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ACTUALIZACIONES / *Review*

NORMAL PHYSIOLOGICAL FUNCTIONS IN TWO ANIMAL SPECIES WITH HIGHLY DIFFERENT VITAMIN D STATUS COMPARED TO THAT OF HUMANS

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Abstract

Mole rats live in permanent darkness, in networks of underground tunnels (which extend up to 1 km in the subsoil), excavated with their incisors, in warm and semi-arid areas of South Africa. Mole rats have an unusually impoverished vitamin D_3 status with undetectable and low plasma concentrations of 25-hydroxyvitamin D_3 and 1α,25-dihydroxyvitamin D_3, respectively. They express 25-hydroxylase in the liver and 1-hydroxylase and 24-hydroxylase in their kidneys. The presence of specific receptors (VDR) was confirmed in the intestine, kidney, Harder's glands and skin. In spite of their poor vitamin D_3 status, the apparent fractional intestinal absorption of calcium, magnesium and phosphate was high, always greater than 90%. Oral supplementation with cholecalciferol to mole rats did not improve the efficiency of gastrointestinal absorption of these minerals. Mole ratsdo not display the typical lesion of rickets: hypertrophic and radiolucent growth cartilages. Histological studies reported normal parameters of trabecular and cortical bone quality.

Marmosets (monkeys of the New World)are not hypercalcaemic, eventhough they exhibit much higher levels of 25-hydroxyvitamin D_3, 1α,25-dihydroxyvitamin D_3 and parathyroid hormonethan that of rhesus monkeys and humans. Fed a high vitamin D_3 intake (110 IU/day/100 g of body weight), a fraction of the experimental group was found to display osteomalacic changes in their bones: distinct increases in osteoid surface, relative osteoid volume, and active osteoclastic bone resorption. These findings suggest that some marmosets appears to suffer vitamin D-dependent rickets, type II.

The maximum binding capacity of the VDR or the dissociation constant of VDR-1α,25$(OH)_2D_3$ complex of mole rats and New World monkeys are distinctly different of VDR isolated from human cells. Health status of those species appears to be adaptations to the mutations of their VDR. Though rare, as mutations may occur at any time in any patient, the overall message of this review to clinicians may be: recent clinical studies strongly suggests that the normality of physiological functions might be a better indicator of the health status than the serum levels of vitamin D metabolites.

Key words: vitamin D_3, 25-(OH) vitamin D_3, 1,25-$(OH)_2$ vitamin D_3, 1α-hydroxylase, 25-hydroxylase, VDR, vitamin D_3 receptor.

Resumen

FUNCIONES FISIOLÓGICAS NORMALES EN DOS ESPECIES ANIMALES CON ESTATUS DE VITAMINA D MUY DIFERENTES DEL ACTUAL EN SERES HUMANOS.

Las ratas topo viven en la oscuridad permanente, en redes de túneles subterráneos excavadas con sus incisivos (que se extienden hasta 1 km en el subsuelo), en áreas cálidas y semiáridas de Sudáfrica. Las ratas topo tienen un estatus de vitamina D_3 inusualmente empobrecido con concentraciones plasmáticas indetectables de 25-hidroxivitamina D_3 y bajas de 1α, 25-dihidroxivitamina D_3. Poseen 25-hidroxilasa en el hígado y 1-hidroxilasa y 24-hidroxilasa en sus riñones. La presencia de receptores específicos (VDR) ha sido confirmada en el intestino, el riñón, las glándulas de Harder y la piel. A pesar de su pobre estatus de vitamina D_3,la absorción fraccional intestinal aparente de calcio, magnesio y fosfato fue alta,

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siempre superior al 90%. La suplementación oral con colecalciferol a las ratas topo no mejoró la eficacia de la absorción gastrointestinal de estos minerales. No muestran la lesión típica del raquitismo: cartílagos de crecimiento hipertróficos y radiolúcidos. Varios estudios histológicos confirman los hallazgos radiológicos y se informan parámetros normales de la calidad ósea trabecular y cortical.

Los titíes (monos del Nuevo Mundo) exhiben calcemias normales con niveles más elevados de 25-hidroxivitamina D_3, 1α,25-dihidroxivitamina D_3 y hormona paratiroidea que los monos rhesus y los seres humanos. Un tercio de un grupo de titíes alimentados con una alta ingesta de vitamina D_3 (110 UI/día/100 g de peso corporal) exhibió cambios osteomalácicos en sus huesos: aumento en la superficie osteoide, volumen osteoide y activa reabsorción osteoclástica. Estos hallazgos sugieren que una fracción de la población de titíes padece raquitismo dependiente de vitamina D, tipo II.

Debido a mutaciones ocurridas hace millones de años, las máximas capacidades de ligamiento del VDR o los valores de la constante de disociación del complejo VDR-1α,25(OH)$_2$D$_3$ de las ratas topo o monos del Nuevo Mundo son muy diferentes de los verificables en receptores aislados de células humanas actuales. El mensaje de esta revisión a los médicos clínicos podría ser: varios estudios clínicos recientes indican que la normalidad de las funciones fisiológicas de un paciente es un mejor indicador de su salud que los niveles séricos de los metabolitos de la vitamina D.

Palabra clave: *vitamina D_3, 25-(OH) vitamina D_3, 1,25-(OH)$_2$ vitamina D_3, 1α-hydroxylasa, 25-hydroxylasa, VDR, vitamina D_3 receptor.*

Introduction

The contents of this review support the hypothesis that robust compensatory mechanisms exist that create tolerance for a wide variation in circulating concentrations of 25(OH) vitamin D (25(OH)D_3) and 1α,25(OH)$_2$ vitamin D_3 (1α,25(OH)$_2$D$_3$) across species populations, suggesting the existence of a complex evolutionary relation between sun exposure, skin color and the vitamin D pathway.

