Proceedings of the Nutrition Society

cambridge.org/pns

The Nutrition Society of Australia 2025 was held at the Gold Coast Convention & Exhibition Centre on 3rd - 5th December 2025

Conference on 'Nourishing Generations: 50 years of the Nutrition Society of Australia'

Symposium 2 - Micronutrient issues

Review Article

Cite this article: Fraser DR and Mason RS (2026). Microbial biosynthesis of vitamin D_2. *Proceedings of the Nutrition Society*, page 1 of 7. doi: 10.1017/S0029665126103073

Received: 19 March 2026
Revised: 29 April 2026
Accepted: 7 May 2026

Keywords:
Microbial biosynthesis; Vitamin D_2; Colon microbiome; Colon absorption

Corresponding author:
David Fraser;
Email: david.fraser@sydney.edu.au

© The Author(s), 2026. Published by Cambridge University Press on behalf of The Nutrition Society. This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted re-use, distribution and reproduction, provided the original article is properly cited.

Microbial biosynthesis of vitamin D_2

David R. Fraser[1] and Rebecca S. Mason[2]

[1]Sydney School of Veterinary Science, Faculty of Science, The University of Sydney, Australia and [2]School of Life and Environmental Sciences, School of Medical Sciences and Charles Perkins Centre, The University of Sydney, Australia

Abstract

In the early history of vitamin D research most of the studies on its chemistry and function were performed with vitamin D_2 which was readily obtained by UV irradiation of ergosterol from yeast. Yet, in the physiological economy of vitamin D for most vertebrates, including humans, fish and especially for birds, vitamin D_3 produced in skin by solar irradiation of 7-dehydrocholesterol, is the natural form of vitamin D. Vitamin D_2, as a dietary supplement, while of comparable potency to vitamin D_3 in most mammals, has been found in nature only when ergosterol in fungi is inadvertently exposed to solar UV radiation. Nevertheless, some herbivorous animals, horses and elephants, seem to maintain adequate vitamin D status with vitamin D_2 rather than vitamin D_3. The source of that vitamin D_2 has been assumed to be the traces derived from ergosterol in endophytic fungi exposed to the sun on grass being consumed. However, outdoor grazing sheep in winter maintained adequate vitamin D status with vitamin D_2, yet no vitamin D_2 could be detected on the grass they were consuming. Bovine rumen contents, fermenting in an artificial rumen, had an increase in vitamin D_2 concentration, particularly when cellulose fibre was added as a fermentation substrate. Furthermore, mice being raised from weaning on a vitamin D-free diet had vitamin D_2 in their colon contents. This review examines anaerobic microbial production of vitamin D_2 in the alimentary tract, in the dark, and describes a natural function for vitamin D_2 in microorganisms and potentially for gut health.

Introduction

This review examines the evidence for the novel idea that microorganisms in the alimentary tract synthesise vitamin D_2. In monogastric animals, including the human, such biosynthesis would be in the contents of the large intestine, which raises the question whether this vitamin D_2 could be beneficial not only for the microorganisms but also for the health of the host animal. If there were a health advantage for gut microbial generation of vitamin D_2, then understanding this metabolic process could suggest strategies, particularly nutritional strategies, to promote its biosynthesis.

Vitamin D, although usually considered as a nutrient, is a precursor of a steroid hormone with widespread regulation of gene expression in many organs and tissues[1]. It is derived from the action of short wavelength solar ultraviolet radiation (290–315 nm) on 7-dehydrocholesterol to produce vitamin D_3 and on ergosterol to produce vitamin D_2 (Fig. 1(a)). For its role as a steroid hormone, vitamin D has to be metabolised first to 25-hydroxyvitamin D [25(OH)D] and then to the functional hormone, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] (Fig. 1(b)). Vitamin D and its metabolites are transported in blood mainly bound to a specific vitamin D-binding protein (DBP), with 25(OH)D being the most plentiful metabolite, and where its concentration is an indicator of vitamin D status.

When vitamin D was discovered, over 100 years ago, its molecular structure was determined using vitamin D_2 derived from UV irradiation of ergosterol from yeast[2,3]. Because vitamin D deficiency causes the bone disease of rickets, vitamin D_2 supplied in the diet was used to investigate the vitamin D effect on bone development[4], and then its more general role in calcium homeostasis[5]. Many studies have shown that vitamin D_2 has comparable, but not identical, biopotency to that of vitamin D_3 in maintaining vitamin D status in humans[6] and also in preventing rickets in rats[7]. Nevertheless, because vitamin D status in most terrestrial vertebrates is determined mainly by the supply of vitamin D_3 by solar UV radiation of 7-dehydrocholesterol in skin[8], and because most foods contain negligible quantities of either vitamin D_2 or vitamin D_3[9], then vitamin D_2 appears to be a chemical curiosity with little natural relevance to the biological role of vitamin D. On the other hand, horses[10] and elephants[11] do not produce vitamin D_3 in their skin when exposed to sunlight and the major vitamin D metabolite in blood is 25$(OH)D_2$. Their vitamin D status has therefore been interpreted as dependent on vitamin D_2 produced from solar irradiated ergosterol in endophytic fungi in the plants they consume.

