

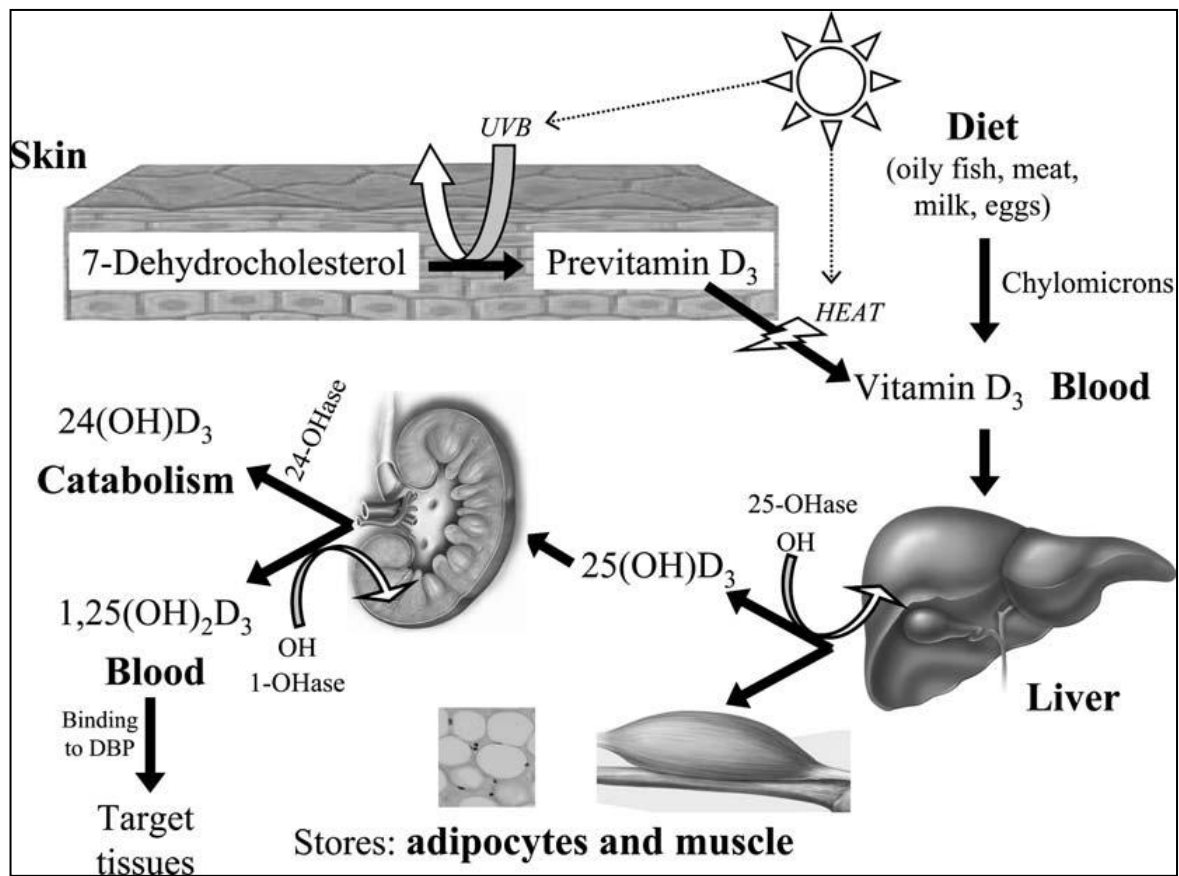
INTRODUCTION

‘Vitamin D’ refers to a mixture of substances, but in particular to the two secosteroids ergocalciferol (vitamin D₂) and cholecalciferol (vitamin D₃). Vitamin D is unusual as a vitamin in that it can be obtained not only from exogenous (dietary) sources, but it can also be produced from endogenous sources, specifically UVB irradiation (sunlight)-mediated hepatic/renal hydroxylation of its precursor, 7-dehydrocholesterol. The active form of vitamin D, calcitriol, binds to a vitamin D receptor (VDR) which then acts as a transcription factor that modulates gene expression in the body (Bouillon et al., 2003).

