

ADVANCES IN VITAMIN D METABOLISM AND CALCIUM HOMOEOSTASIS IN DOMESTIC SPECIES

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SUMMARY

Vitamin D metabolism and function is quantitatively and qualitatively very similar in most mammalian species. Chickens, however, have a higher rate of turnover of vitamin D and are thus more susceptible to vitamin D deficiency if vitamin D supply is inadequate. Intensively reared animals such as pigs and poultry require dietary vitamin D supplements. However, for all other species, vitamin D status is maintained by exposure to solar ultraviolet light. Vitamin D requirements for laying hens are high because of the large quantities of calcium for eggshell formation and also because there is a loss of 1–2 μg of vitamin D in the yolk of each egg. The role of vitamin D in calcium homoeostasis for all species is discussed.

INTRODUCTION

Several thousand research papers on vitamin D and calcium homoeostasis have been published since the discovery in 1970 that vitamin D was metabolically transformed into a steroid hormone, 1,25-dihydroxycholecalciferol (1,25(OH)₂D). Studies have been made in a variety of animals with the assumption that for each species some unique characteristic of vitamin D metabolism would be found. Perhaps surprisingly, such is not the case for there is great similarity in the way vitamin D is handled in all animal species examined.

Apart from intensively reared pigs and poultry, vitamin D is obtained by humans and other animals by exposure of their skin to solar ultraviolet radiation. For all species of land vertebrates there are few feedstuffs which contain more than trace amounts of vitamin D. Indeed, for humans, the only natural dietary sources of vitamin D of any quantitative significance are egg yolk, a few species of fatty fish and dairy products. In Australia, these sources have minimal impact on human vitamin D status which, as for other animals, is mainly determined by exposure to solar ultraviolet light.

Vitamin D (cholecalciferol) formed photochemically in skin from 7-dehydrocholesterol is transported into the circulation bound to a specific vitamin D-binding protein. The subsequent conversion of vitamin D to 25-hydroxyvitamin D [25(OH)D] in the liver and the further hydroxylation of 25(OH)D to 1,25(OH)₂D in the kidney are now so well known that this topic need not be reviewed again here.

INTERPRETATION OF VITAMIN D METABOLITE CONCENTRATIONS

Studies of vitamin D metabolism *in vivo* indicate that the more vitamin D that is given to an animal, the higher the concentration of 25(OH)D in plasma (Fraser 1980). Although the production of 25(OH)D is not directly proportional to vitamin D input, nevertheless 25(OH)D concentration in plasma, within the range 0.025–0.125 μM (10–50 ng/ml), is a good indicator of vitamin D supply. Thus, in temperate geographical regions the plasma concentration of 25(OH)D is highest in late summer and lowest in late winter. This reflects the seasonal change in the intensity of solar ultraviolet-B radiation (290–320 nm) (Fraser 1983). However, even with extensive solar irradiation, in tropical regions or with whole-body exposure beside the sea in summer, the plasma concentration

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of 25(OH)D rises to no higher than 0.2 μM (80 ng/ml). Evidently, the greatest rate of formation of vitamin D in skin cannot maintain 25(OH)D concentrations above this level. Nevertheless, the capacity to synthesize 25(OH)D is considerably greater than the plasma levels produced by ultraviolet irradiation would suggest. Large oral doses of vitamin D, which give rise to signs of vitamin D toxicity, can raise the plasma concentration of 25(OH)D to more than 1 μM (400 ng/ml).

There is remarkable similarity in 25(OH)D concentrations in plasma among different species. Thus cows (Horst and Reinhardt 1982), sheep (Smith and Wright 1981), horses (Smith and Wright 1984), pigs (Goff *et al.* 1984), goats (Hines *et al.* 1986), camels (Shany *et al.* 1978) and chickens (L. Berven pers. comm.) all have 25(OH)D₃ concentrations in plasma of between 0.025 and 0.125 μM .

These observations lead to the conclusion that a key factor determining plasma 25(OH)D concentration in all species is the input of vitamin D to the site of hydroxylation in the liver. Hence, these plasma levels are a good index of vitamin D status and help to discriminate between vitamin D deficiency and other causes of bone disease.