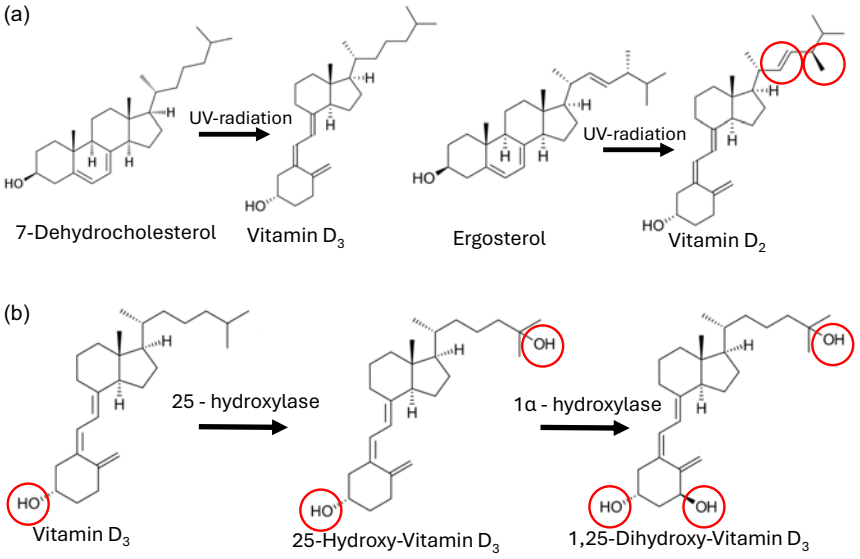


Fig. 1. **(a)** Ultraviolet radiation of 7-dehydrocholesterol produces vitamin D_3. Ultraviolet radiation of ergosterol produces vitamin D_2 which differs from vitamin D_3 by having a 22–23 double bond and a 24-methyl group in the sidechain (indicated with red circles). **(b)** Conversion of vitamin D_3 to a steroid hormone requires an oxygen atom at either end of the long axis of the molecule and another added oxygen to enable specific binding to the vitamin D receptor protein for regulation of gene expression. Vitamin D_3 therefore undergoes 25-hydroxylation and then 1α-hydroxylation (indicated with red circles) to produce the hormonal structure of 1,25-dihydroxy-vitamin D_3.

Studies in ruminants

The vitamin D status of Merino sheep grazing out of doors throughout the year, feeding only on natural pasture, was assessed by measuring the blood plasma concentration of 25(OH)D_2 and 25(OH)D_3 by liquid chromatography-tandem mass spectrometry[12]. The mean total 25(OH)D concentration was 18.1 ng/ml in summer and 23.3 ng/ml in winter. In both seasons the 25(OH)D_2 was a substantial proportion of the total 25(OH)D concentration at 40.9% in summer and 76.8% in winter. Yet vitamin D_2 analysed in the pasture grass on which these sheep were grazing, was below the level of detection.

To explore whether vitamin D_2 was present and could be derived from the alimentary tract of ruminants, the rumen contents of a Friesian cow grazing on the same pasture as the sheep[12] were collected through a surgically created permanent rumen fistula. The liquid obtained by compressing the contents was analysed for vitamin D by liquid chromatography-tandem mass spectrometry. Six samples of fresh untreated rumen liquid contents contained 0.64 ± 0.08 μg vitamin D_2/g dry matter. Such rumen samples were then incubated anaerobically for 24 h in the dark in a 'Rusitec' artificial rumen apparatus[13]. The vitamin D_2 content after incubation had increased by 93.8% to 1.94 ± 0.06 μg/g dry matter. Additional Rusitec experiments on bovine rumen contents with cellulose powder added as a microbial fermentation substrate, showed further increases in vitamin D_2 content. These findings indicated that rumen microorganisms were biosynthesising vitamin D_2.

Studies in monogastrics

To determine whether microbial production of vitamin D_2 could occur also in the alimentary tract of a monogastric animal, weanling C57BL/6 mice, in a room with no ultraviolet light, were fed on a vitamin D-free maize meal diet. After 3 weeks the large intestine contents of these mice were found to contain 0.04 μg vitamin D_2/g dry matter, but analysis of the diet did not detect any vitamin D_2[12]. Earlier studies in which rats were raised and bred on a vitamin D-free diet for two generations, found that although there was no detectable 25(OH)D in the blood plasma of the second-generation rats, the vitamin D hormone, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], was detected at 30–40 pg/ml[14]. The analytical methods for vitamin D metabolites at that time could not distinguish whether this metabolite was derived from vitamin D_2 or from vitamin D_3. It is notable that vitamin D_2 was found to have higher potency than vitamin D_3 when fed to rats, whereas in pigs and chickens the reverse applies[15]. The microbial production of vitamin D_2 in the alimentary tract is therefore postulated as an explanation as to how nocturnal animals that are never exposed to the sun and where their diet is devoid of vitamin D, could maintain adequate vitamin D status. It has been apparent for many years that rats are able to survive and reproduce when maintained on a vitamin D-free diet[16], whereas chickens on such a diet rapidly develop signs of vitamin D deficiency, as vitamin D_2 has very low potency in birds[17].

Potential mechanisms of vitamin D_2 biosynthesis

Because ergosterol from fungi and yeasts is the well-established precursor of vitamin D_2, produced when these organisms are exposed to UV radiation, and because the fungus family of *Neocallimastigomycota* are a component of the gut microbial population[18], an early hypothesis for the biosynthesis pathway was that gut fungi were generating vitamin D_2 by metabolically splitting the 9–10 carbon bond of their endogenously produced ergosterol. However, it is well established that ergosterol biosynthesis requires molecular oxygen[19], so the anaerobic environment of either the rumen of ruminants or the large intestine of monogastrics is not favourable for gut fungi to be generating ergosterol. Thus, if ergosterol were the precursor of the vitamin D_2 being produced by microbial metabolism in the alimentary tract, it would have been derived from the food being consumed, rather than being synthesised by microorganisms in the gut. Microbial metabolic modification of steroid structures is well known. For example, intestinal bacteria have a wide range of steroid metabolising functions such as hydrogenation and dehydrogenation of steroid C-C bonds as well as dehydroxylation of steroid hydroxyl groups[20]. Anaerobic bacteria have also been shown to be capable of steroid hydroxylation[21] which could be a theoretical step in the metabolic cleavage of the 9–10 bond of ring B of ergosterol as the precursor in the biosynthesis of vitamin D_2.