Vitamin D is a group of fat-soluble prohormones which were identified after the discovery of the anti-rachitic effect of cod liver oil in the early part of the 20th century. The vitamin found in cod liver oil was designated “D” following Vitamin A, B and C, which had been discovered earlier (Wolpowitz and Gilcrest, 2006). The two major biologically inert precursors of vitamin D are vitamin D₃ (cholecalciferol) and vitamin D₂ (ergocalciferol) (Vieth, 2004). Vitamin D₃ is formed when 7-dehydrocholesterol in the skin is exposed to solar ultraviolet B (UVB, 290-320 nm), and then converted to previtamin D₃. In a heat-dependent process, previtaminD₃ is immediately converted to vitamin D. Excess UVB rays transform previtamin D₃ into biologically inactive metabolites, tachysterol and lumisterol. Vitamin D₂ is plant derived, produced exogenously by irradiation of ergosterol, and enters the circulation through diet (Wolpowitz and Gilcrest, 2006). Both vitamin D precursors resulting from exposure to the sunshine and the diet are converted to 25-hydroxyvitaminD [25(OH)D] (calcidiol) when they enter the liver (Holick, 2006). In order to be biologically active, additional hydroxylation in the kidneys takes place to form active 1,25-dihydroxyvitaminD [1,25(OH)₂D] which is also known as calcitriol. Then 1,25(OH)₂D is released into the circulation where it binds to vitamin D-binding protein (DBP) until it reaches its target tissue by means of the vitamin D receptors (VDR). Vitamin D 24-hydroxylase (24-OHase) is the enzyme which catalyses the catabolism of the hormone in the kidney. The diagrammatic representation of vitamin-D metabolism is given in Figure 1.1.

The concentration of 25(OH)D is commonly used for measurement of serum vitamin D levels, despite the active form being 1,25-OHD. This is because parathyroid hormone increases the renal conversion when there is an insufficiency, causing the active form to be normal or elevated. However, measurement of 25(OH)D levels may not be accurate in

FIGURE 1.1 VITAMIN D METABOLISM IN THE BODY



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

patients with severe renal diseases (creatinine clearance less than 15 mL/min or undergoing dialysis), as renal conversion of 25(OH)D level is reduced. Hence, parathyroid hormone levels should also be taken into account when identifying vitamin D deficiency (VDD) especially among the subjects with kidney disorders (Fosnight et al., 2008).

The consensus regarding the cut-off level for VDD is still under discussion. It has been suggested previously that VDD in adults should be defined as a serum 25(OH)D level below 50 nmol/L (20 ng/mL). However, further research suggests that optimal serum 25(OH)D status should be maintained above 80 nmol/L (32 ng/mL), because the risk for various medical conditions, including reduced bone density, periodontal disease, colon cancer, hypertension, and impaired lung function, is lowest above this level. The risk of undiagnosed diabetes and impaired glucose tolerance was also found to be lowest when the serum 25(OH)D level was above 83 nmol/L; while that of myocardial infarction was lowest when serum 25(OH)D was above 107 nmol/L (Scragg and Bartley, 2007).

Technically, there are still some uncertainties about the optimal method for measuring vitamin D levels. Multiple methodologies for 25(OH)D measurement (radioimmunoassay, high-performance liquid chromatography, and liquid chromatography tandem mass spectroscopy) are available and subject to variability, which causes difficulties in defining the cut-off value for vitamin D insufficiency or deficiency. Therefore, aiming at a higher 25(OH)D level seems to be clinically safe and reasonable, as it improves vitamin D status with minimal risks (Holick et al., 2011). Laboratories have now increased the lower limit of the reference range to 75 nmol/L (30 ng/mL). In addition, 25(OH)D level varies with seasons, exposure to sunlight, and dietary intake (Rosen, 2011). Therefore, issues such as choice of assay, timing of measurement, and reference range if are not clearly addressed, then the vitamin D levels obtained should be interpreted cautiously.

