr>
<tr>
<td></td>
<td>(139;141;142)</td>
<td></td>
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Table 2. The clinical interpretation of serum 25(OH)D levels and the estimated intakes of vitamin D needed to ensure these levels (1 mcg = 40 IU).

<table>
<thead>
<tr>
<th>Serum 25(OH)D nmol/L</th>
<th>Deficiency (rickets and Osteomalacia)</th>
<th>Insufficiency (increased PTH secretion, osteoporosis)</th>
<th>Sufficiency</th>
<th>Desirable (suppress PTH, optimize Calcium absorption)</th>
<th>Toxic/Therapy (could increase urine and serum calcium)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-25</td>
<td>25-40</td>
<td>40-100</td>
<td>75-160</td>
<td>&gt;220</td>
<td></td>
</tr>
</tbody>
</table>

*Vitamin D3 mcg/day needed to reach the 25(OH)D above:*

- **Food and nutrition board**
  - 0 mcg/d
  - 5-10
  - 5-15
  - Not stated
  - ≥95

- **From Evidence Reviewed**
  - 0-5 mcg/d
  - 10-15
  - 25-100
  - 100-250
  - >100 (>40000 IU)

*a Implications drawn from current National Academy of Sciences nutritional guidelines that the stated intake will deliver the level of adequacy (67). The “adequate intake” recommendations for vitamin D vary according to age: adults <50, 5 mcg/day; 50-70 years, 10 mcg/day; >70 Years, 15 mcg/day.

b Based on literature (69;108;122)*
TABLE 3. Strategies to increase circulating 25(OH)D concentration in adults: Effects of compound, dose, and duration.

<table>
<thead>
<tr>
<th>Compound</th>
<th>25(OH)D increase per mcg/d</th>
<th>DOSE mcg/day</th>
<th>Duration on the dose wks</th>
<th>Absolute increase in 25(OH)D nmol/L Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D</td>
<td>4.1</td>
<td>50</td>
<td>4</td>
<td>206.4 (109)</td>
</tr>
<tr>
<td>25(OH)D</td>
<td>4.0</td>
<td>10</td>
<td>4</td>
<td>40 (109)</td>
</tr>
<tr>
<td>25(OH)D</td>
<td>3.8</td>
<td>20</td>
<td>4</td>
<td>76.1 (109)</td>
</tr>
<tr>
<td>cholecalciferol</td>
<td>1.5</td>
<td>15</td>
<td>52</td>
<td>22 (143)</td>
</tr>
<tr>
<td>cholecalciferol</td>
<td>1.4</td>
<td>20</td>
<td>8</td>
<td>27 (144)</td>
</tr>
<tr>
<td>cholecalciferol</td>
<td>1.1</td>
<td>25</td>
<td>8</td>
<td>28.6 (109)</td>
</tr>
<tr>
<td>cholecalciferol</td>
<td>1.1</td>
<td>21</td>
<td>20</td>
<td>23.4 (145)</td>
</tr>
<tr>
<td>cholecalciferol</td>
<td>0.8</td>
<td>100</td>
<td>52</td>
<td>81 (143)</td>
</tr>
<tr>
<td>cholecalciferol</td>
<td>0.8</td>
<td>25</td>
<td>20</td>
<td>19 (108)</td>
</tr>
<tr>
<td>cholecalciferol</td>
<td>0.7</td>
<td>138</td>
<td>20</td>
<td>102.7 (145)</td>
</tr>
<tr>
<td>cholecalciferol</td>
<td>0.6</td>
<td>275</td>
<td>20</td>
<td>169.8 (145)</td>
</tr>
<tr>
<td>cholecalciferol</td>
<td>0.6</td>
<td>250</td>
<td>8</td>
<td>146 (109)</td>
</tr>
<tr>
<td>cholecalciferol</td>
<td>0.5</td>
<td>100</td>
<td>20</td>
<td>51.8 (108)</td>
</tr>
<tr>
<td>cholecalciferol</td>
<td>0.5</td>
<td>1250</td>
<td>8</td>
<td>643 (109)</td>
</tr>
<tr>
<td>ergocalciferol</td>
<td>0.3</td>
<td>36</td>
<td>104</td>
<td>(43)</td>
</tr>
</tbody>
</table>

1 The results in this table represent recent work not included in Figure 4. These data were assembled to permit comparison of efficacy dose of different strategies for increasing 25(OH)D concentration. The results are sorted in order of decreasing response to the dose, based on the nmol/L increase in 25(OH)D per mcg/day of oral the doses used in these studies.