https://doi.org/10.1017/S0029665126103073 Published online by Cambridge University Press

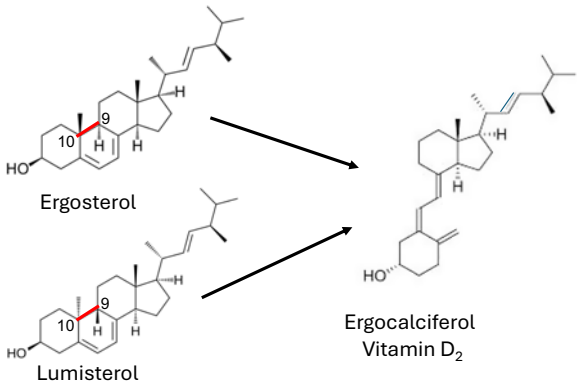


Fig. 2. Molecular structure of isomeric 9α 10β-ergosterol and 9β 10α-lumisterol, the substrates for the proposed microbial biosynthesis of ergocalciferol (vitamin D_2)[27]. The 9–10 carbon bond in ring B that would be cleaved by microbial metabolism is indicated in red.

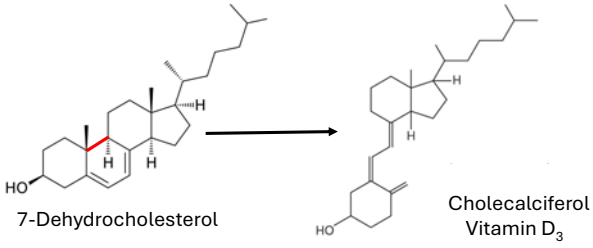


Fig. 3. Molecular structure of 7-dehydrocholesterol, the substrate for the proposed microbial biosynthesis of cholecalciferol (vitamin D_3) by *Faecalibacterium prausnitzii*[29]. The 9–10 carbon bond in ring B that would be cleaved by microbial metabolism is indicated in red.

Therefore, it is postulated that dietary ergosterol could be the substrate for microbial production of vitamin D_2 in the anaerobic environment of the alimentary tract contents. Studies of orally supplied ergosterol have shown that some is absorbed[22,23], but when tritium-labelled ergosterol was given orally to rats, most of the labelled molecules were excreted unchanged in faeces[24]. Hence dietary ergosterol would be available as a potential substrate for microbial metabolism in the large intestine of humans and other monogastric animals. Because endophytic fungi are naturally present in plant seeds[25], then laboratory animal diets based on cereal grains, even if devoid of vitamin D, would contain ergosterol from this fungal presence which would be available for gut microbial metabolism.

Studies on the effect of long-term feeding of ergosterol on cholesterol biosynthesis in rats, reported as a coincidental observation that vitamin D_2 was then detected in blood plasma[26]. In similar experiments where lumisterol$_2$, the 9β,10α-stereoisomer of 9α,10β- ergosterol, was fed to mice, vitamin D_2 was then found in a number of tissues[27] (Fig. 2). Likewise, the feeding of 7-dehydrocholesterol to mice, the natural precursor substrate for vitamin D_3 production by UV irradiation of skin, resulted in increased vitamin D_3 concentration in liver and kidney without any exposure of the animals to ultraviolet lighting[28] (Fig. 3). These reports of increased tissue vitamin D concentration following oral supply of ergosterol, lumisterol$_2$ or 7-dehydrocholesterol are all compatible with the concept of gut microbial enzyme cleavage of the 9–10 bond in the steroid B-ring of these vitamin D precursors to produce the vitamin D molecular structure.

More direct evidence for gut microbial biosynthesis of vitamin D has been claimed for two bacteria isolated from the human large intestine: *Carnobacterium maltaromaticum* and *Faecalibacterium prausnitzii*. Cultures of *C. maltaromaticum* were reported to produce 7-dehydrocholesterol, presumably from cholesterol in the intestinal contents, while *F. prausnitzii* in culture was apparently able to metabolically convert this vitamin D_3 precursor into vitamin D_3 and then furthermore, to the functional metabolites 25(OH)D_3 and 1,25$(OH)_2D_3$[29]. In a randomised controlled trial of human subjects, the daily oral intake of magnesium glycinate for 12 weeks was associated with increased proportions of *C. maltaromaticum* and *F. prausnitzii* in the bacterial population found in rectal swabs from the participants[30]. The postulated explanation for promotion of the growth of these bacteria that were producing and metabolising vitamin D, was that magnesium was acting as an enzyme co-factor in bacterial metabolism and thus stimulating their growth. In another study, the addition of the anaerobic gut bacterium *Bifidobacterium adolescentis* CCFM1447 to in-vitro fermentation culture of mouse faeces was found to increase the concentration of 1,25$(OH)_2$D in those cultures, although the analysis did not determine whether this was the metabolite of vitamin D_2 or vitamin D_3[31]. The suggested mechanism was the promotion of the growth of bacteria producing this vitamin D metabolite.

Why would colon bacteria biosynthesise vitamin D?

The viability of microorganisms in the intestinal contents depends upon their ability to combat noxious antimicrobial substances as well as the defence mechanisms of the host animal. One type of dietary antimicrobial molecule that reaches the large intestine are long chain fatty acids. The growth of Gram-positive bacteria is inhibited, or the bacteria are killed, by the interaction of such fatty acids, particularly unsaturated fatty acids, with the bacterial cell membrane[32]. These long-chain fatty acids destabilise the bacterial cell membrane[33] and promote membrane permeability and ATP leakage from the bacterial cytoplasm[34]. Such physico-chemical action on the cell membrane could be particularly bactericidal for some species in the colon microbiome, and it is suggested that this could partly explain how increasing the dietary intake of olive oil reduces the diversity of the gut microbiota and is linked to a more favourable profile of intestinal bacterial species for prevention of colorectal cancer[35].

