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VITAMIN D STATUS: CURRENT OPINION ON CRITICAL LEVELS FOR PLASMA CALCIUM AND BONE MINERAL HOMEOSTASIS

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Abstract
Currently there is an unprecedented level of interest regarding the purported wide-ranging beneficial effects of an adequate vitamin D status translating into marked increases in test requests for clinical laboratories. The well characterised endocrine pathway of vitamin D metabolism and action is solely responsible for vitamin D regulating plasma calcium and phosphate homeostasis. A large body of data confirm that vitamin D exerts activities within each of the major bone cells and that these same cells are capable of synthesising the active metabolite, 1,25-dihydroxyvitamin D from 25-hydroxyvitamin D. Such data arising from in vitro studies, animal models and clinical sources are consistent with a paradigm that local metabolism of vitamin D by bone cells to form 1,25-dihydroxyvitamin D and its consequent local actions within bone cells exerts an anabolic effect to increase bone mineral status. The data reviewed here provide plausible mechanisms for both catabolic and anabolic actions of vitamin D on bone depending on dietary calcium intake.

INTRODUCTION

Numerous beneficial effects of an adequate vitamin D status on a wide range of clinical conditions have been proposed particularly since the turn of the 21st century. These claims have generated an unprecedented level of interest in the medical media and lay press alike translating into great interest in assessing vitamin D status particularly amongst patients presenting to family or general practitioners. This growth in requests for serum 25-hydroxyvitamin D levels and the consequent issues for the clinical laboratory have recently been well addressed in this journal (1).

A major factor generating this marked interest in vitamin D has been the reports of simple associations between a particular disease state or condition and low vitamin D status. Thus much of this evidence is weak and there are numerous knowledge gaps. It is clearly apparent that an improved vitamin D status can be associated with many attributes of good health relating to mobility and activities promoting sunlight exposure with out the involvement of a direct biological action of vitamin D. However during the 21st century there has been a flowering of knowledge of metabolism of vitamin D within a range of tissues including synthesis of the active metabolite 1,25-dihydroxyvitamin D (1,25D) and activation of the vitamin D receptor (VDR) within the tissue of synthesis. Such a mechanism now provides plausible physiological and molecular mechanisms for a diverse array of activities and tissue responses as has been reviewed recently (2). In this article I will review the current knowledge for the action of vitamin D on plasma calcium and phosphate homeostasis and bone mineral homeostasis with particular focus on the knowledge gaps. Data published in the 21st century are challenging the concept of a single paradigm for the actions of vitamin D. An alternate paradigm involving local bone tissue metabolism of vitamin D with actions different from those observed with the endocrine source of 1,25D provides a plausible mechanism for activities observed in population studies and from randomised controlled trials of vitamin D supplementation to reduce the risk of fracture in the elderly.

The actions of vitamin D to regulate plasma calcium and phosphate homeostasis:

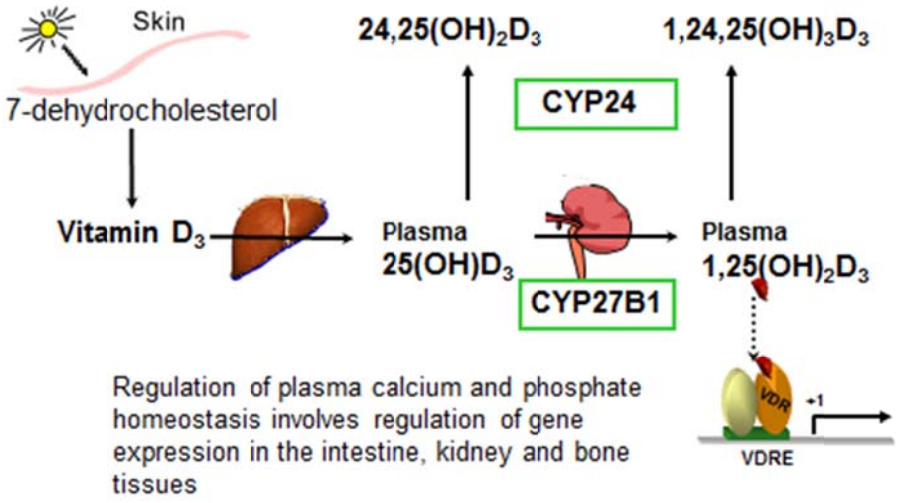

Figure 1.The endocrine pathway of vitamin D metabolism and activity to regulate plasma calcium and phosphate homeostasis. © HA Morris 2011