The vitamin D–endocrine system is thought to play multiple roles in physiologic pathways, such as bone mineral metabolism and the modulation of immune response. In addition, potential links to several chronic conditions, such as hypertension, cancer, and obesity have been described [1-,5].

Vitamin D is acquired from dietary intake and endogenous synthesis after sun exposure. In the liver, vitamin D_3 is converted to 25(OH)D_3[1], which circulates in both bound and unbound fractions and is the metabolite usually measured to determine vitamin D status. Serum 25(OH)D_3 reflects the combination of exposure to sunlight and diet.

Policy guidance with regard to 25(OH)D_3 concentrations is further complicated by large variation between racial-ethnic groups in many countries in the temperate latitudes and by the dearth of information on the concentrations of unbound (bioavailable) fraction of 25(OH)D_3. Part of 25(OH)D_3 is converted by the kidneys to 1α,25(OH)$_2$D$_3$, the biologically active form of vitamin D that acts as a hormone. In spite of the latter fact, because of its greater complexity and cost, the measurement of plasma concentration of 1α,25(OH)$_2$D$_3$ is reserved to complex cases.

This paper review the characteristics of vitamin D status in two species: mole rats (healthy specimens exhibit an unusually impoverished vitamin D_3 status with undetectable and low plasma concentrations of 25(OH)D_3 and 1α,25(OH)$_2$D$_3$) and New World healthy monkeys (with much higher levels of 25(OH)D_3 and 1α,25(OH)$_2$D$_3$ than those found in humans with normal accepted indicator values for Vitamin D nutritional status). These examples emphasize the fact that normal physiological functions can be observed with "abnormal" 1α,25(OH)$_2$D$_3$ levels, if the dissociation constant of the complex VDR-1α,25(OH)$_2$D$_3$ allows the normal performance of those functions. It is expected that the information contained in this review will increase the awareness toward the importance and the as yet poorly known aspects of vitamin D metabolism.

The case of the mole rats

The mole rats inhabit warm and semi-arid areas of South Africa. They live in networks of underground tunnels excavated with their incisors, which extend up to 1 km in the subsoil. The tunnels have no permanent opening on the surface and develop their own microclimate: warm and humid air, with low levels of oxygen. They are herbivores, feeding on tubers and

Figure 1. Mole rats. A: Cryptomys damarensis, B: Heterocephalus glaber,C: Cryptomys hottentotus).

bulbs. They do not drink, they get all the water from their food, which is also a source of minerals.

Mole rats form eusocial colonies. A eusocial colony consists of a single reproductive pair and offsprings with repressed reproductive capacity. The non-reproductive members of the colony spend their time searching for food and maintaining the tunnel system. The colony has a clearly defined hierarchy, with the dominant breeding male, followed by the breeding female, then the non-breeding males and females. In vertebrates, the only known eusocial mammals are the mole rats *Cryptomys damarensis*[6] *y Cryptomys hottentotus*[7] of the family Bathyergidae, and the hairless or Heterocephalus glaber[8] of the family Heterocephalidae. Due to its confirmed longevity (ca. 30 years), the latter has become a very attractive experimental model for studies on cancer resistance and senescence research[9].

In captivity, naked molerats are fed a vegetarian diet. At the San Diego Zoo[10] naked mole rats are fed a commercial pellet made with water, yams, carrots, corn, leafy greens, and fruit.To absorb more of the nutrients from their food, naked molerats eat their own feces. The vegetarian diet is hard to digest, so their intestinal flora aid with digestion.

Plasma levels of metabolites, enzymes of the biosynthetic pathway and Vitamin D receptor (VDR)

Research on the vitamin D_3 status of mole rats followed the publications on the characteristics of this most interesting experimental model[11]. Mole rats have a particularly impoverished vitamin D_3 status. The averaged plasma levels of $25(OH)D_3$ and $1\alpha,250(OH)_2D_3$[12-14] are: <5 ng/ml (n=68) and 17.3.0±6.1 pg/ml (n=68), respectively.

Buffenstein et al.[14] investigated whether the naked mole rat (H glaber)had the same enzymes present in mammals exposed to UVB radiation. Hepatic 25-hydroxylase and kidney 1-hydroxylase and 24R-hydroxylase were detected. The activity of 1-hydroxylase predominates over that of 24R-hydroxylase. After receiving a supra-physiological vitamin D_3 supplement, the1-hydroxylase activity was repressed and the activity of 24R-hydroxylase increased.The administration of phorbol esters (activators of protein-kinase C) produced similar effects to those caused by vitamin D_3 supplementation. The data confirmed that mole rats can convert cholecalciferol in $1\alpha,25(OH)_2D_3$. In addition, the enzymes 1-hydroxylase and 24R-hydroxylase present in the kidneys of these mammals are independently regulated by intracellular signaling pathways involving the protein kinase C.

According to Pitcher et al.[12] when Damara mole rats were housed in the dark, the ratio of the activities of 1-hydroxylase to 25-hydroxylase in the kidney corresponded with those of a vitamin D_3 deficient animal. When these rats received an oral supplement of vitamin D_3 or when they were exposed to sunlight (Table 1), they increased the plasma concentration of $1\alpha,25\ (OH)_2D_3$ with a proportional decrease ($P<0.05$) in the 1-hydroxylase activity and a resulting decrease ($P<0.05$) in the activity ratio 1-hydroxylase /24R-hydroxylase. Despite these changes, the intestinal mode of calcium uptake, plasma levels of total and ionized calcium and phosphate remained unchanged.

Sergeev et al. (1993)[15] investigated whether the tissues of naked mole rats *(H. glaber)* had the $1\alpha,25(OH)_2D_3$ receptor (VDR) and whether the biological responses mediated by VDR in the intestine and the kidney correspond to those found in similar tissues of other mammals. The presence of VDR was investigated in the intestine, kidney, Harder's glands and skin.

