Till few decades ago, vitamin D was thought of only in relation to bone health and calcium homeostasis. Now, medical and nonmedical fraternities across the world are getting increasingly curious and realising the potential role vitamin D plays in health and disease. It stands at the frontline of current scientific endeavors, being a topic of greatest interest to medical researchers all over the globe. A growing body of evidence, implicating hypovitaminosis D as a risk factor for many diseases right from conception throughout lifespan, implies that awareness and management of widespread vitamin D deficiency (VDD) may fetch profound future health benefits. Keeping in mind the research objectives mentioned in previous chapter the review of literature is discussed in reference to the following points:

1. Vitamin-D
    a) Introduction
    b) Metabolism and biological importance in the body
    c) Target organs and action as a hormone in the body
    n) Sources and Recommended Dietary Allowances of vitamin D
    o) Assessment of vitamin-D in serum
2. Vitamin D deficiency (VDD)
    a) Definition & risk factors of VDD
    b) Global prevalence of VDD
    c) VDD in Indian scenario
    d) Causes and consequences of VDD
3. Vitamin D & type-II diabetes mellitus (T2DM)
    a) Introduction of T2DM
    b) Risk factors, consequences and prevalence of T2DM
    c) Vitamin D & T2DM
4. Strategies to combat VDD through vitamin D supplementation
        i. Pharmacological therapy
        ii. Food based approach
        iii. NHE- a tool for lifestyle modification

I. VITAMIN D

A) INTRODUCTION

The discovery of vitamin D and the elimination of rickets as a major medical problem must rank as one of medicine's great achievements (Steenbock, 1924). From the early studies of McCollum and Davis (1913), when the first vitamin was discovered, until 1940, the work leading to the identification of vitamin D and its role in bone formation and prevention of hypocalcemic tetany included many outstanding contributions. Most noteworthy was the work by Sir Edward Mellanby, who demonstrated that rickets could be produced in dogs by feeding them the diet characteristic of Scotland, ie, oatmeal; unknown to Sir Edward Mellanby was the fact that he deprived those dogs of sunlight. Because of the work of McCollum and Davis in discovering fat-soluble vitamin A, Mellanby (1919) attributed the ability of cod liver oil to cure the rachitic condition in dogs as being another property of vitamin A. McCollum et al (1922), very cleverly destroyed the vitamin A activity of cod liver oil by bubbling oxygen through the solution and heating it, but the ability to cure rickets remained in the preparation. McCollum correctly concluded that this represented a new vitamin, called vitamin D. Huldshinsky (1919) and Chick et al (1923) independently demonstrated that rachitic children could be cured with exposure to sunlight or artificially produced ultraviolet light. The puzzle was ultimately solved when Steenbock and Black (1924) discovered that irradiation not only of the skin of animals but also of the food they consumed imparted antirachitic activity to either the animals or their food. Furthermore, Goldblatt and Soames (1923) showed that livers taken from irradiated rats could heal rickets in rats.

Therefore, 2 important discoveries occurred. First, Steenbock and Black (1924) conceived that foods could be irradiated to impart vitamin D and rickets as a major medical problem would disappear. Second, the irradiation of fat-soluble substances extracted from tissues could be used to generate large amounts of vitamin D for later characterization. The most important aspect of vitamin D chemistry centers on its cis-triene structure. This unique structure makes vitamin D and related metabolites susceptible to oxidation, ultraviolet (UV) light-induced conformational changes, heat-induced conformational changes, and attacks by free radicals. The structure of vitamin D_2 was deduced in 1931 by Askew et al (1931), and the structure of vitamin D_3 was determined through synthetic means by Windaus et al (1936). Vitamin D was discovered with many other vitamins and is classed as a vitamin even now.

However, findings from the second half of the 20th century showed that vitamin D is truly a prohormone and not a vitamin.

Vitamin D has been produced by phytoplankton for more than 500 million years (Holick, 1989) and is thought to be the oldest of all hormones whose function initially could have been the protection of ultraviolet-sensitive macromolecules including proteins, DNA and RNA, when these early forms of life were exposed to sunlight for photosynthesis. Later, after the evolution of ocean dwelling animals with vertebral skeletons ventured onto land, the maintenance of calcium homeostasis was a major physiological problem (as opposed to living in the calcium-rich ocean). It was vitamin D that ensured the efficient intestinal calcium absorption from dietary sources and ultimately was essential for the development and maintenance of a calcified mammalian skeleton (Holick, 2011). Obtaining vitamin D from either sunlight or diet is still critical for most vertebrates for their skeletal health (Yoshida, & Stern, 2012; Sai et al., 2011; Lips & Schoor, 2011). Over time, vitamin D has evolved into a hormone having numerous extraskeletal effects by regulating up to estimated 2000 genes (Nagpal & Rathnachalam, 2005; Holick, 2007). Today, the vitamin D receptor (VDR) and the activation enzyme, CYP27B1, have been identified in numerous cell types not involved in calcium and phosphorus homeostasis, suggesting involvement in other body functions.

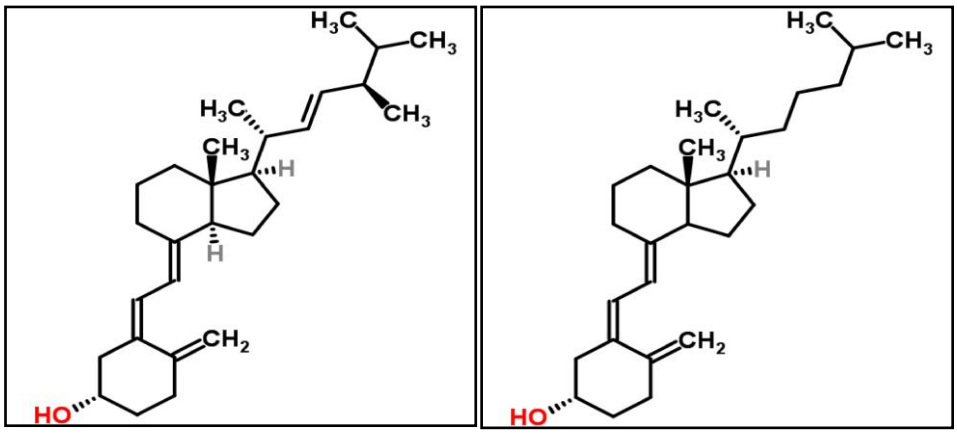
The growing concern of VDD and its relationship with several pathological states has been the recent buzz of the endocrine world, with a few sceptics wondering whether we are overdoing the vitamin D saga. Nevertheless what is obvious is that both the prevalence and incidence of VDD are high and steadily increasing. The human being seems to have eclipsed the sun (sunlight), which is the natural source of vitamin D. Conventionally, the vitamin D endocrine system has been thought to be responsible for musculoskeletal health. It is now recognized that the VDR is ubiquitously present in most tissue types (immune, endocrine [parathormone, beta-cells of pancreas, rennin producing], cardiovascular cells, pulmonary, neural, etc.) that respond to active vitamin D (1,25-dihydroxy (OH)2 vitamin D) exposure (Gupta, 2012).

B) METABOLISM & BIOLOGICAL IMPORTANCE OF VITAMIN D IN THE BODY

An important fact about vitamin D is that, it is required throughout life. It not only is needed for the formation of bone but also likely plays an important role in several other physiologic systems. Vitamin D has two distinct forms: vitamins D_2 and D_3. Vitamin D_2 is a 28-carbon molecule derived from ergosterol (a component of fungal cell membranes), while vitamin D_3 is a 27-carbon derived from cholesterol (Ahmed & Shoker, 2010).Vitamin D is normally produced through robust photolytic process acting on a derivative of cholesterol (ie, 7-dehydrocholesterol) to produce pre-vitamin D, which is then slowly isomerized to vitamin D_3. The chemical structures of these active metabolites are given in Figure 2.1 while Table 2.1 shows the nomenclature for vitamin D precursors and metabolites.

Vitamin D orchestrates (coordinates) the "Ca-vitamin D-Parathyroid hormone endocrine axis". To understand the vitamin D endocrine system one needs to be familiar with the different forms of vitamin D, namely cholecalciferol, calcidiol [25(OH)D], and calcitriol (1,25-OHD). Synthesis of endogenous vitamin D begins in the skin. Exposure of human skin to solar UVB radiation (wavelengths: 290–315 nm) leads to the conversion of 7-dehydrocholesterol to previtamin D_3 in the skin. Previtamin D_3 is then rapidly converted to vitamin D_3 (cholecalciferol) by temperature- and membrane-dependent processes. Excess UVB rays transform previtamin D_3 into biologically inactive metabolites, tachysterol and lumisterol (Wolpowitz & Gilchrest, 2006). Vitamin D_2 and vitamin D_3 from dietary sources is incorporated into chylomicrons, transported by the lymphatic system into the venous circulation (Holick, 2006). Vitamin D (D represents D_2 or D_3) made in the skin or ingested in the diet can be stored in and then released from fat cells. Vitamin D in the circulation is bound to the vitamin D binding protein which transports it to the liver where vitamin D is converted by the vitamin D-25-hydroxylase to 25-hydroxyvitamin D [25(OH)D]. This is the major circulating form of vitamin D that is used by clinicians to measure vitamin D status (Jones, 2007). It is biologically inactive and must be converted in the kidneys by the 25-hydroxyvitamin D-1α-hydroxylase (1-OHase) to its biologically active form 1,25-dihydroxyvitamin D [$1,25(OH)_2D$]. Serum phosphorus, calcium, fibroblast growth factors (FGF-23) and other factors can either increase (+) or decrease (−) the renal production of 1,25(OH)2D. $1,25(OH)_2D$ feedback regulates its own synthesis and decreases the synthesis and secretion of parathyroid hormone (PTH) in the parathyroid glands (Nagpal & Rathnachalam, 2005; Holick, 2007).

FIGURE 2.1: CHEMICAL STRUCTURES OF VITAMIN D_2 AND VITAMIN D_3



Vitamin D2 *(Ergocalciferol)* **Vitamin D3 *(Cholecalciferol)***

TABLE 2.1: NOMENCLATURE OF VITAMIN D PRECURSORS AND METABOLITES

Common Name	Clinical Name	Comments
7-Dehydrocholesterol	Pro-vitamin D_3	Lipid in cell membranes
Cholecalciferol	Pre-vitamin D_3	Photosynthesized in skin or diet
Ergocalciferol	Pre-vitamin D_2	Obtained from diet. Equivalent to vitamin D3 as precursor for active vitamin D
Calcidiol	25-Hydroxyvitamin D Abbreviation: 25[OH]D	Best reflects vitamin D status
Calcitriol	1,25-Dihydroxvitamin D Abbreviation: 1,25[OH]D_2	Active form of vitamin D, tightly regulated

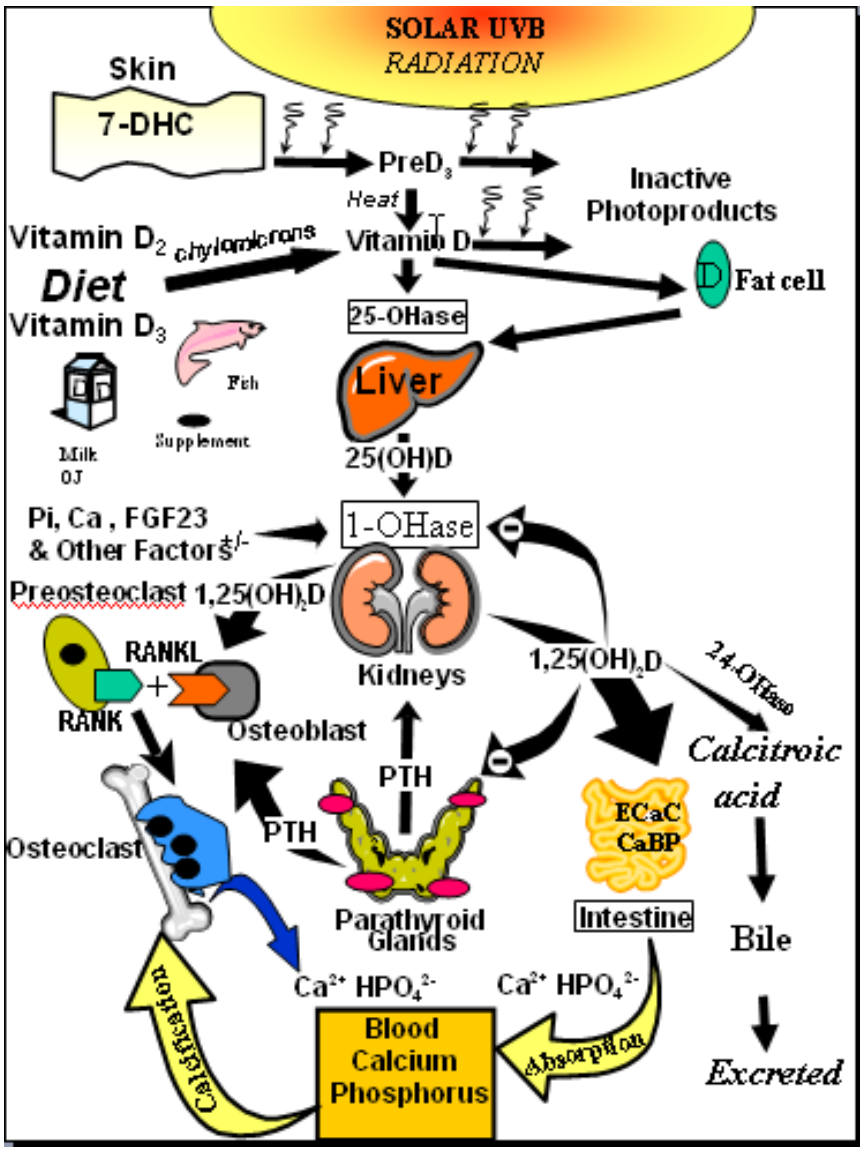
$1,25(OH)_2D$ increases the expression of the 25-hydroxyvitamin D-24-hydroxylase (24-OHase) to catabolize $1,25(OH)_2D$ to the water soluble biologically inactive calcitroic acid which is excreted in the bile (Bosworth et al, 2012). $1,25(OH)_2D$ enhances intestinal calcium absorption in the small intestine by stimulating the expression of the epithelial calcium channel (ECaC) and the calbindin 9K (calcium binding protein; CaBP) (Christakos et al., 2011; Christakos, 2012). $1,25(OH)_2D$ is recognized by its receptor in osteoblasts causing an increase in the expression of receptor activator of NFκB ligand (RANKL). Its receptor RANK on the preosteoclast binds RANKL which induces the preosteoclast to become a mature osteoclast. The mature osteoclast removes calcium and phosphorus from the bone to maintain blood calcium and phosphorus levels. Adequate calcium and phosphorus levels promote the mineralization of the skeleton.

Excess vitamin D formation is prevented from accumulating in the body by the following three processes (Gupta, 2012):

i. On prolonged skin exposure toxic levels of previtamin D_3 are prevented because of conversion to lumisterol and tachysterol, of which lumisterol can be reconverted back to previtamin D_3 should the need arise.
ii. The 24-hydroxylase pathway which breaks down excess vitamin D_2 and D_3.
iii. Negative feedback of $1,25(OH)_2D$ (renal) on the production of 25(OH)D in the liver.

Thus to summarise the vitamin D metabolism, vitamin D obtained from cutaneous synthesis or dietary/supplemental intake, is transported to the fat where it can be stored hydroxylated to 25(OH)D, the major circulating form of vitamin D. 25(OH)D is metabolized in the kidneys by the mitochondrial enzyme 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1) to generate the systemically circulating active form $1,25(OH)_2D$ (Holick et al., 2011; Holick, 2009; Jones, 2007). The renal synthesis of $1,25(OH)_2D$ is regulated by several factors including serum phosphorus, calcium, fibroblast growth factor 23, parathormone and itself. CYP27B1 is also expressed extra renally in a multitude of tissues (Zehnder et al., 2001), including bone, placenta, prostate, keratinocytes, macrophages, T-lymphocytes, dendritic cells, several cancer cells (Lehmann & Meurer, 2010), and the parathyroid gland (Ritter et al., 2012) and enables the production of $1,25(OH)_2D$. $1,25(OH)_2D$ induces its own destruction by rapidly inducing the 25-hydroxyvitamin D-24-hydroxylase (CYP24A1), which leads to the multistep catabolism of both 25(OH)D and $1,25(OH)_2D$ into biologically inactive, water-soluble metabolites including calcitroic acid (Holick, 2007; Bosworth et al, 2012) (Figure 2.2).

FIGURE 2.2: SCHEMATIC REPRESENTATION OF THE SYNTHESIS AND METABOLISM OF VITAMIN D



Source: Holick M.F., 2010

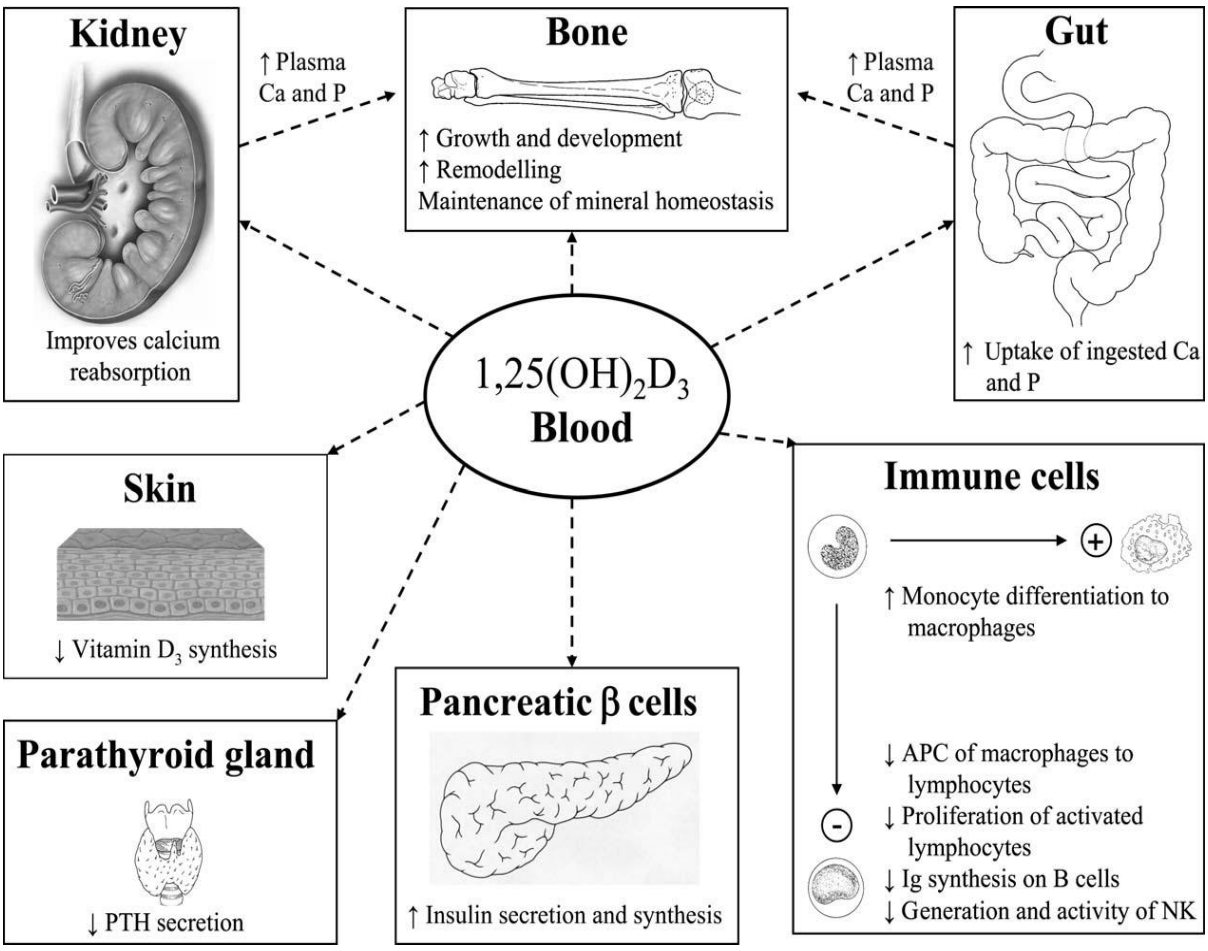
Vitamin D Receptor (VDR)—Distribution and Function

$1,25(OH)_2D$ exerts its effects by binding to a specific nuclear receptor (vitamin D receptor, or VDR), a ligand-dependent transcription factor that belongs to the super-family of steroid-thyroid hormone-retinoid nuclear receptors and that recognizes specific DNA sequences known as vitamin D response elements (DeLuca, 2004). Its widespread distribution across many tissues explains the myriad of physiological actions of vitamin D. By interacting with the VDR, a transcription factor, $1,25(OH)_2D$ regulates directly and indirectly the expression of up to 2000 genes (Nagpal & Rathnachalam, 2005; Holick, 2007), many of whose promoters contain specific vitamin D response elements (VDRE). The VDR partners with other transcription factors, most importantly the retinoid X receptor (RXR) (Carlberg et al, 1993), and coactivators and corepressors provide target gene specificity (McKenna et al., 1999; Smith et al., 2004; Dunlop et al., 2004). A membrane-bound VDR may also exist and mediate more immediate, non-genomic actions of $1,25(OH)_2D$ (Lehmann et al., 2010; Fleet, 1999; Norman, 2006).

The vitamin D hormone functions through a single VDR, which has been cloned for several species including humans, rats, and chickens. It is a member of the class II steroid hormones, being closely related to the retinoic acid receptor and the thyroid hormone receptor. It, like other receptors, has a DNA-binding domain called the C-domain, a ligand-binding domain called the E-domain, and an F-domain, which is one of the activating domains. A single receptor appears to mediate all of the functions of vitamin D, which complicates the preparation of analogs for one specific function rather than another. The human receptor is a 427-amino acid peptide. As mentioned earlier of all of the genes identified to date, the most powerfully regulated is the CYP24 or 24- hydroxylase enzyme, which is responsible for the degradation of vitamin D (Jones et al., 1998).

Scientific research has reported many vitamin D targets, such as heart, stomach, liver, brain, skin, pancreatic islets (β cells), thyroid, parathyroid and adrenal glands and immune cells (Bikle, 1992). Remarkably, some of these tissues and cell types, including brain, activated lymphocytes (T and B cells), macrophages and skin, contain not only the nuclear VDR but also the enzymes required for its synthesis, thereby suggesting alternative non-classical actions (Holick, 2002; Chiu et al., 2004). The major targets and action of vitamin D_3 on peripheral tissues is depicted in Figure 2.3. Vitamin D exerts its actions on target tissues through its binding to the cytosolic / nuclear VDR that functions as a transcriptional activator

FIGURE 2.3: MAJOR TARGETS AND ACTIONS OF VITAMIN D_3 ON PERIPHERAL TISSUES



APC - antigen-presenting capacity; Ig - immunoglobulin; NK- natural killer cells;
PTH - parathyroid hormone

Source: Palomer, González-Clemente, Blanco-Vaca, & Mauricio, 2008

of many genes.

Recent and mounting evidence suggests that this secosteroid hormone plays pleiotropic role influencing numerous bodily processes in addition to calcium metabolism. Vitamin D pleiotropism concept has its origin in two discoveries (Staud, 2005; Holick, 2006). The first is discovery of VDRs in non-osseous tissues. To date VDRs are found in more than 30 tissues including heart, intestine, liver, kidney, lungs, brain, muscle, skin, pancreas and various immune cells. The second is the discovery of enzyme CYP27B1 (capable of converting 25(OH)D into 1,25(OH)2D) in various tissues throughout the body. These findings suggest local autocrine and paracrine role for vitamin D in addition to its role as an endocrine hormone (Vieth, 2004).

The nonskeletal autocrine effects of vitamin D are essentially different from its skeletal effects in that the former operate outside the tight feedback-controlled endocrine loop and are more substrate dependent (Need et al., 1993). This observation gave birth to the concept of maintaining an adequate blood level of vitamin D for regulating its various non-osseous functions. This autocrine pathway of vitamin D, responsible for its nonskeletal effects, has three key features (Staud, 2005)

a) The bulk of the daily metabolic utilization of vitamin D is by way of the peripheral autocrine pathway.
b) Autocrine action always results in expression of the 24-hydroxylase leading to degradation of locally synthesized calcitriol after its action is over, so that no calcitrol which is locally produced enters the circulation.
c) Local concentration of calcitriol required to support various tissue responses are higher than typical serum concentrations of calcitriol. When bound to the vitamin D receptor, calcitriol seems to be just the right key to open up the locked stores of DNA information, allowing cells to produce proteins needed for tissue specific responses. As amount of calcitriol produced locally is substrate dependent, optimal serum level of 25(OH)D is crucial in maintaining ability of the cell to respond to pathological stimuli.

C) SOURCES AND RECOMMENDED DIETARY ALLOWANCES OF VITAMIN D

Humans obtain vitamin D through dietary intake and exposure to sunlight. Thus the main sources of vitamin D are sunlight, supplements and diet. The number of foods naturally containing vitamin D in significant amounts is very limited. Among these are oily fish such as salmon, sardines and tuna, and oils of the liver of some fish such as cod as well as sun-exposed mushrooms (Holick, 2007). Egg yolks are reported to contain vitamin D though the amounts are highly variable. Moreover, the cholesterol content of egg yolks makes it a poor source of vitamin D. Bactrian camel milk is also high in vitamin D, but the content available varies with the species. Vitamin D_2 is found in vegetable sources like sun-exposed yeast and mushrooms also. A list of vitamin D content in different food sources is given in Table 2.2.

To increase the content of vitamin D_2 in mushrooms producers irradiate them with UV radiation (Urbain et al., 2011; Mau et al., 1998). In the 1930s, the fortification of milk, sodas, bread and even beer became popular (Tangpricha et al., 2003); however, after several cases of presumed vitamin D intoxication in infants in the 1950s in Great Britain strict regulations limiting vitamin D fortification to only margarine were introduced in Europe (Holick, 2006; Holick, 2004). Due to a relatively high prevalence of lactose intolerance leading to an avoidance of milk by many adults, the fortification of orange juice in the US was introduced as a novel approach of enhancing the vitamin D status of the public in the 2003 and proved to be as effective as oral supplementation (Tangpricha et al., 2003; Biancuzzo et al., 2010). Other fortified foods include margarine, yogurt, infant formula, butter, cheese and breakfast cereals.

Commercially, vitamin D is available as vitamin D_2 (ergocalciferol) made from plant products and vitamin D_3 (cholecalciferol) made from animal products. Vitamin D_2 and vitamin D_3 are available as oral over-the-counter supplements. In the US, only vitamin D_2 is available as prescription drug (Holick, 2009). Although there has been debate as to whether vitamin D_2 is as effective as vitamin D_3 in maintaining vitamin D status (Armas et al., 2004; Romagnoli et al, 2008; Heaney et al., 2011; Tripkovic et al., 2012), other studies in children and adults have demonstrated that they are equally effective (Biancuzzo et al., 2010; Thacher et al., 2009; Gordon et al., 2008; Rapuri et al., 2004).

Sunlight is the most abundantly available natural source of vitamin D. However the amount of vitamin D production in the skin depends on the incident angle of the sun and thus on

TABLE 2.2: SOURCES OF VITAMIN D_2 AND VITAMIN D_3

Natural sources	
Cod liver oil	~400–1000 IU/tsp vitamin D3
Egg yolk	~20 IU/yolk vitamin D3 or D2
Mackerel, canned	~250 IU/3.5 oz vitamin D3
Salmon, canned	~300–600 IU/3.5 oz vitamin D3
Salmon, fresh farmed	~100–250 IU/3.5 oz vitamin D3, vitamin D2
Sardines, canned	~300 IU/3.5 oz vitamin D3
Shiitake mushrooms, fresh	~100 IU/3.5 oz vitamin D2
Shiitake mushrooms, sun dried	~1600 IU/3.5 oz vitamin D2
Sunlight/UVB radiation	~20,000 IU equivalent to exposure to 1 minimal erythemal dose (MED) in a bathing suit. Thus, exposure of arms and legs to 0.5 MED is equivalent to ingesting ~3000 IU vitamin D3
Tuna, canned	236 IU/3.5 oz vitamin D3
Fortified foods	
Fortified breakfast cereals	~100 IU/serving usually vitamin D3
Fortified butter	56 IU/3.5 oz usually vitamin D3
Fortified cheeses	100 IU/3 oz usually vitamin D3
Fortified margarine	429/3.5 oz usually vitamin D3
Fortified milk	100 IU/8 oz usually vitamin D3
Fortified orange juice	100 IU/8 oz vitamin D3
Fortified yogurts	100 IU/8 oz usually vitamin D3
Infant formulas	100 IU/8 oz vitamin D3
Pharmaceutical Sources in the United States	
Drisdol (vitamin D2)	liquid 8000 IU/mL
Vitamin D2 (Ergocalciferol)	50,000 IU/capsule
Supplemental Sources	
Multivitamin	400, 500, and 1000 IU vitamin D3 or vitamin D2
Vitamin D3	400, 800, 1000, 2000, 5000, 10,000, 14,000, and 50,000 IU

Source: Holick MF, 2007

latitude, season and time of the day. It is highest when the sun is in the zenith and a flattening of the incident angle leads to a reduced vitamin D production (Holick, 2009). Whole body exposure to sunlight with one minimal erythema dose (MED), *i.e.*, the minimal dose leading to pink coloration of the skin 24 h after exposure, leads to vitamin D levels comparable to oral intake of 10,000 to up to 25,000 IU vitamin D_2 (Holick et al., 2011; Holick, 1995). However, sun exposure during most of the winter at latitudes above and below ~33 degrees North and South, respectively, doesn't lead to any production of vitamin D_3 in the skin (Holick et al., 2011; Holick & Chen, 2008). Other factors influencing the cutaneous vitamin D production adversely are an increase in skin pigmentation, aging, especially age >65 years and the topical application of a sunscreen (Holick, 2009).