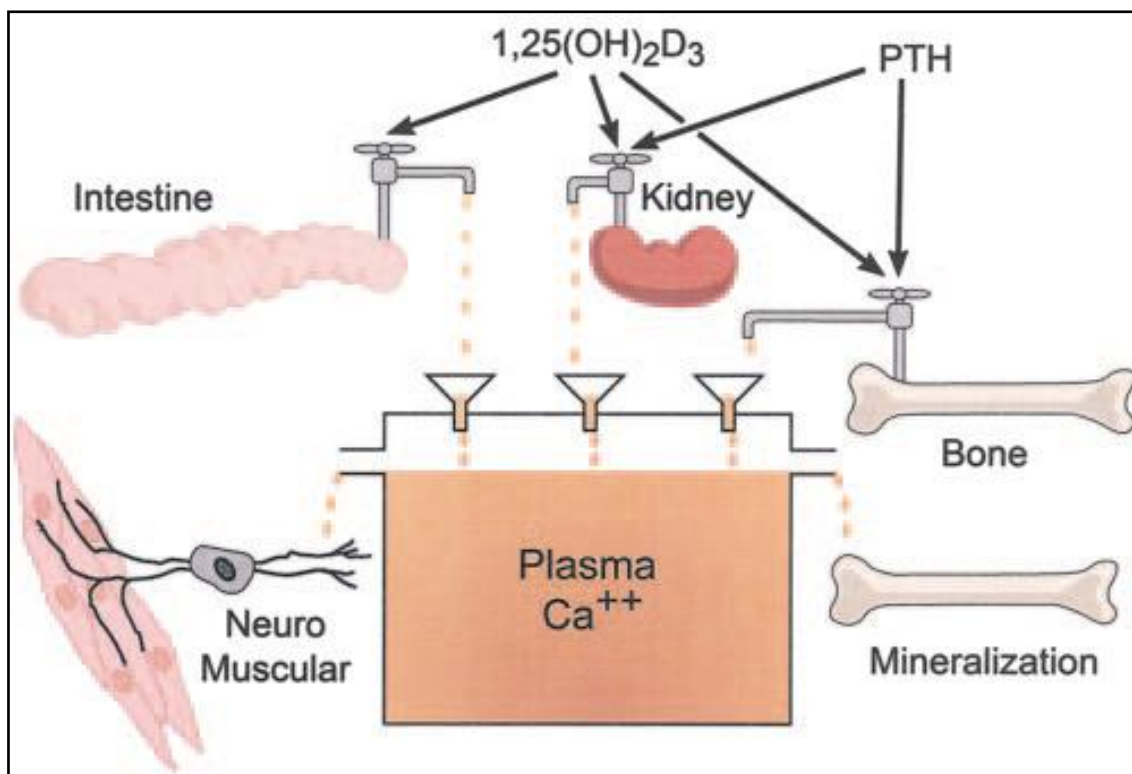
Vitamin D is virtually absent from the food supply. Humans obtain vitamin D usually through exposure to sunlight. Very few foods naturally contain vitamin D. It is not found in plant materials (eg, vegetables, fruits, or grains) and is present in low abundance in meats and other animal food sources, except in rare cases such as fish liver oils and plants such as waxy-leaf nightshade (*Solanum glaucophyllum*) (DeLuca, 2004). Oily fish such as salmon, mackerel, and sardines are some of the good sources. 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 (Zhang & Naughton, 2010). Also a very small

number of foods are fortified with vitamin D such as milk, orange juice and some bread and cereals (Holick, 2004; Tangpricha et al., 2003).

It is now more than 75 years since the discovery of calciferol, known commonly as vitamin D, and of its ability to cure rickets in animals and children. Findings from the second half of the 20th century showed that vitamin D is truly a prohormone and not just a vitamin. The classic function of vitamin D as shown in Figure 1.2 is that, it increases the gut uptake of ingested calcium and phosphorus and improves calcium reabsorption by the kidney, thus resulting in the elevation of both mineral elements in plasma (Deluca & Cantorna, 2001). As a consequence, the major biological actions of this vitamin include maintenance of mineral homeostasis and regulation of bone remodelling (Ogunkolade et al., 2002; Zittermann, 2003). However, recent epidemiologic and clinical studies have indicated that vitamin D may have far more roles than just in the prevention of rickets. A brief summary of the biological consequences are represented in Figure 1.3. Thus diseases such as osteoporosis, muscle weakness, several types of cancer, diabetes, hypertension, and cardiovascular disease may all result from subtle and chronic vitamin D deficiency. Vitamin D is taken for granted and is assumed to be plentiful in a healthy diet. Unfortunately, very few foods naturally contain vitamin D, and only a few foods are fortified with vitamin D. This is the reason why vitamin D deficiency has become epidemic for all age groups.

