INTRODUCTION

The discovery that most tissues and cells in the body have a vitamin D receptor and that several possess the enzymatic machinery to convert the primary circulating form of vitamin D,25-hydroxyvitamin D, to the active form, 1,25-dihydroxyvitamin D, has provided new insights into the function of this vitamin. Of great interest is the role it can play in decreasing the risk of many chronic illnesses, including common cancers, autoimmune diseases, infectious diseases, and cardiovascular disease ***(Holick, 2007)***

Directly or indirectly, 1,25-dihydroxyvitamin D controls more than 200 genes, including genes responsible for the regulation of cellular proliferation, differentiation, apoptosis, and angiogenesis ***(Holick, 2006; Deluca, 2004).***

It decreases cellular proliferation of both normal cells and cancer cells and induces their terminal differentiation ***(Holick et al., 2006).***

Without vitamin D, only 10 to 15% of dietary calcium and about 60% of phosphorus is absorbed. The interaction of 1,25-dihydroxyvitamin D with the vitamin D receptor increases the efficiency of intestinal calcium absorption to 30 to 40% and phosphorus absorption to approximately 80 % ***(Deluca, 2004).***

Vitamin D inadequacy constitutes a largely unrecognized epidemic in many populations worldwide ***(Simonelli et al., 2005)***.

Physical factors that attenuate UV-B exposure, including clothing, sunscreens, and glass shielding, markedly reduce or completely eliminate the production of vitamin D3 in the skin. At latitudes above 37˚N and below 37˚S, sunlight is insufficient to induce cutaneous vitamin D3 synthesis during the winter months ***(Holick, 2004).***

Even in the sunniest areas vitamin D deficiency is common when most of the skin is shielded from the sun. In Saudi Arabia, The United Arab Emirates, Australia, Turkey, India and Lebanon 30-50% of children and young adults had 25-hydroxy vitamin D levels below 20ng/ml ***(Marwaha et al., 2005).***

In Egypt, pilot studies have indicated a 70-80% prevalence of vitamin D deficiency in women in Cairo ***(Matar, 2011)*** and port Saiid ***(El-Daoudy, 2011).***

Aim of the Work

Evaluation of the magnitude of Vitamin D deficiency/ sufficiency in a sample of Healthy Egyptian men between 25-60 years old and correlating that to various work, life style and dietary factors

PHYSIOLOGY OF VITAMIN D

Vitamin D plays a pivotal role in calcium homeostasis and skeletal metabolism throughout life. Classical vitamin D deficiency causes rickets in children and osteomalacia in children and adults ***(Pettifor, 2003).***

Vitamin D is also important for the functioning of many other systems, such as the immune, cardiovascular, and reproductive systems ***(Norman, 2008; Scientific Advisory Committee on Nutrition, 2007).***

Several forms (vitamers) of vitamin D exist. The two major forms are vitamin D_2 or ergocalciferol, and vitamin D_3 or cholecalciferol; vitamin D without a subscript refers to either D_2 or D_3 or both. These are known collectively as calciferol. ***(Dorland's Illustrated Medical Dictionary, 2013)***

Table (1): Forms of vitamin D.

Name	Chemical composition
Vitamin D_1	Molecular compound of ergocalciferol with lumisterol
Vitamin D_2	Ergocalciferol (made from ergosterol)
Vitamin D_3	Cholecalciferol (made from 7-Dehydrocholesterol in the skin).
Vitamin D_4	22-dihydroergocalciferol
Vitamin D_5	Sitocalciferol (made from 7-dehydrositosterol)

(Dorland's Illustrated Medical Dictionary, 2013)

Sources of Vitamin D

1- Food

Very few foods in nature contain vitamin D. The flesh of fatty fish (such as salmon, tuna, and mackerel) and fish liver oils are among the best sources ***(Institute of Medicine, 2010).***

Small amounts of vitamin D are found in beef liver, cheese, and egg yolks. Vitamin D in these foods is primarily in the form of vitamin D_3 and its metabolite 25(OH) D3 ***(Ovesen et al., 2003)***.

Some mushrooms provide vitamin D_2 in variable amounts. Mushrooms with enhanced levels of vitamin D_2 from being exposed to ultraviolet light under controlled conditions are also available. ***(Calvo et al., 2004)***

Fortified foods provide most of the vitamin D in the American diet ***(Instiute of Medicine, 2010).*** For example, almost all of the U.S. milk supply is voluntarily fortified with 100 IU/cup ***(Institute of Medicine,2010***)

2- Sun exposure

Most people meet at least some of their vitamin D needs through exposure to sunlight ***(Institute of Medicine, 2010) (Cranney, 2007)***.