INDIAN PERSPECTIVE

India is a tropical country, and majority of its states receive ample sunlight almost nine months a year. This maybe the reason why it was never thought that VDD would be prevalent in here. But today epidemiological data clearly shows that this condition does exist in our country. Till date it was thought that sunlight received is enough to fulfil the vitamin D needs of the population, but now there is an urgent need to identify new strategies to overcome the deficiency. To the biggest disappointment, yet India has to arrive on its daily recommended dietary allowance for vitamin D. The situation worsens with no current ongoing vitamin D fortification programmes in the country. Though there are few foods which have been fortified with vitamin D in India, however, there is a need to investigate the effectiveness, cost benefit ratio and stability of the fortificants in them.

Vitamin D fortified milk from Amul® (an Indian dairy cooperative, located in Anand, Gujarat, India) is the only fortified milk product found in the general market. It is 4.5% fat, homogenized milk fortified with calcium 150 mg, vitamin A 75 μg and vitamin D 0.5 μg (20 IU), *etc*., per 100 mL. The expiry date of this milk is 120 days if the carton is unopened. Incidentally, with a 10% or more loss per month at 4 °C, there is not much vitamin D left by 120 days. It may be hoped that storage temperatures are always adhered to. But in India this is a remote possibility due to economical and technical limitations. In a brief survey, most retailers reported that the Amul® milk cartons supplied to them were generally one month past expiry date already at the time of delivery and that the demand for this product was very

low. Cost per liter is INR 64 (as in October 2015) as opposed to the cost of unfortified milk (INR 48).

Gujarat has also taken the initiative for fortification of oil with vitamin A and D. But this project (which is carried out by Gujarat state government and sponsored by ICMR and Ministry of Health) demands impact studies to be carried out to check its effectiveness. Also caution is required for the fortified oil should be packed in dark bottles to cut off UV radiation, and prevent oxidation of vitamin. Similarly, Kellogg's breakfast cereals fortified with vitamin D along with other micronutrients are also available. However, the exorbitant prices of these products are essentially prohibitive for consumption by the common people of India (Ritu & Gupta, 2014).

Though very few foods are fortified with vitamin D in India, there is an array of foods which can act as potential vehicle for fortification. A list of foods which can be considered in India by the food industries and manufacturers is given below-

a) Milk: The whole array of different grades of milk available could be fortified—whole milk, toned, double toned and skim milk.
b) Milk curd and yogurt
c) Infant formulas
d) Butter, *ghee* (clarified butter) and oils, to use as spreads or to spike already cooked food.
e) Soy milk, soy curd (tofu), orange juice and mango juice may be fortified to cater to the needs of the lactose intolerant individuals and those who are allergic to milk proteins. Processed cheese also has very low lactose content and is rich in calcium and may be fortified for the benefit of the lactose intolerant. Due to high prevalence of dyslipidemia, metabolic syndrome and cardiovascular diseases in India, these fortified items will also offer healthier choices to the general population.
f) Widely consumed and affordable staple food items such as *chapati* flour, *maida* (all purpose wheat flour, used to make bread and other bakery products), rice and rice flour may be suitable vehicles for fortification strategies in the Indian scenario.

Vitamin D toxicity

Vitamin D intoxication is extremely rare. Studies showed that doses of more than 50,000 IU per day, which raises 25(OH)D to more than 150 ng/ml, is associated with hypercalcemia and hyperphosphatemia (Holick & Garabedian, 2006; Bouillon, 2001; Holick, 2006). Even doses of 10,000 IU of vitamin D_3 per day for up to 5 months did not cause toxicity (Vieth, 2004). However, patients with chronic granulomatous disorders should be cautious with the dose of vitamin D since macrophage production of $1,25(OH)_2D$ causes hypercalcemia and hyperphosphatemia (Holick & Garabedian, 2006; Bouillon, 2001; Holick, 2006). Early symptoms of vitamin D toxicity include gastrointestinal disorders like anorexia, diarrhea, constipation, nausea, and vomiting. Bone pain, drowsiness, continuous headaches, irregular heartbeat, loss of appetite, muscle and joint pain are other symptoms that are likely to appear within a few days or weeks; frequent urination, especially at night, excessive thirst, weakness, nervousness and itching; kidney stones (Schwalfenberg, 2007).

There are three major hypothesis for vitamin D toxicity:

a) Raised plasma $1,25(OH)_2D$ concentrations lead to increased intracellular $1,24(OH)_2D$ concentrations: This hypothesis is not widely supported as many studies revealed that vitamin D toxicity is associated with normal or marginally elevated $1,25(OH)_2D$ (Shepard & Deluca, 1980).
b) Vitamin D intake raises plasma 25(OH)D levels to concentrations that exceed DBP binding capacity, and free 25(OH)D has direct effects on gene expression once it enters target cells: High dietary vitamin D intake alone increases plasma 25(OH)D. The low affinity of $1,25(OH)_2D$ for the transport protein DBP and its high affinity for VDR dominate normal physiology. However, in vitamin D intoxication, overloading by various vitamin D metabolites significantly compromises the capacity of the DBP by allowing other metabolites to enter the cell nucleus (Jones, 2008).
c) Vitamin D intake raises the concentrations of many vitamin D metabolites, including vitamin D itself and 25(OH)D, and these concentrations exceed the DBP binding capacity and release of "free" $1,25(OH)_2D$ which enters target cells (Jones, 2008).

Safety is always an important consideration when formulating recommendations for nutrient intake. The Food & Nutrition Bureau (1997) evaluated the potential for high intakes of vitamin D to produce adverse effects and set a safe Tolerable Upper Intake Level (UL) of

50µg (2000 IU) for vitamin D_3. Using similar methodology, the European Commission Scientific Committee on Food (2002) also identified a vitamin D_3 UL of 50 µg. Through a less quantitative application of the same method, the United Kingdom Expert Group on Vitamins and Minerals (2003) set a vitaminD_3 UL of 25 µg. Selection of a NOAEL for vitamin D is aided by consideration of how serum 25(OH)D concentrations relate to toxicity. More specifically, given the multiple sources of vitamin D (cutaneous biosynthesis, foods, and supplements), the serum 25(OH)D concentrations at which hypercalcemia occurs must be examined to ascertain how overall status relates to toxicity (Vieth, 1999).

D) ASSESSMENT OF VITAMIN D IN SERUM

Practically vitamin D does not naturally occur in foodstuffs that humans eat. The fact is, from an evolutionary standpoint, humans did not require vitamin D in their food supply because over millions of years humans evolved a photosynthetic mechanism in their skin to produce large amounts of vitamin D_3. The problem now is that humans avoid the sun, wear sunscreen and reside in latitudes that we are not programmed to live. To make matters worse, the dietary requirement for vitamin D in adults is also not met. As a result of these factors, it is now important to define a 'normal' circulating 25(OH)D range using various biomarkers of physiology or disease as opposed to a random population Gaussian distribution.

The first use of biomarkers to define 'normal' 25(OH)D levels, started with parameters that affected skeletal integrity such as parathyroid hormone, bone mineral density and intestinal calcium absorption (Vieth et al., 2003; Heaney et al., 2003; Looker & Mussolino, 2008). These parameters demonstrated that a minimum circulating level of 25(OH)D should be at least 32 ng/ml (80nmol) (Hollis & Wagner, 2005; Hollis, 2005). Presently, the 'normal' circulating 25(OH)D level also relies on data based on the other diverse physiological function of 25(OH)D in many autoimmune diseases. For the present it is generally agreed that a normal level of circulating 25(OH)D is 32–100 ng/ml (80– 250nmol). But it should be noted that 32 ng/ml is not an 'optimum' level but a minimum 'normal' level. What constitutes an 'optimum' level remains to be determined and may well be different for different physiological processes.

The assessment of circulating 25(OH)D is rapidly becoming an important clinical tool in the diagnosis and management of many diverse pathologies. But the question is what is its normal circulating level indicating sufficient levels to meet all physiological needs, not

simply skeletal in humans? To define a 'normal' circulating level of a given substance or nutrient usually blood samples from a diverse population are obtained, the substance in question is measured, the data by Gaussian distribution plotted and normality is determined. This method worked well for nutrients such as folate or vitamin E and was precisely how normative circulating levels of 25(OH)D were defined in humans beginning about 40 years ago by Haddad and Chyu (1971). They sampled a population of 'normal' individuals whom were asymptomatic for disease, assessed circulating 25(OH)D and determined a mean value. During this period the 'normal' 25(OH)D range was 10–80 ng/ml. So basically, if one had a heartbeat, one had a 'normal' circulating 25(OH)D level. Fortunately, for the health of all, a 'normal' circulating 25(OH)D is now defined as 32–100 ng/ml. How and why this dramatic change occurred is discussed further. The assessment of circulating 25(OH)D started with the advent of the competitive protein-binding assay (CPBA), to the present the methods which have progressed to radioimmunoassay (RIA), high-performance liquid chromatography (HPLC) and liquid chromatography coupled with mass spectrometry (LC/MS). The various methods are explained briefly below-

Competitive protein-binding assay (CPBA)

This assay assessed circulating 25(OH)D concentrations using the vitamin D-binding protein (DBP) as a primary binding agent and 3H- 25(OH)D3 as a reporter. Although this CPBA was valid, it was also relatively cumbersome. Technicians had to extract the sample with organic solvent, dry it under nitrogen, and purify it using column chromatography. The major difficulty in measuring 25(OH)D is attributable to the molecule itself. 25(OH)D's lipophilic nature renders it especially vulnerable to the matrix effects of any PBA. Anything present in the sample assay vessel that is not present in the calibrator assay vessel can cause matrix effects. These matrix factors change the ability of the binding agent, antibody or binding protein to associate with 25(OH)D in the sample or standard in an equal fashion. When this occurs, it markedly diminishes the assay's validity.

Radioimmunoassay (RIA)

An antigen was designed that would generate an antibody co-specific for 25(OH)D_2 and 25(OH)D_3. In addition, a simple extraction method was also designed that allowed simple non-chromatographic quantification of circulating 25(OH)D (Hollis & Napoli, 1985). In 1985 Immunonuclear Corporation, now known as DiaSorin, introduced this 3H-based RIA as a kit on a commercial basis. This RIA was further modified in 1993 to incorporate a 125I-labeled

reporter and calibrators (standards) in a serum matrix (Hollis et al., 1993). This modification finally made mass assessment of circulating 25(OH)D possible. In that same year this assay became the first FDA-approved device for the clinical diagnosis of nutritional vitamin D deficiency. This test still remains today the only RIA based assay that provides a 'total' 25(OH)D value.

Random-access automated instrumentation

DiaSorin Corporation, Roche Diagnostics, and the now defunct Nichols Institute Diagnostics all introduced methods for the direct (no extraction) quantitative determination of 25(OH)D in serum or plasma using competitive protein assay chemiluminescence technology. In 2004, the DiaSorin Corporation introduced the fully automated chemiluminescence Liaison 25(OHD Assay System (Ersfeld et al., 2004). This Liaison assay uses an antibody as a primary-binding agent as opposed to the human DBP in the Advantage system. Thus, the Liaison is a true RIA method. The Liaison 25(OH)D assay is co-specific for 25(OH)D_2 and 25(OH)D_3, so it reports a 'total' 25(OH)D concentration. DiaSorin recently introduced a second-generation Liaison 25(OH)D assay. This new version has increased functional sensitivity and much improved assay precision. The Liaison 25(OH)D assay is the single most widely used 25(OH)D assay in the world for clinical diagnosis.

Direct physical detection methods

Direct detection methodologies for determining circulating 25(OH)D include both HPLC and LC/MS procedures (Lensmeyer et al., 2006). The HPLC methods separate and quantitate circulating 25(OH)D_2 and 25(OH)D_3 individually. HPLC followed by UV detection is highly repeatable and, in general, most people consider it the gold standard method. However, these methods are cumbersome and require a relatively large sample as well as an internal standard. Sample throughout is slow and is not suited to a high-demand clinical laboratory processing up to 10,000 25(OH)D assays per day. Researchers have recently revitalized LC/MS as a viable method to assess circulating 25(OH)D (Singh et al., 2006). As with HPLC, LC/MS quantitates 25(OH)D_2 and 25(OH)D_3 separately. When performed properly, LC/MS is a very accurate testing method. However, the equipment is very expensive (Maunsell et al., 2005; Chen et al., 2008; Saenger et al., 2006). One unique problem with LC/MS is its relative inability to discriminate between 25(OH)D_3 and its inactive isomer 3-epi-25(OH)D_3. This problem has been especially noticeable in the circulation of newborn infants (Singh et al.,

2006). Next to the DiaSorin assays, LC/MS is the next most utilized procedure for the clinical assessment of circulating 25(OH)D.

Clinically, however, there is no advantage to this separate measurement claim. Not a single scientific publication exists that demonstrates separate $25(OH)D_2$ and $25(OH)D_3$ measurements are superior to a 'total' 25(OH)D value. The DiaSorin RIA has been used to generate all of the 25(OH)D data from the third National Health and Nutrition Examination Survey (NHANES III). The Harvard-based studies, the Health Professionals' Follow-up Study (HPFS) and the Nurses' Health Study (NHS) have been used to establish much of the information in the last decade with regard to the relationship of circulating 25(OH)D levels and various disease states such as cancer, autoimmune, cardiovascular and renal. All of these studies again utilized DiaSorin based assays (Giovannucci et al., 2006; Mikhak et al., 2007; Forman et al., 2007; Martins et al., 2007; Wang et al., 2008; Giovannucci et al., 2008; Chiu et al., 2004; Chonchol & Scragg, 2007).

Thus testing for VDD should be part of any integrative investigation, both for the prevention of ill health and the comprehensive treatment of many illnesses. But the unique problem of estimating total intake of a substance that can be provided in the diet or made in the skin by exposure to sunlight makes it difficult to estimate adequate total intakes of vitamin D for the general population. Accurate food composition data are not available for vitamin D, accentuating the difficulty for estimating dietary intakes. Skin synthesis is equally difficult to estimate, being affected by such imponderables as age, season, latitude, time of day, skin exposure, sun screen use, etc which are eventually the considered risk factors for poor vitamin D status also (http://www.fao.org/3/a-y2809e.pdf).

Measurements of 25[OH]D *versus* 1,25[OH]2D3

Serum 25(OH)D levels is the best available biomarker for the diagnosis of vitamin D deficiency as compared to $1,25(OH)_2D$ because of the following reason and the detailed comparison is given in Table 2.3.

i) Subtle hypocalcemia causes PTH elevations leading to increased 1-α- hydroxylase activity resulting into normal or elevated in face of vitamin D deficiency

ii) Circulating concentrations of $1,25(OH)_2D$ are 100 to 1000 fold less abundant than 25(OH)D

iii) Half life of $1,25(OH)_2D$ is only 4 hours as against 3 to 4 weeks in case of 25(OH)D

iv) 25(OH)D is the storage form of vitamin D (Rathi & Rathi, 2011).

Table 2.3: Calcidiol *versus* Calcitriol

Metabolite function	$25(OH)D_3$	$1,25(OH)_2D_3$
Nutritional Status	Best indicator	Does not indicate nutritional status
Half life	>15 days	<15 h
Stability in serum	Stable	Unstable
Hypovitaminosis D	Indicative (low)	Non-indicative (normal to elevated)
Hypervitaminosis D	Indicative (elevated)	Non-indicative (low to normal or mild elevated)
Calcium regulation	Possible under non-physiological conditions	Tight under physiological condition
PTH regulation	Depends on vitamin D status	Tight
DBP binding	High affinity (releases the free metabolite once DBP is saturated	Low affinity to exert the physiological function
VDR binding	Strongest among metabolite other than calcitriol	High affinity to elicit the biological function

VDR: vitamin D receptor; DBP: vitamin D binding protein; PTH: parathyroid hormone

Source: Alshahrani & Aljohani, 2013

Interpretation of 25(OH)D levels

Along with accurate assessment of serum 25(OH)D levels, its proper interpretation is equally important. Whenever we interpret 25(OH)D levels it should be done in the background factors mentioned above. The most important of them is the solar zenith angle (SZA), minimal erythemal dose (MED) and skin type, UV index and geographical location of the study along with season, skin pigmentation, atmospheric pollution, time of the day, cloud cover, indoor living, dress code etc. Few concepts are explained below-

- Solar Zenith angle is the angular distance between an object in the sky, such as the sun, and an object directly overhead. The zenith angle varies from the time of the day, season and geographic location.
- UV Index is the calculated prediction of the amount of skin damaging UV radiation that will reach a specific location ($1m^2$) during solar noon hour. It is derived from the combination of five elements namely; Latitude, year of the day, total ozone overhead, elevation above sea level and amount of cloud covers.
- Minimal Erythemal Dose (MED) is the amount of sun exposure which causes barely perceptible skin burn (erythema) appears within 24 hours in previously unexposed skin. Skin type of various races are categorized based on skin color(pigmentation), eye color and hair color, reaction to sun whether it freckles, burns, peels, blisters or tans. There are six skin types. Indians come under the skin type V category.
- The quantum of UV – B rays (290 to 310 nm) received by an individual determines the amount of vitamin D synthesized by the skin. Apart from the zenith angle and the skin type other factors in atmosphere affect the UV – B rays. Most UV-B rays are transmitted from 10 am to 2 pm. Cloud retain 10% of the rays, snow absorbs 20% and reflects the rest, the rays increase by 10% per kilometer above sea level, shades reduces the rays by about 50%, only 10% of the outdoor rays will be experienced indoors, sand reflects 25% of the rays and up to 40% of the rays is present at about half a meter depth of water (Harinarayan & Joshi, 2009).

II. VITAMIN D DEFICIENCY (VDD)

A) DEFINITION AND POPULATION AT RISK OF VDD

VDD in children and adults is a clinical syndrome caused by a low circulating level of 25(OH)D. 25(OH)D is the vitamin D metabolite that is measured to assess a patient's vitamin D status. Before serum measurement of vitamin D metabolites became feasible, VDD was suspected in patients with symptoms of bone pain and muscle weakness and was diagnosed by low serum calcium and phosphate levels and elevated alkaline phosphatase activity (Paterson, 1974). In addition, urine calcium excretion in these patients was low. Clinical signs used in the screening of elderly people were those pointing to proximal muscle weakness, such as standing up from a chair. During the last two decades, measurement of serum 25(OH)D has become a common practice for the assessment of vitamin D status and the detection of its deficiency (Preece et al., 1975).

A provocative study in adults who received 50,000 IU of vitamin D_2 once a week for 8 wk along with calcium supplementation demonstrated a significant reduction in their PTH levels when their initial 25(OH)D was below 20 ng/ml (Malabanan et al., 1998). Several, but not all, studies have reported that PTH levels are inversely associated with 25(OH)D and begin to plateau in adults who have blood levels of 25(OH)D between 30 and 40 ng/ml (Chapuy et al., 1996; Holick et al., 2005; Thomas et al., 1998); these findings are consistent with the threshold for hip and non-vertebral fracture prevention from a meta-analysis of double-blind randomized controlled trials (RCT) with oral vitamin D (Bischoff-Ferrari et al., 2010). When postmenopausal women who had an average blood level of 25(OH)D of 20 ng/ml increased their level to 32 ng/ml, they increased the efficiency of intestinal calcium absorption by 45–65% (Heaney et al., 2003). Thus, based on these and other studies, it has been suggested that VDD be defined as a 25(OH)D below 20 ng/ml, insufficiency as a 25(OH)D of 21–29 ng/ml, and sufficiency as a 25(OH)D of 30–100 ng/ml (Holick, 2007). The IOM report (2011) also concluded, based in part on the PTH data, that VDD was defined as 25(OH)D below 20 ng/ml.

VDD can also be defined according to population- based reference limits for serum 25(OH)D or biological indices, *e.g*., hypocalcemia and elevated alkaline phosphatise or PTH levels (health-based limits). The former will depend on the reference population and country, determined by sunshine exposure and nutrition (Lips, 2001).The most common approach is to

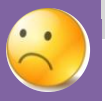
define a sufficient vitamin D state according to reference limits for serum 25(OH)D in healthy adults from the population (*e.g.*, blood donors) sampled throughout the year. However, this depends on climate, sunshine exposure, and clothing habits, leading to large differences between countries.

A functional**,** health based classification could be made using serum PTH. This is easy when serum PTH is increased to above the upper reference limit. However, the increases of serum PTH associated with VDD usually are within the normal reference ranges. In a study in Boston, the seasonal variation of serum PTH was no longer visible when serum 25(OH)D was higher than 90 nmol/liter, leading to the conclusion that serum 25(OH)D should be higher than this level to prevent secondary hyperparathyroidism (Krall et al., 1989). In a French population, serum PTH started to increase when serum 25(OH)D decreased below 78 nmol/liter, leading to a similar conclusion (Chapuy et al., 1997). However, in a large vitamin D study in Amsterdam, the negative relationship between serum PTH and serum 25(OH)D was only significant when serum 25(OH)D was lower than 30 nmol/liter (Ooms et al., 1995). Therefore, different data sets lead to different conclusions. Of course, the differences may partly be due to differences in assays for 25(OH)D.

A parameter of vitamin D insufficiency may be the decrease of serum PTH after vitamin D supplementation. When serum PTH decreases more than 15–20% after vitamin D supplementation, this may point to clinically relevant VDD, leading to bone loss and osteoporosis. In vitamin D supplementation studies, the decrease of serum PTH was 15% in institutionalized elderly (Ooms et al., 1995), and negligible in vitamin D-replete elderly (Himmelstein et al., 1990). When vitamin D and calcium supplementation are combined, serum PTH may decrease up to 50% (Chapuy et al.,1992). Vitamin D supplementation (50,000 IU/wk) with calcium (1,000 mg/d) in 35 elderly patients with a serum 25(OH)D between 25 and 62 nmol/liter decreased serum PTH by 22% (Malabanan et al., 1998). The decrease in serum PTH was significant when baseline serum 25(OH)D was lower than 50 nmol/liter. This is about the serum 25(OH)D level below which serum PTH started to rise in the large study of hospital inpatients (Thomas et al., 1998). Similar conclusions can be drawn from the results in the placebo group of the multicenter raloxifene study consisting of 2,529 women treated with vitamin D (400–600 IU/d) and calcium (500 mg/d). In this study, serum PTH decreased by 12% when baseline serum 25(OH)D was lower than 50 nmol/ liter (Lips et al., 2001).

It may be concluded that it is difficult to delineate sharp diagnostic criteria for vitamin D insufficiency. When the required serum 25(OH)D level is set too high, it will result in a clinically irrelevant diagnosis and unnecessary supplementation. When the required serum 25(OH)D level is set too low, unnecessary bone loss will occur in many patients. The diagnostic cut-offs of levels of serum 25(OH)D to determine the vitamin D status is presented in Table 2.4. A serum 25(OH)D greater than 30ng/mL is known as "sufficiency", levels between 20-30 ng/mL is called the insufficiency stage and lower than 20 ng/mL is called "deficiency". Further the deficiency stage can be defined as "mild VDD" with level 10-20 ng/mL. This is associated with a slightly elevated serum PTH concentration and a mild increase of bone turnover. When serum 25(OH)D is lower than 10 ng/mL, "moderate" VDD is diagnosed. Serum PTH is moderately increased (up to 30%) and high bone turnover is observed. Severe VDD occurs when serum 25(OH)D is lower than 5 ng/mL. In these cases, serum PTH may be increased 30% or more, and a mineralization defect may occur, ultimately leading to frank osteomalacia (Lips, 2001).

TABLE 2.4: DIAGNOSTIC CUT-OFF LEVELS FOR SERUM 25[OH]D

	Stages	Serum 25(OH)D		Serum PTH increase	Bone histology
		nmol/liter	ng/mL		
	**Toxicity**	>250	>100	--	--
	**Sufficiency**	>75	>30	15%	Normal or high turnover
	**Insufficiency**	50- ≤75	20- ≤30	15%	Normal or high turnover
	Mild VDD	25–50	10–20	15%	Normal or high turnover
	**Moderate VDD**	12.5–25	5–10	15–30%	High turnover
	**Severe VDD**	<12.5	<5	> 30%	Mineralization defect Incipient or overt osteomalacia

Adapted from Lips, 2001

According to the Endocrine Society Practice Guidelines a screening for VDD by measuring the 25(OH)D serum level is only recommended for individuals at risk, and not for the general population. The most important risk factors and the major causes are listed below in Figure 2.4. Just as the risk factors and causes of VDD overlap each other, even the signs and symptoms to a large extent are similar. As shown in Figure 2.5, the most common signs and

symptoms are body ache especially back pain, muscle weakness, excess fatigue, sweating specially in the head. They also include depression, heart and kidney disease, Psoriasis-a skin disease, hypertension and obesity. These signs and symptoms are often mistaken as common ailments and ignored and as they are directly or indirectly related to many of the non-communicable diseases, VDD is not suspected as a culprit for their presence.

