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The association between Vitamin D deficiency and diabetes mellitus

A associação entre deficiência Vitamina D e diabetes mellitus

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Abbreviation List

DM: Diabetes Mellitus

T1DM**:** Type 1 Diabetes Mellitus

T2DM: Type 2 Diabetes Mellitus

GDM: Gestational Diabetes Mellitus

1,25 $(OH)_2D$: 1,25-dihydroxyvitamin D

25(OH)D :25-dihydroxyvitamin D

7DHC: 7 -dehydrocholesterol

UVB: Ultraviolet B

DBP: Vitamin D Binding Protein

FNB: Food National Board

IU: International Unit

B: Beta

IL: Interleukin

TNF: Tumor Necrosis Factor

Th1: Type 1 helper

Th2: Type 2 helper

NOD: Non-Obese Diabetic

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Abstract

The main source of vitamin D is synthesized in the body via the action of ultraviolet B radiation on the skin. In addition, although to a lesser extent, some vitamin D is available from food (naturally or fortified) and supplements. The role of vitamin D in maintaining bone health has been known for many years. Recently, however, the discovery that many tissues expressed the vitamin D receptor and are able to transform the 25-hydroxivitamin D into 1,25-dihydroxyvitamin D (active metabolite) has led to the discovery of a role in pathogenesis and prevention of diabetes mellitus. Accumulating evidence links vitamin D deficiency with immunological disturbance in type 1 diabetes mellitus and also linked to abnormal glucose metabolism mainly through effects on the pancreatic β-cell function or on insulin sensitivity in type 2 diabetes mellitus and gestational diabetes mellitus. More randomized clinical studies in humans need to be done in order to prove this potential association. However, several epidemiological studies have shown lower serum 25-hydroxyvitamin D concentrations in diabetic individuals compared to non-diabetic individuals. Contrary to what people may think vitamin D deficiency is a common feature in different populations worldwide due to a number of factors that limit the synthesis of vitamin D by the skin. This deficiency needs to be urgently treated. Nutritionists and other health professionals have an important role in identifying individuals at risk and finding strategies specific for the community they are working on, in order to prevent vitamin D deficiency and the health problems associated to it.

Keywords –Vitamin D Deficiency, Diabetes Mellitus, Vitamin D Receptors, 1,25-dihydroxivitamin D, 25-hydroxivitamin D.

Resumo

A principal fonte de vitamina D é sintetizada no organismo através da acção da radiação ultravioleta B na pele. Além disso, embora em menor grau, alguma da vitamina D está disponível a partir dos alimentos (naturais ou fortificados) e de suplementos. O papel da vitamina D na manutenção da saúde óssea já é conhecido há muitos anos. Recentemente, no entanto, a descoberta de que muitos tecidos expressam o receptor da vitamina D e são capazes de transformar o 25-hidroxivitamina D em 1,25-dihidroxivitamina D (metabolito activo) levou à descoberta do papel da vitamina D na patogenia e na prevenção da diabetes mellitus. Há evidências que associam a deficiência de vitamina D à perturbação imunológica na diabetes mellitus tipo 1, ao metabolismo anormal da glicose, principalmente através dos efeitos sobre a função das células β do pâncreas ou da sensibilidade à insulina na diabetes mellitus tipo 2 e na diabetes mellitus gestacional. Mais estudos de intervenção em seres humanos são necessários, a fim de provar esta potencial associação. Contudo, vários estudos epidemiológicos têm demonstrado menores concentrações séricas de 25-hidroxivitamina D em indivíduos diabéticos em comparação com indivíduos não-diabéticos. Contrariamente ao que as pessoas podem pensar a deficiência em vitamina D é comum em diferentes populações a nível mundial devido a uma série de factores que limitam a síntese de vitamina D pela pele. Esta deficiência que está ocorrendo precisa de ser urgentemente tratada. Os nutricionistas e outros profissionais de saúde têm um papel importante na identificação de indivíduos de risco e na busca de estratégias específicas para a comunidade em que trabalham a fim de evitar a deficiência de vitamina D e os consequentes problemas de saúde associados a essa deficiência.

Keywords – Deficiência de Vitamina D, Diabetes Mellitus, Receptores de Vitamina D, 1,25-dihidroxivitamina D, 25-hidroxivitamina D.

1 Introduction

Diabetes is a chronic condition that occurs when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Hyperglycemia and other related disturbances in the body's metabolism can lead to serious damage to many of the body's systems, especially the nerves and blood vessels. It has become one of the major causes of premature illness and death in most countries, mainly through the increased risk of cardiovascular disease. Cardiovascular disease is responsible for between 50% and 80% of deaths in people with diabetes. Diabetes is a leading cause of blindness, amputation and kidney failure. These complications account for much of the social and financial burden of diabetes [(1)].

There are two basic forms of diabetes, type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). A third type of diabetes, gestational diabetes mellitus (GDM), develops during some cases of pregnancy but disappears after pregnancy [(1)].

The T1DM accounts for only 5–10% of those with diabetes the other 90-95% mainly have T2DM [(1, 2)]. Type 1 diabetes mellitus (T1DM) is a T-cell mediated autoimmune disease that results in the destruction of insulin-producing beta (β)-cells in the pancreas, requiring exogenous insulin for survival [(3)]. The β-cell destruction often begins during infancy and continues over many months or years. By the time, that T1DM is diagnosed, about 80% of the β-cells have been destroyed [(4)]. Although it is acknowledged to be an autoimmune disease, the causes are still considered to be unknown [(5)] . Epidemiologic studies of T1DM

have directed the search for possible genetic predispositions and related environmental factors that are still poorly defined [2, 3]. Some identified environmental risk factors operating early in life include enteroviral infections in pregnant women, older maternal age (39-42 years), preeclampsia, cesarean section delivery, increased birth weight, early introduction of cow's milk proteins and an increased rate of postnatal growth (weight and height) [6, 7]. There is a great necessity to find the cause of this chronic disease because there has been an increase in incidence from one year to the next. It is estimated that currently the incidence is increasing by 3% per year and it is predicted that by 2010 the incidence of T1DM will be 40% higher than it was a decade earlier. This increase can not only be explained by genetic factors something else must be behind this [4]. Several approaches have been tried to prevent T1DM but none of them have been shown to work, and the prevention of T1DM remains an objective for the future [1].

Type 2 diabetes mellitus (T2DM) is a non-insulin dependent diabetes or adult-onset diabetes. These individuals have insulin resistance and usually have a relative insulin deficiency .There are probably many different causes of this form of diabetes. Although the specific etiologies are not known, autoimmune destruction of β-cells does not occur. Most patients with this form of diabetes are obese and obesity itself causes some degree of insulin resistance [2]. At the same time, physical inactivity, both a cause and consequence of weight gain, also contributes to insulin resistance. The problem of obesity and overweight is present in developed countries but is also extending to developing countries, especially in urban areas [1]. T2DM may also occur in individuals who are not obese but that have an increased percentage of body fat distributed predominantly in the

abdominal region, which increases the insulin resistance [2]. Ethnicity is also another risk factor with, higher rates of T2DM reported in people of Asian and African origin, and in indigenous peoples of the Americas and Australasia [1]. T2DM is often shown to be associated with a strong genetic predisposition, more than the autoimmune form T1DM [2]. Therefore, individuals with a family history of diabetes have more probability of developing T2DM. Women who developed diabetes during pregnancy are also more at greater risk of T2DM later in life. Simple lifestyle measures have been shown to be effective in preventing or delaying the onset of T2DM (weight loss, increasing physical activity and having a healthy diet). However, more studies should be done in order to find more preventive measures because the world is facing a growing diabetes epidemic of potentially devastating proportion. At least 171 million people worldwide have diabetes (type 1 and 2) and this figure is likely to increase to 366 million by 2030. In order to stop these numbers from increasing more must be done to see all possible causes of both T1DM and T2DM [1].

