Special Article

Intestinal absorption of vitamin D: a systematic review

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Context: *Vitamin D is frequently prescribed as a supplement, yet its absorption remains poorly understood.* ***Objective:*** *This systematic review was performed to evaluate data on mechanisms involved in the intestinal absorption of vitamin D.* ***Data Sources:*** *PubMed, Embase, and Cochrane Library databases were searched.* ***Study Selection:*** *The following studies were included: experimental laboratory studies of vitamin D absorption through the enterocyte brush-border membrane; absorption tests that used radiolabeled vitamin D; and clinical trials in adults that investigated a single dose of cholecalciferol or ergocalciferol and reported at least 2 measurements of serum cholecalciferol, ergocalciferol, or 25-hydroxyvitamin D.* ***Data Extraction:*** *From 2069 articles identified, 46 met the inclusion criteria.* ***Results:*** *Different methods were employed to evaluate vitamin D absorption. Recent research suggests that vitamin D absorption is not an exclusive simple diffusion process. Vitamin D was better absorbed when it was consumed with fat-containing meals, but absorption also occurred without fat or oily vehicles. Factors that modified cholesterol absorption also altered vitamin D absorption.* ***Conclusion:*** *Vitamin D is probably absorbed through passive diffusion and a mechanism involving membrane carriers, especially cholesterol transporters, although data remain scarce. Some data suggest that fat, when consumed concomitantly with vitamin D, improves vitamin D absorption.*

INTRODUCTION

Vitamin D deficiency is a global concern, particularly because of the importance of vitamin D in bone health and other systemic functions.[1–4] Vitamin D is a steroid prohormone that can be supplied by skin synthesis in humans when skin is exposed to ultraviolet B radiation.[5] Many people, however, require dietary vitamin D supplements, mainly because of low sun exposure. Recently, researchers have shown an increased interest in the physiology and metabolism of vitamin D, especially with regard to the absorption of orally ingested vitamin D.

Until recently, it was accepted that vitamin D was absorbed by means of a simple passive diffusion process. Now, however, it is thought that absorption may occur through complex mechanisms of vitamin D incorporation.

Passive diffusion has been observed with pharmacological doses of vitamin D,[6] and clinical trials have demonstrated that vitamin D was absorbed even when ingested with nonfat meals or nonoily vehicles[7,8] or during fasting.[9]

This systematic review includes studies that evaluated the intestinal absorption of vitamin D. An electronic search of the literature was conducted up to January 2016 using Ovid MEDLINE, Embase, and the Cochrane Library by combining the MeSH terms "vitamin D" and "absorption." Knowing how vitamin D is absorbed, as well as the factors that might interfere

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Key words: absorption, bioavailability, enterocyte, membrane transport, vitamin D.

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Table 1 **PICOS criteria for the inclusion and exclusion of studies**

Criteria	Description
Population	Experimental models of mammalian physiology or human volunteers
Intervention	Administration of cholecalciferol, ergocalciferol, or radiolabeled forms of cholecalciferol or ergocalciferol
Comparison	Plasma concentrations before and after vitamin D administration
Outcomes	Plasma concentrations of cholecalciferol, ergocalciferol, 25-hydroxyvitamin D, or radiolabeled forms of vitamin D
Study design	Experimental laboratory studies, absorption tests using radiolabeled vitamin D, or clinical trials of a single dose of cholecalciferol or ergocalciferol

with absorption, is important to better treat and prevent vitamin D deficiency.

METHODS

This review followed the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines.[10] The Population, Intervention, Comparison, Outcomes, and Study design (PICOS) criteria were used to formulate and narrow the focus of the following research question: What mechanisms are involved in the intestinal absorption of vitamin D (Table 1)?

Identification of studies

Original research studies were identified from the databases PubMed, Embase, and the Cochrane Library, using the combination of medical subject headings (MeSH) "vitamin D" and "absorption." Search details were as follows: ("vitamin D/administration and dosage"[MeSH] OR "vitamin D/pharmacokinetics"[MeSH] OR "vitamin D/physiology"[MeSH]) AND ("absorption"[MeSH terms] OR "absorption"[all fields]).

Criteria for inclusion and exclusion of studies

All original studies in English, Portuguese, and Spanish, published up to January 2016, were considered for this review. The following types of studies were included: experimental laboratory studies of vitamin D absorption through the enterocyte brush-border membrane; absorption tests that used radiolabeled vitamin D; and clinical trials of a single dose of cholecalciferol or ergocalciferol that reported at least 2 measurements of serum cholecalciferol, ergocalciferol, or 25-hydroxyvitamin D [25(OH)D]. Studies were excluded if they were performed in a pediatric sample or were published within a systematic review, book chapter, conference proceedings, correspondence, or authors' comments.

Data extraction

Both authors screened the titles and abstracts identified from the electronic search. In the event the reviewers disagreed about an abstract, the abstract was reevaluated and disagreement was resolved by discussion and consensus. Full texts were obtained and were again evaluated for eligibility by the authors. Additional papers were identified by cross-referencing the texts. Study quality was not rated because of differences in the study design. No meta-analyses were performed.

RESULTS

Studies of vitamin D absorption

From 2069 titles and abstracts, 109 articles were selected for full-text review. Of these, 79 were excluded because of study design. Thus, 30 studies were included, as well as 16 others obtained by cross-referencing articles (Figure 1). All 46 studies fulfilled 1 of the 3 inclusion criteria and, subsequently, were classified into the following groups: 16 laboratory experiments that investigated vitamin D absorption through the enterocyte apical membrane (Table 2[6,11–25]); 9 absorption tests that used radiolabeled vitamin D (Table 3[26–34]); and 21 clinical trials of a single dose of vitamin D, 8 of which were performed in adults with compromised health (Table 4[35–42]) and 13 of which were performed in healthy adults (Table 5[7–9,43–52]).

DISCUSSION

Ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3) are the preparations commonly prescribed to prevent and treat vitamin D deficiency.[53] The former is a plant-derived form of vitamin D and the latter is the animal-derived form of vitamin D, which can be synthesized by irradiation of 7-dehydrocholesterol in the skin by ultraviolet B (UVB) light. Both forms are similar in structure, and both need to be hydroxylated to 25(OH)D within the liver and to 1,25-dihydroxyvitamin D [$1,25(OH)_2D$], the active form, in the kidneys.[54]

Different forms of vitamin D and different methods were used to evaluate vitamin D absorption. Altogether, the selected studies provided insight into the mechanisms involved in vitamin D intestinal absorption.