Tabla 1. Metabolites of vitamin D_3 reported by Pitcher et al.[12] in control, vitamin D_3 supplemented and sunlight exposed Damara mole rats.

	Experimental groups		
Metabolites	**Controls n=10**	**Vit D_3†**	**Sunlight exposure ‡**
25(OH)D_3, ng/ml	<5	30±3	<5
1α,25$(OH)_2D_3$, pg/ml	12±2	35±2	25±3

Figures indicate the mean ± standard error.† 2.5 ng Vit D_3/g of food eaten; n=5. ‡15 min/day/3 weeks,n=5.

The isolated VDRs were biochemically characterized by saturation, sucrose density gradient, DNA binding and competitive ligand analysis. In addition, the homologous positive regulation of the VDR in these tissues and the induction of 25-hydroxylase in the kidney were studied as indicators of the biological responses mediated by VDR. Naked mole rats have VDR in the intestine, kidneys and Harder's glands (a retro-orbital, light-responsive neuroendocrine gland with a similar secretion to that of the pineal gland) but not in the skin. The biochemical characterization of the VDR and the biological responses mediated by VDR in the intestine and the kidney correspond to those found in similar tissues of other mammals. The VDR of Harder's gland is present at a lower concentration, but shows a markedly higher affinity and selectivity towards 1α,25 $(OH)_2D_3$ than that of the intestine and kidneys. Supplementation with vitamin D_3 resulted in a positive regulation of VDR in the intestine and kidney and induced renal 24R-hydroxylase, but had no effect on VDR in Harder's glands. These data showed that naked mole rats had VDR in intestine, kidney and Harder's glands; these VDRs differ in their biochemical characteristics.

Intestinal absorption of calcium, magnesium and phosphate

The mole rats lead a strictly subterranean existence and survive with a strictly herbivorous diet. As stated above (Table 1) they have a naturally impoverished vitamin D status with low plasma concentrations of both 25(OH)D_3 and 1α,25$(OH)_2D_3$.

Published studies in *C. damarensis* report that the apparent fractional intestinal absorption of calcium, magnesium and phosphate was high, always greater than 90%[16]. The high positive retention observed for these elements is attributed to their incisor teeth, which grow without interruption throughout their lives. These teeth wear constantly during the excavation of the tunnels and it is speculated that they represent a mineral "sink", which together with the deposition of excess calcium in the skin (which is regularly renovated), contributes to the maintenance of mineral homeostasis.

The absorption of minerals in the gastrointestinal tract was further investigated in *H. Glaber* and *C. damarensis*[17], measuring the relative rates of transport of radioactive markers, the mode of calcium uptake (Ca), the para-cellular movement and the opening of voltage sensitive Ca channels throughout the gastrointestinal tract. The relative absorption ratio of ^{45} Ca respect to the unabsorbable marker (14C-polyethylene glycol) indicated that more than 88% of the Ca in the diet had been absorbed per day. The greatest absorption occurred in the duodenum within 12 hours of intake. The contribution of the large intestine (caecum and proximal and distal colon) to total Ca absorption was small (<11%). There was only a passive uptake of^{45} Ca in the duodenum (ratio serous: mucosa, S:M = 1). Active uptake occurred in the hindgut (S:M> 2). The absorption of the distal intestine seems to play a role secondary in the absorption of calcium.

Effects of oral administration of cholecalciferol on intestinal absorption of calcium and phosphate in mole rats

Oral supplementation with cholecalciferol[18] to naked mole rats did not improve the efficiency of gastrointestinal absorption of these minerals, but exerts indirect effects on mineral metabolism by increasing food intake. This, in turn, results in a concomitant increase in the daily rate of absorption of calcium and phosphate. The urinary excretion of calcium is reduced by half, while the excretion of inorganic phosphate does not change. The mineral balance is positive, without any obvious pathology.

In fact, serum calcium and phosphate concentrations remain strictly regulated regardless of vitamin D_3 status. They concluded that the mineral balance in naked mole rats is not directly influenced by cholecalciferol administration.

Are mole rats "clinically" vitamin D deficient?

The reviewed evidence support the hypothesis that mole rats have adapted to an environment lacking UV radiation and vitamin D_3 intake, and can obtain enough calcium and other minerals for their needs through processes not dependent on Vitamin D_3.

The expression "vitamin D-deficient animals" appears several times in the above quoted reports. Two papers[19,20] however, report that they do not display the typical lesion of rickets: hypertrophic growth cartilages at the end of their long bones (Figure 2A). The report of Henry et al.[18] contain a series of radiographs (Figure 2B) of male and female naked mole rats (Heterocephalus glaber) that do not display the typical lesion of rickets.

In agreement with the radiological images, histological studies confirm the good quality of bone tissue of adult mole rats bone[19,20]. The growth plate at the epiphyses of long bones are evident in a 2-year-old but is completely resorbed in older animals. This is clear evidence of a well-structured remodeled trabecular bone that extends from the metaphysis to the epiphysis. Trabecular bone morphology is sustained in a 24-years-old naked mole rat; it shows maintenance of trabecular connectivity and no trabecular thinning. Very efficient bone remodeling and maintenance of cortical bone quality was observed. This long term remodeling allows older animals to carry out the same activities

