Review

Can Vitamin D Be Supplied from the Large Intestine?

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Can Vitamin D Be Supplied from the Large Intestine?

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Abstract: The discovery that vitamin D_2 is being generated by anaerobic microbial metabolism in the alimentary tract, raises the question whether such a source of vitamin D could contribute to vitamin D supply for the animal hosting this microbial production system. In ruminants, this microbial generation in the forestomach allows vitamin D_2 to be readily absorbed when it reaches the small intestine, contributing to vitamin D_2 and 25-hydroxyvitamin D_2 [25(OH)D_2] found in their tissues. In monogastric animals like humans, the microbial generation of vitamin D_2 is occurring in the large intestine. There is evidence that vitamin D hydroxy metabolites, delivered to the lumen of the colon can be absorbed. However, the parent vitamin D is more lipophilic than its metabolites, and like lipophilic vitamin K_2 being produced by bacteria in the hindgut, may be poorly absorbed by the colon mucosa. It is now apparent that colon mucosal cells have the proteins megalin and cubilin in their basal membrane. These glycoproteins perform endocytosis of circulating proteins including vitamin D binding protein [DBP]. Inside the cell, DBP binds to cytoplasmic actin and thus provides an array of high affinity binding sites for vitamin D and its functional metabolites. Any traces of vitamin D_2 that may diffuse into the colon mucosal cells from the lumen would thus be retained and accumulate on the DBP-actin. It would then be a substrate for functional hydroxylase metabolism for local endocrine action in these cells, and subsequent delivery of 25(OH)D_2 by diffusion to apo-DBP in the circulation.

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Introduction

The conventional approach to vitamin D, is that it is a micronutrient, one of 4 fat-soluble vitamins, along with vitamins A, E, and K. Dietary vitamin D is absorbed in the small intestine by a passive mechanism in association with dietary fatty acids [1,2,3]. If the diet is low in fat or if there is a defect in the digestion of fat and absorption of dietary fatty acids, then vitamin D along with the other fat-soluble vitamins, fails to enter the small intestine mucosal cells for subsequent transport in blood in lipoprotein or chylomicron lipid. If these conditions persist then fat-soluble vitamin deficiency could develop.

However, vitamin D status in populations around the world is largely determined by the formation of vitamin D_3 in skin by the photochemical action of solar ultraviolet radiation on 7-dehydrocholesterol [4]. Nevertheless, public health policies promote the concept that vitamin D deficiency is to be prevented by the intake of diets supplemented with oral vitamin D [5]. Although vitamin D_3 (cholecalciferol) produced in skin, is the natural form of vitamin D for humans and many other terrestrial animals, vitamin D for dietary supplementation, is often vitamin D_2 (ergocalciferol) (Figure 1), because of the ease of its commercial production by ultraviolet irradiation of ergosterol from yeast [6]. Furthermore, for humans [7] but not all terrestrial mammals [8], the biological activity of vitamin D_2 is comparable, although not identical to that of vitamin D_3 [9].

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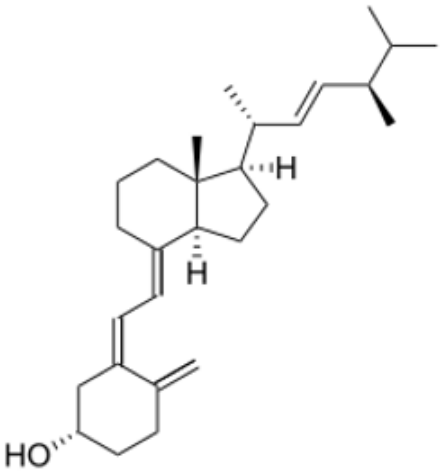
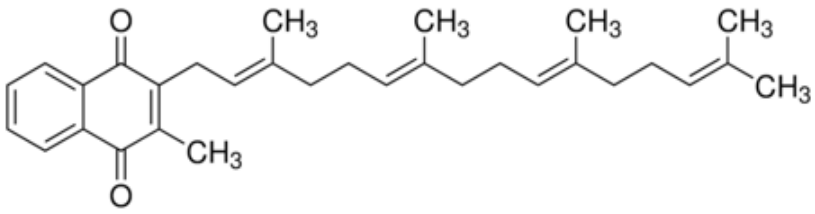


Vitamin D_2 (Ergocalciferol)
Molecular Weight: 396.63



Vitamin K_2 (Menaquinone-4)
Molecular Weight: 444.65

Figure 1. Comparative molecular structures of vitamin D_2 and vitamin K_2 (MK4) of comparable polarity and molecular weights.

