CHOLECALCIFEROL: A PERFECT SYNTHESIS

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Vitamin D activation is realized by means of complex mechanisms included within the physiological regulation of mineral metabolism. This review focuses on metabolic systems that lead to vitamin D synthesis. Evolution has made it necessary to satisfy the needs of increasingly complex organisms, which are moreover located in areas with in areas with ever less calcium availability. Although the mechanism is not yet completely clear, the outline that has emerged – in spite of its complexity – helps us understand the key role that nature has always attributed to this particular vitamin.

VITAMIN D: ONE OR MANY?

Secosteroids are a subclass of tetracyclic steroids in which one of the rings has been "broken" (the prefix "seco-" derives from the Latin *secare*, to cut).

The prototype of these compounds is cholecalciferol (or vitamin D_3), although in reality several secosteroids show such markedly analogous structures that they are grouped under the name of vitamin D (Fig. 1).

In nature, there are essentially two main forms of vitamin D: ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3). Vitamin D_2 is found in plants and derives from irradiation with UVB ultraviolet rays (290-315 nm) of ergosterol or provitamin D_2 (Fig. 1A) [1].

Vitamin D_3, by contrast, is of animal origin and is produced on the skin, thanks to the action of the same kind of UVB irradiation on 7-dehydrocholesterol or provitamin D_3 present on the epidermis (Fig. 1A) [1].

If these are the two main forms of vitamin D, they are not the only ones. Thanks to UVB action, other tetracyclic steroids are converted into secosteroids which are structurally similar to cholecalciferol (Fig. 1B). The best known of these are vitamin D_4 and D_5 [2]. About others we still know little if anything at all, except that they are much less biologically active [2, 3].

Vitamin D_4 is structurally similar to D_3. By means of complex chromatographic techniques, it can be found in certain types of mushrooms (especially in the gills following exposure to sunlight) as well as in marine mollusks and yeasts, in which it is not distinguishable from D_3 in normal doses [2-5]. In mice, it has a much lower capacity (roughly half) than cholecalciferol in healing rickets [4], although its active metabolites have shown antiproliferative and differentiation effects at the cellular level (*in vitro*) similar to those produced by calcitriol [1-25(OH)2 vitamin D_3] [3].

Interesting data are also available for vitamin D_5. It is also of vegetable origin and has been identified (again through chromatographic studies) in some plants, in which, however, its physiological role is still completely unknown. The interest of researchers in vitamin D_5 is connected to the anti-neoplastic capacity of its hydroxylated metabolite in the 1 alpha position (1 alpha-OH vitamin D_5) [7]. *In vivo* and *in vitro* studies have shown its inhibitory effect against mammalian carcinogenesis in mice, with a toxicity framework that is completely negligible. Independently of the dose used, in mice there has in fact been no evidence of the typical toxic effect of hypercalcemia, which is rather seen with calcitriol at doses necessary to have a protective effect against carcinogenesis [7]. Beyond these interesting data on cellular life (proliferation and differentiation), vitamins D_4 and D_5 therefore present calciotropic hormonal activity which is either modest (vitamin D_4) or completely lacking (vitamin D_5); for this reason, they cannot be viewed in the same way as vitamin D_3 [3, 4].

As we know, cholecalciferol normally derives from the transformation of 7-dehydrocholesterol present in the epidermis. This precursor, though, can physiologically follow metabolic paths different from the classical one (Fig. 1C). Under the action of enzymes of the cytochrome P450 superfamily, it can undergo various transformations, even giving rise to the delta-7-steroid family. The latter, again by means of UVB action, can be transformed into secosteroids which are different from and alternative to cholecalciferol (Fig. 1C).