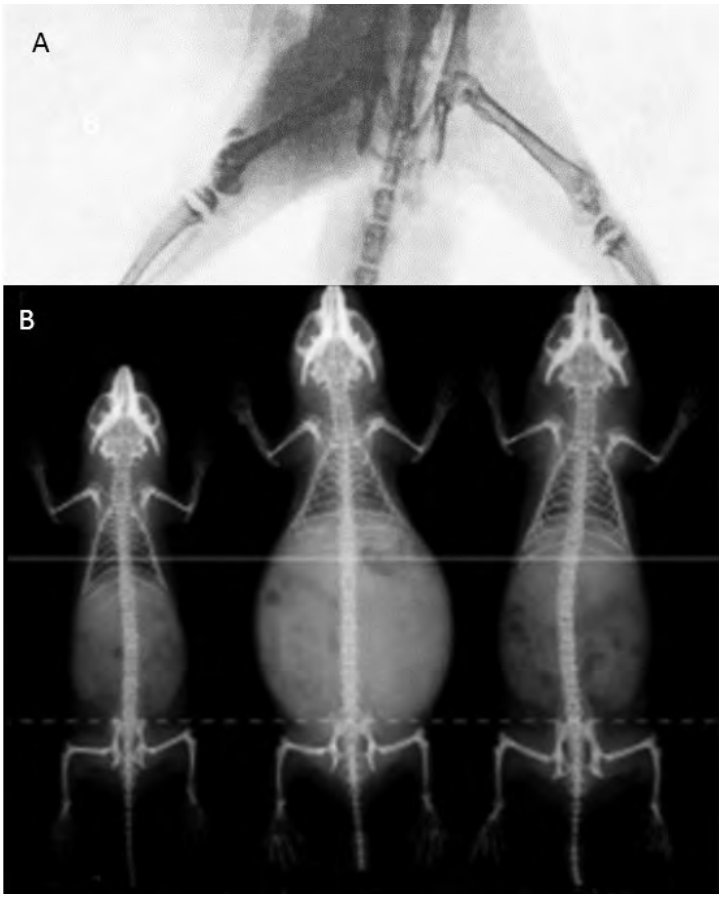

Figure 2. A: Radiograph of hind limbs of a 6 weeks old rat, made rachitic by feeding a rachitogenic diet. B: Radiographs of naked mole rats (Heterocephalus glaber) of both sexes18. Compare with A and note the absence of the typical lesion of rickets.

they did in their youth and may contribute to sustained bone quality in breeding females that continue to reproduce throughout their long lives despite the high mineral demands of both pregnancy and lactation. Cortical bone area and density do not change with age.

The report of Gallagher et al.[21] who treated rachitic rats with 1α,25-$(OH)_2D_3$ gives some probable explanation for the absence of hypertrophic growth plates: "At low levels (1 ng/day) 1α,25-$(OH)_2D_3$ sustained a healing response. Above 5 ng/day administration of 1α,25-$(OH)_2D_3$ resulted in an accumulation of osteoid, giving a histological appearance similar to vitamin D deficiency". The low levels of plasma 1α,25-$(OH)_2D_3$ may explain, paradoxically, the absence of the typical rickets lesions in naked mole rats. The presence of VDR in growth cartilage cells have been reported by Klaus et al.[22]. As referred above, the serum levelsof 25(OH)D_3 were undetectable and those of 1α,25$(OH)_2D_3$ were low. Vitamin D deficiency is an identified condition among high-risk pregnant women[23], though the effects of vitamin D deficiency in human pregnancy are not entirely known [24]. Extrapolating these findings to the mole rats it is evident that if they were clinically vitamin D-deficient, their reproduction might be severely impaired, which is not the case: dominant female breeds continuously through the year, producing approximately every 76 days litters of up to 27 young, with a mean litter size of 9±1[25]. The dominant female could have calvings with 1 to 27 pups. The average was 9.

The physiology of the mole rats points out to two important questions: 1.- The mystery of the synthesis of vitamin D in the obscurity. 2.-How normal physiological functions such as reproduction and breeding are attained with undetectable levels of 25(OH)D_3 and low levels of 1α,25$(OH)_2D_3$. It is likely that the explanation resides in the affinity of 1α,25$(OH)_2D_3$ to the vitamin D receptor (see Table 4).

Synthesis of vitamin D in mammals exposed to UVB irradiation[26]

7-dehydrocholesterol, present in the skin, is a precursor of both cholesterol and vitamin D_3. Normally, one square centimeter of skin contains 25-50 µg of 7-dehydrocholesterol, sufficient to meet the requirements of vitamin D_3. The skin is composed of two main layers: the dermis, formed largely by connective tissue and the epidermis, thinner. The epidermis is 80 to 600 microns thick, it has five cellular strata (from outside to inside): corneal, lucid, granular, spinous and basal. The highest concentrations of 7-dehydrocholesterol in the skin are found in the basal and spinouslayers.

The production of vitamin D_3 will occur mainly at the wavelengths effectively absorbed by 7-dehydrocholesterol. The two most important factors that govern the generation of pre-vitamin D_3 are the amount (intensity) and quality (appropriate wavelength) of the UVB irradiation that receives 7-dehydrocholesterol. During exposure to sunlight, UVB 290-315 nm penetrates the skin and is absorbed by proteins, DNA and RNA, and 7-dehydrocholesterol. When epidermal 7-dehydrocholesterol absorbs solar radiation (Figure 3), the double bonds of the previtamin-D_3 are activated to become vitamin D_3. 7-dehydrocholesterol exists mainly in the plasma membrane of skin cells, associated with the aliphatic chain of fatty acids linked to glycerides.

In this position, the flat and rigid structure of 7-dehydrocholesterol interspersed between the tails of the glycerides is conditioned to become the planar conformer of previtamin-D_3 after exposure to UVB radiation, which is rapidly isomerized to vitamin D_3.

