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Review

Is Vitamin D Redundant in an Aquatic Habitat?

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Summary Certain fish are very rich sources of vitamin D as compared to most of the higher vertebrates which have insignificant amounts of this vitamin. Not only the teleosts, which possess a calcified skeleton, but also the elasmobranchs, which lack calcified skeleton, contain extremely high concentrations of this vitamin, leading to the speculation that the function of vitamin D in fish may be different from its known classical functions in terrestrial animals. Interestingly, the two most common calcemic hormones associated with Ca and P homeostasis in higher vertebrates are either missing [parathyroid hormone (PTH)] or inactive [calcitonin (CT)] in fish. In fact, these hormones appear to have developed after transition of life from water (Ca-P rich environment) to land (environment poor in Ca and P). Thus, living in an aquatic environment with a continuous rich supply of Ca and P, do fish need vitamin D? If so, does it need to be converted to its polar forms? Additionally what are the functions of vitamin D and its metabolites in fish? Since fish stand between the invertebrates and higher vertebrates in evolution, they serve as a unique model for the study of the evolutionary and physiological significance of vitamin D. Investigations have demonstrated that the source of a high amount of vitamin D in them is primarily through their food-chain (plankton). In addition, it appears from the studies in fish that vitamin D perhaps had no physiological function in the calcium-rich aquatic environment, and its metabolism was essentially for catabolic purposes. During the course of evolution, when life started on calcium poor terrestrial environment, vitamin D became functional and its metabolism, an anabolic one, was concerned with calcium homeostasis.

Key Words fish, calcium, evolution, metabolism, vitamin D

Calcium homeostasis

Nature has in a way ensured that calcium and vitamin D are inextricably interlinked. Vitamin D, as it is understood today, carries out the policing job of regulating serum calcium levels in highly evolved mammals. And this regulating mechanism is done with astounding efficiency.

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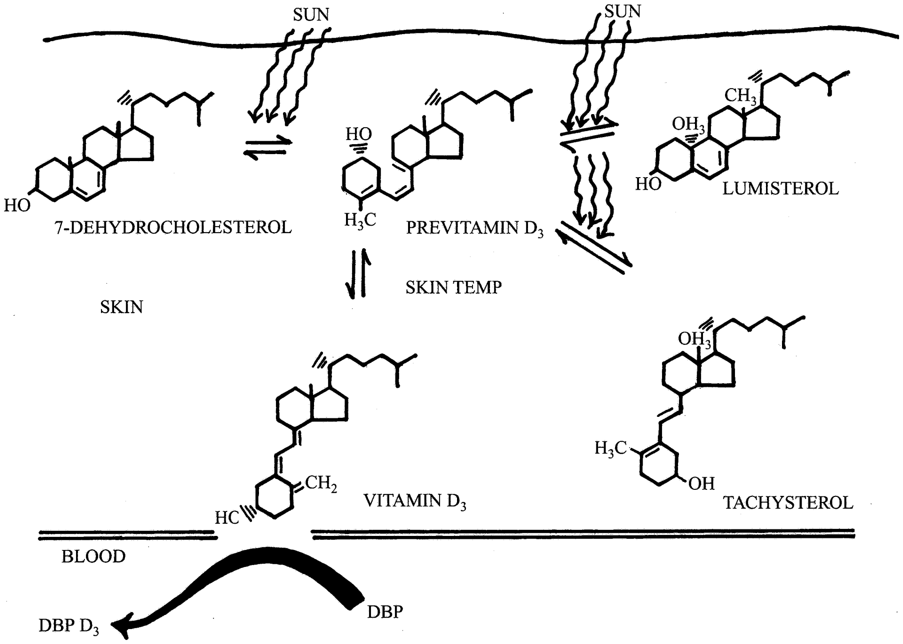

Fig. 1. Photochemical synthesis of vitamin D_3 in the skin of higher animals. DBP, vitamin D binding protein.

Serum calcium level of 2.5 mmol/L is maintained in all life-forms of vertebrates, aquatic and terrestrial. What makes this number sacrosanct? Perhaps this most guarded constant of Nature is the conundrum waiting to be deciphered in the ongoing process of evolution.