Surprisingly, some animals like horses [10] and elephants [11] seem unable to produce vitamin D_3 in their skin and circulating 25-hydroxyvitamin D [25(OH)D] is derived from vitamin D_2. The assumption is that the source of this vitamin D_2 is from endophytic fungi exposed to sun light on the herbivorous diet these animals are eating. Some of the ergosterol in these fungi would thus be converted to vitamin D_2 by solar UV radiation [12].

Vitamin D Presentation to the Colon

As well as oral input, it is now apparent that vitamin D_2 is also being produced metabolically in the alimentary tract by anaerobic microorganisms with no exposure to ultraviolet light [13]. This vitamin D_2 production is occurring in the fermentation chamber of the rumen of ruminant animals, such as sheep and cattle. Metabolic production in this forestomach, allows the vitamin D_2 to be absorbed when the food mass is digested in the small intestine and contributes to the vitamin D_2 and 25(OH)D_2 found in ruminant meat [14]. In monogastric animals like mice, microbial fermentation occurs in the colon and caecum, after food has already been processed in the small intestine. Microbial production of vitamin D_2 has now also been demonstrated in the colon of mice [13], so the question needs to be asked: is vitamin D_2 which is being generated in the large intestine of monogastric animals, including humans, able to be absorbed from that site, and will it then function either locally in the colon mucosa or systemically in the whole body?

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The vitamin D endocrine function, performed by 1,25-dihydroxyvitamin D [1,25(OH)$_2$D], regulates gene expression in many cell types throughout the body. The activity of 1,25(OH)$_2$D has a particular role in the large intestine in maintaining the mucosal cell barrier against bacterial invasion and acting on the immune cells to inhibit inflammation [15]. These functions are protective against inflammatory bowel disease and colon cancer [16]. However, the mucosa of the large intestine has the special feature of a complete capability to convert the parent vitamin D molecule to the hormone 1,25(OH)$_2$D, for its local autocrine or paracrine action in the mucosa.

Colon Mucosal Metabolism of Vitamin D

The human colon mucosal cells [17] contain the two vitamin D 25-hydroxylases, CYP27A1 and CYP2R1, which would in theory convert vitamin D, delivered to the mucosa in the circulating blood, to 25(OH)D. This could then be converted to 1,25(OH)$_2$D by the 1alpha-hydroxylase, CYP27B1, also present in these cells [18,19], for its subsequent local regulatory functions in the colon mucosa. The maintenance of good vitamin D status, with serum concentrations of 25(OH)D >50 nmol/L, thus enables adequate local 1,25(OH)$_2$D production to perform its protective roles in the large intestinal mucosa [16].

Absorption Properties of Colon Mucosa

The absorptive functions of the large intestine mucosa include the uptake of water, electrolytes, and microbially produced short chain fatty acids [20]. But, compared to the wide range of absorptive functions of the small intestine mucosa, the absorption capacity for organic molecules by the colon mucosa is limited. This can be seen from studies on the bacterial production of vitamin K$_2$ (menaquinone) in the lumen of the large intestine. This lipophilic molecule is an essential component of the bacterial electron transport system particularly in Gram-positive bacteria, as well as having a role in animal physiology as an enzyme co-factor in the production of blood-clotting proteins [21]. The chemical structure of vitamin K$_2$ consists of a 2-methyl-1,4-naphthoquinone with a side chain of variable numbers of 5-carbon isoprene units. The shortest side chain has 4 isoprene units and is known as menaquinone-4 or MK-4 (Figure 1), while the longest side chain has 13 such units. In human feces the total vitamin K$_2$ concentration is as high as 34.5 nmol/g dry matter with MK-4 being about 1.4 nmol/g dry matter (0.6 μg/g dry matter) [22]. Compared with the concentration of vitamin D$_2$ in mouse colon contents of 0.04 μg/g dry matter, the concentration of MK-4 is15 times greater.