Over 70 years ago, before the discovery that vitamin D was metabolised to the steroid hormone 1,25$(OH)_2$D, the concept of vitamin D function was that it modified cell membrane properties to increase, for example, the absorption of calcium ions through the intestinal epithelial cells. One strategy for studying this supposed membrane effect was to investigate the action of vitamin D on the cell membrane of bacteria. It was found to have a growth-promoting effect on the Gram-positive *Lactobacillus casei*, particularly when bacterial growth had been inhibited by linoleic or other unsaturated fatty acids[36]. Reversal of the growth inhibition by unsaturated long-chain fatty acids was induced also by tocopherol, lecithin, and various sterols. However, the most potent reversing agents were vitamins D_2 and D_3. It was postulated that this action of vitamin D on the bacterial cell membrane was to restore the functional configuration of the lipoprotein lattice and thus enable the facilitated entry by diffusion of small environmental molecules essential for microbial metabolism and growth[37]. Therefore, one hypothesis would be that the microbial

https://doi.org/10.1017/S0029665126103073 Published online by Cambridge University Press

biosynthesis of vitamin D_2 in the alimentary tract is a mechanism for combatting the antimicrobial action of unsaturated long-chain fatty acids.

Can the colon mucosa absorb vitamin D?

Unlike the wide range of absorptive capacities of the small intestinal mucosal cells, the mucosa of the colon is limited to just absorb water, electrolytes and the short chain fatty acids produced by microbial metabolism[38]. Larger lipophilic molecules like vitamin K_2, produced by Gram-positive bacteria in the colon as a component of their electron transport system[39], and of comparable molecular weight and lipophilicity to vitamin D_2, are little absorbed across the colon mucosa[40]. Hence, by analogy, a steroidal molecule like vitamin D_2 would not be expected to be absorbed into the blood stream across these mucosal cells. Yet, to test whether $1,25(OH)_2D$ could exert its hormonal effect on the colon mucosa if presented from the colon lumen, this metabolite of vitamin D_3 was conjugated through the 25-hydroxy group with glucuronic acid and given orally to mice[41,42]. Because the glucuronic acid in this conjugate prevented absorption of the $1,25(OH)_2D_3$ in the small intestine, the conjugated molecule arrived intact in the large intestine where bacterial glucuronidase released the $1,25(OH)_2D_3$. The colon mucosa responded to this $1,25(OH)_2D_3$ in the lumen contents with an upregulation of the vitamin D 24-hydroxylase gene (CYP24A1), thereby indicating that $1,25(OH)_2D_3$ had been absorbed and was regulating gene expression in the mucosal cells.

A comparable experiment was performed in mice with 25(OH)D_3 conjugated with glucuronic acid through the 3β-hydroxy group[43]. Again, there was evidence that the 25(OH)D_3 released by bacterial glucuronidase in the colon contents, had been absorbed by the mucosal cells, as there was increased local expression of mRNA for the CYP24A1 hydroxylase enzyme.

Although these experiments demonstrated that vitamin D metabolites could be absorbed across the colon mucosa, the more lipophilic vitamin D_2 may not be able to enter the mucosal cells so readily from the colon contents. The basis for a special mechanism that would allow vitamin D_2 to be absorbed across the colon mucosa lies in the properties of the specific vitamin DBP in the circulating blood[44].

DBP has a single vitamin D-specific binding site with highest affinity for 25(OH)D. Although this protein has lower affinity for the parent vitamin D molecule, the specific binding site will still effectively bind unchanged vitamin D[45]. In human plasma the DBP concentration is about 6 μmol/L, but with an adequate 25(OH)D level of 75–100 nmol/L only 1–2% of the vitamin D-specific binding sites are occupied. Another feature of DBP is its rapid turnover in blood with a half-life of 1.7 days. This contrasts with the much longer half-life of plasma albumin of 17.3 days[45]. In addition to the vitamin D-specific binding site, DBP also specifically binds to the filamentous form of the cytoplasmic protein, actin[46].

The relevance of these special characteristics of DBP for the absorption of vitamin D_2 across the colon mucosa are now explained by the development of knowledge of two interactive plasma membrane proteins, megalin and cubilin, in cells of the proximal renal tubule[47], and of skeletal muscle[48], as well as in other tissues. Megalin and cubilin have the function of internalising extracellular proteins into the cell cytoplasm. So, when DBP undergoes endocytosis by the megalin/cubilin mechanism it comes into contact with filamentous actin and binds specifically to this

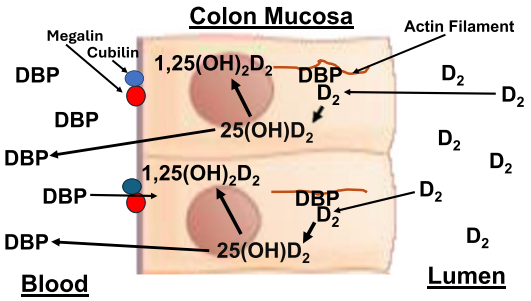


Fig. 4. Special mechanism in the colon mucosal cells for the uptake of vitamin D_2 from the colon lumen contents. Apo-DBP in the circulating blood is taken into the mucosal cells by the endocytosis action of the baso-lateral membrane proteins megalin and cubilin. In the cell cytoplasm DBP binds to actin filaments to provide an array of vitamin D-specific binding sites which accumulate any vitamin D_2 diffusing in from the colon lumen. When the actin-bound DBP undergoes proteolysis, the intracellular vitamin D_2 is released and can then be metabolised to 25(OH)D_2 and subsequently to $1,25(OH)_2D_2$. Because of the high concentration of apo-DBP in the extracellular fluid, with its highest binding affinity for 25(OH)D, some of the newly produced 25(OH)D_2 can diffuse from the cells and be transported into the general blood circulation, bound to DBP.

cytoplasmic protein. Thus, an array of binding sites, specific for the vitamin D molecular structure, is established within cells having the endocytosing proteins, megalin and cubilin in their plasma membrane. It is proposed that this cytoplasmic DBP-actin complex accumulates and retains any vitamin D or 25(OH)D that diffuses into those cells. However, the intracellular DBP soon undergoes proteolysis[49] which releases the bound vitamin D or 25(OH)D free into the cytoplasm. Then, because of the high concentration of apo-DBP in the extracellular fluid, any vitamin D-type molecules within the cell are readily induced to diffuse from the cell, to bind to the extracellular apo-DBP (Fig. 4).