Recent evidence has reported that Vitamin D deficiency predisposes individuals to T1DM and T2DM. This was suggested by several studies that show that vitamin D has several effects on the immune system and is also involved in the Insulin synthesis and secretion therefore suggesting a role in the development of both T1DM and T2DM. Studies have shown that diabetic individuals are more vitamin D deficient than non-diabetic individuals [8]. Vitamin D is a fat-soluble vitamin that is traditionally recognized as a potent regulator of calcium and phosphorus metabolism. Prolonged and severe vitamin D deficiency is known to cause rickets in children and osteomalacia /osteoporosis in adults [9]. Rickets was highly prevalent around the 1900 in large cities and was thought to be eradicated as a

major health problem at the end of the last World War. However, rickets has made an unfortunate comeback and several studies have shown vitamin D deficiency is becoming a global public health problem although it is largely unrecognized [10-13]. It has been estimated that 1 billion people worldwide have vitamin deficiency or insufficiency [9]. Vitamin D deficiency is common in the elderly due to the decreased capacity to produce vitamin D from the skin [14, 15]. A Europe wide survey (excluding Great Britain), of 824 elderly people aged over 70 years showed that 36% of men and 47% of women had low vitamin D status during the wintertime [15]. Some authors defend that elderly people may be vitamin D deficient throughout the year. However, this deficiency does not only affect older people, vitamin D deficiency has also been reported in adolescents and young adults [16]. In a national U.S. study, it was found that the average American spent 93% of their 24-hour day indoors. As time goes by, air conditioning, computers, video games, and extensive television programming become more readily available, increasing time spent indoors. Because of changes in current lifestyles, humans are now more dependent on foods rich in vitamin D and even oral vitamin D supplementation [17].

A study conducted in Edmonton (Canada) showed that low vitamin D status is common among children and adolescents at the beginning of spring and the risk may be higher among older children because vitamin D intake does not adequately rise in proportion with increases in body mass [16]. Remarkably, in the sunniest areas of the world, vitamin D deficiency is also a major health problem. It was found that children in Saudi Arabia, India, Turkey, New Zealand, Israel, Egypt, Hong Kong, China, Libya, Lebanon, Spain, Australia, San Diego, California and the southeastern United States 35–80% are vitamin D deficient. This shows that

we can not assume that just because there is abundant sunlight, all individuals are vitamin D sufficient [11].

Vitamin D deficiency seems to discriminate between races where dark-skinned individual are likely to be more at risk than fair-skinned individuals. This is due to the melanin that is present in greater quantities in dark-skinned individuals, which absorbs the Ultraviolet B (UVB) radiation and thus reducing the vitamin D synthesis [9, 12].

As we can see a number of studies show that vitamin D deficiency in common and strategic measures should be taken to prevent this from happening. Studies have shown that vitamin D may play a role in the prevention of serious chronic diseases, including diabetes mellitus, cardiovascular disease; some inflammatory and autoimmune disorders; as well as some types of cancer. Therefore, showing that vitamin D does not only play a role in calcium homeostasis and bone metabolism but it also has other functions in many parts of the body [18, 19].The focus of this review will be on Vitamin D metabolism, its recommendations and the role it plays on T1DM , T2DM and GDM.

2 Vitamin D and its Metabolism

Vitamin D (calciferol) is a secosteroid compound that can be obtained from food, but most people achieve their vitamin D needs (85-95%) by endogenous synthesis through direct ultraviolet B-mediated synthesis in the skin [5, 20-22]. However if there is insufficient endogenous synthesis, generally caused by limited sun exposure of skin to sunlight, then a dietary supply becomes essential. By the action of UVB light (290-315nm) the B ring of 7-dehydrocholesterol (pro-vitamin D) can be broken to form precholecalciferol (pre-vitamin D), which is rapidly isomerised to vitamin D in a thermo sensitive process [14, 21, 23]. The conformational

changes due to the isomerisation can deliver vitamin D into the circulation, where it is transported by vitamin D-binding protein (DBP) to the liver for further metabolization. In food and dietary supplements, vitamin D exists in the form of either ergocalciferol (vitamin D_2) or cholecalciferol (vitamin D_3) .Both are fat-soluble and once ingested is incorporated into the chylomicron fraction, absorbed through the lymphatic system and transported to the liver where it will also be further metabolized [14, 19]. Vitamin D occurs naturally in a limited number of foods, the highest amounts appear in fatty fish (salmon, sardines, mackerel, and herring, tuna), oils from fish (including codfish liver oil) and in the lowest amounts in red meats, egg yolk, and other animal food products. It is also available in fortified foods (including milk and milk products, juice, bread, margarines and breakfast cereals) [9, 14, 24, 25].

Vitamin D itself is biologically inert and requires two successive hydroxylation reactions, in order to be activated. The first hydroxylation takes in the liver and is carried out by 25-hydroxylases (mitochondrial CYP27A1 and microsomal CYP2R1) which convert vitamin D into 25-hydroxyvitamin D (25(OH)D), also known as calcidiol. The second takes place mainly in the kidneys and is carried out by the e 1α-hydroxylase (mitochondrial CYP27B1) which converts 25(OH)D to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), also known as calcitriol, the active form of vitamin D [8, 20]. The production of $1,25(OH)_2D$ in the kidney is strictly regulated by several factors. The main regulatory factors are $1,25(OH)_2D$ itself, which down regulates its own production; PTH, which stimulates the renal production of the $1,25(OH)_2D$; fetal growth factor 23 and serum concentration of calcium and phosphate [26].

Many extrarenal tissues also express the 1α-hydroxylase, including osteoclasts, pancreatic islets, antigen presenting cells (macrophages/dendrite cells), monocytes, placenta, colon, skin (keratinocytes), mammary(breast), prostate, human brain, endothelium and parathyroid glands [20, 24, 26, 27]. These cells have the ability to produce local concentrations of $1,25(OH)_2D$ which have autocrine and paracrine actions. This extrarenal production plays an important role in modulating immune responses, regulation of cell differentiation, cell proliferation, and apoptosis and thus shows that vitamin D may be involved in other physiological processes, independently of calcium metabolism [14, 18, 21, 27]. This extrarenal production of $1,25(OH)_2D$ is dependent on the circulating precursor levels of 25(OH)D therefore when there is a low serum level of 25(OH)D there will be less extrarenal production [18]. The 1α-hydroxylase present in extrarenal tissues is identical to the renal 1α-hydroxylase, but regulation of its expression and activity is different. The renal 1α-hydroxylase is principally under the control of calcemia and bone signals (such as parathyroid hormone and $1,25(OH)_2D$ itself), the extrarenal 1α-hydroxylase is primarily regulated by immune signals, with interferon-γ and Toll-like receptor agonists being powerful stimulators [8].

Another hydroxylation enzyme which is involved in the vitamin D metabolism is called 24-hydroxylase, which is responsible for the degradation of 25(OH)D and 1, $25(OH)_2D$ [8].

As shown earlier DBP binds and transports vitamin D and its metabolites in plasma. This DBP is synthesized in the liver and circulates at a concentration that is in excess of normal circulating vitamin D metabolite concentration [24]. DBP has a higher affinity for 25(OH)D than $1,25(OH)_2D$. Plasma levels of DBP are 20 times higher than the total amount of vitamin D metabolites, and 99% of vitamin D

compounds are protein bound, mostly to DBP, although albumin and lipoproteins contribute to lesser degrees [18]. The complex 25(OH)D and DBP enters the cell via megalin-mediated endocytosis, the DBP is degraded by the lysozomes, and 25 (OH)D is release and delivered to the mitochondria by a intracellular vitamin D binding protein 3 for hydroxylation, that converts 25(OH)D to $1,25(OH)_2D$ or reenters the circulation bound to DBP. The $1,25(OH)_2D$ produced is transported by the DBP to nuclear vitamin D receptor (VDR) [18]. Only $1,25(OH)_2D$ is metabolically active and exerts its effects mainly by activating the VDR. This VDR is a member of the nuclear receptor super-family of ligand-activated transcription factors which also comprises the thyroid hormone receptors, estrogen receptors, retinoic acid receptors and peroxisome proliferator-activated receptor [8, 20, 21, 23]. Binding of $1,25(OH)_2D$ to the VDR leads to the transcription of genes regulated (over 200 genes) by the $1,25(OH)_2D$. The mechanism is very complex and is only just being unraveled [8]. These VDR's are not only expressed in classical $1,25(OH)_2D$-responsive target tissues (bone, kidney, intestine, parathyroid glands) but are also expressed in a broad range of other tissues (see appendix 1) [26]. The discovery that VDR's are widely expressed in the immune system (activated T cells, macrophages, dendritic cells and the B cells) led to the recognition of the central immunomodulatory role for $1,25(OH)_2D$ and the discovery of VDR in the pancreatic cells led to the recognition of the role of vitamin D in insulin production and secretion [8, 21].

