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CURRICULUM VITAE

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Introduction to Vitamin D: The molecular structure of vitamin D (see Figure 1) is closely allied to that of classical steroid hormones (e. g. progesterone, estradiol, testosterone, glucocorticoids and aldosterone). All of these steroid hormones and also vitamin D are metabolically produced from the same common precursor, namely the sterol cholesterol (1). Technically, vitamin D is a secosteroid. Secosteroids are those in which one of the four ring structures of cholesterol (the cyclopentanoperhydrophenanthrene rings) have undergone breakage of one carbon-carbon bond; in the instance of vitamin D this is the 9,10-carbon bond of rign B.

There is a family of vitamin D-related steroids with variations in the precise structure of the side chain attached to carbon-17. The naturally occurring form of vitamin D is that which has the side chain structure identical to that of cholesterol and 7-dehydrocholesterol; this is known as vitamin D_3 or cholecalciferol. Vitamin D_2, or ergocalciferol, has the side chain of ergosterol and is not a naturally occurring form of the vitamin. Collectively vitamin D_3 plus vitamin D_2 can be termed as the calciferols or simply vitamin D (see Figure 1).

The substance vitamin D_3 is officially classified as being a vitamin, which implies that the body does not have the capability to metabolically produce this substance. The fact that vitamin D_3 is classified as a vitamin is an experimental accident which occurred at the time of its discovery in 1921. By 1925 it was clearly established that vitamin D_3 had a precursor in skin, namely 7-dehydrocholesterol, which when exposed to ultraviolet light or sunlight could be efficiently converted into the molecule vitamin D_3. Thus while it is scientifically correct that vitamin D_3 is not a vitamin, it has proven beneficial to public health to have this molecule made nutritionally available either by food supplementation or incorporation into vitamin capsules. The Recommended Dietary Allowance (RDA) of vitamin D_3 for an adult is 200 IU (5μg) per day or 400 IU for pregnant women and children less than 4 years [Tony, what about elderly?]. However if an individual does not have access to vitamin D supplemented foods, he/she can still meet their nutritional RDA requirement for vitamin D_3 by exposure of their face and hands to sunlight three times per week for approximately 20 minutes[1].

Vitamin D Endocrine System: A totally new era in the field of vitamin D has opened since 1964 with the discovery of the metabolism of vitamin D. Altogether, some 37 metabolites of vitamin D_3 have been isolated and chemically characterized. It is now recognized that there is an endocrine system for processing the prohormone, vitamin D, into its hormonally active daughter metabolite(s) via a two step process involving first the liver which introduces a hydroxyl group on carbon-25 and then the kidney which introduces a second hydroxyl on carbon-1. The molecule vitamin D itself has no intrinsic biological activity. All biological responses attributed to vitamin D are now known to arise only as a consequence of the metabolism of this seco-steroid into its biologically active daughter metabolites, namely, 1α, 25$(OH)_2D_3$ and 24R, 25$(OH)_2D_3$[2].

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Research Interests: Mechanism of action of steroid hormones and in particular, 1,25$(OH)_2$-vitamin D_3; Vitamin D structure-function relationships.