The concentration of 1,25(OH)₂D in plasma (0.072-0.123 nM) (30-50 pg/ml) is 1000-fold lower than that of 25(OH)D. Furthermore, the 1,25(OH)₂D concentration is mainly independent of the supply of the 25(OH)D precursor. A multiplicity of physiological controls have been proclaimed to influence the formation of 1,25(OH)₂D in the cells of the proximal convoluted tubules in the kidney (Fraser 1980). The postulated controlling factors include parathyroid hormone (PTH), calcitonin, prolactin, growth hormone, insulin, glucocorticoids, gonadal steroids, calcium, phosphate, hydrogen ions, potassium and even 1,25(OH)₂D itself. Because of this multitude of factors claimed to regulate the renal 1-hydroxylase there is considerable uncertainty as to how to interpret the controlled production of 1,25(OH)₂D.

Because 1,25(OH)₂D is a hormone, its formation is presumed to be regulated according to the requirements for its endocrine function. The concentration in plasma in general bears no relationship to vitamin D status except in severe deficiency when there is insufficient 25(OH)D precursor to maintain the synthesis of 1,25(OH)₂D.

The standard view of the regulated secretion of 1,25(OH)₂D is that this active metabolite is produced to maintain calcium homeostasis by stimulating target cells in the intestinal mucosa, in the renal tubules, and in bone to increase their transport of calcium. Thus PTH, secreted in response to a fall in extracellular Ca²⁺ concentration, enhances the activity of the renal 1-hydroxylase. The action of the extra 1,25(OH)₂D in "stimulating" the target cells, increases the extracellular Ca²⁺ concentration leading to a fall in PTH secretion. Such a negative feedback control is typical of the endocrine loops which link the secretion and function of peptide hormones. In general, however, steroid hormones have effects which appear to be permissive rather than stimulatory and such negative feedback loops do not occur. It may well be that the function of 1,25(OH)₂D is permissive and not stimulatory, enabling cells to have variable capacity for transporting calcium. This interpretation is compatible with observations on the function of 1,25(OH)₂D and removes the need to find a specific biological role for each factor reported to influence the secretion of 1,25(OH)₂D by the kidney.

Although the production of 1,25(OH)₂D appears to be independent of variation in the supply of 25(OH)D, concentrations of the two metabolites are related during growth (Stern *et al.* 1981) and during the recovery from vitamin

D deficiency (Stanbury 1981). In both these circumstances, the 1-hydroxylase activity is elevated and the amount of 1,25(OH)₂D produced is partly determined by the supply of 25(OH)D. These findings support the concept that the 1-hydroxylase is regulated by varying the accessibility of the 25(OH)D substrate to the enzyme (Fraser 1980). They also reinforce the view that the concentration of 1,25(OH)₂D in plasma cannot be related quantitatively to a required degree of response in target cells.

THE ROLE OF VITAMIN D-BINDING PROTEIN IN PLASMA

The affinity of binding of 25(OH)D to the human vitamin D-binding protein (DBP) ($K_d=6.4 \times 10^{-6}$ M) is higher than for 1,25(OH)₂D ($K_d=3.4 \times 10^{-7}$ M) or for vitamin D (cholecalciferol) itself ($K_d=4.3 \times 10^{-7}$ M) (Haddad and Walgate 1976). This variation in binding affinity probably contributes to marked differences in the half-time or clearance of the metabolites from plasma. The $t_{1/2}$ for 25(OH)D is estimated in humans to be between 15 and 45 days (Clements *et al.* 1987b). In contrast, 1,25(OH)₂D, with its lower affinity for DBP is cleared from human plasma with a half-time of about 5 to 8 hours (Gray *et al.* 1978).

The prolonged time of clearance of 25(OH)D has no apparent parallel with any other plasma constituent. Other endocrine factors are cleared from the circulation in minutes or a few hours. The long time of residence of 25(OH)D in plasma is even more surprising, considering that the transporting DBP is cleared rapidly ($t_{1/2}$ in rabbits = 1.7 days) (Haddad *et al.* 1981). This suggests that DBP, along with its associated 25(OH)D, is taken up by cells, the protein is degraded and 25(OH)D is then released back into the circulation where it binds again to more DBP. The prolonged $t_{1/2}$ of 25(OH)D would thus represent a composite clearance curve derived from the repeated removal and re-entry of 25(OH)D as DBP itself is turned over.

