

# Pharmacology of vitamin D

## Anything new?

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### Keywords

Vitamin D, Apolipoprotein E, intestinal transport, calcidiol plasma levels

### Summary

A main source of food for ancient humans ("hunter-gatherers") was fresh meat. It contains much more 25(OH) vitamin D<sub>3</sub> (25[OH]D<sub>3</sub>) than vitamin D<sub>3</sub>. It seems likely that in northern Europe, where vitamin D is in short supply during the extended winter season, evolutionary forces may have led to optimization of intestinal absorption of 25(OH)D<sub>3</sub>: excellent oral bioavailability (60–80%) and little inter-individual variation. 25(OH)D<sub>3</sub> could be considered the ideal oral "sunshine equivalent" for rapid and reliable restoration of an adequate vitamin D status e.g. in clinical situations. Unless biliary and pancreatic secretion or epithelial function in the small intestine is compromised, vitamin D<sub>3</sub> in "pharmacological doses" is absorbed by 60–100% as a "blind passenger" together with long-chain fatty acids and cholesterol. The question is raised whether very low amounts of the vitamin (as in the diet) are absorbed by a more active ("second order") mechanism. Experimental evidence obtained from cell culture systems indeed suggests that vitamin D<sub>3</sub> can be taken up in part from enterocytes via the same complex, tightly regulated and saturable transport system as is e.g. cholesterol. The ezetimibe drug receptor NPC1L1 may play a role in this process. The Apolipoprotein Epsilon 4 genotype occurs in a north-south gradi-

ent in Europe. Allele frequencies are as high as 30% in Finland and much lower, 5%, around the Mediterranean Sea. The Epsilon 4 genotype may have been selected in the north because it enables more vitamin D to be obtained from food. The association of higher levels of 25(OH)D<sub>3</sub> in humans with the Epsilon 4 genotype, together with evidence from knock-in mice, supports this hypothesis. It is possible, but as yet unproven, that this "lipid-thrifty" genotype is the cause of excess cardiovascular mortality sometimes observed in cohorts with high serum concentrations of 25(OH)D. Latitudinal gradients for mutations in the enzyme delta-7-dehydrocholesterol reductase (DHCR-7) suggest that similar evolutionary adaptations occurred for vitamin D synthesized in the skin following sun exposure.

### Schlüsselwörter

Vitamin D, Apolipoprotein E, intestinaler Transport, Calcidiol-Plasmaspiegel

### Zusammenfassung

Für unsere Vorfahren („Jäger und Sammler“) war Fleisch ein Hauptnahrungsmittel. Es enthält wesentlich mehr 25-(OH)-Vitamin D<sub>3</sub> als Vitamin D<sub>3</sub>. Es ist wahrscheinlich, dass sich im nördlichen Teil Europas wegen fehlender Vitaminbildung (über die Sonne) während der langen Wintermonate die intestinale Resorption des Prohormons evolutionär adaptierte und optimal wurde: Ausgezeichnete orale Bioverfügbarkeit und minimale inter-individuelle Va-

