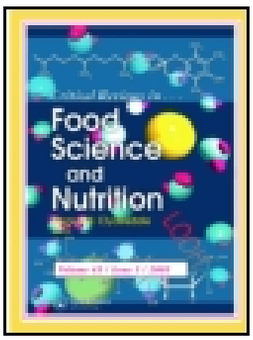

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Vitamin D and pancreas: the role of sunshine vitamin in the pathogenesis of Diabetes Mellitus and Pancreatic Cancer

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Abstract

Increasing evidence suggests that vitamin D exerts multiple effects beyond bone and calcium metabolism. Vitamin D seems to play a role in pancreatic disease, including type 1 and type 2

diabetes mellitus as well as pancreatic cancer. Vitamin D's immune-modulatory action suggests that it could help prevent type 1 diabetes. In type 2 diabetes, vitamin D may influence β-cell function, insulin sensitivity, and systematic inflammation—all characteristic pathways of that disease. Data from observational studies correlated vitamin D deficiency with risk of type 1 and type 2 diabetes. Prospective and ecological studies of pancreatic cancer incidence generally support a beneficial effect of higher 25-hydroxyvitamin D concentration as well as inverse correlations between UVB dose or exposure and incidence and/or mortality rate of pancreatic cancer. This review discusses the literature regarding vitamin D's role in risk of diabetes and pancreatic cancer. The results to date generally satisfy Hill's criteria for causality regarding vitamin D and incidence of these pancreatic diseases.

However, large randomized, blinded, prospective studies are required to more fully evaluate the potential therapeutic role of vitamin D in preventing pancreatic diseases.

Keywords

vitamin D, type 1 diabetes mellitus, type 2 diabetes mellitus, insulin secretion, insulin resistance, insulin sensitivity, pancreatic cancer

Introduction

Vitamin D's classical role is to maintain calcium homeostasis and help regulate bone metabolism (Bhan 2014). However, increasing evidence shows that vitamin D exerts multiple effects on several other organ systems (Deeb et al. 2007; Hossein-Nezhad and Holick, 2013; Leu and Giovannucci 2011; Muscogiuri et al. 2014; van Etten and Mathieu 2005).

Vitamin D_3 (cholecalciferol) derives mainly from photosynthesis in the skin after exposure to solar ultraviolet-B (UVB) radiation, which converts 7-dehydrocholesterol. Small amounts of vitamin D can also be obtained by nutritional intake from dairy products, eggs and wild oily ocean fish. Cholecalciferol itself is biologically inert and requires two successive hydroxylation reactions. The first occurs in the liver, on the C25 position, to form 25-hydroxyvitamin D_3 [$25(OH)D_3$, or calcidiol]. The second takes place in the kidney, in the α position of C1, to form 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$, or calcitriol], which represents the active form of vitamin D (Wootton 2005). $1,25(OH)_2D_3$ acts through binding the nuclear vitamin D receptor (VDR) that heterodimerizes with the retinoid X receptor and mediates the transcription of target genes after binding the vitamin D response element (VDRE). In addition, $1,25(OH)_2D_3$ acts as a chemical messenger to generate non-genomic effects and rapid responses (Bikle 2014).

U.S. Endocrine Society guidelines defined the following criteria for 25(OH)D serum levels (Holick et al. 2011):

- Deficiency, less than 20 ng/mL (less than 50 nmol/L)
- Insufficiency, 21–29 ng/mL (50–74 nmol/L)
- Satisfactory status, 30–100 ng/mL (75–250 nmol/L)

Vitamin D deficiency is common in the general population and several factors may predispose people to a higher risk of developing that state, as geographic distribution and seasonality, skin pigmentation, age and lifestyle.

Epidemiological studies have correlated vitamin D deficiency with pancreatic diseases, such as type 1 and type 2 diabetes mellitus (T1DM and T2DM, respectively) (Muscogiuri et al. 2014; Pittas et al. 2007; Van Belle et al. 2013) and pancreatic cancer (PC) (Wolpin et al. 2012). Examining the literature, this review discusses the relationship between vitamin D status and risk of developing T1DM, T2DM, and PC and evaluates the Hill's criteria for causality regarding vitamin D and incidence of each of these diseases. It also discusses vitamin D’s potential therapeutic role in preventing pancreatic diseases.

Hill's criteria

Hill proposed a set of criteria that can be used to assess whether the evidence from various studies support a causal link between a suspected agent and an outcome in biological systems (Hill, 1965). The important criteria for UVB exposure and vitamin D are strength of association, consistency, temporality, biological gradient, plausibility (e.g., mechanisms), experiment (e.g., clinical trial), and analogy. Later, others added accounting for confounding factors and removing bias (Potischman and Weed, 1999). While not all criteria need be satisfied to claim causality, the more that are, the stronger the case. Hill's criteria have been applied to several health outcomes related to vitamin D: cancer (Grant, 2009), (Mohr et al. 2012), multiple sclerosis (Hanwell and Banwell 2011), periodontal disease (Grant and Boucher, 2010), and cardiovascular disease (Weyland et al. 2014).

Vitamin D and Type 1 Diabetes Mellitus

T1DM is a chronic autoimmune disease that destroys pancreatic β cells, causing insulin insufficiency. To survive, T1DM patients must take insulin (Atkinson and Maclaren 1994; Eisenbarth 1986). Both genetic predisposition and environmental factors underlie the development of T1DM (Atkinson and Eisenbarth 2001). One of the potentially important environmental factors is vitamin D. Vitamin D plays a protective role by regulating components of the immune system and regulating calcium homeostasis, which is important for immune function and insulin secretion (Chakhtoura and Azar 2013; Wolden-Kirk 2011).

In vitro and in vivo studies

In vitro studies have suggested that the active form of vitamin D [$1,25(OH)_2D_3$] protects β cells against proinflammatory cytokines and chronic inflammation, which are involved in cellular stress and apoptosis (Korf et al. 2012; Wolden-Kirk et al. 2014). Indeed, the onset of T1DM is characterized by chronic infiltration in the islets of Langerhans of $CD4^+$ and $CD8^+$ T cells, B lymphocytes, and macrophages (Atkinson and Maclaren 1994; Foulis et al. 1991). $1,25(OH)_2D_3$, or its analogues, can inhibit lymphocyte and macrophage activation, abolish $CD4^+$ expression by inhibiting interleukin 2 and interferon γ, and reduce expression of major histocompatibility complex class II molecules (Ferreira et al. 2014; Riachy et al. 2001).

In vivo studies also confirmed a protective role of vitamin D in T1DM. In animal models of nonobese diabetic (NOD) mice, severe vitamin D deficiency increased risk of developing T1DM (Giulietti et al. 2004). Administering high-dose $1,25(OH)_2D_3$ (5 μg/kg of body weight on alternating days) from early life suppressed insulitis and reduced incidence of T1DM in NOD mice (Mathieu et al. 1994; Riachy et al. 2006; Zella et al. 2003). In contrast to previous studies (Giulietti et al. 2004; Mathieu et al. 1994; Riachy et al. 2006; Zella et al. 2003), which showed

more severe diabetes in vitamin D–deficient NOD mice, Gysemans and colleagues showed that the absence of VDR in knockout NOD mice did not influence the onset, severity, or incidence of diabetes (Gysemans et al. 2008). This discrepancy between the absence of the receptor and the absence of the ligand had already been reported for hair growth (Skorija et al. 2005) and in the immune system (Griffin et al. 2001). The authors speculated that VDRs had a function of their own and that the absence of VDRs resulted in a different phenotype from the absence of the ligand (Gysemans et al. 2008).

A study of rats with inflammation-driven diabetes induced by streptozotocin also showed vitamin D's potential role in T1DM. Administering $1,25(OH)_2D_3$ (250 mg, three times per week) improved diabetes (Del Pino-Montes et al. 2004).

However, very high doses of vitamin D are necessary for humans to achieve the metabolic and immune effect obtained in animal models (Hathcock et al. 2007; Holick et al. 2011). This dose of vitamin D could cause calcemic side effects, such as hypercalcemia and consequent hypercalciuria, and kidney stones when administered long-term. To go beyond these limits, researchers developed synthetic analogues of $1,25(OH)_2D_3$. These analogues had less effect on calcium homeostasis but had the same effect on modulating the immune system. The administration of different vitamin D analogs in NOD mice, such as MC1288 (Casteels et al. 1998) or BXL-219 (Giarratana et al. 2004), prevented T1DM from progressing by inhibiting inflammation even after the initiation of insulitis.

Observational studies

An increasing number of epidemiological studies have associated low serum 25(OH)D levels with several autoimmune diseases (Bellastella et al. 2015; Skaaby et al. 2015). In particular,

patients affected by T1DM reportedly have lower levels of 25(OH)D than healthy control groups (Baumgartl at al. 1991; Bierschenk et al. 2009; Littorin et al. 2006; Pozzilli et al. 2005).

