Abstract

The existence of impaired glucose tolerance in vitamin D-deficient individuals and the discovery of receptors for 1,25-dihydroxyvitamin D₃—the activated form of vitamin D—in islet β cells and immune cells, the main cells involved in the pathogenesis of both types of diabetes, have aroused scientific and clinical interest in the potential role of vitamin D in the pathogenesis of the diseases, but even more so with respect to its therapeutic potential in the prevention of both forms of diabetes. Vitamin D deficiency is detrimental to insulin synthesis and secretion in animal models, as well as in humans, and predisposes them to type 2 diabetes. Interventions with pharmacological doses of 1,25-dihydroxyvitamin D₃ or newer structural analogues can delay onset of type 1 diabetes in non-obese diabetic mice, mainly through immunomodulation, but, to date, no human data are available. Epidemiological studies suggest links between onset of type 1 diabetes and vitamin D deficiency in early life and with certain polymorphisms of the vitamin D receptor. At present, the most important conclusion from the studies on vitamin D and diabetes is that avoiding vitamin D deficiency is a priority not only for calcium and bone issues, but also for diabetes prevention.

Key words: vitamin D, diabetes, β-cell, prevention, vitamin D deficiency, vitamin D receptor polymorphism.

Introduction: vitamin D and its metabolism

Even before the discovery of vitamin D in the 1930s, it was common knowledge that substances in specific foods were crucial for good health. The term vitamin D refers to the secosterols, ergocalciferol (vitamin D₂) and cholecalciferol (vitamin D₃). Vitamin D₂ is produced commercially by irradiation of plant sterols (ergosterol), whereas vitamin D₃ is primarily manufactured in the skin from 7-dehydrocholesterol via photochemical synthesis using ultraviolet B radiation from sunlight and can also be found in food of animal origin. The best food sources are cod liver oil, fatty fish, and egg yolks. In general, adults obtain sufficient vitamin D from skin synthesis and from ingestion of small amounts with food. However, during pregnancy and lactation and for infants and young children (especially in industrialized cities where exposure to sunlight is limited and in dark-skinned children living in northern countries), the synthesis of vitamin D₃ in the skin is insufficient to meet physiological requirements (normal circulating 25-hydroxyvitamin D₃ concentrations between 50 and 150 nmol/L). Under these conditions, dietary intake of vitamin D is essential (>200–400 IU/d [>5–10 µg/d]). Therefore, in many countries food is supplemented with vitamin D.

Vitamin D is the most important regulator of calcium homeostasis in the body by increasing absorption of calcium from food and reducing urinary calcium loss. It exerts important functions in skeletal development and bone mineralization. Yet, vitamin D has no hormone activity itself. Once it enters the blood circulation, either synthesised in the skin or ingested, it is bound by the vitamin D binding protein and transported to the liver for further metabolisation. To become biologically active, vitamin D needs two successive hydroxylations in the liver (at carbon 25) and in the kidney (at α position of carbon 1). In the kidneys, 25-hydroxyvitamin D (25(OH)D) is converted to an activated (1,25-dihydroxyvitamin D; 1,25(OH)₂D) as well as an inactivated (24,25-dihydroxyvitamin D; 1,25(OH)₂D) form (figure 1). The vitamin D hormone, 1,25(OH)₂D, exerts its effects mainly by activating the nuclear vitamin D receptor (VDR), a member of the nuclear receptor super-family of ligand-activated transcription factors, and, when bound to this
receptor, associates with specific recognition sequences called vitamin D-responsive elements, which are present in the promoter of target genes and are involved in regulating their own transcription. The mechanism of this transcriptional regulation is very complex and is only beginning to be unravelled. Classical vitamin D-responsive elements and other responsive sites are being discovered in genes with important functions in the pancreatic β-cells and in genes with key roles throughout the immune system (e.g. cytokines, transcription factors), making vitamin D an attractive molecule to investigate in the context of diabetes treatment. Indeed, the activated form of vitamin D, 1,25(OH)\(_2\)D\(_3\), influences insulin secretion and is an important immune modulator. Furthermore, administration of 1,25(OH)\(_2\)D\(_3\) prevents the development of autoimmune type 1 diabetes, as well as the related insulitis, in non-obese diabetic (NOD) mice. Recently, several case-control studies have found associations between diabetes (type 1 and type 2) risk and restriction fragment length polymorphism of BsmI, ApaI, and TaqI and the exon 2 splice site Fok polymorphism of the VDR. The gene encoding the VDR is located in humans on chromosome 12cen-q12 and shows numerous single nucleotide polymorphisms at the 3’ end of VDR. Various pieces of evidence suggest that vitamin D and its receptor may play a role in the pathogenesis of diabetes mellitus.

