Vitamin D Bioavailability: State of the Art

P. Borel, D. Caillaud, N. J. Cano

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P. BOREL[1-3*], D. CAILLAUD[4], N.J. CANO[4-7]

[1] INSERM, UMR1062, Nutrition, Obesity and Risk of Thrombosis, F-13385, Marseille, France

[2] INRA, UMR1260, F-13385, Marseille, France

[3] Aix-Marseille Univ, Faculté de Médecine, F-13385, Marseille, France

[4] CHU de Clermont-Ferrand, F-63000, Clermont-Ferrand, France

[5] INRA, UMR1019, Unité de Nutrition Humaine, F-63000, Clermont-Ferrand, France

[6] CRNH Auvergne F-63000, Clermont-Ferrand, France

[7] Clermont Université, Université d'Auvergne, F-63000, Clermont-Ferrand, France

Author adress:

Patrick BOREL, Ph.D.

UMR NORT 1260 INRA / 1062 INSERM / Aix-Marseille Université

Faculté de Médecine, 27 boulevard Jean-Moulin, 13385 Marseille Cedex 5, FRANCE.

Phone number: (+33) 4 91 29 41 11; FAX number: (+33) 4 91 78 21 01

E-mail: Patrick.Borel@univ-amu.fr

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List of abbreviations:

25OHD: 25-hydroxy vitamin D, i.e. 25-hydroxy cholecalciferol or/and 25-hydroxy ergocalciferol

$25OHD_2$: 25-hydroxy ergocalciferol

$25OHD_3$: 25-hydroxy cholecalciferol

$1,25(OH)_2D_3$: dihydroxy cholecalciferol

$1,25(OH)_2D_2$: dihydroxy ergocalciferol

FSM: fat-soluble microconstituents, e.g. fat soluble vitamins, carotenoids and phytosterols

Abstract

There has been renewed interest in vitamin D since numerous recent studies have suggested that besides its well-established roles in bone metabolism and immunity, vitamin D status is inversely associated with the incidence of several diseases, e.g. cancers, cardio-vascular diseases and neurodegenerative diseases. Surprisingly, there is very little data on factors that affect absorption of this fat-soluble vitamin, although it is acknowledged that dietary vitamin D could help to fight against the sub-deficient vitamin D status that is common in several populations. This review describes the state of the art concerning the fate of vitamin D in the human upper gastrointestinal tract and on the factors assumed to affect its absorption efficiency. The main conclusions are: (i) ergocalciferol (vitamin D_2), the form mostly used in supplements and fortified foods, is apparently absorbed with similar efficiency to cholecalciferol (vitamin D_3, the main dietary form), (ii) 25-hydroxyvitamin D (25OHD), the metabolite produced in the liver, and which can be found in foods, is better absorbed than the non-hydroxy vitamin D forms cholecalciferol and ergocalciferol, (iii) the amount of fat with which vitamin D is ingested does not seem to significantly modify the bioavailability of vitamin D_3, (iv) the food matrix has apparently little effect on vitamin D bioavailability, (v) sucrose polyesters (Olestra) and tetrahydrolipstatin (orlistat) probably diminish vitamin D absorption, and (vi) there is apparently no effect of ageing on vitamin D absorption efficiency. We also find that there is insufficient, or even no data on the following factors suspected of affecting vitamin D: (i) effect of type and amount of dietary fibre, (ii) effect of vitamin D status, and (iii) effect of genetic variation in proteins involved in its intestinal absorption.
In conclusion, further studies are needed to improve our knowledge of factors affecting vitamin D absorption efficiency. Clinical studies with labelled vitamin D, e.g. deuterated or ^{13}C, are needed to accurately and definitively assess the effect of various factors on its bioavailability.

Introduction

Vitamin D is the generic name for compounds exhibiting the biological activity of cholecalciferol (vitamin D_3). Cholecalciferol is the main dietary source of vitamin D and it is present mostly in foods of animal origin. A significant fraction of vitamin D_3 (estimated at around 80%, but depending on sun exposure) is produced endogenously in the skin from 7-dehydrocholesterol by the action of UV light. Diet can also contain 25-hydroxy cholecalciferol ($25OHD_3$) and trace amounts of dihydroxy cholecalciferol ($1,25(OH)_2D_3$). It is assumed that most people need dietary vitamin D to reach the recommended serum level, i.e. greater than 30 ng/mL (75 nmol/L) (Wimalawansa, 2012). The chemical structures of these vitamin D forms are recalled in **Figure 1**. Very few naturally-occurring foods contain vitamin D. The flesh of fatty fish and fish liver oils are among the best sources. Small amounts of vitamin D are found in beef liver, dairy products and egg yolk. Vitamin D in these foods occurs primarily as vitamin D_3 and its metabolite $25OHD_3$. Some mushrooms provide vitamin D_2 in variable amounts. In some groups, e.g. infants, elderly persons not sufficiently exposed to sunlight, and patients suffering from fat malabsorption, consumption of vitamin D supplements or vitamin D fortified foods are required to meet the daily need, i.e. approximately 2000 IU/day to maintain serum 25(OH)D levels greater than 30 ng/mL (Wimalawansa, 2012), for vitamin D. In supplements and fortified foods, vitamin D can be available as either vitamin D_2 or vitamin D_3. Vitamin D_2 (ergocalciferol) is manufactured by the UV irradiation of ergosterol in yeast, and vitamin D_3 by the irradiation of 7-dehydrocholesterol from lanolin and the chemical conversion of cholesterol. The mechanisms involved in vitamin D absorption and the factors assumed to affect this process have been under-researched. Vitamin D is assumed to follow the fate of lipids in the human upper gastrointestinal tract. It is also assumed that it is secreted in the chylomicrons and then transported to the liver. The absorption efficiency of vitamin D is lower than that of triacylglycerols, and varies widely. Some factors suspected of affecting

its absorption efficiency have been extensively studied, while there is scant or even no data on others. After a review on the fate of vitamin D in the upper human gastrointestinal tract and on recent discoveries concerning mechanisms involved in its absorption, factors known or thought to affect its bioavailability, which is acknowledged as the fraction of ingested vitamin D that is recovered in the blood during the postprandial period after absorption of a known dose of vitamin D, are presented.

Fate of vitamin D in the human upper gastrointestinal tract

The fate of vitamin D in the human upper gastrointestinal tract consists of several sequential steps including physicochemical and enzymatic events. It is assumed that vitamin D absorption is governed by the same intraluminal factors as those that have been described for major lipids (triacylglycerols, cholesterol and phospholipids) (Tso and Fujimoto, 1991). These include emulsification, solubilisation in mixed micelles, diffusion across the unstirred water layer and permeation through the enterocyte membrane.