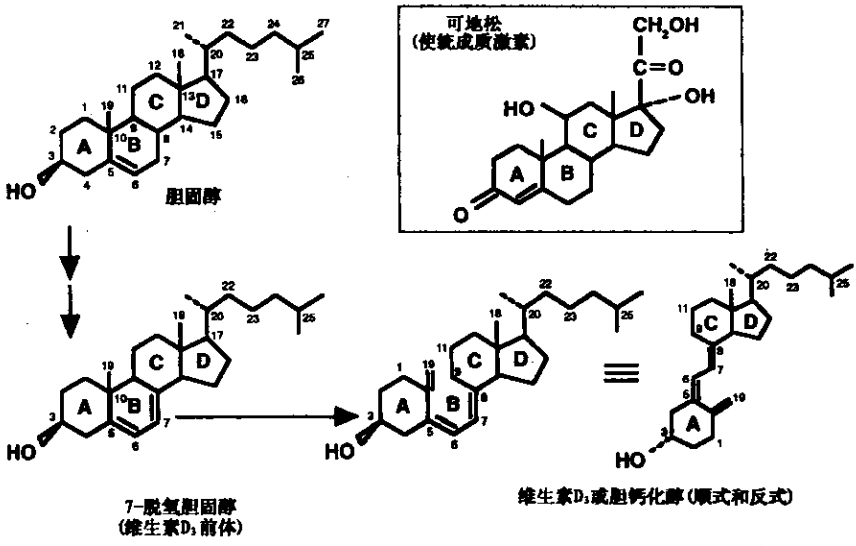

Figure 1: Structural relationship of vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol) with their respective provitamins, 7-dehydrocholesterol and ergosterol. The two structural representations presented at the bottom for both vitamin D_3 and vitamin D_2 are equialent; thse are simply different ways of drawing the same molecule.

Figure 2 presents a summary of the vitamin D endocrine system. A detailed discussion of the vitamin D endocrine system is presented in reference[2]. There are four key components of the vitamin D endocrine system: (a) the parent vitamin D_3 (upper left corner) which is the precursor of the hormone form of vitamin D; (b) the kidney which is the endocrine gland which produce the two steroid hormones $1\alpha,25(OH)_2D_3$ and 24R, $25(OH)_2D_3$; (c) the vitamin D binding protein (DBP) which transports the hormone $1\alpha,25(OH)_2D_3$, vitamin D_3 and other vitamin D metabolites [because of their poor water solubility] through the blood compartment; and (d) the target organs which produces the biological responses that we attribute to vitamin D_3. Thus the DBP delivers the hormone $1\alpha,25(OH)_2D_3$ to its various target tissues. Target tissues, by definition, possess the nuclear receptor for $1\alpha,25(OH)_2D_3$ [known as the vitamin D receptor or VDR]. As a consequence $1\alpha,25(OH)_2D_3$ enters the cell and binds to the VDR in the nucleus of the cell. The VDR + ligand (occupied receptor) then interacts with specific nucleotide sequences on the promoters of genes (vitamin D response elements; VDRE) so that either stimulation or repression of gene transcription occures. This results in the production of more or less messenger RNAs for selective proteins; only those genes which have a VDRE of the are subject to regulation.

The VDR for the hormone $1\alpha,25(OH)_2D_3$ is found not only in the classic target organs (intestine, bone and kidney) but also in at least 32 additional target tissues. Only through the use of modern biochemical and molecular biological techniques, which allowed detection of the VDR in these new locations, was it discovered that the hormone form of vitamin D had far reaching effects in many other tissues (e. g. pancreas, skin, B and T lymphocytes, cancer cells, etc). Also recent results from a number of laboratories indicate that some $1\alpha,25(OH)_2D_3$-mediated biological responses occur too quickly to be explained via the VDR regulation of gene transcription. Thus these rapid responses have been shown to occur within seconds to minutes and have been demonstrated in the intestine, bone osteoblasts, parathyroid cells as well as the pancreas β-cell. The current thinking is that these rapid responses occur as a consequence of $1\alpha,25(OH)_2D_3$ interacting with a different membrane receptor which initiates a different signal transduction system, possibly involving activtion of protein kinase C (PKC) and opening of voltage gated Ca^{2+} channels.