Since the increment in plasma 25(OH)D concentration per mcg dose is at least four times higher for 25(OH)D administration than for vitamin D3 administration, we can conclude that less than 25 percent of vitamin D molecules ever become 25(OH)D. At least three quarters of the molecules of vitamin D that enter the body are removed by some other fate.

The case against ergocalciferol, vitamin D2

Vitamin D is available in two forms for nutritional supplementation, ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3). Vitamin D2, is manufactured by exposing a fat extract of yeast to UV light. Since no metabolite of vitamin D2 is normally detectable in the blood of humans or primates (70;71), I contend that this should be regarded as a drug, and not physiological compound. The present discussion focuses on vitamin D3, cholecalciferol, the form of vitamin D natural present in mammals. Vitamin D3 (from here on, vitamin D) is the more potent form of vitamin D in all primate species and in man (70;71). Comparisons between the two versions of vitamin D (71), and the meta-analysis of effects on 25(OH)D [Figure 4] indicate that vitamin D3 is about 4 times as potent as vitamin D2, i.e. 1 mcg of D3 = approximately 4 mcg of D2. Nonetheless, vitamin D2 continues to be used clinically as if it is equivalent, since official guidelines (67) and pharmacopeias respond slowly to new evidence.

The presumption of equivalence is based on 60-year old studies of rickets prevention in infants – evidence recognized as weak, even at the time (59;72). The older rat data suggesting that vitamin D2 and vitamin D3 were equivalent lose their meaning when it is noted that the rat-line tests last done over 50 years ago were bioassays to establish "units" for the quantity of vitamin D not readily measured in any other way (73). With a bioassay yielding "units", equivalence is not the same as equivalence per milligram or per mole. Furthermore, all species tested show differences between the vitamin D2 and vitamin D3 (74;75). Despite these obvious problems about units, the very conservative approach to official statements has remained unchanged, that one international unit of vitamin D is equivalent to 25 nanograms of either vitamin D2 or vitamin D3 (67;73). In Australia, vitamin D3 has never been licensed for use, and the only nutritional form of vitamin D available there is vitamin D2. Strangely, at the time of this writing, there is no form of vitamin D3 commercially available in France either.

I have summarized the differences between vitamin D2 and vitamin D3 in Table 4. Based on the many major differences between the two, it is clear that unless there is some well-characterized reason to favours vitamin D2 (I am not aware of any), all use of vitamin D for nutritional and clinical purposes should specify cholecalciferol, vitamin D3.

Vitamin D is not a hormone

For most of the 20th century, there was no debate, that vitamin D was a nutrient. It was known as "the sunshine vitamin". Confusion arose when it was realized that the active form of the vitamin D molecule was 1,25(OH)2D (calcitriol), which is a hormone in the true sense of the word. A focus on the inadequacies of the term "vitamin", and a lack of consideration for the term, "hormone", led to the misconception that vitamin D itself might be a hormone instead of a nutrient. Officially
mandated nutritional committee reports in both North America (67) and Europe (76) now state that nutritional vitamin D may be more suitably referred to as a "hormone" instead of nutrient. However, vitamin D is no more a hormone than cholesterol is, because vitamin D is only the raw material needed for synthesis of 1,25(OH)2D. Practitioners not familiar with this ambiguity occasionally administer vitamin D inappropriately, when 1,25(OH)2D or an analog would be appropriate, or vice versa (anyone who specializes clinically in the field of vitamin D knows of examples of such unintended misuse). Ambiguity and the use of jargon in this field has had unfortunate consequences.

Promotion of vitamin D nutrition is hindered by alarmist reactions justifiably associated with administration of any hormone. Use of a hormone implies that natural homeostatic control is circumvented – taken over by the physician. However, vitamin D does not generate an endocrine signal, 1,25(OH)2D and its analogs do this. The purpose of supplementing with vitamin D is to optimize the natural functions of the endocrine/paracrine systems that require it.

UVB light on human skin as a dose of vitamin D.