riation. Daher kann Calcidiol als das ideale orale „Sonnenschein-Äquivalent“ für die rasche und zuverlässige Anhebung eines insuffizienten „Vitamin-D-Status“ z. B. in klinischen Situationen betrachtet werden. Vitamin D<sub>3</sub> wird in pharmakologischen Dosen zwischen 60–100% als „Blinder Passagier erster Ordnung“ absorbiert. Voraussetzungen hierfür sind eine intakte Leber- und Pankreasfunktion sowie ein gesunder Dünndarm. Die Dosis muss entweder mit Fett (Triglyzeride mit langkettigen Fettsäuren) und/oder einer „normalen Mahlzeit“ appliziert werden. Die Frage erhebt sich, wie kleine Mengen von Vitamin D, die üblicherweise in der Nahrung vorkommen, resorbiert werden und ob hier ein „aktiver Prozess“ eine Rolle spielt. Zellkultur-Experimente weisen darauf hin, dass Vitamin D<sub>3</sub> u. a. über die gleichen komplexen und eng kontrollierten Mechanismen aktiv (Prozess „zweiter Ordnung“) in Enterozyten aufgenommen werden kann wie z. B. Cholesterin. Hier spielt u. a. das Rezeptor-Protein für Ezetimibe, NPC1L1, eine Rolle. In Europa besteht ein Nord-Süd-Gradient für das Apolipoprotein E Epsilon 4 Allel (30% in Finnland und etwa 5% um das Mittelmeer). Dieser Genotyp wurde offensichtlich im Norden selektiert, um mehr Vitamin D<sub>3</sub> aus der Nahrung aufzunehmen. Eine klare Assoziation höherer Spiegel von Serum-25-(OH)-Vitamin D mit diesem Allel wurde belegt und in „knock-in“-Mäusen bestätigt. In einigen Studien wurde eine Assoziation von hohen Serumspiegeln von Calcidiol mit Übersterblichkeit gefunden. Ob der (zu Alzheimer und erhöhter kardiovaskulärer Mortalität disponierende) Genotyp Apolipoprotein E Epsilon 4 hier eine Rolle spielt, ist bislang nicht erforscht. Für Mutationen im Enzym Delta-7-Dehydrocholesterin-Reduktase wurde ebenso ein Nord-Süd-Gradient gefunden. Dies weist ebenfalls auf evolutionäre Adaption der Vitamin-D<sub>3</sub>-Synthese in der Haut hin.

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### Was gibt es Neues in der Pharmakologie von Vitamin D?

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Data on the bioavailability of orally applied vitamin D<sub>3</sub> in “pharmacological doses” are straightforward: The vitamin is absorbed almost completely into the general circulation with little inter-individual variation, provided epithelial function in the small intestine and pancreatic as well as biliary secretion are normal. The “vehicle” for the vitamin must contain long-chain fatty acids or it must be given with a “standard meal”. Sunlight-derived vitamin D<sub>3</sub> has a similarly high bioavailability, but always increases – in extreme contrast to the oral route – serum levels of 25(OH)D<sub>3</sub>, depending on prior status. The oral acquisition of very small amounts of the vitamin in the diet is not well understood. Perhaps, an evolutionary selection process of genes responsible for fatty acid and cholesterol absorption has occurred, also favoring vitamin D uptake. Here we will review recent findings which point in this direction.

## Oral bioavailability of vitamin D<sub>3</sub> and calcidiol

### Vitamin D<sub>3</sub>

In the early days of vitamin D research, radiolabelled cholecalciferol was employed to follow oral absorption, metabolism and tissue distribution in human volunteers or patients (1–3). Although by no means following today’s scientific or ethical standards, the conclusions that can be drawn from these studies are: First, the absorption of orally applied vitamin D<sub>3</sub> between 3 µg and 1 mg (40 000 I.U.) is between 60 and 99%, if given with milk, long-chain fatty acid containing triglycerides or a “standard meal”.

Second, the absorption of these doses depends on the bile acid and cholesterol secretion capacity of the liver, is dependent on pancreatic sufficiency and, as mentioned above, is a function of the “vehicle” as also

shown by non-radioactive assays (4). Vitamin D<sub>3</sub> can be regarded as a “blind passenger” traveling with long-chain fatty acids, bile acids and cholesterol in the intestinal tract. It ends up in chylomicrons after passing through enterocytes in an uncontrolled “first-order” process: The dose absorbed is a linear function of the dose applied.

Third, whereas vitamin D<sub>3</sub> resides mainly in adipose tissue, 25(OH) vitamin D<sub>3</sub> in humans (and in some animals, see below) is mainly stored in skeletal muscle. As the authors put it: “by binding to tissue proteins”.