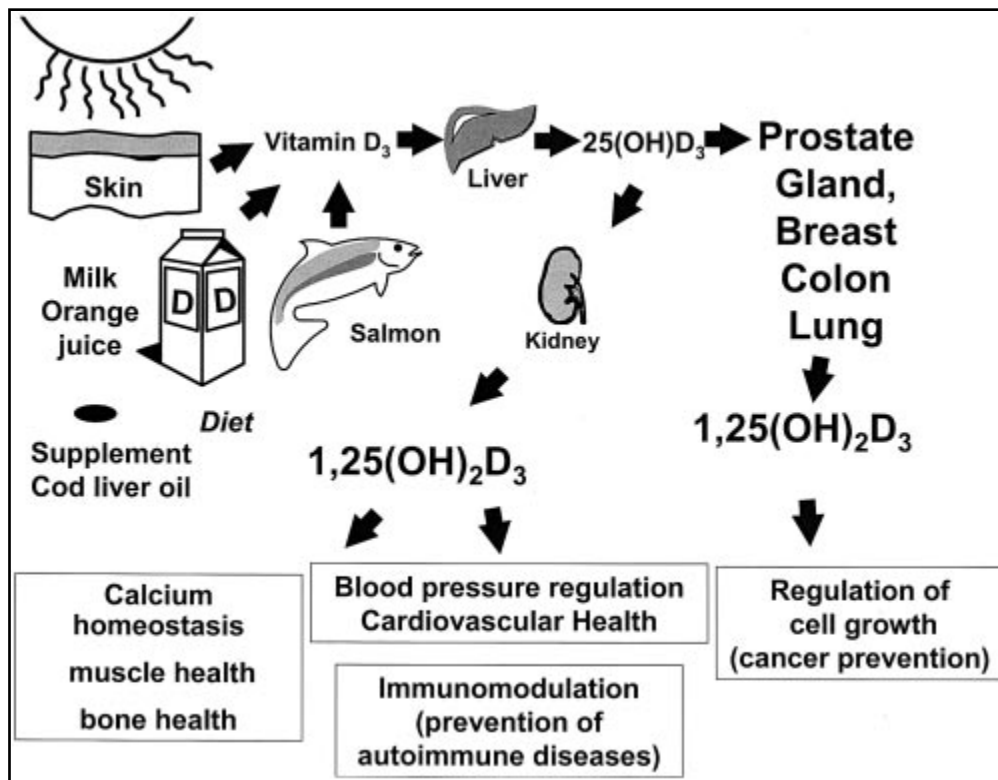
Vitamin D deficiency has been recently defined and recommended by the Institute of Medicine (IOM) as a 25(OH)D of less than 20 ng/ml. Vitamin D insufficiency has been defined as a 25(OH)D of 21–29 ng/ml (Holick, 2007; Heaney, 2004; Malabanan et al., 1998; Heaney et al., 2003; Bischoff-Ferrari et al., 2009; Institute of Medicine, 2011). In accordance with these definitions, it has been estimated that 20–100% of U.S., Canadian, and European elderly men and women still living in the community are vitamin D deficient (Chapuy et al., 1996; Holick et al., 2005; Lips et al., 2006; Holick, 2006; Greene-Finestone et al., 2011). Children and young and middle-aged adults are at equally high risk for vitamin D deficiency and insufficiency worldwide. Vitamin D deficiency is common in Australia, the Middle East, Africa, and South America (Thacher et al., 2006). In the United States, more than 50% of Hispanic and African-American adolescents in Boston (Gordon et al., 2004) and 48% of white preadolescent girls in Maine had 25(OH)D below 20 ng/ml (Sullivan et al., 2005).