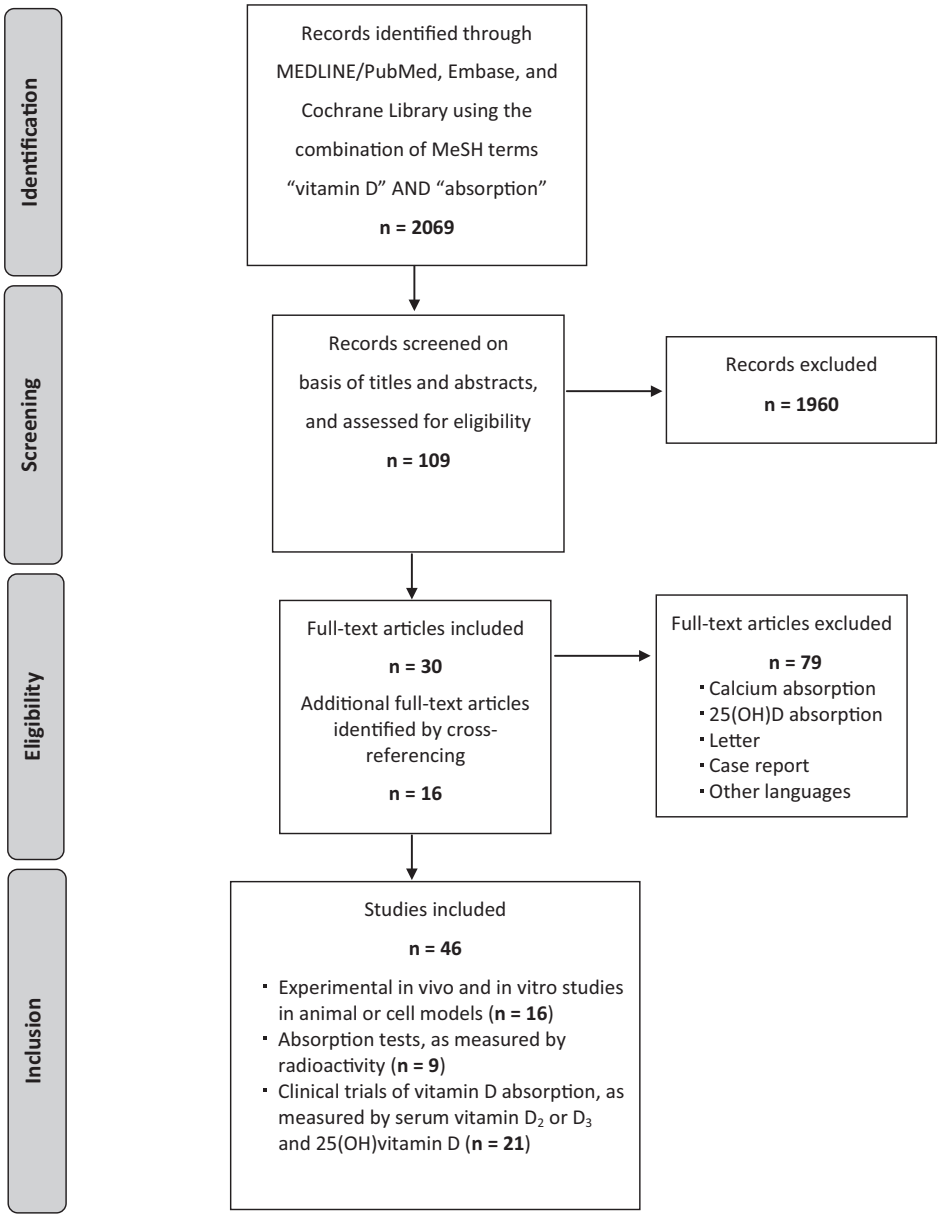

Figure 1 **Flow diagram of the literature search process**.

Basic science research

Table 2 provides an overview of the experimental in vivo and in vitro studies of vitamin D uptake through the apical membrane of the enterocytes.

Current knowledge of vitamin D uptake through the brush-border (apical) membrane of the enterocytes was obtained from experimental studies in the mid-1970s, when Hollander et al.[23,24] demonstrated a linear relationship between the rate of vitamin D absorption and the intraluminal concentration of vitamin D in 2 studies using everted gut sacs and live rats. Moreover, those authors demonstrated that the absorption rate was raised by an increase in either the flow rate of the perfusate or the hydrogen ion concentration, which decreased the resistance of the cell membrane to the diffusion of micelles. These results indicated a lack of saturation kinetics and supported the idea of vitamin D absorption by passive diffusion.

For many years, the mechanistic process of vitamin D intestinal uptake was no longer the emphasis of investigations, and laboratory experiments focused only on factors that could interfere with vitamin D uptake, such as uremia,[19] gastrectomy,[18] aging,[17,21,22] and vitamin D status.[16] For example, the effect of aging on intestinal absorption of vitamin D was evaluated in 3 experimental studies, 2 of which indicated that age had no significant impact on vitamin D absorption in rats.[17,21]

The influence of vitamin D status on the intestinal absorption and body retention of vitamin D was

Table 2 **Experimental in vivo and in vitro studies of vitamin D uptake through the brush-border (apical) membrane of enterocytes**

Reference	Focus of investigations	Methods/experimental model	Outcomes
Goncalves et al. (2015)[11]	Absorption profile of fat-soluble vitamins along the duodenal–colonic axis, and interactions between FSVs during their uptake	Mouse intestine in vivo Caco-2 cells	Fat-soluble vitamins: Location of intestinal absorption in mouse intestine: vitamin A = proximal, vitamin D = median, vitamins E and K = distal In Caco-2 cells: competitive interactions for uptake of vitamins D, E, and K Vitamin A decreased the uptake of other FSVs Vitamin A uptake was not impaired by vitamins D and K and was even promoted by vitamin E
Goncalves et al. (2013)[12]	Effects of fatty acids on vitamin D_3 intestinal absorption: Could FAs modulate vitamin D_3 absorption in the same way they impact cholesterol metabolism?	Analysis of physicochemical properties of micelles formed with different FAs Administration of micelles to human Caco-2 cells Analysis of cholecalciferol uptake and basolateral efflux Analysis of regulation of genes that code proteins involved in lipid absorption	Long-chain FAs in micelles decreased vitamin D_3 uptake in Caco-2 cells; when mixed with other FAs, this decrease was abolished Oleic acid improved vitamin D_3 basal efflux Genes coding for lipid transport proteins such as NPC1L1 and SR-BI were modulated and could partly explain these results
Goncalves et al. (2011)[13]	Absorption of phytosterols and cholecalciferol	Assessment of β-sitosterol's effect on vitamin D_3 postprandial response in mice Evaluation of effects of different sterols on vitamin D_3 micellar incorporation, apical uptake, and basolateral efflux in vitro and ex vivo	β-sitosterol: ↓ Vitamin D_3 bioavailability in mice by 15-fold, $P < 0.05$ Phytosterols: Impaired cholecalciferol incorporation into mixed micelles ↓ Vitamin D_3 apical uptake in Caco-2 cells and mouse intestinal explants No effect on vitamin D_3 secretion at the basolateral side of Caco-2 cells
Reboul et al. (2011)[6]	Is vitamin D intestinal absorption a protein-mediated process? Is there a possible involvement of cholesterol transporters in vitamin D absorption?	Examination of vitamin D_3 apical transport in: Human Caco-2 and transfected HEK cells Wild-type mice and mice overexpressing SR-BI at the intestinal level Mice treated or not with ezetimibe, an NPC1L1 inhibitor	Vitamin D_3 uptake: In Caco-2 cells: uptake was concentration, temperature, and direction dependent and ↓ with coincubation with cholesterol or tocopherol, ↓SR-BI inhibitor, or ezetimibe ↑ SR-BI, CD36, and NPC1L1 transfection of HEK cells ↓ CD36 inhibitor or ezetimibe on CD36 and NPC1L1 transfected HEK cells, respectively Increased in mice overexpressing SR-BI Ezetimibe effect was not significant in mice Similar results were obtained in mouse intestinal explants
van Heek et al. (2001)[14]	Ezetimibe and the absorption of cholesteryl ester, TGs, ethinylestradiol, progesterone, vitamins A and D, and taurocholic acid	Experimental hamster or rat model Biliary anastomosis model was established in rats	Ezetimibe: No effect on absorption of cholesteryl ester, TGs, ethinylestradiol, progesterone, vitamins A and D, or taurocholic acid Elimination of pancreatic function did not affect ability of ezetimibe to block absorption of free cholesterol
Bikhazi & Hasbini (1989)[15]	Vitamin D_3 and 1,25-hydroxyvitamin D_3: their passage across the brush border; their intracellular binding protein translocation, and their subsequent release into the prehepatic systemic circulation	Intestinal perfusion technique for study of $[^{14}C]D_3$ or $[^{3}H]1,25D_3$ absorption through intact jejunal segments of rats Samples of intestinal perfusates, homogenates, and portal blood were assayed for $[^{14}C]D_3$ or $[^{3}H]1,25D_3$ at specified time intervals in control rats and in rats injected with cycloheximide, an inhibitor of protein biosynthesis	In cycloheximide-treated groups: $[^{14}C]D_3$ uptake from the perfusate was not affected ↑ $[^{14}C]D_3$ retention in perfused intestinal segments ↓ $[^{3}H]1,25D_3$ uptake from the perfusate ↑ $[^{3}H]1,25D_3$ intestinal retention Intracellular binding proteins may be involved in transport of $[^{14}C]D_3$ or $[^{3}H]1,25D_3$ through rat enterocytes