Calcium is the 5th most abundant element in the sea, from which life arose. Therefore several vital biological processes, including neuronal excitability, muscle contraction, membrane permeability, hormone release, enzyme activity, and bone mineralization, became dependent on this element during the evolutionary process (*1*).

During the chemical evolution, a steroid would have been formed, and this, on irradiation by solar UV rays, must have resulted in the formation of a secosteroid, calciferol. The foundation for this assumption is that calciferol was found to be present even in primitive living cells. Today, calciferol, known as vitamin D, which is produced in the skin from exposure to sunlight (Fig. 1) (*2–7*), plays an important role in calcium homeostasis in land vertebrates (*8–10*).

The assumption that vitamin D was an important regulator of calcium homeostasis in early evolution may not be true. This is because life evolved in the sea where calcium is abundant and was available at all times in its surroundings. Thus the mechanism for calcium homeostasis and for the conservation of calcium was not needed.

Table 1. Provitamin D and vitamin D in freshwater plankton.

Sample	Concentration (ng/g dry wt)			
	Provit D_2	Provit D_3	Vit D_2	Vit D_3
Phytoplankton	3,889.0	23,581.0	52.5	803.5
Zooplankton	7,167.0	46,238.0	724.0	2,717.0

Values are averages of 3 injections of the sample on HPLC (Ref. *18*).

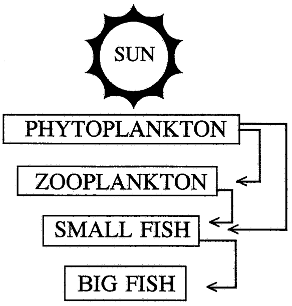

Fig. 2. Food chain as origin of vitamin D in fish.

Environmental calcium and the evolution of life

The evolution of life from salt water (the calcium level in seawater is about 10 mmol/L), to fresh water (calcium concentration ranges from 0.1 to 3.0 mmol/L), and then to dry land (no environmental calcium) required the adaptation from a high-calcium, to a low-calcium, to a no-calcium environment. Throughout the evolution of the vertebrate kingdom, which took place over the immense time span of geological ages, involving the primitive aquatic vertebrates to the most highly evolved terrestrial animals (cartilaginous fish→bony fish→amphibians→reptiles →aves and mammals), the serum calcium level has remained the same (2.5 mmol/L) and is highly regulated (*11*). This is in spite of the shift from a calcium-rich to a calcium-poor environment.

Therefore homeostatic control systems necessarily must be evolved in the various groups of vertebrates to precisely control the serum calcium levels (*1*, *12*), for the vital biological functions of one of Nature's most carefully guarded constants, in calcium-rich and calcium-poor environments.

Source of vitamin D and regulation of calcium in fish

Fish are known to be rich sources of vitamin D [25,000–250,000 IU/g oil (*13–16*)] compared with the higher vertebrates [<1 IU/g oil (*17*)]. This vitamin is mainly derived through diet, i.e., plankton (Table 1), which inhabit the photic zone and thus are capable of photosynthesizing vitamin D (*18*) (Fig. 2). Because vitamin D