The well characterised endocrine pathway of vitamin D metabolism and action is summarised in Figure 1 and is considered to be solely responsible for the contribution of vitamin D to regulate plasma calcium and phosphate. New findings of the interaction between FGF23 and 1,25D and plasma phosphate levels are coming to light providing further insight in this area (3). The identification of the endocrine pathway for activation of vitamin D in the 1970's was a major achievement. The biologically active metabolite, 1,25D in plasma arises from sequential hydroxylations of vitamin D, firstly by the liver to form 25D and then by the kidney to form 1,25D (4). The catabolism of vitamin D metabolites through the action of the 25-hydroxyvitamin-24-hydroxylase (CYP24) has also been demonstrated to be an essential regulatory pathway for vitamin D homeostasis. Under conditions when this enzyme activity is reduced or

ablated the serum half-lives of both 25D and 1,25D are increased and contribute to the development of hypercalcaemia, particularly following vitamin D supplementation (5). This mechanism has recently been identified to account for idiopathic infantile hypercalcemia and other toxic effects of vitamin D (6)

The binding of 1,25D to its highly specific nuclear receptor, VDR, modulates transcriptional activity of vitamin D responsive genes through binding to vitamin D response elements (VDRE's) located within the promoter regions of these genes. Most organs and tissues in the body express the VDR with one study demonstrating that the VDR occupied some 2776 genomic positions modulating the expression of at least 229 genes (7). Another study indicated that approximately 913 genes were responsive to VDR in a squamous cell cancer cell line with 80% of genes being up-regulated and that these effects take some 6 to 12 hours to reach measurable levels (8). 1,25D also initiates rapid responses resulting from activation of mitogen activated protein (MAP) kinases and other intracellular signalling pathways (9). This latter activity demonstrates the rapid (within minutes) activation of various pathways for 1,25D and data are also published that implicate a membrane-specific receptor for 1,25D contributing to these rapidly acting activities. These pathways are considered to operate whether 1,25D arises from the plasma as a result of synthesis by the kidney and acting as an endocrine agent or whether it arises from endogenous synthesis by extra-renal tissues and acts as an autocrine or paracrine agent.

The endocrine mechanism for plasma 1,25D was demonstrated to contribute to maintaining calcium homeostasis in the 1960's (10) and continues to dominate thinking in the field today. The current controversial issue in this area is what is the critical level for serum 25D to maintain plasma calcium homeostasis? An extensive hormonal interaction operates to maintain calcium homeostasis regulated by plasma ionised calcium level acting through the calcium-sensing receptor. The action of this receptor directly modulates the levels of parathyroid hormone (PTH) and calcitonin which in turn act on the kidney, bone and gut (4). Low calcium increases PTH secretion and synthesis whereas high calcium increases calcitonin secretion and synthesis. PTH acts rapidly on the kidney to stimulate renal calcium reabsorption from the glomerular filtrate and in concert with 1,25D it stimulates bone resorption to enhance the flow of calcium from bone into plasma. 1,25D in association with PTH increases the number of osteoclasts by way of an indirect mechanism through increased expression of the osteoblast-derived cytokine, RANKL, which promotes osteoclastic differentiation (11). Expression of the VDR gene is required for 1,25D to stimulate the expression of RANKL in osteoblasts as those derived from VDR knockout mice are unable to stimulate the differentiation of osteoclasts (12). Alternatively it is important to note that in vitamin D deficiency or when vitamin D activity is ablated through gene mutations, hypocalcaemia develops despite a high PTH in humans or rodents (13,14). These findings suggest that PTH requires 1,25D in order to increase osteoclast number and stimulate bone resorption to normalise extracellular fluid (ECF) calcium.