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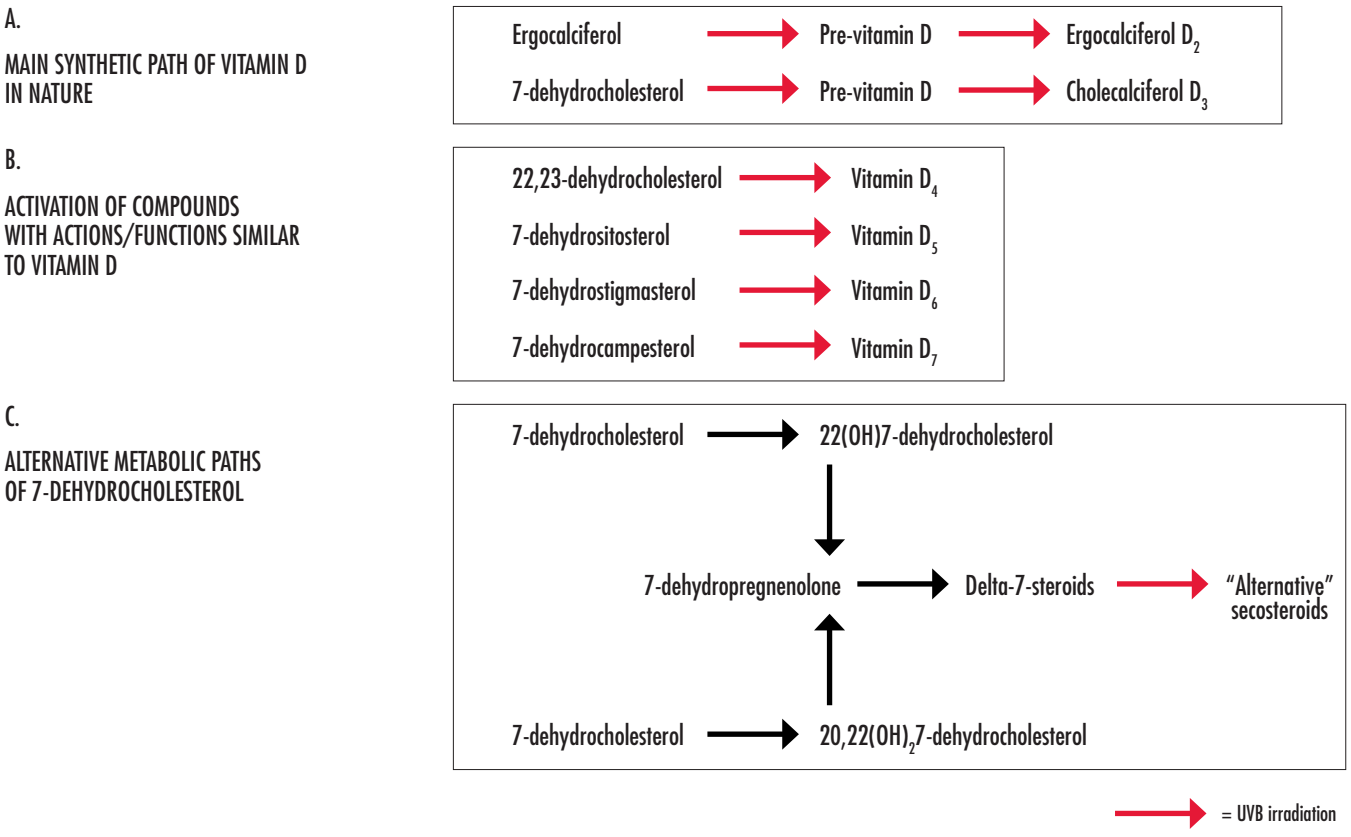

FIGURE 1.
Schematic summary of processes that lead to the synthesis of various secosteroids.

In a way similar to vitamins D_4 and D_5, these compounds can likewise produce metabolites which are biologically active at the cellular level (they can, for example, inhibit the proliferation of melanoma cells in a way similar to calcitriol) [8], without, however, producing any type of endocrine effect or of having consequences on bone metabolism [5].
In sum, ergocalciferol and above all cholecalciferol represent in humans the only efficient substrate – together with and thanks to PTH – to carry out important functions in regulating calcium and phosphorus. All other compounds, even if they often have great molecular similarities and sometimes even analogous autocrine activity, do not at all resemble vitamin D from either a functional or biological point of view in the way in which we normally consider it to be the center of skeletal metabolism.