There is one report in the literature where vitamin D<sub>3</sub> was given to fasting volunteers with water. Surprisingly, in these conditions single-dose pharmacokinetics of vitamin D<sub>3</sub> exhibit extreme variations (▶ Table 1) in AUC<sub>0–120h</sub> (Area Under the Curve) or AUC<sub>0–80h</sub> for 2800 or 5600 I.U. vitamin D<sub>3</sub>, respectively. AUC varies from ~64 to ~1700, about 25-fold. C<sub>max</sub> values, correspondingly, also varied between 1.5 and ~34 ng/ml. Both parameters were unadjusted for base-line levels, which were around 3 ng/ml (5). Apparently, fasting individuals differ in their ability to provide sufficient transport for the “blind passenger”. Sufficient means: basal secretion of cholesterol, bile acids and phospholipids. We suspect that dispositions for (re-)absorbing cholesterol, including bile acid secretion (see e.g. [6]) or the extreme inter-individual variations in fatty acid and cholesterol transporter protein expression along the human intestinal tract (7) may play a role in these conditions.

Variability of vitamin D<sub>3</sub> absorption between fasting individuals receiving water instead of a standard meal or oil with long chain fatty acids must not be confused with the extremely variable responses of the circulating metabolite 25(OH)D after oral, pharmacological doses of vitamin D<sub>3</sub>. For instance, a close inspection of Figure 3 in (8) reveals that of 16 volunteers (5 males, 11 females, average age 74 years, receiving 1600 I.U. daily) 7 (~50%) had no increase or even a decrease of serum 25(OH) vitamin D<sub>3</sub>.

### Oral bioavailability of calcidiol

The oral bioavailability of calcidiol is high (▶ Table 1). It is absorbed via the portal vein,

**Table 1** Properties of vitamin D<sub>3</sub> and calcidiol: **F.W.** = Fasting volunteers; vitamin D<sub>3</sub> given with Water. **M.** = volunteers received milk, or long-chain fatty acids containing triglycerides and/or a standard meal together with the vitamin. **VDBP** = Vitamin D Binding Protein (GC); n. d. = not determined; observed half-life and t<sub>max</sub> (maximum concentration achieved after single oral doses) are dependent on the absorption constant. Hence, the decline in plasma concentration is not identical to the plasma elimination half-life. In addition, „body-half-life“ of vitamin D<sub>3</sub> (and, possibly, 25(OH) vitamin D) is much longer. As an example for intracellular high-affinity calcidiol binding proteins, heat shock protein 70 is mentioned (see text). Direct binding of vitamin D<sub>3</sub> to proteins involved in cholesterol uptake and transport (e.g. NPC1L1) has not been investigated. The EC<sub>50</sub> value for calcidiol refers to the concentration leading to 50% of a maximal response in a model system for non-genomic signal transduction via the “VDR-Alternative-Pocket” of the vitamin D receptor (VDR, see text). A single, still unconfirmed analysis demonstrated high-affinity binding of vitamin D<sub>3</sub> to *smoothed*, a member of the Hedgehog signal transduction pathway (32).

Parameter	Vitamin D <sub>3</sub>	25(OH) Vitamin D <sub>3</sub>
Bioavailability [F]		
● skin (UV-B)	1.0	
● oral		
– oral F. W.	extreme variability (0–?)	0,8
– oral M	0.6–1.0	0,8
Distribution Volume [Vd, Liter/kg]	4	0.12–0.20
Observed Plasma Half-Life after single oral Dose [Days]	2–3	10–12
Dissociation constant (Kd) for VDBP (GC) [nM]	1000	50
Kd for Heat-Shock Protein 70 [nM]	n. d.	0.2
Kd for VDR [nM]	n. d.	1220; EC <sub>50</sub> = 1 nM
Kd for <i>smoothed</i> [nM]	10	n. d.
Kd for NPC1, NPC2, NPC1L1	n. d.	n. d.

exhibits very little inter-individual variation and – in contrast to oral vitamin D<sub>3</sub> – leads to an almost immediate and predictable increase in the circulation (▶ Table 1). If daily or weekly doses of vitamin D<sub>3</sub> are given, it takes several months (!) until a pseudo steady-state between input of the precursor and serum levels of its metabolite calcidiol is reached, not to mention “Non-Responders” (see the above example).