Vitamin D metabolism may begin in the stomach, where foods are subjected to acid conditions and gastric enzymes. There is apparently no data on the sensitivity of the main dietary forms of vitamin D (cholecalciferol, ergocalciferol, $25OHD_3$) to the acidic pH of the stomach. It can be hypothesised that pepsin plays a role in vitamin D absorption by releasing the fraction of vitamin D associated with proteins. Because vitamin D is fat-soluble it can also be hypothesised that a fraction of dietary vitamin D not incorporated in oils, e.g. vitamin D in fish flesh, transfers to the fat phase of the meal during digestion. This might improve vitamin D bioavailability, assuming that vitamin D in oil is more bioaccessible than vitamin D in a complex matrix. It is reasonable to suggest that the transfer efficiency depends on the characteristics of the food matrix and on the presence of fat, but no data is available on this topic. Finally, there is no data on the role of gastric lipase on vitamin D ester hydrolysis, but it

can be suggested that vitamin D esters (Hollis et al., 1996) are at least partially hydrolysed by this lipase. This could be of importance when vitamin D is ingested as vitamin D esters, and when pancreatic lipase activity is not optimal (e.g. neonates and pancreatic insufficiency).

In the duodenum, digestive enzymes, e.g. proteases, amylases and lipases, continue to release vitamin D from the food matrix. It is assumed that vitamin D not incorporated in oils, i.e. present naturally in dietary fat or transferred from a non-food matrix to the fat phase of the meal in the stomach, is transferred from the food matrix to dietary fat and then into micelles, but it is not known whether there is a direct transfer of vitamin D from the food matrix into micelles. In the duodenum, it is assumed that vitamin D esters, if present, are hydrolysed by bile salt stimulated lipase (Lombardo and Guy, 1980), also called carboxyl ester lipase, but it is not known whether other hydrolytic enzymes, such as pancreatic lipase (Mathias et al., 1981a), pancreatic lipase related proteins (Reboul et al., 2006a), or enzymes of the brush border (Mathias et al., 1981b) are also involved in their hydrolysis. It is assumed that vitamin D_3 is solubilised in mixed micelles (Rautureau and Rambaud, 1981) and absorbed by the lymphatic route, while $25OHD_3$, being more polar, is less dependent on bile acids, and being water-soluble, is mainly absorbed *via* the portal route (Maislos et al., 1981). This has been confirmed by an Israeli team who studied the effect of absence of bile salts on the absorption of non-hydroxylated and hydroxylated vitamin D forms. Their results showed that $1,25(OH)_2D_3$ absorption was unaffected by the lack of biliary salts. Conversely, absorption of $25OHD_3$ was decreased and that of vitamin D_3 was fully impaired (Maislos and Shany, 1987). Fat-soluble entities can be theoretically solubilised in structures other than micelles in the gut. More precisely, as vesicles have been observed in the human duodenal lumen during digestion (Staggers et al., 1990), it is hypothesised that they can incorporate fat-soluble substances in their phospholipid membranes (Urano et al., 1987; Kirilenko and Gregoriadis, 1993). However, it is not known whether a significant proportion of the different forms of vitamin D is incorporated in vesicles, and whether this has any effect on their absorption.

The main site of vitamin D absorption in humans is not accurately known, but in the rat, uptake was observed in both jejunum and ileum (Hollander et al., 1978; Reboul et al., 2011), the ileum being the main site of absorption (Reboul et al., 2011). Absorption efficiency of vitamin D_3 given in peanut oil was found to range between 55 % and 99 % (mean 78%) in healthy subjects (Thompson et al., 1966). These rates are similar to those from animal experiments, where rates between 66% and 75% were reported. Vitamin D is detected in the bile. However, the significance of a conservative enterohepatic circulation of vitamin D is controversial. **Figure 2** summarises current knowledge on the fate of vitamin D in the human upper gastrointestinal tract. In addition, although the metabolism of vitamin D lies outside the scope of this review, we need to know how vitamin D metabolism modifies the forms of vitamin D that circulate in the blood; a figure is therefore presented that summarises the current knowledge of vitamin D metabolism in the human body (**Figure 3**).

Absorption mechanisms of vitamin D

From early studies using everted rat gut sacs (Hollander and Truscott, 1976) and perfusion of rat small intestinal loops (Hollander et al., 1978), the mechanism of absorption of the non-hydroxylated forms of vitamin D, i.e. cholecalciferol and ergocalciferol, had been assumed to be a non-saturable passive diffusion process. However, recent results using the human intestinal cell line Caco-2 and HEK transfected cells have shown that intestinal cell membrane proteins are involved in the uptake of these non-hydroxylated forms at the apical side of the enterocyte (Reboul et al., 2011). These proteins are SR-BI (Scavenger Receptor class B type 1), CD36 (Cluster Determinant 36) and NPC1L1 (Niemann-Pick C1-Like 1), also involved in apical uptake of cholesterol and of other lipidic micronutrients: vitamin E (Reboul et al., 2006b; Narushima et al., 2008) and carotenoids (Reboul et al., 2005; van Bennekum et al., 2005; Moussa et al., 2008). The results of Reboul et al. (Reboul et al., 2011) also suggest

that vitamin D uptake is mainly protein-mediated at low, dietary concentrations of vitamin D, while it is also passive at high, pharmacological concentrations. Finally, the fact that cholecalciferol uptake was higher in the rat jejunum than in the duodenum suggests that another transporter specifically expressed in the jejunum might play an important role in vitamin D uptake (Reboul et al., 2011). There is no data concerning absorption mechanisms of the hydroxylated forms of vitamin D. However, it can be hypothesised that the mechanisms are different, because these entities ($25OHD_3$ and $1,25(OH)_2D_3$) are less dependent on bile acids for absorption, and their absorption efficiency is considerably higher, about three times that of the non-hydroxylated forms (Compston et al., 1981). **Figure 4** shows the current knowledge on proteins involved in absorption of vitamin D by human intestinal cells.

Possible factors affecting absorption

Before it can be absorbed, vitamin D has to be released from the food matrix, e.g. fish oil, fish flesh, or mushrooms, in which it is embedded, and be presented to the brush-border in a state such that it can be absorbed by intestinal cells. It is assumed that absorption efficiency depends on an array of variables including: (i) the food matrix, (ii) the composition of the meal, (iii) the activities of digestive enzymes, and (iv) the efficiency of transport across the enterocyte. The mnemonic "SLAMENGHI", listing the factors thought to govern carotenoid absorption (de Pee and West, 1996), was used to review the factors suspected to affect vitamin D absorption. Each letter stands for a factor: Species (molecular forms) of vitamin D, molecular Linkage of vitamin D (e.g. esterification), Amount of vitamin D consumed in a meal, Matrix in which vitamin D is incorporated, Effectors of absorption and bioconversion, Nutrient status of the host, Genetic factors, Host-related factors, and mathematical Interactions. Each SLAMENGHI factor was reviewed by querying the on-line US National Library of Medicine (last review January 31, 2012). It appeared that some factors

had been extensively studied, e.g. the effect of ageing or the effect of diseases that lead to fat malabsorption, while there is no data on other factors, e.g. effect of gender or genetic factors. Results from the available literature are presented here.

a. Species of vitamin D

Although cholecalciferol is the main dietary form of vitamin D, foods can also contain ergocalciferol and 25OHD$_3$. Several studies have compared the bioavailabilities of these two vitamin D species with that of vitamin D$_3$.