THE IMPORTANCE OF SUNLIGHT AND HEAT

As is clearly seen in Figure 1, a key role in vitamin D synthesis is played by solar irradiation. Why? The reason is simple. Only energy from photons in the UV spectrum is able to effect the opening of the B ring in 7-dehydrocholesterol, which is essential for for the formation of the secosteroid prototype cholecalciferol – that is, of cholecalciferol [5]. The same thing obviously holds true for ergocalciferol. Nonetheless, UVB action turns out to be particularly efficient (both in quantitative terms and in the rapidity of transformation) when the precursor which it acts upon is located within the biological membrane. In the experiment shown in Figure 2, UVB irradiated both 7-dehydrocholesterol molecules inserted into a cellular membrane (lizard and human skin) and molecules in a biological solution. The rapidity and extent of the transformation process were much greater when the precursors were structured within a cellular membrane [9]. This explains why this reaction also occurs in biological materials which clearly possess little vitality, such as animal feces or hay [5].
The experiment proposed in Figure 2 also foregrounds the crucial role played by temperature. In fact, in the same structure (precursor in a membrane or in a solution), transformation turns out to be much more efficient at a higher temperature (25°C). Physiologically, 7-dehydrocholesterol, when placed at the cellular membrane level and subjected to UVB action, is transformed into previtamin D_3 (Fig. 1). The last stage of the cutaneous synthesis of vitamin D involves the conversion of previtamin D into vitamin D by means of a process of temperature-dependent isomerization. Previtamin D is not only an unstable molecule that must be "guided" in its transformation toward vitamin D; it is also and above all a biologically inactive compound for which this transformation becomes absolutely crucial.
In the course of the evolution of the species, nature has selected more and more efficient mechanisms. At first, precursors were diluted within cellular cytoplasm with a quite reduced transformation efficiency. Later, the isomerization yield was increased (by over 15 times) by the structure of the precursors within a membrane; finally it was made

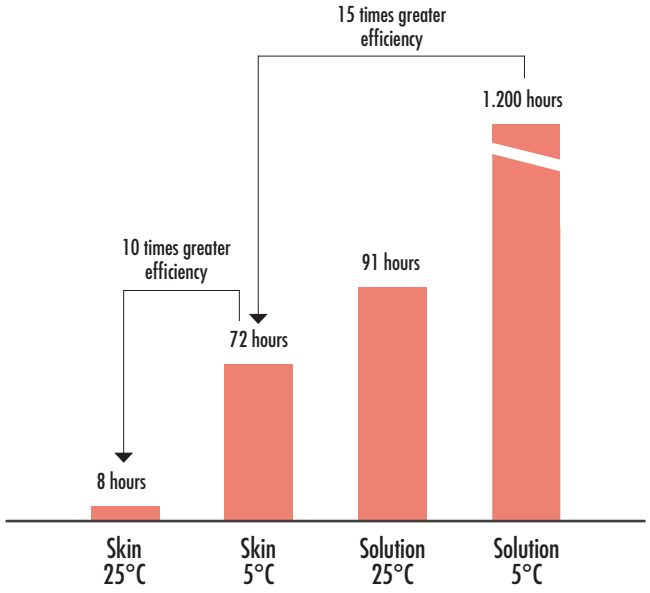

FIGURE 2.
Times of UVB irradiation necessary to transform 50% of 7-dehydrocholesterol into cholecalciferol (vitamin D_3). Comparison of transformation efficiency in different conditions: 1) precursor in biological solution or within cellular membrane (e.g., skin); 2) low and high temperatures. Maximum efficiency is obtained when precursor is within cellular membrane (e.g., skin) at high temperature: this condition is realized in warm-blooded animals (from Holick et al., 1995, modified) [9].

Evolution →

	MARINE VERTEBRATES	AMPHIBIOUS VERTEBRATES	TERRESTRIAL VERTEBRATES
ENVIRONMENT	Oceans	Coasts	Land
CALCIUM AVAILABILIT	High	Medium	Low
ORGANIZATION OF VITAMIN D PRECURSORS	Solution	Membrane	Membrane
TEMPERATURE CORPOREAL	Cold	Cold	Hot
PRE-VITAMIN D ISOMERIZATION	Reduced	**High**	**Very high**

FIGURE 3.
Hypothetical explanation of improvement of vitamin D synthesis mechanisms. In marine animals, the wide availability of calcium rendered improved absorption processes useless. With the gradual movement to sea and ocean layers closer to the surface and especially to land (where calcium is far less available), these mechanisms were perfected over time. At first, vitamin D precursors were arranged within the membrane (skin) such that solar energy could be harnessed. Then, with the evolution of warm-blooded animals, higher temperatures made possible the optimization of the final isomerization process of previtamin D into vitamin D.