In contrast, as exemplified by a recent small study with female volunteers (BMI = 23.5 ± 3.2 kg/m<sup>2</sup>) receiving 500 µg (≈ 1250 nMol) of calcidiol monthly (equivalent to 0.24 ng/kg/d, assuming a mean 70 kg body weight) raised average serum levels from ~45 nM to ~125 nM (difference: 80 nM) already on day 3 and on day 120 (9). The formula derived by (10) predicts that the increase in 25(OH)D<sub>3</sub> should be: 327.5 × 0.24 = 78.6, which is almost too good to be true. Nevertheless, the results indicate the general validity of the equation. If the distribution volume is in the reported range of 0.12 to 0.2 l/kg BW (▶ Table 1), the observed total body increase is in the range of 700 to 1120 nMol. From the total body increase we can calculate an oral bioavailability of 60–90%. Animal experiments indicate a significant pre-systemic catabolism to the 24-(OH) metabolite of calcidiol (11) but data for humans are missing.

25(OH)D<sub>3</sub> (similarly to curcumin) has an additional binding site on the vitamin D receptor (▶ Table 1) termed “VDR-Alternative-Pocket”, VDR-AP (12). Calcidiol can exert with equal EC<sub>50</sub> values as calcitriol so-called rapid (non-genomic) responses which may – in model systems – also amplify gene expression (13). Therefore, long term clinical trials are needed to establish benefits and risks of calcidiol as a complete substitute for vitamin D<sub>3</sub>.

### Calcidiol – optimal supply for our “hunter ancestors”

It takes many months to build up calcidiol levels after pharmacological doses of vitamin D<sub>3</sub>. One wonders how our ancestors in northern Europe coped with the problem of vitamin D<sub>3</sub> supply given the much smaller amounts present in their food. Most likely, the hunters’ major source was calcidiol in meat.

Indeed, content of 25(OH)D<sub>3</sub> in voluntary muscle (“meat”) is high and in steaks up to 25 ng/g wet weight, if cows are raised on pasture in summer (14). The high content cannot be explained by plasma contamination and suggests intracellular binding proteins. A member of heat-shock protein family (hsp70) binds calcidiol with high affinity (15), but there probably are more candidates among other sterol (and oxy-sterol) binding proteins. Speculative, but interesting is that other natural steroid hormones (e.g. estradiol) have a very significant first-pass-effect. In contrast, the pro-hormone calcidiol (similar to calcitriol) is not destroyed before reaching the general circulation. Higher affinity and plasma concentration (“avidity”) of the vitamin D binding protein may have resulted from an evolutionary selection process to obtain more of the hormone precursor from food.

### A saturable (second order) process for absorption of dietary vitamin D<sub>3</sub>

#### Is Vitamin D<sub>3</sub> not only a blind passenger?

Dietary cholesterol and vitamin D<sub>3</sub> for humans are obtained exclusively from animal sources. Saltwater fish can also supply it, but mainly in esterified form. Vitamin D<sub>3</sub> content in steaks (animal meat) is between 0.8 and 16 ng/g fresh tissue (14) and in lamb cuts up to ~1 ng/g (16). Meat and fish contain ~0.5 mg cholesterol per gram of fresh tissue. The ratio cholesterol: vitamin D<sub>3</sub> is of such a magnitude that even the most sophisticated transport and sorting system cannot distinguish between the two. Can vitamin D<sub>3</sub> enter the systemic circulation by specific uptake systems, responsible for cholesterol (17) and long-chain fatty acid transport?

Reboul et al. (18) report that vitamin D<sub>3</sub> (and D<sub>2</sub>) transport in Caco-2 cells is a saturable, direction- and temperature-dependent (“second-order”) process. In their experiments, cells were grown on transwells as a monolayer; apical chambers were exposed to mixed micelles containing (lyso-) phospholipids, oleic acid, 100 µM chole-