Because vitamin D_3 is thermodynamically

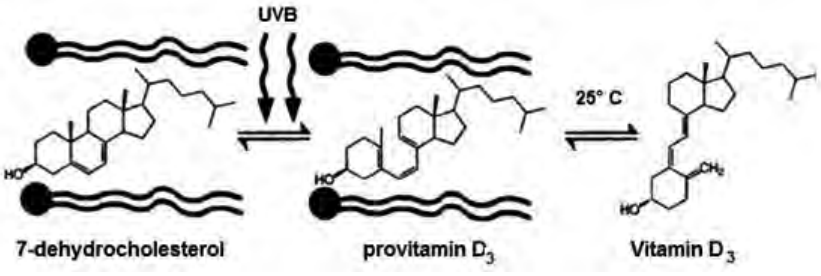

Figure 3. Conversion of 7-dehydrocholesterol to pre-vitamin D3. Redrawn from Wackerand Holick[26].

more stable and also more flexible, it is expelled from the plasma membrane into the extracellular space and diffuses into the capillary bed of the dermis where it binds to the vitamin D_3 transport protein (DBP) for transport to the liver. In approximately 8 hours, the previtamin-D_3 of the skin is converted to vitamin D_3[26,27]. The totality of vitamin D_3 produced in the skin binds to the DBP and remains in the circulation 2-3 times more than the one incorporated by oral intake.

By exposure to sunlight only ca. 15% of 7-dehydrocholesterol can be converted to previtamin-D_3. Any additional exposure will result in the conversion into lumisterol$_3$ and tachysterol$_3$. Therefore, the excessive exposure of a human being to the sun will not produce vitamin D_3 poisoning.

Its biosynthesis has two stages. The first stage occurs in the skin and requires UV irradiation. In a first step 7-dehydrocholesterol is converted into previtamin D_3, a second step is the thermal isomerization to vitamin D_3. In the second stage, the liver transform cholecalciferol into 25(OH)-vitamin D_3, which will become 1α,25-$(OH)_2D_3$ in the kidney (Figure 4).

As shown in Figure 3, Vitamin D in nature can only come from the sunlight-mediated photolysis of 7-dehydrocholesterol to provitamin D. Holick et al.[27] published results of experiments emphasizing the evolutionary importance for the membrane enhancement of the production of vitamin D_3 in the skin of poikilothermic animals: “As vertebrates evolved in the fertile oceans and began to venture onto the earth's surface, they were confronted with a major problem. Whereas the fertile oceans contained a high amount of calcium, thereby satisfying their calcium requirement, the earliest vertebrates on land ventured into an environment that was deficient in calcium. Vitamin D was absolutely essential to enhance the efficiency of the gastrointestinal track to absorb dietary calcium to maintain a structurally sound, mineralized skeleton. However, the first terrestrial vertebrates were cold-blooded (poikilothermic), and therefore, were faced with a problem in making vitamin D in their skin”.

Living in the obscurity, can mole rats produce vitamin D_3 by a non-photochemical pathway? Norman and Norman[28] suggested four possible mechanisms. It should be appreciated that their proposal is based upon an understanding of organic chemistry: there is as yet no evidence to support their existence though they have been shown to occur in other biological systems. Their suggestions may serve as the basis for discussion and study of the synthesis of vitamin D_3 in these animals.

The first example of an enzyme-catalyzed pericyclic process came with the characterization of chorismate mutase [which catalyzes the (3,3)-sigmatropic rearrangement of chorismic acid to prephenate in microorganisms]. Two other mechanisms are described that depend upon the enzymatic epoxidation of 7-dehydrocholesterol. A second enzyme could then catalyze the opening of the epoxides by acid

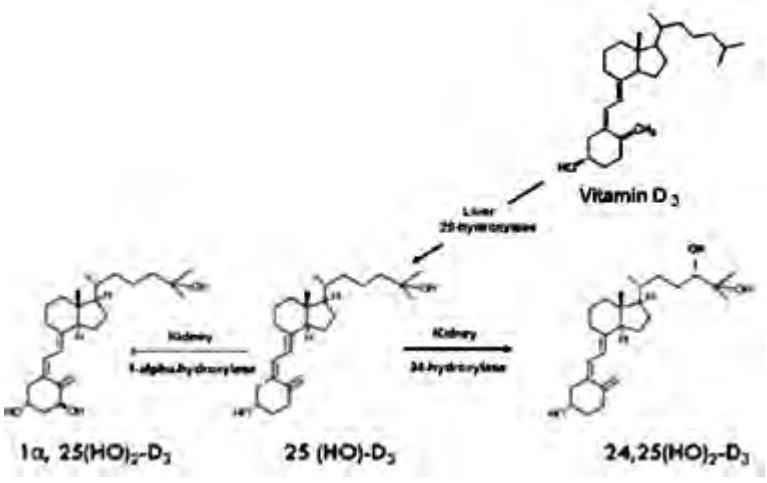

Figure 4. Scheme of the second stage in the synthesis of 1α,25$(HO)_2D_3$ in mammals exposed to sunlight. Redrawn from Wacker and Holick[26].

catalysis. In both mechanisms, the product of the enzymatic process is previtamin D_3, which could then thermally isomerize to vitamin D_3. Taking into account that steroid biosynthesis has been shown to proceed from squalene via a selective enzymatic epoxidation, and acid-catalyzed cyclization. It is appears reasonable to assume that such pathways may have also evolved in animals lacking access to light to catalyze the conversion of 7-dehydrocholesterol to previtamin D_3. Their proposal invoke the binding of previtamin D_3 to enzymes that could replace the effect of UVB radiation. The result of enzyme binding is the formation of a complex in which the attractive forces holding the components together are generally non-covalent and, thus, energetically weaker than covalent bonds. The presence of the necessary enzymes has yet to be demonstrated in the skin of these animals.

Once formed, previtamin D_3, a thermodynamically unstable molecule, undergoes a temperature-dependent isomerization to vitamin D, investigated by Holick et al.[27]. The temperature requirement is satisfied by mole rats. According Roberts [29] these animals do not use automatic metabolic control means to regulate body temperature. Their core temperature is in the range of 29-32°C. Urison and Buffenstein [30] reported that body temperature in early pregnancy was similar to non-pregnant animals. Only at the 8th week of pregnancy the body temperature was 2.5±0.6 °C greater than in non-pregnant mates. Herold et al.[31], through the implantation of an intra-peritoneal radio-telemetry system, reported that the naked mole rat has a distinct body temperature and activity rhythm that is not coupled to environmental conditions. Under their experimental conditions, body temperature ranged from 30 to 32°C. The slow rate of conversion of previtamin D_3 to vitamin D_3 in cold-blooded vertebrates would have had disastrous consequences because the vitamin D_3 formation rate most likely would have been below the rate of previtamin D_3 degradation.