- Ergocalciferol *vs.* cholecalciferol: In a first study, bioavailability was evaluated by administering single doses of 50,000 IU of either cholecalciferol or ergocalciferol to 20 healthy male subjects. The two forms produced similar initial rises in serum 25OHD over the first 3 days, suggesting equivalent intestinal absorption (Armas et al., 2004). In a second study (Holick et al., 2008), healthy subjects received either placebo, or 1000 IU vitamin D$_3$, or 1000 IU vitamin D$_2$, or a mixture of 500 IU vitamin D$_3$ and 500 IU vitamin D$_2$. 25OHD increased similarly in all the vitamin D supplemented groups, again suggesting similar bioavailabilities of the two forms. A consistent result was obtained recently when vitamin D$_2$ and vitamin D$_3$, incorporated in orange juice or in a supplement, gave similar serum 25OHD responses (Biancuzzo et al., 2010). However, two clinical studies (Romagnoli et al., 2008; Khazai et al., 2009a) suggested a higher bioavailability of vitamin D$_3$ than of vitamin D$_2$. However, there was a confounding variable in the study of Khazai et al. (Khazai et al., 2009a): vitamin D$_3$ was in a powder-based supplement while vitamin D$_2$ was in oil. Furthermore, the subjects had cystic fibrosis, and the effect of the vehicle in which vitamin D is solubilised can be more critical, especially when it is oil, in subjects with fat malabsorption than in healthy subjects. The study of Romagnoli et al. (Romagnoli et al., 2008) found that vitamin D$_3$ was apparently

almost twice as potent as vitamin D_2 in increasing serum 25OHD after 60 d. However, as discussed by the authors themselves, the 25OHD increased in parallel, suggesting a comparative absorption. Thus the difference in 25OHD levels after 60 d was attributed to a more rapid metabolism/clearance of vitamin D_2 than of vitamin D_3 metabolites (see below). The best way to avoid the effect of liver and kidney metabolism on blood vitamin D concentration is to compare absorption in cultures of human intestinal cell. This was recently done (Reboul et al., 2011) and it was shown that vitamin D_3 and vitamin D_2 were absorbed with similar efficiencies by Caco-2 cells.

The biological equivalence between vitamin D_3 and vitamin D_2 is still a matter of debate (Armas et al., 2004; Khazai et al., 2009b; Binkley et al., 2012; Youssef et al., 2012). Thus the fact that absorption efficiencies of vitamin D_3 and vitamin D_2 are apparently similar contrasts with the finding in the clinical study by Armas et al. (Armas et al., 2004) that serum 25OHD continued to rise in the vitamin D_3-treated subjects, peaking at 14 d, while 25OHD fell rapidly in the vitamin D_2-treated subjects and was not different from baseline at 14 d. Consequently, area under the curve (AUC) to day 28 was 151 nmol.d/L for vitamin D_2 and 512 for vitamin D_3. It was concluded that vitamin D_2 potency was less than one third that of vitamin D_3. This conclusion is supported by three studies from another team who showed that treatment with vitamins D_2 and D_3 in the same doses produced considerably different serum concentrations of vitamin D metabolites (Tjellesen et al., 1985b; Tjellesen et al., 1986), and not the same action of the two vitamins on bone metabolism (Tjellesen et al., 1985a), and also by the results of a study with very large doses of vitamin D_2 and D_3 given to elderly subjects (Romagnoli et al., 2008). The mechanisms explaining the different biological effects of vitamin D_2 and D_3 are apparently not linked to their first hydroxylation by the liver, as similar increases in serum 25OHD levels were observed after intravenous injection of either vitamin D_2 or D_3. Other mechanisms have been suggested, e.g. different affinities of $1,25(OH)_2D_2$ and

1,25OHD_3 for the VDR nuclear receptor, but these hypotheses remain to be addressed in dedicated studies.

- 25OHD_3 *vs.* cholecalciferol and ergocalciferol: The relative bioavailability of vitamin D_3 and 25OHD_3 is well-documented. In healthy subjects, the total amount of [3H]vitamin D_3 recovered in plasma 6 h after [3H]25OHD_3 intake was considerably higher after [3H]25OHD_3 than after [3H]cholecalciferol intake. Conversely, a much higher percentage of [3H]vitamin D_3 was found in the chylomicron fraction after ingestion of [3H]vitamin D_3 than after ingestion of [3H]25OHD_3 (Compston et al., 1981). This apparent discrepancy was explained by the fact that vitamin D_3 absorption occurs *via* chylomicrons, while that of 25OHD_3 occurs *via* the portal route. However it is also possible that a significant fraction of newly absorbed cholecalciferol and ergocalciferol is quickly stored in adipose tissue leading to lower blood concentrations of these forms as compared to 25OHD_3. The higher bioavailability of 25OHD_3 than of vitamin D_2 and D_3 was confirmed in another study in which ten times more vitamin D, either as ergocalciferol or as cholecalciferol, than 25OHD_3 was required to produce equivalent plasma 25OHD_3 concentrations (Stamp et al., 1977). As patients suffering from diseases that lead to fat malabsorption also malabsorb vitamin D (Lo et al., 1985; Heubi et al., 1989; Lark et al., 2001), several studies were dedicated to assessing whether 25OHD_3 could be used to improve vitamin D absorption in these patients: the results were positive. The absorption of cholecalciferol and 25OHD_3 was compared in normal subjects and in patients with mild and severe cholestatic liver disease. Results showed that absorption of 25OHD_3 was greater than that of cholecalciferol in all three groups (Sitrin and Bengoa, 1987). In another study in patients with Crohn's disease and resections of the small bowel, it was also observed that 25OHD_3 was better absorbed than cholecalciferol (Leichtmann et al., 1991).

In conclusion, 25OHD is better absorbed than the non-hydroxylated forms cholecalciferol and ergocalciferol. These forms are apparently absorbed with the same efficiency, but metabolism and biological activities of the hydroxylated metabolites of vitamin D_2 and D_3 are apparently different, and lead to an apparent lower biological activity of vitamin D_2 compared with vitamin D_3.

b. Molecular Linkage

Dietary vitamin D is non-esterified, but because some supplements may contain vitamin D esters (Hollis et al., 1996), the question arises of whether esterification affects vitamin D bioavailability. Only one study is dedicated to comparing the relative absorption efficiency of free vitamin D and vitamin D esters (Hollis et al., 1996). In this study, performed at various postnatal ages, the abilities of vitamin D_3-palmitate and non-esterified vitamin D_2 to elevate circulating vitamin D_3 and vitamin D_2, respectively, were compared. It was concluded that the two forms approached equivalence when the gastrointestinal tract was mature. Conversely, vitamin D_3-palmitate bioavailability was lower below age 10 days, probably due to the lack of maturation of the digestive tract. This confirms the need for an efficient digestion of lipids to efficiently absorb vitamin D, in particular vitamin D esters.