(continued)

Table 2 **Continued**

Reference	Focus of investigations	Methods/experimental model	Outcomes
Lorentzon & Danielsson (1985)[16]	Influence of vitamin D status on the intestinal absorption and body retention of vitamin D	Rats were kept on a diet deficient in vitamin D for 2 mo Randomly assigned to 1 of 3 groups and were given different amounts of vitamin D for 9 d [^{3}H]D_3 was administered intragastrically Serum radioactivity was recorded after various periods of time Animals were kept in metabolic cages, and urine and feces were collected	Vitamin D–deficient rats: ↑ Serum radioactivity, mostly confined to 25(OH)D and 1,25D fractions ↓ Radioactivity in 3-d fecal collection
Hollander & Tarnawski (1984)[17]	Aging and intestinal absorption of vitamin D_3	Absorption and mucosal accumulation of vitamin D were measured using single-pass technique in male rats 9–101 wk of age	Vitamin D_3 absorption was higher at 41 wk of age than at 9 wk of age and remained relatively constant thereafter
Meyer et al. (1984)[18]	Intestinal absorption of cholecalciferol in gastrectomized rats	Rats were gastrectomized, and intestinal absorption and fecal excretion of cholecalciferol were studied following administration of [^{3}H]D_3 by subcutaneous injection or via gastric tube Radioactivity in feces and serum was measured	Gastrectomized rats: ↓ [^{3}H]D_3 intestinal absorption ↑ [^{3}H]D_3 fecal excretion
Vaziri et al. (1983)[19]	Uremia and vitamin D_3 intestinal absorption	In vivo perfusion technique was used to determine rate of intestinal absorption of vitamin D_3 in uremic and normal rats	Uremic rats: Rate of vitamin D_3 absorption less than that in control animals ($P < 0.001$)
Sitrin et al. (1982)[20]	Vitamin D absorption vs 25(OH)D absorption	Administration of physiological amounts of vitamin D and 25(OH)D in vivo into jejunal sacs in rats with thoracic and bile duct cannulas	Absorption of 25(OH)D greater than absorption of vitamin D The majority of absorbed vitamin D and 25(OH)D was transported from the intestine in portal blood rather than in lymph When luminal fluid contained 2.5 mM oleic acid and monoolein, the presence of taurocholate did not affect total intestinal absorption of vitamin D or 25(OH)D but increased recovery of vitamin in lymph
Fleming & Barrows (1982)[21]	Aging and intestinal absorption of vitamins A and D	Rats were fasted for 12 h and then anesthetized and treated with 1.6 μCi [^{3}H]vitamin A and 1.0 μCi ^{4}C vitamin D_3 via stomach tube	Age did not decrease absorption of either vitamin A or vitamin D Pattern of distribution of the dose of vitamins A and D also did not differ significantly as a function of age
Holt & Dominguez (1981)[22]	Aging and intestinal absorption of vitamin D_3, fatty acids, and monoglycerides	[^{3}H]-trioleylglycerol and [^{14}C]D_3 were perfused intraduodenally for 5 h in aged and young adult rats	Aged rats: ↑ In the intestinal content of vitamin D_3 at all perfusion rates when compared to young adult controls ↑ Trioleylglycerol at the higher perfusion rates Intestinal re-esterification of absorbed [^{3}H]-labeled fatty acid and partial glycerides was unchanged, so impaired intestinal triglyceride formation did not cause the increased content of tissue [^{3}H]-labeled lipids No accumulation of [^{14}C]D_3 metabolites was detected
Hollander et al. (1978)[23]	Absorption of physiological concentrations of vitamin D_3	Rat was anaesthetized and its abdomen opened; catheters were used to isolate proximal jejunal and distal ileal segments Intestinal loops were replaced into peritoneal cavity and abdomen was closed Animal was allowed to awaken and was placed in a restraint cage	[^{3}H]D_3 uptake: ↑ Concentration and ↑ rate of uptake: linear relationship in jejunum and ileum ↑ By increases in H^+ concentration or perfusate's flow rate ↓ By addition of 2–5 mM FAs of varying chain length and degrees of saturation

(continued)

Table 2 **Continued**

Reference	Focus of investigations	Methods/experimental model	Outcomes
			Increasing sodium taurocholate concentration in perfusate did not change ileal absorption rate but did decrease jejunal absorption rate
Hollander & Truscott (1976)[24]	Mechanism and site of vitamin D_3 intestinal uptake in rat bowel sacs	Everted rat small bowel sacs from 3 different regions of the small bowel were incubated in a micellar medium containing [^{3}H]D_3 in pharmacological concentrations	[^{3}H]D_3 uptake: ↑ Concentration and ↑ rate of uptake: linear relationship Uptake in proximal and medial small bowel greater than that in distal small bowel ($P < 0.01$)
Thompson & Thompson (1969)[25]	Cholestyramine and the absorption of radioactive vitamin D and calcium in rats	Absorption of 10 μg [^{3}H]D_3 and 1 mg ^{47}Ca after addition of cholestyramine to diet in an amount sufficient to cause steatorrhea, measured in treated rats vs in control rats	[^{3}H]D_3 uptake: ↓ By the addition of cholestyramine to diet ^{47}Ca absorption was similar in both control and cholestyramine-fed rats

Abbreviations: [^{14}C]D_3, [^{14}C] carbon-14 labeled cholecalciferol; CD36, cluster determinant 36; FAs, fatty acids; FSVs, fat-soluble vitamins; [^{3}H]1,25D_3, 25-[^{3}H]hydroxyvitamin D_3; [^{3}H]vitamin A, [1-^{3}H (N)] vitamin A; HEK, transfected human embryonic kidney; NPC1L1, Niemann-Pick C1-like 1; 25(OH)D, 25-hydroxyvitamin D; SR-BI, scavenger receptor class B type I; TGs, triglycerides.

evaluated by Lorentzon and Danielson.[16] Radiolabeled cholecalciferol ([^{3}H]D_3) was administered intragastrically to rats fed previously with different amounts of vitamin D. Animals with vitamin D deficiency accumulated high levels of serum radioactivity, which were to a great extent confined to polar fractions of 25(OH)D and 1,25$(OH)_2$D, with less radioactivity found in the 3-day fecal collection from these animals. These results suggested that intestinal absorption of vitamin D might be greater in states of vitamin D deficiency.

In a wider perspective, Bikhazi and Hasbini[15] investigated the brush-border mechanistic passage of vitamin D and its metabolite 1,25$(OH)_2$D. Radiolabeled cholecalciferol ([^{14}C]D_3) and 1,25$(OH)_2$D ([^{3}H]1,25D_3) were measured in intestinal perfusates and portal blood samples of rats injected with cycloheximide, an inhibitor of protein and chylomicron synthesis. The amount of [^{14}C]D_3 lost from the perfusate was similar in the experimental and control groups. The treated rats, however, showed a drastic increase in [^{14}C]D_3 retention in the intestine and a reduction in [^{14}C]D_3 portal plasma. The authors concluded that cholecalciferol might be transferred through the cytosol by carrier-binding proteins. Moreover, they observed that apical membrane absorption was different for cholecalciferol and 1,25$(OH)_2$D, since [^{3}H]1,25D_3 uptake from the perfusates was significantly reduced in cycloheximide-treated rats.[15]

Sitrin and Bengoa[27] demonstrated that 25(OH)D, another polar metabolite of vitamin D, had an improved absorption pattern compared with cholecalciferol, both in patients with chronic cholestatic liver disease and in healthy persons. Although the mechanistic processes are not clear, other experiments in humans and in rats suggested that intestinal absorption of 25(OH)D was less dependent on chylomicron production and release than was intestinal absorption of cholecalciferol.[20,29]