Each of these activities operates to restore the low plasma ionised calcium to its homeostatic level. PTH also acts on the kidney to stimulate transcription of the gene coding for the enzyme which converts 25D to 1,25D, 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1) increasing the levels in frank hypocalcaemia with secondary hyperparathyroidism by some 100 fold (15). It is this mechanism that can maintain serum 1,25D in the normal range or at adequate levels to maintain intestinal calcium absorption in the face of significant falls in serum 25D. Thus evidence from postmenopausal women with various levels of serum 25D considered to be in the depleted range (ie 40 nmol/L or lower) indicate that blood ionised calcium, serum 1,25D levels and intestinal radiocalcium absorption do not fall significantly until serum 25D is below 20 nmol/L (16). These data suggest that the critical level of serum 25D to maintain the endocrine actions of 1,25D to maintain plasma calcium homeostasis is 20 nmol/L or greater.

The actions of vitamin D to regulate bone mineral homeostasis:

The level of bone mineral at any one time is the result of at least two opposing cellular actions, bone formation by osteoblasts and bone resorption by osteoclasts. Rickets in children (or osteomalacia in adults when bone growth has ceased) is the index disease for vitamin D deficiency. It arises from a defect, or more accurately a delay, in mineralisation. Therefore an action of vitamin D to enhance mineralisation has long been considered. Currently this concept is highly controversial and remains the subject of much debate. Strong data have been generated from rodent models and human disease to indicate that dietary deficiency or genetic ablation of vitamin D activity results in hypocalcaemia, hypophosphataemia, hyperparathyroidism and rickets (17). Correction of the hypocalcaemia, hypophosphataemia and secondary hyperparathyroidism corrects the rickets. Such a result is often accompanied by a statement that the bone is now "normal" (17). Therefore it has been concluded that the action of vitamin D on bone mineral can be completely accounted for by its actions to maintain plasma calcium and phosphate homeostasis with no requirement for direct actions of vitamin D on bone cells to maintain or increase bone mineral status. The calcaemic action of 1,25D particularly in conjunction with PTH to stimulate osteoclastogenesis and bone resorption as discussed above is a direct endocrine action of 1,25D on bone cells to reduce bone mineral in order to maintain plasma calcium levels.

In contrast to these findings a large body of data have been and continue to be reported on the expression of VDR by each of the major types bone cells and on effects of 1,25D in vitro to inhibit proliferation of osteoblast-like cells and to stimulate osteoblast maturation and mineralisation (18). Osteoblasts as well as osteocytes and osteoclasts express the VDR and the CYP27B1 and CYP24 enzyme genes as well as a number of vitamin D responsive genes associated with mineralisation in osteoblasts including type I collagen, alkaline phosphatase and osteocalcin and other genes necessary for osteoblast and osteocyte maturation amongst a much larger number of proteins. Furthermore there is evidence including clinical and rodent model studies suggesting that vitamin D is likely to exert activities directly on bone cells to improve or maintain bone mineral status (19). Clinical studies since the early 1980's have reported that an increased risk of hip fracture amongst the elderly occurs at mean levels of serum 25D of some 40 nmol/L (20), a level now considered as adequate for maintaining plasma calcium homeostasis. Furthermore population studies from the US have demonstrated that bone mineral density increases with increasing serum 25D levels reaching a plateau at serum 25D levels of approximately 75 nmol/L (21). Data from randomised clinical trials of vitamin D supplementation and fracture incidence indicate that anti-fracture efficacy at the hip or other non-vertebral sites is not achieved until serum 25D levels of 75 nmol/L are achieved (22).

Interesting confirmatory data for a direct anabolic action of vitamin D on bone have arisen from animal model studies. Mouse models in which vitamin D activity has been ablated either by knocking out the gene for the VDR or the CYP27B1 enzyme, as stated previously develop rickets when fed a normal calcium diet. When the dietary calcium and the phosphate levels are markedly increased sufficient to normalise plasma calcium and phosphate levels, the rickets phenotype is rescued and as mentioned above, many researchers have described the bone as 'normal' (17). One research group has extended the feeding of the 'rescue' diet to these genetically modified mice for a longer period (16 weeks instead of 10 weeks) (23). On careful examination of their skeleton they confirmed that the rickets had been rescued. However these mice had lower levels of normally mineralised bone than wild type mice; that is they had osteoporosis. As well the gene knockout mice had fewer osteogenic bone marrow cells although there was no difference in the number of osteoclasts in the bone of wild type and gene knockout mice. Thus these data support the concept that vitamin D activity is necessary for normal bone mineral levels at least in adult mice due to the production of an optimal number of osteoblast forming cells and osteoblast activity.