even more productive and rapid (over 10 times so) by high temperatures (≥ 25°C) [5, 9]. This gradual optimization of processes of vitamin D synthesis connected to the action of light and temperature finds a possible explanation in the history of the evolution of vertebrates (Fig. 3) [5]. Large quantities of calcium were present in fertile oceans, more than enough to satisfy the needs of the first marine vertebrates. During evolution, however, animals began to move toward ocean layers closer to the surface and then onto land, an environment totally lacking in calcium. At first, solar energy was used, thanks to the precursors on the level of the skin (cold-blooded animals), with a clear improvement in synthetic efficiency. This was then further improved in warm-blooded animals thanks to the catalytic action of temperature (Fig. 3).

ENDOGENOUS FACTORS THAT CONDITION VITAMIN D SYNTHESIS

Melanin is an excellent solar filter able to block ultraviolet radiation, including UVB rays, which are necessary for vitamin D synthesis. This then explains why colored populations have a less efficient synthesis of vitamin D [10].
Age is a critical factor as well. Unfortunately, the concentration of 7-dehydrocholesterol in skin is progressively reduced as one ages [11]. For this reason, an elderly person has a decisively lower response in terms of cutaneous vitamin D synthesis with respect to a young person with the same exposure to sun [10]. Supplementation therefore represents the only way to satisfy vitamin D requirements in the elderly.

PROTECTION FROM EFFECTS OF EXCESSIVE SUN EXPOSURE

Solar irradiation is fundamental for the transformation of 7-dehydrocholesterol into pre-vitamin D. But what happens in the case of prolonged exposure to the sun? In reality, previtamin D also feels the effects of UVB radiation: in cases of overexposure, it undergoes further photolysis, with the formation of inactive compounds such as lumisterol and tocopherol (Fig. 4A, B). It should also be mentioned that cholecalciferol, once synthesized on the skin, must be rapidly captured by the cycle and removed. Otherwise, if it undergoes renewed exposure to the sun, it will also be subject to further photolysis, with the production of inactive final compounds (Fig. 4A) [10].