It is now apparent that the epithelial cells of the colon mucosa have megalin[50] and cubilin[51] in their baso-lateral plasma membrane. Therefore, DBP endocytosed by the megalin/cubilin process, could accumulate on the actin filaments in the cytoplasm of those mucosal cells and could provide high-affinity binding sites for any vitamin D-type molecules that diffuse into those cells. This hypothesis explains how $1,25(OH)_2D$ delivered in the colon contents would have been taken up by the mucosal cells and was then able to activate a vitamin-D-regulated gene[41,42]. Likewise, in the experiments where 25(OH)D-3-glucuronide was given orally to mice, any of the 25(OH)D released by bacterial glucuronidase that then diffused from the colon contents into the mucosal cells[43], would have accumulated on the DBP/actin complex. However, because colon mucosal cells also have the 1-alpha-hydroxylase enzyme, CYP27B1[52,53], some of the accumulated 25(OH)D, after being released from DBP after this protein underwent proteolysis, would have been converted in those cells to $1,25(OH)_2D$ and would thus have activated the gene coding for the mRNA of the CYP24A1 hydroxylase enzyme.

In the intestine, it is proposed that any traces of microbially produced vitamin D_2 that diffuse into the colon mucosal cells would accumulate on the DBP-actin filament complex. When the DBP in this complex undergoes proteolysis, the vitamin D_2 that is released would then become a substrate for CYP27A1 or CYP2R1, the vitamin D 25-hydroxylases, also present in these mucosal cells[54]. Because the vitamin D-specific binding site of DBP has highest affinity for 25(OH)D, then much of the 25(OH)D_2 being generated by the mucosal cell 25-hydroxylases, would be induced to diffuse from those cells and be carried away on extracellular DBP

https://doi.org/10.1017/S0029665126103073 Published online by Cambridge University Press

in the circulating blood. The high concentration of apo-DBP in the extracellular fluid has been shown to be a key factor in transferring vitamin D_3 into blood after its formation in skin cells[55,56]. Some of the 25(OH)D_2 synthesised from the microbially-generated vitamin D_2 substrate would be converted to 1,25(OH)$_2D_2$ to perform its paracrine or autocrine role in those cells. However, quantitatively more of the absorbed vitamin D_2, after conversion to 25(OH)D_2, would be contributing to the vitamin D status of the human or other host monogastric animal. In contrast, vitamin D_2 being generated by microbial metabolism in the forestomach rumen of ruminant animals would be delivered to the small intestine where it can be readily absorbed.

Vitamin D role in maintaining health of the intestinal mucosa

Many epidemiological studies in populations have found an association between vitamin D deficiency and inflammatory bowel disease and colorectal cancer[57–59]. The explanation for this link is said to lie with the known protective functions of vitamin D through its endocrine metabolite, 1,25(OH)$_2$D, when bound in cells to its receptor protein, VDR[60]. This endocrine factor then acts on gene expression in maintaining the tight junctions between mucosal epithelial cells, thus preventing invasion by pathogenic bacteria, as well as demonstrating many other components of this protective action of 1,25(OH)$_2$D in the intestinal mucosa. It has a role in reducing inflammation through binding to the VDR in T- and B-lymphocytes and macrophages, and downregulating pro-inflammatory cytokines, as well as promoting the secretion of anti-inflammatory cytokines[61]. In addition, 1,25(OH)$_2$D has an antitumour function by inhibiting neoplastic cell proliferation, induction of cell differentiation, and the inhibition of tissue invasion and metastasis of cancerous cells[62].

Good vitamin D status is therefore considered to have this protective role against diseases of the colon mucosa by the delivery of 25(OH)D through the blood circulation to the mucosal cells. There it would be converted to 1,25(OH)$_2$D by the CYP27B1 1α-hydroxylase, to then perform its many protective functions as a paracrine or autocrine factor in gene regulation. The discovery of microbial biosynthesis of vitamin D_2 in the large intestine now suggests an alternative mechanism of supporting vitamin D protection against diseases of the colon mucosa. It is now apparent that the colon mucosal cells have the ability to absorb and retain vitamin D_2 from the large intestinal contents, and to convert it to 25(OH)D_2 and then to 1,25(OH)$_2D_2$ for its local endocrine functions[44]. The microbial generation of vitamin D_2 in quantities to meet this endocrine role in the colon mucosa, would therefore depend upon the intake of appropriate dietary components in food that stimulate the growth and metabolic activity of the micro-organisms that are generating effective quantities of vitamin D_2 in the lumen of the large intestine.