Further independent data from other mouse models support this concept that vitamin D activity within mature osteoblast cells exerts an anabolic action on bone tissue. The OSVDR transgenic mouse line was prepared to over express the human gene for VDR only in mature osteoblasts and some osteocytes by utilising the promoter region of the human osteocalcin gene to regulate VDR transgene expression (24). Adult OSVDR mice demonstrate a strong bone phenotype compared with wild type mice with an increase of both cortical and trabecular bone volumes of some 15% at a number of sites in the skeleton. The increased bone volume is a result of both an increase in bone formation and decreased bone resorption (24). A similar but distinct transgenic mouse line (OSC mice) has recently been prepared in which the human CYP27B1 gene is expressed under the control of the human osteocalcin promoter. These OSC mice demonstrate increased synthesis of 1,25D from 25D only in mature osteoblasts and some osteocytes. These mice demonstrate increased trabecular bone volume in females at 20 weeks of age (25). These latest results have only been published as abstracts and are only preliminary at this time. Further data on the activities of the bone cells are required.

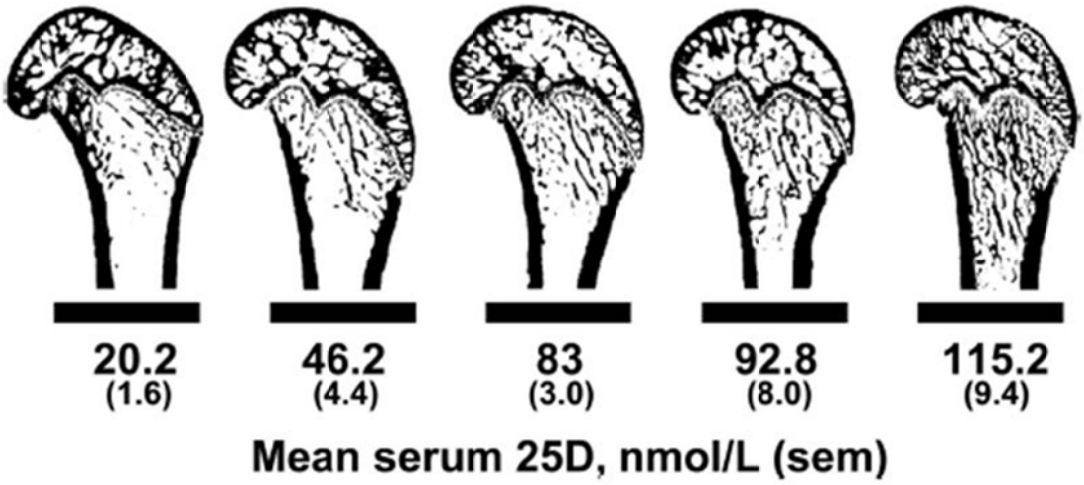

Figure 2. Representative von-Kossa stained sections of the distal femur from 30 week-old rats maintained with serum 25D levels ranging from 20.2 to 115.2 nmol/L for 20 weeks. Note that the distal metaphyseal trabecular bone (bone volume as a ratio to total volume (BV/TV)) is significantly increased as the 25D level increased. (© John Wiley & Sons 2008 reproduced from reference 22 with permission of the publishers John Wiley & Sons, Inc.)

Another line of inquiry has utilised dietary studies in rodent models to demonstrate that at levels of serum 25D between 20 and 80 nmol/L, trabecular bone volume is reduced over 3 months as a result of increased bone resorption from increased osteoclastogenesis due to increased bone expression of the key osteoclastogenic cytokine, RANKL (Figure 2), (26). At 25D levels below 20 nmol/L and dietary calcium of 4%, osteomalacia is observed. At values above

20 nmol/L the mineralisation lag time is normal, which therefore excludes osteomalacia. In these animals it was only the serum 25D level and not serum 1,25D or PTH which significantly correlated with trabecular bone volume, osteoclast surface or bone RANKL mRNA levels.