FIGURE 2.4 RISK FACTORS FOR VITAMIN D DEFICIENCY

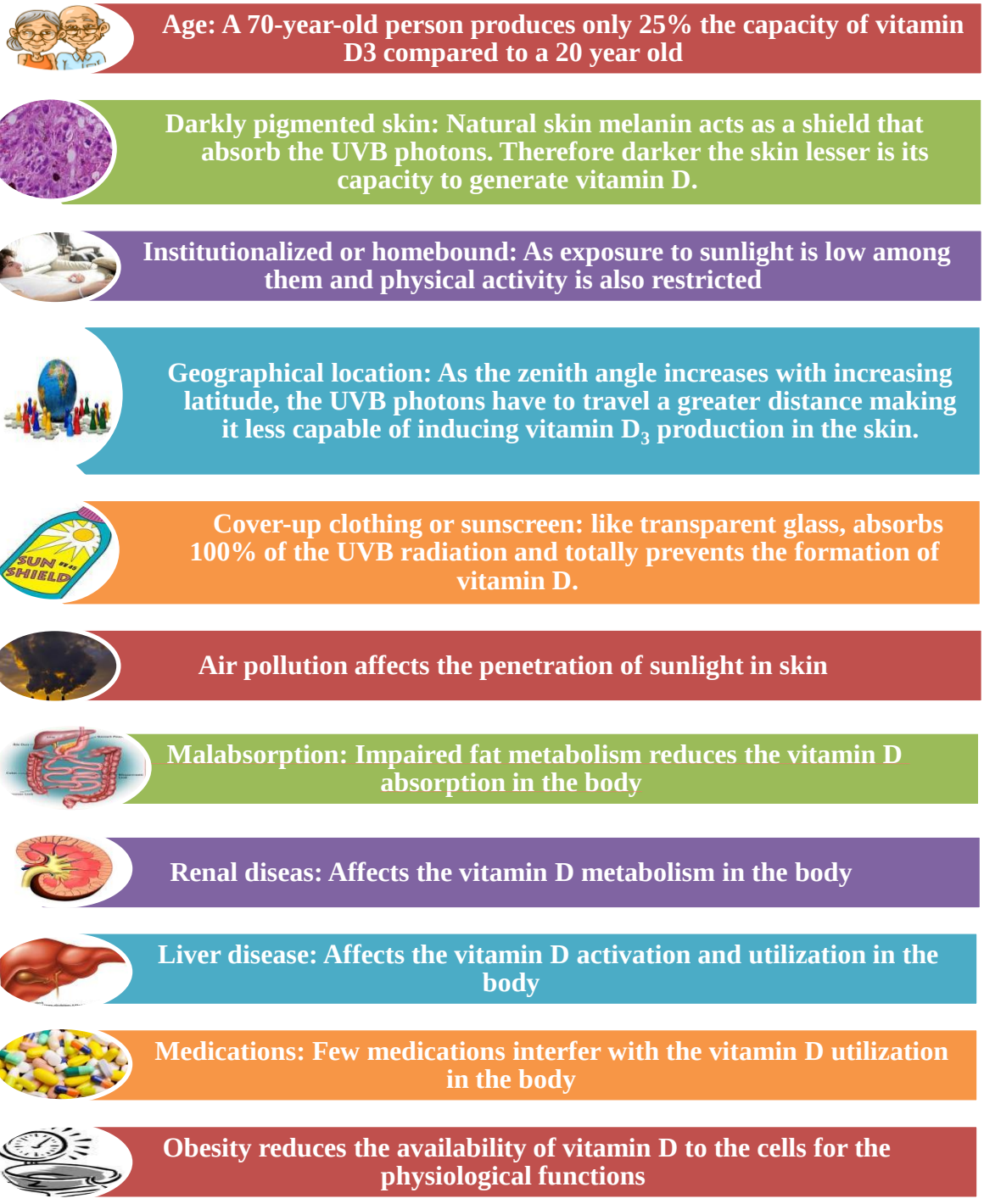
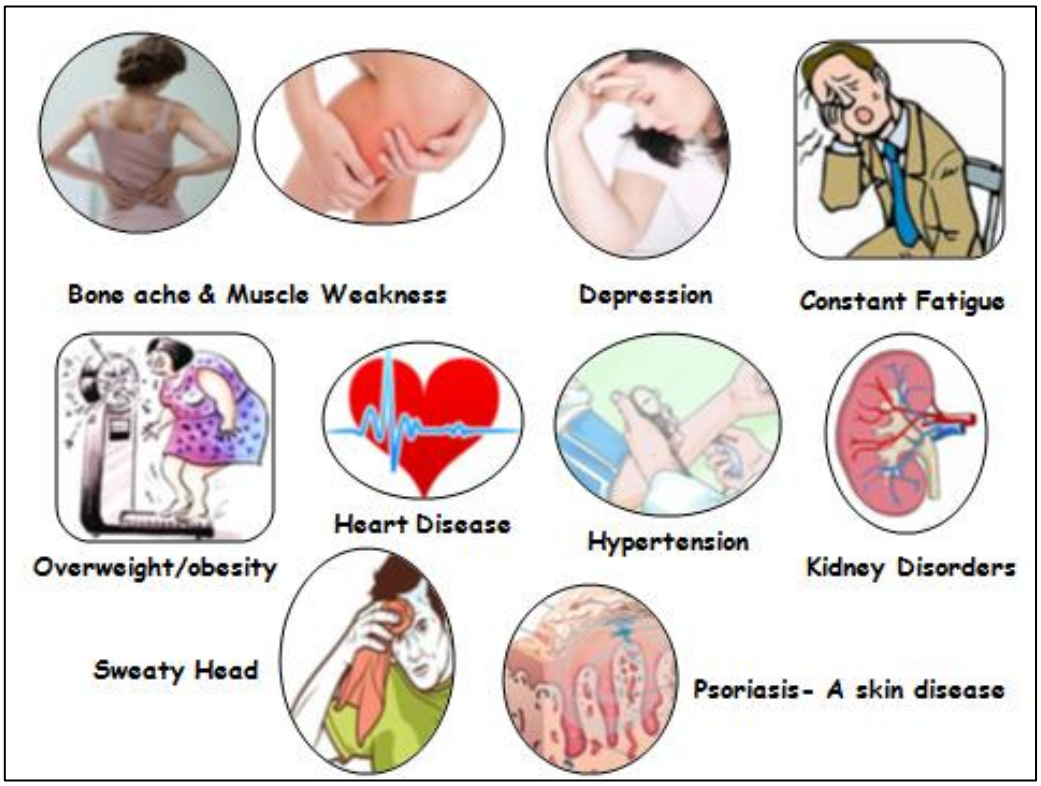


FIGURE 2.5: SIGNS AND SYMPTOMS OF VITAMIN D DEFICIENCY



Source: www.google.com/signsandsymptomsofvitaminDdeficiency

B) PREVALENCE OF VDD- GLOBAL VIEW

Despite the numerous reports of the association of vitamin D with a spectrum of development, disease treatment and health maintenance, VDD is common. Originating in part from the diet but with a key source resulting from transformation by exposure to sunshine, a great deal of the population suffers from vitamin D deficiency. Recent data indicates that VDD is pandemic, even the healthy and the young are not spared. It is universally accepted that the circulating level of 25-hydroxyvitamin D should be used as an indicator of vitamin D status. As discussed earlier VDD is diagnosed when 25(OH)D <20 ng/mL, vitamin D insufficiency is defined as 25(OH)D of 21–29 ng/mL, and 25(OH)D >30 ng/mL is considered sufficient, with 40–60 ng/mL being the preferred range. Vitamin D intoxication usually doesn't occur until 25(OH)D >150 ng/mL (Holick et al., 2011).

According to studies in Canada, 30%–50% of children and adults are vitamin D deficient (Holick et al., 2012). Studies in Indian school children revealed a prevalence of severe VDD (<9 ng/mL) in more than 35% (Marwaha et al., 2005) and over 80% of pregnant women in India had 25(OH)D levels <22.5 ng/mL (Sachan et al., 2005). Also reports from Africa (Prentice et al., 2009), Australia (Mei et al., 2007), Brazil (Maeda et al., 2007), Middle East (Sedrani, 1984; Fuleihan, 2010) Mongolia (Rich-Edwards, 2011) and New Zealand (Rockell et al., 2006) documented a high risk for VDD in both adults and children (Prentice, 2009; Holick et al., 2012). Based on these findings, it has been estimated that 1 billion people worldwide are vitamin D deficient or insufficient (Holick et al., 2012). Reported incidence of VDD defined as a 25(OH)D <20 ng/mL around the globe including Australia, Canada, China, India, Korea, Malaysia, Middle East, Mongolia, New Zealand, North Africa, Northern Europe and United States is depicted in Figure 2.6.

The National Health and Nutrition Examination Surveys 2001–2006 showed a prevalence of vitamin D deficiency of 33% (Holick et al., 2012; Looker et al., 2011). CDC's National Center for Environmental Health used the DiaSorin radioimmunoassay kit (DiaSorin Inc, Stillwater, MN) to provide data on serum 25(OH)D concentrations for the National Health and Nutrition Examination Survey (NHANES) (Hollis, 1996). Prevalence estimates for various age groups are shown in Figure 2.7. Three cut offs for serum 25(OH)D concentrations because of the controversy regarding the definition of vitamin D insufficiency have been used (DeLuca, 2004; Zehnder et al., 2001; Lehmann & Meurer, 2010). National Center for Health Statistics (NCHS) found that 5% of NHANES participants had values

FIGURE 2.6 GLOBAL INCIDENCE OF VITAMIN D DEFICIENCY (Defined as a 25(OH)D <20 ng/mL)

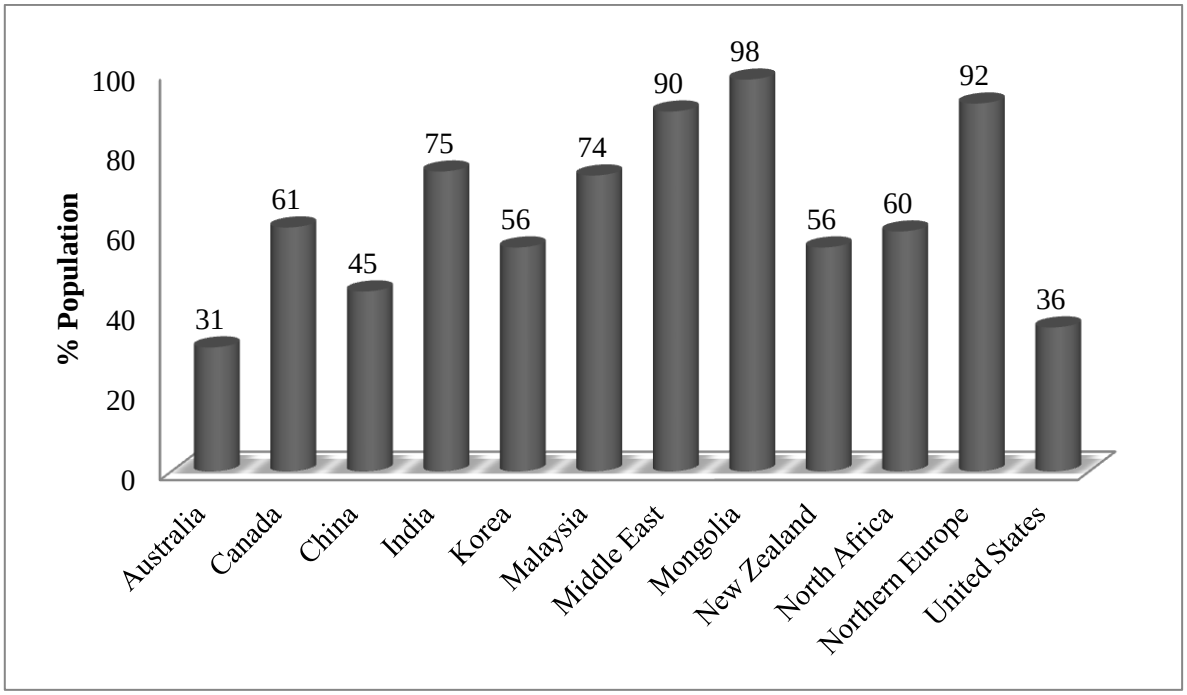
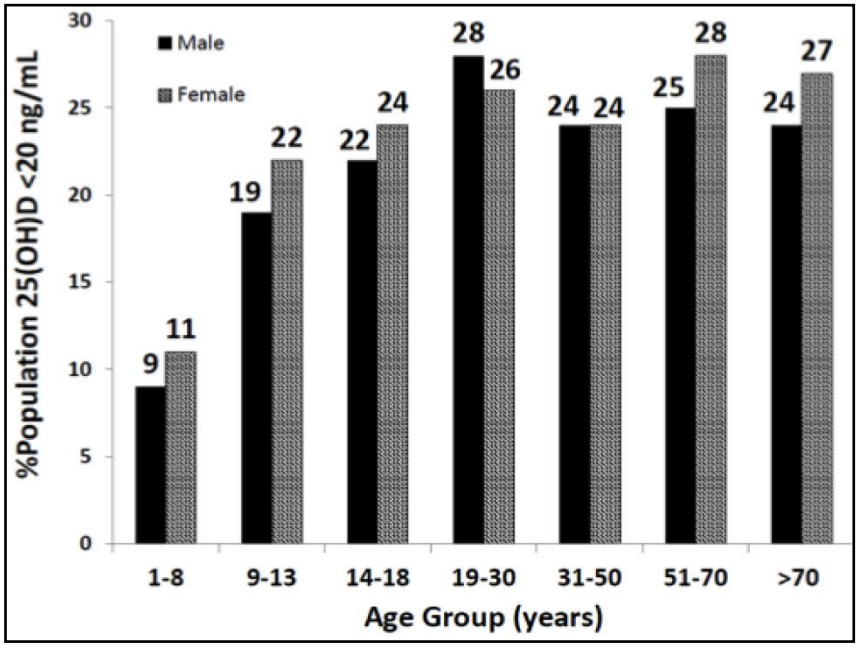


FIGURE 2.7: PREVALENCE AT RISK OF VITAMIN D DEFICIENCY DEFINED AS 25(OH)D <20 ng/mL BY AGE AND GENDER



United States, 2001–2006

Source: Wacker & Holiack, 2013

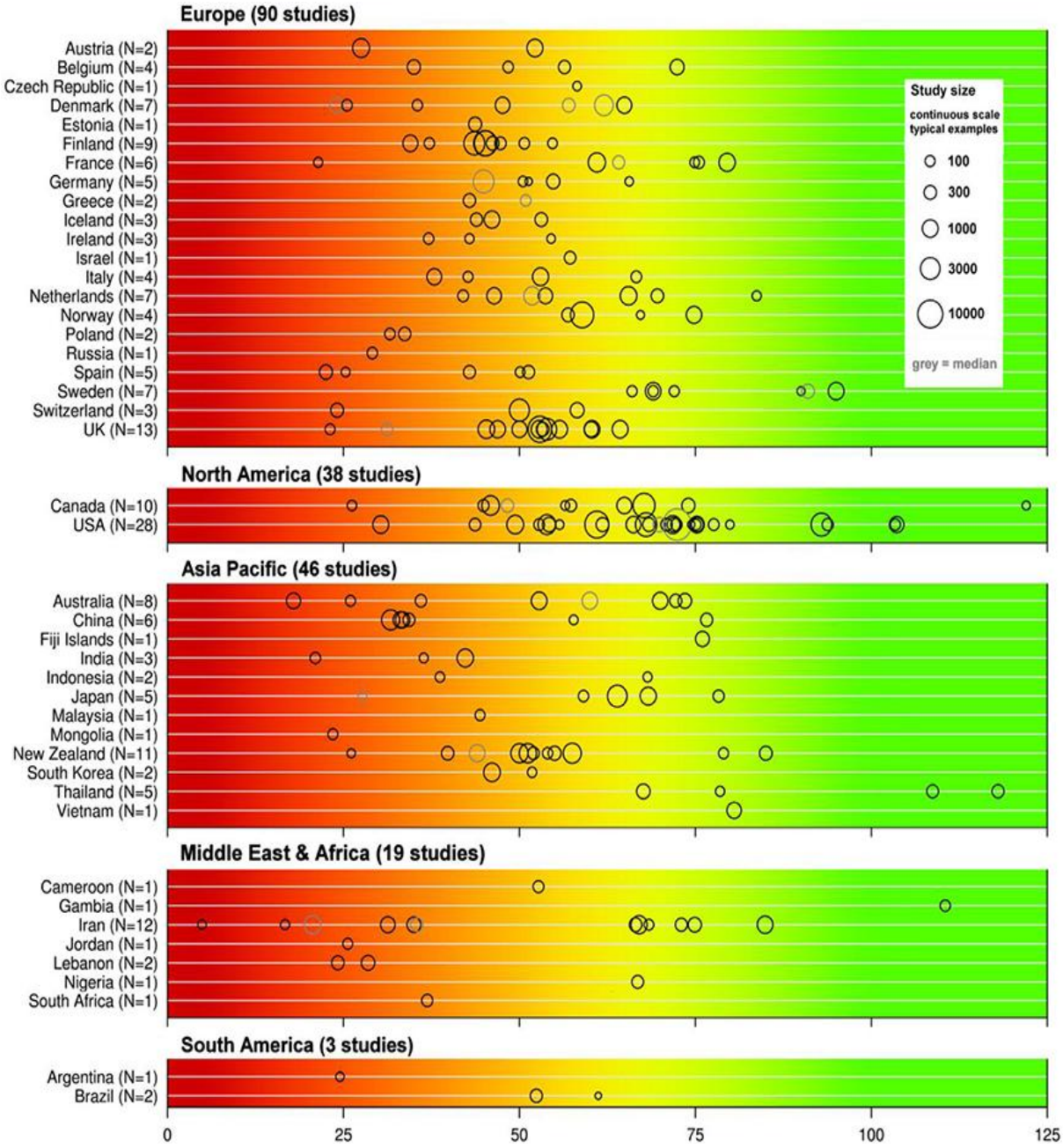
below the traditional cut off of<27.5 nmol/L (11 ng/mL). The prevalence of values<27.5 nmol/L was ≤1%for infants and children aged ≤11 y, 5% for adolescents aged 12–19 y, and 6% for adults aged ≥20 y. The use of higher cut offs resulted in considerably higher prevalence rates of low 25(OH)D values (Yetley, 2008).

A systematic review of vitamin D status in populations worldwide recently published contained data on a total of 1,68,389 participants from 44 countries. The sample size of individual studies ranged from 11 to 18,462 participants with a median of 316 (inter quartile range 117–861), with majority of studies reporting data on males and females. The review reported a significant variability in the estimates of 25(OH)D values across the studies with mean and median values ranging from 4·9 to 136·2 nmol/l and 20·7 to 91·0 nmol/l, respectively. An astonishing 88·1% of the samples presented in the present review had mean 25(OH)D values below 75 nmol/l (<30 ng/ml), 37·3% had mean values below 50 nmol/l (<20 ng/ml) and 6·7% had mean values below 25 nmol/l (<10 ng/ml). The details about the country/region and serum 25(OH)D levels are given in Figure 2.8. As can be seen from the figure, most of the studies reported vitamin D levels falling in the red and yellow colour zone i.e. <75 nmol/l thus indicating deficiency and insufficiency respectively (Hilger et al., 2014).

A cross-sectional study of 634 healthy volunteers aged 18-50 years was performed to measure serum 25(OH) D and parathyroid hormone in Boston. The results revealed that 39% of subjects had 25(OH)D ≤20 ng/ml and 64% had 25(OH)D ≤30 ng/ml. Predictors of lower 25(OH)D levels included male sex, black or Asian race, and lack of multivitamin use (p<0.001 for each predictors). Lower 25(OH)D levels were associated with increased risk of elevated parathyroid hormone. Thus the authors concluded that low 25(OH)D levels in healthy adults may confer risk of skeletal disease. Black and Asian adults were at increased risk of deficiency (Mitchell et al., 2012).

Another cross sectional study of 77 healthy Japanese women, age 19 to 66 y, and working in nursing homes was conducted. The investigation included blood tests, forearm bone mass measurements, and a lifestyle questionnaire. The proportion of subjects younger than 30 years who had serum 25(OH)D concentrations < 30 nmol/l was 42.1% and was significantly higher (p <0.001) than the proportion of those 30 years and older (10.3%). There was a weak but significant linear association between serum 25(OH)D concentrations and forearm bone

FIGURE 2.8: MEAN/MEDIAN 25(OH)D VALUES, BY GEOGRAPHICAL REGION AND COUNTRY



Note: medians (○) are shown where mean values (o) are not reported; Study size is indicated by circle size. The background colour scheme is intended to reflect the current uncertainty around the definition of thresholds for deficient, insufficient and adequate 25(OH)D levels. Mean/median values falling within the intensely red zone are most consistent with severe vitamin D deficiency; those in the green zone reflect adequate vitamin D levels. Values within the yellow zone are those thought to be indicative of insufficiency.

Source: Hilger et al., 2014

mineral content ($R2$= 0.114, p= 0.0052) but not between serum 25(OH)D concentrations and bone mineral density (Nakamura et al., 2000). Similarly a multi centeric study carried out in five metropolitans in Iran to measure serum vitamin D and other biochemical variables among 2396 healthy males revealed that about 68.8% of participants suffered from VDD. Vitamin D levels were the highest in Bushehr (n= 111, 40.3%) (p< 0.05) while Tehran had the highest prevalence of VDD (n= 380, n= 85.7%). Geographical zone independently predicted vitamin D status (p< 0.05). There was not any association among age (r= 0.035, p> 0.05), physical activity (r= 0.023, p> 0.05), and exposure of face & hands to sunlight (r= 0.022, p> 0.05) with vitamin D levels (Rahnavard et al., 2010).

Institute of Medicine (IOM) and the American Academy of Pediatrics (AAP) in 1997 defined VDD in infants and children as a serum 25(OH)D level below 11 ng/mL but level below 20 ng/mL are now considered insufficient. High prevalence rates are reported in otherwise healthy infants, children and adolescents (Holick, 2008; Gordon et al., 2008; Lee et al., 2007), and also from diverse countries around the world including India (Huh & Gordon, 2008). In the randomized intervention study among 290 healthy schoolgirls (6-17 y), 124 from lower socioeconomic strata (LSES) (attending government schools) and 166 from upper socioeconomic strata (USES) (attending private schools) in Delhi, about 93.7% the girls were found to be vitamin D deficient at baseline with suboptimal serum 25(OH)D levels of 31.2 and 29.1 nmol/l respectively (Marwaha et al., 2010). In 2003, the AAP Committee on Nutrition and section on Breastfeeding advocated 200 IU per day of vitamin D intake for children of all ages (Calikoglu & Davenport, 2003; Holick, 2006) but now the recommendation is 400 IU per day for all infants, children and adolescents (Misra et al., 2008) till they are not getting this amount from alternative sources.

The analysis of vitamin D metabolites in adipose tissue is rare due to difficulties in extracting and separating the metabolites from tissue matrices. Heaney et al. (2009) estimated adipose 25(OH)D content in a 70 kg woman with 24.5 kg fat mass and suggested a total of 114.5 nmol 25(OH)D was distributed within this tissue compartment. In an exploratory study which was a part of a larger human clinical trial, 20 overweight and obese adult subjects of age 20–50 years without any other metabolic complications were enrolled. The objective of the present study was to determine whether 25(OH)D is detectable in subcutaneous white adipose tissue (SWAT) in overweight and obese persons enrolled in a twelve week energy restricted diet. Only two subjects had adequate serum 25(OH)D concentrations (≥50 nmol/L) at

baseline. Of the remaining participants with sub-adequate serum 25(OH)D, eight had deficient serum 25(OH)D concentrations (<30 nmol/L). Body composition was assessed by dual energy x-ray absorptiometry. subcutaneous white adipose tissue (SWAT) 25(OH)D concentrations were 5.8 ± 2.6 nmol/kg tissue and 6.2 ± 2.7 nmol/kg tissue pre- and post-intervention. There was a significant positive association between SWAT 25(OH)D concentration and serum 25(OH)D concentration (r = 0.52, P < 0.01). The researchers concluded that no significant changes in SWAT 25(OH)D_3 or serum 25(OH)D after a 6% loss of total body weight and 13% reduction in total fat provided the first human evidence that adipose 25(OH)D does not likely contribute to serum 25(OH)D with moderate weight loss. Weight loss alone is not sufficient to increase serum 25(OH)D and increases in dietary or dermal biosynthesis of vitamin D appear to be the most critical contributors to in vitamin D status (Piccolo et al., 2013).

In another cross sectional study among 601 non-diabetic young and middle-aged (35-60 years), urban Chinese population to examine the association between serum vitamin D concentrations and cardiometabolic risk factors it was observed that VDD or insufficiency was present in 66% of the tested population, and serum 25(OH)D levels were lower in subjects who were overweight/obese or suffered metabolic syndrome when compared to individuals of healthy weight without metabolic syndrome (24.08 ± 8.08 vs 31.70 ± 11.77 ng/ml, 21.52 ± 6.9 vs 31.74 ± 10.21 ng/ml respectively). 25(OH)D was inversely associated with waist circumference, fasting glucose, fasting insulin, triglycerides and LDL-cholesterol, and it was positively associated with HDL-cholesterol in a multivariable-adjusted regression model. Thus it was concluded that low vitamin D concentration was associated with indices of adiposity and cardiometabolic risk factors in the population (Yin et al., 2012).

A population study among 5,714 men and women, aged 30–79 years, from the Health 2000 Survey representing the Finnish population was conducted with the aim to investigate the associations between serum 25(OH)D concentration and sociodemographic, lifestyle and metabolic health-related factors. Serum 25(OH)D concentration was determined by radioimmunoassay and sociodemographic, lifestyle and metabolic factors were determined by questionnaires. The mean serum 25(OH)D concentration was 45.3 nmol/l and it varied between categories of socio-demographic, lifestyle and metabolic health variables. Older age, being married or cohabiting and higher education were related to higher serum 25(OH)D concentration. Those with the healthiest lifestyle estimated by a lifestyle index based on body

mass index, physical activity, smoking, alcohol consumption and diet had 15.8 nmol/l higher serum 25(OH)D concentration compared to those with the unhealthiest lifestyle. Of the indicators of metabolic health, only waist circumference and HDL cholesterol were significantly associated with 25(OH)D after adjustment for socio-demographic, lifestyle and other metabolic health factors. Thus it was concluded that serum 25(OH)D concentration was associated with a multitude of sociodemographic, lifestyle and metabolic health factors thus suggesting that such factors confound associations observed between serum 25(OH)D concentration and chronic diseases (Jääskeläinen et al., 2013).

Seasonal variations also affect the cutaneuous production of vitamin D. In winters vitamin D-synthesizing UVB radiations do not reach the surface of the earth. Keeping this in mind a 1-year longitudinal observational study was conducted to determine seasonal variation in vitamin D status in 54 healthy adolescent girls (11–13 years) and 52 elderly community-dwelling women (70–75 years) living in Denmark, and to quantify the impact of sun exposure and intake on the seasonal changes in vitamin D status. The participants were examined three times (winter–summer–winter). Serum 25(OH)D concentration and vitamin D intake were measured at each visit. Sun exposure was measured during summer. Serum 25(OH)D concentrations (winter, summer, winter) were median (25, 75 percentiles) 23.4 (16.5, 36.4), 60.3 (42.7, 67.7), 29.5 (22.2, 40.4) and 47.2 (27.3, 61.1), 67.3 (35.1, 79.2), 50.5 (32.7, 65.5) nmol/l for girls and women, respectively. The usual sun habits were determinant (p=0.002) for change in vitamin D status from winter to summer. Vitamin D intake from supplements (p =0.0001) and diet (p =0.002) were determinants for change in vitamin D status from summer to winter. Thus it was concluded that low vitamin D status among adolescent girls and elderly women improved during the summer (Andersen et al., 2013).

Ultra-orthodox lifestyle, which encourages modest dress and indoor activity in few communities, represents a risk factor for VDD. The vitamin-D status of religious Jewish males according to sun exposure and outdoor activity, and the correlation between serum 25(OH)D and PTH level was determined in a cross-sectional study among 74 young adult males in Jerusalem. Yeshiva-A ultra orthodox students (aged 20.1± 0.6) wear traditional clothing, live in dormitories and stay mostly indoor. Yeshiva-B ultra orthodox students (aged 33.0±4.2) dress similarly but have regular outdoor activities. Yeshiva-C religious students (aged 19±2.0) participate in a mixed army/Yeshiva program. Weekly outdoor activity time and degree of sun exposure were estimated by questionnaire. The results revealed that serum

25(OH)D was 8.9±3.6, 10.2±5.7 and 21.7±10.4 ng/ml (mean±SD) in Yeshiva A, B and C. 25(OH)D was correlated with degree of sun exposure (r=0.54, p< 0.0001) and inversely correlated with PTH (r=−0.3, p=0.01). PTH was normal in 87% of vitamin D-deficient subjects from Yeshiva-A and Yeshiva-C (mean age 20), compared to 52% of Yeshiva-B students (mean age 33). Bone mineral density studied in a random subset (n=14) of vitamin D-deficient subjects showed Z-scores of −1.5±1.0, −1.8±0.8, −2.1±0.4 in femoral neck, spine and radius. Thus conclusions drawn were- severe VDD was extremely prevalent in ultra-Orthodox males. Despite rare secondary hyperparathyroidism, they represented an important previously unrecognized high-risk group for metabolic bone disease (Tsur et al., 2011).

Thus from the above literature it is evident that VDD is rampant around the global and is observed in all walks of life, different age groups, in both the genders and is also prevalent in various metabolic conditions. The vitamin D status and its deficiency are also seen to be affected by socioeconomic status, seasonal variations, dress code, and even to amount of sunlight exposure.

C) VDD- INDIAN SCENARIO

India, is located between 8.4 and 37.6 ˚N latitude and majority of its regions receive ample amount of sunlight throughout the year (Babu & Calvo, 2010). So, it has been a general belief that rickets and vitamin D deficiency are uncommon problems in India because of abundant sunshine. There is, however, now increasing evidence that this is not true. But in the Indian scenario, vitamin D sufficiency cannot be attained by depending on adequate sun exposure. For Indian skin tone, minimum “direct sun exposure” required daily is more than 45 min to bare face, arms and legs to sun’s UV rays (wavelength 290–310 nm). With the exception of those who perforce need to work outdoors in the sun, most Indians do not get adequate sun exposure to produce sufficient amounts of vitamin D endogenously. Indian social and or religious norms related to public modesty dictate that most parts of an individual’s body, irrespective of gender, be covered. The not so D-lightful price of urbanization- in big cities a majority of people live in very high population density areas. They perforce live in overcrowded tenements, which are closely packed and 3–4 stories high. Consequently, direct sunlight does not reach inside most parts of the dwellings, thereby disallowing any sun exposure to an individual in the privacy of one’s home. Additionally, lack of space offers limited options for outdoor activities. Atmospheric pollution of

metropolitan India also factors in with respect to vitamin D status (Agarwal et al., 2002). Use of sunscreen creams and umbrellas do not help either. The extreme discomfort of the scorching heat associated with most sunny days of Indian summer and (not to mention) the undying desire of most Indians to attain a fairer skin complexion instantly extinguish any desire for sun exposure, and a person's primary focus is on finding ways to avoid the sun, at all costs. In the blazing heat of India these two concerns score very high and the quest for vitamin D sufficiency takes a backseat, always (Ritu & Gupta, 2014).

Thus all the focus is on a new emerging global threat called VDD which is not merely rickets or osteomalacia but a huge hidden problem now being unraveled and is reaching epidemic proportions in India. The problem has increased to alarming proportions after new definition of 25OHD levels and its impact on bone health (Harinarayan & Joshi, 2009). The first laboratory documentation of vitamin D status was in a group of patients with primary hyperparathyroidism and control population (Harinarayan et al., 1995). Later other reports ensued.

Countrywide studies (Table 2.5) have reported VDD in as high as 70%–100% of ostensibly healthy individuals. High prevalence of VDD was reported from northern to southern and western to eastern India, in ostensibly healthy children, adolescents, young adults and those ≥50 years old. All over India, VDD was highly prevalent in pregnant women and lactating mothers. Vitamin D status of these mothers correlated well with their neonates and their exclusively breastfed infants. Subjects from rural and urban areas presented a similar picture. Relatively, fish are a rich source of vitamin D. The residents of Bengal (eastern India) eat more fish compared to the rest of the Indians. Surprisingly, their vitamin D status appears to be just as poor as in the rest of the country (Baidya et al., 2012). Similarly, even healthy young soldiers with sufficient intake of calcium, adequate sun exposure and regular exercise regimen were found to be vitamin D deficient (Goswami et al., 2000; Tandon et al., 2003), as were young sportswomen (Marwaha et al., 2011). Among resident doctors from Mumbai (western India) (Multani et al., 2010) and also doctors from eastern India (Baidya et al., 2012), most were vitamin D deficient. VDD was also observed in most of 2119 healthcare professionals studied from all over India (Beloyartseva et al., 2012). Evidently, countrywide prevalence of VDD is undeniable.