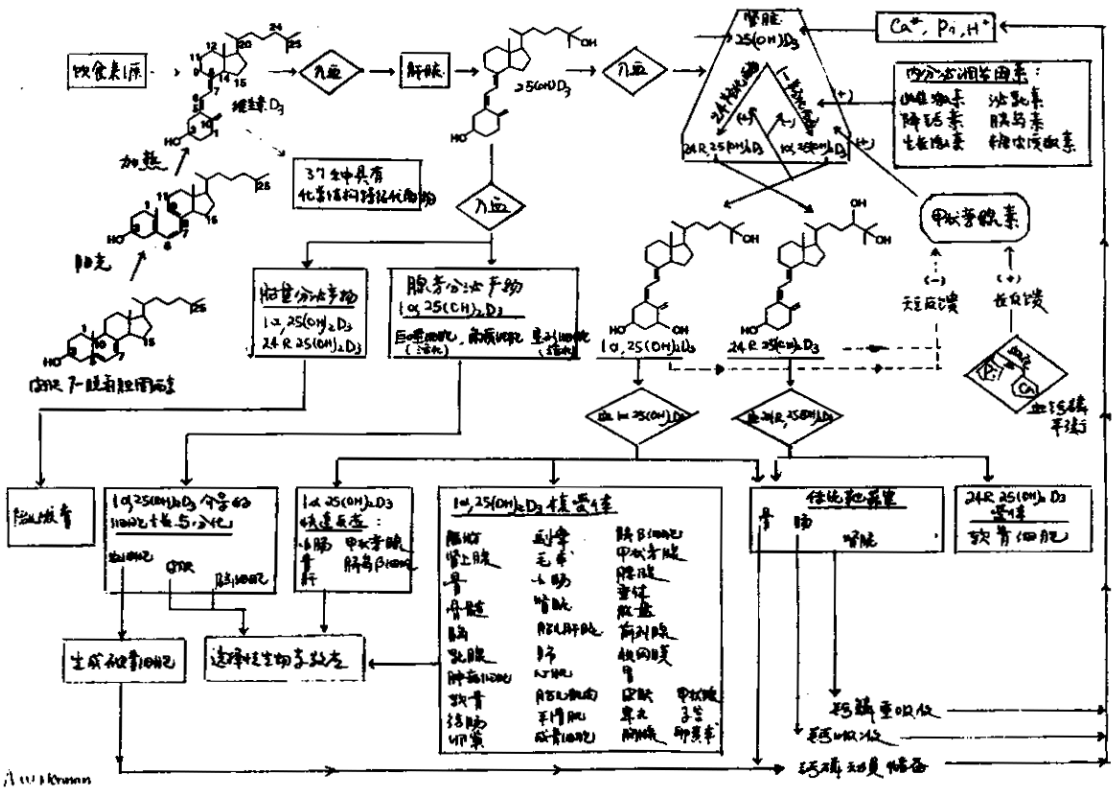

Figure 2 Summary of the vitamin D endocrine system. The key components are the prohormone, vitamin D_3; the kidney functioning as an endocrine gland to produce the two steroid hormones, namely 1α, 25 $(OH)_2D_3$ and 24R, 25$(OH)_2D_3$; and the target organs, both classical (intestine, bone and kidney and the approximately 32 other tissues which possess the nuclear receptor for 1α, 25$(OH)_2D_3$

While interaction of 1α, 25$(OH)_2D_3$ with its VDR is believed to be responsible for most of the important biological responses attributable to the parent vitamin D, there is also evidence that some parent vitamin D responses involve the second renal hormone 24R, 25 $(OH)_2O_3$. Receptors for 24R, 25 $(OH)_2D_3$ have been demonstrated in cartilage cells and in bone fracture-healing callus (2). These new developments are currently under intensive investigation

The steroid hormone 1α, 25 $(OH)_2D_3$ is produced only in accord with strict physiological signals dictated by the calcium "demand" of the organism; a bimodal mode of regulation has been suggested. Thus, under normal physiological circumstances, both renal dihydroxylated metabolites [1α, 25 $(OH)_2D_3$ ad 24R, 25 $(OH)_2D_3$] are secreted and are circulating in the plasma. Parathyroid hormone (PTH) is the major stimulator of the 25 $(OH)D_3$-1-hydroxylase, [the kidney enzyme that produces 1α, 25 $(OH)_2D_3$]. In instances of hypocalcemia PTH will become elevated and stimulate the production of more 1α, 25 $(OH)_2D_3$. Conversely, when serum Ca^{2+} is normal, PTH secretions is diminished, and there is a reduction in the production of 1α, 25$(OH)_2D_3$ and an increase in the production of 24R, 25 $(OH)_2D_3$. There is evidence for a "short feedback loop" for both of these metabolites to modulate and/or reduce the secretion of PTH. There is also some evidence that other endocrine modulators such as estrogens, androgens, growth hormone, prolactin, and insulin may affect the renal production of 1α, 25 $(OH)_2D_3$. Thus, the kidney is clearly an endocrine gland, in the classic sense, which is capable of producing in a physio-

logically regulated manner appropriate amounts of 1α, $25(OH)_2D_3$.