A large observational study, the Diabetes Autoimmunity Study in the Young (DAISY) took place in Denver, USA, evaluated children with increased risk of development T1DM (Simpson et al. 2011). The authors showed that neither dietary intake of vitamin D nor 25(OH)D levels in infancy or throughout childhood were associated with the risk of islet autoimmunity (IA). Moreover, progression to T1DM in IA-positive children was not associated with vitamin D intake or 25(OH)D levels. However, there were several weakness in this study. Shortcoming number one is that the dietary vitamin D intake was too low to have a measureable effect. The authors did not administrated cholecalciferol but they only measured the dietary intake of vitamin D using food frequency questionnaire for children aged 2-9 years, and the youth/adolescent questionnaire for children aged 10-12 years. Thus, vitamin D intake was not homogeneous among the studied population. Shortcoming number two is that the results for 25(OH)D are not well described. The authors found about a 20-24 nmol/L difference between those who developed T1DM and those who did not. However, there were significant differences in HLA-DR phenotypes between those who did and did not develop T1DM.

Recently, Raab and colleagues (2014) demonstrated that children with multiple islet autoantibodies (pre-T1DM state) and children newly diagnosed with T1DM have lower 25(OH)D levels than children with negative autoantibodies. The authors showed that 25(OH)D serum levels in childhood were not associated with progression to T1DM (Raab et al. 2014). This study appears to be stronger than the Simpson et al. 2011 gives the limitations of the study. They did not appear to do a multivariate analysis of the findings.

Observational studies find that low 25(OH)D concentrations are a risk factor for developing T1DM. For example, in a study of military service members in the U.S., odds ratios for incidence of T1DM "by quintile of serum 25(OH)D, from lowest to highest, were 3.5 (95% CI 2.0, 6.0), 2.5 (1.5, 4.2), 0.8 (0.4, 1.4), 1.1 (0.6, 2.8) and 1.0 (reference) (p (trend) <0.001). The quintiles (based on fifths using serum 25(OH)D concentration in the controls) of serum 25(OH)D in nmol/l, were <43 (median 28), 43-59 (median 52), 60-77 (median 70), 78-99 (median 88) and ≥100 (median 128)." (Gorham et al. 2012).

A meta-analysis of prospective eight observational studies found a pooled odds ratio of 0.71 (95% CI, 0.51, 0.98) for development of T1DM anywhere from 1 to 31 years with respect to vitamin D intake (Dong et al. 2013).

However, some studies argued that vitamin D deficiency is a consequence of metabolic derangements in T1DM. Subjects affected by T1DM are more sensitive to UV radiation and have less skin pigmentation than healthy controls, which suggests that reduced sun exposure could account for the low level of vitamin D in these patients (Ziegler et al. 1990). A multiple regression analysis in children affected by T1DM and aged <14 years in 51 regions worldwide demonstrated an inverse correlation among T1DM incidence, latitude, and seasonal pattern ($p<0.05$) (Mohr et al. 2008) —thereby highlighting the tight relationship of vitamin D levels with sun exposure in subjects with T1DM.

Recent studies correlated polymorphisms of vitamin D metabolism–related genes (such as *DHCR7/NADSYN1* and *CYP27B1)* with low 25(OH)D level and associated them with a higher risk of developing islet autoimmunity and later diabetes (Cooper et al. 2011; Frederiksen et al. 2013).

Interventional studies and clinical trials

Several studies showed that supplementation with vitamin D in early life reduced the risk of development T1DM (Hypponen et al. 2001; Stene et al. 2003). As vitamin D supplementation for infants in Finland decreased, T1DM incidence rates increased. There was a nearly linear increase in incidence rates for those under the age of 15 years from 1965 to 1992 after which the rate of increase nearly doubled; vitamin D supplementation for infants was 2000 IU/d in 1964, dropping to 1000 IU/d in 1975 and 400 IU/d in 1992 (Hypponen 2010). This finding is associational and does not prove causality, but is consistent with causality. Two retrospective studies from Norway associated use of cod liver oil —an important dietary vitamin D source in high-latitude countries— in the first year of life of newborns or during pregnancy with a lower risk of developing T1DM (Stene et al. 2000; Stene et al. 2003). These results were supported by EURODIAB (European Community-sponsored Concerted Action on the Epidemiology and Prevention of Diabetes), the first case–control study on this topic. That study showed that children who received vitamin D supplementation during the first year of life had a 33% reduced risk of developing T1DM in comparison with non-supplemented children (EURODIAB Study Group 1999).

Ongoing trials assess how vitamin D supplementation affects established T1DM and associated outcomes (Table 1). Two clinical trials are investigating the safety and tolerability of the coadministration of vitamin D plus the glutamic acid decarboxylase–based vaccine Diamyd alone (NCT02352974) or in combination with etanercept (NCT02464033), a tumor necrosis factor inhibitor, in T1DM patients. Another trial (NCT02407899) evaluated the efficacy of vitamin D combined with saxagliptin and insulin against that of insulin alone in adults with

latent autoimmune diabetes. To our knowledge, no ongoing clinical trials are investigating how vitamin D supplementation affects T1DM development and no trials are registered to investigate the effect of $1,25(OH_2)D_3$ analogs on T1DM prevention.

The available observational studies show that an adequate level of 25(OH)D should be guaranteed in children at high risk of developing T1DM. However, a need exists for results from large randomized controlled trials (RCTs) that support the role of supplementation with vitamin D or its analogues in preventing T1DM or improving metabolic control in subjects with T1DM.

Hill's criteria for T1DM

Strength of association: A meta-analysis of eight observational studies (two cohort studies and six case-control studies) found a pooled odds ratio of 0.71 (95% CI, 0.51, 0.98) for development of T1DM anywhere from 1 to 31 years with respect to vitamin D intake (Dong et al. 2013). The study with the highest vitamin D supplementation, 2000 IU/d (Hypponen et al. 2001) found the greatest reduction in risk.

Consistency: Of the eight observational studies, five found significant inverse correlations between vitamin D supplementation, one found a non-significant inverse correlation, and two found a non-significant direct correlation (Dong et al. 2013).

Temporality: The cohort study with the strongest finding (odds ratio for supplementing with vitamin D during the first year of life = 0.22 (95% CI, 0.05, 0.93) demonstrates temporality (Hypponen et al. 2001).

Biological gradient: A meta-analysis of eight observational studies found a pooled odds ratio of 0.71 (95% CI, 0.51, 0.98) for development of T1DM anywhere from 1 to 31 years with respect to vitamin D intake (Dong et al. 2013).

Plausibility: Vitamin D has been shown to have effects on the immune system related to T1DM as well as slow decline of residual β-cell function (Gabbay et al. 2012). Vitamin D regulates components of the immune system and regulating calcium homeostasis, which is important for immune function and insulin secretion (Chakhtoura and Azar 2013; Wolden-Kirk 2011).

Experiment: Supplementation of those with new onset T1DM with 2000 IU/d vitamin D_3 used as adjunctive therapy with insulin was associated with a protective immunologic effect (increased chemokine ligand 2 and regulatory T cells and reduced production of stimulated C-peptide) and slower decline of residual β-cell function (Gabbay et al. 2012).

Analogy: T1DM often occurs in those with multiple sclerosis, which is linked to vitamin D deficiency (Tettey et al. 2015).

Thus, Hill's criteria for causality are reasonably well satisfied for vitamin D in reducing risk of T1DM. However, clinical trials finding that vitamin D supplementation reduces risk of T1DM appear to be lacking.

Vitamin D and Type 2 Diabetes Mellitus

T2DM is one of the most common chronic conditions and is associated with serious morbidity, increased mortality, and high health care cost (Kharrobi and Darwish 2015; Liebl et al. 2015). Evidence from several animal and human studies suggests a potential protective role of vitamin D in the development of T2DM (Pittas et al. 2007).

Mechanism of action

Several mechanisms could explain the link between vitamin D and the risk of development T2DM. Indeed, vitamin D could influence β-cell function, insulin sensitivity, and systematic

inflammation (Fig. 1), all pathways that characterized T2DM. Moreover, numerous studies showed the expression of VDRs in pancreatic β-cells and in all insulin-responsive tissues (Harinarayan 2014; Muscogiuri et al. 2014; Pittas et al. 2007).

$1,25(OH)_2D_3$ could direct stimulate insulin secretion because of the presence of VDRE, the vitamin D response element, in the insulin gene promoter of pancreatic β-cell (Fig.1) (Johnson et al. 1994; Maestro et al. 2003). VDRE activates the transcription of insulin gene and of several other genes involved in cellular growth, cytoskeletar organization and intracellular junctions of β-cell (Wolden-Kirk et al. 2012). Therefore the expression of 1-α-hydroxylase in β cells generates a locally production of $1,25(OH)_2D_3$, which creates a paracrine effect (Bland et al. 2004). Increased insulin secretion after vitamin D supplementation was shown in both *in vitro* and *in vivo* studies (Cade et al. 1986; Chertow et al. 1986; Norman et al. 1980). A study on vitamin D–deficient rats showed a reduction of 48% in insulin secretion after 30 minutes of perfusion with glucose and arginine compared with rats supplemented with vitamin D before the procedure (Norman et al. 1980). Therefore, an in vivo study showed that mice lacking VDR had impaired insulin secretion (Zeitz et al. 2003). Human studies demonstrated an increase of insulin secretion during OGTT (oral glucose tolerance test) in diabetic patients (Inomata et al. 1986) and in patients at high risk to development diabetes (Bouche et al. 1995) after administration of vitamin D. In contrast, Borissova and colleagues (2003) found an increase only in the first phase of insulin secretion during intravenous glucose tolerance test in diabetic women after supplementation of cholecalciferol for one month; no change was found in the second phase of insulin secretion. The major weakness of these clinical studies were the small cohort of evaluated patients and the short time of vitamin D treatment. A recently study on a larger cohort of patients

with T2DM and deficiency level of 25(OH)D did not showed a correlation among overnight fast glucose, insulin and 25(OH)D level (Al-Shoumer et al. 2013). However, the authors did not perform any stimulation test to assess a better evaluation of insulin secretion and did not implement vitamin D treatment.