c. Amount consumed in a meal

It is generally assumed that like other vitamins, the absorption efficiency of vitamin D decreases with increasing dose. The effect of the dose of vitamin D on its absorption efficiency was studied in the rat. In both the jejunum and ileum, a linear relationship was found between the absorption rate of the vitamin and its intraluminal concentration (Hollander et al., 1978). This suggests that at least in the range of the studied concentrations, vitamin D

absorption efficiency does not significantly decrease with dose. This result is supported by the result of a clinical study in which 116 subjects were placed in nine treatment groups that ingested vitamin D_3 (25, 250 or 1250 µg/d for 8 weeks), $25OHD_3$ (10, 20 or 50 µg/d for 4 weeks) or $1,25(OH)_2D_3$ (0.5, 1.0 or 2.0 µg/d for 2 weeks). Results showed that serum levels of all three forms increased linearly with the dietary dose, showing a similar efficiency of absorption, at least in the range of doses studied (Barger-Lux et al., 1998). A recent study, performed in the human intestinal cell line Caco-2, has shown that at low concentrations of vitamin D_3 in micelles, i.e. between 0.1 and 2-3 µmole/L, higher than the physiological concentration in the human duodenum, calculated to be about 0.01 µmole/L (Reboul et al., 2011), the uptake of both vitamin D_3 and D_2 was saturable, while it was linear at high pharmacological concentrations, i.e. up to 10 µmole/L. This result supports the involvement of intestinal proteins in vitamin D absorption at dietary concentrations and a passive diffusion that becomes preponderant at pharmacological concentrations.

d. Matrix in which vitamin D is incorporated

This factor is thought to be important because it is assumed that vitamin D needs to be extracted from its food or supplement matrix to become bioaccessible, i.e. to become solubilised in micelles and available for absorption.

Vitamin D bioavailability in foods was measured in some foods only, i.e. meat, mushrooms, orange juice, milk and fortified cheese. This was done in different studies and it is therefore difficult to compare the values obtained. In a clinical study, the relative bioavailability of vitamin D_2 from pig meat sources was estimated to be about 60% compared with a vitamin D_2 supplement (van den Berg, 1997). In two studies, performed in the rat, it was concluded that vitamin D_2 from UV-irradiated mushrooms (*Agaricus bisporus* or *Lentinula edodes*) was bioavailable (Jasinghe et al., 2005; Koyyalamudi et al., 2009), but there

was no comparison of the bioavailability with any other matrix. The bioavailability of vitamin D_2 from mushrooms (*Cantharellus tubaeformis*) was confirmed in a clinical study involving 27 volunteers. The volunteers were randomly divided up into three groups of nine persons. For 3 weeks, group 1 received mushrooms providing 14 µg ergocalciferol/d with their lunch, group 2 received an ergocalciferol supplement providing 14 µg/d, and group 3 received no supplementation. Serum 25OHD concentrations at 3 weeks were higher in groups 1 and 2 than in group 3, and did not significantly differ between groups 1 and 2, suggesting that the vitamin D_2 in mushrooms had the same bioavailability as the vitamin D_2 in supplement (Outila et al., 1999). A recent controlled trial confirmed this finding, showing that vitamin D_2 from UV-B-irradiated button mushrooms had the same bioavailability as that of a vitamin D_2 supplement (Urbain et al., 2011). A clinical study found similar bioavailabilities of 25000 UI vitamin D_2 ingested with either milk (whole or skimmed) or 0.1 mL corn oil on toast (Tangpricha et al., 2003), suggesting no effect of milk nutrients on vitamin D bioavailability. Four years ago, a clinical study compared the bioavailability of vitamin D_3 from fortified wheat bread, fortified rye bread and a supplement (Natri et al., 2006). Both fortified breads increased serum 25OHD as effectively as the supplement. It was concluded that bread matrix did not significantly affect vitamin D bioavailability. The bioavailability of vitamin D in bread was confirmed recently in a study that showed that vitamin D2-rich yeast baked into bread was bioavailable and improved bone quality in vitamin D-deficient rats (Hohman et al., 2011). Recently, a clinical study involving 80 subjects was dedicated to comparing the bioavailability of vitamin D_3 between fortified cheeses and a supplement (liquid vitamin D_3). It was concluded (from measurement of blood 25OHD) that vitamin D_3 was equally bioavailable from both sources (Wagner et al., 2008). Finally, a recent study showed that vitamins D2 and D3 were equally bioavailable in orange juice and in supplement (Biancuzzo et al., 2010).

Concerning vitamin D bioavailability in supplements, the effect of the vehicle substance used in vitamin D supplements on vitamin D bioavailability was recently reviewed

(Grossmann and Tangpricha, 2010). It is concluded that although limited studies are available, vitamin D in an oil vehicle is more bioavailable than when incorporated in a powder-based vehicle (cellulose or lactose) or in ethanol. However, a recent clinical trial has concluded that vitamin D is more bioavailable from a lactose capsule than from an oily drop formulation (Coelho et al., 2010).

In summary, most data suggest that food matrix has no marked effect on vitamin D bioavailability. The effect of supplement matrix is less clear.

e. Effectors of absorption

A very recent study found that taking vitamin D supplement with the largest meal of the day improved vitamin D absorption (Mulligan and Licata). This suggests that one or more components of the meal, or gut enzymes secreted after meal intake, improve vitamin D absorption. However, another study did not confirm this finding, showing no significant difference in blood 25OHD levels between subjects consuming a vitamin D supplement with or without food (Wagner et al., 2008). As foods contain a multitude of substances (nutrients, micronutrients, fibre, phytochemicals, etc.) it is likely that the different results of these two studies were due to the different composition of the meals. To evaluate whether food components can affect vitamin D bioavailability, it is necessary to perform dedicated experiments to study the effect of each food component. Some studies presented here have used this approach.

I. Dietary lipids

Lipids are assumed to improve absorption of fat-soluble microconstituents (FSM) by several mechanisms. First, they can facilitate the release of FSM from food matrices by

providing a hydrophobic phase where FSM can be solubilised. Second, because lipids stimulate biliary secretion, and consequently micelle production, they can increase the proportion of micellarised FSM, i.e. of FSM available for absorption. Third, lipid digestion products, e.g. fatty acids, monoglycerides and lysophospholipids, are micelle components and so the more lipids digested, the more micelles are available to solubilise FSM (Hofmann, 1963). Finally, by inducing chylomicron synthesis, lipids can enhance FSM transport outside the enterocytes and thus prevent the accumulation of vitamin D in enterocytes. This in turn will theoretically increase FSM absorption. Several characteristics of dietary lipids are thought to affect FSM absorption: (i) the amount of triacylglycerols ingested with vitamin D, (ii) the species of fatty acids that constitute triacylglycerols (iii) the amounts of phospholipids, d) the species of phospholipids, and (iv) the emulsification of lipids. However, data on the effect of only two of these characteristics is available.