Among otherwise healthy adolescents and adults in India, along with low serum 25(OH)D levels, a significant number of subjects also revealed clinical manifestations of VDD.

TABLE 2.5: VITAMIN D STATUS OF OSTENSIBLY HEALTHY INDIANS

Locale	Study Subjects		25(OH)D (ng/mL) Mean (SD)	Vitamin D Status		Reference
				% deficient	% sufficient	
Northern India						
Kashmir (34.3° N)	Total *N* = 92	Adults, age 28.15 (4.9) years	--	83	--	Zargar 2007
	N = 64	M	15.06 (12.0)	76.6	--	
	N = 28	F	5.51 (4.42)	94.4	--	
	N = 50	Urban subjects	11.26 (9.65)	85.7	--	
	N = 42	Rural subjects	12.84 (12.39)	80	--	
		Occupation:				
	N = 17	Rural/farmer, 17 M/0 F	--	70.6	--	
	N = 23	Government employee, 20 M/3 F	--	69.6	--	
	N = 15	Household, 0 M/15 F	--	100	--	
	N = 23	Medical professional, 16 M/7 F	--	91.3	--	
	N = 14	Student 11 M/3 F	--	85.3	--	
Punjab (31.1° N) & Haryana (29° N)	Total *N* = 90	Adults, paramilitary soldiers. Adequate diet, sun exposure and physical exercise.	--	--	--	Tandon 2003
	N = 40	M, in winter, age 22.7 (2.8) years.	18.4 (5.3)	--	--	
	N = 50	F, in summer, age 23.4 (3.1) years.	25.3 (7.4)	--	--	
Chandigarh (30.7° N)	Total *N*= 329	Young urban adults, age 18–25 years, at the end of summer	52.9 (33.7)	--	72.5	‡ Ramakrishnan 2011
	N = 237	Subjects from the same cohort at the end of winter	31.8 (21.1)	--	50.7	

Delhi (28.3° N)	*N* = 31	Urban adults, M, age 25 (5) years, soldiers, winter	18.87 (4.69)	--	--	Goswami 2000
	N = 15	Urban adults, 10 M + 5 F, age 43 (16) years, depigmented 2000 [55] persons, winter	7.28 (4.49)	--	--	
	N = 19	Urban adults, 11 M + 8 F, age 23 (5) years, physicians and nurses, winter	3.19 (1.39)	--	--	
	N = 19	Urban adults, 11 M + 8 F, age 24 (4) years, physicians and nurses, summer	7.17 (3.19)	--	--	
	N = 29	Urban adult F/mothers, age 23 (5) years, low income, summer	8.76 (4.29)	--	--	
	N = 29	Newborns of the mothers studied, 16 M + 13 F, summer	6.68 (1.99)	--	--	
Agota village (29° N) 80 km from Delhi	Total *N* = 57	Rural Adults	14.56 (9)	68.5	--	Goswami 2008
	N = 32	M, rural, age 42.8 (16.6) years	17.68 (9.76)	--	--	
	N = 25	F, rural, age 43.4 (12.6) years	10.76 (6.86)	--	--	
Delhi (28.3° N)	Total *N*=186	Young adults F, age 18.6 (1.3) years	12.96 (9.84)	--	--	Marwaha 2011
	N = 90	Sports-girls from colleges	21.2 (7.57)	--	--	
	N = 96	College girls	5.16 (3.08)	100	0	
Delhi (28.3° N)	Total *N*=642	Urban adults, middle income group	7 (4.08)	--	--	Goswami 2009
	N = 244	Adult M, age 31.4 (13.4) years	7.2 (3.64)	--	--	
	N = 398	Adult F, age 35.1 (13.4) years	6.88 (4.36)	--	--	
Delhi (28.3° N)	Total *N*=105	Urban adults, middle income group, age 43.3 (9.7) years	9.8 (6.0)	94.3	--	Vupputuri 2006
	N = 51	M, indoor workers	10.8 (6.8)	--	--	
	N = 54	F, housewives	8.8 (4.9)	--	--	

Delhi (28.3° N)	Total *N*= 404	Adolescents, F, urban, age 12.3 (3.4) years	12.74 (6.17)	90.8	--	Puri 2008
	N = 193	Low income level	13.84 (6.97)	89.6	--	
	N = 211	Higher income level	11.75 (5.07)	91.9	--	
Delhi (28.3° N)	Total *N*=5137	Adolescents, urban, school children, age 10–18 years	11.8 (7.2)	--	--	Marwaha 2005
	N = 3089	Lower income level, 1079 M, 2010 F	10.4 (0.4)	92.6	--	
	N = 2048	Higher income level, 968 M, 1080 F	13.7 (0.4)	84.9	--	
Delhi (28.3° N)	Total *N*= 664	Urban adolescent F, school girls, age 12.8 (2.7) years	11.4 (5.8)	--	--	Marwaha 2007
	N = 369	Lower income level, age 12.8 (2.7) years	11.1 (5.2)	--	--	
	N = 295	Higher income level, age 12.7 (2.6) years	11.8 (6.4)			
Delhi (28.3° N)	Total *N*= 1346	Urban adults ≥50 years, 643 M, 703 F, age 58 (9.5)years (range 50–84 years)	9.79 (7.61)	91.2	2	Marwaha 2011
	N = 995	Age group 50–65 years	9.72 (7.75)	91.3	1.8	
	N = 351	Age group >65 years	9.99 (7.2)	91.2	2.2	
Delhi (28.3° N)	*N* = 1346	Adults, ≥50 years, age 58 (9.5) years, 48% M, 52% F	9.8 (7.6)	91.3	1.9	Garg 2013
	N = 1829	Adolescents, 45% M, 55% F, age 13.3 (2.5) years	8.3 (5.2)	96.9	0.5	
Delhi (28.3° N)	*N* = 521	Pregnant women, lower-middle income level, age 24.6 (2.8) years	9.28 (4.88)	96.3	--	Marwaha 2011
	N = 342	Lactating mothers, from the above group 6–8 weeks postpartum	7.84 (3.32)	99.7	--	
	N = 342	Exclusively breastfed Infants	8.92 (4.2)	98.8	--	
Delhi (28.3° N)	*N* = 180	Lactating mothers	10.88 (5.8)	--	--	‡ Seth 2009
	N = 180	Exclusively breastfed infants, 2–24 weeks old	11.56 (8.3)	--	--	

Delhi (28.3° N)	*N* = 26	Infants, urban, age 16 ± 4.1 months, 15 M/11 F, low income families, high air pollution area	12.4 (7)	--	--	Agarwal 2002
	N = 31	Infants, urban, 15.9 (3.8) months, 15 M/16 F, low income families, low air pollution area	27.1 (7)	--	--	
Delhi (28.3° N)	*N* = 60	Lactating mothers 25.0 (2.0) years	9.06 (4.78)	98.3		Mehrotra 2010
	N = 60	Breastfed infants, 3.0 (0.14) months	9.03 (4.63)	100	0	
Delhi (28.3° N)	*N* = 98	Lactating mothers 23.1 (3.3) years	Median 9.8 (5.0–13.8)	--	--	Jain 2011
	N = 98	Breastfed infants, 58.2% M, age 13.6 (2.2)weeks	Median 10.1 (2.5–17.1)	--	--	
Delhi (28.3° N)	*N* = 220	Infants, low birth-weight, at birth	Median 6.5 (4.0–54.5)	93	--	Agarwal 2012
	N = 127	Infants, low birth-weight, at 3 months	Median 11.1 (4.0–78.0)	72.4	--	
	N = 116	Infants, normal birth-weight, at birth	Median 5.8 (4.0–26.6)	94.8	--	
	N = 77	Infants, normal birth-weight, at 3 months	Median 8.2 (4–29.7)	83.1		
	N = 216	Mothers of low birth-weight infants, at term	Median 5.6 (4.0–38.3)	93.5	2.3	
	N = 116	Mothers of normal birth-weight infants at term	Median 5.8 (4.0–21.1)	96.6	1.7	
Lucknow, 26.8° N	*N* = 92	Urban adults, 67 F, 25 M, age 34.2 (6.7) years, hospital staff	12.3 (10.9)	78.3	--	Arya 2004
Lucknow, 26.8° N	Total *N*= 207	Pregnant women before labor, low and middle income group, age 24.0 (4.1) years.	14 (9.3)	--	--	Sachan 2005
	N = 140	Urban F	14.0 (9.5)	--	--	
	N = 67	Rural F	14.1 (8.9)	--	--	
	N = 207	Neonates/cord blood	8.4 (5.7) 95.7	--	--	

Varanasi (25.3° N)	*N* = 200	Adults, M, ≥50 years, age 62.61 (7.64) years	18.96 (10.23)	58	13.5	Agarwal 2013
Southern India						
Tirupati (13.4° N)	Total *N*=1285	21% M, 79% F	--	--	--	Harinarayan 2008
	N = 205	Rural adults, age 43 years. 53% M, 47% F	M 23.73 (0.8) F 19 (0.89)	M 44 F 70	M 16.5 F 1	
	N = 941	Urban adults, age 46 years. 14% M, 86% F Hospital staff & relatives	M 18.54 (0.8) F 15.5 (0.3)	M 62 F 75	M 12 F 6	
	N = 70	Rural children, age 13 years. 48% M, 52% F	M 17 (1.3) F 19 (1.59)	M 76.5 F 72.2	M 8.8 F 13.9	
	N = 69	Urban children, age 13 years. 43% M, 57% F	M 15.57(1.2) F 18.5 (1.66)	M 81.5 F 62.9	M 3.7 F 11.4	
Tirupati (13.4° N)	Total *N* =191	Semi-urban women	--	--	--	Harinarayan 2011
	N = 55	Reproductive F age 37.42 (0.72) years	15.70 (1.38)	76.3	7.3	
	N = 136	Postmenopausal F, age 53.29 (0.72) years	17.70 (0.94)	66.9	10.3	
Mysore (12.3° N)	*N* = 559	Pregnant women at the 30th week of pregnancy, age 24 years	Median 15.12 (9.6–23.4)	66.5	--	Farrant 2009
Eastern India						
Kolkata (22.5° N)	*N* = 40	Doctors, 39 M, 1 F, age 52.22 (10.91) years	13.02 (4.77	92.5	2.5	Baidya 2012
Western India						
Mumbai (18.9° N)	*N* = 42	Pregnant women 37th week of pregnancy, age 20–35 years, middle income group	22.99 (10.93)	--	--	Bhalala 2007
	N = 42	Cord blood/neonates	19.36 (9.57)	--	--	
	N = 35	Infants, 3 months old, exclusively breastfed	18.19 (9.74)	--	--	

Mumbai (18.9° N)	Total *N*= 1137	Young urban adults, age 30-38 (3.55) years	17.4 (9.1)	--	7.2	Shivane 2011
	N = 558	M	18.9 (8.9)	--	9.7	
	N = 579	F	15.8 (9.1)	--	4.8	
Pune (18.5° N)	*N* = 110	Slum toddlers, age 2.6 (0.7) years		--	--	Ekbote 2010
	N = 50	25 M, 25 F, outdoors (daily sun exposure of 15–60 min or more)	45.24 (31.88)	--	--	
	N = 60	31 M, 29 F, indoors	3.84 (10.64)	--	--	
Pune (18.5° N)	*N* = 71	Urban children, 36 M, 35 F, age 2.8 (0.6) years, all income groups	11.94 (12.61)	--	--	Ekbote 2011
Pune (18.5° N)	*N* = 50	Adolescent girls, low income group, age 14.7 (0.10) years	Median 9.36 (5.4–12.76)	--	--	Khadilkar 2010
Pune (18.5° N)		Women: housewives, working women, retired	--	--	--	Kadam 2010
	N = 80	Premenopausal F, age 45.6 (4.8) years	9.68 (4.56)	--	--	
	N = 92	Postmenopausal F, age 54.0 (7.1) years	10.76 (6.8)	--	--	
Pune (18.5° N)	Total *N* =214	Premenarchal school girls, low income group	24.6 (10.4)	34.2	--	Kadam 2011
	N = 134	Age 8–9 years	24.36 (10.32)	--	--	
	N = 80	Age 10–12 years	25.12 (10.64)	--	--	
All over India						
18 cities spread all over India	*N* = 2119	Adults, medical and paramedical personnel, 72% M, 28% F, age 42.71 (6.8) years.	14.35 (10.62)	79	6	Beloyartseva 2012
		No significant differences were found either between men and women, or northern and southern India.				

All 25(OH)D values have been shown in ng/mL. To convert from nM to ng/mL, nM values were divided by (2.5). Vitamin D deficiency is defined as 25(OH)D < 20 ng/mL, insufficiency as 20–29 ng/mL and sufficiency as ≥30 ng/mL; ‡ Information available from the abstract of the article. Age of the subjects is in mean age (SD) years, unless otherwise indicated.

Biochemical evidences of suboptimal bone health are: elevated alkaline phosphatase, a surrogate marker for increased bone turnover, and elevated PTH levels (secondary hyperparathyroidism or SHPT). ALP and PTH were often reported in research papers, especially with respect to their correlation with vitamin D status. DEXA is the most accurate and convincing evidence of bone density. The results revealed that most studies did not show any correlation of bone mass density (BMD) with vitamin D status. Notably, among 90 adults, who were 20–30 year-old soldiers from Indian paramilitary forces, with adequate nutrition, sun exposure and physical exercise, BMD was lower when compared to Caucasians. Among men, osteopenia was noted in 50% at the lumbar spine, 35% at the hip, and 50% at the forearm. Additionally, 10% of men had osteoporosis of the lumbar spine. Among women, osteopenia was noted in 32% at the lumbar spine, 14% at the hip and 21% at the forearm. The authors speculated that the effect of childhood malnutrition may have contributed to lower peak bone mass accumulation in these subjects (Tandon et al., 2003). BMD studies emphatically underline the need for adequate nutrition, sun exposure and physical exercise from the very beginning of one's life, to attain peak bone mass, and later to maintain it. Indubitably, vitamin D status in India is grim and needs to be reckoned with.

Vitamin D sufficiency by dietary intake is the only tenable solution for Indians. However, this solution itself has a barrage of problems. There are many nutritional factors attributing to high prevalence of vitamin D deficiency in India (Ritu & Gupta, 2014)-

1) Most dietary sources of vitamin D have very low vitamin D content. Most of the food items rich in vitamin D are of animal origin. Most Indians are vegetarians. Commonly, a dietary source of vitamin D for vegetarians is milk, provided milk has been fortified with vitamin D. Milk is rarely fortified with vitamin D in India. The vitamin D content of unfortified milk is very low (2 IU/100 mL). Additionally, milk and milk products are unaffordable to the socioeconomically underprivileged. Another concern in India is the rampant dilution and/or adulteration of milk and milk products.
2) Low calcium in Indian diet: Low dietary intake of calcium in conjunction with vitamin D insufficiency is associated with secondary hyperparathyroidism (SHPT). 24 hydroxylase is the key enzyme of vitamin D catabolism and is regulated by 1,25(OH)2D, PTH and FGF23 (Fibroblast Growth Factor 23) levels. Overproduction of FGF23 can result in increased morbidity associated with vitamin D deficiency (Liao, 2013). Most studies reported calcium intake much lower than the RDA (Recommended Daily Allowance)

defined by the Indian Council of Medical Research (ICMR). ICMR's RDA for calcium intake in India is lower than that of the western world.

3) Intake of caffeine from tea and coffee is very high in India. Most Indians consume milk as part of their tea or coffee. The proportion of milk is very low in these drinks. Thus calcium intake through these beverages is low. Vitamin D is stable during cooking. It is stable up to 200 °C. However, thermal stability of vitamin D is an inverse function of both temperature and time. In India, milk is boiled for several minutes before consumption. In India most of the times, beverages like tea and coffee are boiled for several minutes to get the right flavor. This boiling may reduce the content of any vitamin D that there may have been left after boiling of the milk itself. Therefore, these beverages may not contribute significantly to either calcium or vitamin D intake in Indians.
4) High prevalence of lactose intolerance in India is a major deterrent pertaining milk consumption, further lowering intake of calcium and vitamin D in these individuals.
5) Notably, nearly all studies pertaining vitamin D status in healthy subjects reported a high phytate/calcium intake ratio. Indians require a higher intake of calcium in their diet to lower the phytate/calcium intake ratio. Dietary habits in India have changed significantly. Many people remove a substantial proportion of bran from whole wheat flour before kneading to improve texture and fluffiness of *chapatis*. Consumption of white bread is also very high. Most people prefer processed, split and polished pulses to whole seeds due to the ease of shorter time required for cooking and the consequent lowered expense of cooking fuel.
6) Cooking practices in India: Indians in general adhere to traditional cooking styles and practices, irrespective of their migration to any part of the world. In tropical climate perishable food items putrefy quickly. Consumption of uncooked fresh produce, especially vegetables, milk, *etc.*, is generally considered ill-advised. As in the rest of the world, in India too, slow cooking is widely practiced. This culinary practice, however, is ill-advised bearing in mind the thermal instability of many vitamins. As mentioned earlier vitamin D is degraded at temperatures above 200 °C. Its thermal stability is inversely related to temperature and time. Cooking gas flame reaches temperature above 1900 °C and coal stove heat reaches 300–700 °C. Water boils at 100 °C. Baking is done mostly above 175 °C but the temperature in the food does not reach such high

temperatures, therefore stability of vitamin D during baking is well within acceptable range (Natri et al., 2006).

Pertaining shallow and deep-frying of food, most cooking fats and oils have smoke points above 180 °C. Shallow and deep frying of foods is very popular in India. When foods are fried, vitamin D in the food comes out into the cooking medium and is thermally degraded (Lu et al., 2007). Pressure cooking temperatures vary depending on the pressure withstood by the cooker used and may range from 100 °C to 120 °C. Short-time (as short as possible) pressure cooking is definitely advisable to retain at least some of the thermally more stable essential nutrients in cooked food, including vitamin D.

7) Poor nutrition resulting from poverty: Perforce, in the sordid context of poverty, focus on a balanced diet is always on the back burner. It is very convenient to attribute dietary patterns or cooking traditions, than face a reality as grim as poverty. Factually, balanced diet is only an occasional treat to the impecunious.

Thus the concluding thoughts on the growing concern about VDD in India are discussed herewith. The major limitation of the available data is to translate it to the whole country with more than a billion populations. Even though data regarding the VDD has started pouring in from all directions of the country, still many sects of people remain untouched. There is limited data of populations residing at higher latitudes and altitude (soldier in Himalayas, northeastern part of India), in deserts of Rajasthan, fisherman in the sea coasts, and the rural population who are below the poverty line (Harinarayan & Joshi, 2009). Studies covering them should be undertaken on priority basis to develop a database of vitamin D status in Indian population. There is urgent need to prioritize development of national level programs to make available, quality-regulated and affordable vitamin D supplements and vitamin D fortified foods to the Indian populace. Very importantly, the government needs to implement measures to educate the Indian populace about the current status of vitamin D in India and also the modes to attain vitamin D sufficiency (Ritu & Gupta, 2014).

D) CAUSES AND CONSEQUENCES OF VITAMIN D DEFICIENCY

Suddenly, vitamin D is no longer ‘just a vitamin’ and a deficiency has serious effects on the functional integrity of the whole system. Ethnical and gender differences in skin pigmentation indicate the evolutionary importance of a sufficient vitamin D supply. The

varying degrees of depigmentation that evolved in order to permit UVB-induced synthesis of previtamin D_3 when hominids migrated outside the tropics can be considered as a compromise solution to the conflicting physiological requirements of vitamin D synthesis. An evolutionary selection pressure towards a lighter skin coloration going along with a higher ability to produce vitamin D seems not only to be exerted by living in geographic regions with a lower UV intensity but also by being female. Gender differences in skin pigmentation with females being lighter skinned than males in all populations for which data about the skin reflectance was available could be explained by the higher needs of vitamin D during pregnancy and lactation (Jablonski & Chaplin, 2000).

Vitamin D synthesis is affected by latitude, atmospheric pollution, clothing, melanin pigmentation and sunlight exposure. Any one of these factors can be etiological for vitamin D deficiency apart from decreased vitamin D intake as breast feeding, maternal vitamin D deficiency and unusual diets; defects in vitamin D metabolism as low calcium intake, intestinal malabsorption and genetic variation (Harinarayan & Joshi, 2009). There are many factors which can explain the paradox of hypovitaminosis D. These factors or causes overlap with the risk factors as stated earlier in the chapter. Generally, they can be divided into two groups: UVB-related deficiency and medical/physical condition-related deficiency.

UVB-related deficiency

- **The elderly:** The elderly, due to the decreased presence of skin 7-dehydrocholesterol which is the precursor for UVB mediated synthesis of vitamin D, are particularly at risk of vitamin D deficiency. Moreover, reduced mobility or institutionalization that discourages sun exposure, reduced renal production of 1,25 dihydroxyvitamin D as well as decreased intake of fortified foods pose great difficulties in vitamin D formation in body (Lips, 2001; Lips, 2007).

- **Dark skin:** People with dark skin have great amounts of melanin in their epidermis. Melanin competes with 7-dehydrocholesterol for absorption of UVB photons. Therefore, people of dark color are less efficient in producing vitamin D than are whites. It is reported that a person with skin type 5/6 (dark skin) requires 10-50 times the exposure to sunlight to produce the same amount of vitamin D as does a white person with skin type 2/3 (Heaney, 2003). Epidermal melanin (a natural sunscreen) on one hand reduces the risk of skin cancer induced by UVR but on the other hand, reduces cutaneous vitamin D

synthesis. An Asian Indian would require 3 times the sun exposure than light-skinned person to produce equivalent amount of vitamin D (Holick et al., 2005). It is interesting to note that women of all population have lighter skin than men, presumably because of increased vitamin D needs during pregnancy and lactation (Hanley & Davison, 2005).

- **Season, latitude, and the time of day:** It has been established that the ozone layer can absorb UVB radiation above 290 nm which is responsible for generating previtamin D_3. Zenith angle, defined as the angle of the sunlight reaching the Earth's surface, decides the thickness of ozone layer which sunlight needs to penetrate. The thicker the ozone layer is, the fewer amounts of UVB photons can reach the earth, thus few previtamin D_3 can be produced. Zenith angle is dependent on factors such as time of day, season of the year, and latitude. Thus those factors have great effects on vitamin D production (Holick et al., 2008; Glendenning et al., 2009). For example, residents of Boston (42°N), Edmonton, Canada (52°N) and Bergen, Norway (61°N) cannot produce sufficient quantities of vitamin D in their skin for 4, 5, and 6 months, respectively (Vieth, 1999). Modern day life style changes have significantly reduced the total duration of sun exposure in children. UV-B, having shorter wavelength, tend to scatter earlier or later in the day and hence cutaneous vitamin D synthesis is maximum between 10 AM to 3 PM, the time when most of the people are indoors. Exposure of only face, hands and arms due to clothing versus whole body is associated with marked differences in vitamin D synthesis (Holick et al., 2005). Cloud cover, increasing water vapour and industrial pollution can reduce the amount of UV-B that reaches the earth's surface (Heaney, 2004).

- **Sunscreen users:** Sunscreens can efficiently absorb UVB radiation. This dramatically prevents the interaction of UVB with 7-dehydrocholesterol, the process of previtamin D_3 generation. It has been shown that when used properly, a sunscreen with a sun protection factor of 8 reduces the production of previtamin D_3 by 95%, and 99% by a sun protection factor of 15 (Matsuoka et al., 1987; Holick, 1994).

However caution is required when UV light/sunlight is used for vitamin D production. UVB irradiation does not appear to suit some individuals who develop headache, nausea and possibly vomiting and rise of temperature after exposure. Also people with sensitive skin may react strongly to UV rays, thus are unsuitable for UV treatment.

Medical/physical condition-related deficiency

- **Fat malabsorption:** As a fat-soluble vitamin, vitamin D requires the presence of dietary fat in the gut for absorption. Certain pathological conditions, such as Crohn's disease, cystic fibrosis (CF), celiac disease, surgical removal of part of the stomach or intestines are associated with fat malabsorption and thus may lead to vitamin D deficiency. For example, CF patients suffer from pancreatic exocrine insufficiency. This results in malabsorption of fat-soluble vitamins, including vitamin D. CF patients, depending on the degree of exocrine insufficiency, absorb approximately 50% less vitamin D than normal (Lo et al., 1985).

- **Anticovulsant use:** Anticonvulsants, also called antiepileptic drugs, have been used to treat epileptic seizures and bipolar disorder. It is well recognized that long-term use of some antiepileptic drugs, including phenobarbital, phenytoin, and carbamazepine and the antimicrobial agent rifampicin (RIF) can result in osteomalacia (Pack et al., 2002; Andress et al., 2002; Karaaslan et al., 2000). The induction of the catabolism of 1,25-dihydroxyvitamin D by these drugs is thought to contribute to their deleterious side effects.

- **Chronic kidney disease:** In order to become biological active vitamin D, kidney plays an important role in this transforming process. Chronic kidney disease such as patients with stage 4 or 5 chronic kidney disease, as well as those requiring dialysis, leads to an inability to make sufficient 1,25-dihydroxyvitamin D which has a direct effect in inhibiting parathyroid hormones expression (Dusso et al., 2006; Correa et al., 2002). Thus 1,25-dihydroxyvitamin D3 intake is needed to maintain calcium level in blood as well as to control parathyroid hormone levels.

- **Obesity:** It has been known for a long time that obese people are prone to be vitamin D deficient since they have lower 25-hydroxyvitamin D levels (Hey et al., 1982; Hyldstrup et al., 1993). A number of studies proved that the vitamin D3 precursor 7-dehydrocholesterol levels in the skin of obese people were not significantly different from non-obese people (MacLaughlin et al., 1985). One explanation was that the subcutaneous fat, which is known to store vitamin D, sequestered more of the cutaneous synthesized vitamin D, which results in less release of vitamin D from the skin into the circulation in the obese subject than non-obese subject (Wortsman et al., 2000).

- Dietary factors like very low calcium intake and high fibre diet may deplete vitamin D stores. Even genetic factors like increased 25(OH)D-24-hydroxylase (leading to degradation of vitamin D) activity in South Asians (Khadilkar et al., 2007) are also among the various explanations of hypovitaminosis D in sunny countries.

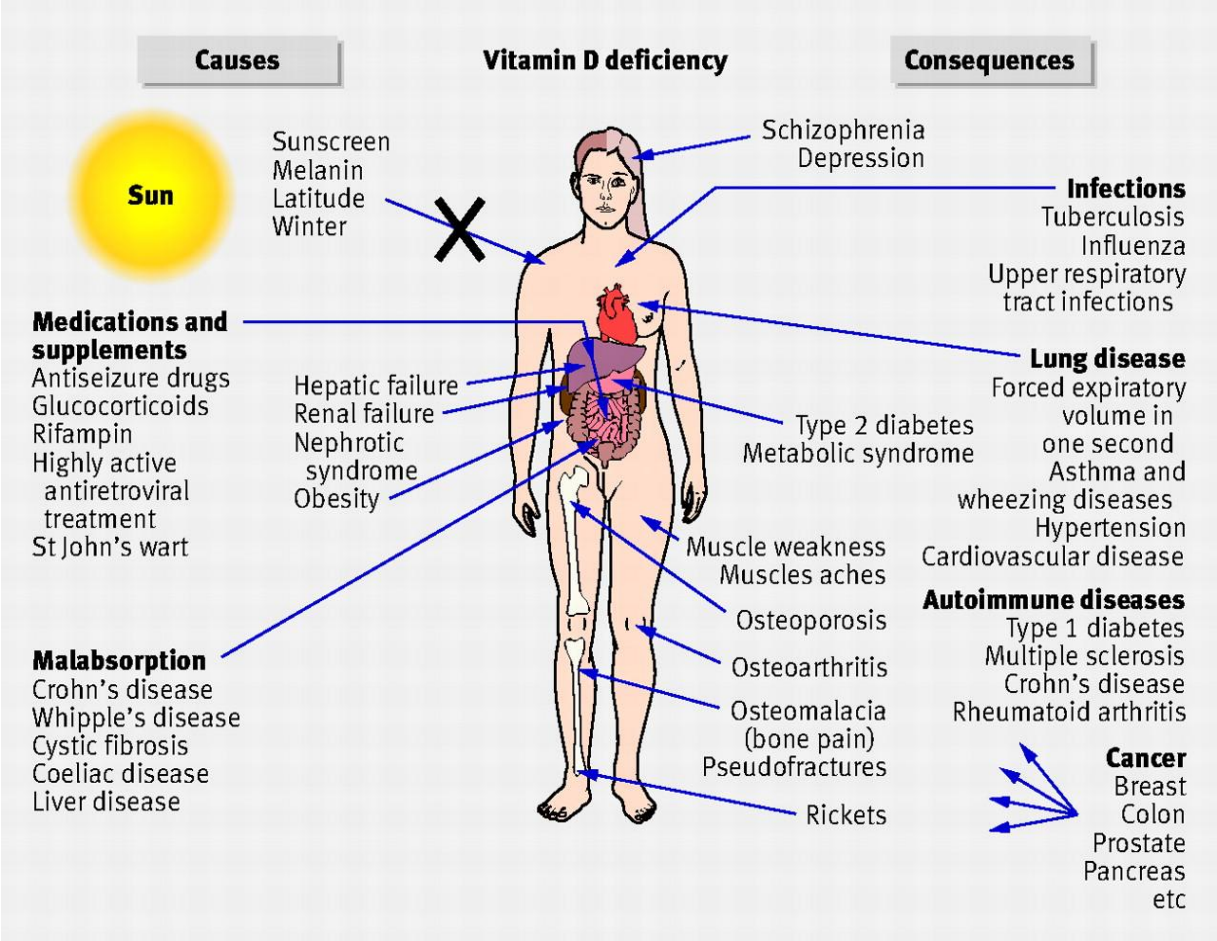
Health Consequences of Vitamin D Deficiency

The major role of vitamin D in vertebrate animals and humans is to increase the absorption of calcium and phosphate for the mineralization of the skeleton. Low vitamin D levels lead to insufficient calcium absorption, and this has clinical implications not only for bone health but also for most metabolic functions. Recently biochemical studies have implicated vitamin D deficiency in many chronic diseases including, but not limited to, infectious diseases, autoimmune diseases, cardiovascular diseases, diabetes and cancer. Numerous epidemiological publications support the extraskeletal benefits of vitamin D and they cannot be ignored. The schematic representation of the major causes for vitamin D deficiency and its potential health consequences is given in Figure 2.9 and explained henceforth.