More than 30 years after the first experiments about intestinal uptake of vitamin D, researchers observed that cholesterol and vitamin D had similar absorption mechanisms.[6,11–13] These studies demonstrated that cholesterol and factors that are known to interfere with cholesterol uptake, such as phytosterols and long-chain fatty acids, also reduced the absorption of vitamin D in experimental models.[12,13,25]

Great advances in knowledge about cholesterol absorption have been made in recent decades. It is now well recognized that cholesterol absorption involves protein membrane transporters.[55] The most important cholesterol membrane carrier is the Niemann-Pick C1-like 1 carrier (NPC1L1),[55] followed by scavenger receptor class B type I (SR-BI),[56] cluster determinant 36 (CD36), and ATP-binding cassette transporter A1.[57]

Table 3 **Studies examining intestinal absorption of radiolabeled vitamin D in humans**

Reference	Characteristics of participants	Objective	Intervention	Plasma radioactivity measured at post intervention times	Peak plasma radioactivity	Vitamin D absorption
Leichtmann et al. (1991)[26]	Crohn disease + bowel resection (n = 12) CG (n = 4)	Compare absorption of $[^3H]D_3$ with that of $[^3H]25(OH)D_3$, administered separately	$[^3H]D_3$ or $[^3H]25(OH)D_3$ 5.9–8.9 10^{-9} μCi + 100 μg vitamin D + dietary formula	$[^3H]$ total: 0 h, 2 h, 4 h, 8 h, 12 h, and 24 h	$[^3H]$ total: 12 h after $[^3H]D_3$ 8 h after $[^3H]25(OH)D_3$	$[^3H]D_3 < [^3H]25(OH)D_3$ CD + BR < CG (both presentations) ↑ BR ↓ absorption\
Sitrin & Bengoa (1987)[27]	Chronic cholestasis, female, 36–63 y (n = 8) Chronic cholestasis, male, 44 y (n = 1) CG (n = 5)	Compare absorption of $[^3H]D_3$ with that of $[^3H]25(OH)D_3$, administered simultaneously	8–10 μCi of $[^3H]D_3$ + 8–10 μCi of $[^3H]25(OH)D_3$ 8 + 100 μg vitamin D + dietary formula	$[^3H]D_3$, $[^3H]25(OH)D_3$: 0 h, 4 h, 8 h, 12 h, and 24 h	$[^3H]D_3$:12 h $[^3H]25(OH)D_3$:8 h	$[^3H]D_3 < [^3H]25(OH)D_3$ $[^3H]D_3$: severe cholestasis (n = 4) < mild cholestasis (n = 5) = CG $[^3H]25(OH)D_3$: similar in all groups Not correlated with basal serum 25(OH)D
Danielsson et al. (1982)[28]	Primary biliary cirrhosis, female, 41–71 y (n = 8) CG (n = 8)	Evaluate absorption, metabolism, and excretion of $[^3H]D_3$	$[^3H]D_3$ 12.5 μCi + 2000 IU D_3 orally after 48 h: 12.5 μCi $[^3H]D_3$ + 2000 IU D_3 IV	$[^3H]D_3$, $[^3H]25(OH)D_3$: 1 d, 2 d, 3 d, 4 d, 5 d, and 6 d	$[^3H]D_3$: 6 h $[^3H]25(OH)D_3$: 6 d	PBC < CG ↑ Steatorrhea ↓absorption Metabolism of D_3 to $25(OH)D_3$ similar between PBC and CG
Compston et al. (1981)[29]	Healthy young men (n = 12)	Compare appearance of $[^3H]D_3$ with that of $[^3H]25(OH)D_3$ in chylomicron fraction of plasma and plasma	0.23 MBq of $[^3H]D_3$ (n = 4) 0.148 MBq of $[^3H]25(OH)D_3$ (n = 5) 0.33 MBq of $[^3H]25(OH)D_3$ (n = 3)	$[^3H]$ in the chylomicron fraction: 2 h, 3 h, 4 h, and 6 h	Not evaluated	Administration of $[^3H]D_3$ resulted in higher $[^3H]$ activity in the chylomicron fraction than $[^3H]25(OH)D_3$ Administration of $[^3H]25(OH)D_3$ caused earlier appearance of $[^3H]$ activity in plasma
Davies et al. (1980)[30]	Gastrectomy (n = 5) Short bowel (n = 5) Celiac disease (n = 3) CG (n = 5)	Compare absorption of $[^{14}C]D_3$ with that of $[^3H]25(OH)D_3$, administered concurrently	$[^{14}C]D_3$ 2 μCi + $[^3H]25(OH)D_3$ 8 μCi + meal and milk	$[^{14}C]D_3$, $[^3H]25(OH)D_3$: 2 h, 4 h, 8 h, 12 h, and 13 d	$[^{14}C]D_3$: 6–24 h $[^3H]25(OH)D_3$: 4–12 h	Patients with steatorrhea had malabsorption of both vitamin D_3 and $25(OH)D_3$, which was greater for vitamin D_3 than $25(OH)D_3$. The decrease in absorption was inversely associated with steatorrhea
Barragry et al. (1978)[31]	Healthy adults, 30–58 y (n = 22) Hospitalized elderly, 68–94 y (n = 20) Adults with malabsorption, 31–66 y (n = 5)	Experiment 1: pilot evaluation of $[^3H]D_3$ absorption Experiment 2: compare $[^3H]D_3$ absorption and compare metabolism of D_3 with that of $25(OH)D_3$ in healthy young adults and in hospitalized elderly adults	Experiment 1: $[^3H]D_3$ 1–3.5 μCi + meal: 08 g fat, after 3 h (n = 2) 50 g fat, at 0 h (n = 2) 50 g fat, after 8 h (n = 1) Experiment 2: $[^3H]D_3$ 6 μCi + meal with 30 g of fat, at 0 h (n = 47)	Experiment 1: $[^3H]D_3$: 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, and 9 h Experiment 2: $[^3H]D_3$ and $[^3H]25(OH)D_3$: 1 h, 2 h, 3 h, 4 h, 5 h, and 6 h	Experiment 1: 8 g after 3 h: 6 h 50 g at 0 h: 9 h 50 g after 8 h: 9 h Experiment 2: Last measurement taken at 6th hour, so peak was not determined	Experiment 2: Absorption in hospitalized elderly and adults with malabsorption was less than in healthy persons $[^3H]25(OH)D_3$: response was less in hospitalized elderly

(continued)

Table 3 **Continued**

Reference	Characteristics of participants	Objective	Intervention	Plasma radioactivity measured at post intervention times	Peak plasma radioactivity	Vitamin D absorption
Krawitt et al. (1977)[32]	Primary biliary cirrhosis (n = 6) CG (n = 4)	Evaluate [^{3}H]D_3 absorption	[^{3}H]D_3 8 μCi + meal with milk + [^{14}C]D_3 2 μCi intravenously	[^{3}H] total, [^{14}C]D_3, [^{14}C]25(OH)D: From 10 min until 13 d	Not reported	PBC with steatorrhea (n = 4) < PBC without steatorrhea (n = 2) and in CG
Schaefer et al. (1972)[33]	Patients taking anticonvulsant drugs (n = 8) CG (n = 5)	Compare [^{14}C]D_3 absorption in patients taking anticonvulsant with that in healthy individuals	[^{14}C]D_3 10 μCi + meal with milk	[^{14}C] total: 4 h, 8 h, 24 h, 72 h, and 144 h	[^{14}C]D_3: 8–24 h	Plasma radioactivity and shape of radioactive peak and decline were similar in both groups
Thompson et al. (1966)[34]	Gastrointestinal disease (n = 10) CG (n = 9)	Apply a protocol for the evaluation of [^{3}H]D_3 absorption in humans	[^{3}H]D_3 1.5–55 μCi + milk	[^{3}H] total: 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, 1 d, 2 d, 3 d, and 4 d	[^{3}H] total: 6–12 h	Celiac disease and patients with steatorrhea ≤ CG Radioactivity after 5 h: 45%–100% in chylomicron fraction

Abbreviations: BR, bowel resection; CD, Crohn disease; CG, control group; [^{14}C]D_3, [^{14}C] carbon-14 labeled cholecalciferol; [^{14}C] 25(OH)D, [^{14}C] carbon-14 labeled 25 hydroxycholecalciferol; IV, intravenous; [^{3}H]D_3, [^{3}H] cholecalciferol; [^{3}H]25(OH)D_3, [^{3}H] 25-hydroxycholecalciferol; MBq, megabecquerel; μCi, microcurie; PBC, primary biliary cirrhosis.