How much MK-4 then gets absorbed across the colon mucosa? Studies with radioactively labelled vitamin K presented into the lumen of the colon in rats indicated that very little was absorbed, and the absorbability declined even further as the number of side-chain isoprene units increased [23,24]. Even though colonic bacteria are producing quantities of vitamin K$_2$ in amounts that would more than meet the needs of this micronutrient in human physiology, very little is available because of its very limited absorption across the colon mucosa.

Colon Absorption of Vitamin D Metabolites

How then would even smaller quantities of vitamin D$_2$ being generated by microbial metabolism in the large intestine have any chance of contributing to vitamin D status of a human or other terrestrial mammals? An ingenious method of testing the possible uptake of vitamin D and its metabolites by the large intestinal mucosa, is to provide oral intakes of these molecules which have been linked to glucuronic acid. When 1,25(OH)D$_3$ was conjugated with glucuronic acid through the 25-hydroxy group (Figure 2A) and given orally to rats it passed along the length of the small intestine without any absorption because uptake into mucosal cells was prevented by the conjugated glucuronic acid. On arrival in the large intestine, bacterial glucuronidase released 1,25(OH)D$_3$ [25]. This hormonal form of vitamin D was presumably absorbed by the colon mucosal cells because there was a resultant upregulation of the vitamin D 24-hydroxylase gene (CYP24) in those cells [25,26]. Likewise, when the 3β-glucuronide of 25(OH)D$_3$ (Figure 2B) passed into the lumen of the mouse

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colon, 25(OH)D_3 was released by cleavage of the glucuronide ligand and there was a consequent increase in mRNA for the CYP24 enzyme [27].

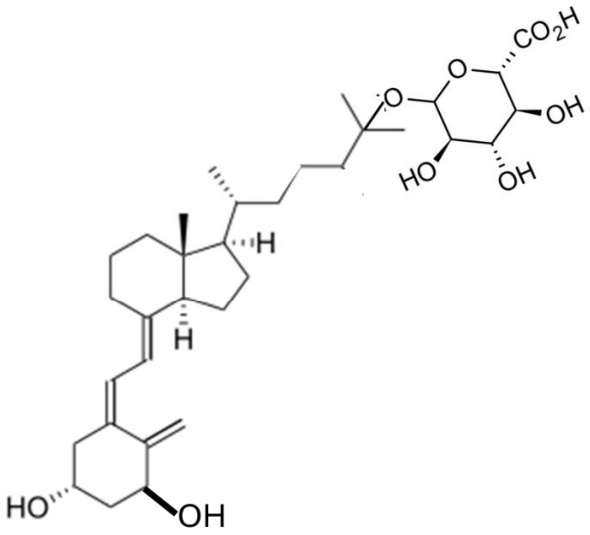

Figure 2. A: Molecular structure of 1,25-dihydroxyvitamin D_3 – 25-glucuronide.

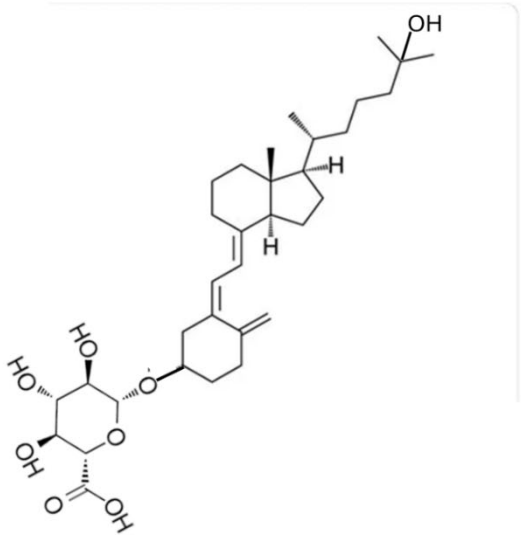

Figure 2. B: Molecular structure of 25-hydroxyvitamin D_3 – 3-glucuronide.