1) Vitamin D and Skeletal Health

Rickets, osteomalacia and osteoporosis are terms related to skeletal deformities and widely prevalent all over the world. Vitamin D sufficiency is pivotal for normal skeletal development both *in utero* and in childhood, and for achieving and maintaining bone health in adults (Holick & Chen, 2008). The most well recognized function of 1,25(OH)2D involves regulation of calcium and phosphorus balance for bone mineralization and remodeling. Without adequate levels of 1,25(OH)2D in the bloodstream, dietary calcium cannot be absorbed. The absence of vitamin D results in not more than 10–15% of calcium absorption, which is increased by approximately 40% for calcium and 80% for phosphorous once the vitamin D levels are in the "sufficient" range. Low calcium levels lead to an increase in serum PTH concentration, which leads to increased tubular reclamation of calcium in kidneys and resorption from the skeleton at the cost of lowering bone density. Approximately 40%–60% of total skeletal mass at maturity is accumulated during childhood and adolescence. Rickets results from inadequate mineralization of growing bone. Thus it is a childhood disease and it is manifested as bone deformities, bone pain and weakness. Biochemical abnormalities consistently include hypophosphatemia, elevated alkaline phophatase levels and serum 25(OH)D levels are usually below 5 ng/mL (Ritu & Gupta, 2014). In adults low

FIGURE 2.9: SCHEMATIC REPRESENTATION OF THE MAJOR CAUSES FOR VITAMIN D DEFICIENCY AND POTENTIAL HEALTH CONSEQUENCES



Source: Wacker & Holiack, 2013

25(OH)D and high PTH also lead to a low serum calcium×phosphorus product, resulting in osteomalacia, *i.e.*, a defective mineralization of the collagen matrix causing a reduction of structural support thus increasing risk of fracture.

Results from the National Health and Nutrition Examination Survey III (NHANES III) showed that bone density in the hip was directly related to the serum 25(OH)D level in both genders of all ethnicities (Adams & Hewison, 2010; Bischoff-Ferrari et al., 2009). Osteoporosis has a prevalence of ~1/3 in women 60–70 years of age and of ~2/3 in women 80 years of age or older. An osteoporosis-related fracture will be experienced by one in eight men over age 50 years in their lifetime (Khosla et al, 2008). The link between VDD and osteoporosis has been well established especially in the elderly. Reduced bone mineral density (BMD) increases the risk of fractures, which significantly contributes to morbidity and mortality of older persons. According to data from the Women's Health Initiative (Cauley et al., 2008), the odds ratio of risk for hip fracture was inversely related to the serum 25(OH)D level. There's evidence that patients with 25(OH)D levels >30 ng/mL have a lower risk of fracture. Several studies have been conducted to evaluate the effect of vitamin D supplementation on the fracture risk, with some studies showing a significant reduction of the risk of fractures while others didn't (Bischoff-Ferrari et al., 2012). A meta-analysis of more than 30,000 participants did show that supplementation with vitamin D (≥792 IU/day) led to a significant reduction in the risk of fracture; the risk of hip fracture was reduced by 30%, the risk of any non-vertebral fracture by 14% (Grant et al., 2005; Meyer et al., 2002; Jackson et al., 2006; Pfeifer et al., 2009).

2) Muscle weakness

Muscle weakness is also a prominent feature of VDD. Patients with nonspecific muscle weakness, muscle aches and pains have also been found with vitamin D inadequacy. Impaired muscle function is known to cause an increased number of falls which can lead to hip fractures. 1,25(OH)2D is thought to modulate muscle function via the VDR, which seems to be expressed in skeletal muscles, by regulating gene transcription and promoting *de-novo* protein synthesis (Bischoff-Ferrari, 2012). However, the existence of a VDR in muscle cells is discussed highly controversially, as a more recent study failed to detect the VDR in muscle cells and as the antibodies used for immunocytochemical staining to detect the VDR in previous studies have been shown to be not exclusively specific for the VDR and could explain potentially false-positive results in these previous studies (Wang & DeLuca, 2011).

VDD is associated with diffuse muscle pain, muscle weakness, predominantly in the proximal muscle groups, and a reduction in performance speed. This is caused by muscle atrophy of mainly type II muscle fibres (Janssen et al., 2002). Proximal muscle weakness in severe VDD could also be caused by secondary hyperparathyroidism and resultant hypophosphatemia. There is a positive association between 25(OH)D, lower extremity function, proximal muscle strength and physical performance (Wicherts et al., 2007; Bischoff-Ferrari, 2012). Muscle strength and postural and dynamic balance (Bischoff-Ferrari et al., 2006) were increased by vitamin D supplementation. The effect of vitamin D supplementation on the risk of falls was examined in a randomized, controlled multi-dose study, showing that the supplementation of 800 IU/day lowered the adjusted-incidence rate ratio of falls by 72% compared to those taking placebo over 5 months (Broe et al., 2007). A meta-analysis of 8 randomized controlled trials (n = 2426) showed that supplemental vitamin D of 700–1000 IU/day or a serum 25(OH)D of ≥24 ng/mL reduced the risk of falls by 19% and 23% respectively. No benefit was observed with lower supplemental doses or lower serum 25(OH)D concentrations (Bischoff-Ferrari et al., 2009).

3) Cancer

Living at higher latitudes with lower UV exposure and thus lower vitamin D production is associated with an increased risk for the occurrence of a variety of cancers and with an increased likelihood of dying from them, as compared to living at lower latitudes. A review of ecological studies associating solar UVB exposure-vitamin D and cancers found strong inverse correlations with solar UVB irradiance for 15 types of cancer: bladder, breast, cervical, colon, endometrial, esophageal, gastric, lung, ovarian, pancreatic, rectal, renal, and vulvar cancer; and Hodgkin's and non-Hodgkin's lymphoma (Grant, 2012). Retrospective and prospective epidemiologic studies showed that when 25(OH)D levels were <20 ng/mL there was a 30%–50% increased risk of developing and dying of colorectal, prostate, breast, pancreatic, and esophageal cancer (Ritu & Gupta, 2014).

These associational studies have certain limitations regarding the establishment of a causality between vitamin D status and a reduced risk of cancer, e.g., as low serum 25(OH)D levels are also linked with confounding factors related to higher cancer risk, including obesity (vitamin D is sequestered in adipose tissue), and lack of physical activity (correlated with less time outdoors and less solar exposure) (Manson et al., 2011). However, a population-based, double-blind, randomized placebo-controlled trial of 4 years duration with more than

thousand postmenopausal women, whose principal secondary outcome was cancer incidence, showed that the supplementation with calcium (1400–1500 mg/day) and vitamin D_3 (1100 IU/day) reduced the relative risk (RR) of cancer by ~60% ($p < 0.01$). The repetition of a cancer free survival analysis after the first 12 months revealed, that the relative risk for the calcium + vitamin D group was reduced by ~77% (confidence interval [CI]: 0.09–0.60; $p < 0.005$) (Lappe et al., 2007).

Experimental evidence supports a reduction of risk of many cancers through the action of $1,25(OH)_2D$ in suppressing the proliferation and stimulating differentiation of cancer cells. 1,25(OH)2D exerts anti-proliferative effects on cancer cells by promoting cyclin-dependent kinase (CDK) inhibitor synthesis, and by influencing several growth factors and their signalling pathways including insulin-like growth factor 1 (IGF-1), transforming growth factor β (TGFβ), Wnt/β-catenin, MAP kinase 5 (MAPK5) and nuclear factor κB (NF-kB) (Fleet et al., 2012) 1,25(OH)2D3 might exert anti-carcinogenic effects by promoting various pro-apoptotic mechanisms including the down regulation of the anti-apoptotic gene Bcl-2 and by up-regulating of the pro-apoptotic gene Bax, 1,25(OH)2D3 induces differentiation, partly by reducing the expression of the *c-myc* oncogene. It regulates the prostaglandin (PG) metabolism and signalling, thus decreasing PG-mediated promotion of carcinogenesis (Krishnan & Feldman, 2011). It suppresses tumor angiogenesis, e.g., mediated by 1,25(OH)2D's effects on the PG synthesis and by regulating the expression of crucial factors controlling the angiogenesis. 1,25(OH)2D3 suppresses tumor invasion and metastasis by various mechanisms Other effects mediated by 1,25(OH)2D are thought to be the induction of autophagy as process to trigger the death of cancer cells and to block tumor growth and by inducing enzymes involved in antioxidant defense mechanisms and DNA-repair (Fleet et al., 2012). 1,25(OH)2D also regulates androgen and estrogen receptor signaling, thereby inhibiting tumor growth of some sex hormone dependent tumors such as prostate and breast cancer.

4) Immunity

The plethora of effects of vitamin D on regulating the immune system plays a role in fighting infectious diseases. Vitamin D enhances the innate immunity against various infections especially tuberculosis, influenza and viral upper respiratory tract infections. Vitamin D has shown to inhibit the following actions:

i. T cell proliferation, in particular the TH1 (T-helper cell 1) response (cytotoxic) by reducing gammainterferon and interleukin 2.
ii. Costimulatory molecules CD40, CD80, CD86 on dendritic cells which leads to reduced secretion of IL-12 (critical for Th1 development).
iii. Differentiation of B cell precursors into plasma cells thereby reducing immunoglobulin production.
iv. TH17 response: They are a subset of T helper cells that produce interleukin 17 (IL-17) considered developmentally distinct thought to play a key role in autoimmune disease.
v. Antigen presenting dendritic and macrophage cells. Vitamin D deficiency impairs macrophage ability to mature, produce macrophage-specific surface antigens, lysosomal enzyme acid phosphatase, and to secrete hydrogen peroxide, which is essential to their antimicrobial function.

It has shown to up-regulate the following:

i. Protective TH2 (T-helper cell 2) response by increasing IL-4,5 and 10.
ii. T-cell regulatory cells (Treg): Regulatory T cells actively suppress activation of the immune system and prevent pathological self-reactivity, i.e., autoimmune disease (Gupta, 2012).

Autoimmune disorders: VDD has been implicated in pathogenesis of systemic lupus erythematosus, type 1 diabetes mellitus, multiple sclerosis, inflammatory bowel diseases, and rheumatoid arthritis. Up to 77% of patients with multiple sclerosis and >60% with rheumatoid arthritis have vitamin D levels <55 nmol/l (Gupta, 2012). Type 1 diabetes (T1D) is caused by autoimmune destruction of pancreatic β cells, which eventually leads to insulin-dependent diabetes. A meta-analysis of observational studies showed a 30% reduction in risk of T1D in children receiving vitamin D supplements (Zipitis & Akobeng, 2008).

In a meta-analysis of observational studies, eight studies (two cohort studies and six case-control studies) on vitamin D intake during early life and three studies (two cohort studies and one case-control study) on maternal vitamin D intake during pregnancy were identified. The pooled odds ratio for T1D comparing vitamin D supplementation with non-supplementation during early life was 0.71 (95% CI, 0.51–0.98). Similar results were observed in the case-control subgroup analysis but not in the cohort subgroup analysis. The pooled odds ratio with maternal intake of vitamin D during pregnancy was 0.95 (95% CI, 0.66–1.36). The authors concluded that vitamin D intake during early life may be associated

with a reduced risk of T1D. However, enough evidence for an association between maternal intake of vitamin D and risk of T1D in the offspring was not available (Dong et al., 2013).

Multiple sclerosis is an autoimmune disease in which the body's immune system attacks myelin, a key substance that serves as a nerve insulator and helps in the transmission of nerve signals. It has been long recognised that annual and winter hours of sunlight have been proved to have the strongest negative correlation with the prevalence of multiple sclerosis. One explanation is that the increase of vitamin D results from sunlight exerting a protective effect (Raghuwanshi et al, 2008; Cantorna, 2008). Studies also found that individuals with multiple sclerosis tend to have insufficient vitamin D levels.

Infections

- **Tuberculosis:** As early as in the 19th century, cod liver oil (a rich source of vitamin D) was used for treating tuberculosis (TB). Skin exposure to sunlight was an effective therapy for treating Mycobacterium infections of the skin. Cross-sectional studies showed that patients with TB have lower 25(OH)D levels in comparison with control subjects (Chan et al., 1994). In a study among South Asians it was found that low vitamin D status resulting from a vegetarian diet is an independent risk factor for active TB (Strachan et al., 1995). In a survey conducted in London approximately 56% had undetectable levels of vitamin D, and an additional 20% had levels below 9 ng/ml. Vitamin D has shown to inhibit the growth of *Mycobacterium* tuberculosis via activation of the Toll-like receptor (TLR2/1) which reduces its viability within macrophages and monocytes (Ustianowski et al., 2005).

  A study to compare the vitamin D group of pulmonary tuberculosis patients with a placebo group in terms of clinical improvement, nutritional status, sputum conversion, and radiological improvement was carried out among 67 tuberculosis patient. The subjects were randomised to receive vitamin D (0.25 mg/day) or placebo in a double blind method, during the 6th initial week of TB treatment. 100% of the vitamin D group and only 76.7% of the placebo group had sputum conversion (p=0.002). However the sputum conversion had no correlation with the hemoglobin level, blood clotting time, calcium level, lymphocyte count, age, sex, and nutritional status. There were more subjects with radiological improvement in the vitamin D group (Nursyam et al., 2006).

- **Skin infections** *(staphylococcus aureus pseudomonas auroginosa):* Keratinocytes treated with vitamin D have been shown to have substantially more killing abilities toward epidermal bacteria mediated by enhanced cathelicidin expression in human epidermal keratinocytes. Following epidermal injury 1,25-hydroxylase activity in the skin increases suggesting the contributory role of vitamin D in maintaining structural skin integrity by fighting infection.
- **Viral:** Vitamin D sufficiency is an independent predictor of sustained virological response following antiviral therapy given for chronic hepatitis C infection. An increased associated with the common cold and flu has also been seen with low vitamin D levels with severe infections requiring intensive care associated with the lowest range of vitamin D levels (<20 ng/ml) (Gupta, 2012).

5) Cardiovascular Risk

Cardiovascular diseases (CVDs), including heart failure and coronary artery disease are a major cause of morbidity and mortality worldwide. There is accumulating epidemiological evidence from observational studies suggesting that CVDs are associated with vitamin D deficiency and that adequate vitamin D may be beneficial for preventing cardiovascular disease. The prospective Intermountain Heart Collaborative Study with more than 40,000 participants revealed that 25(OH)D <15 ng/mL compared to 25(OH)D >30 ng/mL was associated with highly significant increases in the prevalence of type 2 diabetes mellitus, hypertension, hyperlipidemia, and peripheral vascular disease, coronary artery disease, myocardial infarction, heart failure, and stroke ($p < 0.0001$), as well as with incident death (all-cause mortality was used as primary survival measure), heart failure, coronary artery disease/myocardial infarction ($p < 0.0001$), stroke ($p = 0.003$), and their composite ($p < 0.0001$) (Anderson et al., 2010).

Kims et al. (2008) in NHANES reported that vitamin D <20 ng/mL was associated with increased prevalence of self reported coronary heart disease, heart failure, and peripheral vascular disease. Giovannucci et al. (2008) in the US Health Professionals Follow-up Study reported that vitamin D <15 ng/ml was associated with twofold increased rate of myocardial infarction) while Wang et al. (2008) in Framingham Offspring Study observed that vitamin D<10 ng/ ml was associated with a 1.80-fold increase rate of developing the first cardiovascular event compared with subjects with levels >15 ng/ml. Similarly Pilz et al. (2008) also observed that vitamin D levels <10 ng/ml was associated with three to five times

risk of sudden cardiac death or heart failure during a 7-year follow-up period. Individuals spending less time exercising outdoors in the sun, e.g., have a higher risk of developing cardiovascular diseases, and those individuals also will likely have lower 25(OH)D levels coincidentally (Reid & Bolland, 2012; Tall, 2002).

The primary mechanisms proposed for the cardiovascular benefit are

- Renin and angiotensin down-regulation: Vitamin D has shown to regulate the rennin–angiotensin system (RAS) and vitamin D receptor absent mice have shown to exhibit high-blood pressure, cardiac hypertrophy, and polyuria. Human studies have shown that vitamin D acts as an endocrine inhibitor of the RAS. Vitamin D insufficiency (15.0–29.9 ng/ml) and deficiency (<15.0 ng/ml) having higher circulating angiotensin II levels and a significantly blunted renal plasma flow response to infused angiotensin II suggesting that low plasma vitamin D may upregulate RAS and induce hypertension contributing to coronary heart disease (Li, 2011).
- Cardiomyocyte: Vitamin D may directly affect myocardial contractility by reducing intramyocardial calcium and may protect against atherosclerosis through the inhibition of macrophage cholesterol uptake and foam cell formation, reduced vascular smooth muscle cell proliferation, and reduced expression of adhesion molecules in endothelial cells and through inhibition of cytokine release from lymphocytes (Wang et al., 2008).

6) Hypertension

Vitamin D supplementation has shown down regulate the RAS and reduce systemic blood pressure. An upregulated RAS has been implicated in the development of beta-cell dysfunction and insulin resistance all contributing the development of hypertension (Vaidya & Williams, 2012). Short-term vitamin D supplementation (800 IU) was shown to decrease systolic blood pressure by approximately 9% (Pfeifer et al., 2001) confirming the possible role of vitamin D in cardiovascular protection.

Reduced blood pressure has been found in people taking oral supplementation of vitamin D. In humans, skin exposure to UVB, which is the major source of vitamin D formation, has been linked with lower blood pressure. In a study, patients with hypertension were exposed to UVB radiation three times a week for 3 months. Results showed that 25(OH)D levels increased by approximately 180%, and both systolic and diastolic blood pressure reduced by 6 mm Hg (Krause et al., 1998). In contrast, a large prospective study of men and women

found no association between intake of vitamin D from diet and supplements and hypertension incidents (Forman et al., 2005). In a recent review, ten RCTs were summarized for their outcomes to gain an appreciation of the recent causative evidence linking vitamin D and endothelial function. Only two studies showed an improvement in flow mediated dilatation with vitamin D. Three other studies reported decreases in C-reactive protein, platelet activation inhibitor-1, tissue plasminogen activator or B type natriuretic peptide. Recent evidence from good quality RCTs did not support a beneficial effect of vitamin D on vascular reactivity. Thus future intervention studies may need to target a higher vitamin D status and longer duration to determine whether the vitamin has a regulatory role in endothelial function. Prospective studies could involve dose response trials that target a range of status values and maintain that target value for at least six months (Alyami et al., 2014).

7) Obesity

Obesity, another condition associated with cardiovascular disease, is also associated with a lower vitamin D status due to a sequestration and volumetric dilution of the lipophilic vitamin D in the fat tissue. The NHANES has shown that there is a growing prevalence of vitamin D deficiency and obesity. Numerous studies have shown an inverse relationship between the two. A serum vitamin D level of less of < 50.0 (20 ng/ml) nmol/l has shown to be significantly associated with new-onset obesity (defined as waist circumference of ≥88 cm for women and ≥102 cm for men) in both in adults and children/adolescents. In the latter the highest incidence of obesity was found with a serum vitamin D of <17 ng/ml (Mai et al., 2012; Pacifico et al., 2011). One of the possible mechanisms explaining the association is that a larger fat mass promotes greater storage of vitamin D effectively reducing bioavailable vitamin D.

8) Type-2 Diabetes Mellitus

Type 2 diabetes mellitus (T2DM) is marked by insulin resistance (IR). In IR insulin is adequately or overproduced by pancreatic β cells, but is ineffectively utilized by the target cells of adipose, hepatic and skeletal muscles tissues. As a response to hyperglycemia, β cells further increase insulin production leading to hyperinsulinemia, which is often indicative of a pre-T2DM stage. Hyperinsulinemia is associated with hypertension, obesity, dyslipidemia, and glucose intolerance.

The relationship between vitamin D status and T2DM is seen in many observational studies which is also supported experimentally. A meta-analysis examining the association between

vitamin D status or vitamin D supplementation, and incident T2DM showed that individuals with 25(OH)D levels >25 ng/mL compared to those with 25(OH)D <14 ng/mL had a 43% lower risk of developing T2DM and that a vitamin D supplementation with >500 IU/day compared to <200 IU/day reduced the risk by 13% (Mitri et al., 2011). A prospective study following-up more than 2000 participants showed, that the risk of progression from prediabetes to diabetes was reduced by 62% when comparing the highest quartile of 25(OH)D levels with the lowest quartile (Holick, 2012; Deleskog et al., 2012). This could be explained by experimental findings indicating that vitamin D exerts various antidiabetic effects. The VDR is expressed in pancreatic beta cells and $1,25(OH)_2D$ stimulates insulin secretion. Improvement in vitamin D status also leads to an improvement of insulin sensitivity, mediated for example by upregulation of insulin receptors (Pilz et al., 2011) and modulates inflammation, which is also thought to play a role in T2DM (Holick, 2012). This would indirectly reduce cardiovascular risk as uncontrolled glycaemia is a major risk factor for CVD.

9) Metabolic Syndrome

Metabolic syndrome (MS) is characterized by several abnormalities including impaired glucose tolerance, blood lipid derangements, and prothrombotic and pro-inflammatory states. Circulating 25(OH)D has been inversely associated with abdominal adiposity, hypertriglyceridemia, and hyperglycemia. Thus vitamin D status may indirectly affect MS development. In a case-control study a total of 375 Iranian women with waist circumference (WC) >88 cm were examined to find 100 who met MS criteria according to the NCEP-ATP III criteria. Of those without MS, 100 age- and residence area-matched women were selected as a control group. There was no significant difference in serum 25(OH)D or intact parathyroid hormone (iPTH) between the two groups. Serum hsCRP concentration was significantly higher in the MS group, compared to the controls (3.4 ± 3.3 vs 2.0 ± 1.9 mg/L, $p<0.001$). When data were categorized according to vitamin D status, in the MS group significantly higher plasma glucose concentrations were observed in subjects with vitamin D deficiency compared to those with insufficiency or sufficiency (104.0 ± 11.7, 83.0 ± 11.3 and 83.2 ± 9.9 mg/dL, respectively, $p<0.001$). In stepwise regression analysis, 25(OH)D was the main predictor of both hsCRP and plasma glucose. Thus vitamin D status may, at least in part, be a determining factor of systemic inflammation and the related metabolic derangements of MS (Salekzamani et al., 2011).

In a cross-sectional sample of 441 Indians, aged 39.7±12.8 years (237 men and 204 women) with 27.9% prevalence of MS, vitamin D insufficiency (12.5 to <50 nmol/l) and hypovitaminosis D (50 to <100 nmol/l) were present in 65.6 and 31.1% of participants, respectively. Multivariate regression analysis indicated a positive relationship between 25(OH)D and β-cell function (homeostasis model assessment (HOMA)-B; β=0.245, p=0.006), whereas regression coefficients for fasting glucose (β=0.262, p=0.794), insulin (β=−0.140, p=0.889) and HOMA-IR (β=−0.119, p=0.172) were insignificant. Gender-stratified analysis showed no linear trend for increasing quintiles of 25(OH)D with prevalence of MS or its components (p>0.05). Although highly prevalent, vitamin D insufficient status was not associated with MS or IR in Asian Indians of either gender. Thus further investigation to test the association between 25(OH)D levels, MS is required (Majumdar et al, 2011).

10) Non-alcoholic Fatty Liver Disease

Non-Alcoholic Fatty Liver Disease (NAFLD) is an increasing global health concern, with an estimated prevalence of 20%–30% in Western countries and 15% in Asian countries (Bellentani et al., 2010). NAFLD is tightly linked with obesity and is regarded as the hepatic manifestation of the metabolic syndrome. Prevalence of NAFLD among individuals with type 2 diabetes is estimated to be 65%–70%, which is more than twice the prevalence among people without diabetes (Leite et al., 2009).

The mechanism of the association of vitamin D and NAFLD is believed to be related to oxidative stress and inflammation. These two diseases share inflammation as the common pathogenic mechanism. Because inflammation and oxidative stress might act as the common pathogenic mechanisms of NAFLD and vitamin D deficiency, and as both diseases are associated with insulin resistance, T2DM and cardiovascular disease, studies have been recently performed to examine the relationship of vitamin D levels with the development of NAFLD (Katz et al., 2010; Roth et al., 2012; Barchetta et al., 2011). As NAFLD and metabolic syndrome are considered to stem from the same pathogenic paradigm, the association of vitamin D deficiency with NAFLD may be related to its possible link with abdominal obesity and metabolic syndrome. Targher et al. (2007) reported that decreased vitamin D as 25(OH)D concentrations in patients with biopsy-proven NAFLD were significantly ($p < 0.001$) and independently associated with increased histological severity of hepatic steatosis and fibrosis. Sprague-Dawley rats fed a high-fat/high-fructose corn syrup

diet deficient in vitamin D showed greater hepatic steatosis and up-regulated gene expression of markers of oxidative stress and inflammation, compared to the group with sufficient vitamin D (Roth et al., 2012). However, there is insufficient evidence to recommend that patients with NAFLD consume more than the RDA for vitamin D (600 IU/day for 19–70 year old males and females, and 800 IU/day for adults over 70 years). Future investigations should examine the biological role of the vitamin in NAFLD pathogenesis, and whether increasing the amount through diet or supplementation to that beyond the RDA has therapeutic effects.

Thus from the above literature it is clear that vitamin D because of its actions via VDR; which are present in almost all vital organs, is crucial for maintaining the metabolic equilibrium of the body. VDD has been equally identified as a consequence for extra skeletal health too. The most recent to be added in the list is NAFLD. However among all the consequences the most sought after is type-2 diabetes mellitus, because of its alarming prevalence which keeps the researchers on toes to find out new management techniques. What is type-2 diabetes mellitus, what are its causes and consequences and what is its relationship with vitamin D is discussed in detail further.