Reboul et al.[6] evaluated vitamin D uptake in human Caco-2 cells, which are models of human intestinal epithelium often employed in the assessment of intestinal transport of lipids, and in human embryonic kidney (HEK) 293T cells, which are validated models of SR-BI, CD36, and NPC1L1 overexpression. Vitamin D uptake was higher in transfected HEK 293T cells and decreased after administration of carrier-specific inhibitors. Both ezetimibe, a pharmacological NPC1L1 inhibitor, and an SR-BI inhibitor similarly reduced the uptake of vitamin D in Caco-2 cells. In live mice treated with ezetimibe, vitamin D absorption decreased nonsignificantly in a study conducted by van Heek et al.[14]

Gonçalves et al.[12] found that long-chain fatty acids, which modulate cholesterol absorption, interfered with vitamin D absorption. They postulated a possible modification in the micellar electrical charge or a modulation of cholecalciferol outflow through the basolateral membrane of enterocytes as a potential mechanism for the reduction in vitamin D absorption. Another possible explanation offered was the competition between free fatty acids and cholecalciferol for the same transporter, especially CD36 or SR-BI, since these 2 proteins are known to be involved in fatty acid uptake.

Phytosterols are commonly employed to reduce dietary cholesterol absorption, and the same research group evaluated, both in vitro and ex vivo, the effects of different sterols on micellar incorporation of cholecalciferol, apical uptake of cholecalciferol, and basolateral efflux.[13] In mice, cholecalciferol bioavailability was 15-fold lower in the presence of β-sitosterol ($P < 0.05$). The significantly impaired cholecalciferol incorporation into mixed micelles (from −16% to −36%. depending on the sterol micellar composition) and the significantly lowered cholecalciferol apical uptake (from −13% to −39%) were cited by the authors as possible causes for the decreased vitamin D bioavailability. They also observed vitamin D and phytosterol competition for a common membrane transporter, supposedly NPC1L1.

Clinical research: radiolabeled absorption tests

Administering radiolabeled forms of vitamin D and then monitoring vitamin D levels in blood, tissues, urine, and feces is the most direct way to evaluate vitamin D absorption. Studies of intestinal absorption of radiolabeled forms of vitamin D in humans are shown in Table 3 and will be described in this section.

Thompson et al.[34] were the first group to describe a protocol for the assessment of vitamin D absorption in humans. The protocol consisted of administering a single oral dose of [^{3}H]D_3 to healthy individuals or to patients with different gastrointestinal diseases and then monitoring total plasma radioactivity over a 4-day

Table 4 **Studies examining absorption of a single oral dose of vitamin D in adults with compromised health**

Reference	Characteristics of participants	Objective	Intervention	Serum vitamin D measured at postintervention times	Vitamin D absorption in adults with compromised health vs in controls
Farraye et al. (2011)[35]	Quiescent Crohn disease (n = 37) Healthy (n = 10)	Compare vitamin D absorption in healthy people with that in Crohn disease patients, and determine whether Crohn location and previous surgeries interfere with vitamin D absorption	D_2 50 000 IU	D_2: 0 h, 12 h	In patients with Crohn disease: Peak D_2 level was 30% lower, $P < 0.01$ Considerable variability between participants Location of disease, past surgery, or surgery type was not predictive of decreased absorption
Aarts et al. (2011)[36]	Obese premenopausal women, 20–50 y Pre- and post-RYGB surgery (n = 14)	Create a method to quantify changes in vitamin D_3 absorption after RYGB	D_3 50 000 IU, pre-RYGB D_3 50 000 IU, post-RYGB	D_3 pre-RYGB: 0 d, 1 d, 2 d, 3 d, and 14 d D_3 post-RYGB: 0 d, 1 d, 2 d, 3 d, and 14 d	In post-RYGB patients: D_3 peak time was not affected ↓ Mean D_3 in 26.6% ± 3.7, $P = 0.02$ Considerable variability between participants
DiVasta et al. (2011)[37]	Young women with anorexia nervosa (n = 12) Controls (n = 12)	Examine serum response to oral vitamin D_2 in malnourished adolescents with anorexia nervosa	D_2 50 000 IU	D_2, D_3, 25(OH)D, and 1,25-D: 0 h, 6 h, and 24 h and weekly for 4 wk	In patients with anorexia nervosa: D_2: Similar baseline levels, peaked later, trajectories converged at 24 h, and returned to baseline by end of week 1 25(OH)D: Similar baseline levels, similar trajectories, peaked at 24 h, and returned to baseline by end of week 3 ↑ D_3 levels at baseline and throughout 4-wk period ↓ 1,25-D, with no changes throughout 4-wk period in either group
von Restorff et al. (2009)[38]	Vitamin D–deficient elderly patients during admission for musculoskeletal pain, bone disease, or gait changes (n = 33)	Evaluate 25(OH)D levels after a high vitamin D dose	D_3 300 000 IU + Calcium 500–1000 mg	25(OH)D: 0 mo, 3 mo, and 6 mo	In elderly patients, mean 25(OH)D (± SD): Baseline = 15 nmol/l (± 5.5) Month 3 = 81.4 nmol/l (± 29.7) Month 6 = 69 nmol/l (± 17.9)
Lark et al. (2001)[39]	Cystic fibrosis (n = 10) Healthy (n = 10)	Assess absorption of vitamin D, 25(OH)D response, and compare healthy persons vs cystic fibrosis patients	D_2 100 000 IU + A meal + Pancreatic enzymes	D_2 and 25(OH)D: 0 h, 5 h, 10 h, 24 h, 30 h, and 36 h	In cystic fibrosis patients:[a] Considerable variability in absorption ↓ D_2 in 50%, $P < 0.001$ ↓ 25(OH)D, $P = 0.0012$
Wortsman et al. (2000)[40]	Obese (n = 19) Normal BMI (n = 19)	Determine if obesity alters absorption of vitamin D_2	D_2 50 000 IU	D_2 and 25(OH)D: 0 h, 5 h, 10 h, 15 h, 20 h, and 25 h after D_2 administration	In obese patients: BMI was inversely correlated with peak serum D_2 concentrations after D_2 intake ($r = -0.56$, $P = 0.007$)

(continued)

Table 4 **Continued**

Reference	Characteristics of participants	Objective	Intervention	Serum vitamin D measured at postintervention times	Vitamin D absorption in adults with compromised health vs in controls
Clemens et al. (1986)[41]	Institutionalized elderly, 57–88 y (n = 7) Young adults, 22–28 y (n = 8)	Study the effect of age on vitamin D absorption	D_2 50 000 IU	D_2: 0 h, 4 h, 8 h, 16 h, 24 h, 48 h, and 72 h	In institutionalized elderly persons and young adults: Similar peak plasma levels: at 8–16 h Similar trajectories: levels returned to baseline on day 3
Lo et al. (1985)[42]	Malabsorptive syndromes (n = 7) Controls (n = 7)	Develop a test for clinical evaluation of vitamin D absorption	D_2 50 000 IU	D_2 and 25(OH)D: 0 h, 4 h, 8 h, 12 h, and 1 d, 2 d, 3 d, and 14 d	In patients with malabsorptive syndromes: Similar results in 2 of 7 patients D_2 peaked at 12 h Levels returned to baseline on day 3 No absorption detected in 5 of 7 patients

Abbreviations and symbols: BMI, body mass index; D_2, ergocalciferol or vitamin D_2; D_3, cholecalciferol or vitamin D_3; 25(OH)D, serum 25-hydroxyvitamin D; 1,25-D, 1,25-dihydroxyvitamin D; RYGB, Roux-en-Y gastric bypass; SEM, standard error of the mean; ↑, increased; ↓, decreased.
[a]No control group.

period. They found higher plasma radioactivity levels 6 to 12 hours after $[^3H]D_3$ administration, with lower plasma radioactivity concentrations in patients with steatorrhea or celiac disease.