Although these experimental results are good indications that the colon mucosa is able to absorb 25(OH)D_3 and 1,25$(OH)_2D_3$ they don't conclusively indicate that the more lipophilic parent vitamin D_2 would also be absorbed. The 25-hydroxylated vitamin D metabolite has long been postulated to be delivered to the large intestine naturally as a glucuronide conjugate, which is generated in the liver and excreted in bile into the duodenum. It has been suggested that this output of 25(OH)D-glucuronide was part of an enterohepatic circulation of 25(OH)D [28]. The output of such

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glucuronides in bile, along with a range of other polar catabolites of vitamin D, has long been known following injection of radioactively labelled vitamin D [29].

However, when 7 μg ^{14}C-labelled vitamin D_3 was injected intravenously into humans with bile duct cannulations, most of the radioactive substances excreted in bile were polar metabolites and only about 4% of the radioactivity was in the form of 25(OH)D_3 or its glucuronide conjugate [30]. Such experiments present the liver with a bolus input of vitamin D, as indeed does oral intake of vitamin D in food or supplements. In contrast, when vitamin D_3 is formed by ultraviolet radiation in skin it trickles very slowly into the circulation and likewise enters the liver at the same rate as its metabolite 25(OH)D_3 is being released back into blood [31]. Hence 25(OH)D glucuronide would only be delivered to the colon from the relatively unphysiological supply of vitamin D from oral ingestion.

The Intracellular Role of Vitamin D-Binding Protein

The key component determining the delivery of vitamin D and its metabolites around the body is a specific vitamin D-binding protein (DBP) in the circulating blood [32]. This protein has a single binding site for the vitamin D molecular structure. For human DBP the binding affinity is highest for 25(OH)D_3 with a 10-fold lower affinity for 1,25(OH)$_2D_3$ [33,34]. Although the affinity for vitamin D_3 itself is lower again than those of its metabolites [34], DBP still has the capacity for the specific binding of unchanged vitamin D. The concentration of DBP in human plasma is approximately 6 μmol/L, so with a normal 25(OH)D concentration of 50-100 nmol/L only about 1-2% of DBP in the circulation has a 25(OH)D ligand in its binding site [32]. The vast majority of apo-DBP therefore ensures that the vitamin D metabolites are tightly associated with DBP which prevents their uncontrolled diffusion into cells.

A special mechanism exists which causes endocytosis of extracellular proteins into various cell types. This mechanism consists of two proteins, megalin and cubilin in the plasma membrane of cells, such as those of the proximal renal tubules [36] and of skeletal muscle [37]. Megalin, a large transmembrane glycoprotein, in association with membrane cubilin, binds DBP and other extracellular proteins and transfers them into the cell cytoplasm. DBP, as well as having a single binding site for the vitamin D molecular structure, also has a specific binding site for the protein actin [32]. When DBP is internalised by the megalin/cubilin mechanism into skeletal muscle cells, it binds to cytoplasmic actin and thus provides an array of intracellular binding sites which by binding 25(OH)D allows this vitamin D metabolite to accumulate when traces diffuse into the cell [37]. Because the vast majority of DBP molecules in the extracellular fluid are in the apo-DBP state, the vitamin D-binding site on DBP being internalised by the megalin mechanism would be vacant, so that 25(OH)D would only enter the cell by simple diffusion, rather than being transported already bound to DBP. The actin-bound DBP in skeletal muscle cells has a short residence time and soon undergoes proteolysis [38]. The 25(OH)D, thus released from the DBP cytoplasmic actin complex, then diffuses out of the cell under the influence of the high concentration of apo-DBP in the extracellular fluid.

Absorption Mechanism for Vitamin D_2 in Colon Mucosal Cells

The discovery that colon mucosal epithelial cells have both megalin [39] and cubilin [40] in their plasma membrane thus provides a mechanism for internalisation of DBP from the circulation into those cells. This DBP, as in skeletal muscle cells, would bind to cytoplasmic actin in the colon epithelial cells, and would create an array of high affinity specific binding sites to allow any unbound 25(OH)D or vitamin D in the circulation which diffuses into those cells to be retained and be available as substrates for the 1-hydroxylase or 25-hydroxylase vitamin D metabolising enzymes. The high concentration of apo-DBP in the extracellular fluid would then also induce 25(OH)D to exit the mucosal epithelial cells.