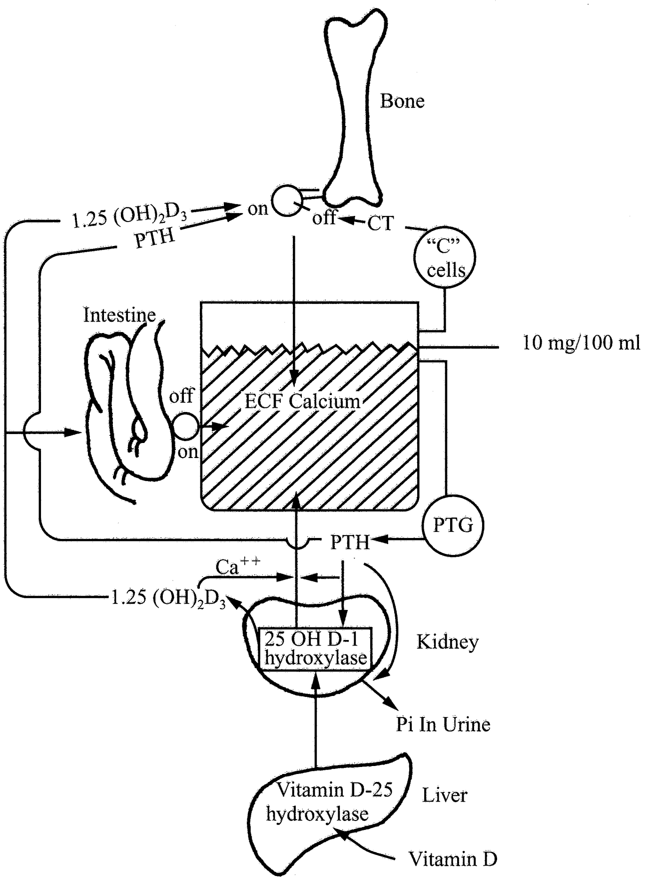

Fig. 3. The calcium homeostatic system, including the vitamin D endocrine system. $1,25(OH)_2D_3$, 1,25-dihydroxy vitamin D_3; CT, calcitonin; PTH, parathyroid hormone; PTG, parathyroid gland.

is a lipid soluble, nonpolar compound, it must have accumulated, resulting in its high amount in fish. This raises the question of whether vitamin D has any function in fish? As we know, in a calcium-phosphorus-poor terrestrial environment, the precise control of plasma calcium is dependent on the vast reservoir of the element in the skeleton. A regulation of calcium involves vitamin D (via its active metabolite, 1,25-dihydroxy vitamin D_3 [$1,25(OH)_2D_3$] a steroid hormone), parathyroid hormone (PTH), and calcitonin (CT) (*1*, *12*) (Fig. 3). When a continuous and virtually inexhaustible supply of calcium and phosphorus is ensured by the aquatic environment, fish may not require vitamin D for the regulation of these minerals. Apart from this, the calcemic hormone PTH is absent in fish, and this seems to have developed very much later after fish in the evolution process (*12*). Furthermore, several studies have shown a lack of relationship between the vitamin D content of fish and their calcemic/phosphatemic status (*19–21*); moreover, the administration of vitamin D/metabolites shows no effect on known parameters in fish dependent on vitamin D (*22*). Thus it appears that vitamin D may not have a role in the calcium and phosphorus regulation in fish. This raises a very pertinent query, "Is vitamin D silent in fish, and if it is, why?"

Calcified skeleton as a reservoir of calcium

As the regular and constant availability of calcium and phosphorus from the sea became ensured, a reservoir for these minerals was found unnecessary; thus some fishes, such as the cyclostomes, chimaerids, and elasmobranchs, developed a completely cartilaginous skeleton, but the bones of many teleosts became relatively acellular and metabolically inactive, not useful for homeostatic control (*1*). During the course of evolution, when life shifted on to low calcium (0.1 mmol/L) fresh water, two options were left for the animals: either to shift the vital biological calcium-dependent functions to some other chemical that is constantly available, or to ensure a constant supply of calcium within their bodies by conservation. The animals seem to have selected the second option, and this led to the development of bone, a calcified endoskeleton, to serve as a large reservoir of calcium and phosphorus, and this is of great importance in the history of evolutionary events.

Because a continuous supply of these minerals was still ensured from the surrounding environment, vitamin D seems unnecessary to regulate them. In support of this, the high ionic strength of Ca^{2+} in marine cyclostomes (0.77) and marine elasmobranch (0.29) lowers the chemical reactivity of calcium and prevents crystal formation (*11*).

Thus if the ionic strength of the physiological fluid is the crucial determinant of tissue calcification, and if vitamin D apparently had no role in this process, why do fish metabolize vitamin D?