3 Vitamin D recommendations

The first recommended dietary allowance (RDA) for vitamin D for Americans in 1941 was 400 international unit (IU). This value derived from an observation that this amount, which was found in a teaspoon of codfish liver oil,

was sufficient to prevent rickets. In 1997, the recommended intake level by the Food and Nutrition Board (FNB) was set as an adequate intake (AI) value rather than an RDA [27].

Table 1- DRI: dietary reference intakes; reference [28].

Current Vitamin D recommendation (DRI) for Canada and the United States for 1997

Age group	Level recommended (μg)
0–6 months	5 (200 IU)
7–12 months	5 (200 IU)
1–3 years	5 (200 IU)
4–8 years	5 (200 IU)
9–18 years	5 (200 IU)
19–30 years	5 (200 IU)
31–50 years	5 (200 IU)
51–70 years	10 (400 IU)
>70 years	15 (600 IU)

The reason why it changed from RDA to AI was because of the lack of scientific data necessary to set an RDA. In order to set an RDA, one must first determine from the published literature an estimated average requirement (EAR), which is a measure of the intake needs of 50% of a specified group. Therefore, since there was not sufficient data to draw an EAR, the RDA could not be drawn [28]. Since publication of the AI, much has been learned regarding the metabolism of vitamin D allowing an EAR to be set so that an RDA may be derived [27, 28]. However, it is very difficult to determine an accurate value for an EAR due to the fact that sunlight exposure is difficult to quantify and that foods have variable quantities of vitamin D. However, it is crucial to set EAR and RDA values in order to facilitate planning and nutrition initiatives [27].

The aim of the 1997 AI is based on the need to maintain serum 25(OH)D levels, in the absence of sunlight, at or above 27.5 nmol/L for most age groups in order to prevent rickets or osteomalacia. However, it has been shown that higher serum concentrations (75-80 nmol/L) are needed in order for vitamin D to support paracrine and autocrine functions [26, 28]. If an adult consumes the AI of 200 IU/day

the circulating 25(OH)D levels usually remain unchanged, especially during winter months therefore showing that this quantity is not enough. In a study adult submariners where supplemented with 600 IU/day for 6 months with no sunlight exposure. The results showed that not even 600 IU/day of vitamin D managed to maintain circulating 25(OH)D in a sufficient level [17].

The FNB of the National Academy of Sciences states that the upper limit (UL) is 2000 IU and the lowest observed adverse effect level is 3800 IU [29, 30]. A recent review applied the risk assessment method used by the FNB in order to try to update the safe tolerable UL for vitamin D. The conclusion that they obtained was that the UL for vitamin D for adults could be 10000 IU/day without any adverse effects. This indicates that the margin of safety for vitamin D consumption for adults could be around 10 times higher than any current recommended intakes [29]. Another study was conducted in order to assess the efficacy and safety of prolonged vitamin D intakes of 1000 and 4000 IU/day. The results showed that a intake of 1000-4000 IU/day of vitamin D/day managed to increase serum 25(OH)D without adverse effects and therefore was considered to be safe [30]. Until a consensus is made on the recommendations of vitamin D, one thing can be ascertained from several studies, being that the present AI are inadequate in order support the autocrine and paracrine functions of this vitamin and it should be reviewed and changed [27, 28].

4 Defining vitamin D status

Serum 25(OH)D is considered to be the best biochemical marker of vitamin D status as it reflects the amount ingested in the diet (including that from supplements and vitamin D fortified food products) and the amount produced in the skin in response to UVB radiation exposure [14, 19, 28]. Although $1,25(OH)_2D$

represents the active form of the vitamin, it is not a good indicator of the vitamin D status due to the tight regulation of its production as well as a relatively short half-life(4-6hours) [14, 19].

Controversy exists, due to insufficient evidence, on the cut-off points used to define vitamin D status [28, 31]. Most agree that a 25(OH)D concentration <50nmol/L is an indication of vitamin D deficiency, whereas a 25(OH) D concentration of 51-74nmol/L, is considered to indicate insufficiency and concentration of 75nmol/L is considered to be sufficiency. Vitamin D intoxication typically does not occur until 25(OH)D concentrations are >375nmol/L[9, 32]. Hipervitaminosis D (hypercalcemia and hyperphosphatemia) has been reported with serum 25(OH)D concentration ranging from 700-1600nmol/L [31]. The lack of consensus as to the serum vitamin D serum status is due to the variability in assays for 25(OH)D [31].The first assays used to measure 25(OH)D was the competitive protein binding format with the DBP as the binder. The advantage of this assay was that DBP recognized 25(OH)D_2 equally as well as 25(OH)D_3 but it also recognized other vitamin D metabolites, including 24,25 dihydroxyvitamin, 25,26-dihydroxyvitamin D, and the 25,26-dihydroxyvitamin D-26, 23-lactone. In 1985, a radioimmunoassay (RIA) was developed for 25(OH)D. This assay (Diasorin essay) also recognized 25(OH)D_2 as well as 25(OH)D_3 but it also recognized 24,25$(OH)_2$D and other polar metabolites. Thus, both the DBP and the RIA assays typically overestimated 25(OH)D levels by approximately 10–20%. In the mid 1970s high-performance liquid chromatography (HPLC) was applied to the 25(OH)D assays. HPLC was able to remove interfering vitamin D metabolites and therefore was considered to be the golden standard but the limitation was that it was not a very manageable assay, and thus, was not routinely used by reference laboratories. Later a liquid chromatography tandem

mass spectroscopy was used for the direct measurement of 25(OH)D in human serum. This assay quantitatively measured both $25(OH)D_2$ and $25(OH)D_3$ [33]. Using different methods, we may find different results even if we analyze the same sample. Thus, the cutoff value for 25(OH)D used to define the vitamin D status must be defined in terms of appropriate assay methods [27]. Membership in the international Vitamin D Quality Assessment Scheme (DEQAS), an international quality-control program, and the availability of a standard serum from the National Institute of Standards and Technology should help investigators to reduce the variability among laboratories [14, 34].

So after knowing what serum levels are considered to be sufficient (>75nmol/L) the question is, what is the intake needed in order to reach this sufficiency? A 6 months prospective study was conducted in order to answer this question. In order to know the intake needed they took into consideration the wide dose-response curve and basal 25(OH)D concentration and found that a dose of 3800 IU/day for those with serum 25(OH)D above 55nmol/L and a dose of 5000 IU/day for those below 55nmol/L was need [31]. Other studies show that an intake of 500-1000IU/day is needed to maintain serum level of 75nmol/L. Although in sunlight deprived subjects a daily intake of 4000IU/day may be needed [21].

Other authors defend that healthy adults and children can obtain enough vitamin D by being exposed to sunlight in the face and hands for 2 hours per week. However, they say that there are certain individuals that are more at risk of becoming vitamin D deficient (elderly, dark skinned, pregnant women, breastfeeding women and early childhood) therefore, may need extra food supplementation in order to keep normal serum levels of 25(OH)D [14, 21].

The lack of sun exposure is known to be the primary cause of low serum 25(OH)D. However, as stated earlier even with adequate sun exposure low serum 25(OH)D levels can be found. Table 2 (Appendix 2) shows the various factors that affect the vitamin D status in an individual [35].

5 Role of vitamin D on the pathogenesis of T1DM

The existence of VDR in activated T lymphocytes, antigen-presenting cells (APCs) (macrophages and dendritic cells), and thymus tissue raised the idea that 1,25$(OH)_2$D might function as an immunomodulator [20, 36, 37]. The fact that immune cells (activated macrophages and dendritic cells in particular) also contain the enzyme 1α-hydroxylase, which is necessary for the final activating step in the conversion of 25(OH)D to the metabolically active molecule, shows that these cells are able to synthesize and secrete 1,25$(OH)_2$D which is able to target the immune system [8].