Conclusion

Dietary vitamin D_2 has been the conventional explanation for the presence of a significant proportion of total plasma 25(OH)D as 25(OH)D_2 in the blood of ruminants. The ability of micro-organisms in the rumen of ruminant animals to biosynthesise vitamin D_2 is a source of vitamin D to the host animal when absorbed from the small intestine. This microbial supply of vitamin D_2 provides an alternative mechanism to the dietary delivery of this chemical form of vitamin D from fungi exposed to the sun in pasture grass. In monogastric animals, including humans, it is likely that the capability of microorganisms in the hind gut to metabolically produce vitamin D_2 and for the colon mucosal cells to have the ability to absorb this locally generated vitamin D_2, is a source of vitamin D for its local autocrine functions in the mucosa, as well as contributing to total 25(OH)D for the systemic vitamin D functions throughout the body. The metabolic pathway for this microbial biosynthesis, and the characterisation of the organisms involved in different animal species await the findings of further research.

Acknowledgements. D.R.F. and R.S.M. are grateful to Alex Chaves and Mark Rybchyn for their technical expertise and special knowledge in the discovery of the microbial biosynthesis of vitamin D_2 in ruminants and mice. The authors thank the organisers of the 2025 Annual Scientific Meeting of the Nutrition Society of Australia for the invitation to write this review.

Author contributions. D.R.F. and R.S.M. generated the ideas to be considered in this review. D.R.F. wrote the first draft, and both authors critically edited the manuscript.

Financial support. This research review received no funding from any research agency or commercial organisation.

Competing interests. D.R.F. and R.S.M. declare that there are no conflicts of interest.

Ethical statement. Not applicable.

References

1. Jones G (2022) Review: 100 years of vitamin D: historical aspects of vitamin D. *Endocr Connect* **11**(4), e210594. doi: 10.1530/EC-21-0594.
2. Windaus A & Thiele W (1935) Über die constitution des vitamin D_2. *Ann Chem* **521**, 160–175.
3. Heilbron IM, Jones RN, Samant KM, *et al.* (1936) Studies in the sterol group. XXIV. The constitution of calciferol. *J Chem Soc* 905–907.
4. Nicolaysen R & Jansen J (1939) Vitamin D and bone formation in rats. *Acta Paediat Stockholm* **23**, 405–433.
5. Nicolaysen R & Eeg-Larsen N (1953) The biochemistry and physiology of vitamin D. *Vitam Horm* **11**, 424–444.
6. van den Heuvel EG, Lips P, Schoonmade LJ, *et al.* (2024) Comparison of the effect of daily vitamin D_2 and vitamin D_3 supplementation on serum 25-hydroxyvitamin D concentration (total 25(OH)D, 25(OH)D_2, and 25(OH)D_3) and importance of body mass index: a systematic review and meta-analysis. *Adv Nutr* **15**, 100133. doi: 10.1016/j.advnut.2023.09.016.
7. Bekemeier H (1959) A comparison of the peroral antirachitic effectiveness of vitamin D_2 and vitamin D_3 in prophylactic and therapeutic experiments on rats. *Acta Biol Med Ger* **3**, 109–113.
8. Macdonald HM (2013) Contributions of sunlight and diet to vitamin D status. *Calcif Tissue Int* **92**, 163–1076. doi: 10.1007/s00223-012-9634-1.
9. Byrdwell WC (2009) Comparison of analysis of vitamin D_3 in foods using ultraviolet and mass spectrometric detection. *J Agric Food Chem* **57**, 2135–2146. doi: 10.1021/jf803398u.
10. Azarpeykan S, Dittmer KE, Gee EK, *et al.* (2016) Influence of blanketing and season on vitamin D and parathyroid hormone, calcium, phosphorus, and magnesium concentrations in horses in New Zealand. *Domest Anim Endocrinol* **56**, 75–84. doi: 10.1016/j.domaniend.2016.03.003.
11. Childs-Sanford SE, Makowski AJ & Wakshlag JJ (2020) The vitamin D status of Asian elephants (*Elephas Maximus*) managed in a northern temperate climate. *J Zoo Wildl Med* **51**, 1–12. doi: 10.1638/2019-0097.
12. Chaves AV, Rybchyn MS, Mason RS, *et al.* (2024) Short communication: metabolic synthesis of vitamin D_2 by the gut microbiome. *Comp Biochem Physiol Part A* **295**:111666. doi: 10.1016/j.cbpa.2024.111666.
13. Terry SA, Ramos AFO, Holman DB, *et al.* (2018) Humic substances alter ammonia production and the microbial populations within a RUSITEC fed