III. VITAMIN D AND TYPE-II DIABETES MELLITUS (T2DM)

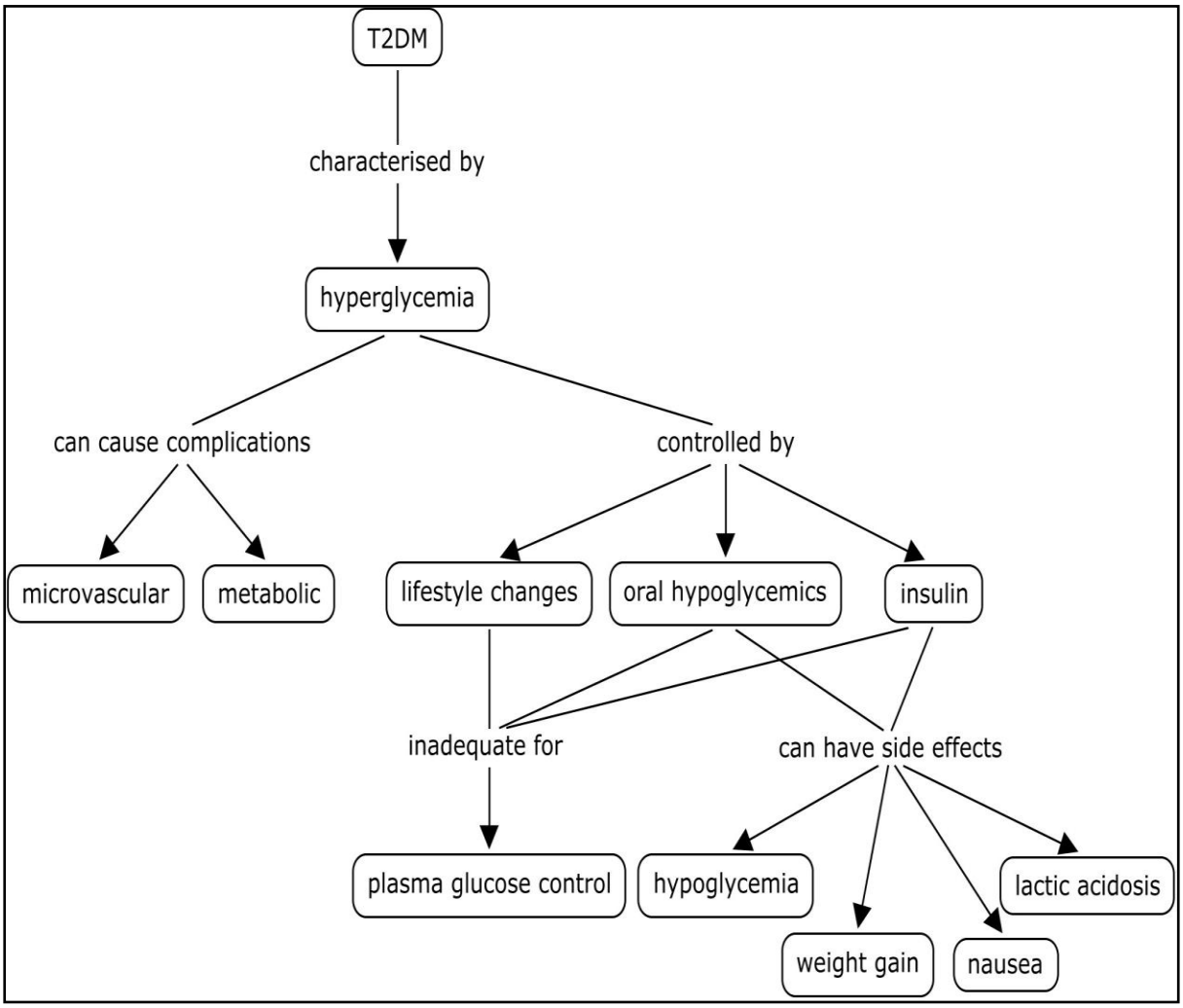
A) T2DM - AN INTRODUCTION

The term *Diabetes*, literally meaning 'siphon', was coined by Aretaeus of Cappediocia, in reference to the polyuria characteristic of uncontrolled disease. He linked the disease to a 'siphon', sucking water out of the body through the urine (Schadewaldt, 1987). In ancient India, Diabetes was known as *prameha* (pra:excess, meha:urine), a term used to refer to the disease even today in certain Indian languages. The Charak Samhita, recognises 20 types types of *prameha*, which if not treated, can lead to *Madhumeha* (madhu: honey, meha: urine; literally sweet urine, an unambiguoud description of diabetes). The ancient Indian texts also recognised the existence of two distinct types of *Madhumeha*: i) krisha (lean; coresponding to type-I diabetes) and ii) Sthula (obese; type-II diabetes mellitus). Thus ancient Indian physicians were acquainted with the classification, etiology and treatment of diabetes as long ago as 1500 BC (Parivallal, 2007).

At the beginning of the 20th century, T2DM was a disease of the better-off populations of the more advanced nations in the world. However, by the 2nd half of the century, it has become clear that no nation or ethinc group was exempt from the disease. Countries like India, China and the Arab nations of the Middle East, which experience rapid economic growth after centuries of deprivation, found themseleves at the epicenter of a global epidemic, which they were ill-equipped to handle. With explosive increase in its prevalence, diabetes mellitus as a chronic medical disorder, is to be reckoned on par with hypertension and atherosclerosis. A versatile disease, diabetes, in view of its frequent clinical and epidemiological link with the other two, constitues a health problem of paramount concern for a very large proportion of the worlds population.

Diabetes mellitus comprizes of a group of chronic metabolic disorders involving the principal metabolic fuels, carbohydrates, fats as well as proteins. The disorder results from absolute or relative deficiency in insulin secretion often along with the defect in insulin action. Primarily diabetes evolves from interaction of genetic and environmental factors resulting in autoimmune destruction of the β-cells or progressive deterioration in β-cells capacity on the face of gross defects in insulin action. The diagramatic representation of the general etiology of T2DM is shown in Figure 2.10.

FIGURE 2.10: DIAGRAMATIC REPRESENTATION OF ETIOLOGY OF T2DM



Source: www.google.com/etiologyoftype2diabetes

B) T2DM - RISK FACTORS, CONSEQUENCES AND PREVALENCE

Diabetes mellitus, a pan metabolic disorder is characterized by chronic hyperglycemia. A syndrome rather than a disease entity, diabetes is classified according to whether hyperglycemia is the primary feature or is a part of some other disorder. David Barker in his fetal programming suggested that as the growing fetus depends on the mother for its nutritional needs, and therefore any disturbance in the maternal nutritional status or its supply to the fetus will adversely impact fetal growth. The available nutrition is utilized for the growth of the fetal brain, which is vital for survival thus compromising the growth and functions of 'less important' insulin sensitive organs like the pancreas, liver and skeletal muscles. These organs then fail to function optimally in later life leading to disease (Figure 2.11). These individuals have higher adiposity, insulin resistance and increased risk of T2DM, similar to obese individuals, but at lower levels of BMI (Hales and Barker, 1992). Thus this fetal programming proves to be the first cause of chronic non-communicable diseases as well as the promising strategy for primary prevention of these conditions.

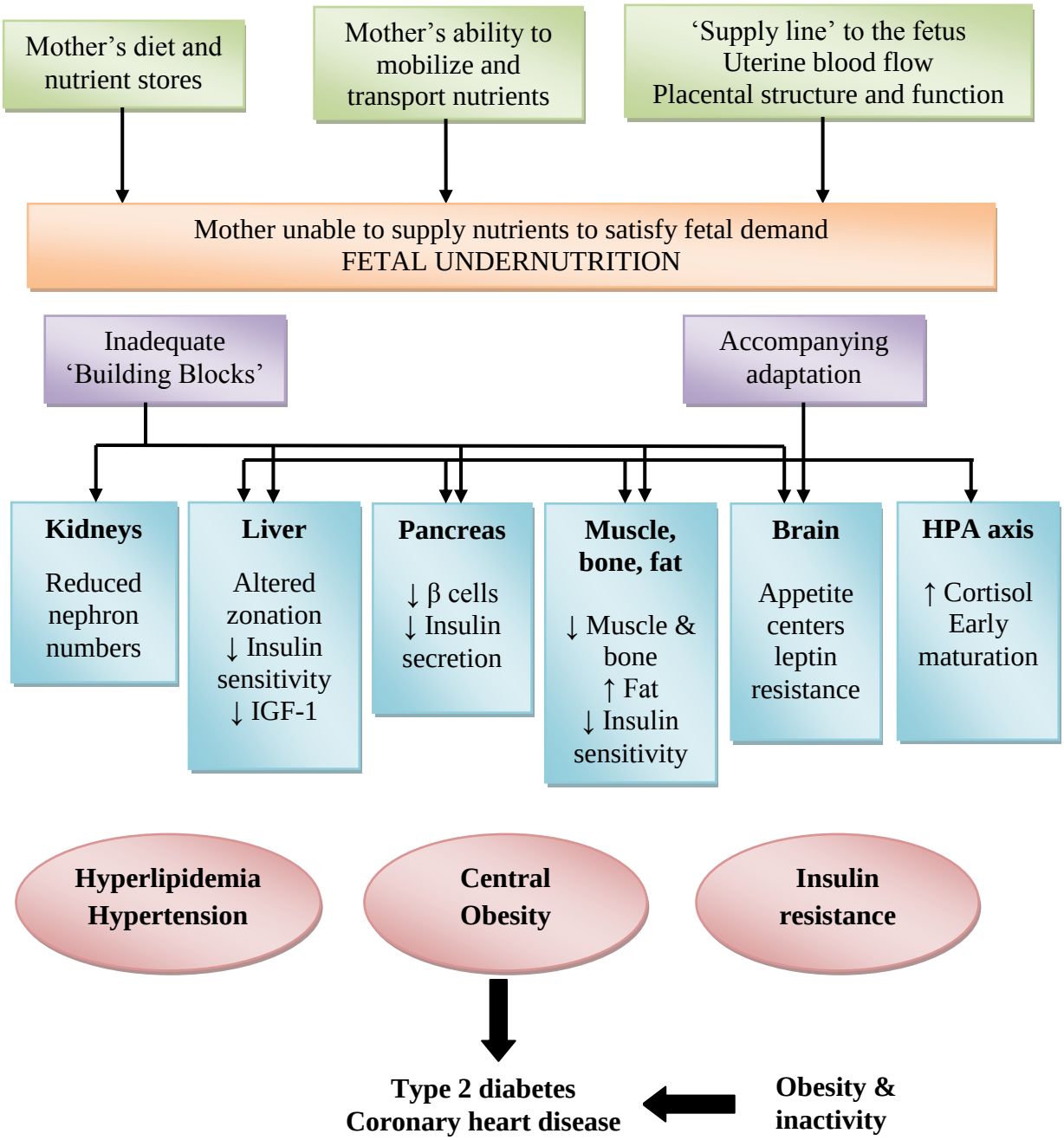
The important risk factors for diabetes are

i. **Urbanization:** The developing countries are undergoing rapid urbanization and migration of population to the urban areas. Internal rural to urban migration is yet another factor adversely impacting lifestyle factors such as reduction in physical activity, unhealthy changes in dietary habits, and increasing adiposity and obesity.

ii. **Racial predisposition:** A racial predisposition to diabetes is evident from the Indian migrant studies which showed that the Asian population living in different parts of the world had higher prevalence of diabetes compared with the coinhabitants of other races. Recent studies have highlighted the fact that even internal migration within a country, causing affluence and sedentary lifestyle, unmasks the tendency for diabetes in the Asian races (Ramachandran et al, 2010).

iii. **Genetic risk:** Both the thrifty genotype and thrifty phenotype hypothesis appear to have etiological roles in the development of diabetes in the Asian population. The combination of gestational diabetes, in uteronutritional imbalance, childhood obesity, and overnutrition in adulthood continues to fuel the epidemic in Asian countries undergoing rapid nutritional transition (Yajnik, 2009). In India, nearly 75% of T2DM patients have first degree family history of diabetes (Mohan et al, 1996).

FIGURE 2.11: BARKERS FETAL PROGRAMMING HYPOTHESIS



IGF-1: Insulin-like growth factor

Source: Fetal Programming of T2DM. In: Textbook of Diabetes Mellitus (3rd Edition, 2014)

iv. **Aging:** Diseases of the elderly, such as diabetes, hypertension, CVD and cancer have become more common due to aging of the population. Prevalence of diabetes is known to increase linearly with increasing age. However younger onset of diabetes has been noted in Asian Indians in several studies (Vijayakumar et al, 2009). The age criteria as a risk factor for T2DM is persons over 45 years, but for South Asians it is above 35 years.

v. **Anthropometric characteristics:** An excess body fat especially concentrated within the abdomen has a range of potential harmful effects. It includes increased risk for diabetes, blood pressure, dyslipidemia, insulin resistance, coronary artery disease and some forms of cancer. For diabetes, obesity and specifically abdominal obesity are a major risk factor. Several studies have shown that an average BMI level in Asians ranges between 20-23 kg/m^2 and the risk of metabolic disease increases progressively above 22 kg/m^2 (WHO, 2000). The cut-off for waist circumference, which is an index of upper body adiposity, is also lower in the Asian population (Snehalatha et al, 2003).

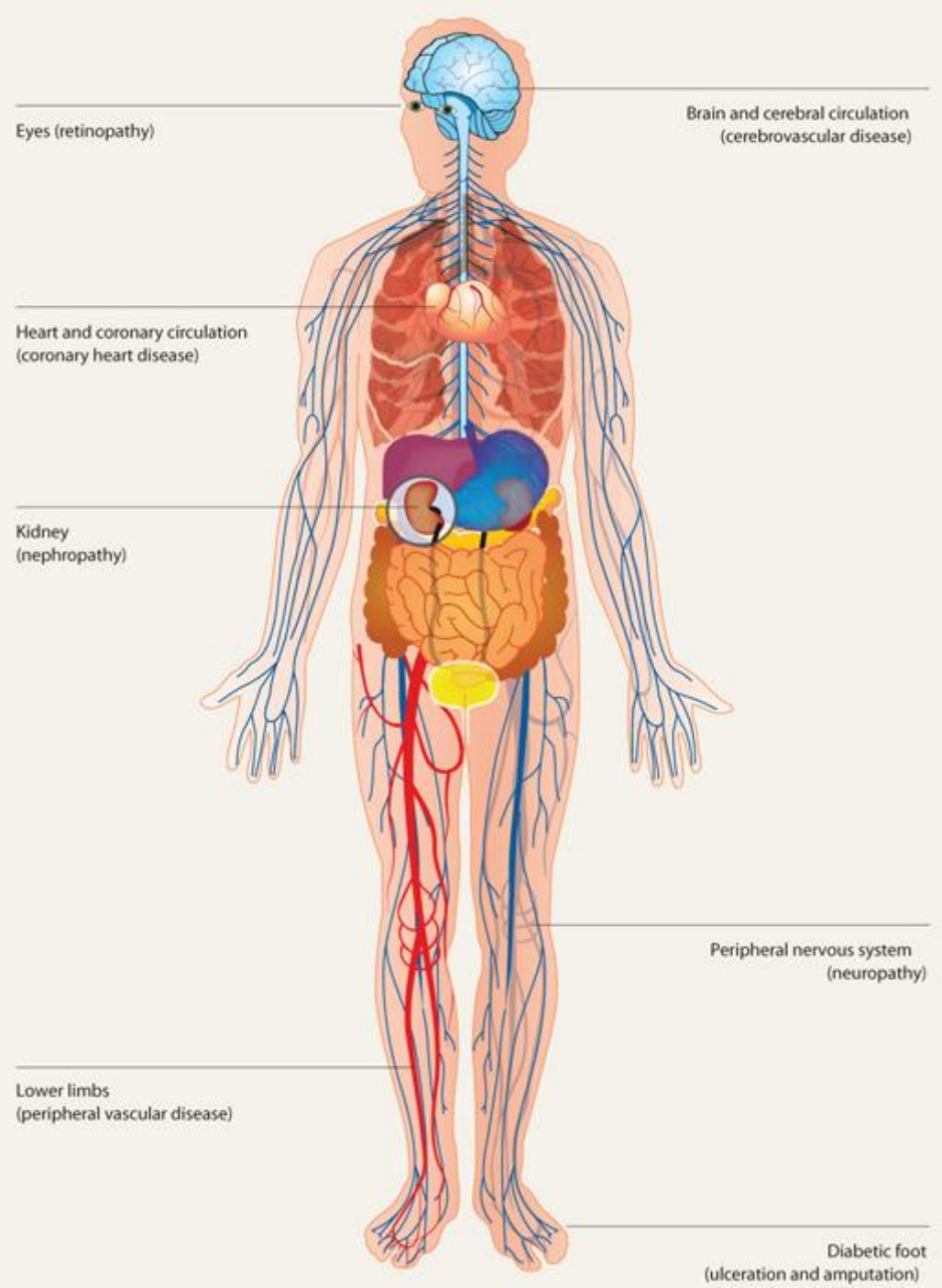
vi. **Insulin resistance:** Despite having lean body, insulin resistance is found to be a characteristic feature in Asian Indians and is adversely affected by even small increments in body weight (Ramachandran et al, 2010).

Although by definition, diabetes is characterized by elevated glucose concentrations; its impact on both the health of individuals and on the health care systems is almost entirely due to the long term 'complications' of diabetes which affect almost every system in the body, but particularly the eyes, kidneys, heart, feet and nerves. T2DM is a progressive disease and hampers the quality of life of the patients due to micro and macrovascular complications (Figure 2.12). Diabetes related vascular complications can be broadly classified as:

- **Microvascular complications:** It affects the retina (retinopathy), kidney (nephropathy) and the peripheral nerves (neuropathy)

  Diabetic retinopathy is considered the most specific complication of diabetes and is one of the hallmarks of the disorder. Diabetic nephropathy is the leading cause of end-stage renal disease (ESRD). It affects 20-30% of type-1 and type-2 diabetic patients. It has been estimated that 20% of T2DM patients reach ESRD during their lifetime (Ayodele et al, 2004). While diabetic neuropathy affects nearly 50% of all diabetic subjects and is considered to be the main cause of morbidity. Neuropathy occurs with the same frequency

FIGURE 2.12: MAJOR COMPLICATIONS OF DIABETES MELLITUS



Source: International Diabetes Federation (IDF) Atlas, 6th Edition (2013)

in both type-1 and type-2 diabetes suggesting a common etiology mechanism based on chronic hyperglycemia. People with diabetic nephropathy in developing countries incur huge costs largely due to foot complications (Shobhana et al, 2000).

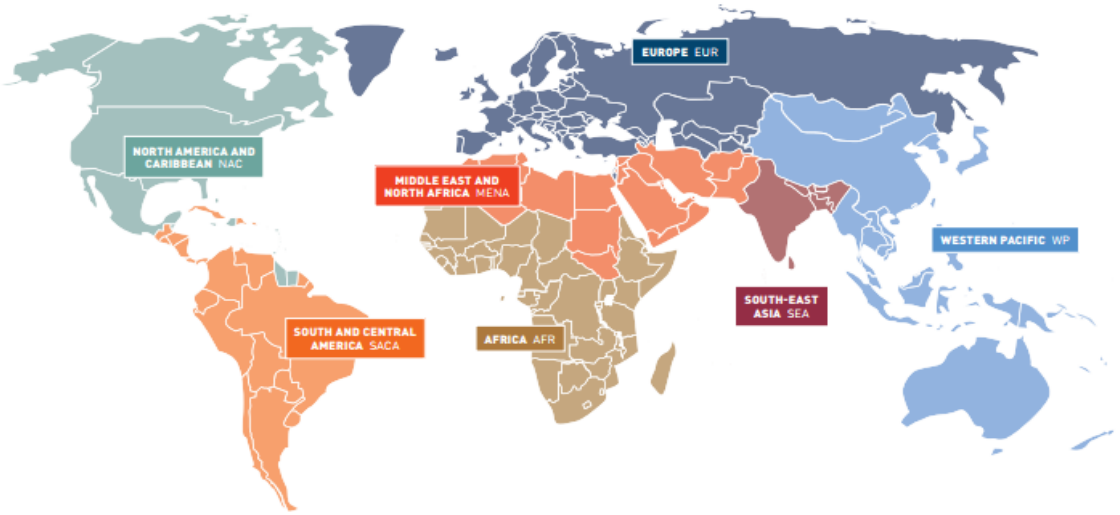
- **Macrovascular complications:** It affects the heart (cardiovascular disease), brain (cerebrovascular disease) and the peripheral arteries (peripheral vascular disease).
  Diabetes mellitus is an independent risk factor for cardiovascular disease. This is because T2DM is a component of the metabolic cluster, which is associated with other risk factors like insulin resistance, dyslipidemia, hypertension, abdominal obesity and prothrombotic state (Reaven, 1993). Indeed, of all diabetic complications, the most dangerous and life threatening is cardiovascular disease. Strokes are the third commonest cause of mortality in diabetic patients after heart disease and cancer. Patients with diabetes have a higher frequency of stroke, and also a poorer prognosis after a stroke (Kurukulasuriya et al, 2006). Peripheral vascular disease (PVD) in diabetic patients is frequently asymptomatic and may present with ischemic foot ulceration or gangrene with no previous claudication. The occlusive lesions usually occur in more distal vessels such as the tibialis and peroneals (Gibbons, 1987).

- **Hypertension** is a common comorbid condition in diabetes and both type-1 and type-2 patients are prone to develop it. It substantially increases the risk of both macrovascular and microvascular complication in diabetes. About 50% of diabetic individuals in India have hypertension (Singh et al, 1996). Recently, the screening India's twin epidemic (SITE) cross-sectional study conducted in 10 Indian states (Singh et al, 1998) reported that diabetes and hypertension were coexistent in 20.6% patients, which demonstrates that the burden of diabetes and hypertension is on the rise in India.

Diabetes mellitus is one of the most common chronic diseases across the world and number of diabetic patients is on rise. IDF's most recent estimates indicate that 8.3% of adults – 382 million people – have diabetes, and the number of people with the disease is set to rise beyond 592 million in less than 25 years. This equates to approximately three new cases every 10 seconds, or almost 10 million per year. The worst part is that almost 175 million of cases currently are undiagnosed, and these people with diabetes are progressing towards complications unawares. The new estimates show an increasing trend towards younger and younger people developing diabetes, a trend that is very worrisome for future generations. The majority of the 382 million people with diabetes are aged between 40 and 59, and 80% of

them live in low- and middle-income countries like India. In South-East Asia region 72.1 million (8.3%) are suffering from diabetes which will increase to a staggering figure of 123 million (9.4%) by 2035 (Figure 2.13).

FIGURE 2.13: IDF REGIONS AND GLOBAL PROJECTIONS OF THE NUMBER OF PEOPLE WITH DIABETES (20-79 YEARS), 2013 AND 2035



IDF REGION	2013 MILLIONS	2035 MILLIONS	INCREASE %
Africa	19.8	41.4	109%
Middle East and North Africa	34.6	67.9	96%
South-East Asia	72.1	123	71%
South and Central America	24.1	38.5	60%
Western Pacific	138.2	201.8	46%
North America and Caribbean	36.7	50.4	37%
Europe	56.3	68.9	22%
World	**381.8**	**591.9**	**55%**

All types of diabetes are on the increase, in particular type 2 diabetes: the number of people with diabetes will increase by 55% by 2035. An additional 21 million cases of high blood glucose in pregnancy are estimated to contribute to the global burden of diabetes. That is equivalent to 17% of live births to women in 2013 that had some form of high blood glucose in pregnancy. In human as well as financial terms, the burden of diabetes is enormous, provoking 5.1 million deaths and taking up some USD 548 billion dollars in health spending (11% of the total spent worldwide) in 2013. The ICMR-INDIAB national study reported that there are 62.4 million people with T2DM and 77 million people with pre-diabetes in India (Anjana et al, 2011). These numbers are projected to increase to 101 million by the year 2030.

India is in the midst of an ever increasing epidemic of diabetes mellitus. T2DM accounts for more than 90% of all patients with diabetes in India. The ICMR study (1972-1975) carried out in 6 representative cities was the first systematic nationwide collaborative study on the prevalence of diabetes. The prevalence of diabetes was found to be 2.8% in rural and 5% in urban population above age of 40 years (Sen, 1983). A series of epidemiological studies carried out by the Diabetes Research center and the Madras Diabetes Research Foundation in Chennai showed that the prevalence of impaired glucose tolerance (IGT) increased from 5.2% and 2.0% respectively from 1984 to 14.3% and 10.2% respectively in 2004 among urban Indians. The CURES showed that while the diabetes prevalence continues to rise in urban India that of IGT has begun to decrease (Mohan et al, 2006). ICMR-INDIAB study, a national study to determine the prevalence of diabetic and prediabetic in India, reported a prevalence of 10.4% in Tamilnadu, 8.4% in Maharashtra, 5.3% in Jharkhand, 13.6% in Chandigarh (Anjana et al, 2011). The Prevalence of Diabetes in India Study (PODIS) carried out in 77 centers reported a standardized prevalence rate in the total, urban and rural population of 4.3%, 5.9% and 2.7% respectively (Sadikot et al, 2004).

Gujarati people are presumed to have high prevalence of CAD risk factors: Obesity, metabolic syndrome, diabetes, hypertension, dyslipidemia because of traditional Gujarati food and less physically active lifestyle. Gujarat is having the second highest number of diabetics in the country after Tamil Nadu with around 10 per cent of the total diabetic population that is close to 50 lakh. Koria et al (2013) reported the prevalence of diabetes in Ahmedabad city as 7.33% with majority of the study population (53.64%) between 45-60 years. While in rural Ahmedabad the prevalence was found to be 2.6%, with highest (6.7%) prevalence in age group 21-30 and 51-60 years. Diabetes was significantly associated with hypertension, family history of diabetes and sedentary working (Agarwal et al., 2014). In a comparative study Iyer et al. (2011) reported the prevalence of diabetes and hypertension to be higher in Godhra (19% and 36% respectively) as compared to Vadodara city (12% and 24% respectively) among middle aged subjects (mean age of 43.2 + 15.8 yrs). Family history of diabetes, high BMI, waist circumference, hypertension, physical inactivity, smoking, alcohol, tobacco abuse, low intake of fruits and vegetables and low intake of green leafy vegetables emerged as predictor variables. Another study among women (30-65 years) in Vadodara city reported the prevalence of diabetes to be 6.1% and about 25.7% had insulin resistance (HOMA 2) (Elayath & Iyer, 2012).

Thus the data reveals that there is a need to minimize the negative impact of diabetes in human health through early recognition, diagnosis and management of risk factors as well as of disease. The preventive strategies for T2DM included: 1) Primary prevention which refers to the prevention of the onset of the disease 2) Secondary prevention refers to early diagnosis and treatment of the disease so as to prevent complications and 3) Tertiary prevention which refers to limiting physical disability resulting from the complications and institution of rehabilitation measures. Genetic and environmental factors are of equal importance in causation of diabetes. The modifiable environmental risk factors like obesity, unhealthy dietary habits (high carbohydrate, fat, low fibre diet), lack of physical activity, smoking, excess alcohol consumption should targeted and altered in a favourable manner (Marble, 1971). A sustained intervention, at both an individual and public health level, will be necessary to achieve this. Doctors and researchers still have a great deal to learn about the pathogenesis of type 2 diabetes and how best to use the therapies available, although great progress has been made in clarification of their modes of action. In the coming years hopefully the knowledge and approaches needed to reduce the global harm of type 2 diabetes, not only through management of the disorder more effectively with a combination of non-pharmacological and pharmacological approaches, but also through prevention of the disease and identification of new strategies to directly target its complications will be generated (Kahn et al., 2014).

More recently, animal and human studies have suggested that vitamin D is a potential modifier of diabetes risk. Vitamin D has been shown to play an important role in the disorders of glucose and insulin metabolism. It has been observed that vitamin D with calcium supplementation produces a significant decrease in fasting glucose and insulin resistance in patients with impaired fasting glucose and vitamin D supplementation has been suggested to have a role in improving and even preventing diabetes in humans (Stene et al., 2000; EURODIAB study, 1999). This evidence gives hope for a new avenue of primary prevention for both type-1 and type-2 diabetes mellitus.

C) VITAMIN D AND TYPE-II DIABETES MELLITUS

Type 2 diabetes can be prevented by adoption of a healthy diet and lifestyle. However, many people have difficulties complying with dietary and lifestyle changes, so the quest for more convenient ways to reduce diabetes risk continues. Vitamin D has been the focus of much interest in this regard (Muscogiuri et al., 2014). Due to the presence of both 1-α-hydroxylase and VDR in pancreatic β cells, vitamin D is important for insulin synthesis and release. Moreover, vitamin D is also involved in insulin sensitivity by controlling calcium flux through the membrane in both β cells and peripheral insulin-target tissues (Wolden-Kirk et al., 2011). Pancreatic islets have both VDR and vitamin D-dependent calcium-binding proteins (CaBP), suggesting a role for vitamin D in insulin secretion. Its effect on the β cells is by increasing insulin response to glucose stimulation, but it does not affect basal insulin secretion (Bourlon et al., 1999). Vitamin D deficiency may also impair insulin secretion through its associated increase in PTH levels. However whether insulin secretion is influenced by the direct action of vitamin D through its receptor, or through changes in calcium, or PTH, is a matter of ongoing studies. Epidemiological studies in human beings consistently show that low blood concentrations of 25(OH)D, a marker of vitamin D status are associated with an increased risk of T2DM (Table 2.6). These studies, however, cannot distinguish causation from association because of possible uncontrolled confounding and reverse causation.