In any discussion of vitamin D pharmacology or dosage, it would be a major oversight to ignore the role of sunshine, particularly its UVB component. As described elsewhere in this book (Chapter 3), the synthesis of vitamin D is self-limiting reaction, reaching an equilibrium after 25-20 min of summer UVB exposure for people with white skin, and producing no net increase in vitamin D production after that (77). Darker skin requires longer exposure but the potential yield of vitamin D is the same. Exposure of full skin surface to UVB light, in an amount less than erythemal, is equivalent to a vitamin D consumption of about 250 mcg (10 000 IU/d) (78-81). Lifeguards in the United States and in Israel, as well as farmers in the Caribbean all exhibit serum 25(OH)D concentrations greater than 100 nmol/L (82-84). Furthermore, even regular short periods in sun-tan parlors consistently raise serum 25(OH)D to beyond 80 nmol/L (85) (36;86;86-90). The highest 25(OH)D concentrations in the groups of adults acquiring vitamin D physiologically (via UV exposure) range up to 235 nmol/L (36;82), and none of these studies imply that such 25(OH)D levels have caused hypercalcemia. Since humans evolved as naked apes, whose native habitat was within 30 degrees latitude of the equator, I contend that our genome was selected under conditions of abundant vitamin D supply (69). As such, it is reasonable to think that the substantially lower levels of 25(OH)D prevalent among modern humans have been accompanied by biological compromises, such as increased PTH secretion and altered cellular metabolism. By now, these compromises may have been detrimental to the health of modern humans for so long, that we are no longer in a position to realize it.

Barger-Lux and Heaney studied the effect of sunshine on outdoor workers in the US Midwest, relating it to the vitamin D intakes needed to bring about the 25(OH)D levels observed. They concluded that the summertime supply from sunshine was approximately 2800 IU/d (70 mcg/d). Despite this supply during summer, it did not ensure sufficiency through the winter, when 25(OH)D was less than 50 nmol/liter in 3 of 26 subjects and less than 75 nmol/liter in 15 of 26 subjects.

### Table 4. The case against vitamin D2, compared to vitamin D3

<table>
<thead>
<tr>
<th>Vitamin D2</th>
<th>Vitamin D3</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not detectable in humans or primates unless administered from an artificial source</td>
<td>The natural metabolite generated within skin and the oils of fur</td>
<td>(146)</td>
</tr>
<tr>
<td>Vitamin D binding protein has lower affinity for vitamin D2 than for vitamin D3 and its metabolites</td>
<td>(147)</td>
<td></td>
</tr>
<tr>
<td>Generates metabolites for which there is no vitamin D3 equivalent</td>
<td>(148)</td>
<td></td>
</tr>
<tr>
<td>Microsomal 25-hydroxylase does not act on it</td>
<td>Substrate for both microsomal and mitochondrial 25-hydroxylases</td>
<td>(149;150)</td>
</tr>
<tr>
<td>Per mole of dose, 25(OH)D increases by less than with vitamin D3</td>
<td>(71)</td>
<td></td>
</tr>
<tr>
<td>The 25(OH)D response to vitamin D2 is less in the elderly than in younger adults</td>
<td>25(OH)D response to vitamin D3 is the same for young vs older adults</td>
<td>(144;151) (143)</td>
</tr>
<tr>
<td>All known cases of iatrogenic toxicity with vitamin D involved the vitamin D2 form</td>
<td>All known adult cases of toxicity with vitamin D3 have been unintentional, &quot;industrial&quot; accidents</td>
<td>(69;100) (111) (99)</td>
</tr>
<tr>
<td>Less stable in dose preparations</td>
<td></td>
<td>(trang1998) (71)</td>
</tr>
</tbody>
</table>
A complete understanding of the pharmacokinetics of the vitamin D system has eluded researchers. This is because of the technical issue of measuring the nanomolar quantities of vitamin D potentially embedded within tissues or excreted in catabolized forms. It is extremely difficult to detect or to measure vitamin D and its metabolites when they exist among great excesses of other lipids. Perhaps the most careful study into the fate of physiological amounts of cholecalciferol was reported by Lawson et al. (91;92). They exposed rats with shaved skin to ultraviolet light (UVB), and measured vitamin D and 25(OH)D in tissues at various times afterwards. Although adipose tissue concentration of vitamin D was never greater than the plasma concentration, it contained the largest exchangeable pool of vitamin D. Recovery of vitamin D3 in adipose was less than 5 percent of the amount produced within the skin (91), and this low recovery was attributed to vitamin D excretion into the bile. Lawson et al estimated that the volume of distribution of unmetabolized vitamin D3 was approximately four liters per kg (based on concentration decay curves from plasma and total amounts recovered from tissues). Vitamin D is not detectable in the adipose tissue of normal rats (92;93), but with administration of pharmacologic doses (94), or shaving of fur to increase yield 5-fold (91), vitamin D is detectable. Brouwer et al estimate the half-life of vitamin D in rat adipose tissue to be 96 days, which I find plausible because it compares with the functional half-life of 25(OH)D in humans (69). In contrast, Lawson et al estimated the vitamin D in adipose tissue to have a half-life of 13.8 days by (91). The more rapid half-life reported by Lawson et al was likely due to the younger age of the rats.