a mixed Hay - concentrate diet. *Front Microbiol* **9**, 1410. doi: 10.3389/fmicb.2018.01410.
14. Clements MR & Fraser DR (1988) Vitamin D supply to the rat fetus and neonate. *J Clin Invest* **81**, 1768–1773. doi: 10.1172/JCI113518.
15. Horst RL, Napoli JL & Littledike ET (1982) Discrimination in the metabolism of orally dosed ergocalciferol and cholecalciferol by the pig, rat and chick. *Biochem J* **204**, 185–189. doi: 10.1042/bj2040185.
16. Halloran BP & DeLuca HF (1979) Vitamin D deficiency and reproduction in rats. *Science* **204**, 73–74. doi: 10.1126/science.432628.
17. Drescher D, DeLuca HF & Imrie MH (1969) On the site of discrimination of chicks against vitamin D_2. *Arch Biochem Biophys* **130**, 657–661. doi: 10.1016/0003-9861(69)90084-8.
18. Wang Y, Youssef NH, Couger MB, *et al.* (2019) Molecular dating of the emergence of anaerobic rumen fungi and the impact of laterally acquired genes. *mSystems* **4**, e00247–19. doi: 10.1128/mSystems.00247-19.
19. Lees ND, Skaggs B, Kirsch DR, *et al.* (1995) Cloning of the late genes in the ergosterol biosynthetic pathway of Saccharomyces cerevisiae–a review. *Lipids* **30**, 221–226. doi: 10.1007/BF02537824.
20. Groh H, Schade K & Hörhold-Schubert C (1993) Steroid metabolism with intestinal microorganisms. *J Basic Microbiol* **33**, 59–72. doi: 10.1002/jobm.3620330115.
21. Szaleniec M, Wojtkiewicz AM, Bernhardt R, *et al.* (2018) Bacterial steroid hydroxylases: enzyme classes, their functions and comparison of their catalytic mechanisms. *Appl Microbiol Biotechnol* **102**, 8153–8171. doi: 10.1007/s00253-018-9239-3.
22. Hanahan DJ & Wakil SJ (1953) Studies on absorption and metabolism of ergosterol-C. *Arch Biochem Biophys* **44**, 150–158. doi: 10.1016/0003-9861(53)90018-6.
23. Glover J, Leat WM & Morton RA (1957) Sterol metabolism. 2. The absorption and metabolism of [14C] ergosterol in the guinea pig. *Biochem J* **66**, 214–222. doi: 10.1042/bj0660214.
24. Tsugawa N, Okano T, Takeuchi A, *et al.* (1992) Metabolism of orally administered ergosterol and 7-dehydrocholesterol in rats and lack of evidence for their vitamin D biological activity. *J Nutr Sci Vitaminol (Tokyo)* **38**, 15–25. doi: 10.3177/jnsv.38.15.
25 Sun X, Sharon O & Sharon A (2023) Distinct features based on partitioning of the endophytic fungi of cereals and other grasses. *Microbiol Spectr* **11**(3), e0061123. doi: 10.1128/spectrum.00611-23.
26. Kuwabara N, Sato S & Nakagawa S (2023) Effects of long-term high-ergosterol intake on the cholesterol and Vitamin D biosynthetic pathways of rats fed a high-fat and high-sucrose diet. *Biol Pharm Bull* **46**, 1683–1691. doi: 10.1248/bpb.b23-00348.
27. Kotwan J, Kühn J, Baur AC, *et al.* (2021) Oral intake of lumisterol affects the metabolism of vitamin D. *Mol Nutr Food Res* **65**, 2001165. doi: 10.1002/mnfr.202001165.
28. Kühn J, Hirche F, Geissler S, *et al.* (2016) Oral intake of 7-dehydrocholesterol increases vitamin D_3 concentrations in the liver and kidney. *J Steroid Biochem Mol Biol* **164**, 199–204. doi: 10.1016/j.jsbmb.2015.12.017.
29. Li Q, Chan H, Liu W-X, *et al.* (2023) *Carnobacterium maltaromaticum* boosts intestinal vitamin D production to suppress colorectal cancer in female mice. *Cancer Cell* **41**, 1450–1465. doi: 10.1016/j.ccell.2023.06.011.
30. Sun E, Zhu X, Ness RM, *et al.* (2025) Magnesium treatment increases gut microbiome synthesizing vitamin D and inhibiting colorectal cancer: results from a double-blind precision-based randomized placebo-controlled trial. *Am J Clin Nutr* **122**, 1185–1194. doi: 10.1016/j.ajcnut.2025.09.011.
31. Yu X, Tian G, Wang Y, *et al.* (2025) *Bifidobacterium adolescentis* CCFM1447 effectively alleviates osteoporosis by enriching intestinal flora capable of vitamin D conversion. *Front Nutr* **12**:1647671. doi: 10.3389/fnut.2025.1647671.
32. Raychowdhury MK, Goswami R & Chakrabarti P (1985) Effect of unsaturated fatty acids in growth inhibition of some penicillin-resistant and sensitive bacteria. *J Appl Bacteriol* **59**, 183–188. doi: 10.1111/j.1365-2672.
33. Shin S, Tae H, Park S, *et al.* (2023) Lipid membrane remodeling by the micellar aggregation of long-chain unsaturated fatty acids for sustainable antimicrobial strategies. *Int J Mol Sci* **24**, 9639. https://doi.org/10.3390/ijms24119639.
34. Shin S, Yu J, Tae H, *et al.* (2024) Exploring the membrane-active interactions of antimicrobial long-chain fatty acids using a supported lipid bilayer model for gram-positive bacterial membranes. *ACS Appl Mater Interfaces* **16**, 56705−56717. https://doi.org/10.1021/acsami.4c11158.
35. Memmola R, Petrillo A, Di Lorenzo S, *et al.* (2022) Correlation between olive oil intake and gut microbiota in colorectal cancer prevention. *Nutrients* **14**(18), 3749. doi: 10.3390/nu14183749.
36. Kodicek E (1956) Fat-soluble vitamins. *Ann Rev Biochem* **25**, 497–536. doi: 10.1146/annurev.bi.25.070156.002433.
37. Kodicek E (1956) The effect of unsaturated fatty acids, of vitamin D and other sterols, on gram-positive bacteria. In *Biochemical Problems of Lipids*, pp. 401–406 (G. Popjak and Le Breton, editors]. London: Butterworth Scientific Publications.
38. Kunzelmann K & Mall M (2002) Electrolyte transport in the mammalian colon: mechanisms and implications for disease. *Physiol Rev* **82**, 245–289. doi: 10.1152/physrev.00026.2001.
39. Kurosu M & Begari E (2010) Vitamin K2 in electron transport system: are enzymes involved in vitamin K2 biosynthesis promising drug targets? *Molecules* **15**, 1531–1553. doi: 10.3390/molecules15031531.
40. Groenen-van Dooren MM, Ronden JE, Soute BA, *et al.* (1995) Bioavailability of phylloquinone and menaquinones after oral and colorectal administration in vitamin K-deficient rats. *Biochem Pharmacol* **50**, 797–801. doi: 10.1016/0006-2952(95)00202-b.
41. Goff JP, Koszewski NJ, Haynes JS, *et al.* (2012) Targeted delivery of vitamin D to the colon using β-glucuronides of vitamin D: therapeutic effects in a murine model of inflammatory bowel disease. *Am J Physiol Gastrointest Liver Physiol* **302**, G460–G469. doi: 10.1152/ajpgi.00156.2011.
42. Koszewski NJ, Horst RL & Goff JP (2012) Importance of apical membrane delivery of 1,25-dihydroxyvitamin D_3 to vitamin D-responsive gene expression in the colon. *Am J Physiol Gastrointest Liver Physiol* **303**, G870–G878. doi: 10.1152/ajpgi.00149.2012.
43. Reynolds CJ, Koszewski NJ, Horst RL, *et al.* (2020) Role of glucuronidated 25-hydroxyvitamin D on colon gene expression in mice. *Am J Physiol Gastrointest Liver Physiol* **319**, G253–G260. doi: 10.1152/ajpgi.00355.2019.
44. Fraser DR (2025) Short communication: can vitamin D be supplied from the large intestine. *Comp Biochem Physiol Part A* **300**,111784. doi: 10.1016/j.cbpa.2024.111784.
45. Bouillon R, Schuit F, Antonio L, *et al.* (2020) Vitamin D binding protein: a historic overview. *Front Endocrinol (Lausanne)* **10**, 910. doi: 10.3389/fendo.2019.00910.
46. Van Baelen H, Bouillon R & De Moor P (1980) Vitamin D-binding protein (Gc-globulin) binds actin. *J Biol Chem* **255**, 2270–2272. doi: 10.1016/S0021-9258(19)85885-4.
47. Nielsen R, Christensen EI & Birn H (2016) Megalin and cubilin in proximal tubule protein reabsorption: from experimental models to human disease. *Kidney Int* **89**, 58–67. doi: 10.1016/j.kint.2015.11.007.
48. Rybchyn MS, Abboud M, Puglisi DA, *et al.* (2020) Skeletal muscle and the maintenance of vitamin D status. *Nutrients* **12**(11), 3270. doi: 10.3390/nu12113270.
49. Haddad JG, Fraser DR & Lawson DE (1981) Vitamin D plasma binding protein. Turnover and fate in the rabbit. *J Clin Invest* **67**, 1550–1560. doi: 10.1172/jci110186.
50. Ternes SB & Rowling MJ (2013) Vitamin D transport proteins megalin and disabled-2 are expressed in prostate and colon epithelial cells and are induced and activated by all-trans-retinoic acid. *Nutr Cancer* **65**, 900–907. doi: 10.1080/01635581.2013.805422.
51. Steegenga WT, de Wit NJ, Boekschoten MV, *et al.* (2012) Structural, functional and molecular analysis of the effects of aging in the small intestine and colon of C57BL/6J mice. *BMC Med Genomics* **5**, 38. doi: 10.1186/1755-8794-5-38.
52. Lagishetty V, Chun RF, Liu NQ, *et al.* (2010) 1alpha-hydroxylase and innate immune responses to 25-hydroxyvitamin D in colonic cell lines. *J Steroid Biochem Mol Biol* **121**, 228–233. doi: 10.1016/j.jsbmb.2010.02.004.
53. Cross HS, Nittke T & Kallay E (2011) Colonic vitamin D metabolism: implications for the pathogenesis of inflammatory bowel disease and colorectal cancer. *Mol Cell Endocrinol* **347**, 70–79. doi: 10.1016/j.mce.2011.07.022.
54. Vantieghem K, Overbergh L, Carmeliet G, *et al.* (2006) UVB-induced 1,25(OH)$2D_3$ production and vitamin D activity in intestinal CaCo-2 cells