The case of the primates of the New World

A very brief overwiew on the serum levels of $1\alpha,25(OH)_2D_3$ in primates of the New World (first 5 genus in Figure 5) shows the opposite picture reported above for the vitamin D metabolism of mole rats [32]. Please note that the levels of $1\alpha,25(OH)_2D_3$ are shown in a logarithmic scale.

A report by Ziegler et al. [32] (Table 2) confirm the difference in the levels of blood metabolites between New and Old World monkeys and humans, using the most sensitive, specific and reliable method available for measuring vitamin D and its metabolites (liquid chromatography in tandem with mass spectrometry (LC-MS/MS). These methods are superior to the usual assays, which cannot distinguish between vitamin D_2, vitamin D_3 and their hydroxylated forms.

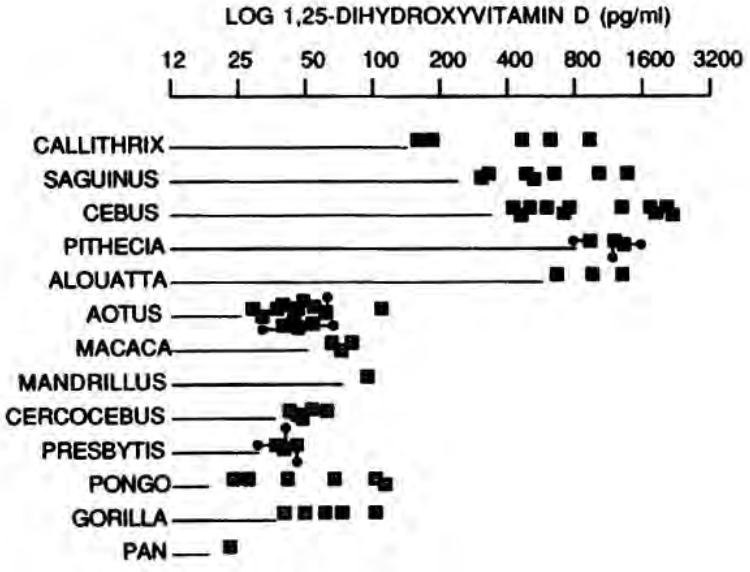

Figure 5.- Serum levels of 1α,25-dihydroxyvitanmin D in primates of theNew World (first five listed genus) and the Old World. Redrawn from Gacadand Adams[33].

As shown in Table 3, compared with the rhesus monkeys, marmosets are not hypercalcemic though exhibit very high levels of 25-hydroxyvitamin D_3, 1α,25$(OH)_2D_3$ and parathyroid hormone. Some histological details of bone tissue are discussed below.

It is known that in higher vertebrates, renal bio-synthesis of 1α,25$(OH)_2D_3$ is tightly regulated by the circulating levels of calcium, phosphorus, parathyroid hormone,estrogens, 1α,25$(OH)_2D_3$ and other peptide and steroid hormones. All the factors involved in the 1α,25$(OH)_2D_3$ increased serum levels in the marmosets have not yet been identified (Takahashi et al. [34]).

Relationship between the biological effects of 1α,25$(OH)_2D_3$ and characteristics of its receptor

1α,25$(OH)_2D_3$ (and many other steroid hormones) generate biological responses both by regulating gene transcription (the classic genomic responses) and by rapidly activating a variety of signal transduction pathways at or near the plasma membrane (rapid or non-genomic responses). The genomic responses to 1α,25$(OH)_2D_3$ result from its stereospecific interaction with its nuclear receptor which binds 1α,25$(OH)_2D_3$ with high affinity constant.

Immunohistochemical and physicochemical characterization of 1α,25$(OH)_2D_3$ receptors. Despite some differences in molecular weight of VDRs, certain domains have conserved structure and function. Antisera against the chick avian protein display extensive cross reactivity with the mammalians forms of the protein[35]. An examination of the recognition patterns of 3 monoclonal antibodies interacting with the avian receptor revealed that only one of them was unique for the chick protein, the other two were equally reactive with receptors from human, primate, porcine, rat and mouse sources[35,36]. In addition, investigated with immunochemical techniques, VDRs appears to be identical in all tissues.

Extensive research has shown that 1α,25$(OH)_2D_3$ receptors sediment in high salt sucrose gradients as molecules of 3.0 to 3.7 S [37,38]. The avian receptor sediments between 3.3-3.7 S whereas mammalian receptors display lower sedimentation coefficients 3.1-3.2 S [39,40]. Once isolated the protein from tissues or cell cultures

Tabla 2. Plasma levels of 25-hydroxyvitamin D_3 and 1α,25-dihydroxyvitamin D_3 measured by LC-MS/MS] in primates of the New and Old World. Ziegler et al. [32]

Species	Individuals	25$(OH)D_3$, ng/ml	1α,25$(OH)_2D_3$,pg/ml
Marmoset	Callithrix jacchus (NWm)	394.8±43.5 (25)	694.8±52.3 (25)
Rhesus	Macaca mulata (OWm)	154.8±5.5 (25)	205.9±18.5 (25)
Cynomolgus	Macaca fascicularis (OWm)	164.8±639 25)	192.8±14.9 (25)
Human	Homo sapiens	57.0±6.6 (14)	55.3±6.1 (20)

Figures indicate the mean ± standard error (N). NWm: New World monkeys. OWm: Old World monkeys

Tabla 3. Serum concentrations of calcium, phosphorus, vitamin D metabolites and parathyroid hormone in Rhesus monkeys and marmosets (Takahashi et al.[34]).