The presence of the DBP-actin complex in the colon epithelial cells would also explain how the small quantities of 25(OH)D and 1,25(OH)$_2$D delivered to the lumen of the colon would be absorbed, retained and be functional in those cells [26,27]. The high concentration of DBP in the circulation also has a role in inducing vitamin D_3 produced in the skin keratinocytes to exit those cells for subsequent

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delivery to the liver [41] This was confirmed when DBP-knockout rats were found to be unable to release vitamin D_3 produced in those skin cells [42]. Therefore, by analogy, vitamin D_2 being generated by microbial metabolism in the lumen of the colon would also be induced by intracellular DBP to accumulate in the mucosal cells, even if only traces were to diffuse slowly across the luminal membrane. Furthermore, this internalised vitamin D_2 would then be available as substrate for the 25-hydroxylase enzymes in those cells and subsequently for the 1-hydroxylase. Thus, vitamin D_2 generated in the lumen of the colon, would become available to meet the requirements for the autocrine or paracrine functions of $1,25(OH)_2D$ in the mucosal tissue. Because blood in the submucosal capillaries of the colon would have a much higher concentration of apo-DBP than that in the mucosal cell cytoplasm, $25(OH)D_2$ being produced in those cells would be induced to diffuse into the extracellular fluid and thence be transported, bound to DBP into the general circulation (Figure 3). Such an affinity-diffusion process in the colon mucosa of elephants and horses could also be an explanation for the origin of $25(OH)D_2$ as the main form of vitamin D in the blood of those species.

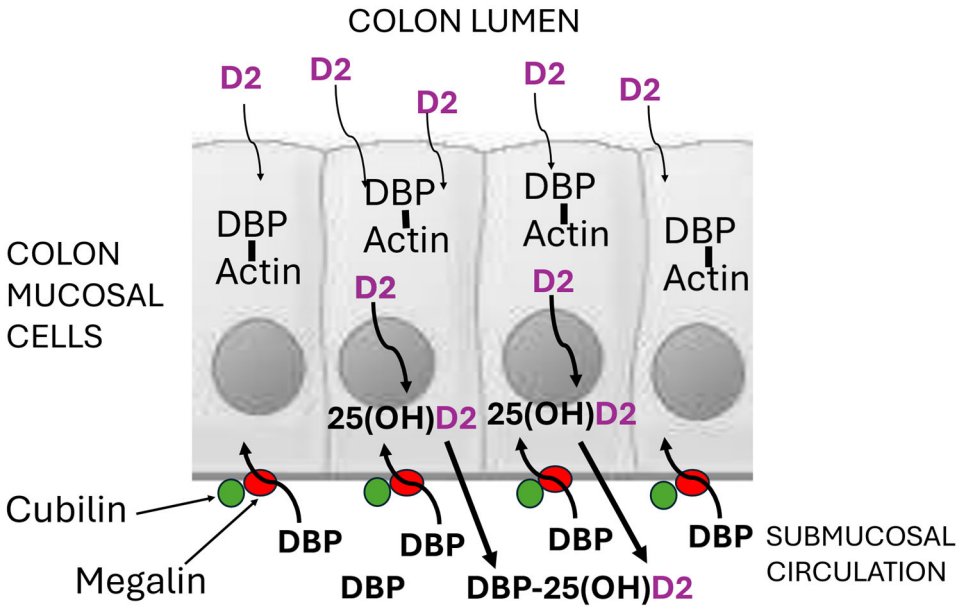

Figure 3. Schematic diagram of colon mucosal cells with megalin and cubilin in the basal membrane, enabling apo-DBP in the submucosal circulation to enter the cells by endocytosis. Within the cells, apo-DBP binds to cytoplasmic actin to provide an intracellular array of vitamin D-specific binding sites which retain the traces of microbially produced vitamin D_2 diffusing in from the lumen of the colon. This intracellular vitamin D_2 would then be metabolised to 25-hydroxyvitamin D_2 and then to 1,25-dihydroxyvitamin D_2 by CYP2R1 and CYP27B1 hydroxylase enzymes within those cells. The high concentration in the submucosal blood supply of apo-DBP with its high affinity for 25(OH)D, would also induce $25(OH)D_2$ to diffuse from the cells into the circulation.

Conflicts of Interest: The author declares no conflict of interest.

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