FIGURE 1.2 ROLE OF VITAMIN D HORMONE IN MINERALIZING THE SKELETON



Source: Jones, Strugnell & DeLuca, 1998

FIGURE 1.3 BIOLOGICAL CONSEQUENCES OF VITAMIN D DEFICIENCY IN THE BODY



Source: Holick, 2004

In addition, 42% of African-American girls and women aged 15–49 years throughout the United States had a blood level of 25(OH)D below 15 ng/ml at the end of the winter (Nesby-O'Dell et al., 2002), and 32% of healthy students and physicians at a Boston hospital had 25(OH)D below 20 ng/ml (Tangpricha et al., 2002). Pregnant and lactating women who take a prenatal vitamin and a calcium supplement with vitamin D remain at high risk for vitamin D deficiency (Hollis and Wagner, 2004). Thus vitamin D inadequacy does indeed constitute an epidemic in many populations across the world and has been reported in healthy children, young adults, and middle aged and elderly adults.

India being a sunny country earlier was never considered under cloud of vitamin D deficiency, but to its dismay, series of studies from different parts of the country have pointed towards widespread VDD in Asian Indians of all age groups including toddlers, school children, pregnant women and their neonates and adult males and females residing in rural or urban areas (Goswami, Mishra, & Kochupillai, 2008).

Goswami et al. in 2000, used sensitive and specific assay to measure serum 25(OH)D in apparently healthy subjects in Delhi, and showed that significant hypovitaminosis D was present in upto 90 % of them. Harinarayan et al.(2011) reported VDD in women of reproductive age group (76 %) and in post menopausal women (70%) with serum 25(OH)D levels <20ng/ml in south India. Similarly Marwaha et al.(2011), in a study of 1346 apparently healthy individuals above 50 years from north India reported VDD with serum 25(OH)D levels below 20 ng/ml in 91.2% of the population and vitamin D insufficiency (serum 25(OH)D 20-<30 ng/ml) in 6.8% of the population. In the study population of 290 healthy schoolgirls (6-17 y) of Delhi, 93.7% girls (97.5% vs. 90.9% in Lower Socioeconomic Strata and Upper Socioeconomic Strata, respectively) were found to be vitamin D deficient.

Apart from these, there are other studies conducted in India on various socio-economic groups, different ages, on both genders and different race, as well as different disease states, such as primary hyperparathyroidism reporting widespread vitamin D deficiency/insufficiency in India (Harinarayan & Joshi, 2009; Harinarayan et al., 1995). There are many factors which can explain this paradox of hypovitaminosis D inspite of abundant sunshine like duration and timing of sun exposure, amount of skin exposed, atmospheric pollution, skin pigmentation, sunscreen use, dietary and genetic factors (Rathi & Rathi, 2011).

Vitamin D is utilized by the body through various vitamin D receptors (VDR) present on many tissues and organs in the body. VDR has opened up new roles for vitamin D far beyond

the traditional function of maintaining calcium-phosphate homeostasis. New evidence has now established that vitamin D deficiency is also linked with the pathogenesis and/or progression of several disorders, including cancer, hypertension, multiple sclerosis, diabetes and cardiovascular diseases (Zhang & Naughton, 2010).

Diabetes mellitus is a serious metabolic disorder highly prevalent among all walks of life. International Diabetes Federation's (IDF) 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 (Figure 1.4 A & B). At the beginning of 20th century, diabetes mellitus 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 ethnic group was exempt from the disease. Countries like India, China and the Arab nations of the Middle East, which experienced rapid economic growth after centuries of deprivation, found themselves at the epicentre of a global epidemic. Recent data shows these countries featuring in the top ten countries in respect to the number of people with diabetes. India stands second in the list with a high population of 65.1million adults living with diabetes mellitus (Figure 1.4 (A)).