Since vitamin D is present in the body naturally, and it is not drug, it impossible to start with completely deprived individuals to do appropriate studies of pharmacokinetics. Furthermore the component of nutritional interest is 25(OH)D, and this is a metabolite of vitamin D3. Studies using isotopic techniques show that in humans, molecules of 25(OH)D have a plasma half-life of about 10 days (56;95). However, a more practical measure of the half-life of 25(OH)D is reflected in the rate at which 25(OH)D decays. Follow-up of 25(OH)D concentrations in adults intoxicated with vitamin D3 suggest that the functional in vivo half life is of the order of several months (98-100).

During summer, we accumulate vitamin D3 and store it, so that supplies for vitamin D do not become completely depleted during the winter months. Within three days of a dose of vitamin D3, very little of the original vitamin D is detectable in plasma of rats (101) or humans (102), while most vitamin D entering the circulation appears to be excreted unmetabolized into the bile. The highest total concentrations of vitamin D and its metabolites occur in plasma. However, since plasma represents only 2.5% of body mass, larger pools of vitamin D3 and 25(OH)D exist in adipose and muscle (103-105).

Vitamin D leaves tissue stores, and is utilized to sustain 25(OH)D concentrations over several months. When there is a continuous supply of vitamin D, like the situation for people who regularly expose a large proportion of their skin surface to tropical sunshine, an equilibrium is reached that maintains a balance between vitamin D stored within body compartments and the removal from tissue stores, its metabolism, and clearance. Under these physiologic circumstances 25(OH)D concentrations in plasma sustain levels of more than 200 nmol/L (69). At these levels of vitamin D nutrition, there has never been a concern raised that sudden loss of adipose tissue would either raise 25(OH)D or predispose to vitamin D toxicity. Likewise, despite 70 years of experience with the use of super-physiological amounts of vitamin D, there has never been a report of a sudden excess of vitamin D caused by release from adipose stores.

Storage Of Vitamin D In The Body

It is thought that since vitamin D is a fat-soluble vitamin, it must show preferential accumulation in adipose tissue (106;107). Two studies showed that following a defined dose of vitamin D or sunshine the rise in 25(OH)D was less for obese individuals than for people who weighed less. These studies did not show that adipose tissue concentrated vitamin D, and they failed to account for the obvious effect of a larger body compartment size, which should produce a lower concentration of anything, regardless of whether adipose plays a role or not. In our study using vitamin D3 doses of 100 µg/day in adults, we found no correlation between weight and serum 25(OH)D (108). At physiological doses, cholecalciferol (unmetabolized vitamin D3) distributes widely into tissues, not just to adipose, but to skeletal muscle and organs as well (91;105). As stated above, turnover of vitamin D stored in tissues produces a long half-life of vitamin D in the body, of about 2 months.