Ultraviolet (UV) B radiation with a wavelength of 290–320 nanometers penetrates uncovered skin and converts cutaneous 7-dehydrocholesterol to previtamin D_3, which in turn becomes vitamin D_3 ***(Institute of Medicine, 2010)***. Season, time of day, length of day, cloud cover, smog, skin melanin content, and sunscreen are among the factors that affect UV radiation exposure and vitamin D synthesis ***(Institute of Medicine, 2010)***. Perhaps surprisingly, geographic latitude does not consistently predict average serum 25(OH)D levels in a population. Ample opportunities exist to form vitamin D (and store it in the liver and fat) from exposure to sunlight during the spring, summer, and fall months even in the far north latitudes ***(Institute of Medicine, 2010).***

Complete cloud cover reduces UV energy by 50%; shade (including that produced by severe pollution) reduces it by 60% ***(Wharton, 2003).***

UVB radiation does not penetrate glass, so exposure to sunshine indoors through a window does not produce vitamin D ***(Holick, 2005)***.

Sunscreens with a sun protection factor (SPF) of 8 or more appear to block vitamin D-producing UV rays, although in practice people generally do not apply sufficient amounts, cover all sun-exposed skin, or reapply sunscreen regularly ***(Wolpowitz and Gilchrest, 2006).*** Therefore, skin likely

synthesizes some vitamin D even when it is protected by sunscreen as typically applied.

The factors that affect UV radiation exposure and research to date on the amount of sun exposure needed to maintain adequate vitamin D levels make it difficult to provide general guidelines. It has been suggested by some vitamin D researchers, for example, that approximately 5–30 minutes of sun exposure between 10 AM and 3 PM at least twice a week to the face, arms, legs, or back without sunscreen usually lead to sufficient vitamin D synthesis and that the moderate use of commercial tanning beds that emit 2%–6% UVB radiation is also effective ***(Holick, 2007)***.

Individuals with limited sun exposure need to include good sources of vitamin D in their diet or take a supplement to achieve recommended levels of intake.

Despite the importance of the sun for vitamin D synthesis, it is prudent to limit exposure of skin to sunlight ***(Wolpowitz and Gilchrest, 2006)*** and UV radiation from tanning beds ***(International Agency for Research on Cancer, 2006).***

Vitamin D Bioactivation

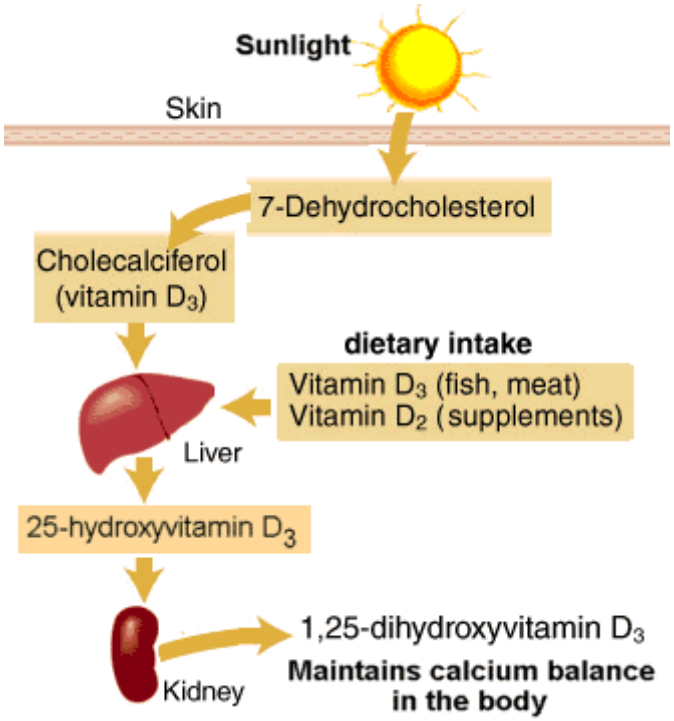

Figure (1): Steps of vitamin D bioactivation ***(Ramos et al., 2010).***

Vitamin D can be obtained from the diet and by the action of sunlight on the skin. Exposure of the skin to the UV rays of sunlight induces the photolytic conversion of 7-dehydrocholesterol to previtamin D_3 followed by thermal isomerization to vitamin D_3 ***(Holick et al., 1977).*** Only a few natural food sources contain significant amounts of vitamins D_2 and D_3, but many foods are now fortified with vitamin D. Nonetheless, vitamin D insufficiency persists in most of the world including North America and Europe due to nutritional

deficit and perhaps to avoidance of sunlight and the use of sunscreens ***(Calvo and Whiting, 2003)***.