Plasma levels	Rhesus monkeys (n=5)	Marmosets (n=15)
Calcium, mg/dl	9.4±0.3	8.4±0.2†
Phosphorus, mg/dl	4.3±0.5	4.5±0.2†
25$(OH)D_3$, ng/ml	50±4	478±108†
1α,25$(OH)D_3$, pg/ml	93±17	491±93†
Parathyroid hormone ng/ml	0.23±0.06	1.75±0.2‡

Figures indicate the mean ± standard error.
† P<0.05 and ‡ P<0.005 when compared with rhesus monkeys, respectively.

(leukocytes, skin fibroblasts) the following characteristics can be determined. A) the dissociation constant of the complex steroid-receptor 40 (Kd; units: nM, the smaller the dissociation constant value, the higher the affinity between ligand and protein); B) the minimum and maximum ligand binding capacities (βmax, fmoles of 1α,25$(OH)_2D_3$/mg of protein), and C) binding of the complex steroid-receptor to DNA in vitro.

Comparison of the dissociation constant of the complex receptor-1α,25$(OH)_2D_3$ and their maximum ligand binding capacity between the marmosets and mole rats.

Inspection of Table 4 reveals that differences between the dissociation constant of the complex receptor-1α,25$(OH)_2D_3$ and their maximum ligand binding capacity between marmosets and mole rats, which that appears related to the differences in circulating levels of the secosteroid.

The average Kd of marmosets is nearly ten times larger than that for humans (0.24) with a lower maximum binding capacity. In other terms the affinity between 1α,25$(OH)_2D_3$ and the receptor and the maximum binding capacity is lower for marmosets than for humans. This may be an argument to explain the health status of marmosets with a vitamin D status very different with that of humans. The case of mole rats is not easy to evaluate with the available data.

The marmoset is not hypercalcemic in spite of the extremely high circulating levels of 1α,25$(OH)_2D_3$. The resistance to the high levels of 1α,25$(OH)_2D_3$ was investigated by Takahashi et al. [34] concluding that the intestinal epithelium of the marmosets contain a receptor for the hormone which was very similar to that from the rhesus monkeys, but a) it was only one-sixth as abundant in the marmosets as in the rhesus monkeys, b) occupied receptors appear to be a small fraction of the total number of receptors, and c) the activity of the 1a,25$(OH_2D_3$-receptor complex bound to DNA-cellulose was very low.

Yamaguchi et al.[48], compared the bones (X-rays and histological examinations) of common marmosets fed a high vitamin D_3 intake (110 IU/day/100 g of body weight) vs. rhesus monkeys (Old World monkey) fed a diet with normal vitamin D content (5 IU/day/100 g of body weight). Three out of 20 marmosets were found to have osteomalacic changes in their bones distinct increases in osteoid surface, relative osteoid volume, and active osteoclastic bone resorption, whereas non-osteomalacic marmosets had no increase in osteoid tissues in their bones. None of the marmosets, either osteomalacic or non-osteomalacic, was

Tabla 4. Dissociation constants (Kd, nM) and their maximum ligand binding capacity (β_{max}, fmoles/mg of protein) of the binding of 1α,25$(OH)_2D_3$ to its receptor in cells of several species.

References	Species	Tissue	Kd	β_{max}
Liberman et al.[41]	Marmosets	Lymphocytes	2.20	7
Sergeev et al.[15]	Mole rats	Intestinal mucosa	0.75	380
		Kidney	0.18	80
		Harderian glands	0.97	28
		Skin	<0.015	nd
Li et al.[43]	Homo	Skin	0.22	nr
Costa et al.[44]	Homo	Muscle, myoblasts	0.11	nr
		Muscle, myotubes	0.08	nr
Liberman et al.[42]	Homo	Lymphocytes	0.27	45
Norman AW[45]	Homo	Not indicated	≈ 0.5	nr
Megoouh F et al.[46]	Homo	Adenocarcinoma	0.15	10
Eisman et al.[47]	Homo	MCF 7, breast cancer cell line	0.11	nr

Figures indicate the mean ± standard error (N). NWm: New World monkeys. OWm: Old World monkeys

hypercalcemic despite the extremely high circulating levels of 1α,25$(OH)_2D_3$. However, the serum 25$(OH)D_3$ and 24R,25$(OH)_2D_3$ levels were significantly lower in the osteomalacic than in the non-osteomalacic marmosets. These results suggest that the marmoset is likely to exhibit osteomalacic bone changes despite the high daily production of vitamin D_3. These changes resemble those in vitamin D-dependent rickets, type II.

The vitamin D-dependent rickets type II[49], which occurs in humans and in marmosets, points to the importance of the 1α,25$(OH)_2D_3$ receptor in mediating the biologic effects of vitamin D[50]. The disorder is characterized by rickets or osteomalacia, which is present despite marked increases in circulating 1α,25$(OH)_2D_3$ and results from abnormalities in the receptor for 1α,25$(OH)_2D_3$. Studies with cultured skin fibroblasts demonstrated that vitamin D-dependent rickets type II is a heterogeneous disorder. Liberman et al.[42] concluded that in spite of consanguinity, severe resistance to 1α,25$(OH)_2D_3$ resulted from five or six distinct genetic mutations in six kindreds (Table 5).

Closing remarks

Nearly half of the papers quoted in this review, reporting data of the mole rats and New World monkeys, were published before 1998 by investigators with easy access to these animals.