An important confounder when interpreting data from *in vivo* studies could be the evaluation of serum calcium level because it plays a crucial role in the glucose-stimulated insulin secretion and in insulin action (Fig.1) (Bergsten et al. 1994; Hangström et al. 2007). Indeed, a non-genomic effect of vitamin D is the increase in cytosolic calcium level, which stimulates the insulin secretion. That mechanism is modulated by the activation of two different signaling pathways mediated by protein kinase A (PKA) (Bourlon et al. 1997) and protein kinase C (PKC) (Fig.2) (Billaudel et al. 1995). $1,25(OH)_2D_3$ could activate the PKA that mediates the phosphorylation of different proteins, including the L-type voltage-dependent calcium channels and proteins necessary for the exocytotic mechanism, thus increasing the insulin secretion (Ammälä et al. 1993). Moreover, $1,25(OH)_2D_3$ could activate phospholipase C (PLC), which cleaves phosphoinositides into inositol 1,4,5-trisphosphate (IP_3), involved in calcium release from the endoplasmic reticulum, and diacylglycerol (DAG) that mediates the activation of the PKC. The activated PKC phosphorylates the K_{ATP} channels and the L-type voltage-dependent calcium channels. The activation of the K_{ATP} channels causes the depolarization of the cytoplasmatic membrane, which opens the T-type and the L-type voltage-dependent calcium channels resulting in an increase of the intracellular calcium level (Doyle and Egan 2003). The PKC also mobilizes the secretory vesicles that, together with the increase of calcium level, promotes insulin secretion (Doyle and Egan 2003). The increased calcium concentration causes the secretory response of

insulin through the activation of calcium-calmodulin-dependent protein kinase II (CaMKII), a protein localized at the insulin secretory granules, which promotes the phosphorylation of several proteins involved in the mobilization and in the exocytosis of insulin granules. It has been suggested that the raised intracellular calcium level activates also the insulin gene expression via CREB (Calcium Responsive Element Binding protein) (Dalle et al. 2011). Furthermore, $1,25(OH)_2D_3$ also regulates the expression of calbindin-D_{28k}, a cytosolic calcium-binding protein expressed by β cells that stimulates insulin secretion by regulating intracellular calcium mobilization (Johnson et al. 1994; Sooy et al. 1999). An *in vitro* study showed that in calbindin-D_{28k} transfected pancreatic β-cells, calbindin inhibited the free radical formation induced by cytokines and protected β-cells against degeneration (Christakos et al. 2003).

In addition to effects on pancreatic β-cells, $1,25(OH)_2D_3$ may target insulin-responsive tissues, such as liver, skeletal muscle and adipose tissue (Fig. 1). Several studies associate vitamin D deficiency and insulin resistance (Chiu et al. 2004; Tai et al. 2008), a condition in which peripheral tissues fail to respond to ordinary levels of circulating insulin to maintain normal glucose homeostasis. As a compensatory mechanism, insulin secretion increases and contributes to a progressive failure of the β-cells (Caro et al. 1987). Vitamin D may directly influence insulin sensitivity by stimulating expression of insulin receptors (IRs) on target tissues (Maestro et al. 2000). Contrasting data has been showed regarding the stimulation of IRs by vitamin D in the liver. George and colleagues (2012) demonstrated an increased IRs expression in the liver of streptozotocin-induced diabetic rats after vitamin D supplementation. On the contrary, different studies in streptozotocin-induced diabetic rats (Calle et al. 2008) and in mice model fed on a high-fat diet or low-fat diet (Alkharfy et al. 2013) did not found any alteration in

the liver expression of IRs after vitamin D treatment. Several evidence supported that vitamin D plays an important role in skeletal muscle function (Fig.1) (Girgis et al. 2013). In VDR knockout (VDRKO) mice, the absence of the VDR is associated with atrophy fibers, poor musculoskeletal performance in behavioral tests, and marked changes in gait (Kalneff et al. 2004). Similar results were observed in rats with vitamin D deficiency, which presented an alteration of muscle function when compared with vitamin D-replete animals (Rodman and Baker 1978). However, both studies do not separate the effects of muscle vs. whole-body deletion of VDR, therefore other confounding factors may cause the alteration of muscle function observed in these animal models. More strong seems to be the correlation between vitamin and the peroxisome proliferator-activated receptor δ (PPARδ), a transcription factor involved in regulating fatty acid metabolism and mobilization. The activation of PPARδ by $1,25(OH)_2D_3$ improve the free fatty acid-induced insulin resistance in skeletal muscle (Dunlop et al. 2005). Indirectly, vitamin D could decrease insulin resistance in skeletal muscle through the regulation of cellular calcium concentration (Fig.2). The increased calcium level in muscle cells enhanced the recruitment of Glucose transporter type 4 (GLUT4) to the cell membrane (Wright et al. 2004). However, in a recent study in mice fed on a high-fat diet or low-fat diet, the authors did not find any differences in IRs and GLUT4 gene expression after supplementation with vitamin D also in liver, muscle and adipose tissue (Alkharfy et al. 2013). An important mechanism of action of $1,25(OH)_2D_3$ in adipose tissues is the reduction of inflammation and the change of adipokine secretion, with a decrease of IL6, IL1β and TNFα (Fig.1) (Mutt and al. 2014). $1,25(OH)_2D_3$ could also enhance insulin resistance through the inhibition of the renin-angiotensin-aldosterone system (RAAS), which is a well-known inhibitor of insulin action in peripheral tissues (Wei et al. 2008).

Vitamin D may decrease systemic inflammation (Cannell et al. 2015; van Etten and Mathieu 2005), which plays an important role in the pathogenesis of T2DM. In particular, vitamin D protects β cells from apoptosis and cytokine-induced insulin resistance by modulating the expression and activation of cytokines and inhibiting activation of NF-κB (Gysemans et al. 2005). Moreover, vitamin D also inhibits dendritic cell differentiation and immune activation by a reduction of MHC class II complex expression in cells surface, and inhibits the production of cytokine pro-inflammatory, such as IL6, IL1, IL12 and TNFα (Fig.1). This lead to a regulates T-lymphocyte activity with a shift in T cell polarization from T helper (Th)1 to Th2 (van Etten and Mathieu 2005).

Observational studies

Many observational studies in humans have evaluated the association between 25(OH)D levels and risk of T2DM. Most such studies reported a correlation between vitamin D deficiency and T2DM. However, confounding factors that influence T2DM and are linked to vitamin D status—such as age, diet, and lifestyle—may have influenced the results of these studies (Mitri et al. 2011; Pittas et al. 2007). In particular, obesity is a major confounder since a high BMI represents the most important contributor to the development of T2DM (Kahn et al. 2006) and most diabetic patients have high BMI. In contrast, serum 25(OH)D level is inversely correlated with BMI (Muscogiuri et al. 2010), because of vitamin D is a liposoluble vitamin that can be sequestered by adipose tissue. Therefore, patients affected by T2DM usually have an unhealthy lifestyle and consequent reduction of cholecalciferol formation in the skin. Thus, BMI became an important confounder when valuating data linking 25(OH)D level and T2DM (Vimaleswaran et al. 2013). For example, Grimnes and colleagues (2010) showed that baseline serum 25(OH)D

was inversely associated with subsequent T2DM in a population-based 11-year follow-up study, but this correlation disappeared after adjustment for BMI. On the contrary, another study continued to find a strong correlation between 25(OH)D level and insulin sensitivity index and β-cell function performing an hyperglycemic clamp, independent of confounding factors such as ethnicity, BMI, WHR, systolic and diastolic blood pressure and season (Chiu KC et al. 2004). Nevertheless, the authors did not find any differences in terms of serum 25(OH)D level at baseline. Due to dilution of 25(OH)D level in obese patients, vitamin D replacement therapy needs to be adjusted for body size to achieve the desired serum 25(OH)D concentrations (Drincic et al. 2012).