1,25(OH)$_2$D$_3$ Metabolism

The high potency of 1,25$(OH)_2D_3$ in elevating serum calcium and phosphate levels requires a mechanism to attenuate its activity. This is accomplished within virtually all target cells by the 1,25 $(OH)_2$ D_3-inducible vitamin D 24-hydroxylase, which catalyzes a series of oxidation reactions at carbons 24 and 23, leading to side chain cleavage and inactivation. Mice lacking a functional 24-hydroxylase gene have high serum 1,25$(OH)_2D_3$ levels due to the decreased capacity to degrade it ***(Messerlian et al., 1997).***

24-Hydroxylase is regulated in a reciprocal manner to 1α-hydroxylase. Its activity and expression are increased by phosphate ***(Tangpricha et al., 2002)*** and reduced by PTH ***(Henry and Norman, 1984).***

Reference Intakes

Intake reference values for vitamin D and other nutrients are provided in the Dietary Reference Intakes (DRIs) developed by the Food and Nutrition Board (FNB) at the Institute of Medicine of The National Academies (formerly National Academy of Sciences) ***(Institute of Medicine, 2010).***

DRI is the general term for a set of reference values used to plan and assess nutrient intakes of healthy people. These values, which vary by age and gender, include:

- Recommended Dietary Allowance (RDA): average daily level of intake sufficient to meet the nutrient requirements of nearly all (97%–98%) healthy people.
- Adequate Intake (AI): established when evidence is insufficient to develop an RDA and is set at a level assumed to ensure nutritional adequacy.
- Tolerable Upper Intake Level (UL): maximum daily intake unlikely to cause adverse health effects.

(Institute of Medicine, 2010)

The FNB established an RDA for vitamin D representing a daily intake that is sufficient to maintain bone health and normal calcium metabolism in healthy people. RDAs for vitamin D are listed in both International Units (IUs) and micrograms (mcg); the biological activity of 40 IU is equal to 1 mcg (Table 2). Even though sunlight may be a major source of vitamin D for some, the vitamins D RDAs are set on the basis of minimal sun exposure ***(Institute of Medicine, 2010)***.

Table (2): Recommended Dietary Allowances (RDAs) for Vitamin D.

Age	Male	Female	Pregnancy	Lactation
0–12 months*	400 IU (10 mcg)	400 IU (10 mcg)		
1–13 years	600 IU (15 mcg)	600 IU (15 mcg)		
14–18 years	600 IU (15 mcg)	600 IU (15 mcg)	600 IU (15 mcg)	600 IU (15 mcg)
19–50 years	600 IU (15 mcg)	600 IU (15 mcg)	600 IU (15 mcg)	600 IU (15 mcg)
51–70 years	600 IU (15 mcg)	600 IU (15 mcg)		
>70 years	800 IU (20 mcg)	800 IU (20 mcg)		

**** Adequate Intake (AI) (Institute of Medicine, 2010)***

Biological actions of vitamin D

Classic Vitamin D-Responsive Tissues

Intestine

Vitamin D is essential to enhance the efficiency of the small intestine to absorb dietary calcium and phosphate. Epithelial calcium transport is stimulated by $1,25(OH)_2D_3$ by induction of ***(Hoenderop et al., 2000).***

1. The apical calcium channel (TRPV6 or TRPV5) that enhances calcium entry

2. The cytosolic calcium binding protein (CaBP; calbindin) that facilitates calcium movement across the cell and
3. The basolateral plasma membrane calcium ATPase (PMCA1) that pumps calcium from the cell.

The initial calcium uptake is the rate-limiting step in intestinal calcium absorption and highly dependent on vitamin D ***(Vesely and Juan, 1984.).***

$1,25(OH)_2D_3$ also increases active phosphate transport through stimulation of the expression of the Na-P_i cotransporter ***(Yamamoto et al., 1999)*** and changes in the composition of the enterocyte plasma membrane that increase fluidity and phosphate uptake. Little is known, however, concerning the molecular mechanisms involved in the extrusion of phosphate across the basolateral membrane into the circulation ***(Kurnik and Hruska, 1985).***

Skeleton

Vitamin D is essential for the development and maintenance of a mineralized skeleton. Vitamin D deficiency results in rickets in young growing children and osteomalacia in adults ***(Amling et al., 1998).***

Furthermore, the $1,25(OH)_2D_3$-VDR system was revealed to be critical for the normal coupling of bone remodeling. Both osteogenesis and osteoclastogenesis were impaired in the $1,25(OH)_2D_3$-VDR-defective mutants(***Panda* et al*., 2001)**.