The amounts of vitamin D recoverable from tissue stores account for only a fraction of the dose administered (91). The animal data indicate that more than 3/4ths of the molecules of vitamin D that enter the body are catabolized and excreted without ever being stored in tissues, and without ever becoming 25(OH)D. The human data also support this. In humans, when vitamin D or 25(OH)D are given over the long-term, to achieve an equilibrium concentration of 25(OH)D, it takes more than 4 times as much vitamin D to produce the same 25(OH)D plateau (109). By definition, at that plateau in 25(OH)D, exchange of vitamin D with tissues is at equilibrium where release of stored vitamin D equals storage of new vitamin D. Still the 4-fold difference in efficacy at sustaining 25(OH)D exists when comparing effects of doses of 25(OH)D and vitamin D. That is, the difference in efficacy between 25(OH)D and vitamin D at sustaining plasma 25(OH)D concentrations cannot be explained by deposition of vitamin D into storage sites. The difference in efficacy at sustaining 25(OH)D can only explained by loss of vitamin D entering the circulation to fates other than 25-hydroxylation or storage.
VITAMIN D TOXICITY AND SAFETY ISSUES.

Amounts of vitamin D substantially greater than physiologic amounts (>250 mcg/day) are toxic because they preoccupy circulating vitamin D binding protein (DBP) and force the percent of vitamin D that is free and unbound to increase (69;110). At toxic doses, the freely circulating vitamin D, along with its metabolites, can accumulate not only in adipose (94) but also in muscle (105). The 100 µg/d vitamin D we have used in adults is physiologic and far below what would be needed to change the free fraction of vitamin D or its circulating metabolites (54). The average capacity of human plasma DBP to bind vitamin D and its metabolites is 4700 nmol/L (54), and this exceeds by 20 times the physiologic total concentration of its vitamin D-derived ligands.

The vast majority of cases of vitamin D intoxication have involved vitamin D2 (69). The situations involving vitamin D3, to date, have been industrial accidents (99;110;111) or poisonings from an unknown source (100). In our case, we assayed blood levels of vitamin D and its metabolites by chromatography and found that despite record-high 25(OH)D concentrations in humans (2400 nmol/liter), they were still small in comparison to a large excess of vitamin D3 (17,000 nmol/liter) suggesting that the capacity of liver to hydroxylate vitamin D is limited.

Like anything that has an effect on living things, vitamin D can be harmful if taken in excess. I contend that the ratio of the physiologically effective dose vs the toxic level for vitamin D is similar to the safety margin of many other nutrients (including even water). The reason vitamin D has been perceived as toxic was probably because daily ingestion in the milligram range has caused harm. In contrast, milligram amounts of other nutrients are benign. Toxicity in normal adults requires intake of more than 1000 mcg/day (40,000 IU/day), which reflects amounts of vitamin D that are four times more than the 250 mcg/day can be acquired naturally by sunshine (69). In what I see as an overreaction to the potential for toxicity with vitamin D, the current recommendation (called an “Adequate Intake” in North America) for adults under age 50 represents a homeopathic dose of about 2% of what adults with white skin would be making within 20 min of summer sun. In other words, the fear of vast excess has resulted in physiologically miniscule intake recommendations for adults.

Concentrations of 1,25(OH)2D are not increased much by vitamin D intoxication. This reflects the high level of regulation of this hormone via both its synthesis and catabolism. Nonetheless, vitamin D toxicity is probably manifest by the excessive levels of “free” 1,25(OH)2D, displaced from its carrier protein, DBP, by the vast excess of other vitamin D metabolites (112). This excess was confirmed by studies looking into “free” 1,25(OH)2D concentrations in vitamin D intoxicated individuals (110). This excess of metabolite over binding capacity was also confirmed by the high total of vitamin D and 25(OH)D concentrations (19500 nmol/L) in a patient intoxicated after consuming over a million units (>25 mg) daily for many months (100).

We recently reported a safety evaluation of vitamin D3 supplementation of normal adults, involving daily consumption of 100 mcg (4,000 IU). Contrary to the report by the Narang (113) that was used by the Food and Nutrition Board to establish the 50 mcg/d (2,000 IU/day) upper limit for vitamin D intake, this dose produced no detectable change in serum or urine calcium (108;114). The 25(OH)D results for this sort of study should be looked at in the context of the lowest and the highest 25(OH)D level attained with each dose. The objectives for establishing nutritional guidelines focus on the lowest level of 25(OH)D “ensured” by the given dose, while avoiding the possibility of risking an excess (108;115).