In the Women's Health Study (Liu et al. 2005), an intake of vitamin D and calcium was associated with lower incidence of metabolic syndrome than in control group. However, there are several weakness in this large study. First, the authors did not use a low exposure control group. Indeed patients randomized to placebo treatment, had a high intake if calcium. This elucidates why there was a minimum effect on bone mineral density and fracture after supplementation with calcium. Then, the dose of vitamin D used for the supplementation (400 IU daily) was too low to produce an effect. Therefore, most of patients enrolled for the trial had a insufficiency 25(OH)D level at baseline and the authors did not measured the final concentration after the treatment, necessary to evaluated if patients reach a normal 25(OH)D range (Lappe and Heaney 2012).

Another prospective study from U.S., the Nurses' Health Study, showed a 33% lower risk for incidence of T2DM in women who had the highest intake of both vitamin D and calcium supplementation (Pittas et al. 2006). A study from different cohort, the Mini-Finland Health

Study (Knekt et al. 2008), reported a relative risk of 0.6 to develop T2DM in patients with lowest 25(OH)D levels in comparison with those with the highest 25(OH)D levels. In a meta-analysis that includes 21 observational studies, Song and colleagues found a relative risk for T2DM of 0.62 (95% confidence interval [CI], 0.54–0.70) for people with lower 25(OH)D level in comparison with those with higher level, as well as a 4% reduction of T2DM risk for each increment of 10 nmol/L in 25(OH)D levels (Song et al. 2013). The data indicated a plateau above 75 nmol/L. This association was not influenced by sex, duration of follow-up, study sample size, diabetes diagnostic criteria, or 25(OH)D assay method. Similar results were found also in a smaller meta-analysis that showed an odds ratio (OR) of 1.50 (95% CI, 1.33–1.66) for the development of T2DM in patients with lower 25(OH)D levels (Afzal et al. 2013).

A recent Mendelian randomization study on 104,488 people evaluated inter-individual variability in circulating 25(OH)D levels caused by common alleles variants of four genes involved on vitamin D synthesis and metabolism (*DHCR7*, *CYP2R1*, *DBP*, and *CYP24A1*). That study also evaluated a causal association of 25(OH)D levels with T2DM (Ye et al. 2015). The authors reported an OR for T2DM of 1.01 (95% CI, 0.75–1.36; $p = 0.94$) for each 25-nmol/L reduction in genetically related 25(OH)D concentration. These results were compared with those from a meta-analysis of 22 observational studies regarding the association of 25(OH)D and T2DM, which showed a relative risk of 1.21 (95% CI, 1.16–1.27; $p = 7.3 \times 10^{-19}$). The authors concluded that the inverse correlation between 25(OH)D levels and T2DM was not causal and that efforts to increase 25(OH)D levels might not reduce risk of diabetes—as would be expected from observational studies (Ye et al. 2015).

Clinical trials

Various RCTs investigated how supplementation with vitamin D or its analogues affects T2DM-related parameters. However, drawing definitive conclusions from these trials is difficult because of several limitations. Some of those studies evaluated mainly outcomes on glycemia, some were underpowered or were post hoc analyses, some varied the supplementation of vitamin D, and some used supraphysiological vitamin doses. For example, some trials found that vitamin D supplementation had no effect on glycemic parameters (Jorde and Figenschau 2009; Nilas and Christiansen 1984), whereas the concomitant supplementation of vitamin D and calcium improved fasting plasma glucose levels (Harinarayan et al. 2013). In contrast, in the Women's Health Initiative study (de Boer et al. 2008), the combination of vitamin D and calcium supplementation did not reduce risk of incident T2DM and had no effect on fasting glycemia and insulin resistance. All these studies give contrasting data about the effect of vitamin D and calcium supplementation on T2DM development. A better designed 2-by-2 factorial, double-masked, placebo-controlled trial (NCT00436475) evaluated the impact of vitamin D supplementation, with or without calcium, improved β-cell function and insulin sensitivity in a cohort of ninety-two patients with high risk of T2DM (Mitri et al. 2011). Patients randomly assigned to receive 2000 IU of cholecalciferol daily or matching placebo and, within each category, to receive 800 mg of calcium carbonate daily for 16 weeks. The authors demonstrated an improved of β-cell function after supplementation with cholecalciferol; no differences were found in insulin sensitivity in any group. Therefore, the supplementation with calcium alone did not have any significant effect. The major weakness of this study was the short term of the supplementation with cholecalciferol and calcium.

A recent double-blind RCT (NCT00812578) showed no significant changes in insulin

sensitivity and inflammatory markers and minimum improvement of insulin secretion after vitamin D supplementation in a small cohort of 16 patients affected by T2DM (Kampmann et al. 2014). The authors use an adequacy dose in the treatment group: 11200 IU daily of oral cholecalciferol for 2 weeks, followed by 5600 IU daily for 10 weeks. However, the time of exposure may have been too short to demonstrate any kind of effect in insulin sensitivity and β-cells function. Therefore, even though a difference in 25(OH)D serum level at baseline and at the end of the study was shown in treated patients, there was no difference in 25(OH)D level among treated and control group at baseline and after 12 weeks. According to this result, treated group did not reach a 25(OH)D level achieved to have any effect. Therefore, we could deduce that the treatment used was insufficiency to conclude that vitamin D supplementation did not cause alteration in insulin sensitivity and β-cell function.

A systemic review and meta-analysis of RCTs concluded that insufficient evidence existed to recommend supplementation with vitamin D to improve glycemic control and prevent diabetes (George et al. 2012). Another meta-analysis of 35 RCTs with a total of 43,407 patients confirmed these conclusions (Seida et al. 2014). The authors found no evidence for using vitamin D supplementation to reduce insulin resistance and hyperglycemia in prediabetic patients or in patients with established T2DM and to prevent T2DM development.

A more recent systematic review by Poolsup and colleagues (2015) analyzed ten RCTs and concluded that vitamin D had no beneficial effect in improving insulin resistance. However, there are several points of weakness in this systematic review. The authors included trials used cholecalciferol and one trial with unknown form of vitamin D (Irai et al. 2012), thus meaning that the interventional treatment of the included studies was different and evidence showed that

the metabolism of vitamin D_2 might be less effective than that of vitamin D_3 (Avenell et al. 2005). In addition, in some of those studies, there was oral calcium supplementation at different dose in treated group alone or in both treated and in placebo group. Moreover, one of the included study used a large bolus of intramuscular injection of 300,000 IU monthly of vitamin D (Irai et al. 2012) and it is well known that the intramuscular vitamin D may be less bioavailable than when administered orally (Smith al. 2007). Indeed, from a pharmacokinetic view, a large bolus of vitamin D would rapidly (in a few days) be absorbed, much would excreted and become undetectable in the serum. In addition, the duration of the treatment is not homogeneous: it was from 8 weeks to 7 years. In particular, four studies had a duration of less than 4 months, which is a period that may be too short to obtain any kind of effect after vitamin D treatment. Another weakness in that not all trials had reported on the attained serum concentration of 25(OH)D serum levels post-treatment. The 25(OH)D measured at a single time point could not be considered as having attained therapeutic levels. Moreover, in most studies was not present a low exposure group and, according to Lappe and Heaney (2012), vitamin D supplementation could not show significant effect in replete subjects because they had nearly enough of vitamin D level concerned. Finally, only eight trials reported the homeostatic model assessment (HOMA) of insulin resistant results, such as for the 2-hours plasma glucose after oral glucose tolerance test (OGTT), which represent the two primary outcome of the systematic analysis. These kinds of meta-analysis are likely to bring an erroneous clinical message to the general practitioners or diabetologist that are not familiar with these aspects.

The lack of a beneficial effect in several observational and randomized studies could be due to the suboptimal dose of vitamin D used, the short follow-up (12 months or less) or the

treatment with different form of vitamin D (cholecalciferol, calcidiol or calcitriol) or the different routes of administration. In addition, most of these studies did not report the 25(OH)D serum level reached after the supplementation. Moreover, serum 25(OH)D concentration was measured at a single time point and could not be considered as having attained therapeutic level. Another weakness is that most studies did not assess parameter of insulin secretion and sensitivity, as well as 25(OH)D level, with several confounding factor such as BMI, age, sex, lifestyle and waist circumference or did not evaluated serum calcium level. Vitamin D replacement therapy needs to be adjusted for confounding factors to achieved the desired 25(OH)D serum level. The lack of a low exposure group of 25(OH)D was another important weaknesses in most of these studies. Heaney and colleagues demonstrated in a large cohort of non-diabetic adult Canadians that supplementation with vitamin D in population with lower 25(OH)D baseline level is more effective than in patient with normal level (Heaney et al. 2013). The authors found that insulin response was correlated with 25(OH)D level after adjusting for BMI, waist circumference, weight, age and sex, in patient with a 25(OH)D baseline level from 40 to 90 nmol/L. According to these results, we may consider the adequacy and the relevance of vitamin D supplementation in the prevention for patients with high risk to development T2DM and with baseline 25(OH)D serum level less than 80-90 nmol/L.