- Amount of fat (triacylglycerols)

Surprisingly, adding 2.5 mM fatty acids of varying chain length and degree of saturation resulted in a decrease in the rate of vitamin D_3 absorption in rats (Hollander et al., 1978). This suggests that conversely to what is observed for other fat-soluble vitamins (vitamin E and β-carotene), fat may diminish vitamin D absorption. However, four clinical studies did not find any significant effect of fat on vitamin D bioavailability. The first one, mainly designed to compare the bioavailability of vitamin D in two beverages (orange juice and milk), showed that the fat content of milk did not significantly affect vitamin D_2 bioavailability in 18 adults (Tangpricha et al., 2003). The second study did not find any difference between vitamin D_3 fortified cheddar cheese (~33% fat) and vitamin D_3 fortified low-fat cheese (~7% fat) in their ability to increase blood 25OHD (Wagner et al., 2008). The third study found no difference between vitamin D in multivitamin tablets and vitamin D in fish oil in their ability to increase serum 25OHD (Holvik et al., 2007). In the fourth trial,

consumption of vitamin D with 2 g fish oil once weekly did not improve vitamin D absorption (Korkor and Bretzmann, 2009). We note that in a recent clinical study (Raimundo et al., 2011), it was concluded that a high-fat meal (25.6 g fat/meal) increased vitamin D absorption compared with a low fat meal (1.7 g). However, a close look at the results obtained reveals that there was no effect of either the low fat or high fat meals, which contained 50000 UI vitamin D_3, on serum 25OHD 7 days after intake. Furthermore, it was not a crossover study and the authors acknowledged that some of the subjects may have had secondary hyperparathyroidism. Thus the available data suggests that the amount of fat ingested with vitamin D has no major effect on vitamin D bioavailability.

- Type of fatty acid (within triacylglycerols)

The pioneering work on this topic was performed by Hollander's team (Hollander et al., 1978). It was shown in rat perfusate intestine that addition of fatty acids of varying chain length and degrees of saturation, i.e. butyric, octanoic, oleic and linoleic, resulted in a decrease in the rate of vitamin D_3 absorption. More precisely, the inhibitory effects of the oleic and linoleic acids were higher than that of octanoic acid in the distal part of the intestine. The authors suggested that unlike medium-chain fatty acids, which are not incorporated in micelles, long-chain fatty acids hinder vitamin D absorption by causing enlargement of the micellar size, thereby slowing their diffusion towards the enterocyte. However, this result was not confirmed in a clinical trial: absorption of a pharmacological dose of vitamin D_3 was compared when ingested with either medium-chain triacylglycerols or long-chain triacylglycerols (peanut oil). Results showed that the serum levels of vitamin D_3 were significantly higher after administration in peanut oil (long-chain triglycerides) than after administration in medium-chain triglycerides. (Holmberg et al., 1990). Concerning the comparison between different types of fatty acids, a recent clinical study has concluded that

diets rich in monounsaturated fatty acids may improve the effectiveness of vitamin D_3 supplements in healthy older adults, while those rich in polyunsaturated fatty acids may reduce it (Niramitmahapanya et al., 2011).

Taken together, these results suggest that the type of fatty acid can affect vitamin D bioavailability. However, further studies are required to draw firm conclusions on the compared effects of each type of fatty acid on vitamin D bioavailability.

II. Dietary Fibre

Dietary fibre has been suspected of affecting FSM absorption by several mechanisms: (i) by affecting micelle formation; (ii) by altering emulsification and triacylglycerol lipolysis (Pasquier et al., 1996) and thus affecting the release of FSM embedded in fat droplets, and (iii) by increasing the viscosity of the chyme and thus limiting the diffusion of FSM containing micelles to the brush border. Compston suggested that the high dietary fibre intake of Asian immigrants caused vitamin D bioavailability to diminish, thus explaining the higher incidence of rickets and osteomalacia in this population (Compston, 1979). However, there are only two clinical studies on the effect of fibre on vitamin D absorption. The first study compared the rate of plasma disappearance of radiolabelled 25OHD in healthy volunteers on either normal or high-fibre diets (20 g/d). The authors observed that the mean plasma half-life of 3H-25OHD_3 in the high-fibre group was 19.2 ± 1.7 d, significantly shorter than in the group on normal diet (27.5 ± 2.1 d). They suggested that fibre enhanced elimination of 25OHD by an action in the intestinal lumen (Batchelor and Compston, 1983). However, a recent clinical study did not find any significant differences between vitamin D fortified low-fiber wheat bread (3 g/100 g) and high-fiber rye bread (12 g/100 g) consumed for 3 weeks, in their ability to increase blood 25OHD in 41 healthy subjects (Natri et al., 2006). However, the authors

acknowledged that there was no significant difference in daily total fiber intake among the two groups of subjects, because they were allowed to eat other breads. In conclusion, there is too little data to conclude on the effect of this factor on vitamin D bioavailability.

III. Inhibitors of lipid absorption

Since obesity is a major health problem, several antiobesity drugs have been proposed to diminish the absorption of triacylglycerols and cholesterol. Because of the closely similar fate of lipids and vitamin D in the gastrointestinal lumen, it has been hypothesised that these drugs can also impair vitamin D absorption. It has been shown that tetrahydrolipstatin (orlistat), a non-absorbed inhibitor of gastric and pancreatic lipases, and olestra, a sucrose polyester used as a fat substitute, reduce absorption of vitamin D (Schlagheck et al., 1997; McDuffie et al., 2002). Phytosterols, the plant sterols used to diminish cholesterol absorption, might also impair vitamin D absorption by impairing its solubilisation in mixed micelles. This hypothesis was supported both by results obtained in rats that showed a decrease in both blood and liver vitamin D levels after dietary levels of stanol esters for 13 wks (Turnbull et al., 1999), and by recent results obtained *in vitro* and in mice that showed that phytosterols compete with vitamin D_3 for incorporation into mixed micelles as well as for apical uptake (Goncalves et al., 2011). Surprisingly, phytosterols were claimed to have no effect on vitamin D status in different clinical trials [32, 33]. However, in all these studies, vitamin D status was evaluated as plasma 25OHD, which is highly variable depending on factors such as season and sun exposure. As a consequence, 25OHD levels can display a high variability between the beginning and end of the study or between the different groups (Gylling et al., 2010). In addition, the intervention periods may have been too short to reveal a significant effect on vitamin D homeostasis. Finally, we note that a close look at one of these studies reveals a significant lowering effect of a sitostanol ester enriched margarine on vitamin D

plasma level after a long-term treatment (Gylling et al., 1999). We note that the negative effect of inhibitors of lipid absorption on absorption of fat soluble vitamins can be offset by adding vitamin supplements to the diet. This was demonstrated in a clinical study in 102 subjects in whom serum concentrations of vitamin E and $25OHD_2$ were restored to control concentrations by adding 2.1 mg d-α-tocopheryl acetate and 0.06 µg ergocalciferol per gram of olestra, respectively, to the diet (Schlagheck et al., 1997).