In order to provide evidence that vitamin D affects the risk of developing T1DM, several studies have been done using animal models such as non-obese diabetic (NOD) mice. These NOD mice are genetically susceptible to diabetes and experience disease pathogenesis similar to humans with hyperglycemia, polyuria, polydipsia, glucosuria, insulitis, and depend on exogenous insulin to live. Such characteristics have made the NOD mouse an excellent and widely accepted model of human T1DM [8].

A study conducted by Mathieu *et al* reported that 1,25$(OH)_2$D has been shown to reduce T1DM onset in NOD mice. He administrated 5μg/kg of 1,25$(OH)_2$D in the NOD mice and found that the diabetes incidence in NOD mice at 200 days was reduced to 8% in the 1,25$(OH)_2$D-treated group versus 56% in the control group [38, 39]. A study conducted by Zella *et al* reported that a vitamin D deficient state

alone potentiated diabetic onset in the NOD mouse. These authors also found that daily dietary supplementation with 2000 IU of $1,25(OH)_2D$ from weaning completely prevents diabetes through 200 days of age, regardless of vitamin D status before the study [40]. They suggested that oral administration of $1,25(OH)_2D$ or preferably a nonhypercalcemic analog would be clinically relevant for the prevention of T1DM in humans [37].

A study conducted by Giulietti *et al*. reported that vitamin D deficiency in early life might increase T1DM in NOD mice. These authors found that at after 250 days 35% male and 66% female vitamin D–deficient mice were diabetic compared to 15% and 45% of the control mice. In the vitamin D–deficient mice a defect in the cytokine profile might have been the reason for the triggering of the T1DM [41].

Vitamin D is considered to be a potential environmental and genetic risk factor for T1DM [37].

Vitamin D as a Environmental risk factor

Seasonal and geographical factors are both known, as risk factors, for T1DM and vitamin D deficiency and several epidemiological studies have reported that. The probability of developing T1DM has been shown to be about 400 times more likely in a child living in Finland (latitude 61ºN) than a child living in Venezuela (latitude 8ºN) [4, 42]. An ecological study analyzed the relationship between UVB radiation, and age-standardized (<14years) incidence rate of T1DM in children, according to 51 regions of the world. The results showed that the incidence rate was higher in regions that were more distant from the equator (higher latitude), where UVB radiation is lower, than in those closer to the equator (lower latitudes), where UVB radiation is much higher (see appendix 3) [5].

A seasonal variation of T1DM diagnosed cases is also usually observed with the largest proportion of T1DM cases diagnosed during autumn-winter and the lowest during the summer. Could this be due to the sunlight exposure and vitamin D status that are highest in the summer and lowest during autumn and winter? Some studies explain this increase in incidence during winter months due to a diminished exposure to UVB radiation which leads to a decreased vitamin D production [(43)]. A recent retrospective/prospective study was conducted in the province of Newfoundland to try to investigate the temporal association between average daily UVB radiation and T1DM. This was the first study to relate incidence of T1DM to UVB data acquired from satellite data. In this study, all newly diagnosed children with T1DM were included from the year 1987 to 2005 and the incidence trends were compared with UVB trends. Results showed that monthly incidence of diagnosis of T1DM had an inverse relationship with the UVB trends where there was a lower summer incidence and higher winter incidence. These results strengthen even more the evidence that the incidence of T1DM is positively correlated with UVB radiation [(3)].

Similar seasonal and geographic variations have also been suggested for the variation of serum 25(OH)D levels. The 25(OH)D levels seem to be highest in the summer and lowest during autumn and winter [(37)]. An author defends that many cases of T1DM might be prevented by raising the serum 25(OH)D levels of infants and children living at high latitudes to levels in the range of 125-150nmol/l (50-60ng/ml) which is the serum 25(OH)D levels have been observed in lifeguards in the United States during summer [(5)]. The problem is that in order to reach these serum levels people must increase their sun exposure or their vitamin D intake to higher levels than the current recommendations. Baumgartl *et al*. reported that

serum 25(OH)D levels measured throughout the year are lower in patients newly diagnosed with T1DM than in healthy controls [37]. Several observational studies have been done throughout recent years which show that vitamin D supplementation during pregnancy and early childhood may offer protection against the development of T1DM because it prevents this vitamin D deficiency [28]. In certain countries, such as Finland, Sweden and Norway, the exposure to sunlight is below optimal for infants and pregnant women, therefore supplementation is recommended. However, this recommendation is not done in many other countries [5, 6]. Breast-milk is a poor source of vitamin D and so the vitamin D status of the newborn is dependent on the stored vitamin D acquired from the mother during pregnancy or dietary supplements before weaning [6].

In a cohort study in Colorado, the intake of vitamin D during the third trimester of pregnancy was assessed in the mothers of 233 children. These children were then followed for an average of 4 years. The results showed that maternal intake of vitamin D from food had a protective effect against the appearance of islet cell auto-antibodies that are associated with the development of T1DM. However, this effect was restricted to vitamin D intake in food. The sun exposure should have also been evaluated in order to see if it would have had any impact on the results [5]. Similar results were also found in the Diabetes Autoimmunity Study in the Young (DAISY) where an inverse relationship was found between the presence of islet auto-antibodies in offspring and maternal dietary vitamin D intake during pregnancy [44].

A case-control study done in Norway by Stene *et al* (2000) showed a strong negative association between mothers taking codfish liver oil during pregnancy and the risk of T1DM in their children. The problem with these results is that we

do not know whether this protective effect against T1DM is due to the vitamin D or due to the n-3 fatty acids [Eicosapentaenoic (EPA) and Docosahexaenoic (DHA)] present in the codfish liver oil, or whether it is due to both [(6)]. Codfish liver oil is a rich source of n-3 fatty acids (EPA and DHA) that have anti-inflammatory effects, which could be potentially relevant in the etiology of T1DM. However, it is also an important source of vitamin D so the question to be answered is whether it is vitamin D the one that provides this protective effect or is it the n-3 fatty acids [(45)]. Stene *et al* (2003) later conducted a large case-control study to investigate whether the use of dietary codfish liver oil in the first year of life was associated with lower risk of T1DM among children. In this study, parents of 545 diabetic children and 1668 population-based controls responded to mailed questionnaires, which included questions about their children's intakes of vitamin D supplements and codfish liver oil during the first year of life. The Codfish liver oil given to the infants at least 5 times a week was associated with a significant reduction in diabetes risk compared to those that were not supplemented. However, there was no evidence of a protective effect of other vitamin D supplements whether taken 1 to 4 times per week or even 5 or more times per week. The reason for the different associations of codfish liver oil and other vitamin D supplements with diabetes risk is unclear. Could this prove that it is the n-3 fatty acids that has the protective effect and not the vitamin D? The authors suggest that a possible reason for these results is that vitamin D in codfish liver oil may be more bioavailable than vitamin D in other forms[(45)]. However, this question remains to be answered. The problem codfish liver oil is that it contains variable amounts of vitamin D and usually contains high amounts of vitamin A that in high quantities causes toxicity so caution should be taken when recommending it [(46)].

A large case-control study (EURODIAB) was conducted in 7 European countries, where parents of 820 diabetic children and 2335 population-based controls were interviewed to determine whether or not their children had been given vitamin D supplementation during the first year of life. The results showed that risk for T1DM by age 15 was reduced by about a third in supplemented versus unsupplemented children. The favorable association with vitamin D persisted after adjusting for certain confounding factors [(47)].

In Finland, Hypponen et al undertook a birth cohort study, where 10 366 children who were born in 1966 were followed through to 1997, with the primary outcome being a diagnosis of T1DM. This study found that children who had oral vitamin D intake of 2000 IU/day had a lower risk of developing T1DM compared with children who consumed lower amounts. In this cohort study the serum 25(OH)D was measured and showed that, those who had rickets diagnosed early in life (thus more likely to be those with the lowest amount of vitamin D) were more likely to develop T1DM. In addition, those that were supplemented more regularly or had higher doses of vitamin D supplement displayed a reduced risk of developing T1DM [(48)].