Studies of genetic variants that specifically affect 25(OH)D concentration can provide another route to draw causal inference. The results of a recent mendelian randomisation study made use of the inter individual variability in circulating 25(OH)D concentrations caused by common genetic variants and tried to estimate the unconfounded, causal association between 25(OH)D concentration and risk of T2DM. Mendelian randomisation analysis requires genetic variants to be related to a main exposure, but not to potential confounders. Because allocation of genetic variants at conception is independent of behavioural and environmental factors, their associations with disease are less likely to be affected by confounding or reverse causation. On the basis of the expression of four genetic variants that affect plasma 25(OH)D concentrations, single nucleotide polymorphisms of *DHCR7* (related to vitamin D synthesis), *CYP2R1* (hepatic 25-hydroxylation), *DBP* (also known as *GC*; transport), and *CYP24A1* (catabolism), Ye and colleagues reported that for each 25 nmol/L reduction in genetically determined 25(OH)D concentration, the odds ratio for T2DM was 1.01 (95% CI 0.75–1.36;

TABLE 2.6: HUMAN STUDIES THAT ASSOCIATE VITAMIN D WITH T2DM

Reference	Study design	Subjects included	Main outcome
Mattila et al., 2007	Cohort (Mini-Finland Health Survey)	4097 individuals followed-up for 17 years	The highest *vs* the lowest serum 25OHD: RR = 0.70; 95% CI = 0.42–1.16); *p* for trend = 0.07).
Grimnes et al., 2010	Cohort (Tromsø Study)	4157 non-smokers & 1962 smokers followed-up for 11 years	Baseline serum 25OHD was inversely associated with type 2 diabetes.
Pittas et al., 2006	Cohort (Nurses' Health Study)	83,779 women followed-up for 20 years	The highest *vs* the lowest category of vitamin D intake from supplements: RR = 0.87; 95% CI = 0.75–1.00; *p* for trend = 0.004).
Knekt et al., 2008	Nested case-control	412 cases and 986 controls	The highest *vs* the lowest quartiles of serum 25OHD: OR = 0.28 (95% CI = 0.10–0.81) in men and OR = 1.14 (95% CI = 0.60–2.17) in women.
Laaksonen et al., 2010	Meta-analysis	Polled data from 2 cohort studies with 8627 individuals aged 40–79 years.	The highest *versus* the lowest serum 25OHD: RR = 0.66; 95% CI = 0.50–0.87.
Liu et al., 2010	Cohort (Framingham Study)	3066 (1402 men and 1664women) followed-up for 7 years	A higher 25OHD serum levels is associated with decreased risk of type 2 diabetes.
Pittas et al., 2010	Nested case-control	608 cases and 559 controls.	The highest *vs* the lowest serum 25OHD quartile: OR= 0.52; 95% CI = 0.33–0.83.
Tahrani et al., 2010	Cross-sectional	210 individual aged more than 40	VDD was more common in diabetic compared to control.
Dalgard et al., 2011	Cross-sectional	668 individuals aged70–74 years	Serum 25OHD < 50 nmol/L doubled the risk of newly diagnosed type 2 diabetes.
Gagnon et al., 2011	Cohort (AusDiab study)	5200 individuals; mean age 51 years.	Each 25 nmol/L increment in serum 25OHD was associated with a 24% reduced risk of type 2 diabetes (OR = 0.76;95% CI = 0.63–0.92).
Brock et al., 2011	Cross-sectional	2465 subjects.	Serum 25OHD ≥ 80 nmol/L *versus* ≤37 nmol/L in Caucasians: OR = 0.5; 95% CI = 0.1–0.7.
Mitri et al., 2011	Systematic review of 7 observational Cohort studies	238,424 individuals aged 30–75 years	Vitamin D intake >500 *vs* <200 UI: risk of T2DM 13% lower. Serum 25OHD level (>25 ng/mL *versus* <14 ng/mL):risk of T2DM 43% lower.

p=0.94). This null result is in notable contrast to the association noted in observational studies. The researchers also meta-analysed 22 prospective observational studies and report a pooled relative risk of 1.21 (95% CI 1.16–1.27; $p=7.3\times10^{-19}$). Taken together, these findings suggest that the inverse association for 25(OH)D concentration and type 2 diabetes is not causal and further investigations to identify causal factors that might increase 25(OH)D concentration and also reduce the risk of type 2 diabetes are required (Ye et al., 2015). Physical activity might be a strong confounder, because it reduces risk of T2DM but is independently related to sunlight exposure and therefore vitamin D status. Existing observational studies generally recorded self-reported physical activity, which is subject to errors and residual confounding. Similarly adiposity is usually estimated with BMI, which does not include sufficient information about fat distribution. This issue could be important because low 25(OH)D concentration might be especially associated with central adiposity.

One of the major challenges is to find strategies for the early detection of individuals at high risk of type 2 diabetes. There are few studies addressing the issue of whether low serum 25(OH)D concentrations can predict the development of type 2 diabetes. Studies have shown that low serum levels of IGF-binding protein-1(IGFBP-1) in men and women with normal glucose tolerance (NGT) strongly predict risk of T2DM and prediabetes (impaired fasting glucose, impaired glucose tolerance or the two combined) at a follow-up 8–10 years later (Lewitt et al., 2008; Lewitt et al.,2010). In addition, a combination of high concentrations of 25(OH)D and IGF-1 has been associated with a low risk of the metabolic syndrome (Hypponen et al., 2008).

Deleskog et al (2012), also investigated if serum 25(OH)D concentrations would predict the development of prediabetes and T2DM, either on their own or when combined with serum concentrations of IGF-1 or IGF-binding protein-1, which may interact with 25(OH)D among participants aged 35–56 years without known T2DM. Participants who had prediabetes or T2DM at follow-up 8–10 years later were selected as cases; these were then age and sex matched to controls with normal glucose tolerance (NGT) at both baseline and follow-up. High serum 25(OH)D concentrations predicted a reduced risk of T2DM in individuals with prediabetes (OR 0.52, 95% CI 0.30, 0.90), but not NGT. In both genders, progression from prediabetes to diabetes was reduced by about 25% per 10 nmol/l increase in 25(OH)D. There were no significant interactions between 25(OH)D and IGFBP-1 or IGF-1 in terms of risk of diabetes. However IGFBP-1 was a better indicator than 25(OH)D thus suggesting that

vitamin D supplementation should be evaluated for the prevention of T2DM in prediabetic individuals.

Deficient vitamin D status has also been associated with decreased insulin secretion and insulin resistance, the hallmarks of type 2 diabetes mellitus. To test the hypothesis that low plasma 25(OH)D is associated with increased risk of type 2 diabetes in the general population 9841 white individuals from the Copenhagen City Heart Study were studied in a prospective cohort study and followed for up to 29 years. Researchers observed an association of low plasma 25(OH)D with increased risk of type 2 diabetes (log-rank trend, $p=2x10^{-7}$ and $p=4x10^{-10}$) which was also substantiated in a meta-analysis. The multivariable adjusted hazard ratios of type 2 diabetes were 1.22 (95% CI 0.85–1.74) for 25(OH)D <5 vs ≥20 µg/L and 1.35 (1.09 –1.66) for lowest vs highest quartile. Also, the multivariable adjusted hazard ratio of type 2 diabetes for a 50% lower concentration of 25(OH)D was 1.12 (1.03–1.21); the corresponding hazard ratio for those ≤58 years old was 1.26 (1.15– 1.41). Finally, in a meta-analysis of 16 studies, the odds ratio for type 2 diabetes was 1.50 (1.33–1.70) for the bottom vs top quartile of 25(OH)D (Afzal et al., 2013).

Vitamin D has got various pleiotropic effects such as suppression of cell mediated immunity, regulation of cell proliferation, stimulation of neurotropic factors like nerve growth factor, neurotropin, suppression of RAAS, reduction of albuminuria, immunomodulatory, anti‑inflammatory and antiangiogenic effects. Thus it is implicated in many ways in the pathogenesis of diabetic retinopathy, neuropathy and nephropathy. Robinson et al (2011) in his study among older women found that vitamin D were significantly lower in those diabetics who had microvascular complications. In another study the mean vitamin D_3 concentrations fell with increasing severity of diabetic retinopathy (Aksoy et al., 2000). Soderstorm et al, (2012) reported that vitamin D insufficiency was associated with the adjusted composite measure of neuropathy. Recently Bajaj et al., (2014) in a cross‑sectional case‑control study among 18 T2DM patients (18‑70 years), evaluated the correlation of vitamin D levels with microvascular complications. The mean vitamin D was lower in T2DM than healthy subjects (19.05 vs. 27.19 ng/ml). Prevalence of VDD and insufficiency was found to significantly higher in diabetics when compared to healthy subjects (p=0.0001). VDD was found to be significantly associated with neuropathy (χ2=5.39, p=0.020), retinopathy, (χ2=6.6, p=0.010) and nephropathy (χ2=10. 52, p=0.001). Lower levels of vitamin D were found to be associated with increasing prevalence of combinations of

microvascular complications namely neuropathy with retinopathy (p=0.036), neuropathy with nephropathy (p=0.029), retinopathy with nephropathy (p=0.022) and neuropathy with retinopathy with nephropathy (p=0.0001).

The mechanistic connection between T2DM and vitamin D is thought to be the immunomodulatory actions of the later (Mathieu et al., 2005). Since the activation of inflammatory pathways interferes with normal metabolism and disrupts proper insulin signaling, it is also hypothesized that vitamin D could influence glucose homeostasis by modulating inflammatory response. However, human studies investigating the impact of vitamin D supplementation on inflammatory biomarkers of subjects with or at high risk of developing T2DM are scarce and have generated conflicting results. Based on available clinical and epidemiological data, the positive effects of vitamin D seem to be primarily related to its action on insulin secretion and sensitivity and secondary to its action on inflammation (Chagas et al., 2012). The beneficial effects of vitamin D on T2DM are also less definitive, as revealed in a systematic review of longitudinal cohort studies reporting associations between vitamin D status and incident T2DM and randomized controlled trials of vitamin D supplementation (Mitri et al., 2011). The review authors concluded that 'lower vitamin D status and intake were associated with higher risk of incident T2DM in observational studies; however, the effect of vitamin D supplementation on glycemic outcomes was not evident in small underpowered trials or post hoc analyses of larger trials. Thus the available data is insufficient to support the contention that T2DM can be improved by raising vitamin D concentration. Future studies specifically designed to investigate the role of vitamin D on T2DM using inflammation as the main outcome are urgently needed in order to provide a more robust link between vitamin D, inflammation and T2DM.

A systematic review including fifteen trials to systematically review the evidence for the effect of vitamin D supplementation on glycaemia, insulin resistance, progression to diabetes and complications of diabetes revealed that combining all studies, no significant improvement was seen in fasting glucose, HbA1c or insulin resistance in those treated with vitamin D compared with placebo. For patients with diabetes or impaired glucose tolerance, meta-analysis showed a small effect on fasting glucose (-0.32 mmol ⁄ l, 95% CI -0.57 to -0.07) and a small improvement in insulin resistance (standard mean difference -0.25, 95% CI -0.48 to -0.03). No effect was seen on glycated haemoglobin in patients with diabetes and no differences were seen for any outcome in patients with normal fasting glucose. Insufficient

data were available to draw conclusions regarding micro- or macrovascular events; two trials failed to show a reduction in new cases of diabetes in patients treated with vitamin D (George et al., 2012).

However many epidemiological studies have shown favourable results for vitamin D supplementation as a potential and inexpensive therapy not only to decrease the risk, but also to improve glycemic parameters in T2DM patients. In subjects at high risk of type 2 diabetes and with baseline serum 25OHD level of 26.5 nmol/L, vitamin D supplementation (2000 UI once daily) was associated with improved β cell function in adults (Mitri et al., 2011). In a randomized, controlled, double-blinded intervention study, insulin resistant and vitamin D deficient (serum 25OHD < 50 nmol/L) subjects supplemented with vitamin D (4000 UI, daily, for 6 months) had improved serum 25OHD level, insulin sensitivity and insulin resistance when compared to controls, while no effects were observed on lipid profile, C-reactive protein and insulin secretion (Von Hurst et al., 2010). Similarly, in another randomized controlled trial, type 2 diabetes patients with baseline serum 25OHD concentration <50 nmol/L treated with a single dose of vitamin D (100,000 or 200,000 UI) had lower systolic blood pressure than controls, but HOMA-IR was significantly improved only in subjects who received the highest dose (Witham et al., 2010).

Experimental studies have also demonstrated several antihypertensive and vascular protective effects of vitamin D, such as suppression of the renin angiotensin aldosterone system, beneficial modulation of classic cardiovascular risk factors, and anti-atherosclerotic properties including improvements of endothelial function. Additional neuroprotective actions of vitamin D have also been reported. In line with this, epidemiological studies have largely shown that vitamin D deficiency is an independent risk factor for arterial hypertension and strokes. Data from randomized controlled trials (RCTs) are, however, limited and less promising, with currently no confirmation that vitamin D reduces stroke incidence. Whereas some RCTs suggest that vitamin D supplementation might modestly reduce blood pressure, this has not been consistently observed in all studies. It is, therefore, premature to recommend vitamin D supplementation for the prevention and treatment of arterial hypertension and stroke. Nevertheless, the fact that patients with arterial hypertension and cerebrovascular disease are at a relatively high risk of vitamin D deficiency, and therewith associated musculoskeletal diseases can serve as a rationale for the evaluation, prevention and treatment of vitamin D deficiency in these patients (Kienreich et al., 2013).

Similarly vitamin D status has been linked to the risk of cardiovascular disease (CVD). 19 independent studies with 6123 CVD cases in 65,994 participants were included for a meta-analysis. In a comparison of the lowest with the highest 25(OH)D categories, the pooled relative risk was 1.52 (95% confidence interval, 1.30–1.77) for total CVD, 1.42 (95% confidence interval, 1.19–1.71) for CVD mortality, 1.38 (95% confidence interval, 1.21–1.57) for coronary heart disease, and 1.64 (95% confidence interval, 1.27–2.10) for stroke. These associations remained strong and significant when analyses were limited to studies that excluded participants with baseline CVD and were better controlled for season and confounding. A fractional polynomial spline regression analysis to assess the linearity of dose–response association between continuous 25(OH)D and CVD risk revealed that the CVD risk increased monotonically across decreasing 25(OH)D below ≈60 nmol/L, with a relative risk of 1.03 (95% confidence interval, 1.00–1.06) per 25-nmol/L decrement in 25(OH)-vitamin D (Wang et al., 2012).

Thus though the data on the potential links between vitamin D and T2DM and its related co-morbidities (CVD risk and hypertension) is unclear, there is a strong possibility of vitamin D playing a crucial role in its pathophysiology. Hence preventative interventions will prove to be more cost-effective than the medications for diabetes care and so there is significant motivation to find ways to help reduce the progression as well as possibly delay the onset of T2DM with the novel strategy involving vitamin D.

IV. STRATEGIES TO COMBAT VDD

A growing body of evidence, implicating hypovitaminosis D as a risk factor for many diseases right from conception throughout lifespan, implies that awareness and management of widespread vitamin D deficiency may fetch profound future health benefits. When environmental, social, or physiological circumstances prevent adequate exposure to sunlight, dietary compensation must occur to maintain serum 25(OH)D levels. For those countries in which high levels of fatty fish are not consumed, the richest natural source of vitamin D, the only alternative to increasing their exposure to natural or artificial UV light is to fortify their food or to use vitamin supplements. But what is the best strategy to increase vitamin D intake and to improve 25(OH)D status in vulnerable populations? Promotion of supplementation targeted to the risk groups is a primary consideration. Food fortification is another consideration, but this strategy has a tendency to only benefit the general population and to not improve the intake of specific groups at risk (Nowson & Margerison,2002; Serra-Majem, 2004), while Nutrition Health Education Program for targeted audience can improve both nutrition and health status.

A) PHARMACOLOGICAL THERAPY

Vitamin D is a good example of a vitamin that is finding its rightful place in preventive medicine. The focus of integrative medicine is on supporting health and improving function. If the functional integrity of the body can be improved, spontaneous healing will occur and fewer drugs will be necessary and related consequences may even be prevented.

To prevent VDD, the Institute of Medicine (IOM) 2011, recommends, that infants should immediately receive a daily supplementation of vitamin D of 400 IUs during the first year of life. Individuals between 1 and 70 years should receive 600 IU of vitamin D daily and adults >70 years should receive a daily dose of 800 IU vitamin D. The serum 25(OH)D level increases for every 100 IU/day by~0.6–1.0 ng/mL. The doses recommended by IOM will likely increase the 25(OH)D level to 20 ng/mL, which they considered to be adequate for bone health, but not to levels >30 ng/mL, as recommended by the Endocrine Society.

Keeping this in mind the Endocrine Society's Practice Guidelines recommended supplementation and treatment strategies for patients with vitamin D deficiency depending on

age and underlying medical conditions. For vitamin D deficient infants 0–1 years old, a treatment with 2000 IU/day of vitamin D_2 or vitamin D_3 or with 50,000 IU of vitamin D_2 or vitamin D_3 once weekly for 6 weeks was suggested, followed by maintenance therapy of 400–1000 IU/day. Vitamin D deficient children aged 1–18 years who are vitamin D deficient, treatment with 2000 IU/day of vitamin D_2 or vitamin D_3 or with 50,000 IU of vitamin D_2 or vitamin D_3 once a week, both for at least 6 weeks, was suggested, followed by maintenance therapy of 600–1000 IU/day is suggested. Vitamin D deficient adults should be treated with 50,000 IU of vitamin D_2 or vitamin D_3 once a week for 8 weeks or with ~6000 IU/day of vitamin D_2 or vitamin D_3, followed by maintenance therapy of 1500–2000 IU/day. In obese patients, patients with malabsorption syndromes, and patients on medications affecting vitamin D metabolism, two to three times higher doses are (at least 6000–10,000 IU/day) of vitamin D to treat vitamin D deficiency are recommended, followed by maintenance therapy of at least 3000–6000 IU/day (Holick et al., 2011). The Endocrine Society's Clinical Practice Guidelines also recommended sensible sun exposure, which for most individuals is the main physiological source of vitamin D, and encouraged taking a daily vitamin D supplement to ensure adequate 25(OH)D levels. The supplementation details given by both IOM and the Endocrine society are given in Table 2.7.

Vitamin D supplements in various strengths are available in developed countries as over the counter medicines. In India vitamin D is available in selective doses. Information was collected from 10 pharmacists, from an upper middleclass locality of Delhi. Supplements commonly available were- D_3 (cholecalciferol), $1,25(OH)_2D_3$ and 1 alpha hydroxylvitamin D_3 (alfacalcidol). Some formulations had calcium too. Multivitamin formulations were also available and contained about 400 IU of D_3. None of the pharmacists had heard of D_2 supplements. D_3 supplement of 60,000 IU is the highest selling one and is available in powder form in sachets or as oil-based capsules. Recommended dose on the label is once per week. The sachets indicate that half a sachet per week may also be taken. According to some pharmacists, many clinicians recommended 1 sachet daily for 10 days, followed by 1 sachet/week for 5–6 weeks to 1 sachet/week forever. The other vitamin D supplements mentioned were present in lower doses (0.25 μg or 500 IU) Calcium supplementation is generally recommended with vitamin D intake by the clinicians. The cost of a single dose of 60,000 IU of vitamin D_3 was about INR 30 (Ritu & Gupta, 2014).

TABLE 2.7: RECOMMENDATIONS OF THE INSTITUTE OF MEDICINE AND THE ENDOCRINE SOCIETY PRACTICE GUIDELINES FOR DAILY VITAMIN D SUPPLEMENTATION

Life Stage Group	IOM Recommendations		Endocrine Society's Recommendations	
	RDA	UL	Daily Allowance (IU/day)	UL
Infants				
0 to 6 months		1000 IU (25 µg)	400-1000	2000
6 to 12 months		1500 IU (38 µg)	400-1000	2000
Children				
1–3 years	600 IU (15 µg)	2500 IU (63 µg)	600-1000	4000
4–8 years	600 IU (15 µg)	3000 IU (75 µg)	600-1000	4000
Males				
9-13 years	600 IU (15 µg)	4000 IU (100 µg)	600-1000	4000
14-18 years	600 IU (15 µg)	4000 IU (100 µg)	600-1000	4000
19-30 years	600 IU (15 µg)	4000 IU (100 µg)	1500-2000	10,000
31-50 years	600 IU (15 µg)	4000 IU (100 µg)	1500-2000	10,000
51-70 years	600 IU (15 µg)	4000 IU (100 µg)	1500-2000	10,000
>70 years	800 IU (20 µg)	4000 IU (100 µg)	1500-2000	10,000
Females				
9-13 years	600 IU (15 µg)	4000 IU (100 µg)	600-1000	4000
14-18 years	600 IU (15 µg)	4000 IU (100 µg)	600-1000	4000
19-30 years	600 IU (15 µg)	4000 IU (100 µg)	1500-2000	10,000
31-50 years	600 IU (15 µg)	4000 IU (100 µg)	1500-2000	10,000
51-70 years	600 IU (15 µg)	4000 IU (100 µg)	1500-2000	10,000
>70 years	800 IU (20 µg)	4000 IU (100 µg)	1500-2000	10,000
Pregnancy				
14-18 years	600 IU (15 µg)	4000 IU (100 µg)	600-1000	4000
19-30 years	600 IU (15 µg)	4000 IU (100 µg)	1500-2000	10,000
31-50 years	600 IU (15 µg)	4000 IU (100 µg)	1500-2000	10,000
Lactation*				
14-18 years	600 IU (15 µg)	4000 IU (100 µg)	600-1000	4000
19-30 years	600 IU (15 µg)	4000 IU (100 µg)	1500-2000	10,000
31-50 years	600 IU (15 µg)	4000 IU (100 µg)	1500-2000	10,000

*Mother's requirement 4000-6000 (mother's intake for infant's requirement if infant not receiving 400 IU/day)

IU=International Units; RDA=Recommended Dietary Allowance; UL=Upper Intake Level

Source: Wacker & Holiack, 2013

VDD is thought to be common among pregnant women, and has been found to be associated with an increased risk of pre- eclampsia, gestational diabetes mellitus, preterm birth, and other tissue-specific conditions. However, there is no evidence of an effect on either maternal pre-eclampsia in pregnant women receiving vitamin D plus calcium supplementation or on the risk of having a low birth weight infant (less than 2500 g) in pregnant women receiving vitamin D supplementation alone compared with pregnant women not receiving supplementation or receiving a placebo. Pregnant women who received vitamin D supplementation alone had significantly higher serum concentrations of 25-hydroxyvitamin D at term compared with those not receiving supplementation or placebo as reported in the Guideline: Vitamin D supplementation in pregnant women by WHO (2012). Thus, vitamin D supplementation is not recommended during pregnancy to prevent the development of pre-eclampsia and its complications (*strong recommendation*). In addition, due to the limited evidence currently available to directly assess the benefits and harms of the use of vitamin D supplementation alone in pregnancy for improving maternal and infant health outcomes, the use of this intervention during pregnancy as part of routine antenatal care is also not recommended (*conditional recommendation*). It suggested that research priorities to improve the body of evidence on the benefits or harms of this intervention among pregnant women should be identified, at the basic, epidemiological and programmatic level.

VDD is clearly being implicated in virtually every disease state ranging from cardiovascular disease to autoimmune disorders. Although a clear association exists between several pathology states and vitamin D deficiency it remains to be seen if vitamin D supplementation can slow down, halt or even reverse the disease processes with which they are associated. Few of the vitamin D supplementation studies are listed below.

In an open‐label study in 137 subjects with prediabetes, the effect of 12 months of vitamin D supplementation on glycemic parameters and progression of prediabetes to diabetes in an ethnically homogeneous Kashmiri population was evaluated. Subjects were randomized to receive in addition to standard lifestyle measures, either vitamin D 60,000 IU weekly for 4 weeks and then 60,000 IU monthly (*n*=69) or no vitamin D (*n*=68). Fasting plasma glucose (FPG), 2h plasma glucose and A1C levels were estimated at 0, 6 and 12 months. At 12 months, A1C levels were significantly lesser (5.7%±0.4%) in the vitamin D supplemented group when compared with non-vitamin D supplemented (6.0%±0.3%). Similarly, FPG (97±7) and 2h plasma glucose (132±16) were significantly less in vitamin D supplemented

group as compared with non-vitamin D supplemented group (FPG =116±6 and 2h plasma glucose=157±25) at 12 months. Nine out of 65 in non-vitamin D supplemented and seven out of 64 in the vitamin D supplemented group developed diabetes (Kuchay et al., 2015).

Vitamin D supplementation plays a beneficial role in hypertension too. 100 hypertensive patients (group I) were given conventional antihypertensive drugs while another 100 patients (group II), in addition, were supplemented with Vitamin D3 (33,000 IU, after every 2 weeks, for 3 months). Besides diastolic and systolic blood pressure, serum calcium, phosphorous, alkaline phosphatase, albumin, albumin-corrected calcium, and 24 h urinary creatinine levels were estimated in both the groups before the start of treatment and after 3 months. Vitamin D supplementation showed a more significant decrease in systolic blood pressure. This group also showed a significant increase in serum calcium as well as albumin-corrected calcium with a decrease in phosphorous. Results of the study confirmed that vitamin D supplementation has a role in reducing blood pressure in hypertensive patients and that it should be supplemented with the antihypertensive drugs (Goel & Lal, 2011).

Similarly the effects of 8 weeks of supplementation with vitamin D_3 (cholecalciferol) and calcium on blood pressure and biochemical measures of bone metabolism were studied among 148 elderly women (mean±SD age, 74±61 yr) with 25(OH)D level below 50 nmol/L. They received either1200 mg calcium plus 800 IU vitamin D_3 or 1200 mg calcium/day. Compared with calcium, supplementation with vitamin D_3 and calcium resulted in an increase in serum 25(OH)D of 72% (p<0.01), a decrease in serum PTHof 17% (p=0.04), a decrease in systolic blood pressure (SBP) of 9.3% (p=0.02), and a decrease in heart rate of 5.4% (p=0.02). Sixty subjects (81%) in the vitamin D3 and calcium group compared with 35 (47%) subjects in the calcium group showed a decrease in SBP of 5 mm Hg or more (p= 0.04). No statistically significant difference was observed in the diastolic blood pressures of the calcium-treated and calcium- plus vitaminD3-treated groups (p=0.10). Thus it was concluded that a short-term supplementation with vitamin D3 and calcium is more effective in reducing SBP than calcium alone. Also that inadequate vitamin D3 and calcium intake could play a contributory role in the pathogenesis and progression of hypertension and cardiovascular disease in elderly women (Pfeifer et al., 2001).

19 independent studies with 6123 CVD cases in 65,994 participants were included for a meta-analysis. In a comparison of the lowest with the highest 25(OH)D categories, the pooled

relative risk was 1.52 (95% confidence interval, 1.30–1.77) for total CVD, 1.42 (95% confidence interval, 1.19–1.71) for CVD mortality, 1.38 (95% confidence interval, 1.21–1.57) for coronary heart disease, and 1.64 (95% confidence interval, 1.27–2.10) for stroke. These associations remained strong and significant when analyses were limited to studies that excluded participants with baseline CVD and were better controlled for season and confounding. A fractional polynomial spline regression analysis was used to assess the linearity of dose–response association between continuous 25(OH)D and CVD risk. The CVD risk increased monotonically across decreasing 25(OH)D below ≈60 nmol/L, with a relative risk of 1.03 (95% confidence interval, 1.00–1.06) per 25-nmol/L decrement in 25(OH)D. Thus this meta-analysis demonstrated a generally linear, inverse association between circulating 25(OH)D and risk of CVD (Wang et al., 2012).

VDD is highly prevalent in patients with obesity, and many studies have demonstrated the significant effect of calcitriol on adipocytes. A review on the relationship between vitamin D and obesity suggested that genetic studies provide opportunities to determine which proteins link vitamin D to obesity pathology. However, some studies demonstrated no effect of vitamin D on weight change and energy expenditure and little improvement in cardiovascular risks with cholecalciferol and cholecalciferol supplementation showed no effect on cytokines and markers of inflammation in obese subjects. Therefore, further investigation of vitamin D supplementation in obese patients is needed (Khanh & Thi, 2013).

Similarly a systematic review and meta-analysis conducted on high quality, randomized controlled trials (RCTs) that had supplemented vitamin D without imposing any caloric restriction also concluded that vitamin D supplementation did not decrease measures of adiposity in the absence of caloric restriction. A potential confounding by age and gender was encountered. 12 studies which provided the required data for the meta-analysis reporting either body weight, body mass index (BMI), fat mass (FM), percentage fat mass (%FM) or lean body mass (LBM) were included.. Vitamin D supplementation did not influence the standardized mean difference (SMD) for body weight, FM, %FM or LBM. A small but non-significant decrease in BMI (SMD = −0.097, 95% confidence interval: [−0.210, 0.016], p = 0.092) was observed. Meta-regression confirmed that neither the absolute vitamin D status achieved nor its change from baseline influenced the SMD of any obesity measure. However, increasing age of the subjects predicted a shift in the SMD for FM towards the placebo

treatment, whereas a greater percentage of women in these studies favoured a decrease in FM following vitamin D (Pathak et al., 2014)

To assess the beneficial and harmful effects of vitamin D supplementation for prevention of mortality in healthy adults and adults in a stable phase of disease a systematic review of 56 randomised trials with 95,286 participants (age 18-107 years) was conducted. The mean proportion of women was 77%. Vitamin D decreased mortality in all 56 trials analysed together (5,920/47,472 (12.5%) vs 6,077/47,814 (12.7%); RR 0.97 (95% confidence interval (CI) 0.94 to 0.99); p=0.02; I_2 = 0%). When different forms of vitamin D were assessed in separate analyses, only vitamin D_3 decreased mortality (4,153/37,817 (11.0%) vs 4,340/ 38,110 (11.4%); RR 0.94 (95% CI 0.91 to 0.98); p=0.002; I_2 = 0%; 75,927 participants; 38 trials). Vitamin D_2, alfacalcidol and calcitriol did not significantly affect mortality. Vitamin D_3 statistically significantly decreased cancer mortality (RR 0.88 (95% CI 0.78 to 0.98); p=0.02; I_2 = 0%; 44,492 participants; 4 trials) (Bjelakovic et al., 2011).

The above studies do confirm that vitamin D supplementation is and should be considered as one of the novel strategies toward prevention and control of T2DM, its related co-morbidities and many other clinical conditions. However for every disease the results are conflicting. This calls for in-depth and well managed randomised interventional trials to be conducted to study the association of vitamin D status with the diseases and also to study the impact of vitamin D supplementation in favourably altering the medical problems and various biochemical parameters.