Clinical disorders: Human clinical disorders related to vitamin D can be considered to arise because of one of the following: (a)altered availability of the parent vitamin D; (b)altered conversion of vitamin D to its two principal daugter metabolites, 1α, $25(OH)_2D_3$ and 24R, $25(OH)_2D_3$; (c) conditions that may be due to variations in organ responsiveness to these dihydroxylated metabolites; and (d) perturbations in the integrated interactions of these metabolites with PTH and calcitonin. A wide variety of organs have diseases related to vitamin D: the intestine (malabsorption), the parathyroid gland (hyper-and hypoparathyroidism), the kidney (chronic renal failure), the skin (psoriasis), and the bone (osteomalacia, rickets, and osteoporosis). All of these, in their own way, reflect a disturbance in or a malfunction of the body's normal endocrine processing of vitamin D and its interaction with the other calcemic hormones.

Osteoporosis: Osteoporosis is most simply characterized as a state of insufficiently calcified bone; the poor bone mineralization can frequently result in fractures of the vertebra, hip or arm. Osteoporosis is the most common generalized disorder of bone and affects hundreds of millions of women and men worldwide. Osteoporosis normally begins in middle life and becomes progressively more frequent with advancing age, when it manifests itself with a bone fracture.

As summarized in Table 1, osteoporosis can be classified from a pathophysiological perspective as resulting from: (a)insufficient dietary vitamin D intake or sunlight exposure (designated as vitamin D insufficiency), (b)estrogen deficiency in females which is associated with the menopause [designated as type I]; or (c) the aging process in both women and men which occurs because of changes in calcium homeostasis [designated as type II][3,4].

Table 1 Osteoporosis Classification

Category of Osteoporosis	Description	Site of major fracture(s)
Vitamin D insufficiency	Inappropriately low dietary intake of vitamin D_3 or sun light exposure	Hip and vertebra
Type I	Estrogen deficiency in females (after the menopause	Vertebra and forearm
Type II	Aging process in both males and females (60—90 years of age)	Vertebra and proximal femur

As discussed by Meunier there are clinical differences between being vitamin D deficient versus vitamin D insufficient[4]. The term "deficient" is defined as empty; clinically a person who is vitamin D deplete, will develop rickets (child) or osteomalacia (adult), not osteoporosis. In contrast, the term "insufficient" is defined as lacking in something necessary for completeness; clinically an elderly person who has suboptimal vitamin D nutrition may develop osteoporosis. Practically speaking, vitamin D deficiency versus vitamin D insufficiency is defined according to the blood concentrations of $25(OH)D_3$. Vitamin D deficiency occurs with serum $25(OH)D_3$ levels from 0-10nmol/liter, whereas in vitamin D insufficiency, the plasma $25(OH)D_3$ levels range form 10—50 nmol/liter. In contrast, in the healthy vitamin D replete state, the serum $25(OH)D_3$ levels fall in the range of 50—200 nmol/liter.

Vitamin D Endocrine System and Osteoporosis: Evaluation of the vitamin D endocrine system as presented in Figure 2 and the pathogenesis of osteoporosis as classified in Table 1, and with consideration to the information and concepts presented in references (3—7), it is possible to identify six significant linkages between these two entities (see Table 2). Each of these topics will be briefly discussed.

Table 2 Linkages between osteoporosis and the vitamin D endocrine system

⇒ Vitamin D availability
⇒ $1\alpha,25(OH)_2D_3$ availability
⇒ Receptor for $1\alpha,25(OH_2)D_3$
⇒ Parathyroid hormon
⇒ Intestinal calcium absorption
⇒ Bone remodeling: osteoclasts vs. osteoblasts

It is obvious that the concentration of plasma vi-

tamin D and 25(OH)D_3 will determine the ability to produce the hormone 1α,25$(OH)_2D_3$. When the plasma 25(OH)D_3 level falls below 30nmol/liter, there is a documentable impairment in bone mineral formation[4]. Importantly, several studies have shown that plasma levels of 25(OH)D_3 decrease with age. Thus by age 70 there is frequently a 50% reduction of the age 30 level of 25(OH)D_3 in both men and women[3,4]. In fact 25—50% of the elderly in Europe were found to be vitamin D insufficient and for those elderly persons who do not leave their homes (no sunlight) 75% were vitamin D insufficient.