sterol and 0.01–10 µM vitamin D. In these conditions, the maximal transport rate for both vitamin D<sub>3</sub> and D<sub>2</sub> was ~110 pmol/h/mg of protein, with half-maximal concentrations for saturable uptake of ~0.2 µM. The authors demonstrated that uptake and transport were unidirectional (from apical to basolateral) and that at “pharmacological” concentrations (>2 to 4 µM), vitamin D uptake was no longer saturable but linearly related to the concentration, as seen in human studies. In addition, they demonstrated weak inhibition by ezetimibe glucuronide in the Caco-2 cell system and increased vitamin D<sub>3</sub> uptake in human embryonic kidney (HEK) cells when Niemann-Pick C1-like 1 (NPC1L1) was overexpressed. The latter protein may not be the sole transporter as others (e.g. scavenger receptor class B type I), when overexpressed in HEK cells, facilitated vitamin D directional transport as well. Surprisingly, within the limits of the concentration range of cholesterol in the micelles (zero to 200 µM), there was little competition with 0.5 µM vitamin D<sub>3</sub>.

In a second publication, (19) β-sitosterol and cholesterol impaired the micellar concentration of vitamin D<sub>3</sub>. Furthermore, force-feeding mice with vitamin D<sub>3</sub> (100 µg) and 10 mg of β-sitosterol reduced the plasma concentrations of cholecalciferol dramatically. Vegetarians may possibly suffer from low absorption of vitamin D<sub>3</sub> in supplements – but this still remains to be proven.

Caco-2 cells are not an ideal system to study the direct role of NPC1L1 in sterol transport, as most of it is expressed intracellularly. Merck researchers have developed cell lines in which flux of sterols (bound to albumin) can be studied as a function of NPC1L1 expression on the apical plasma membrane (20). In these optimized cells, both cholesterol and β-sitosterol flux are exquisitely sensitive to β-lactame-based ezetimibe analogues and ezetimibe glucuronide. Such a system could be useful to study NPC1L1-mediated vitamin D uptake in a more direct manner. The sterol binding domains of NPC1L1 (21, 22) and NPC1 can be also directly explored with respect to affinity and specificity for vitamin D.

Uptake into enterocytes is a necessary but by no means sufficient step for absorp-

tion (i.e. entry into the systemic circulation). Phytosterols are a good example: they are taken up but enter the general circulation only in minute amounts (23).

### Phytosterols, serum 25(OH)D levels and HDL-cholesterol

The idea is intriguing that for the very low amounts of food-derived (dietary) vitamin D<sub>3</sub>, saturable uptake processes play a significant role. In this context, please note that low and high absorbers of dietary cholesterol differed most significantly in their serum HDL-levels: High absorbers had higher levels (24, 25). It is tempting to speculate that the high absorbers import vitamin D<sub>3</sub> twice as well as the low absorbers. In 22 cross-sectional studies, serum 25(OH)D levels were positively associated with HDL-C (26). Do higher levels of serum 25(OH)D and phytosterols reflect better absorption of dietary sterols including vitamin D? If so, there may be no causal relationship between (phytosterol or) 25(OH)D levels and HDL-C.

### Genome-wide-association studies, serum calcidiol levels and Apolipoprotein E

Genome-wide association studies so far have identified three main players deter-

mining vitamin D status in populations (27), among them two enzymes involved in vitamin D metabolism and catabolism (► Fig. 1) or determining the steady-state level of the precursor 7-dehydrocholesterol in the skin (3-β-hydroxysterol-delta-7-reductase, DHCR-7). The third player is the vitamin D Binding protein (VDBP=GC) which transports cholecalciferol from the skin into the general circulation and calcidiol from the intestine to the liver as well as to other tissues and cells. The carrier frequency of DHCR-7 mutations in Caucasians, which can be as high as 2.3%, was suggested to be advantageous for obtaining vitamin D<sub>3</sub> from the sun (28). Correlations between serum 25(OH)D levels and carriers are not yet investigated. As humans in addition to UV-B may obtain vitamin D<sub>3</sub> (or 25[OH]D<sub>3</sub>) orally, one wonders whether correlations to transport systems in the intestine and the lipoproteins involved in cholesterol and lipid traffic as well as bile acid production would also show up in these studies.