B) FOOD BASED APPROACH

Vitamin D is an essential ingredient in everyone's daily diet unless they get a significant amount of sun exposure without sunscreen. Fortification-together with other strategies-can be useful tool for increasing vitamin D intake in diet and ultimately the vitamin D status. Fortified foods are those to which one or more essential nutrients have been added, whether or not it is normally contained in the food, for the purpose of preventing or correcting a demonstrated deficiency. Food fortification is a much more economically viable approach compared to vitamin D supplementation. While the cost of fortified food items will be more than unfortified foods, it will be lower than supplementation. Food fortification may be a better choice compared to supplementation strategies, especially when targeting those who need it the most—women (including non-pregnant, pregnant and lactating), infants, children

and senior citizens. Adaptability to fortified food by the consumers is much better than to supplementation. Food fortification requires relatively less change in food habits and preferences, leading to better efficacy of fortification programs, lowered cost to the consumer and a larger profit to the food manufacturers. However it is critical to identify an appropriate food vehicle, usually a food staple that would preferentially increase the intake of the target group. Use of staple foods such as *bread* flour, rice, milk, *etc.*, for fortification may have certain advantages over other fortification matrices.

At present, the number and variety of vitamin D fortified foods available on the market differs significantly between countries. The relative deficit of vitamin D fortified foods can be partly attributed to the country-specific policies on food fortification which are not yet unified. Most individual countries have their own national policies. Mandatory or voluntary fortification and fortified foods, which the consumer needs, also have to comply with nutritional, regulatory, food safety and technical issues. In the past, there has been some confusion concerning the specific regulations that govern the addition of vitamin D to foods in the United States. These regulations have been confused with the fortification or nutritional quality guidelines for foods generally recognized as safe (GRAS) (US Food and Drug Administration). Vitamin D is an affirmed GRAS ingredient [21 Code of Federal Regulations (CFR) 184.1950]. Vitamin D is also affirmed as GRAS for use in infant formula [21 CFR 184.1950 (c) (2)] and as an optional ingredient in margarine [21 CFR 184.1950 (c) (3)].

Vitamin D is a fat-soluble vitamin with the potential for toxicity if it is chronically consumed at very high doses; therefore, in Canada and the United States, the addition of vitamin D to foods is very carefully regulated. Canada currently has mandatory fortification of foods, through the Canadian Food and Drug Regulations (Health Canada). In the case of milk, fluid milk “shall contain added vitamin D in such an amount that a reasonable daily intake of the milk contains not less than 300 IU and not more than 400 IU of vitamin D” (Health Canada. Food & Drug Act B.08.003). Fluid milk in Canada is labeled as providing 44% of the recommended daily intake (of 400 IU) per 250-mL serving. All margarines in Canada are fortified with vitamin D (530 IU/100 g). Unlike Canada, where fortification with vitamin D is mandatory for designated foods, the addition of vitamin D to eligible foods in the United States is optional in most cases, with the exception of fortified milk. In the United States, milk and ready-to-eat cereals are the predominant food sources of vitamin D. However cross-sectional studies suggest that current US/Canadian fortification practices are not effective in

preventing hypovitaminosis D, particularly among vulnerable populations during the winter, whereas supplement use shows more promise. Recent prospective intervention studies with higher vitamin D concentrations provided evidence of safety and efficacy for fortification of specific foods and use of supplements (Calvo et al., 2004).

Increases in serum 25(OH)D from vitamin D–fortified foods may be influenced by a number of factors, including total vitamin D intake, bioavailability, and the actual vitamin D content within the fortified food source, the assay used, and the baseline 25(OH)D concentrations. In a systematic review aimed to determine the effects of vitamin D–fortified foods on serum 25(OH)D concentrations, nine RCTs (n=889 subjects) were included, of which 8 consistently showed a significant beneficial effect of food fortification on 25(OH)D concentrations. All 9 trials included community-dwelling participants. Specifically, the vitamin D dietary interventions included fortified milk, nutrient-dense fruit- and dairy-based products , fortified orange juice, fortified cheese, and fortified bread. The factorial RCT had 2 other intervention groups: an exercise program and a combined program of exercise and nutrient dense products. The individual treatment effects ranged from 14.5 (95% CIs: 10.6, 18.4) nmol/L to 34.5 (17.64, 51.36) nmol/L (3.4–25 µg vitamin D/day). Thus it was concluded that though most trials were small in size and inadequately reported allocation concealment, results showed that vitamin D–fortified foods improved vitamin D status in adults (O'Donnell et al., 2008).

In Germany, vitamin D intake from food and synthesis in the skin is low, which leads to low 25(OH)D serum concentrations. General vitamin D food fortification is still prohibited in Germany, although the European Commission published a regulatory framework to harmonize addition of vitamins to foods. Thus Brown et al. (2013) undertook a study to develop a vitamin D fortification model, taking into account all vitamin D sources with the goal to fulfil requirements of intake recommendations or preferable 25(OH)D serum concentrations. Researchers developed a mathematical bottom-up model of 25(OH)D serum concentrations based on data about vitamin D sources of the German population such as sunlight, food and supplements for all federal states taking seasonal and geographical variations into account. This model was used to calculate the optimal fortification levels of different vitamin D carriers in two approaches. First required fortification levels based on fixed intake recommendations from e.g. the IOM or the DGE were calculated and second based on achieving certain 25(OH)D serum concentrations. The results showed that to lift

25(OH)D serum concentration in Germany to 75 nmol/L, e.g. 100 g bread has to be fortified with 11.3 μg during winter, resulting in a daily vitamin D intake of 23.7 μg. Bread seems to be a suitable carrier for base supply. Hence it was concluded that with the model in hand, it was possible to conceive vitamin D fortification strategies for different foodstuffs and model its impact on 25(OH)D serum concentrations.

Thus it is feasible to enhance a variety of foods with vitamin D; however, the biological significance of consumption of these foods needs to be assessed. In order to assess the efficacy of vitamin D enhanced products in increasing serum 25(OH)D concentration, a number of randomized controlled trials (RCTs) have been conducted in recent years. The RCTs which provided at least 10μg of vitamin D_2 or D_3 each day with foods that were enhanced with vitamin D are summarized in Table 2.8.

In general, the majority of the studies concentrated on markers of bone metabolism as well as vitamin D status and as a consequence most analysed serum 25(OH)D, PTH and calcium concentration; some included collection of a three day food diary. Most of the interventions were three to twelve weeks duration and were performed during winter months, when the cutaneous synthesis of vitamin D is low and does not contribute to circulating levels of 25(OH)D. Two out of nine studies had a longer duration of twenty-four months. Across the nine studies volunteers were generally healthy, representing both genders and age groups ranging from 18 to 87 years. Two long term studies (24 months) used milk fortified with vitamin D_3 to deliver 10 μg or greater per day (Chee et al., 2003; Daly et al., 2006). In both studies, 25(OH)D concentrations increased for the supplemented group compared to baseline; however, the relative increases varied between the studies (25.0% *vs.* 7.4%) which may be as a result of very different study populations. It should be noted that in the study by Daly and colleagues a 20% decrease in serum 25(OH)D was reported for the control group. Both studies concluded that vitamin D was bioavailable and improved 25(OH)D status and markers of bone turnover. Similarly, daily intake of 25 μg of vitamin D_3 in a yogurt drink with or without added calcium significantly increased serum 25(OH)D3 concentrations after 12 weeks by 75.0% and 67.6%, respectively, compared to a decrease of 10.6% in the control group. In addition, the glycaemic status in diabetic patients was improved (Nikooyeh et al., 2011). Acceptance of the drink was high among volunteers and no adverse effects were observed.

TABLE 2.8: CHANGE IN SERUM 25(OH)D AFTER INTAKE OF FOODS ENHANCED WITH VITAMIN D

Food source	References	Daily dose (µg) vitamin D (µg vitamin D/100 g product)	Duration and population	Study groups (product portion)	% serum 25(OH)D change from baseline
Fortified milk	Chee et al., 2003	10 µg (2.2 µg/100 g)	24 months 50–65 years, postmenopausal females *n* = 200	1. vitamin D3 + Ca-fortified skimmed milk 2. Usual diet	25.0 # 4.1 #
	Daly et al., 2006	20 µg (5.0 µg/100 g)	24 months 50–87 years, ambulatory community-living (male) *n* = 167	1. Vitamin D3 + Ca-fortified UHT milk, reduced fat 2. Usual diet	7.4 # −19.9 #
Fortified yogurt drink	Nikooyeh et al., 2011	25 µg (5.0 µg/100 g)	12 weeks, October-March 30–60 years, diabetic subjects (fasting blood glucose ≥126 mg/dL) *n* = 90	1. Vitamin D3-fortified yogurt drink 2. Vitamin D3 + Ca-fortified yogurt drink 3. Plain yogurt drink	75.0 67.6 −10.6
Fortified cheese	Johnson et al., 2005	15 µg (17.6 µg/100 g)	2 winter months ≥60 years, subjects (total serum cholesterol <240 mg/dL) *n* = 110	1. Vitamin D3-fortified process cheese 2. Placebo process cheese 3. No cheese	−8.7 10.0 5.6
	Wagner et al., 2008	100 µg * (2083.3 or 1690.8 µg/100 g) **	8 weeks, January–April 18–60 years, healthy subjects *n* = 80	1. Vitamin D3-fortified regular fat cheddar cheese *** 2. Vitamin D3-fortified reduced fat cheddar cheese 3. Vitamin D3 supplement to be taken with food 4. Vitamin D3 supplement to be taken without food 5. Placebo regular fat cheddar cheese 6. Placebo supplement	128.8 120.7 106.5 111.0 −7.8 ##

Fortified orange juice	Tangpricha et al., 2003	25 μg (10.4 μg/100 g)	12 weeks, commenced in March 22–60 years, healthy subjects *n* = 30	1. Vitamin D3 + Ca-fortified orange juice 2. Placebo Ca-fortified orange juice	150.0 45.0
	Biancuzzo et al., 2010	25 μg (10.6 μg/100 g)	11 weeks, commenced in February 18–79 years, healthy subjects *n* = 105	1. Vitamin D3 orange juice ^ + placebo capsule 2. Vitamin D2 orange juice + placebo capsule 3. Vitamin D3 capsules + placebo orange juice 4. Vitamin D2 capsules + placebo orange juice 5. Placebo capsule + placebo orange juice	71.5 67.1 42.9 65.1 −8.6
UV enhanced mushrooms	Urbain et al., 2011	100 μg * (191.0 μg/100 g) **	3 weeks + 2 weeks follow up, January–March Healthy female and male <45 years *n* = 27	1. Vitamin D2 soup + placebo orange juice *** 2. Placebo soup + vitamin D2 supplement in orange juice 3. Placebo soup + placebo orange juice	50.0 # 76.7 # −28.9 #
Fortified bread	Natri et al., 2006	10 μg (27.3 μg/100 g)	3 weeks, February–March 25–45 years, healthy females (25(OH)D < 58.1 nmol/L) *n* = 41	1. Vitamin D3-fortified wheat bread (85 g) 2. Vitamin D3-fortified rye bread 3. Regular wheat bread + vitamin D3 supplement 4. Regular wheat bread	65.0 ### 59.0 ### 78.0 ### −1.2 ###

* equivalent to a daily dose; ** ingested in one weekly dose; *** all portions served once a week; # measurement at the end of the intervention; ## combined placebo groups; ### estimated baseline 25(OH)D; ^ all orange juice in this study contained 350 mg Ca/236.6 mL

Fortification of cheese with vitamin D has been examined in two separate eight-week studies showing diverse results. In the study by Johnson and colleagues, consumption of fortified cheese (15 μg vitamin D) resulted in an 8.7% decrease in their serum 25(OH)D (Johnson et al., 2005). This was an unexpected finding that the authors attribute to a higher baseline value of 25(OH)D in the supplemented group. Additionally no changes in serum PTH were found. The second study showed an approximate 120% increase in serum 25(OH)D concentration after a weekly dose of 700 μg vitamin D fortified cheese (Wagner et al., 2008). In this study serum PTH decreased and there were no changes in serum calcium concentration. Overall, cheese could be a feasible food for the improvement of vitamin D status, but a more realistic amount and vitamin D dose should be used in future studies. To summarise, vitamin D was bioavailable from all studied dairy products; enhanced milk and yogurt or a very high weekly intake of enhanced cheese improved vitamin D status, whilst consumption of 15 μg of vitamin D in cheese was insufficient to produce a significant increase in serum 25(OH)D.

Due to lactose intolerance or low dairy product consumption in some countries, new foods should be considered as possible vehicles for enhancement with vitamin D. Two RCTs using orange juice fortified with 25 μg of vitamin D showed a positive effect on serum 25(OH)D concentration. In the study of Tangpricha et al. (2003), 25(OH)D increased by 150% after 12 weeks supplementation. The second study reported that ingestion of 25 μg vitamin D_2 and D_3 fortified orange juice for 11 weeks resulted in a rise in serum 25(OH)D concentration by 67.1% and 71.5%, respectively (Biancuzzo et al.,2010). Serum PTH decreased only in the first study, while calcium concentration remained stable in both interventions. No adverse effects were reported in either study. The fortification of orange juice with vitamin D seems a promising method of boosting serum 25(OH)D concentration. However, replication of the above studies using a daily dose in line with the RDA for vitamin D is warranted.

The food and beverage industry plays a positive role in helping people make smart healthier choices. The Coco-Cola company introduced Minute Maid Premium orange juice fortified with calcium in 1986. A significant gap in the intake of vitamin D compared with recommended levels was identified based on the National Health and Nutrition Examination Survey (NHANES III, 1988–1994) and Continuing Survey of Food Intakes by Individuals (CSFII 1994–1996, 1998), when the Company worked to create a vitamin D database to estimate mean intakes of the entire United States population. Findings a study was undertaken to assess whether or not vitamin D would be bioavailable when added to calcium-

fortified orange juice indicated that vitamin D was bioavailable, and the company submitted a food additive petition to the Food and Drug Administration (FDA) to allow for the addition of vitamin D to calcium-fortified juices and juice drinks. In 2003, the FDA approved the food additive petition, and Minute Maid Premium orange juice fortified with calcium plus vitamin D was launched (Short, 2005).

Another non-dairy vitamin D enhanced product that has received attention recently is mushrooms. Vitamin D_2 from wild grown mushrooms was well absorbed and bioavailable in humans. Urbain et al. (2011) investigated the effect of a weekly dose of 700 μg of vitamin D from vitamin D_2-enhanced mushrooms in a soup and reported a 50% increase in serum 25(OH)D concentration. There were no changes in serum PTH and calcium concentration and no adverse effects were observed. Additionally, the soup was well tolerated by volunteers. This study demonstrated that vitamin D from mushrooms was bioavailable and shows promise as an alternative to supplements in maintaining serum 25(OH)D concentration.

Bread fortified with vitamin D could also serve as a good source of vitamin D due to its common consumption. To date, only one RCT has investigated the effect of supplementation of wheat and rye bread with 10 μg of vitamin D_3 per daily 85 g portion (equivalent to four thin slices). In this study serum 25(OH)D increased by approximately 60% after 3 weeks in both groups that received fortified bread. No differences in serum PTH were found, while calcium concentration decreased in the fortified rye bread group. In conclusion, fortified bread increased serum 25(OH)D concentration, with rye and wheat bread equally effective (Natri et al., 2006).

Finally, the aforementioned RCTs show the potential of a number of vitamin D enhanced foods to increase vitamin D status. A comparison between the studies is difficult to perform because of variability in the population characteristics (*i.e.*, age, gender), dose of vitamin D provided, intervention period and methods used for 25(OH)D determination. With respect to intake of vitamin D supplements it has been demonstrated that the frequency of intake has an impact on 25(OH)D serum concentrations (Che et al., 2008), illustrating the imperative need to study this in the vitamin D enhanced foods. Overall, in the enhanced food a higher dose was more effective in increasing 25(OH)D concentration; however, for short term studies (3 weeks) the increase seemed to be independent of dose. Milk, yogurt, orange juice, mushrooms and bread proved to be good matrices for supplementation with vitamin D at the

level of 10 μg (milk, bread), 25 μg (orange juice and yogurt) or or 100 μg (mushrooms). More studies are needed to assess the effectiveness of vitamin D fortified cheese, with the use of a more feasible serving size. Finally, more RCTs, using these foods, should be conducted to investigate the link between vitamin D and other disease markers beyond those related to bone health and vitamin D status (Mahony et al., 2011).

As there is no national vitamin D fortification program available in India and neither is any food product checked for efficacy of fortification except few brands of breakfast cereals and milk, in the literature discussed so far no study has been reported from India. It is evident that the dietary intake of vitamin D is negligible due to low consumption of foods rich in vitamin D and the absence of fortification or supplementation in India. Correction of widespread vitamin D deficiency/insufficiency through fortification of flour or development of supplementation programs could significantly impact future health care costs and incidence of chronic disease in all Indians. Wheat flour is the major component of chapattis, a bread staple consumed by vegetarians as well as omnivores, by all ages, socioeconomic backgrounds and by the rural and urban populations in India. The stability of vitamin D fortification of grain products to long shelf life, stability to high baking temperatures and excellent bioavailability has been observed in previous studies. Unlike pasteurized, fortified milk which is not widely consumed in India, flour fortification with vitamin D requires no refrigeration or special distribution and processing and is cost effective (Newmark et al., 2004)

The growing evidence for increased vitamin D deficiency in infants manifested as rickets or other bone deformities will hopefully motivate the policy makers to initiate much needed guidelines for vitamin D supplementation and/or fortification. Indian government offers cereals, *chapati* flour, rice, lentils, *etc*., at subsidized rates to the socioeconomically underprivileged citizens. Additionally, cost of these products is generally tightly regulated by the government. Thus, no large fluctuations are observed in the cost of these food items in the open market also. This will ensure continued consumption of these foods if fortified with vitamin D, by those who avail them and will result in better and sustained economical feasibility of the fortification programs in the long run.

C) NHE - A TOOL FOR LIFESTYLE MODIFICATION

Nutritional deficiencies are a major problem and are a relatively easy way to improve function and help the system move back from dysfunction to improved function and healing. The prevention of ill health requires that clinicians/nutritionists understand what it means to optimise the functional integrity of the physiological systems of the body. It is importance emphasise the causes and consequences of vitamin D insufficiency as it may not have a classical presentation easy to diagnose. The reason for this is that most patients do not only have a single nutrient deficiency but a complex of nutrient deficiencies, including, for example, deficiencies of magnesium, vitamin A, C, E and selenium. How these complexes of deficiencies play themselves out is dependent on gene expression and environmental issues, particularly toxins in the environment, cigarette smoking and drugs, which may all drive the process in different directions. One person's vitamin D deficiency may move towards a hip fracture and another towards cancer.

While prevention of disease by optimising health using nutrients and other lifestyle approaches is a major tool of integrative medicine, the same understanding also applies to the management of disease. Behind every disease is a range of dysfunctional systems. It is these dysfunctional systems that are the primary focus of an integrative approach. One of the ways to correct these dysfunctional systems is by optimising function. The simple act, for example, of making sure that vitamin D is not deficient in a patient will go a long way in improving function and health and possibly decrease the need for multiple drug use. It is not good enough to simply make a diagnosis of disease and treat the disease without a thorough history of diet, stress, exercise and other environmental factors contributing to ill health. The treatment of ill health should always include the optimisation of health (Brom & Dip, 2010).

Nutrition and Health Education Behavioural Change Communication (BCC) programs to targeted audience can improve both nutrition and health status. Nutrition education for professionals- (agriculture, medical, social scientists and others), needs to be strengthened. Innovative strategies need to be developed and tested not only to improve knowledge and attitudes but practices as well. Behavioural modification modules are needed. Nutrition education policy is nonexistent. Awareness among school children, teachers, consumers and women has to be enhanced. Women empowerment is essential to improve diets and overall health of the family.

A healthful eating pattern, regular physical activity, and often pharmacotherapy are key components of diabetes management. Among this lifestyle modification is the primary mode of therapy in T2DM. The American Diabetes Association (ADA) also recognizes the integral role of nutrition therapy in overall diabetes management and has historically recommended that each person with diabetes be actively engaged in self-management, education, and treatment planning with his or her health care provider, which includes the collaborative development of an individualized eating plan. Medical nutrition therapy (MNT) is not synonymous with diabetes self-management training (DSMT). DSMT is an education and training program that helps patients' self-manage diabetes, whereas MNT consists of more individualized diagnosis, therapy, and counselling related to nutrition. MNT provide "more intensive nutrition counselling and a therapy regimen that relies heavily on follow-up and feedback to assist patients with changing their behaviour (s) over" (Daly et al, 2009).

Sullivan et al (2013) in his study to examine outcomes in adult patients with T2DM who received diabetes counselling and education (C/E) services compared with those who did not confirmed that diabetes C/E is associated with improved glycemic control, but further analyses are needed to evaluate long-term cost-effectiveness of diabetes counselling and education. A matched, retrospective cohort study of 17,483 C/E recipients and 17,470 non-C/E controls was followed for up to 12 months. Outcomes included glycemic control (glycosylated hemoglobin A1C levels <7.0%), hypoglycemic events. Compared with the non-C/E group, patients in the C/E group had significantly lower A1C (7.7% vs 7.2%) and were more likely to achieve glycemic control at 6 months' follow-up; they were also more likely to have a hypoglycemic event.

Sumamo et al (2011) in a report to synthesize evidence from randomized controlled trials (RCTs) on the effectiveness of lifestyle interventions to control progression of type 2 diabetes, progression to diabetes from metabolic syndrome, or recurrence of breast cancer and prostate cancer included 20 unique RCTs (plus 80 associated publications). The editors concluded that comprehensive lifestyle interventions that include exercise, dietary changes, and at least one other component are effective in decreasing the incidence of type 2 diabetes mellitus in high risk patients and the benefit extends beyond the active intervention phase. In patients who have already been diagnosed with type 2 diabetes, there is some evidence to suggest long-term benefit on microvascular and macrovascular outcomes, although the evidence is from one trial of high risk diabetic patients and included pharmacotherapy. This

RCT reported that, at 13 years post intervention, the lifestyle intervention group had fewer nonfatal strokes, reduced incidence of retinopathy, reduced progression of autonomic neuropathy, and reduced incidence of nephropathy. A number of studies reported positive effects for lifestyle interventions on changes in body composition, metabolic variables, physical activity, and dietary intake; however, the results were not always statistically significant and were not always sustained following the end of the active intervention. While for Metabolic syndrome, four studies reported that lifestyle interventions decreased the risk of developing type-2 diabetes. Thus comprehensive lifestyle interventions appear to have a positive impact on behavioral outcomes including exercise and dietary intake, as well as a number of metabolic variables, at least in the short-term in all populations addressed in the report.

In many parts of the Asia-Pacific region, diabetes prevalence has increased and has become a major risk factor for cardiovascular disease. The phenomenon seems predicated on insulin resistance (IR), partly attributable to an early impact of abdominal (visceral) adiposity than in Caucasian populations. Food intake along with physical activity and emotional stress are all determinants of glycaemic status. The nutritional management of diabetes is best served by counselling changes in a sociocultural context and step-wise fashion by negotiation rather than prescription. It needs to be accompanied by advice to engage in regular physical activity, both aerobic and strength training. The same concept applies to the prevention of abdominal adiposity and T2DM in the Asia-Pacific region, but with particular reference to protective regional food (Wahlqvist, 2001).

The traditional Indian practise of 'Surya Namaskar' is an excellent combination of 12 yoga *asanas* (which maintain the flexibility of all tendons and joints in the body), with dynamic aerobic exercise deserves to be promoted universally. Certain *asanas* or postures such as *'Dhanurasana'* or *'Ardhamatsyendrasana'* have been identified as being helpful in the control of diabetes. If these *yogasanas* are practised in morning sunlight they will serve the dual benefit of glycemic control and fulfilling the vitamin D requirement.

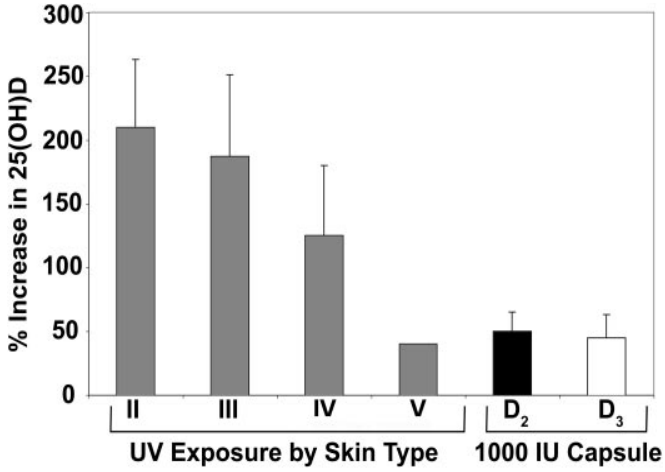
Thus recent epidemiological data have suggested that the majority of cases of T2DM could be avoided by behaviour modification. More recently, animal and human studies have suggested that vitamin D is a potential modifier of diabetes risk and vitamin D replenishment can delay or alter the risk factors of T2DM as well. Hence developing a nutrition health counselling material, involving both the concepts of vitamin D and its potential link with

T2DM and related consequences seems to be a useful tool. One important aspect of increasing the vitamin D levels naturally is adequate exposure to sunlight. This also forms the crucial angle of lifestyle modification. It has been estimated that for every 100 IU of vitamin D ingested, there is an increase in the blood level of 25(OH)D of 1 ng/ml (2.5 nmol/L) (Heaney et al., 2003; Holick et al., 2008). Limited sensible exposure to sunlight or ultraviolet B radiation is more effective in raising blood levels of 25(OH)D than 1000 IU vitamin D_3 taken daily for adults of most skin types (Holick et al., 2007).

Many studies support the concept that one full-body exposure to sunlight can be equivalent to an oral vitamin D intake of 250 mg (10 000 IU). Stamp (1975) compared oral vitamin D to the effects of ultraviolet light treatment sessions and found that the rise in 25(OH)D was the same in subjects treated with ultraviolet light as in those given 250 mg (10 000 IU) vitamin D/d. Holick et al. (2008), studied the comparison of the percentage increase in serum 25(OH)D levels of healthy adults who were in a bathing suit and exposed to suberythemal doses (0.5 MED) of ultraviolet B radiation once a week for 3 months with healthy adults who received either 1000 IU of vitamin D_2 or 1000 IU of vitamin D_3 daily during the winter and early spring for a period of 11 wk. Fifty percent increase represented approximately 10 ng/ml from baseline 18.3 to 28.4 ng/ml. Skin type is based on the Fitzpatrick scale: Type II always burns, sometimes tans; type III always burns, always tans; type IV sometimes burns, always tans; type V never burns, always tans. Data are means±SEM (Figure 2.14).

The majority of Asians indigenous to India range in skin type from IV to V and may require from two to three times longer exposure duration than lighter skinned Europeans (types I, II and III) to synthesize the same level of vitamin D (Webb & Engelsen, 2006). These estimates are also based on considerable skin exposure (face, arms and legs), which may often be concealed by the modest traditional dress in India. Vitamin D is a critical nutrient in breast milk, whose level is very sensitive to maternal diet and sun exposure (Lonnerdal, 1986). Mothers with adequate circulating vitamin D levels during pregnancy may provide sufficient vitamin D in milk for up to 8wk after delivery (Fraser, 1995).

FIGURE 2.14: COMPARISON OF THE PERCENTAGE INCREASE IN SERUM 25(OH)D LEVELS



Reflecting the poor vitamin D status of mothers worldwide, human breast milk is usually very low in vitamin D, containing approximately 25 IU/L vitamin D (>0.5 mg/L). A typical infant consumes about 800–1000mL/day (Collier et al., 2004), which makes sun exposure the main source of vitamin D for infants who are exclusively breast fed and not receiving supplements.

Thus the literature suggests that counselling would act as a key component in bringing a positive behavioural change among the high risk population and show a favourable impact by adopting a healthy lifestyle. The strategy should focus on concepts related to healthy eating habits, increased and regular physical activity and proper medication routine. Increased exposure to sunlight should also be emphasised as it is the natural and most abundantly available source of vitamin D more so among Indians, who are lucky to get it throughout the year and also because of the alarmingly increased prevalence of T2DM and hypovitsminosis D among them.

SUMMARY

Thus to summarize the above literature:

- Vitamin D is unique because it is synthesized in the body and it functions as a hormone with the help of VDRs present in more than 30 tissues in the body.
- Vitamin D occurs naturally in a limited number of foods in highest amounts in fatty fish and in low amounts in meats and other animal food products. It is also available in fortified foods (including milk and milk products, margarines, and breakfast cereals) and as supplements in various doses.
- Vitamin D synthesis is affected by latitude, atmospheric pollution, clothing, melanin pigmentation & sunlight exposure.
- Serum 25-hydroxyvitamin D (Calcidiol) is the most reliable indicator of vitamin D adequacy of an individual.
- Vitamin D deficiency is now considered a global epidemic, affecting all walks of life.
- Major reasons for vitamin D deficiency identified in India are skin complexion, poor sun exposure due to long indoor working hours, vegetarian food habits & lack of vitamin D food fortification program in the country.
- Vitamin D & its deficiency is linked with pathogenesis and/or progression of several disorders, including diabetes, hypertension, and CVDs through various VDR present on many tissues and organs in the body.
- There is consistent evidence supporting that vitamin D status is related to and is important to regulate some pathways related to type 2 diabetes development.
- Vitamin D supplementation can prove to be a cost effective public health measure in preventing or delaying the cardio-metabolic aberrations.
- Adopting healthy dietary practices and lifestyle modifications such as regular exercise, adequate exposure to sunlight and proper medications can positively influence ones' quality of life.