This very long residence of 25(OH)D in extra-hepatic tissues could explain the ability of vitamin D reserves to be maintained for long periods of time. Studies in children have shown that only a few hours exposure to summer sunlight is able to produce sufficient vitamin D to avoid deficiency for several months (Poskitt *et al.* 1979). In contrast, in chickens, where affinity of DBP for 25(OH)D is 2-3-fold lower than in mammals (DeLuca *et al.* 1988), the rate of removal of 25(OH)D is 3 times faster so that without a continuous supply of vitamin D, deficiency rapidly develops (L. Berven pers. comm.).

Therefore, the association of 25(OH)D with DBP in the circulation provides a reserve of the precursor for 1,25(OH)₂D formation, it provides an accurate index of vitamin D status, and it provides a means for investigating the cause of vitamin D deficiency by studying the kinetics of 25(OH)D turnover in plasma.

CALCIUM HOMOEOSTASIS

Calcium homoeostasis is effectively the process of maintaining a constant extracellular Ca²⁺ concentration. In all vertebrate species this desired concentration is close to 1.25 mM (5 mg/ml) (Urist 1963). In land vertebrates, the means of achieving this constancy are to increase the absorption capacity of the small intestine and to mobilize calcium from bone when the Ca²⁺ concentration tends to fall. The main mechanism for preventing the Ca²⁺ concentration from rising above the desired level is to increase excretion of calcium by the kidney.

The vitamin D metabolite 1,25(OH)₂D is the primary regulating factor determining changes in intestinal absorption capacity and bone resorption to

maintain the extracellular Ca^{2+} concentration. In comparison to its action in intestine and bone, vitamin D appears to have a quantitatively minor influence on calcium transport in the kidney (Costanzo 1974).

Although the action of $1,25(\text{OH})_2\text{D}$ is frequently described as “stimulating” intestinal calcium absorption and bone calcium resorption, this interpretation is possibly too simplistic. Most studies to identify the function of vitamin D in intestine and bone have made use of vitamin D-deficient animals. When such animals are repleted with vitamin D there is, after several hours delay, an increase in the absorption capacity for calcium across the mucosa of the small intestine. Likewise, as the abnormal rachitic bone is repaired, there is mobilization of mineral from bone.

Both these responses to vitamin D are inevitable consequences when the abnormal state of vitamin D deficiency is corrected. Because the ability to maintain calcium homeostasis is impaired in vitamin D deficiency, then correction of this deficiency will activate mechanisms which control extracellular Ca^{2+} concentration. In animals with an adequate vitamin D status the action of $1,25(\text{OH})_2\text{D}$ on extracellular Ca^{2+} concentration appears to be that of a permissive factor, enabling cells in intestine and bone to have the capacity to transport Ca^{2+} . This transporting mechanism could then be modified by other short-term regulators according to the immediate needs for calcium homeostasis. Evidence has been found which suggests that PTH (Nemere and Norman 1986), growth hormone (Chipman *et al.* 1980) and even calcitonin (Jaeger *et al.* 1986) could be short-term modifiers of vitamin D-dependent active transport of calcium in the intestine.

A similar interpretation can be made for the role of $1,25(\text{OH})_2\text{D}$ in bone. Experiments with bone in organ culture demonstrate that $1,25(\text{OH})_2\text{D}$ is a potent stimulator of osteoclastic bone resorption *in vitro* (Raisz *et al.* 1972). This reinforces the long-held view that the function of $1,25(\text{OH})_2\text{D}$ in bone is to “stimulate” resorption. If the concentration of $1,25(\text{OH})_2\text{D}$ in blood is experimentally raised in human subjects, then an enhanced rate of bone resorption is indeed found, providing that the subjects are eating a low calcium diet (Maierhofer *et al.* 1983). Yet, if the supply of dietary calcium is adequate, an increase in serum level of $1,25(\text{OH})_2\text{D}$ has no stimulatory effect on the resorption of bone (Maierhofer *et al.* 1984). These observations again suggest that $1,25(\text{OH})_2\text{D}$ has a permissive role, enabling bone cells to transport calcium. A homeostatic increase in bone resorption could be mediated by PTH which activates the vitamin D-dependent calcium transport process when extracellular Ca^{2+} concentration falls. Perhaps the function of $1,25(\text{OH})_2\text{D}$ in bone may be a general one of giving cells the capability to handle Ca^A so that normal growth and turnover of bone takes place. Such a role could apply also to the action of $1,25(\text{OH})_2\text{D}$ in cells not directly concerned with whole-body calcium homeostasis.