Fifteen ongoing clinical trials are designed to test the effect of vitamin D on T2DM (Table 1). Only eight of them are specifically designed to test the role of vitamin D in the development and metabolism of T2DM. Among them, only one study (NCT01942694) is evaluating the effect of vitamin D supplementation on the onset of T2DM in people at risk for the disease. Patients will be randomized to take 4000 IU daily of vitamin D or placebo for two

years. Four studies are evaluating the metabolic effect of vitamin D supplementation on oral glucose tolerance (NCT01726777 and NCT01856946) or insulin resistance (NCT02098980) or both (NCT02112721) in patients at risk of developing T2DM. The other three studies are investigating the effect of vitamin D supplementation versus a placebo arm of metabolic control in T2DM patients. In particular, they are evaluating insulin secretion (NCT02112721), insulin resistance (NCT01889810), and the change in HbA1c, glycated hemoglobin (NCT01991054).

Although evidence suggests that vitamin D deficiency represents a risk factor for the development of T2DM, large, well-designed, RCTs are required to better define the correlation between vitamin D and T2DM and its potential clinical role in preventing and treating diabetes.

Hill's criteria analysis for T2DM

Strength of association: The relative risk for incidence of T2DM from 21 prospective studies, high vs. low 25(OH)D quantile, is 0.62 (0.54-0.70) (Song et al. 2012).

Consistency: Out of 21 prospective studies, high vs. low quantile of 25(OH)D concentration was significantly inversely correlated with incidence of T2DM in 10 studies, non-significantly inversely correlated in nine studies, and non-significantly directly correlated in two studies (Song et al. 2012)

Temporality: Prospective studies find an inverse correlation between serum 25(OH)D concentration and incidence of T2DM (Song et al. 2012).

Biological gradient: The meta-analysis of incidence of T1DM with respect to 25(OH)D concentration in prospective studies showed increasing risk as 25(OH)D concentration decreased below 75 nmol/L (Song et al. 2012).

Plausibility: The mechanisms by which vitamin D might reduce the risk of T2DM were reviewed

recently (Mitri and Pittas, 2014). They include supporting insulin secretion: The "majority of observational studies, [find] that vitamin D is positively correlated with insulin sensitivity and its role is mediated both by direct mechanism through the availability of vitamin D receptors in several tissues and indirectly through the changes in calcium levels (Al-Shoumer and Al-Essa, 2015)

Experiment: Clinical trials for vitamin D supplementation in reducing the incidence of T2DM or having any impact on glucose control and insulin resistance in general populations (Pilz et al. 2013), (Al-Shoumer and Al-Essa, 2015). However, a clinical trial in Iran, where vitamin D deficiency is common, have shown that vitamin D plus calcium supplementation can decrease fasting glucose (Shab-Bidar et al. 2011). The baseline 25(OH)D concentration was 38 nmol/L. The successful result was most likely due to the low baseline concentration, as suggested by the meta-analysis of clinical trials of vitamin D and biomarkers of inflammation, where 50% of the trials with baseline 25(OH)D concentration <49 nmol/L found beneficial effects while only 26% of those with higher baseline concentrations did (Cannell et al. 2015). Most clinical trials have not been properly designed according to the guidelines proposed by Heaney (Heaney, 2014).

Analogy: Health outcomes with similar findings for the criteria include cardiovascular disease (Weyland et al. 2014).

Confounding factors: Obesity is an important risk factor for T2DM; those with high body mass index generally have low 25(OH)D concentrations. While studies of 25(OH)D and incidence of T2DM try to correct for body mass index, it is possible that it is not fully corrected for (Pilz et al. 2013).

Evaluation by others: Several reviews have concluded that there is good evidence from several points of view that vitamin D reduces risk of T2DM but that there is still some uncertainty regarding this relation due to the lack of strong clinical trials (Pilz et al. 2013), (Al-Shoumer and Al-Essa, 2015). However, the failure is likely due to poor clinical trial design.

Thus, Hill's criteria for causality are reasonably well satisfied for vitamin D in reducing risk of T2DM.

Vitamin D and Pancreatic Cancer

PC is a relatively common disease with a low survival rate since it is generally diagnosed at stage III and IV. Thus, finding ways to prevent PC is worthwhile. Vitamin D is an attractive candidate to try to reduce risk of PC. Vitamin D has several mechanisms that reduce the risk of cancer and increase survival after initiation, including effects on cellular differentiation, proliferation, and apoptosis as well as antiangiogenic and antimetastasis properties (Moukayed and Grant 2013). In addition, incidence and/or mortality rates of about 15 cancers have been found inversely correlated with indices of UVB dose or exposure, with the most likely reason being production of vitamin D (Moukayed and Grant 2013).

This paper reviews findings from several types of studies used to assess vitamin D's role in reducing risk of PC:

- Case–control studies of oral vitamin D intake
- Prospective studies of PC incidence with respect to serum 25(OH)D concentration
- Prospective studies of PC incidence with respect to predicted 25(OH)D concentration

- Case–control studies of solar UV exposure
- Ecological studies of PC mortality rates with respect to solar UVB doses

A review of case–control studies of PC with respect to oral vitamin D intake was recently published (Waterhouse et al. 2015). In a study of 2880 cases and 7502 controls, an increase in dietary take of 100 IU/day was associated with a 13% increased risk of PC (OR = 1.13 [95% CI, 1.07–1.19], $p < 0.001$). From four studies regarding taking vitamin D supplements above or below 400 IU/day, no significant association was found.

Several prospective studies have examined PC incidence with respect to serum 25(OH)D concentration (Table 2). One was of smokers in Finland from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study of Finnish men (Stolzenberg-Solomon et al. 2006). The multivariate OR for the highest vs. lowest 25(OH)D concentration was 2.92 (95% CI, 1.56–5.48), $p = 0.001$. Some concerns regarding this study exist, including that all the cases had been smokers aged 50–69 years who were randomly assigned within each of the 14 study centers to receive α-tocopherol (50 mg, as DL-α-tocopheryl acetate), β-carotene (20 mg), α-tocopherol plus β-carotene, or placebo daily for 5–8 years (median, 6.1 years) in a double-blinded fashion (Albanes et al. 1996). The follow-up period was up to 16.7 years, so that 25(OH)D concentrations could change considerably during the long follow-up time (Grant 2011). The fact that a study of colon cancer incidence with respect to 25(OH)D concentration with the same cohort found a increased OR for the highest vs. lowest 25(OH)D concentration (OR = 2.11 [95% CI, 1.20–3.69], $p = 0.01$) when all other studies find inverse correlations (Grant 2011) raises concerns about the studies from this cohort.

A study from the Prostate, Lung, Colorectal, and Ovarian (PLCO) Screening Trial with

up to an 11.7-year follow-up found an insignificant direct correlation between 25(OH)D concentration and incidence of PC (Stolzenberg-Solomon et al. 2009). However, when the cohort was divided into regions of low and high residential sun exposure, those in the low region had a significantly higher PC incidence rate for high vs. low 25(OH)D concentration, whereas those living in the high region had an insignificantly increased risk. The authors noted that 25(OH)D concentration was a combination of vitamin D_2 and D_3. Thus, a likely explanation for the finding was that those people living in the region with low sun exposure were taking vitamin D supplements and may have started taking them shortly before enrolment and blood draw. Support for this hypothesis is found in two studies of frailty with respect to 25(OH)D concentration in the U.S. One study with men found an inverse correlation between 25(OH)D concentration and frailty (Ensrud et al. 2011), whereas a study with women found a U-shaped relation, associating both low and high 25(OH)D concentration with increased risk (Ensrud et al. 2010). In the U.S., elderly women are much more likely than elderly men to be told to take vitamin D supplements. Additional support for this hypothesis comes from a large study of 25(OH)D_2 and 25(OH)D_3 as a function of location in the U.S. For those with total 25(OH)D level greater than 125 nmol/L, the amount of 25(OH)D_2 varied: 75% in the north, 15% in the center, and 10% in the south (Kroll et al. 2015). When physicians prescribe vitamin D in large doses, it is usually ergocalciferol (vitamin D_2). Also, vitamin D is often prescribed in response to finding a vitamin D–deficiency condition or diseases such as osteopenia or osteoporosis.

A pooled analysis from the Vitamin D Pooling Project—which included 952 cases and 1333 controls from China, Finland, and the U.S., with mean follow-up time near 9 years—found a marginally nonsignificantly increased risk for those with levels greater than 100 nmol/L versus

50–75 nmol/L (Stolzenberg-Solomon et al. 2010). Two studies from the U.S.—the PILCO study and one from the Washington, DC, area involving 12 cases and three controls—had the highest ORs. Thus, inclusion of people who started taking vitamin D supplements late in life is a likely explanation for the finding of this study.

Another pooled study included 501 PC cases and 1175 matched controls obtained from five large prospective cohort studies organized by Harvard University. Participants consisted of both male and female U.S. health professionals with up to 25.3 years of follow-up (Wolpin et al. 2012): "Participants in quintiles two through five had multivariable-adjusted ORs (95% confidence intervals) of 0.79 (0.56–1.10), 0.75 (0.53–1.06), 0.68 (0.48–0.97), and 0.67 (0.46–0.97; p_{trend} = 0.03), respectively, compared with the bottom quintile." Given the reduction in OR with increasing follow-up time for breast and colorectal cancer (Grant 2011), lower ORs would probably have been found if 25(OH)D concentration had been measured during the follow-up periods.