IV. Microconstituents

As the main dietary source of vitamin D, i.e. cholecalciferol, can be ingested together with other vitamin D species (vitamin D_2, vitamin D esters, etc.) and other lipid micronutrients (other fat-soluble vitamins, carotenoids and phytosterols), and because common mechanisms of absorption are involved (Reboul and Borel, 2011), it has been hypothesised that interactions can occur that affect their absorption (Reboul and Borel, 2011). A study performed in our laboratory in Caco-2 cells supports this hypothesis by showing that high concentrations of vitamin D_2 significantly impair cholecalciferol absorption (Reboul et al., 2011). However, this competition should be re-assessed *in vivo*, as conflicting results have been published: in a first clinical study, oral administration of 400 IU/d of vitamin D_2 for 1 week led to very small variations of plasma vitamin D_3 and its metabolites (Matsuoka et al., 1989). Furthermore, in a recent study, a mixture of 500 IU of vitamin D_3 and 500 IU of vitamin D_2 led to a similar rise in 25OHD as 1000 IU of vitamin D_3 (Holick et al., 2008), suggesting no inhibition of D_3 absorption by D_2 at relatively low doses. Conversely, in another study, vitamin D_2 treatment (4000 IU/d for 8 weeks) decreased blood $25OHD_3$ and $1,25(OH)_2D_3$ (Tjellesen et al., 1986). Finally, a recent study in cows has shown that vitamin D_3, given as an oral bolus of 250 mg (1.0×10^7 IU) after vitamin D_2, is less efficient in increasing the plasma status of $25OHD_3$ than the same dose of vitamin D_3 given without

previous vitamin D_2 administration (Hymoller and Jensen, 2011). Thus high doses of vitamin D_2 supplements might decrease vitamin D_3 absorption, but available data are in different species and therefore further studies are needed to definitely conclude on the effect of high doses vitamin D_2 on vitamin D_3 absorption in humans.

V. Milk and milk-derived products

The bioavailability of vitamin D naturally present in milk has never been measured, probably owing to the low concentration of the vitamin in it (about 25 IU of vitamin D/L human milk). By contrast, studies were performed to measure bioavailability of vitamin D in fortified milk or cheeses. In a first study in 18 subjects, it was shown that milk, either whole or skimmed, did not significantly modify vitamin D_2 bioavailability compared with its intake without milk (Tangpricha et al., 2003). In a second study in young and older adults, it was observed that vitamin D bioavailability was higher when incorporated in cheese than when solubilised in water (Johnson et al., 2005). In a third study, dedicated to comparing vitamin D bioavailability between fortified cheeses and supplement, it was concluded that vitamin D was equally absorbed between fortified cheese and water (Wagner et al., 2008). In conclusion, it appears that milk or cheese do not significantly affect vitamin D bioavailability.

VI. Absorption enhancers

Some substances, e.g. β-cyclodextrine, nanoparticles and compounds able to form micelles, are used to improve absorption efficiency of liposoluble drugs or fat-soluble vitamins. Their ability to enhance absorption efficiency of vitamin D was assessed in some studies. The first absorption enhancer studied was a water-soluble form of vitamin E (tocopheryl succinate polyethylene glycol 1000 (TPGS). This substance was a good candidate

for improving vitamin D absorption as it is able to form micelles that can solubilise lipophilic compounds including vitamin D. This was assessed in a clinical study with eight children (aged 5 mo to 19 y) suffering from severe chronic cholestasis. Results showed a significantly higher absorption of vitamin D administered in a mixture with TPGS (Argao et al., 1992). The ability of β-cyclodextrine to enhance absorption of vitamin D was studied in rats. Results showed a 2.5-fold increase in absorption compared with vitamin D alone (Szejtli et al., 1983).

f. Nutrient status of the host

As vitamin D has numerous biological effects, and as large amounts of vitamin D can be toxic, it can be hypothesised that as observed for vitamin A (Lobo et al., 2010), vitamin D absorption is regulated by vitamin D status. Surprisingly, only one study in rats has been dedicated to testing this hypothesis. In this study it was observed that the intestinal absorption of vitamin D_3 and its hydroxylation in the liver were higher in vitamin D-deficient rats than in vitamin D-treated ones (Apukhovskaia et al., 1990). This very interesting finding requires support from further experiments.

g. Genetic factors

A line of results has suggested that genetic variants play a key role in modulating 25OHD blood concentrations (Orton et al., 2008; Fu et al., 2009; Sinotte et al., 2009; Ahn et al., 2010; Karohl et al., ; McGrath et al., 2010). The fact that proteins are involved in cellular uptake of vitamin D raises the question of the impact of modulations in the expression or activity of these proteins on blood and tissue concentrations of vitamin D. The expression and activity of proteins can be modulated by several factors, including genetic ones. Genetic variations in or near genes that encode proteins may affect protein expression, e.g. a genetic

variation that affects the binding of a transcription factor in the promoter region of a gene (Hernandez-Romano et al., 2009), or protein activity, e.g. a genetic variation that leads to a functional modification in the amino acid sequence of the protein, and in turn the ability of these proteins to accurately perform their function in the metabolism (Lindqvist et al., 2007; Borel, 2011). However, no result has been published on this exciting topic.

h. Host-related factors

A line of evidence suggests that host-related factors (mainly age and diseases) can have an effect on vitamin D absorption. Thus several studies have been performed to assess their effect in order to optimise RDA as a function of age and disease.

I. Age

Ageing, by affecting numerous physiological processes, may directly or indirectly affect vitamin D absorption. More precisely, age-related alterations of gastrointestinal tract functions (Vellas et al., 1991; Russell, 1992; Ikuma et al., 1996) may modify the efficiency of vitamin D absorption, as suggested for vitamin E (Borel et al., 1997). As most elderly people have a low vitamin D status, it has been hypothesised that absorption efficiency of vitamin D is less efficient in elderly persons than in young adults.

The first study dedicated to testing this hypothesis was performed in 20 elderly women, most of whom were vitamin D-depleted. Because the plasma [3H]cholecalciferol response after oral ingestion was significantly lower than that of a group of younger female subjects, it was suggested that there was a defect in intestinal absorption of cholecalciferol in the elderly (Barragry et al., 1978). However, subsequent studies on this topic did not confirm this finding: a study in rats pointed to a normal absorption of vitamin D_3 in ageing animals

(Hollander and Tarnawski, 1984). In another study, serum vitamin D_2 concentrations were compared after oral administration of 50,000 IU of vitamin D_2 in both healthy vitamin D-sufficient elderly subjects and young adults. Again, no evidence of malabsorption of vitamin D in the elderly subjects was observed (Clemens et al., 1986). In a third study, involving four young (23 to 50 yr) and four older (72 to 84 yr) subjects, peak serum 25OHD and 25OHD areas under the curve were not significantly different between the younger and older adults (Johnson et al., 2005). Finally, a study by Harris and coworkers involving 25 young men (18 to 35 yr) and 25 older men (62 to 79 yr) supplemented with 800 IU of vitamin D_3 per day (Harris and Dawson-Hughes, 2002), found that the magnitude of blood 25OHD increase was identical between the two age groups. In conclusion, there is apparently no significant effect of ageing on vitamin D absorption efficiency. The frequent lower vitamin D status in elderly than in young subjects may be due to lower dietary intakes, lower exposure to sunlight, lower efficiency of vitamin D synthesis in the skin or lower hydroxylation of vitamin D by the liver.