There are four possible mechanisms to describe the age-related decrease in plasma 25(OH)D_3 levels: (a)many elderly subjects may not consume the recommended dietary allowances(RDA)for vitamin D_3; (b)elderly individuals may have an impaired intestinal absorption of vitamin D; (c)exposure to sunlight is often reduced in the elderly population than in the young and also the aging process may adversely affect the irradiation process that converts skin 7-dehydrocholesterol into vitamin D_3; and (d) there may be an effect of age so as to increase the metabolic clearance rate of 25(OH)D_3 which would then impair the ability to produce adequate quantities of 1α, 25$(OH)_2D_3$ from a lowered serum 25(OH)D_3 level.

A second linkage between the vitamin D endocrine system and osteoporosis occurs because of the well documented reduction in plasma levels of 1α, 25$(OH)_2D_3$ which occurs particularly after age 65[3]. There are five possible mechanisms to describe the age-related reduction in plasma 1α,25$(OH)_2D_3$ levels. (a) A reduction in the plasma transport protein, DBP, with aging would lower the "free" concentration of 1α, 25$(OH)_2D_3$. However there is no evidence that this occurs in the elderly population. (b) A marked reduction in the serum 25(OH)D_3 levels, as in vitamin D deficiency, would significantly impair the production of 1α, 25$(OH)_2D_3$. However as discussed above, the elderly population is more likely to be vitamin D insufficient than deficient. (c)The onset of the menopause which creates an estrogen deficiency might contribute to the age-related reduction in 1α,25$(OH)_2D_3$ levels, however there is no clear data to support this possibility. (d)The aging process may decrease the levels of the 25(OH)D_3-1-hydroxylase enzyme present in the kidney. Thus this key enzyme could become less responsive to the stimulatory effects of parathyroid hormone(PTH)or growth hormone which would result in a reduction in the amount of 1α,25$(OH)_2D_3$ produced; there is evidence to support this possibility[3]. (e)There may be an effect of aging to increase the metabolic clearance rate of 1α,25$(OH)_2D_3$. This would have the consequence of decreasing the plasma levels of 1α,25$(OH)_2D_3$. While there is evidence of this effect in rats, there is no convincing data in man.

A third connection between the vitamin D endocrine system and osteoporosis relates to the possibility of an age related reduction in the levels of the nuclear receptor for 1α,25$(OH)_2D_3$(VDR)in key target organs such as the intestine or bone. Clear evidence has been published that the intestinal levels of VDR decrease with age[6]. Thus in an elderly person there could be a reduction in the fractional absorption of dietary calcium due to the fall in intestinal levels of the VDR.

Several studies have documented that in the aging process there is a modest increase in the plasma levels of immunoreactive PTH[3]. The consequences of the increased levels of PTH could be either to increase the kidney production of 1α,25$(OH)_2D_3$(through elvent of the 25(OH)D_3-1-hydroxylase enzyme activity) or to stimulate bone calcium resorption directly at the osteoblast-osteoclast level. It is not clear what is the stimulatory signal for the increased PTH levels, but a small reduction in serum calcium with aging is the most likely. Alternatively there could be a change in the set-point for suppression of PTH secretion by serum calcium, which would imply that there would be a higher PTH secretion at any given ionized calcium concentration.

There is clear evidence that the efficiency of intestinal calcium absorption decreases with age[3,4-6]. Also it is known that the primary form of vitamin D which stimulates intestinal calcium absorption is 1α,25$(OH)_2D_3$. There are four possible mechanisms which can explain a decreased calcium absorption with aging. (a) A reduction in serum levels of 1α,25$(OH)_2D_3$ will result in an obligatory reduction in intestinal calcium absorption. (b)There can be an age-related decrease in intestinal responsiveness to 1α,25$(OH)_2D_3$. This could be due to an age-related decrease in the VDR levels.

(c)In the female after the menopause, the absence of estrogen can result in a reduction in the efficiency ICA. Estrogen therapy is known to increase ICA. (d)Reductions in the kidney output of $1\alpha,25(OH)_2D_3$ will reduce intestinal Ca^{2+} absorption.