https://doi.org/10.1017/S0029665126103073 Published online by Cambridge University Press

and in THP-1 macrophages pretreated with a sterol Delta7-reductase inhibitor. *J Cell Biochem* **99**, 229–240. doi: 10.1002/jcb.20910.
55. Haddad JG, Matsuoka LY, Hollis BW, *et al.* (1993) Human plasma transport of vitamin D after its endogenous synthesis. *J Clin Invest* **91**, 2552–2555. doi: 10.1172/JCI116492.
56. Duchow EG, Cooke NE, Seeman J, *et al.* (2019) Vitamin D binding protein is required to utilize skin-generated vitamin D. *Proc Natl Acad Sci U S A* **116**, 24527–24532. doi: 10.1073/pnas.1915442116.
57. Mouli VP & Ananthakrishnan AN ((2014) Review article: vitamin D and inflammatory bowel diseases. *Aliment Pharmacol Ther* **39**, 125–136. doi: 10.1111/apt.12553.
58. Ananthakrishnan AN, Cheng SC, Cai T, *et al.* (2014) Association between reduced plasma 25-hydroxy vitamin D and increased risk of cancer in patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol* **12**, 821–827. doi: 10.1016/.j.cgh.2013.10.011.
59. Meeker S, Seamons A, Maggio-Price L, *et al.* (2016) Protective links between vitamin D, inflammatory bowel disease and colon cancer. *World J Gastroenterol* **22**, 933–948. doi: 10.3748/wjg.v22.i3.933.
60. Sun J & Zhang YG (2022) Vitamin D receptor influences intestinal barriers in health and disease. *Cells* **11**, 1129. doi: 10.3390/cells11071129.
61. Fekete M, Lehoczki A, Szappanos Á, *et al.* (2025) Vitamin D and colorectal cancer prevention: immunological mechanisms, inflammatory pathways, and nutritional implications. *Nutrients* **17**(8), 1351. doi: 10.3390/nu17081351.
62. Muñoz A & Grant WB (2022) Vitamin D and cancer: an historical overview of the epidemiology and mechanisms. *Nutrients* **14**(7), 1448. doi: 10.3390/nu14071448.

https://doi.org/10.1017/S0029665126103073 Published online by Cambridge University Press