Regulation of bone synthesis of 1,25D

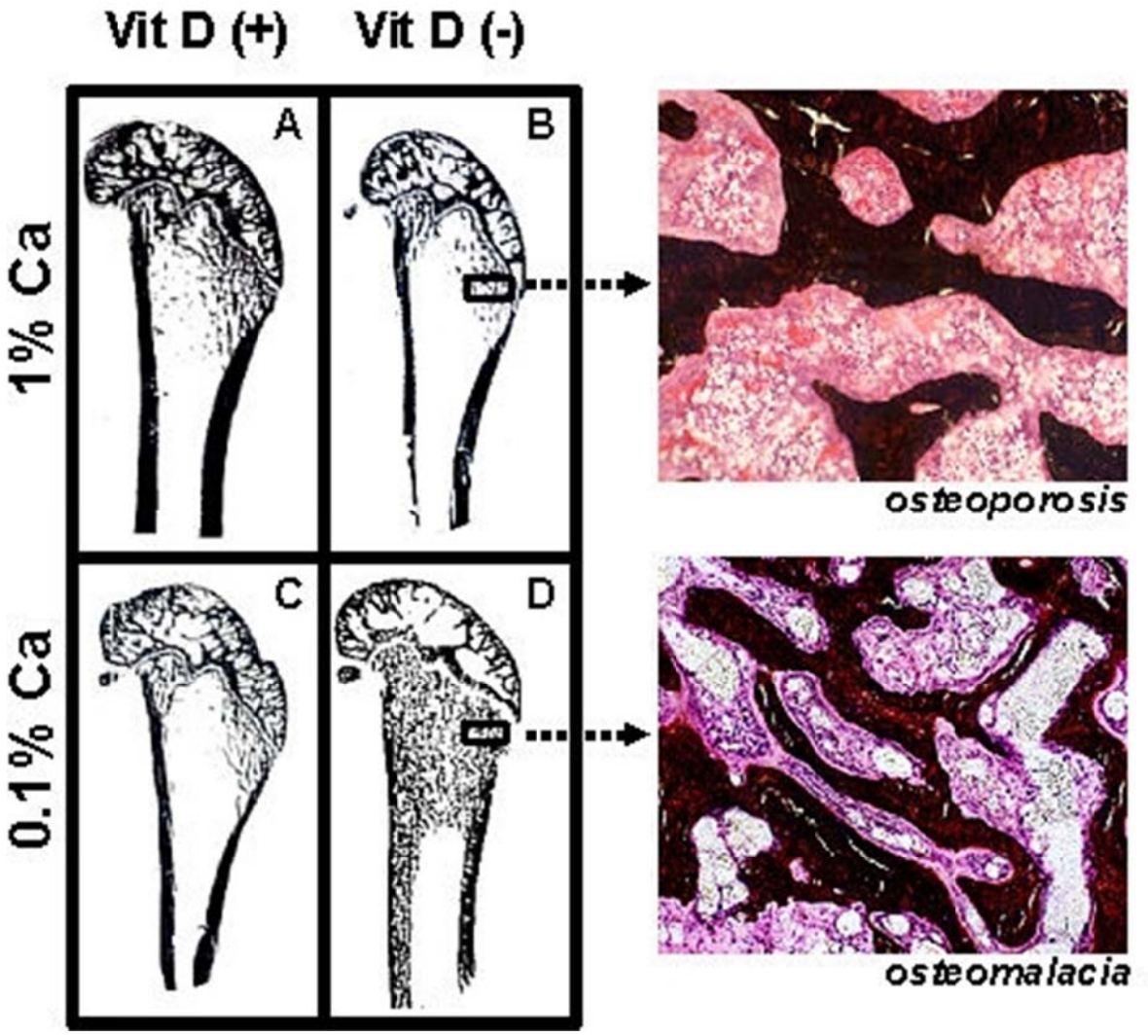

Figure 3. Longitudinal sections (Von Kossa stained) of 9-month old Sprague-Dawley rat distal femora following 3 months feeding either A. 1% calcium/20 IU vitamin D3/day; B. 1% calcium/0 IU vitamin D3/day; C. 0.1% calcium/20 IU vitamin D3/day; D. 0.1% calcium /0 IU vitamin D3/day. Highly trabecularized bone with osteomalacia (D) is evident in contrast to reduced trabecular bone volume (B &C) compared to A. © H A Morris 2010