Type 2 diabetes mellitus (T2DM) accounts for more than 90% of all patients with diabetes worldwide. The prevalence of diabetes in adults is showing an upward trend (6.4% in 2010 to an estimated 7.7% in 2030). Asians, particularly from Indian subcontinent have received greater attention in diabetes studies, because of their migration in large numbers (Shaw et al., 2010). The factors implicated in causation of T2DM in Asian Indians apart from geographic regions and migration are age gender, obesity, diet, physical inactivity, ethnic susceptibility, genetic factors, parity, stress and insulin resistance. The complications of T2DM are also a major cause of morbidity and mortality worldwide. T2DM if left untreated can lead to a multitude of microvascular and macrovascular conditions such as retinopathy, nephropathy, neuropathy and cardiovascular disease. T2DM is closely related to high incidence of elevated blood pressure, central adiposity and dyslipidemia. All these factors ultimately contribute towards high prevalence of Metabolic Syndrome among the diabetic patients. Hence clinicians and nutritionists should aim to achieve as tight a control of diabetes as possible without compromising the safety by adopting preventive lifestyle measures.

FIGURE 1.4 (A) NUMBER OF PEOPLE WITH DIABETES BY IDF REGION, 2013

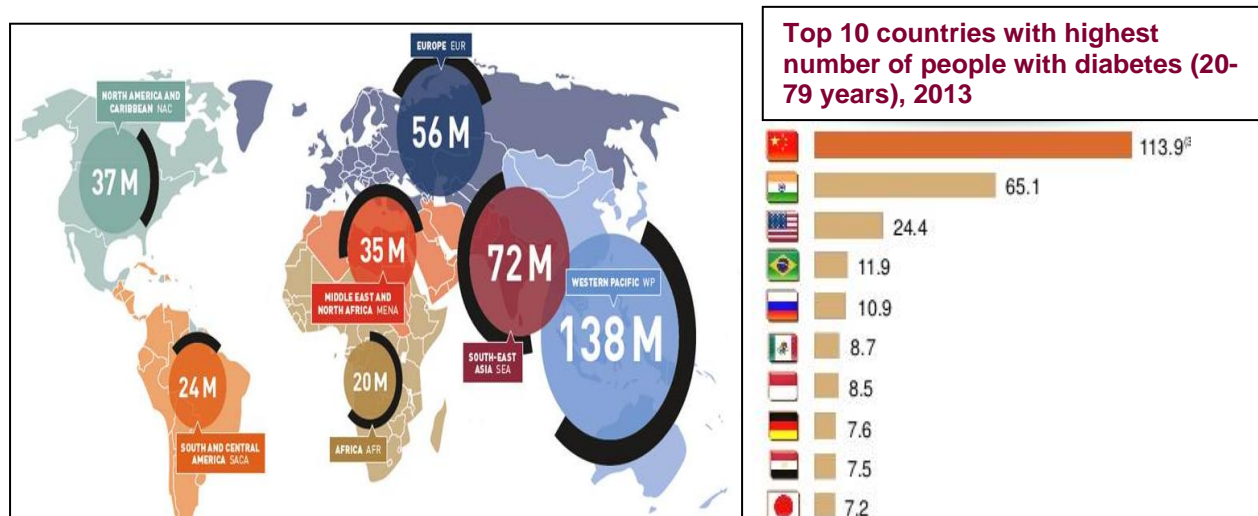
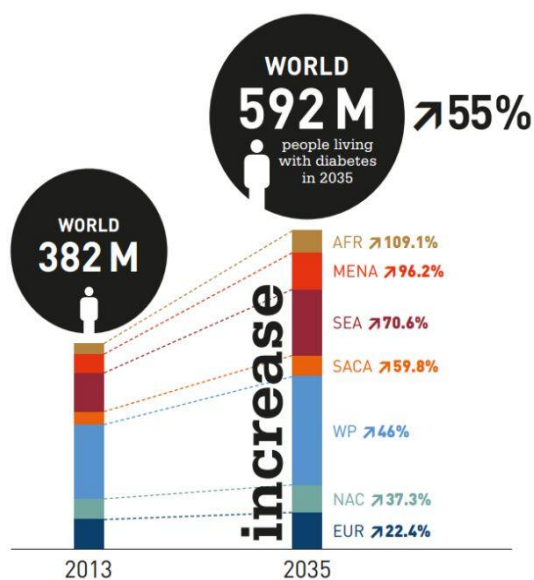


FIGURE 1.4 (B) EXPECTED INCREASE IN PREVALENCE OF DIABETES



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

Despite the array of tools at disposal to tackle the disease – effective drug therapies, advanced technology, ever-improving education and preventive strategies – the battle to protect people from diabetes and its disabling, life-threatening complications is being lost and there is still a need felt to identify newer strategies to combat this epidemic. Vitamin D, a recently identified tool shows promising potential in prevention and management of diabetes. There is growing evidence supporting the fact that vitamin D status is important to regulate some pathways related to diabetes development, thus making VDD more evident in diabetic subjects. Since the activation of inflammatory pathways interferes with normal metabolism and disrupts proper insulin signalling, it is hypothesized that vitamin D could influence glucose homeostasis by modulating inflammatory response.