### Apolipoprotein Allele ε4 – better bones but earlier death?

In 2003, Lars Ulrik Gerdes (29) published an opinion paper in which he speculated that the geographical distribution of the apolipoprotein E (APOE) allele ε4 in Europe (south to north gradient) protected

against vitamin D deficiency. Eisenberg et al. (30) recently analyzed the worldwide frequencies of the ε4 allele of apolipoprotein E gene under the hypothesis that this allele would protect against low cholesterol levels. They concluded that natural selection has been responsible for the observed frequencies – both south and north of the equator. The relative effects of skin colour and UV-B irradiance were not considered. Interestingly, when increasing elevation (which increases skin vitamin D production via higher UV-B levels) was included in the various models (in order to account for lower temperature) the result was opposite to the expected but is clearly in support of the vitamin D hypothesis. If the association between APOE ε4 status and serum 25(OH)D levels in a general population sample and a small number of subjects for an interventional study are investigated, APOE ε4 carriers had significantly higher levels, especially when APOE ε2 allele carriers were excluded. APOE ε4 carriers had lower PTH and higher serum calcium levels (31). In support of these findings, knock-in mice (APOE 4) had significantly higher serum 25(OH)D levels (~71 nM) than found in wild-type (~28 nM), APOE-2- or APO-3-mice. Indirect evidence was provided for increased calcitriol effects including higher femoral calcium and increased expression levels of genes involved in calcium absorption. Most interestingly, there was increased bile production, higher expression levels of CYP 7A1 (key enzyme in bile production) and higher mRNA expression for vitamin D binding protein.

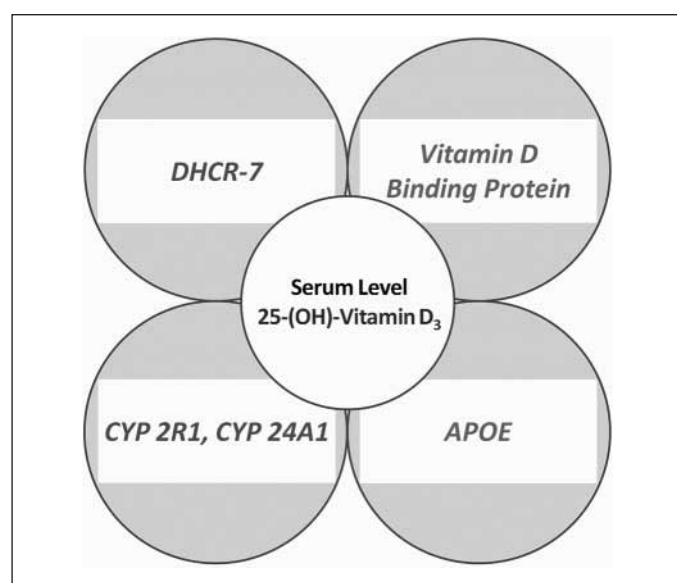
Taken altogether, the association data in humans and the phenotype of the APOE ε4 knock-in mice establish the APO ε4 allele as novel modifier of the vitamin D status.

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### Conflict of interest

The author declares that there is no conflict of interest.



**Fig. 1**  
Gene loci so far identified for 25(OH) vitamin D serum levels



**Note added in proof**

The view expressed above, namely that 25(OH) vitamin D<sub>3</sub> (and not vitamin D<sub>3</sub>) is the ideal oral „sunshine equivalent“ was mainly based on pharmacokinetic data. A comparative, double-blind study (33) with otherwise healthy postmenopausal women but an average baseline level of calcidiol of 13.2 ng/ml not only confirms now the superior bioavailability and almost immediate action for the prohormone but surprisingly suggests that there may be major differences to oral vitamin D<sub>3</sub> with respect to pharmacodynamics (improved muscle function, lowering of systolic blood pressure, decreases of markers of innate immunity). Speculative explanations are that the majority of oral vitamin D<sub>3</sub> (the fate of which is still unknown) may be metabolized into a more „antagonistic“ compound or that it triggers as a ligand (hedgehog pathway?) activities which may not be identical and even opposite to calcidiol.

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