One study was reported based on predicted 25(OH)D concentration. In this approach, a model of 25(OH)D concentration based on serum 25(OH)D concentrations for a portion of the cohort is developed with respect to geographical location, skin pigmentation, oral vitamin D intake, and leisure time spent outdoors (Giovannucci et al. 2006). Thus, this index reflects combined vitamin D from oral intake and solar UVB exposure. In a study involving 575 cases with predicted 25(OH)D concentration based on data from 1986 and monitored through 2006, the risk ratio for 32.6 ng/mL was 0.67 (95% CI, 0.46–0.96) compared with 23.4 ng/mL, p_{trend} = 0.03. If those diagnosed in the first 2 years were omitted, the relative risk dropped to 0.66 (95% CI, 0.46–0.96), p_{trend} = 0.02 (Bao et al. 2012). Again, this study provides good support for the

role of vitamin D in reducing risk of PC.

Several ecological and related studies have investigated the role of risk of PC incidence and/or mortality rate with respect to solar UVB doses or exposure. Vitamin D production is the most likely explanation for the link between UVB exposure and reduced risk of cancer (Moukayed and Grant 2013). A multifactorial U.S. study based on mortality rates for 1950–1969 and 1970–1994 found inverse correlations for 15 cancers, including PC (Grant and Garland 2006). Both lung cancer, an index of smoking combined with diet –direct- and solar UVB doses –inverse- were significantly correlated with PC for men, but only lung cancer for women (Table 3). Other factors found related to PC mortality rates were alcohol consumption, Hispanic heritage, and poverty status; however, they were not consistently associated for men and women for two time periods, 1950-69 and 1970-94.

An ecological study in Japan found a significant increase in PC mortality rates with increasing latitude (Table 3) (Kinoshita et al. 2007). Global solar radiation was found to be strongly inversely correlated with PC mortality rates and daily maximum temperature weakly inversely correlated.

Diets high in animal products and added sweeteners are also an important risk factor for PC (Grant, 2013). However, intra-country variations of diet are generally less pronounced than variations in UVB doses and smoking rates so are not expected to significantly influence the geographical variations in PC mortality rates.

A study of PC incidence versus ambient solar UV radiation (UVR) was conducted based on data from six U.S. states (California, Florida, Louisiana, New Jersey, North Carolina, and Pennsylvania) and two metropolitan areas (Atlanta and Detroit) (Lin et al. 2012). This study

found a U-shaped relation, with an intermediate UVR region having the lowest incidence rate. Two weaknesses of this study are that the UV index included a sizable portion of its value from the non-UVB region, which could affect the attribution to UVB and vitamin D production, and that the analysis included no other factors.

A study in Australia assessed UVR and skin sun sensitivity from questionnaires answered by 496 PC cases and 589 controls (Tran et al. 2013). As shown in Table 3, three measures of UV exposure and/or vitamin D production found significant inverse correlations with PC incidence rates. Use of UVR in Australia is a good approximation to UVB since both indices have similar latitudinal and longitudinal variations. The situation is unlike that in the U.S., where UVB doses are highest in the southwest and lowest in the northeast because of geographic variations in surface elevation, stratospheric ozone burden, and aerosol and cloud burdens.

Another type of study relates cancer incidence rates to occupational solar UVB exposure by using a new index: lip cancer incidence rate less lung cancer incidence rate (Grant 2012). From 1960 to 2005, the five Nordic countries had 1.4 million male cancer cases and 1.36 female cancer cases in 54 occupational categories (Pukkala et al. 2009). Because both lip and lung cancer are linked to smoking, the analysis used the combination of the two cancers. However, many women wear lipstick, so an independent UVB index for women could not be developed. Occupations with the highest fraction of outdoor workers, such as farmers, foresters, and gardeners, had the lowest risk of many cancers. Both melanoma and nonmelanoma skin cancer rates inversely correlated with this UVB index. Both cancers are more strongly correlated with long-wave UV (UVA), and occupational exposure to UV is not a risk factor for melanoma. As Table 3 shows, the UVB index was significantly inversely correlated with PC incidence either in

combination with lung cancer or by itself.

In total, studies related to oral vitamin D intake find an increased incidence rate of PC for higher vitamin D intake. Prospective studies of PC incidence with respect to serum 25(OH)D concentration at time of enrollment in the study generally support a finding of a beneficial effect of higher 25(OH)D concentration. This conclusion incorporates the assumption that the reason for direct correlation between 25(OH)D concentration and PC incidence in higher latitude regions is due to cases having started taking vitamin D supplements shortly before blood draw. Ecological and related studies nearly always find inverse correlations between UVB dose or exposure and incidence and/or mortality rate of PC. Such studies have several advantages, such as large numbers of cases and using long-term UVB indices.

To our knowledge, no clinical trials have reported outcomes related to PC for vitamin D intake. PC is a relatively rare cancer, making it difficult to obtain enough cases to calculate the risk.

Hill's criteria analysis for pancreatic cancer

Strength of association: the analysis of results from five cohorts found a 33% (95% confidence interval, 3-54%) reduction in PC incidence for high vs. low 25(OH)D concentration (Wolpin et al. 2012). In addition, several ecological studies found significant inverse correlations between indices of solar UVB doses and PC incidence and/or mortality rate (Moukayed and Grant 2013).

Consistency: The findings of inverse correlations between prediagnostic 25(OH)D concentration and incidence of PC in the U.S. are in general agreement for low-latitude regions (Stolzenberg-Solomon et al. 2009) and for the entire U.S. (Wolpin et al. 2012). The

inconsistent findings in high-latitude studies in Finland (Stolzenberg-Solomon et al. 2006) and high latitudes in the U.S. (Stolzenberg-Solomon et al. 2009) are attributed to those with high 25(OH)D concentrations starting to supplement with vitamin D shortly before enrolling in the study. Evidence for this conclusion comes from differences for men and women for frailty with respect to 25(OH)D concentrations (Ensrud et al. 2010, 2011) and that those with higher 25(OH)D concentrations in the northern states are more likely to have taken vitamin D_2, which is prescribed by physicians (Kroll et al. 2015). In addition, geographical ecological studies nearly always find an inverse relationship between PC incidence or mortality rates with respect to solar UVB doses (Moukayed and Grant, 2013).

Temporality: The prospective study involving five cohorts investigated incidence of PC with a follow-up time of up to 25.3 years (Wolpin et al. 2012).

Biological gradient: Both ecological and observational studies show inverse relations between solar UVB indices and/or 25(OH)D levels and incidence and/or mortality rates of PC (Moukayed and Grant, 2013; Wolpin et al. 2012)

Plausibility (mechanisms): the mechanisms by which vitamin D reduces risk of cancer are well known and include effects on cellular differentiation, progression and apoptosis, angiogenesis and metastasis and inflammation (Krishnan and Feldman, 2011), (Moukayed and Grant 2013).

Experiment: Two clinical trials found significant reduced all-cancer incidence for those taking vitamin D and calcium compared to a placebo or calcium (Bolland et al. 2011; Lappe et al. 2007). Clinical trials have also found that vitamin D reduces biomarkers of

inflammation (Cannell et al. 2015). No trial has looked at incidence of PC specifically, which would be difficult to do given the low incidence of PC.

Analogy: Vitamin D has been found to significantly reduce the risk of all and breast and non-significantly reduce colorectal cancer in clinical trials (Bolland et al. 2011; Lappe et al. 2007), and PC incidence and/or mortality rates have similar inverse correlations with respect to solar UVB doses as breast and colorectal cancer (Moukayed and Grant 2013).

Thus, Hill's criteria for causality are well satisfied for vitamin D in reducing risk of pancreatic cancer.

Conclusion

Vitamin D deficiency is associated with pancreatic diseases involved with both the endocrine function of the pancreas, such as T1DM and T2DM, and tumor disease. Observational studies find an inverse correlation between 25(OH)D levels and the prevalence of both types of diabetes. Prospective and ecological studies of PC incidence generally support a finding of a beneficial effect of higher prediagnostic 25(OH)D concentration as well as inverse correlations between UVB dose or exposure and incidence and/or mortality rate of PC. These observational studies suggest a potential therapeutic role of vitamin D to decrease the risk of T1DM and T2DM, improve the metabolic profile of T2DM, and decrease the incidence of PC. The recommended 25(OH)D levels for many health outcomes including those related to the pancreas are above 75 nmol/L with possible additional protection at higher concentrations up to 250 nmol/L with minimal risks (Holick et al. 2011). Many physicians are already measuring 25(OH)D concentrations of their patients and recommending vitamin D supplementation. It is likely that physicians will increasingly consider vitamin D for diabetics and prediabetics, especially in those

patients which present level of 25(OH)D lower than 75 nmol/L.