The plasma 1α,25$(OH)_2D_3$ levels of New World monkeys is higher than that of humans and Old World primates. At first sight, this datum suggests end-organ resistance to the hormone. However, the dissociation constant of VDR-1α,25$(OH)_2D_3$ complex or the maximum ligand binding capacity of VDR in mole ratsand New World monkeys are distinctly different from the receptors isolated from cell types obtained from humans (Table 4). Health status (and survival) of these species, then, appears to be due to mutations in the VDR. For New World monkeys, the necessary mutation is presumed to have occurred approximately 60 million years ago[42].

Though rare, as mutations may occur at any time (Table 5), the message of this review to clinicians may be stated as: regarding the health status of a patient, the normality of physiological functions is a better indicator than the levels of vitamin D metabolites. In support of this assertion I would like to call the attention of the reader to a large, well-designed clinical Vida Study designed to evaluate basic health history with focus of cardiovascular conditions and diabetes[50].

The report of Durazo et al.[50] and the literature quoted therein (which includes the NHANES 2003-2005 study) show that robust compensatory mechanisms exist that create tolerance for wide variation in plasma concentrations of 25(OH)D across populations. Concern therefore exists as to whether relatively low concentrations are associated with adverse health effects (see Table 7), a fact that explains the inclusion of the word “challenge” in the title of Durazo et al. paper[50].

Inspection of Table 6 confirms the correlation between latitude (directly related to UV irradiation) and the average plasma concentration of 25(OH)vitamin D_3.

Tabla 5. Summary of the nuclear uptake and cytosol binding of 1α,25$(OH)_2D_3$ by fibroblasts cultured from human skin of normal subjects and six kindred suffering of vitamin D-dependent rickets type II[42].

		Intact Normal fibroblasts		Cytosol of fibroblasts	
		Affinity* nM	**Nuclear uptake, sites per cell**	**Affinity* nM**	**Nuclear uptake, sites per cell**
Normal subjects	n=20	0.5	10300	0.13	8900
Six kindred with rickets resistance type II	n=2	unmeasurable	unmeasurable	unmeasurable	unmeasurable
	n=1	decreased	Decreased	decreased	Decreased
	n=2	unmeasurable	Unmeasurable	≈ normal	≈ normal
	n=1	normal	Normal	normal	Normal

**The method used probably solubilizes receptors from the cytosolic or nuclear compartments of the cell.*

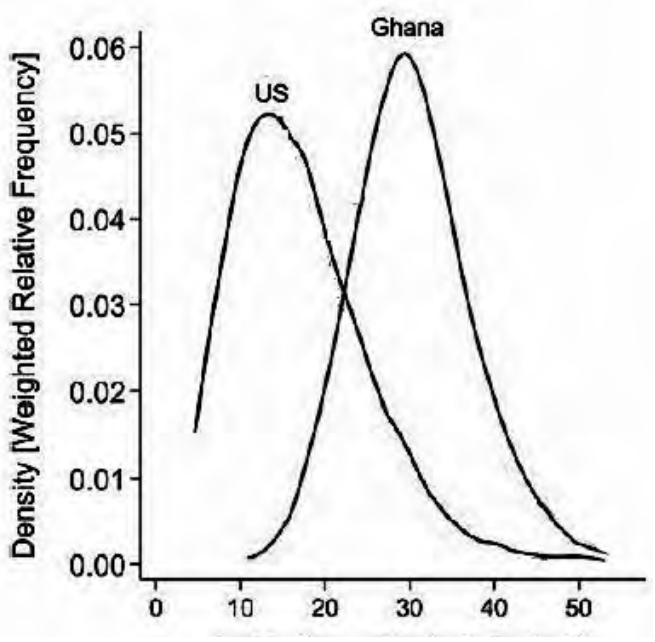

Figure 6. Distribution of plasma values of 25(OH) vitamin$_3$ (expressed as weighted relative frequencies), in subsamples of subjects from Kumasi (Ghana) and Chicago (USA).

Tabla 6. Serum 25(OH)Vitamin D_3 *, Erythema index‡ and Melanin index‡ values in populations of African origin at different latitudes (Durazo et al., 2014)

	SERUM 25(OH)D_3		SKIN COLOUR		
City, country, latitude	**N**	**ng/ml**	**N**	**E-index**	**M-index**
Chicago, USA, 41° N	323	16.5±7.6	235	96.3±10.9	21.0±4.0
Cape Town, South Africa, 34°S	452	23.2±8.1			
Kingston, Jamaica, 17°N	238	28.9±7.1			
Kumasi, Ghana, 6° N	490	29.8±6.8	148	66.6±15.1	19. 4±6.4
Victoria, Seychelles, 4°S	419	29.2±7.8			

**25(OH)Vitamin D_3 values exclude 25(OH)Vitamin D_2 and 3-epimer of 25(OH)Vitamin D_3.*
‡ Measurements of these indexes were performed in the axillary fossa, a sun non exposed area.

Tabla 7. Prevalences of three levels of total plasma 25(OH) vitamin D* in a sample of inhabitants of Chicago (USA) and Kumasi (Ghana). (Durazo et al., 2014).

City	At risk of deficiency,% <12 ng/mL	At risk of inadequacy,% ≥12 ng/mL and <20 ng/mL	Sufficiency,% ≥20 ng/mL
Chicago, USA, n=497	28.8	39.8	31.4
Kumasi, Ghana, n=494	0.2	4.6	95.2

**Total 25(OH)vitamin D is the sum of 25(OH)Vitamin D_3 , 25(OH)Vitamin D_2 and 3-epimer of 25(OH)Vitamin D_3.*

Durazo et al. also measured skin pigmentation from a non-sun-exposed area of the body (the axillary fossa) in a subsample of inhabitants of Kumasi (Ghana) and Chicago (USA). No significant correlation (P>0.05) was observed between the E-index (eryhthema-index) or M-index (melaninindex) measured on the axillary fossa and the plasma concentration of25(OH) vitamin D_3 (Figure 6) suggesting a more complex, and yet undefined, evolutionary relationship between skin color and the vitamin D pathway.

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