Defective control of receptor activator of NF-κB ligand (RANKL)-receptor activator of NF-κB (RANK) interactions by an altered $1,25(OH)_2$ D_3-VDR system contributes to abnormal coupling in bone turnover. Osteoblasts express a surface ligand, RANKL, which can bind either RANK or an osteoblasts-derived soluble decoy receptor, OPG. The binding of RANKL to RANK induces a signaling cascade that results in differentiation and maturation of osteoclasts ***(Kitazawa et al., 2003).***

$1,25(OH)_2D_3$ as well as PTH and prostaglandins stimulate RANKL expression ***(Kitazawa et al., 2003),*** but $1,25(OH)_2D_3$ also inhibits OPG production with a corresponding increase in osteoclastogenesis and osteoclast activity. $1,25(OH)_2D_3$ regulates osteoclastogenesis by reciprocal regulation of receptor activator of NF-κB (RANK) ligand (RANKL) and osteoprotegerin (OPG). $1,25(OH)_2D_3$-VDR increases the expression of RANKL on the surfaces of the osteoblast ***(Kondo et al., 2004).***

RANKL interaction with its receptor, RANK, promotes maturation of osteoclast progenitor cells to mature osteoclasts, the bone-resorbing cells. $1,25(OH)_2D_3$-VDR also represses the expression of OPG, a decoy receptor that binds RANKL and prevents RANK-mediated osteoclastogenesis ***(Narayanan et al., 2004).*** Consequently, the $1,25(OH)_2D_3$-VDR system appears necessary for maximal PTH-induced osteoclast production ***(Takemoto et al., 2003).***

Kidney

The most important endocrine effect of $1,25(OH)_2D_3$ in the kidney is a tight control of its own homeostasis through simultaneous suppression of 1α-hydroxylase and stimulation of 24-hydroxylase and very likely through its ability to induce megalin expression in the proximal tubule ***(Liu et al., 1998).***

$1,25(OH)_2D_3$ involvement in the renal handling of calcium and phosphate continues to be controversial due to the simultaneous effects of $1,25(OH)_2D_3$ on serum PTH and on intestinal calcium and phosphate absorption, which affect the filter load of both ions.$1,25(OH)_2D_3$ enhances renal calcium reabsorption and calbindin expression and accelerates PTH-dependent calcium transport in the distal tubule, the main determinant of the final excretion of calcium into the urine and the site with the highest VDR content. The effect of $1,25(OH)_2D_3$ in improving renal absorption of phosphate in the presence of PTH may not be due to a direct action of the sterol on the kidney ***(Kitazawa et al., 2003).***

$1,25(OH)_2D_3$ administration attenuates the development of glomerulosclerosis and the progression of albuminuria through PTH-independent antiproliferative actions ***(Russell et al., 1998).*** $1,25(OH)_2D_3$-induced decreases in podocyte loss and podocyte hypertrophy may also contribute to the less pronounced albuminuria and glomerulosclerosis ***(Kuhlmann et al., 2004).***

Parathyroid glands

PTH stimulates the production of 1,25(OH) $_2$D. In turn 1,25(OH)$_2$ D inhibits the production of PTH ***(Cantley et al., 1985).*** The regulation occurs at the transcriptional level. Within the promoter of the PTH gene is a region that binds the VDR and mediates the suppression of the PTH promoter by 1,25(OH)$_2$D ***(Mackey et al., 1996).***

Calcium alters the ability of 1,25(OH)$_2$ D to regulate PTH gene expression. Calcium is a potent inhibitor of PTH production and secretion, presumably acting through the calcium receptor on the plasma membrane of the parathyroid cell. Low dietary calcium has been shown to increase calreticulin levels in the parathyroid gland ***(Sela et al., 1996),*** the protein calreticulin binds to nuclear hormone receptors including VDR and inhibits their activity ***(Wheeler et al., 1995).***

The ability of 1,25(OH)$_2$ D to inhibit PTH production and secretion has been exploited clinically in that 1,25(OH) $_2$D and several of its analogs are used to prevent and/or treat secondary hyperparathyroidism associated with renal failure ***(Bikle, 2009).***

Nonclassic Vitamin D Actions

Genetic, nutritional, and epidemiological evidence links abnormalities in the vitamin D endocrine system with disorders unrelated to calcium homeostasis, ranging from hypertension