Vitamin D metabolism in fish

Vitamin D must have entered fish accidentally through a diet that had plenty of it (*18*), and it does not seem to have any known physiological function (*22*) in a calcium-phosphorus-rich aquatic environment. Thus this may be a needless compound that was present in the diet and that entered the fish that way. Therefore it must be disposed of, since it has no function and as a lipid compound gets unnecessarily deposited. Because vitamin D is a nonpolar lipid compound, fish must metabolize it into polar forms to facilitate its disposal through excretion. The enzymes needed for the hydroxylations probably then evolved in the liver (Table 2) (*23*, *24*) to catabolize vitamin D into polar forms. Thus the metabolism of vitamin D in fish is primarily meant only for a catabolic purpose (Fig. 4).

In the evolutionary process, as the scene shifted from a calcium-phosphorus-rich water world to calcium-phosphorous-poor terrestrial habitats, the vertebrates had no other choice but to meet the requirements of these minerals through dietary sources. Then to maintain a regular supply of calcium in the terrestrial environment,

Table 2. 25-Hydroxylase and 1α-hydroxylase in liver and kidney of tilapia.

Tissue	25-Hydroxylase		1α-Hydroxylase	
	% conversion	Enzyme activity (fmol of $25OHD_3$ formed/mg protein/h)	% conversion	Enzyme activity (fmol of $1,25(OH)_2D_3$ formed/mg protein/h)
Liver	3.0 ±0.17***	3.0 ±0.20	11.2 ±0.07***	12.0 ±0.31***
Kidney	1.3 ±0.12	3.0 ±0.21	1.8 ±0.09	4.0 ±0.22

Values are mean ± SE of three observations (Ref. *24*).
$^{***}p < 0.001$.

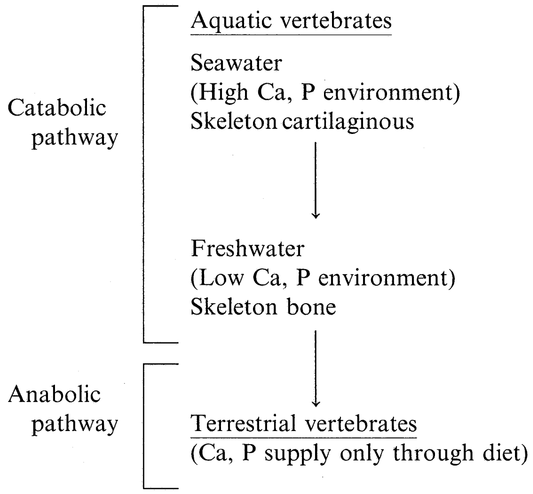

Fig. 4. Evolutionary importance of the vitamin D endocrine system.

calcium-regulating hormones seem to have evolved. At this juncture, vitamin D must have become functional by using the same metabolic pathway to maintain the extracellular calcium-phosphorous homeostasis in an internal environment containing calcified endoskeleton (bone). Thus bone attained significance as a large store for calcium and phosphorous for a close cycle of the servo system for the minute-to-minute control of these minerals. This was accompanied by the evolution of an extensive system of bone cells that responded rapidly to PTH, CT, and $1,25(OH)_2D_3$. Thus this important element (calcium) is now regulated by these three hormones. The metabolism of vitamin D thus acquired a newer meaning as an anabolic one in the calcium and phosphorus homeostasis (Fig. 4). Fish, the very primitive vertebrates, could only metabolize it (*23*, *24*) and not utilize it (*19–24*), and it is the higher animals that learned to use this vitamin (*8*, *9*).

Vitamin D metabolism from catabolic to anabolic purpose during evolution

Thus vitamin D metabolism to the polar forms by the vitamin D_3 25-hydroxylase and 25-hydroxy vitamin D_3 1-α-hydroxylase enzymes appears to be the same in lower aquatic vertebrates and in higher land animals. It is interesting, though, that it seems to be of a catabolic purpose in a calcium-phosphorous rich aquatic environment, and it turned the same pathway to an anabolic purpose when the environment was rendered poor in these minerals. Therefore we hypothesize that during the evolution of life, there was a "molecular economy" in a living system wherein the same catabolic pathway changed to the beneficially anabolic, depending on the need (*25*).

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