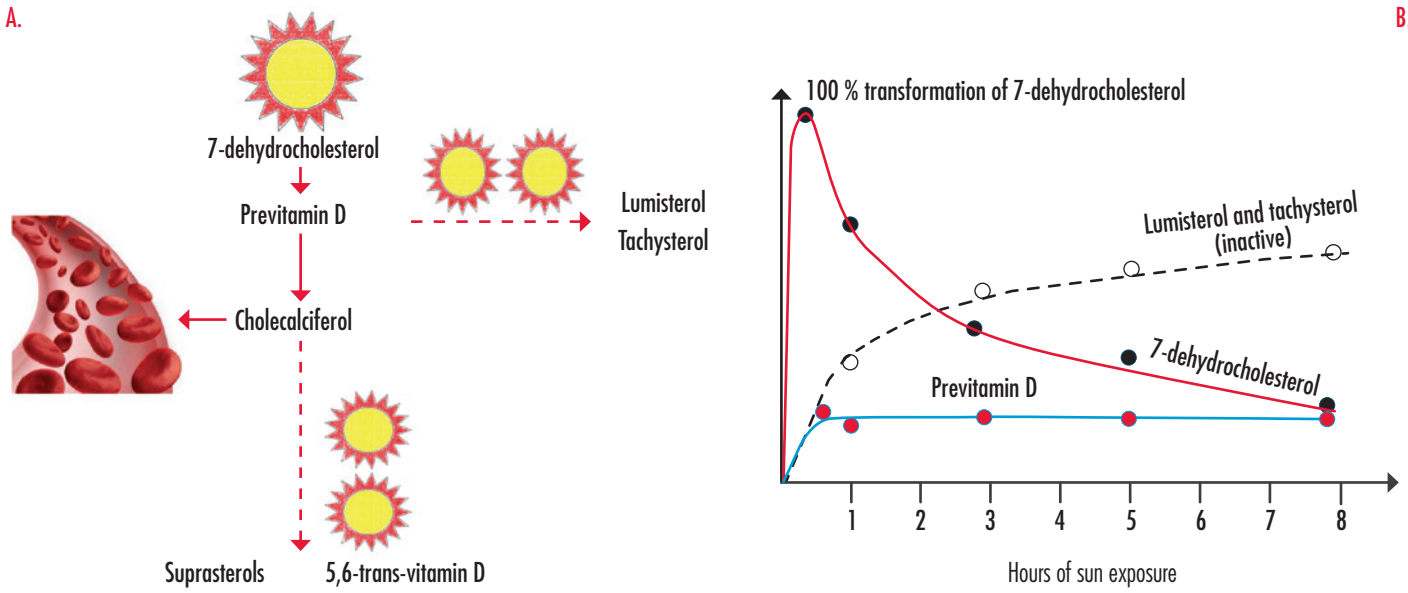

FIGURE 4.
Protection mechanisms against risk of excessive vitamin D synthesis in case of prolonged exposure to sun. A) Sun activates normal synthetic path of vitamin D (solid arrows). In case of prolonged exposure to sun (double suns), alternative paths are activated (dotted arrows). If exposed to UVB rays, previtamin D is transformed into inactive compounds (lumisterol and tachysterol). If cholecalciferol is not rapidly removed from the epidermis and enters the bloodstream, it is transformed under the action of UVB rays into inactive terminal compounds (suprasterol and 5,6 trans-vitamin D). B) Exposure to sun produces rapid (within 30 minutes) and complete transformation of 7-dehydrocholesterol, which is followed by a rapid increase of previtamin D. If exposure is prolonged, no further increase in previtamin D occurs but only an increased production of different, inactive metabolites (lumisterol and tachysterol) (from Holick, 1995, modified) [10].

CONCLUSIONS

The process leading to vitamin D synthesis appears to be particularly complex, as are, after all, the other features of its metabolism. There are many compounds with great structural similarities to cholecalciferol, yet none of these (with the exception of ergocalciferol of plant origin) is so biologically active to be considered vitamin D. This vitamin undoubtedly represents an extraordinary system to meet the needs of an improved intestinal absorption of calcium, a process that during the course of evolution became indispensable as vertebrates gradually moved from seas and oceans (where the availability of calcium was more than sufficient) to land.

For this reason, our organism created a perfect synthetic machine whose aim is to produce – according to need – the only truly efficient compound: cholecalciferol. Unfortunately, the synthesis does not always meet individual requirements, especially in elderly and fragile persons, for whom supplementation is therefore indispensable.

References

1. Holick MF. *High prevalence of vitamin D inadequacy and implications for health.* Mayo Clin Proc 2006;81:353-73.
2. Phillips KM, Horst RL, Koszewski NJ, et al. *Vitamin D_4 in mushrooms.* PLoS One 2012;7:e40702.
3. Tsugawa N, Nakagawa K, Kawamoto Y, et al. *Biological activity profiles of 1alpha,25-dihydroxyvitamin D_2, D_3, D_4, D_7, and 24-epi-1alpha,25-dihydroxyvitamin D_2.* Biol Pharm Bull 1999;22:371-7.
4. Keegan RJ, Lu Z, Bogusz JM, et al. *Photobiology of vitamin D in mushrooms and its bioavailability in humans.* Dermatoendocrinol 2013;5:165-76.
5. Göring H. *Vitamin D in nature: a product of synthesis and/or degradation of cell membrane components.* Biochemistry (Mosc) 2018;83:1350-7.
6. Silvestro D, Villette C, Delecolle J, et al. *Vitamin D_5 in arabidopsis thaliana.* Sci Rep 2018;8:16348.
7. Mehta RG. *Stage-specific inhibition of mammary carcinogenesis by 1alpha-hydroxy-vitamin D_5.* Eur J Cancer 2004;40:2331-7.
8. Zmijewski, MA, Li W, Chen J, et al. *Synthesis and photochemical transformation of 3β,21-dihydroxypregna-5,7dien-20-one to novel secosteroids that show anti-melanoma activity.* Steroids 2011;76:193-203.
9. Holick MF, Tian XQ, Allen M. *Evolutionary importance for the membrane enhancement of the production of vitamin D_3 in the skin of poikilothermic animals.* Proc Natl Acad Sci USA 1995;92:3124-6.
10. Holick MF. *Environmental factors that influence the cutaneous production of vitamin D.* Am J Clin Nutr 1995;61:638S-645S.
11. MacLaughlin J, Holick MF. *Aging decreases the capacity of human skin to produce vitamin D_3.* J Clin Invest 1985;76:1536-8.