There are about half a dozen major vitamin D supplement trials underway around the world trying to determine whether taking up to 2000 IU/d vitamin D reduces the risk of many adverse health outcomes (Bendik et al. 2014). Some of the studies will likely produce data on incidence of the three diseases discussed in this paper. Results are due before 2020. However, the findings are likely to be limited for a number of reasons including that most of the participants likely have above population mean 25(OH)D concentrations at baseline (Guessous, 2015). More clinical trials are warranted to determine what role vitamin D_3 supplementation plays in reducing the symptoms or progression of the three diseases.

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Table 1. Ongoing clinical trials on vitamin D intervention in T1DM and T2DM

ClinicalTrials.gov Identifier	Primary outcome	Phase	Vitamin D form and dose intervention	Study duration	Diabetes type
NCT02464033	Evaluate tolerability of a combination therapy with Diamyd, vitamin D, and etanercept	II	2,000-IU Vitamin D per os per day + etanercept (Enbrel) injected subcutaneously 0.8 mg/kg of body weight once a week + 2 subcutaneous injections of 20 μg of Diamyd	6 mos	T1DM
NCT02352974	Safety of giving Diamyd directly into lymph glands in combination with an oral vitamin D regimen	I	2,000-IU Vitamin D (calciferol) daily in oral solution + GAD-Alum (Diamyd) 4-μg subcutaneous injection at three occasions	4 mos	T1DM
NCT02407899	Change of fasting C-peptide from baseline in LADA patients treated with	IV	Insulin + saxagliptin 5-mg tablets vs. insulin + saxagliptin 5-mg tablets + vitamin D_3 drop	2 yrs	T1DM

	saxagliptin and vitamin D_3 plus insulin or insulin alone		2,000 IU daily vs. insulin		
NCT01942694	Effect of vitamin D supplementation on the onset of T2DM in people at risk for the disease	*	Vitamin D (cholecalciferol) oral soft-gel pill 4,000 IU daily vs. placebo	48 mos	T2DM
NCT02112721	Improvement of insulin secretion and/or insulin resistance in overweight/obese individuals	IV	Start dose of 2,500 μg (100,000 IU) of vitamin D (Ostelin, Reckitt Benckiser), then 100 μg/day (4,000-IU 4 tablets) vs. placebo	16wks	T2DM
NCT02132442	Effect of vitamin D intake on the severity of fatty liver	III	Ergocalciferol 50,000 IU per week for 6 wks, then biweekly for 6 mos vs. placebo	8 mos	T2DM
NCT01991054	Change in HbA1c (%) in study groups	IV	Vitamin D_3 120,000 IU per month vs. placebo	6 mos	T2DM

NCT02416193	Cognitive functioning	II	50,000-IU cholecalciferol once weekly (high dose) vs. 5,000-IU cholecalciferol once weekly (low dose)	3 mos	T2DM
NCT00736632	Hypertension	*	Cholecalciferol 4,000-IU orally daily + calcium carbonate 500 mg orally twice daily vs. placebo + calcium carbonate 500 mg twice daily	16 wks	T2DM
NCT01726777	Oral glucose tolerance in subjects with increased risk of developing diabetes	II	28,000-IU vitamin D once per week vs. placebo	24 wks	T2DM
NCT01889810	Change in insulin resistance	*	3,000-IU (75 µg) vitamin D_3 per day vs. placebo	26 wks	T2DM
NCT01904032	Effect of vitamin D	II	50,000 IU weekly	6 mos	T2DM

	supplementation on depressive symptoms based on Center for Epidemiologic Studies Depression score		Vitamin D_3 vs. 5,000 IU weekly Vitamin D_3		
NCT02098980	Insulin resistance in individuals at risk for developing T2DM	*	Vitamin D supplement 4,000 IU/day vs. placebo	6 mos	T2DM
NCT01736865	Disposition index by the insulin secretion sensitivity index-2	II III	One cholecalciferol pill daily vs. placebo	12 mos	T2DM
NCT01635062	Renal-vascular tissue renin-angiotensin system activity	*	Calcitriol and lisinopril vs. placebo	3 wks	T2DM
NCT02410005	24-h urine albuminuria	II III	Losartan 50 mg twice daily vs. losartan 50 mg twice daily + calcitriol	12 mos	T2DM

			0.25 μg daily		
NCT01856946	Change in oral glucose tolerance in obese adolescent	*	Two 2,000-IUvitamin D_3 pills (total 4,000 IU) daily	6 mos	T2DM
NCT01673204	Changes in renal function with proteinuria	IV	Calcitriol 0.5 μg orally once daily vs. placebo	12 mos	T2DM

*missing data. HbA1c, glycated hemoglobin; LADA, latent autoimmune diabetes in adults; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; yrs, years; mos, months; wks, weeks.

Table 2. Prospective studies with respect to 25(OH)D concentration

Location	Conditions	25(OH)D concentration (nmol/L)		OR (95% CI), high vs. low	Reference
		Low	High		
Finland	Smokers, up to 16.7 yrs follow-up	25.6	83.2	2.92 (1.56–5.48), p_{trend} = 0.001	Stolzenberg-Solomon et al. 2006
USA	Cohort followed up to 11.7 yrs	<45.9	>82.3	1.45 (0.66–3.15), p = 0.49	Stolzenberg-Solomon et al. 2009
USA	Cohort followed up to 11.7 yrs, region of low residential sun exposure	<49.3	>78.4	4.03 (1.38–11.79), p = 0.015	Stolzenberg-Solomon et al. 2009
USA	Cohort followed up to 11.7 yrs, region of high residential sun exposure	<49.3	>78.4	1.29 (0.50–3.96)	Stolzenberg-Solomon et al. 2009
Various*	Approx. 9 yrs follow-up	<25	50–75	0.98 (0.66–1.40)	Stolzenberg-Solomon et al. 2010
Various*	Approx. 9 yrs follow-up	50–75	>100	2.14 (0.93–	Stolzenberg-

				4.92)	Solomon et al. 2010
USA	>0 yrs after blood draw	37.7	93.6	0.73 (0.50–1.05), p_{trend} = 0.05	Wolpin et al. 2012
USA	>5 yrs after blood draw	37.7	93.6	0.63 (0.14–0.99) p_{trend} = 0.02	Wolpin et al. 2012

*Vitamin D Pooling Project. 95% CI, 95% confidence interval; OR, odds ratio; yrs, years.

Table 3. Pancreatic cancer incidence or mortality rate with respect to latitude or UV exposure.

Location	Measure	Finding	Reference
USA	Solar UVB dose for July, other factors; period 1950–1969, men	(β_{UVB} = –0.39, p = 0.02), ($\beta_{lung\ cancer}$ = 0.53, p = 0.002), adj. R^2= 0.39, *	Grant and Garland 2006
USA	Solar UVB dose for July, other factors; period 1970–1994, men	(β_{UVB} = –0.46, p = 0.005), ($\beta_{lung\ cancer}$ = 0.59, *), adj. R^2 = 0.41, *	Grant and Garland 2006
	Solar UVB dose for July, other factors; period 1950–1969, women	(β_{UVB} = –0.74, p = 0.02), (β_{alc} = 0.42, p = 0.007), $\beta_{Hisp.}$ = 0.41, p = 0.004), (β_{pov} = 0.40, p = 0.02), adj. R^2= 0.50, *	Grant and Garland 2006
	Solar UVB dose for July, other factors; period 1970–1994, women	(β_{UVB} = –0.34, p = 0.06), ($\beta_{lung\ cancer}$ = 0.37, 0.008), adj. R^2 = 0.27, p = 0.003	Grant and Garland 2006
Japan	Standardized mortality rate vs. global solar radiation, Mj/m^2/day, males	R = –4.35 (SE = 1.20)	Kinoshita et al. 2007
Japan	Standardized mortality rate vs. global solar radiation, Mj/m^2/day, females	R = –5.02 (SE = 1.25)	Kinoshita et al. 2007

Nordic countries	Occupation, incidence, men	β_{UVB} = –0.39, p = 0.001, $\beta_{smoking}$ =0.81, *), adj. R^2 =0.57, *	Grant 2012
Nordic countries	Occupation, incidence, men	β_{UVB} = –0.6, *, adj. R^2 =0.35, *	Grant 2012
USA	Incidence, ambient UVR, 236.8–253.7 vs. <186.3 J/m^2	OR = 0.79 (95% CI, 0.68–0.91)	Lin et al. 2012
USA	Incidence, ambient UVR, >253.7 vs. <186.3 J/m^2	OR = 0.95 (95% CI, 0.83–1.09), p_{trend} = 0.009	Lin et al. 2012
Australia	Incidence, cumulative daily UV in adulthood, high vs. low	OR = 0.46 (95% CI, 0.26–0.84), p_{trend} = 0.01	Tran et al. 2013
Australia	Incidence, skin color, light vs. dark	OR = 0.49 (95% CI, 0.35–0.69), p_{trend} = 0.001	Tran et al. 2013
Australia	Incidence, skin burn, burn badly vs. no burn	OR = 0.48 (95% CI, 0.32–0.72), p_{trend} = 0.04	Tran et al. 2013

*p < 0.001. 95% CI, 95% confidence interval; R, regression coefficient; UVB, solar ultraviolet-B; UVR, solar ultraviolet radiation; UV: solar ultraviolet.