Vitamin D as a genetic risk factor

In addition to the environmental role of vitamin D on T1DM risk, certain allelic variations in the VDR may also be of genetic risk for T1DM [(49)]. However, published findings have been conflicting. Vitamin D deficiency often runs in families, suggesting that genetic variation might account for differences in vitamin D concentrations; however, the genes regulating vitamin D concentrations remain to be identified. Genetic variation occurs in nearly all genes of the vitamin D system, but most investigations have studied more the polymorphisms of the VDR

gene. VDR polymorphisms have been associated with increase the susceptibility to T1DM in Caucasians, in Bangladeshi Indians, and in Japanese, although such association was not found in a combined scale analyses from the UK, Romania and Finland and a study conducted in Portugal [20, 50]. In a study conducted by Motohashi *et al*. found an association between a VDR gene polymorphism and acute onset of T1DM, regardless of the presence or absence of islet-associated autoantibody [51]. However in a meta-analysis that combined all data from papers published from 1997 to December 2005 showed no association between VDR gene polymorphisms and T1DM risk showed [52].The initial studies used only the polymorphic FokI, BsmI, ApaI and TaqI variants but recently more polymorphisms have been identified [20].

Other allelic variants such as genetic variants of the DBP and α-hydrolylase have also been found to increase the susceptibility to T1DM but have been less studied. The role of the genetic factor is still debatable. Therefore more studies are needed in order to prove this association, however it should be taken into consideration [20, 51].

5.1 Possible mechanisms of vitamin D action on T1DM

As mentioned earlier T1DM is a chronic progressive autoimmune disease that affects genetically prone individuals. The autoimmune process is an inflammation response targeted specifically at the β-cells in the islets of Langerhans, causing their mass reduction and dysfunction [53]. Progression of T1DM has been shown to involve infiltration into pancreatic islet cells by several types of immune cells including antigen-presenting cells (APCs - such as macrophages and dendritic cells), CD4+, and CD8+ T cells, and B cells [51].

In this autoimmune process, the helper T cells (Th0) that derive from the $CD4^+$ T cells have a central role. The microenvironment in which Th0 cells develop determine which of 2 subtypes predominates (Th1 or Th2).The Th1 and Th2 cell responses regulate each other and, during “normal” immune responses, the organism responds with a balance of the 2 subtypes. Th1 cells secrete interferon γ (IFN-γ), interleukin-2 (IL-2) and tumor necrosis factor α (TNF-α) which activate cell-mediated immunity, that is, cytotoxic and inflammatory responses mediated by T cells, natural killer cells, and macrophages. Th2 cells secrete cytokines (IL-4, IL-5, IL-6, IL-9, IL-10, IL-13) which activate humoral immunity, that is, antibody production. In autoimmune diseases like T1DM, Th1 cells are misdirected against self-proteins, which result in β-cell destruction of the pancreatic islets of Langerhans [43, 51]. Prevention of this autoimmune destruction requires early intervention in order to prevent the β-cells destruction from happening. Therefore, more studies should be done in order to figure out all possible factors that initiate this process [53].

A unique feature of $1,25(OH)_2D$ as a immunomodulator is the fact that it not only interacts with T cells, but it also target the APCs [8]. T cells (particularly of the Th1 type) are affected by $1,25(OH)_2D$ which suppresses their proliferation and cytokine production. It has been shown that $1,25(OH)_2D$ decreases the secretion of interleukin IL-2 (a important T-cell differentiation factor), TNF-α and IFN-γ by Th1 and promote the Th2 cytokine production of IL- 5, IL-4 and IL-10 production, therefore tilting the T cell response towards Th2 dominance and inhibits the Th1 response [21, 43]. In VDR knockout mice there was more Th1 cytokine secretion and less of Th2 cytokines. Therefore, in the absence of $1,25(OH)_2D$ or its receptors the T cells compartment has a potentially stronger Th1 phenotype [54]. Therefore,

$1,25(OH)_2D$ by binding to VDR may act as a transcriptional regulator of Th cell cytokines synthesis and Th cell differentiation and therefore it is involved in the pathogenesis of T1DM [43]. The APC also plays an important role in the immune function of $1,25(OH)_2D$. The cytokines produced by the APCs for the recruitment and activation of T cells are directly influenced by $1,25(OH)_2D$ [8, 37]. The dentritic cells (DCs) for example only have antigen presenting capacity when they are in its mature state. The differentiation and maturation of DCs into a potent APC's is inhibited by $1,25(OH)_2D$ and its analogs [37]. The mature DCs have cell-surface Major Histocompatibility Class II molecules and accessory signals for T cell activation and are able to produce higher levels of IL-12. When helper T cells (Th0) are exposed to mature DCs and the IL-12, the Th1 cells predominate over Th2 cells activating macrophages and cytotoxic T cells, which in turn can directly destroy the β-cells of the pancreas. When $1,25(OH)_2D$ is increased it has been shown to inhibit DCs from maturing and prevents this process from happening [49]. $1,25(OH)_2D$ seems to also suppresses the antigen presenting capacity of the macrophages but in vitro, it is known to stimulate the phagocytosis and killing of bacteria by macrophages [8].

Taken together, these observations suggest a physiological role for $1,25(OH)_2D$ in the immune system, with a tightly regulated secretion of $1,25(OH)_2D$ by macrophages and dendritic cells upon immune stimulation on the one hand and a direct inhibitory effect of the molecule on antigen presentation and T cell proliferation and cytokine secretion on the other hand. These immune effects are typically mediated through the binding of $1,25(OH)_2D$ to VDR since these receptors are present in all of these immune cells [8].

6 Role of vitamin D on the pathogenesis of T2DM

The prevention and treatment of T2DM is a public health concern in many health systems. T2DM is usually due to resistance to insulin action in the setting of inadequate compensatory insulin secretory response. It does not emerge in all individuals with insulin resistance but rather only in those with a defect in insulin secretory capacity (presumably genetic) in which pancreatic insulin secretion fails to compensate for the insulin resistance and hyperglycemia occurs [(55)]. This hyperglycemia is first exhibited as an elevated postprandial blood glucose caused by insulin resistance at the cellular level and then is followed by a elevation in fasting glucose concentrations [(56)]. In T2DM, there may be a progressive loss of pancreatic islet β-cells resulting in insulin deficiency and the need to administrate insulin to compensate this loss [(55)].

Both environmental and genetic factors seem to contribute to the development of the disease [(36)]. Potential modifiable environmental risk factors for T2DM have been identified, the major one being obesity, particularly central obesity [(55)]. Recent evidence has shown that vitamin D could also be an environmental or genetic factor that may play a role in the pathogenesis of T2DM [(57)].

Vitamin D as a environmental risk factor

The association that vitamin D has with T2DM is suggested by a seasonal variation in glycemic control, which has been reported in patients with T2DM as being worse in winter. This may, at least in part, be due to prevalent hypovitaminosis D in the winter. In a population-based study done Hungary, managed to report seasonal pattern in the onset of T2DM, more cases were diagnosed in winter than in the summer months [(38)]. It has been suggested that vitamin D decreases the risk of developing T2DM and therefore individuals with

hypovitaminosis are more at risk of developing the disease. A study reported that a London Bangladeshi population (with higher risk of developing T2DM) have lower serum vitamin D levels compared with the British Caucasian population (with a lower risk of developing T2DM) suggesting that vitamin D status might contribute to the pathogenesis of the disease. Short-term vitamin D replenishment in the Bangladeshi Asian population increased insulin secretion without any glycemic alterations, but with longer vitamin D treatment the glycaemias were also improved [(36)].

Hypovitaminosis D is also common in obese individual and obesity as already stated is a risk factor of T2DM. This hypovitaminosis D observed in obese individuals is due to the storage of vitamin D (liposoluble vitamin) in the adipose tissue where it is no longer bioavailable [(36)].In subjects with obesity, elevated PTH levels have also been reported. This hyperparathyroidism may contribute to the production of insulin intolerance since PTH inhibits the synthesis of vitamin D thus contributes to insufficient circulating 25(OH)D levels [(36, 58)]. Therefore treating the vitamin deficiency in obese individuals we may be able to improve glucose tolerance and decrease the chance of T2DM to develop [(36)].