The official safety limit for vitamin D intake without supervision by a physician is referred to as the “upper limit” (UL) (114;116). This is the amount of vitamin D that the general public can take safely on a long-term basis with no anticipation of harm. Guidelines in both North America (67) and Europe (76) have established the UL as 50 µg (2000 IU)/day. This is a very conservative value that seems to remain the same, even though the evidence shows that higher intakes are safe. The value of 50 µg (2000 IU)/day has remained unchanged since it was mentioned in the 1968 Recommended Dietary Allowance publication as a dose approaching harmful level (117). To sustain the very conservative approach of making minimal changes to past recommendations, the only thing to change has been the safety margin applied, not the UL. For example when the "no observed adverse effect level" (the highest dose shown to have no harmful effect) was 2400 IU/day, based on a 1984 study (113), the safety factor applied by the United States food and nutrition Board was 1.2. When
subsequent data were published indicating that 4000 international unit/day was safe, the safety margin was increased by The European Commission to a value of 2.0 (76). Recent evidence in men shows that eight weeks of supplementation with 275 mcg/day of vitamin D does not affect circulating calcium concentration (i.e., the dose is non-calcemic, and causes no harm) (118). Even with the application of a safety factor of 2.75, this would suggest that 100 mcg (4000 IU) of vitamin D could be a safe adult UL for vitamin D. I predict that past patterns will hold true for official guidelines, and that the UL for vitamin D will remain unchanged at 50 µg (2000 IU)/day, and as a response to changing data, only the safety factor deriving that UL will change, to 5.5.

The weight of published evidence on toxicity shows that the lowest dose of vitamin D proven to cause hypercalcemia in some healthy adults is 1000 mcg (40,000 IU) per day of the vitamin D2 form (69) (Figure 4). This translates to 1000 micrograms, or 1 milligram, taken daily for many months. If a consumer wanted to achieve this toxic dose, he or she would need to take forty of the 1000 unit pills (the highest dose available in North America without a prescription) every day for many months.

Ten years ago, a dairy in the Boston area, servicing 10,000 households, made prolonged, gross errors in fortifying milk with hundreds of thousands of units (several milligrams) per quart. The case was published quickly (119) and covered by the media. The more rigorous epidemiological follow-up was published later. That showed that the situation contributed to two deaths of susceptible elderly (111). While hypercalcemia did occur, it was not widespread. By far the most susceptible group to the excess vitamin D was women over age 65 years of age, suggesting that diminished renal function may play a role. The average 25(OH)D concentration of the confirmed cases of vitamin D toxicity was 900 nmol/L (214 ng/mL) (111); in comparison, physiologically attained 25(OH)D concentrations reach 235 nmol/L safely, without hypercalcemia.

When physiologically higher vitamin D nutrition is associated with hypercalcemia, this reflects aberrant control of 25(OH)D-1-hydroxylase. This would reflect either primary hyperparathyroidism, where PTH continuously stimulates the enzyme in the kidney (50), or granulomatous disease, where peripheral tissue loses ability to regulate the 1-hydroxylase that normally serves a paracrine role (69;120).

If people with abundant sun exposure (25(OH)D > 150 nmol/L) the pre-supplement supply of vitamin D could be equivalent to about 100 mcg/d (121). An additional physiological dosage by mouth of 100 mcg/day of vitamin D would still be less than the dose of vitamin D shown to be safe in a recent study (122). As a further example of this point, we reported that 25 mcg/d of vitamin D resulted in average 25(OH)D concentrations of 69 nmol/L, while four times that amount increased 25(OH)D concentrations by only another 27 nmol/L (108). The increment with each additional amount of vitamin D becomes progressively smaller as the pre-dose 25(OH)D level increases. Thus, a further 100 mcg/d would add marginally to what I regard as the inconsequential risk due to the 250 mcg/d vitamin D supply that is physiological because it is obtainable through sun exposure. Since a long-term vitamin D consumption of at least 1000 mcg/d would be needed to cause hypercalcemia, there is a large margin of safety with 100 mcg/d. (I would welcome any of discussion of evidence implicating harm with vitamin D3 (not D2) in adults at doses below 1000 mcg/d. There is simply nothing published about this, except in infants.)