Similarly, different malabsorptive conditions such as severe cholestasis,[27,28,32] short bowel disease,[26,30] and inflammatory bowel disease[26] were evaluated in 6 subsequent studies that included control groups. These diseases did not seem to interfere with the time to reach the peak plasma level of vitamin D, with the highest concentrations observed about 6 to 24 hours after oral administration.[26–32] However, they did seem to modify absorption, with lower plasma levels observed in patients with malabsorptive conditions than in control groups. The wide range of time in which the vitamin D plasma peaks were detected was possibly related to different study protocols, different vitamin D plasma measurements, and other noncontrolled factors such as different degrees of malabsorption or different gastrointestinal transit times in patients.

Parallel to patients with malabsorptive disease, hospitalized elderly patients also had lower plasma radioactivity concentrations compared with healthy adults.[31] On the other hand, plasma radioactivity and the shape of the radioactive peak and decline were similar in patients taking anticonvulsants and in healthy individuals.[33]

In addition, the intestinal absorption of labeled cholecalciferol was compared with that of labeled 25(OH)D ($[^3H]25(OH)D_3$) in 3 different studies involving patients with multiple gastrointestinal diseases and control groups.[26,27,30] Administration of $[^3H]25(OH)D_3$ resulted in higher serum radioactivity levels, which also peaked faster than after the administration of labeled cholecalciferol. Therefore, apparently 25(OH)D is better absorbed than its less polar precursor (cholecalciferol or ergocalciferol) in individuals with inflammatory bowel disease or cholestasis. Additionally, Compston et al.[29] observed, in healthy men, that 25(OH)D absorption might be independent of bile acids, with some absorption occurring directly into the portal vein.

In another study, Danielsson et al.[28] investigated whether hepatic hydroxylation is altered in patients with primary biliary cirrhosis. They assessed levels of radiolabeled 25(OH)D ($[^3H]25(OH)D_3$) in serum after $[^3H]D_3$ administration in women with primary biliary cirrhosis and in a control group. The response was similar in both groups, with a gradual increase in $[^3H]25(OH)D_3$ levels observed as $[^3H]D_3$ levels declined, showing that hepatic hydroxylation of vitamin D was not impaired in that population.

Clinical trials

Clinical trials using a single nonlabeled dose of ergocalciferol or cholecalciferol showed results to those of

Table 5 **Studies examining absorption of a single oral dose of vitamin D in healthy individuals**

Reference	Characteristics of participants	Objective	Intervention and groups	Serum vitamin D measured at postintervention times	Vitamin D absorption
Silva et al. (2015)[43]	Young men and women (n = 51)	Evaluate if NPC1L1 cholesterol transporter is involved in D_3 absorption	D_3 50 000 IU: + Meal containing 15 g of fat + Ezetimibe (n = 24) or placebo (n = 27)	25(OH)D: 0 d and 14 d	↑ D_3 ≈ in ezetimibe and placebo groups, $P = 0.26$
Dawson-Hughes et al. (2015)[7]	Men and postmenopausal women, > 50 y (n = 50)	Evaluate if D_3 is best absorbed with dietary fat or with increased amounts of MUFAs/PUFAs	D_3 50 000 IU: + Fat-containing meal (n = 19) + Nonfat meal (n = 31) + Meal with high MUFA/PUFA ratio (n = 20) vs meal with low MUFA/PUFA ratio (n = 11)	D_3: 0 h, 10 h, 12 h, and 14 h	↑ D_3 absorption: greater with fat-containing meal than with nonfat meal High-MUFA/PUFA group = low-MUFA/PUFA group Plasma peak: 12 h
Raimundo et al. (2015)[8]	Young men and women (n = 64)	Compare 25(OH)D levels after D_3 is taken with or without fat-containing meals	D_3 50 000 IU or placebo: Vitamin D was given with meals containing 30 g of fat (n = 15), 15 g of fat (n = 17), or 0 g of fat (n = 15) Placebo was given with meals containing 30 g of fat (n = 5), 15 g of fat (n = 5), or 0 g of fat (n = 7)	25(OH)D: 0 d and 14 d	↑ 25(OH)D: D_3 group> placebo group, $P < 0.001$ 15 g and 30 g fat meals > 0 g fat meal, $P < 0.05$ 15 g fat meal ≈ 30 g fat meal
Dawson-Hughes et al. (2013)[44]	Men and postmenopausal women, 50–59 y (n = 62)	Evaluate whether a meal and its fat content influence D_3 absorption and 25(OH)D levels	D_3 50 000 IU: + No meal (n = 21) + Meal containing 11.1 g fat (n = 20) + Meal containing 35.2 g fat (n = 21)	D_3: 0 h and 12 h 25(OH)D: 0 d, 30 d, and 90 d	↑ D_3: 11.1 g fat meal > 32.2 g fat meal and 0 g fat meal ↑ 25(OH)D: Approx. equal in all groups
Raimundo et al. (2011)[45]	Young men and women (n = 30)	Compare 25(OH)D levels after D_3 is ingested with high-fat vs low-fat meals	D_3 50 000 IU: + Meal containing 25.6 g fat (n = 15) + Meal containing 1.7 g fat (n = 15)	25(OH)D: 0 d, 7 d, and 14 d	↑ 25(OH)D: After 14 d: 25.6 g fat meal > 1.7 g fat meal, $P < 0.01$
Denker et al. (2011)[9]	Adults, 18–85 y (n = 88)	Evaluate the pharmacokinetic parameters of D_3 and ALN, taken alone or in combination	D_3 2800 IU, with/without ALN (n = 28) D_3 5600 IU, with/without ALN (n = 60)	D_3: − 24 h, − 18 h, − 12 h, − 6 h, 0 h, 2 h, 3 h, 5 h, 7 h, 9 h, 12 h, 16 h, 24 h, 36 h, 72 h, 96 h, and 120 h	↑ D_3: Absorption with ALN ≈ absorption without ALN
Wagner et al. (2008)[46]	Adults, 18–60 y (n = 80)	Investigate (1) whether D_3 is bioavailable from fortified hard cheeses and (2) whether food affects D_3 bioavailability after 8 weekly servings of fortified cheese or D_3 supplement	D_3 28 000 IU, in fortified cheese/placebo: Cheddar (n = 20/n = 10) Low-fat cheese (n = 10/n = 10) D_3 28 000 IU, as supplement: With food (n = 20) Without food (n = 10)	25(OH)D: 2 wk, 4 wk, 6 wk, and 8 wk	↑ 25(OH)D: Approx. equal in all groups that received D_3, $P > 0.50$

(continued)