Some other studies have shown that vitamin D combined with calcium supplementation is also known to decrease the risk of T2DM [(57, 59, 60)]. One of these studies, the Nurses Health Study, reported that a daily intake of >800IU of vitamin D and >1,200mg of calcium was associated with a 33% lower risk of T2DM compared to a daily intake of <400IU of vitamin D and 600mg of calcium [(59)]. Another study done in older individuals with impaired fasting glucose, shows that combined calcium and vitamin D supplementation attenuated the increase in glycaemia and insulin resistance that occur with aging. This study provides a

preliminary support for the important role of vitamin D and calcium supplementation in lowering the risk of progression of diabetes in individuals with glucose intolerance [60].

Vitamin D as a genetic risk factor

The VDR seem to be present in the pancreatic tissue and mutations in the VDR genes have been shown to contribute to the genetic preposition of T2DM. Studies in mice expressing functionally inactive mutant VDR showed a pronounced impairment in oral glucose tolerance and insulin secretory capacity, together with a reduction in pancreatic insulin mRNA levels in normally fed animals. Studies done on humans also reported that allelic variations in the VDR gene modulated β-cell function. For example in a cohort study of nondiabetic Bangladeshi subjects (a population considered at risk for T2DM) showed that polymorphic BsmI, ApaI and TaqI variants influenced insulin secretion in response to an oral glucose tolerance test [61].

Other polymorphisms beside those on VDR genes have also been studied. These polymorphisms include those on DBP (transports 25(OH)D) and 1α-hidroxylase (converts 25(OH)D to its active metabolite 1,25$(OH)_2$D) genes. Few studies have been done on the polimormisms on the 1α-hidroxylase gene. However, polimormisms on DBP genes have been suggested to affect the availability of active vitamin D forms in β-cells and insulin secretion. Nonetheless, this effect has only been found in certain ethnic groups [36]. Like in T1DM more studies need to be done in order to prove this association.

6.1 Potential mechanisms of vitamin D on T2DM

For glucose intolerance and T2DM to develop, defects in pancreatic β-cells function, insulin sensitivity, and systematic inflammation are often present. Evidence has been found that vitamin D has a role in all these mechanisms [57].

6.1.1 Effects of vitamin D on pancreatic β-cell function

In the recent years, is has been suggested that vitamin D is essential for normal insulin release in response to glucose. Vitamin D deficiency present in both humans and animal models is shown to alter insulin synthesis and secretion [36]. There is evidence that vitamin D may stimulate pancreatic insulin secretion directly and indirectly. The direct effect takes place when $1,25(OH)_2D$ binds to the nuclear VDR; which is found in a variety of tissues, including the pancreatic islet β-cells [62]. The VDR is considered to be the master regulator of transcription and is shown to activate the protein biosynthesis in pancreatic islets therefore increasing the insulin secretion [20, 36]. In order to understand the role of vitamin D in β-cell function, transgenic VDR knockout mice have been generated. In these mice the circulating insulin concentrations is reported to be lower and blood glucose concentrations higher. However, data from VDR-knockout mice are conflicting because the genetic backgrounds of these knockout mice appear to be crucial, because VDR-knockout mice with a different genetic background have shown to have normal β-cell function [8, 20].

The indirect effects of vitamin D may be mediated via regulating extracellular calcium and calcium flux through the β-cell. Insulin secretion is a calcium-dependent process; therefore, alterations in calcium flux can have adverse effects on β -cell secretory function. Vitamin D insufficiency has been shown to alter the

balance between the extracellular and intracellular β-cell calcium pools, which may interfere with normal insulin release, mainly in response to glucose [57].

In a study conducted by Bourlon *et al* it was shown that 1,25$(OH)_2$D could activate the de novo biosynthesis of insulin in islets from vitamin D-deficient rats after the stimulation of the islets with glucose, increasing the rate of conversion of proinsulin to insulin. In this study they could not establish whether this acceleration of the conversion from proinsulin to insulin was a direct or an indirect effect of vitamin D or of calcium, since this process is calcium-dependent [63]. In a case study conducted by Kumar *et al* observed that when the patient was vitamin D deficient was supplemented with 2000 IU, an improvement in glucose tolerance was observed. The authors stated that this improvement in glucose tolerance was mainly due to improvement in β-cell insulin secretary capacity. This report shows the importance of vitamin D on the regulation of insulin secretion [64].

6.1.2 Effects of vitamin D on insulin resistance

Vitamin D may have an effect on insulin sensitivity either directly, by stimulating the expression of insulin receptor and thereby enhancing insulin responsiveness for glucose transport, or indirectly via its role in regulating extracellular calcium and ensuring normal calcium influx through cell membranes and adequate intracellular cytosolic calcium pool. Intracellular cytosolic calcium is essential for optimal insulin-mediated functions in insulin-responsive tissues such as skeletal muscle and adipose tissue. Changes in intracellular cytosolic calcium in primary insulin target tissues may contribute to peripheral insulin resistance [57]. In one study with healthy adults, there was a positive correlation of 25(OH)D concentration with insulin sensitivity and a negative effect of hypovitaminosis D on β-cell function, even after correction for confounding factors such as body

composition [65]. In another study however found no relationship between vitamin D status and glucose control [66].

6.1.3 Effects of vitamin D on systemic inflammation

Inflammatory factors have often been associated with insulin resistance and β-cell failure, both of which are key features of T2DM. An increase in acute-phase proteins (C-reactive protein), cytokines (TNF-α, TNF-β, IL-6) and mediators associated with endothelial dysfunction has been reported in T2DM [36].

Vitamin D may improve insulin sensitivity and promote β-cell survival by directly modulating the generation and effects of cytokines [57]. Vitamin D is reported to downregulate the production of several cytokines: IL2, IL6, IL12, interferon-γ, TNF-α, TNF-β. To date, however, it remains to be elucidated whether the systemic inflammation observed in T2DM might be influenced by the immune properties of vitamin D [36].

7 Role of vitamin D on the pathogenesis of GDM

Gestational diabetes mellitus is defined as glucose intolerance, with its onset occurs or is first recognized during pregnancy representing, on average,3–8% of all pregnancies [25]. Because GDM is a state of insulin resistance like T2DM and relative insulin insufficiency, it parallels T2DM in many aspects [67]. It has long been known that vitamin D deficiency is prevalent among pregnant women and is shown to be common in both healthy pregnant women and pregnant women with GDM [36]. In a study conducted by Clifton-Bligh *et al* in Australia found that maternal 25(OH)D concentrations are inversely related to fasting glucose and insulin concentrations. However, the authors did not find a significant relative risk of gestational diabetes in women with low 25(OH)D levels

because vitamin D deficiency was also shown to be common in healthy pregnant women. A serum 25(OH)D concentration < 50 nmol/l was present in 48% of this cohort study and similarly high rates have also been described in pregnant women in other populations [(68)].

In a cross-sectional study, serum concentrations of 25(OH)D measured at the time of GDM screening test were significantly lower in GDM women than in normal glucose tolerance pregnant women. The results managed to show a positive correlation of 25(OH)D concentration with insulin sensitivity and vitamin D deficiency could be a sign of insulin resistance [(25)]. In a recent study suggested that maternal vitamin D deficiency in early pregnancy is significantly associated with an elevated risk for GDM. This association remained statistically significant even after controlling for established risk factors such as maternal age, family history of T2DM, race/ethnicity and pre-pregnancy BMI [(69)]. Few studies have been done in order to see the influence of vitamin D in glucose homeostasis during pregnancy and the development of GDM. However, pregnancy is a critical stage to prevent Vitamin D deficiency even if the role that Vitamin D has on GDM has not been proved. Vitamin D status of a newborn is largely determined by the mother status and therefore if vitamin D levels are low during pregnancy, the newborn also will have low 25(OH)D status. Low stores of vitamin D have been shown to affect the prevalence of T1DM therefore this should be avoided [(70)].

8 Critical analyses and Conclusion

Diabetes is one of the fastest growing chronic diseases worldwide that contribute to a great number of deaths. The fact that the number of people with diabetes might be doubled in the next 25 years is an extremely concerning thought. Due to this increase in prevalence there is an urgent need to discover and

implement preventive measures to address this growing epidemic. Several studies have tried to figure out all possible causes for of T1DM in order to find preventive measures but none of these measures have shown to be effective. However, for T2DM simple measures have been found to be effective in preventing or delaying the onset of this disease. Vitamin D deficiency has been considered to be an environmental and genetic factor that affects the onset of T1DM, T2DM and GDM. It has been shown that individuals that are vitamin D deficient have an increased probability of developing diabetes mellitus. This review presents a number of studies on animals and humans that have managed to prove that.