One concern sometimes expressed, is that if adipose tissue were to break down, a sudden influx of vitamin D from adipose might be toxic (123). I want to address this issue. In both rats and cattle, high doses of vitamin D are needed before vitamin D ends up as detectable in adipose tissue (94;105). Despite being present in “significant” amounts in tissues, storage in tissues is not efficient. As a proportion of what enters the body via the skin or the diet, the amounts of vitamin D stored in adipose are a fraction of the total. In normal humans, adipose tissue content of vitamin D has been reported to be as high as 116 ng/g (approx 5 IU/g adipose) (92). In cattle intoxicated with 7.5 million IU vitamin D (to cause hypercalcemia, in an experimental process to activate proteases to make beef more tender after slaughter), muscle levels of vitamin D reached 91 ng/g tissue (4 IU/g). The highest tissue level reported in those animals was in the liver, which contained vitamin D at 979 ng/g (39 IU/g) (105). The point here is, that while there is “meaningful” storage of vitamin D in tissues, all the evidence to date indicates that only a fraction of any vitamin D dose ends up in tissues to be withdrawn at later times.

If there were a sudden breakdown of 1 Kg of adipose tissue, or liver, this would release as much as 979 mcg (39000) IU of vitamin D into the body. A toxic excess of vitamin D would require the break down daily of one Kg of adipose tissue that had been primed with by prior vitamin D intoxication, with daily adipose catabolism to continue for several weeks. When toxic doses of vitamin D are administered, the effect will be manifest during the period of administration. There is no evidence that enough residual vitamin D can be stored in adipose tissue that vitamin D toxicity could possibly arise at some later time, because of weight loss.

**SUMMARY**

To conclude, I return to the pharmacological questions posed at the start of this chapter, and offer Table 5 as a way to address the issues, based on the material in this chapter.
<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a. What is the disease indication for the drug?</td>
<td>Fracture prevention; preservation of bone mineral density; normalization of PTH levels</td>
</tr>
<tr>
<td>1b. What kind of clinical or health effects should we be looking for, based on preclinical animal and laboratory research?</td>
<td>Disease prevention: cancer, diabetes, multiple sclerosis, high blood pressure, fibromyalgia.</td>
</tr>
<tr>
<td>2a. What are the most useful approaches to delivering the drug to people: the vehicle,</td>
<td>Encouragement to expose a large percent of skin to summertime sunshine, 10 min daily. Fortification of foods to physiologically meaningful levels of vitamin D, consumption of supplement preparations with vitamin D.</td>
</tr>
<tr>
<td>2b. The dosage, route of administration, and Dosage depends on target 25(OH)D desired. We can assume a rule of thumb, that vitamin D at 1 mcg/day increases 25(OH)D by 1nmol/L, after 8 months of use. Oral vitamin D is probably more effective at increasing than injection.</td>
<td></td>
</tr>
<tr>
<td>2c. Interval between doses?</td>
<td>Since the half-life for decline in 25(OH)D is effectively 2 months, doses of vitamin D could be given monthly (we use weekly in our studies). Less frequent dosing than once every 2 months will generate large fluctuations in 25(OH)D concentrations that may not be desirable, because the enzymes involved in the regulation of vitamin D metabolism are functioning in a 1st order relationship with substrate.</td>
</tr>
<tr>
<td>3. What is the desirable target for the plasma concentration, what dose would be needed to attain or ensure this?</td>
<td>Current consensus points to a goal of ensuring that 25(OH)D levels be higher than 70-100 nmol/L. Note that to ensure this level for those with the weakest response to vitamin D, we need to aim for an average 25(OH)D concentration of about 120 nmol/L. These objectives are achieved with a dose that averages 100 mcg/day for all adults.</td>
</tr>
<tr>
<td>4. What, if any, are the biological markers to monitor toxicity, and what are our criteria for determining therapeutic effectiveness? What is the &quot;therapeutic index&quot; the ratio between toxic vs beneficial dose levels?</td>
<td>Hypercalcemia is the classic criterion for toxicity of vitamin D and its metabolites or their analogues. “Non-calcemic” doses are considered “safe”. The most sensitive clinical index of excessive vitamin D-related effects is probably hypercalcuria, which would logically occur at lower doses than hypercalcemia. Of greater concern for the long-term use of vitamin D, its metabolites or analogs should be effects on soft-tissue calcification, within aorta, kidney or other tissues. These effects may be seen radiologically in humans, or by direct measure in pre-clinical animal studies.</td>
</tr>
</tbody>
</table>
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