Any function of bone as a calcium reservoir must, of necessity, be a limited one in mammals. Some calcium is released from bone to compensate for the loss in milk during lactation in women (Lamke *et al.* 1977) and dairy cows (Ramberg *et al.* 1970). However, extensive mineral mobilization would affect the mechanical properties of bone and diminish its structural strength. Hence, the main long-term control of calcium homeostasis is at the level of absorption of calcium by the small intestine.

The regulated, vitamin D-dependent transport pathway accounts for about 75% of calcium absorbed by the small intestine from a diet adequate in calcium (Sheikh *et al.* 1988). The mechanism by which $1,25(\text{OH})_2\text{D}$ promotes this absorption capacity is still uncertain despite the well-known action of $1,25(\text{OH})_2\text{D}$ to induce the synthesis of a specific calcium-binding protein

(CaBP, molecular weight = 8800) in the intestinal mucosa (for review see Lawson 1985).

An elegant analysis by Bronner (1987) of the kinetics of calcium transport now suggests that CaBP facilitates the inward flux of Ca^{2+} by amplifying the intracellular calcium gradient between the brush border and basolateral poles of the mucosal cells. "This interpretation of experimental and theoretical values for transcellular calcium movement is the most convincing analysis to date for the mechanism of action of $1,25(\text{OH})_2\text{D}$ on the absorption of calcium.

Apart from endocrine control of absorption, another factor determining the supply of calcium to meet homeostatic requirements is variation in the availability of calcium from the diet. The actual proportion of dietary calcium which is utilized is seldom more than 50% in humans and usually, in adults, is no more than 30% (Nordin *et al.* 1979). Values for domestic animals have not been determined. The formation of complexes with phytate (Wise 1983), oxalate (Johnston 1952) and unavailable carbohydrate (dietary fiber) (James *et al.* 1978) decreases the accessibility of calcium to the absorptive surface of the small intestine in monogastric animals. Therefore, when the intake of dietary calcium is low and it is in an unavailable form, any increase in the absorptive capacity of the small intestine will be ineffective in raising the supply of calcium.

It is difficult to explain how calcium homeostasis can be maintained in animals consuming diets which are both low in calcium and where the calcium is apparently unavailable for absorption by the small intestine. Experiments with rats (Favus 1985) and humans (Grinstead *et al.* 1984) have demonstrated that calcium can be absorbed from the large intestine and that the capacity for absorption is increased by $1,25(\text{OH})_2\text{D}$. Because bacteria in the large intestine are able to break down any fiber and phytate that has resisted digestion in the small intestine, the calcium complexes carried into the colon could become available for absorption from the large intestine. A persistently low calcium intake with low theoretical availability may in fact induce an adaptive increase in calcium absorption from the large intestine. The efficiency of utilizing calcium in these circumstances may be greater than has previously been considered likely. Such a mechanism may explain the availability of calcium to monogastric herbivores such as horses.

THE INFLUENCE OF CALCIUM SUPPLY ON VITAMIN D STATUS

Studies with rats have shown that vitamin D deficiency can be induced by feeding a diet where the calcium content or availability is low (Clements *et al.* 1987a). Calcium deprivation promotes mild hyperparathyroidism which stimulates the production of $1,25(\text{OH})_2\text{D}$. However, the increased utilization of $25(\text{OH})\text{D}$ for $1,25(\text{OH})_2\text{D}$ synthesis is not the direct cause of depletion of vitamin D reserves. The extra $1,25(\text{OH})_2\text{D}$ has now been shown to enhance the metabolic inactivation of $25(\text{OH})\text{D}$ in the liver in rats (Clements *et al.* 1987a). A similar enhanced destruction of $25(\text{OH})\text{D}$, related to elevated concentrations of $1,25(\text{OH})_2\text{D}$ in plasma, has also been found in humans (Clements *et al.* 1987b).

Therefore, a deficiency in the availability or supply of calcium could lead to induced vitamin D deficiency. The enhanced metabolic destruction of $25(\text{OH})\text{D}$ in the liver is not thought to be a physiological mechanism for regulating hepatic vitamin D metabolism. Rather, this destruction would appear to be secondary to some other primary effect of $1,25(\text{OH})_2\text{D}$ on liver function. Thus, an adequate supply of calcium may be as important for maintaining vitamin D status as it is for meeting the needs of calcium homeostasis.

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