Some people may think that vitamin D deficiency is not common due to the fact that it is produced by the skin with sunlight exposure, but several studies have shown that vitamin D deficiency has become a worldwide epidemic problem that is present in all ages. This deficiency is increasing due to the fact that people cover themselves with clothing and spend a lot of time closed in buildings and less time outdoors. The skin cancer campaigns also contributed to the decrease in sunlight exposure. The question is: should health professionals advise individuals to ‘‘return to nature’’ and be exposed to the sun more often so that 25(OH)D concentrations raise to a sufficient level? Controversy exists on whether to rely only on sunlight radiation to meet vitamin D needs. The reason for this is that solar radiation contains both UVB (responsible for the vitamin D synthesis and sunburn) and UVA (accountable for photoaging). Both UVB e UVA leads to skin cancer. The problem with extended UV exposure is that it also increases the risk of skin cancer or melanomas. When sunscreen is used it blocks the absorption of both these rays therefore protecting against cancer but decreasing/inhibiting the vitamin D production. A key question is, whether a threshold exists for meeting individuals

vitamin D needs through UVB exposure while minimizing the risk of several types of skin cancer (eg. basal cell carcinoma, squamous cell carcinoma, and melanoma) [(24)]. Until this question is answered, caution is needed when recommending more sunlight exposure in order to meet vitamin D needs but one thing is certain total sunlight exclusion should not be recommended but the most dangerous hours of exposure should be avoided.

In countries with higher latitudes where UV exposure is not sufficient for the production of Vitamin D food sources are extremely important. In nature, very few foods have vitamin D. Almost all of the human intake of vitamin D from foods comes from fortified milk products and other fortified foods such as breakfast cereals. Both natural and fortified foods have a variable quantity of vitamin D. Therefore, human diets do not provide sufficient vitamin D to maintain serum 25(OH)D concentrations in the absence of sun exposure. As a result, supplementation may be needed. Several studies have shown that administration of high doses of vitamin D had no adverse effect. The problem with these studies is that most of them are observational studies that do not provide proof of causality. In order to draw up real conclusions well designed interventional studies are required in order to ascertain if improving 25(OH)D concentrations by utilizing supplements will have a beneficial effect on the immune system in T1DM and on the glycemic control in T2DM and in GDM. These studies need to be done before embarking on long-term interventions with supplements. These studies should also take into consideration the baseline 25(OH)D concentration, because the impact of supplementing will be greater when individuals are vitamin D deficient than in those with sufficient levels. Many studies did not measure this before conducting the study and therefore could not see the real effect that the

supplementation had. Properly designed dose-response studies should also be done in order to see the necessary dose to have beneficial health effects, such as the effects on T1DM, T2DM and GDM.

In order to explore the effects of $1,25(OH)_2D$ on diabetes mellitus in humans the development of safe structural analogues will be required. Analogs that manage to dissociate the effect of vitamin D on calcium metabolism (calcaemic effect) from the effect that it has on the immune system, insulin production and insulin sensitivity (non-calcaemic effect). Some novel analogues have been developed with a potent effect on the immune system and on the pancreatic function but with less interference on calcium metabolism. Thus allowing higher doses to be administrated. These analogues are currently being analyzed for their therapeutic potential [(20)]. However, before intentional preventive and/or therapeutic measures can be developed there is an urgent need to determine the RDA and reach a consensus on what is considered to be deficiency, insufficiency and sufficiency. Several studies throughout the review used different cutoff points for 25(OH)D, which make it more difficult to evaluate and compare their results. A consensus must also be reached on which essays should be used to determine the 25(OH)D concentration. Different results have been obtained when using different essays even when the sample is the same. This will lead to incorrect estimation of the 25(OH)D concentrations and therefore incorrect diagnosis of vitamin D deficiency.

In conclusion, vitamin D deficiency exists and is becoming a concerning health problem. Several studies have shown the health benefits that vitamin D has and the effects it has on Diabetes Mellitus. Current recommendations should be reviewed in order to see if any changes must be made. The public should be made aware of the potential health benefits of vitamin D in order to take certain

precautions. Health professionals need to “broaden their horizon” and stop thinking of vitamin D as a vitamin but instead as hormone. Maybe if doctors recommend moderate but secure exposure to sunlight this could be beneficial in preventing vitamin D deficiency. Nutritionists also have an extremely important role in creating strategies to incorporate vitamin rich diets in their community of work. The food sources become more important in the winter months and countries farther from the equator where very little or no vitamin production occurs. The needs of vitamin D will vary from country to country (according to latitude) therefore the policies have to be adjusted accordingly. In some countries the winters are longer and the needs will be higher. Individual strategies are also needed, because not all individuals have the same sunlight exposure and individuals have different capacities in producing vitamin D. More attention is needed on the individuals who are considered to be more at risk of vitamin D deficiency. These individuals include pregnant women, breast-feeding women, infants, dark-skinned individuals, obese individuals and the elderly. Serum 25(OH)D could be analyzed in some these individuals in order to see if vitamin D requirements are met. If not, health professionals should provide safe strategies to bring concentrations to what is considered sufficient.

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Appendix

Appendix 1

Table 1-Tissues that express the vitamin D receptor for the steroid hormone 1,25 $(OH)_2D$; reference[26].

TISSUE DISTRIBUTION	
Adipose	Muscle, embryonic
Adrenal	Muscle (smooth)
Bone	Osteoblast
Bone marrow	Ovary
Brain	Pancreas β cell
Breast	Parathyroid
Cancer cells	Parotid
Cartilage	Pituitary
Colon	Placenta
Epididymis	Prostate
Hair follicle	Retina
Intestine	Skin
Kidney	Stomach
Liver(fetal)	Testis
Lung	Thymus
Lymphocytes(B&T)	Thyroid
Muscle, cardiac	Uterus

Appendix 2

Table 2 - Causes of vitamin D deficiency, Reference [9, 14, 71]

Causes	*Effects*
Reduced skin synthesis	
Sunscreen use- absorption of UVB radiation by sunscreen	Reduced Vitamin D synthesis- SPF 8 by 92,5%. SPF 15 by 99%
Skin pigmentation-absorption of UVB radiation by melanin	Reduced Vitamin D synthesis by as much as 99%
Aging-reduction of 7-DHC in the skin	Reduces Vitamin D synthesis by about 75% in a 70 year old
Season, latitude and time of the day- number of solar UVB photons reaching the earth depending on the zenith angle of the sun (the more oblique the angle, fewer UVB photos reach the earth)	Above 35º north/south latitude, little or no vitamin D can be produced in the winter months.
Atmospheric pollution-reduces the direct exposure to the UVB radiation	
Patients with skin grafts from burns-marked reduction of 7-DHC in the skin	Decreases the amount of vitamin D the skin can produce.
Decreased bioavailability	
Malabsorption- reduced in fat absorption, resulting from cystic fibrosis, celiac disease, Whipple´s diseases, Crohn´s disease, bypass surgery, medication that reduces cholesterol absorption, and other causes.	Impairs the body's ability to absorb vitamin D
Obesity- sequestration of vitamin D in the body fat	Reduce availability of vitamin D
Increased catabolism	
Anticonvulsants, glucocorticoids, , and antirejection medication-binding to the steroid and xenobiotics receptors	Activates the destruction of 25(OH)D and $1,25(OH)_2D$ to inactivate calcitriol acid
Breast-feeding	
Poor Vitamin D content in human milk	Increases infant risk of vitamin D deficiency when breast milk is the only source of nutrition
Decreased synthesis of 25(OH)D	
Liver failure	
Mild-to-moderate dysfunction	Causes Malabsorption of vitamin D, but production of 25(OH)D is possible.
Dysfunction of 90% or more	Results in inability to make sufficient 25(OH)D
Increased urinary loss of 25(OH)D	
Nephrotic syndrome-loss of 25(OH)D bound to Vitamin D binding protein in urine	Results in substantial loss of 25(OH)D to urine
Decreased synthesis of $1,25(OH)_2D$	
Chronic kidney disease	
Stages 2 and 3 (estimated glomerular filtration rate, 31 to 89 ml/min/1.73 m2)	