The final linkage between the vitamin D endocrine system and osteoporosis is changes in the process of bone remodeling. The steady state amount of bone calcium is determined by the balance between osteoclast mediated resorption and the osteoblast mediated bone formation process. In osteoporosis there is evidence that there is a decreased rate of bone formation. Thus over time the amount of calcium in the skeleton will decrease. The rate of bone formation, of course, is linked to the availability of calcium (intestinal absorption) and to the circulating levels of $1\alpha,25(OH)_2D_3$. Because of the presence of the VDR in the osteoblast, it is a target organ for $1\alpha,25(OH)_2D_3$.

Summary: In type I osteoporosis after the menopause the primary stimulus of the vitamin D endocrine system that occurs is the absence of estrogen. Estrogen deficiency is believed to cause a fundamental change in bone metabolism. The resulting loss of bone mineral leads to an increased urinary calcium excretion and mild hypocalcemia. This will lead to a decreased PTH section and results in a lowered serum level of $1\alpha,25(OH)_2D_3$ which has the ultimate consequence of decreasing intestinal calcium absorption. Thus over time, there is an inevitable continued loss of bone mineral that is characteristic of osteoporosis.

In type II osteoporosis, the process of aging results in a decrease in plasma levels of $25(OH)D_3$ as a consequence of reduced sunlight exposure and reduction in skin levels of 7-dehydrocholesterol, and a decrease in the activity of the 1α-hydroxylase activity. Collectively this will result in a decreased production of $1\alpha,25(OH)_2D_3$, a decreased intestinal calcium absorption. and secondary hyperparathyroidism which stimulates the bone loss characteristic of osteoporosis.

Treatment of osteoporosis with vitamin D and/or $1\alpha,25(OH)_2D_3$: The rationale for treatment of osteoporosis in any individual will clearly depend upon the physician's evaluation of the patient to determine what is the nature of the dysfunction, if any, of the vitamin D endocrine system. In the event of nutritional vitamin D deficiency or insufficiency, then use of physiological replacement dosages of vitamin D_3 is the therapy of choice. Small daily doses of 1000 International Units (IU) of vitamin D_3 will easily provide the appropriate dosage level. Torgerson *et al.* have evaluated the cost effectiveness of preventing hip fractures in the elderly population using dietary vitamin D and calcium supplementation[7].

However there is evidence that many patients with type I and type II osteoporosis have an impairment in the metabolism of vitamin D_3 to its hormonal form, $1\alpha,25(OH)_2D_3$. In this circumstance it is appropriate to consider treatment with replacement doses of $1\alpha,25(OH)_2D_3$. Tilyard *et al.* have described a comprehensive study of treatment of postmenopausal women who had experienced a vertebral crush fracture with two daily doses of 0.25 micrograms of $1\alpha,25(OH)_2D_3$ [Rocaltrol][5]. Their conclusion after three years of treatment was that $1\alpha,25(OH)_2D_3$ was effective in preventing the occurrence of additional crush fractures[5].

Reference

1 Norman AW, Vitamin D. chap. 12. In: Ziegler EE, Filer LJ, eds. Present knowledge in nutrution (PKN7), 7th edn. International Life Sciences Institute, 1996; 120-129.

2 Bouillon R, Okamura WH, Norman, AW. Structure-function relationships in the vitamin D endocrine system. Endocr Rev, 1995, 16: 200-257.

3 Eastell R, Riggs BL. Vitamin D and osteoporosis., chap. 44. In: Feldman D, Glorieux FH, Pike JW, eds. Vitamin D. Academic Press, 1997; 695-711.

4 Meunier P. Prevention of hip fractures by correcting calcium and vitamin D insufficiencies in elderly people. Scand J. Rheumatol. 1996, 25: 75-278.

5 Tilyard MW, Spears GFS, Thomson J, et al. Treatment of postmenopausal osteoporosis with calcitriol or calcium. New Engl J Med, 1992, 326: 357-362.

6 Åkesson K, Lau KHW, Baylink DJ. Rationale for active vitamin D analog therapy in senile osteoporosis. Calcif. Tissue Int. 1997, 60: 100-105.

7 Torgerson DJ, Kanis JA. Cost-effectiveness of preventing hip fractures in the elderly population using vitamin D and calcium. Quarterly J. Med. 1995; 88: 135-139.