In a cross-sectional study among 210 south Asian type-2 diabetic subjects in UK aged >40 y, VDD was more common in diabetic compared to control (Tahrani et al., 2010). 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, thus confirming the association of low plasma 25(OH)D with increased risk of type-2 diabetes (Afzal, Bojesen, & Nordestgaard, 2013).

A review indicated that vitamin D deficiency may predispose to glucose intolerance, altered insulin secretion and type 2 diabetes (Pittas et al., 2010), either through a direct action via VDR activation or indirectly via calcemic hormones and also via inflammation (Thorand et al., 2011). Due to the presence of both 1- α -hydroxylase and VDR in pancreatic β cells, vitamin D is important for insulin synthesis and release (Pittas et al., 2010). Mitri, Muraru, & Pittas (2011), in a systematic review, confirmed such evidence by evaluating vitamin D intake and 25-hydroxyvitamin D (25OHD) levels. In 8 observational studies, vitamin D intake >500 international units (IU)/day decreased the risk of type 2 diabetes by 13% compared with vitamin D intake <200 IU/day. Individuals with the highest 25OHD status (>25 ng/mL) had a 43% lower risk of developing type 2 diabetes (95% confidence interval 24–57%) compared with those in the lowest group (<14 ng/mL).

Based on the data from epidemiological studies, vitamin D supplementation is considered to be a potential and inexpensive therapy not only to decrease the risk, but also to improve glycemic parameters in type 2 diabetic patients (Nazarian et al., 2011). In an open-label study among 8 subjects with VDD and impaired fasting glucose, administration of 10,000 IU vitamin D3 daily for 4 weeks, showed a decrease in Acute Insulin Response to Glucose ($p=0.011$) and increase in insulin sensitivity ($p=0.012$). If these findings are repeated in a

randomized, double-blind study, the results indicate that orally administered dose of vitamin D3 supplementation improves insulin sensitivity in subjects with impaired fasting glucose and suggests the supplementation to be an inexpensive public health measure to prevent or delay the progression of pre-diabetes to diabetes (Takiishi et al., 2010).

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 recent study among 100 type-2 diabetic subjects aged 30-70 years, 24% were vitamin D deficient. After treatment with 50,000 IU of vitamin D3 orally per week for eight weeks significant improvements were seen in serum fasting plasma glucose, insulin and in HOMA-IR (Talaie, Mohamadi, & Adgi, 2013).

In a randomized double-blind clinical trial on 42 diabetic patients, after 3 months of a single intramuscular injection of 300,000 IU of vitamin D3 showed a significant increase in serum 25(OH)D levels ($p=0.007$), however HbA1c, anthropometric factors and HOMA-IR in intervention group stayed constant (Heshmat et al., 2012). Daily intake of vitamin D-fortified yogurt (either with or without added calcium) improved serum 25(OH)D levels and glycemic status in type 2 diabetic patients with baseline 25OHD serum level of 44.5 nmol/L (Nikooyeh et al., 2011).

Similarly a growing body of data suggests that low 25-hydroxyvitamin D levels may adversely affect cardiovascular health. Vitamin D deficiency activates the renin-angiotensin-aldosterone system and can predispose to hypertension and left ventricular hypertrophy. In a double-blind, parallel group, placebo-controlled randomized trial on 34 type-2 diabetic subjects, with a mean age 64y and baseline 25(OH)D level of 38.3 nmol/l, a single dose of 100,000 IU vitamin D2 was administered. The supplementation increased 25(OH)D levels by 15.3 nmol/l relative to placebo and significantly improved flow mediated vasodilatation of brachial artery by 2.3%. Vitamin D supplementation significantly decreased systolic blood pressure by 14mm Hg compared to placebo ($p=0.001$), thus showing improvement of endothelia function in diabetic subjects (Sugden et al., 2008).