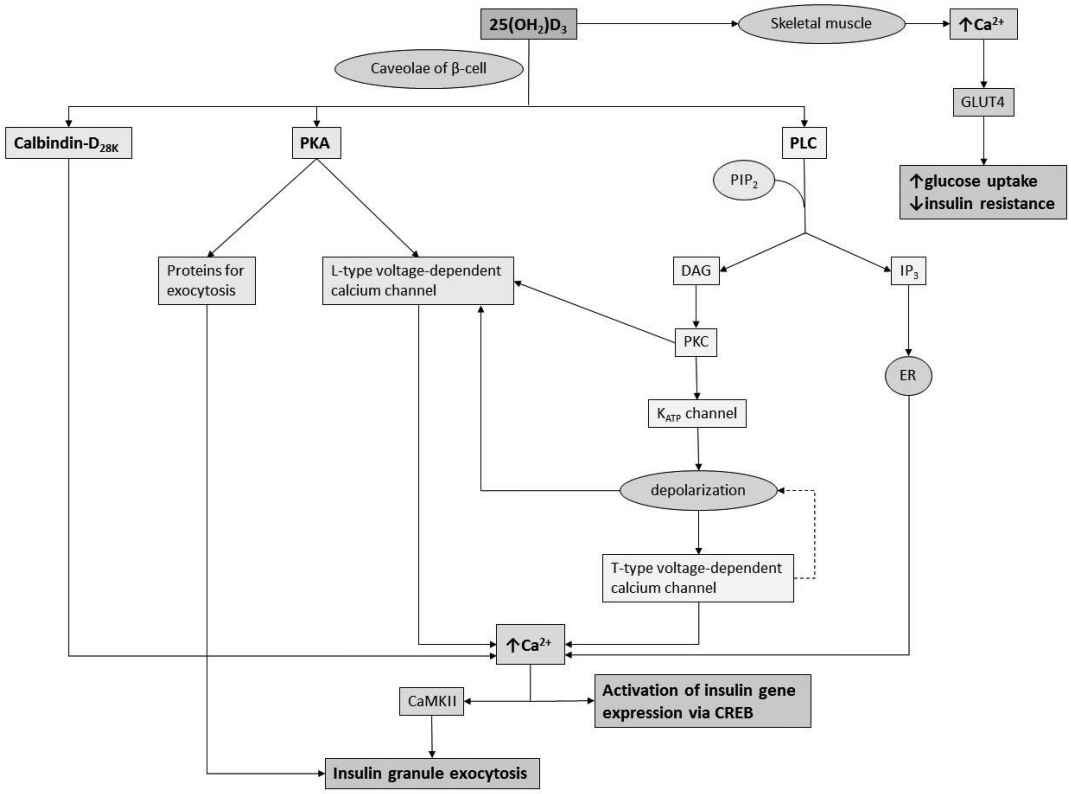

Figure 1. Potential mechanisms by which vitamin D may influenced T2DM. Vitamin D could influences T2DM through several pathways distinguished into three major mechanisms: 1) Stimulates insulin secretion by its interaction with VDRE localized in the promoter region of insulin gene, or stimulates indirectly the secretion of insulin granules through the regulation of intracellular calcium concentration. 2) Stimulates insulin sensitivity through the regulation of IRs expression in target cells and the activation of PPAR-δ in skeletal muscle and adipose tissues, which is involved in fatty acid metabolism. Therefore $25(OH_2)D_3$ regulates the correct function of skeletal muscle and reduces inflammation and changes adipokine secretion in adipose tissue. Indirectly, $25(OH_2)D_3$ inhibits the RAAS, with an improvement of insulin resistance. 3) Regulates systemic inflammation through an inhibition of dentritic cells differentiation and

proinflammatory cytokines, such as TNFα, IL6, IL1, IL2 and IFNγ. Thus results in a indirect shift in T cells polarization from T helper (Th) 1to Th2 and in a reduction of macrophage infiltration. Abbreviation: 1αOHase: 1-α-hidroxylase; Ca^{2+}: calcium; DC: Dentritic Cell; ER: Endoplasmatic Reticulum; IP_3: inositol 1,4,5-trisphosphate; IR: insulin receptor; PKA: Protein kinase A; PKC: Protein Kinase C; PPAR-δ: Peroxisome Proliferator-Activated Receptor Gamma; RAAS: Renin-Angiotensin-Aldosteron system; ROS: reactive oxygen species; RXR: Retinoid X Receptor; Th: T helper limphocite; VDR: Vitamin D Receptor; VDRE: vitamin D responsive element

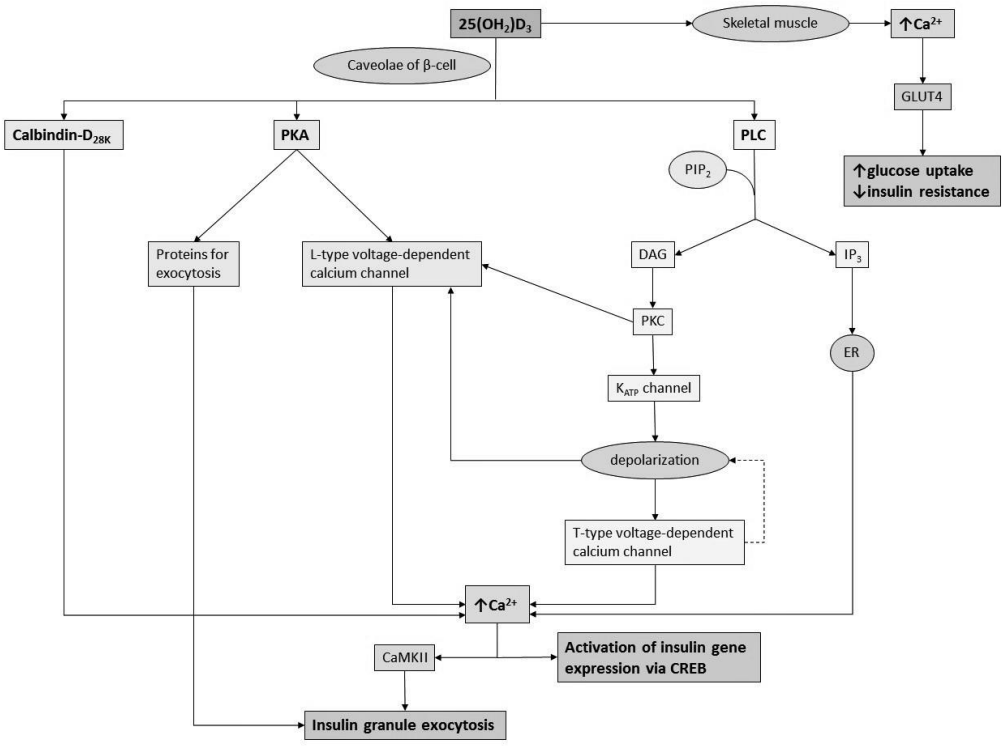

Figure 2. Metabolic pathway of calcium and vitamin D on glucose metabolism. $25(OH_2)D_3$ may modulates the increase of intracellular calcium level by the activation of two different signaling pathways mediated by two different protein kinase: PKA and PKC. The PKA mediates the phosphorylation of different proteins, including the L-type voltage-dependent calcium channels and proteins necessary for the exocytotic mechanism. $1,25(OH)_2D_3$ activates the PLC, which cleaves PIP_2 into IP_3, involved in calcium release from the endoplasmic reticulum, and DAG that mediates the activation of the PKC. The PKC phosphorylates the K_{ATP} channels and the L-type voltage-dependent calcium channels causing the depolarization of the cytoplasmatic membrane and the opening of the T-type voltage-dependent. These events increases intracellular calcium. The PKC also mobilizes the secretory vesicles. The increase of intracellular calcium concentration lead to the activation of CaMKII, a protein localized at the insulin secretory

granules, which promotes the phosphorylation of several proteins involved in the mobilization of insulin granules resulting in exocytosis. Therefore, the increased of intracellular calcium level activates also the insulin gene expression via CREB. Furthermore, $1,25(OH)_2D_3$ also regulates the expression of calbindin-D_{28k}, a cytosolic calcium-binding protein, which stimulates insulin secretion by regulating intracellular calcium diffusion. Finally, the increased calcium level in muscle cells enhanced the recruitment of GLUT4 to the cell membrane, resulting in a decrease of insulin resistance. Abbreviation: 1αOHase: 1-α-hidroxylase; Ca^{2+}: calcium; CaMKII: calcium-calmodulin-dependent protein kinase II; CREB: Calcium Responsive Element Binding protein; DAG: diacylglycerol; ER: Endoplasmatic Reticulum; GLUT4: Glucose transporter type 4 IP_3: inositol 1,4,5-trisphosphate; K_{ATP} channels: ATP-sensitive potassium channel; PIP_2: phosphoinositides; PKA: Protein Kinase A; PKC: Protein Kinase C (PKC); PLC: phospholipase C.