Table 5 **Continued**

Reference	Characteristics of participants	Objective	Intervention and groups	Serum vitamin D measured at postintervention times	Vitamin D absorption
Ilahi et al. (2008)[47]	Elderly adults, 61–84 y (n = 20) Adults, 27–47 y (n = 10) CG, 63–91 y (n = 10)	Evaluate response of 25(OH)D levels after a single dose of D_3	D_3 100 000 (20 elderly + 10 adults) Placebo (n = 10)	25(OH)D: 0 d, 1 d, 3 d, 5 d, 7 d, 14 d, 21 d, 28 d, 42 d, 56 d, 70 d, 84 d, 96 d, and 112 d	↑ 25(OH)D: Plasma peak: 7 d No toxic levels were detected. There was a progressive decline in 25(OH)D levels, with no difference at 84 d or more, when compared with the CG
Johnson et al. (2005)[48]	Adults, 23–50 y (n = 4) Elderly adults, 72–84 y (n = 4)	Compare bioavailability of vitamin D_2 in cheese vs in water, and determine whether absorption differs between younger and older adults	Single acute feedings of: D_2 10 000 IU in cheese D_2 10 000 IU in water Procedures repeated with the other delivery vehicle on the day 14	D_2: 0 h, 6 h, 12 h, and 24 h	↑ D_2: D_2 in cheese, 15 ± 1 ng/mL per 10 000 IU > D_2 in water, 2 ± 0.4 ng/mL/10 000 IU; $P < 0.001$ D_2 from cheese and from water: younger groups ≈ older groups
Armas et al. (2004)[49]	Men, 20–61 y (n = 30)	Test whether D_3 is more effective than D_2 at increasing 25(OH)D	D_2 50 000 IU (n = 10) D_3 50 000 IU (n = 10) No supplement (n = 10)	D_2 or D_3: 0 d, 1 d, 3 d 25(OH)D: 0 d, 1 d, 3 d, 5–7 d, 14 d, and 28 d	↑ 25(OH)D: D_3-treated group > D_2-treated group Began to decline earlier in D_2-treated group ↑ D_2 and D_3: D_3-treated group ≈ D_2-treated group
Holmberg et al. (1990)[50]	Adults, 22–46 y (n = 24)	Compare absorption of D_3 dissolved in peanut oil, which contains long-chain TGs, with absorption of D_3 in a medium-chain TG	D_3 20 640 IU (long-chain TGs/medium-chain TGs): With a meal (n = 6/n = 6) Under fasting conditions (n = 6/n = 5)	D_3 and 25(OH)D: 0 h, 2 h, 4 h, 6 h, 8 h, 10 h, and 12 h 1 d, 2 d, 7 d, 14 d, and 28 d	↑ D_3: Plasma peak: 8–24 h Fasting: medium-chain TGs < long-chain TGs With lunch: medium-chain TGs ≈ long-chain TGs ↑ 25(OH)D: Plasma peak: 7 d Approx. equal in all groups
Whyte et al. (1979)[51]	Young adults (n = 38)	Compare 25(OH)D levels after a single PO, SC, IV, or IM dose of vitamin D preparations	D_2 50 000 IU PO (n = 5) 200 μg/kg D_3 SC (n = 3) 200 μg/kg D_3 IM (n = 3) 200 μg/kg D_2 IM (n = 6) 100 μg/kg D_2 IV (n = 4) 100 μg/kg D_3 IV (n = 17)	25(OH)D: 0, 4, 6, 8, 10, 21, 28, 35, and 42	↑ 25(OH)D: D_2 IV group ≈ D_3 IV group IV and PO groups: rapid increase compared with SC and IM groups 7 wk after injection: increasing absorption in SC and IM groups vs decreasing absorption in IV and PO groups

(continued)

Table 5 **Continued**

Reference	Characteristics of participants	Objective	Intervention and groups	Serum vitamin D measured at postintervention times	Vitamin D absorption
Ellis & Cook (1978)[52]	Indian immigrants with low 25(OH)D levels (n = 7) Europeans, 19–33 y (n = 8)	Assess D_3 absorption in Indians and in persons of European descent	D_3 40 000 IU: + Milk	25(OH)D: 0 d, 1 d, 5 d, 9 d, 20 d, 46 d, 97 d, and 191 d	↑ 25(OH)D: Plasma peak in Europeans: 6 d Plasma peak in Indians: 10 d

Abbreviations and symbols: ALN, alendronate; Approx., approximately; CG, control group; D_3, cholecalciferol or vitamin D_3; 25(OH)D, serum 25-hydroxyvitamin D; IM, intramuscular; IV, intravenous, MUFAs/PUFAs, monosaturated fatty acids/polyunsaturated fatty acids; NPC1L1, Niemann-Pick C1-like 1; PO, orally; SC,subcutaneous; TGs, triglycerides; ≈, approximately equal to; ↑, increased; ↓, decreased.

studies using radioactive forms of ergocalciferol. As ergocalciferol is rarely found in the human diet and is not synthesized by human skin, it is more suitable than cholecalciferol for use in vitamin D absorption tests. The aim of these studies was, in general, to assess the impact of diseases (Table 4) or the influence of dietary compounds (Table 5) on vitamin D absorption.

Impact of disease on vitamin D absorption. Lo et al.[42] were the first group to evaluate the plasma levels after a high dose of nonlabeled ergocalciferol, calling it a challenge test. A capsule containing 50 000 IU of ergocalciferol was offered to 7 patients with clinical fat malabsorptive conditions (cystic fibrosis, Crohn disease, villous atrophy, scleroderma, and ulcerative colitis) and to 7 healthy volunteers. In the healthy group and in 2 patients with malabsorption, plasma ergocalciferol levels increased within 4 hours and peak concentrations were reached by 12 hours, gradually declining to baseline levels within 3 days. In marked contrast, there was no increase in ergocalciferol levels in the other 5 patients with malabsorption.

Similarly, Lark et al.[39] and Farraye et al.[35] conducted ergocalciferol challenge tests in patients with cystic fibrosis and Crohn disease, respectively. Like Lo et al.,[42] both groups also found lower ergocalciferol levels when the unhealthy groups were compared with their respective control groups. It is important to note that there was wide variability in the responses of individual persons.

Besides analyzing ergocalciferol concentrations, Lark et al.[39] examined the 25(OH)D response to a single oral dose of 100 000 IU of ergocalciferol in cystic fibrosis and control groups over 36 hours. In both groups, maximal levels of ergocalciferol were detected in plasma around 24 hours after ergocalciferol administration. As serum ergocalciferol levels gradually declined, serum 25(OH)D levels increased in the control group. In the cystic fibrosis group, 25(OH)D concentrations did not increase significantly at any time.

Roux-en-Y bariatric surgery also altered cholecalciferol absorption in a study by Aarts et al.[36] The difference between the baseline level and the highest postabsorptive cholecalciferol level after a single dose of 50 000 IU of cholecalciferol decreased from 92.0 ± 6.5 to 63.5 ± 10.3 nmol/L after bariatric surgery ($P < 0.02$), although results varied markedly between patients.

On the other hand, young women with anorexia nervosa had a similar response to a single dose of oral ergocalciferol compared with healthy-weight controls, despite their severe malnutrition, in a study by DiVasta et al.[37] Moreover, obesity did not seem to interfere with vitamin D absorption. Peak ergocalciferol concentrations, the difference between peak and basal

concentrations, and mean serum 25(OH)D levels were similar in obese and normal body mass index groups in a study by Wortsman et al.[40] However, body mass index was inversely correlated with peak serum ergocalciferol concentrations after ergocalciferol intake ($r = -0.56$; $P = 0.007$). The authors concluded that obesity-associated vitamin D insufficiency is likely due to the decreased bioavailability of vitamin D deposited in body fat compartments.

Aging also did not seem to interfere with vitamin D absorption. Clemens et al.[41] found similar plasma ergocalciferol peak levels after a single oral dose of 50 000 IU of ergocalciferol in young volunteers and in institutionalized elderly with normal kidney function. Additionally, von Restorff et al.[38] analyzed serum 25(OH)D levels after a single oral dose of 300 000 IU of cholecalciferol in elderly persons with vitamin D deficiency. This intervention increased serum 25(OH)D concentrations in most patients to at least 50 nmol/L, and 48% of patients reached the desirable level of at least75 nmol/L at 3 months. Despite a decline at 6 months, mean 25(OH)D levels were still more than 4 times higher when compared with baseline levels.

Overall, these results highlight the need for caution when interpreting data about vitamin D absorption in health disorders, since several other factors not directly related to the intestinal uptake of vitamin D might interfere with the plasma vitamin D response. For instance, lower serum 25(OH)D levels are commonly found in inflammatory conditions, even when the gastrointestinal tract is not affected.[58] Possible reasons for lower serum 25(OH)D levels include an altered concentration or activity of the 25-hydroxylase enzyme, a decrease in the concentration of vitamin D–binding protein, or higher rates of metabolic clearance of 25(OH)D.