II. Diseases/digestive tube surgery

The intestinal absorption of vitamin D requires normal digestive functions, and so subjects with impaired fat absorption caused by any of several diseases (obstructive jaundice, pancreatic insufficiency, cystic fibrosis or adult coeliac disease) have been suspected of impairing vitamin D absorption. This is supported by the results of several studies: serum vitamin D_2 and D_3 were undetectable in infants and children (age 4-22 mo) with extrahepatic biliary atresia, whose portoenterostomy failed to produce bile flow, despite supplements of 2500-5000 IU/day (Heubi et al., 1990). Also, serum vitamin D_2 concentrations were undetectable despite oral supplementation with 2,500 to 50,000 IU per day of vitamin D_2 in children with cholestasis since infancy (Heubi et al., 1989). Finally, cystic fibrosis patients absorbed less than one third (Farraye et al.), or one half (Lark et al., 2001) the amount of oral

vitamin D_2 that was absorbed by healthy subjects. However, we note that the negative effect of these diseases on vitamin D bioavailability can be partially corrected by sun exposure (Robberecht et al.) or by using hydroxylated vitamin D. Patients with intestinal fat malabsorption syndromes have a relatively well-preserved absorption of $25OHD_3$, which is absorbed directly *via* the portal vein (Leichtmann et al., 1991).

The effect of gastric surgery on vitamin D bioavailability has been studied by Aarts et al. (Aarts et al., 2011). This team showed that peak serum cholecalciferol levels were about 30% lower after Roux-en-Y gastric bypass than before.

III. Obesity

As obesity is usually associated with vitamin D insufficiency, a study was dedicated to assessing the effect of obesity on the cutaneous production of vitamin D_3 and on vitamin D bioavailability, evaluated by the blood response in vitamin D after a vitamin D-rich meal. Obese and matched lean control subjects received either whole-body ultraviolet radiation or a pharmacologic dose of vitamin D_2 orally. Results showed that body mass index was inversely correlated with serum vitamin D_3 concentrations after irradiation, and with peak serum vitamin D_2 concentrations after vitamin D_2 intake. It was concluded that obese subjects had lower blood concentrations of vitamin D (coming either from the skin or the food) because of its deposition in the body fat compartment (Wortsman et al., 2000), and not because of lower skin synthesis or bioavailability. This was supported by a very recent study suggesting that dilution of ingested or cutaneously synthesised vitamin D in the large fat mass of obese patients explains their typically low vitamin D status (Drincic et al., 2012).

i. Mathematical Interactions

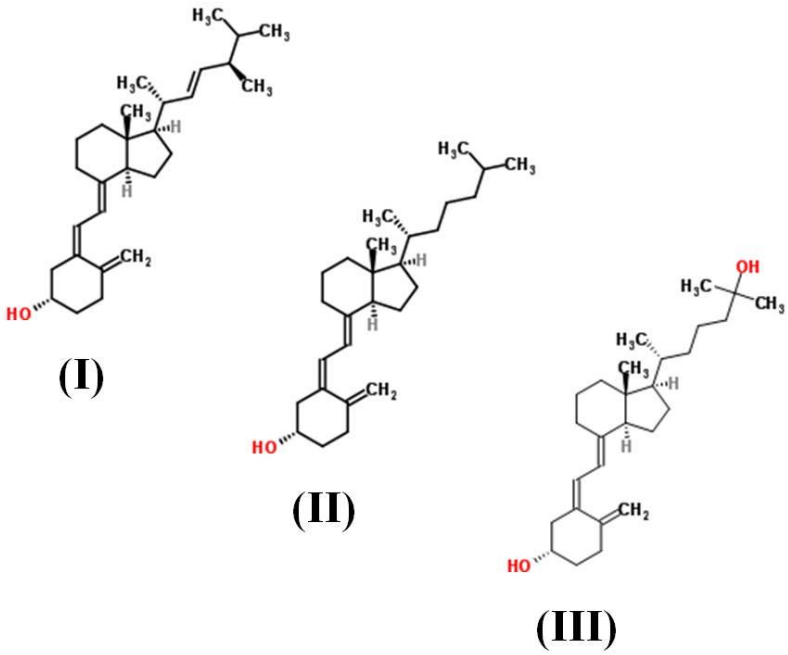
These are the interactions that can occur when several factors are involved (West and Castenmiller, 1998). One recent study (Khazai et al., 2009a) shows a good example of interactions between factors: in this clinical study, bioavailability of vitamin D_3 was better than that of vitamin D_2, but this was probably not due to any difference in absorption efficiency of the two forms of vitamin D (see chapter on the S factor), but rather to an effect of the vehicle in which vitamin D was incorporated (M factor), an effect probably exacerbated in patients with fat malabsorption (H factor).

Conclusions

This review shows that the intraluminal fate of vitamin D and the fundamental mechanisms involved in vitamin D absorption are far from fully understood. It also shows that although the effects of some factors on vitamin D bioavailability are well documented, little data is available on numerous factors that may affect vitamin D bioavailability, e.g. genetic factors, effect of dietary fibre, and effect of vitamin D status. Clinical studies with labelled vitamin D (deuterated or ^{13}C) are needed to accurately and definitively assess the effects of these factors on vitamin D bioavailability.