Hyperphosphatemia increases fibroblast growth factor 23,which decreases 25(OH)D-1α-hydroxylase activity	Causes decreased fractional excretion of phosphorus and decreased serum levels of $1,25(OH)_2D$
Stages 4 and 5 (estimated glomerular filtration rate <30 ml/ min/1.73 m2)	
Inability to produce adequate amounts of $1,25-(OH)_2D$	Causes hypocalcemia, secondary hyperparathyroidism, and renal bone disease
Heritable disorders — rickets	
Pseudovitamin D deficiency rickets (vitamin D–dependent rickets type 1) — mutation of the renal 1α- hydroxylase gene (CYP27B1)	Causes reduced or no renal synthesis of $1,25(OH)_2D$
Vitamin D–resistant rickets (vitamin D–dependent rickets type 2) — mutation of the vitamin D receptor gene	Causes partial or complete resistance to $1,25(OH)_2D$ action, resulting in elevated levels of $1,25(OH)_2D$
Vitamin D–dependent rickets type 3 — overproduction of hormoneresponsive- element binding proteins.	Prevents the action of $1,25(OH)_2D$ in transcription, causing target-cell resistance and elevated levels of $1,25(OH)_2D$
Autosomal dominant hypophosphatemic rickets — mutation of the gene for fibroblast growth factor 23, preventing or reducing its breakdown	Causes phosphaturia, decreased intestinal absorption of phosphorus,hypophosphatemia, and decreased renal 1α-hydroxylase activity, resulting in low-normal or low levels of $1,25(OH)_2D$
X-linked hypophosphatemic rickets — mutation of the PHEX gene, leading to elevated levels of fibroblast growth factor 23 and other phosphatonins	Causes phosphaturia, decreased intestinal absorption of phosphorus, hypophosphatemia, and decreased renal 1α-hydroxylase activity, resulting in low-normal or low levels of $1,25(OH)_2D$
Acquired disorders	
Tumor-induced osteomalacia — tumor secretion of fibroblast growth factor 23 and possibly other phosphatonins	Causes phosphaturia, decreased intestinal absorption of phosphorus,hypophosphatemia, and decreased renal 1α-hydroxylase activity, resulting in low-normal or low levels of $1,25(OH)_2D$
Primary hyperparathyroidism — increase in levels of parathyroid hormone, causing increased metabolism of 25(OH)D to $1,25(OH)_2D$	Decreases 25(OHD levels and increases $1,25(OH)_2D$ levels that are high-normal or elevated.
Granulomatous disorders, sarcoidosis, tuberculosis, and other conditions, including some lymphomas — conversion by macrophages of 25(OH)D to $1,25(OH)_2D$	Decreases 25(OH)D levels and increases $1,25(OH)_2D$ levels
Hyperthyroidism — enhanced metabolism of 25(OH)D	Reduces levels of 25(OH)D

Appendix 3

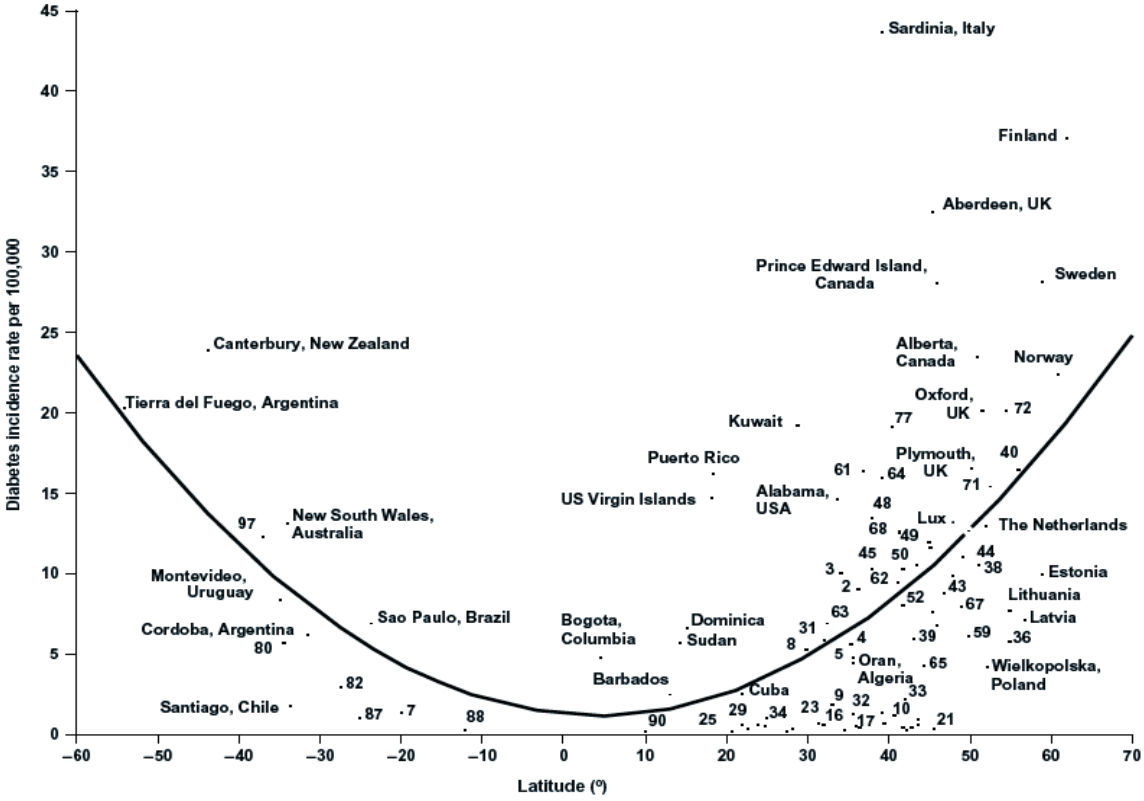

Figure 1: Age-standardised incidence rates of type 1 diabetes per 100,000 boys <14 years of age, by latitude, in 51 regions worldwide, 2002. Data points are shown by dots; names shown adjacent to the dots denote location, where space allows. Where space was limited, numerical codes (below) designate location. Source: data from WHO DiaMond [3]. Lux., Luxembourg. Numerical codes for areas: 2. Beja, Tunisia; 3. Gafsa, Tunisia; 4. Kairoan, Tunisia; 5. Monastir, Tunisia; 7. Mauritius; 8. Wuhan, China; 9. Sichuan, China; 10. Huhehot, China; 16. Nanjing, China; 17. Jinan, China; 21. Harbin, China; 23. Changsha, China; 25. Hainan, China; 29. Hong Kong, China; 31. Israel; 32. Chiba, Japan; 33. Hokkaido, Japan; 34. Okinawa, Japan; 36. Novosibirsk, Russia; 38. Antwerp, Belgium; 39. Varna, Bulgaria; 40. Denmark; 43. France; 44. Baden, Germany; 45. Attica, Greece; 48. Sicily, Italy; 49. Pavia, Italy; 50. Marche, Italy; 52. Lazio, Italy; 59. Krakow, Poland; 61. Algarve, Portugal; 62. Coimbra, Portugal; 63.Madeira Island, Portugal; 64. Portalegre, Portugal; 65. Bucharest,Romania; 67. Slovakia; 68. Catalonia, Spain; 71. Leicestershire, UK;72. Northern Ireland, UK; 77. Allegheny, PA, USA; 80. Avellaneda,Argentina; 82. Corrientes, Argentina; 87. Paraguay; 88. Lima, Peru;90. Caracas, Venezuela; 97. Auckland, New Zealand. Data points not labelled because of space constraints: 11. Dalian, China; 12. Guilin, China; 13.Beijing, China; 14. Shanghai, China; 15. Chang Chun, China; 18. Jilin, China; 19. Shenyang, China; 20. Lanzhou, China ; 22. Nanning, China; 24. Zhengzhou, China ; 26. Tie Ling, China; 27.Zunyi, China ; 28. Wulumuqi, China; 35. Karachi, Pakistan; 37. Austria ; 46. Hungary ; 51. Turin, Italy; 53. Lombardia, Italy; 66. Slovenia; 79. Chicago, USA. Reference [5]