All of these data are consistent with a model that local metabolism of vitamin D by bone cells to form 1,25D and its local action within bone cells can increase bone mineral status (27). So what factors regulate vitamin D metabolism in bone tissue? This is currently an area of intense research. Unlike the kidney, PTH does not increase the synthesis of 1,25D in bone (28) and there is direct evidence that PTH treatment of bone cells does not activate the promoter region of the human gene for CYP27B1 (29). In fact high PTH levels are associated with decreased synthesis expression of the CYP27B1 gene in bone tissue (28). Perhaps the most important finding in this area is that increased dietary calcium intake increases the expression of the CYP27B1 gene in bone by three-fold and reduces the expression of this gene in the kidney (30). Certainly rodent studies have found that optimal bone status is dependent on adequate

vitamin D and dietary calcium status rather than either one alone (21). When rats are fed either an adequate vitamin D level but low dietary calcium or vice versa they develop osteoporosis and when fed both a low vitamin D and calcium diet they develop osteomalacia (Figure 3).

DISCUSSION

The data reviewed here provide a plausible model for both catabolic and anabolic actions of vitamin D on bone depending on the dietary calcium intake. With either an inadequate dietary calcium intake or low vitamin D status with a marginal dietary calcium intake, increased activity of the plasma calcium and phosphate homeostatic mechanisms, including endocrine vitamin D activity, are required to maintain plasma calcium and phosphate levels within physiological levels. This involves increased activity of PTH when serum 25 D levels fall below 60 nmol/L (32) presumably arising from stimulation of the parathyroid gland calcium-sensing receptor as a result of a slight fall in plasma ionized calcium level. However it is also possible that the increased PTH secretion arises as a direct result of the fall in serum 25D (33). The increased PTH acts to elevate the level of CYP27B1 enzyme in the kidney with the increased enzyme activity allowing continued renal synthesis of 1,25D and maintenance of serum 1,25D levels while serum 25D levels fall into the depleted levels (eg levels below 60 nmol/L). The maintenance of serum 1,25D optimises intestinal calcium and phosphate absorption and the interaction between PTH and serum 1,25D stimulates osteoclastogenesis and bone resorption increasing the flow of calcium and phosphate into the plasma compartment. This mechanism is able to maintain plasma calcium and phosphate levels until serum 25D levels fall below 20 nmol/L at which time the substrate levels are too low to maintain serum 1,25D levels. Consequently intestinal calcium absorption decreases coincident with the development of hypocalcaemia (16). Perhaps most importantly it is apparent that both PTH and serum 1,25D are required for adequate bone resorption because the development of hypocalcaemia indicates that even when PTH levels are very high there is inadequate flow of calcium from the bone compartment to maintain normocalcemia in the plasma compartment (15). Under these conditions the bone disease of rickets/osteomalacia develops.

When dietary calcium intakes are adequate to meet all the demands of the calcium economy the data reviewed above suggest that in concert with an adequate vitamin D status (eg, serum 25D levels greater than 75 nmol/L), increased levels of bone CYP27B1 enzyme activity ensure greater synthesis of 1,25D by bone cells associated with reduced circulating PTH levels. These conditions suppress RANKL expression, osteoclastogenesis and bone resorption. There are data to suggest that bone formation variables are also increased but it is unclear whether such changes are direct effects of 1,25D on osteoblasts or indirect effects of suppressed osteoclast activity (26).

These latest findings provide further light on the delicate relationship between plasma calcium homeostasis, bone mineral homeostasis and vitamin D activities involving either endocrine or bone autocrine /paracrine activities. These findings also provide a plausible mechanism for at least two critical levels for serum 25D, one for calcium homeostasis and a second for bone mineral homeostasis. To maintain plasma calcium homeostasis, a serum 25D level of 20 nmol/L or greater appears to be sufficient to provide adequate substrate for the renal CYP27B1 enzyme level. This lower level is adequate because the renal CYP27B1 enzyme level is increased by increasing levels of PTH when serum 25D levels fall below 60 nmol/L (32). In contrast bone CYP27B1 enzyme levels are not increased by PTH and therefore higher levels of serum 25D as substrate (ie serum 25D 75 nmol/L or greater) are required for bone cells to produce sufficient bone tissue 1,25D to suppress osteoclastogenesis. Furthermore adequate dietary calcium intake is also required to increase bone CYP27B1 enzyme levels even at these higher levels of serum 25D.

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