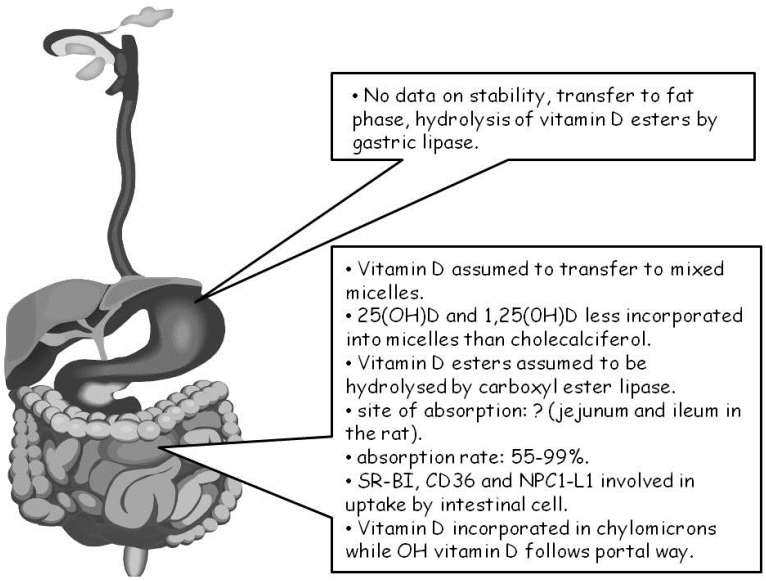
Figures

Figure 1



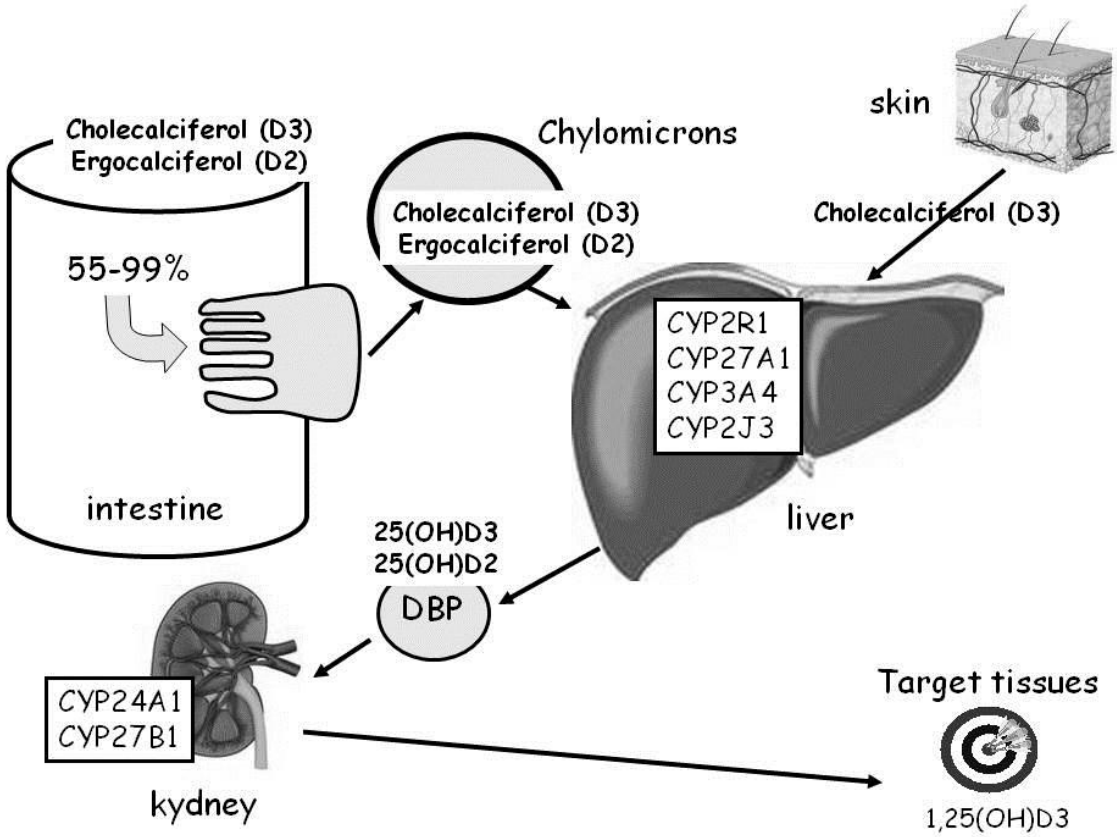
Chemical structures of natural dietary forms of vitamin D: (I) ergocalciferol (vitamin D_2), (II) cholecalciferol (vitamin D_3), (III) 25-hydroxy-cholecalciferol.

Figure 2



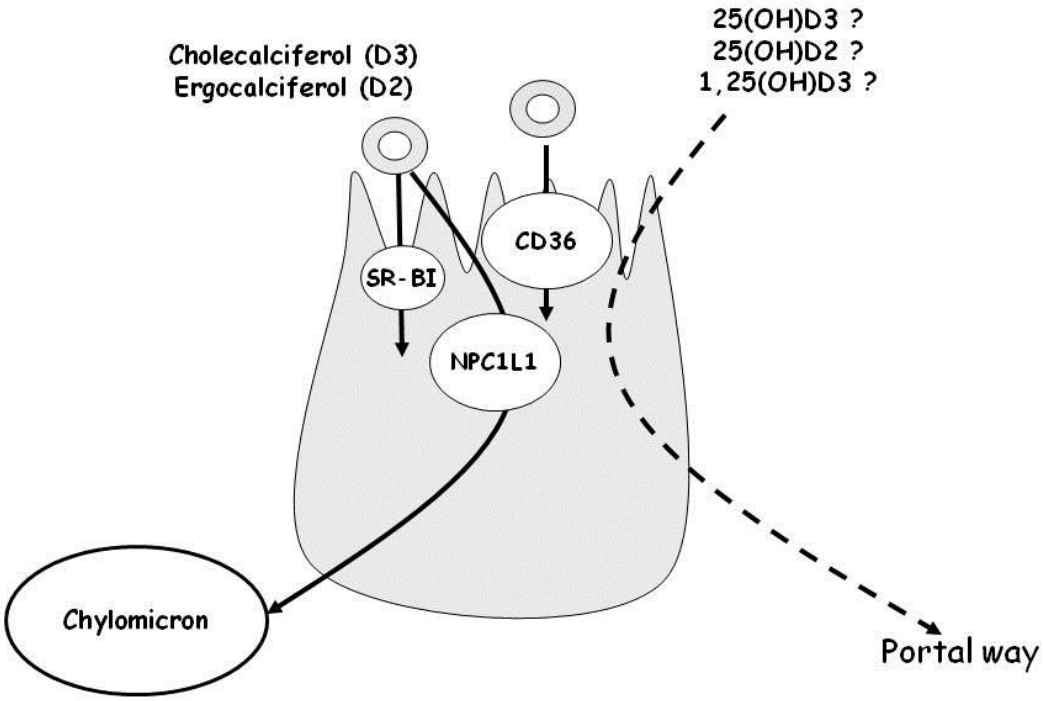
Some important knowledge and lack of knowledge, on the fate of vitamin D in the human upper gastro-intestinal tract lumen.

Figure 3



Vitamin D metabolism in the human body. $25(OH)D_3$: 25-hydroxy cholecalciferol. $25(OH)D_2$: 25-hydroxy ergocalciferol. $1,25(OH)_2D_3$: dihydroxy cholecalciferol, DBP: vitamin D binding protein. CYPxyzw: cytochrome P450, family x, subfamily y, polypeptide zw.

Figure 4



Current knowledge on vitamin D absorption by human intestinal cells. From the paper of Reboul et al. (Reboul and Borel, 2011). SR-BI: scavenger receptor class B type 1. CD36: Cluster Determinant 36. NPC1L1: Niemann-Pick C1-like 1. The dashed arrow means that there is no data on the molecular mechanisms involved in absorption of the hydroxy metabolites of vitamin D. It has been hypothesized (Reboul and Borel, 2011) that protein mediated uptake takes place at dietary doses of vitamin D while passive diffusion probably occurs at high, non-physiological doses.

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