Epidemiologic studies have also recently linked vitamin D deficiency with increased risk of major adverse cardiovascular events (Wang et al., 2008). Epidemiological studies report that the rates of coronary heart disease, diabetes, and hypertension, like vitamin D deficiency, increase in proportion to increasing distance from the equator (Rostand, 1997).

Looking at the devastating consequences of VDD, it is very important to either prevent or treat it in the high risk population. The Institute of Medicine (IOM) recommends that individuals between 1-70 y should receive 600 IU of vitamin D daily. However this dose will likely increase the 25(OH)D level to 20ng/ml, which are considered to be adequate for bone health, but not to levels >30ng/ml, as recommended by the Endocrine Society. Hence the Endocrine Society recommended in its Practice Guidelines that adults >18 y should receive a daily supplementation of 1500-2000 IU (upto 10,000 IU is safe) for the prevention of VDD (IOM, 2011; Holick et al., 2011).

The literature discussed so far does signal towards a global epidemic related to vitamin D deficiency and its consequences and has thus evoked few questions which are as mentioned below:

1. What is the prevalence of vitamin D deficiency in the adult population in Vadodara?
2. What are the predictor variables of vitamin D deficiency in the study population?
3. What is the association of hypovitaminosis-D in type-II diabetes mellitus?
4. Is vitamin D supplementation effective in altering the metabolic aberrations among type-II diabetic subjects?
5. For how long can the increased levels of serum vitamin-D be sustained after supplementation?
6. What life-style changes can be adopted to restore healthy levels of vitamin D in the body?

The questions posed lead to the framing of rationale for the research to be undertaken which is as given below-

Rationale of the present study

The literature cited above does speculate the role of vitamin-D in the progression of various diseases and medical conditions, but there still exists a wide gap to judge its role in the pathogenesis of these diseases. The prevalence of vitamin D deficiency in the adult population with multiple disease profile also needs to be mapped thoroughly. The extent to which serum vitamin D gets affected in the presence of these conditions needs to be addressed. The combined effect of these diseases on vitamin D status of an individual and/or the role of vitamin D deficiency as a causative factor of the conditions needs to be investigated further.

As the prevalence of vitamin D deficiency is reported to be high among the diabetic subjects, it is very important to frame intervention strategies which are low in cost but effective in raising the vitamin D levels in the population. Providing vitamin D supplementation might prove to be a cost effective public health measure in preventing, or at least delaying the cardio-metabolic aberrations related to diabetes mellitus. But still there remains some controversy regarding the form and dose of vitamin D to be supplemented to attain the desired blood level of 25(OH)D for both bone health and reducing risk for VDD associated acute and chronic diseases and also to check the sustainability of increased serum vitamin-D levels after supplementation.

There is also a need felt to address the key components of lifestyle and behaviour change in relation to serum vitamin D levels and its consequences through a well made nutrition health education material to counsel and educate the high risk population.

Hence to address the above queries and keeping the rationale in mind, the present research was framed with the following broad objectives-

- ❖ To map the prevalence of vitamin D deficiency (VDD) among free living adult population and subjects with type-II diabetes mellitus in Vadodara city.
- ❖ To identify the determinants of vitamin-D status and study its association with clinical conditions like overweight/obesity, hypertension & cardiovascular disease.
- ❖ To study the metabolic aberrations in relation to vitamin D deficiency in the study populations.
- ❖ To study the efficacy of vitamin-D supplementation dose on serum vitamin-D status and cardio-metabolic profile of the subjects with type-II diabetes mellitus.
- ❖ To study the washout effect of the vitamin-D supplementation on anthropometry, HbA1c levels and lipid profile of the subjects after eight weeks of post supplementation.
- ❖ To compare the vitamin-D status of the diabetic and non-diabetic populations and see the difference in biophysical measurements, physical activity pattern and nutrient intake of the subjects and levels of various biochemical parameters.
- ❖ To develop nutrition health education material for restoring and maintaining a healthy vitamin-D status among the subjects.