Vitamin D absorption in healthy individuals. Although measurement of serum 25(OH)D could be less sensitive to variations in vitamin D absorption than measurement of serum calciferol, especially during sickness, it has been proven to be a good tool for evaluating both vitamin D status and the effect of vitamin D supplementation in healthy people.[47,52]

Ellis and Cooke[52] were the first group to evaluate the serum 25(OH)D levels in healthy individuals before and at intervals after a single oral dose of 40 000 IU of cholecalciferol. They compared the 25(OH)D response in 2 groups—7 Indian immigrants and 8 Europeans—and found an immediate increase in serum 25(OH)D concentrations after the oral dose in both groups. Over the first 5 days, the mean increase was greater in the Indians than in the Europeans, possibly due to lower 25(OH)D baseline levels in the Indian group.

Subsequently, Armas et al.[49] evaluated the pharmacological effects of cholecalciferol and ergocalciferol by administrating 12 weekly doses of 50 000 IU of the respective calciferols to 20 healthy male volunteers. Both calciferols produced similar rises in the serum concentration of the administered vitamin, indicating equivalent absorption. Similar initial rises in serum 25(OH)D were observed over the first 3 days, but 25(OH)D continued to rise in the cholecalciferol-treated men, peaking after 14 days, while serum 25(OH)D fell rapidly in the ergocalciferol-treated men and was not different from baseline after 14 days. The authors suggested that the potency of ergocalciferol is less than one-third that of cholecalciferol.

In a different approach, Whyte et al.[51] compared the effect of single doses of vitamin D_2 or D_3 administered orally, subcutaneously, intramuscularly, or intravenously to healthy volunteers on 25(OH)D levels. Similar increases in serum 25(OH)D levels were noted after both intravenous D_2 and intravenous D_3. Subcutaneous and intramuscular injection of vitamin D in oil resulted in delayed serum 25(OH)D increases compared with oral and intravenous administration.

Moreover, Ilahi et al.[47] evaluated the pharmacological effects of a single large dose of cholecalciferol (100 000 IU) in healthy people. Peak plasma concentrations of cholecalciferol were reached 8 to 24 hours after administration, while the highest plasma 25(OH)D concentrations were observed 7 days after cholecalciferol administration. It is interesting to note that the peak plasma concentrations of 25(OH)D were reached faster in this study of a single 100 000 IU cholecalciferol dose than in the earlier study of a single 50 000 IU cholecalciferol dose.[47]

Recent studies aimed to evaluate the influence of fat-containing meals on the absorption of cholecalciferol.[7,8,44,45] Raimundo et al.[8,45] conducted 2 randomized controlled trials about the influence of dietary fat on the absorption of a single 50 000 IU oral dose of cholecalciferol. In the more recent study, young medical doctors were divided into 3 groups according to the fat content of meals (0 g, 15 g, or 30 g of fat) given with the cholecalciferol dose or placebo.[8] Mean serum 25(OH)D concentrations were higher in those who received lunch containing at least 15 g of fat, but the group that received the fat-free meal also showed increased 25(OH)D levels when compared with the placebo group.

Following laboratory experiments in which polyunsaturated fatty acids were demonstrated to decrease vitamin D absorption, Dawson-Hughes et al.[7] tested whether the ratio of monounsaturated fatty acids to polyunsaturated fatty acids in food modified vitamin D absorption in men and postmenopausal women, all

older than 50 years. They found that the ratio had no significant influence on vitamin D absorption in this population.

Holmberg et al.[50] showed that cholecalciferol was absorbed more efficiently in the fasting state when incorporated in peanut oil containing long-chain fatty acids than when incorporated in a medium-chain triglyceride, although there was no difference between the 2 formulations when cholecalciferol was administered with food.

Johnson et al.[48] investigated whether the bioavailability of ergocalciferol in cheese was similar to that of ergocalciferol dissolved in water and compared the absorption in younger vs older adults. They concluded that ergocalciferol was absorbed more efficiently from cheese than from water and that age had no impact on absorption. Conversely, Wagner et al.[46] demonstrated, by measuring serum 25(OH)D levels, that the bioavailability of cholecalciferol in fortified cheese and in a liquid supplement, taken either with or without food, was similar.

Denker et al.[9] evaluated the absorption of cholecalciferol doses as low as 5600 IU and 2800 IU. Doses were administered following an overnight fast, and participants continued to fast for 2 hours. The aim of the study was to evaluate if the alendronate component of an alendronate/cholecalciferol combination tablet was bioequivalent to a 70-mg alendronate tablet and if the pharmacokinetic parameters of cholecalciferol were similar with and without alendronate. The bioavailability of alendronate and cholecalciferol was similar in the combination tablet and when administered alone. Notably, cholecalciferol absorption was observed even with nonfat meals and nonoily vehicles.

Clinical trials addressing factors that might interfere with vitamin D absorption are scarce. Silva et al.[43] conducted a randomized, double-blind, placebo-controlled trial intended to determine if the NPC1L1 cholesterol transporter participates in vitamin D absorption. They examined the effect of ezetimibe on serum 25(OH)D levels 14 days after a single oral dose of 50 000 IU of cholecalciferol in healthy young volunteers. The mean change in serum 25(OH)D 14 days after cholecalciferol was not affected by ezetimibe. As already noted, similar results were demonstrated in live mice, supporting the notion that NPC1L1 probably is not an important vitamin D transporter.[6]

Currently, ezetimibe is the only pharmacological inhibitor of cholesterol transporters available for clinical use. This is probably why no clinical studies evaluating the effect of inhibition of other cholesterol transporters, such as SR-BI and CD36, on vitamin D absorption have been performed yet.

CONCLUSION

This systematic review was designed to consolidate and interpret data on the mechanisms of vitamin D absorption, with emphasis on vitamin D uptake through the apical membrane of enterocytes. The reviewed studies revealed some distinctive aspects of vitamin D bioavailability that should be considered when treating or preventing vitamin D deficiency. Ergocalciferol and cholecalciferol, the vitamin D preparations commonly prescribed, are both rapidly absorbed after oral intake, with maximum plasma levels detected around 24 hours after administration. Levels of 25(OH)D increase gradually, peaking at approximately 7 to 14 days, depending on the dose of vitamin D administered. Cholecalciferol seems to be more potent than ergocalciferol, resulting in more sustainable levels of serum 25(OH)D. Absorption is improved when vitamin D is given with fat-containing food and is impaired by intestinal fat malabsorption. Neither aging nor obesity, however, alters vitamin D absorption.

Different forms of vitamin D and various methods were employed to evaluate the absorption of vitamin D. Clinical trials using a single nonlabeled dose of ergocalciferol or cholecalciferol showed results similar to those of studies using radiolabeled vitamin D. Although measurement of serum 25(OH)D is less sensitive than measurement of serum cholecalciferol for detecting minor variations in vitamin D absorption, especially during illness, it was demonstrated to be a good tool for evaluating the effect of vitamin D supplementation in healthy people. Moreover, recent in vivo experiments and in vitro studies demonstrated that vitamin D absorption is not exclusively a simple diffusion process, as previously assumed, but rather a mechanism that involves membrane carriers. Cholesterol transporters may also be responsible for vitamin D uptake, since factors interfering with cholesterol absorption also interfered with vitamin D absorption in laboratory and clinical studies. However, although NPC1L1 is the major cholesterol transporter, it did not seem to play a fundamental role in vitamin D absorption in studies using live rodents or in a clinical trial in which ezetimibe was administered to healthy adults.

This systematic review is limited by the heterogeneity of the included studies, since different methods were used to evaluate vitamin D absorption. Despite recent increased interest in the mechanisms of vitamin D absorption, data on this topic, especially from clinical research, remain scarce. This review provides insight for future research, revealing in particular the need to both identify vitamin D membrane transporters and to translate basic research findings into clinical research.

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