Vitamin D and its metabolites: effects on the insulin-secreting β-cell in the pancreas

As reported, as early as the 1980s, vitamin D deficiency inhibits pancreatic secretion and turnover of insulin (but not of other pancreatic hormones), leading to impaired glucose tolerance. Whether the impairment observed is due to vitamin D deficiency per se or is secondary to low calcium is still a matter of debate. Later on, it was demonstrated that replacement therapy with vitamin D was able to reverse these abnormalities (concomitantly with improved calcium handling within β-cells). Meanwhile, several groups demonstrated that vitamin D, and especially its activated metabolite, 1,25(OH)\(_2\)D\(_3\), but not 24,25(OH)\(_2\)D\(_3\), are involved in controlling the function of the endocrine pancreas, especially of the insulin secreting cells in the pancreas. These effects of 1,25(OH)\(_2\)D\(_3\) are mainly studied in relation to its genomic actions. However, in the mid 1990s, a plasmalemmal receptor for 1,25(OH)\(_2\)D\(_3\) was proposed to mediate the non-genomic effects of the hormone on insulin secretion, in particular the rapid and sustained increase in cytosolic Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)), which seemed to depend on Ca\(^{2+}\) mobilisation from inner stores and extracellular Ca\(^{2+}\) entry. It is clear that calcium per se is important for insulin secretion, as well as for correction of the glucose intolerance seen in vitamin D deficiency. We demonstrated that islets from NOD mice with vitamin-D deficiency in early life (but with normal Ca\(^{2+}\) levels) had perfectly normal insulin synthesis and secretion after glucose exposure.
Also, the effector portion of the vitamin D machinery could be demonstrated in β-cells in the form of vitamin D-dependent calcium binding protein or calbindin-D28K. Intriguingly, calbindin-D28K was reported to modulate depolarisation-stimulated insulin release via regulation of [Ca2+]i and, in addition, protected β-cells from cytokine-induced cell death. In the pathogenesis of type 1 diabetes, β-cell apoptosis (and necrosis) provoked by cytokines and other inflammatory agents might play an important role. 1,25(OH)2D3 not only alters the effects of cytokines on β-cell function, but also changes the induction of surface markers and secretion of cytokines and nitric oxide induced in the β-cells by these cytokines. We recently reported that 1,25(OH)2D3 modulates the expression of chemokines and cytokines in pancreatic islets, but can not protect them from direct cytokine-induced killing. These data may have direct implications for the in vivo effects of 1,25(OH)2D3 and its analogues in prevention of type 1 diabetes observed in animal models.

Vitamin D and its metabolites: clinical implications

Based on the observations in vitro and in animal models of vitamin D deficiency in vivo, clinical trials have been performed to determine the effects of vitamin D or 1,25(OH)2D3 on glucose metabolism. There are several reports demonstrating that vitamin D-depleted humans have reduced insulin secretion. Recently, data from a pilot study examining vitamin D deficiency in type 1 and type 2 diabetes demonstrated that vitamin D deficiency is more common in type 2 diabetes than in type 1 diabetes, independently of age, sex, or insulin therapy. In several reports, vitamin D replacement therapy (and Ca2+) was able to reverse the vitamin D-deficient state and restore glucose tolerance. However, in vitamin D-depleted humans, vitamin D supplements of 2,000 IU per day or a single intramuscular injection of 100,000 IU were administered, making comparisons between these studies hazardous. Further studies are needed to better understand the clinical significance of the observed vitamin D deficiency and to investigate response to vitamin D replacement therapy.

Interesting are the studies where the effects of 1,25(OH)2D3 repletion in the relatively 1,25(OH)2D3-deficient state of uraemia were investigated. Insulin resistance is a typical feature of uraemia. Allegra et al. clearly demonstrated reduced insulin resistance in uraemic patients after 1,25(OH)2D3 therapy. However, repletion of 1,25(OH)2D3 could not completely reverse glucose intolerance. Orwoll et al. performed an interesting pilot study on possible clinical applications of 1,25(OH)2D3 in a situation of impaired insulin secretion without vitamin D deficiency. Clear effects of short-duration therapy with 1,25(OH)2D3 on parameters of calcium metabolism were noted, but this study was unable to determine whether hypovitaminosis D increased the risk of developing type 2 diabetes, as is suggested by other authors. Recently, another pilot trial showed beneficial effects of vitamin D supplements on first phase insulin secretion in type 2 diabetic women. However, Taylor and Wise reported three cases of British Asians with vitamin D deficiency and type 2 diabetes in which vitamin D supplementation led to increased insulin resistance and deteriorated glycaemia control.

In type 1 diabetes, several epidemiological studies describe a correlation between a north-south gradient and the incidence of disease, as well as an inverse correlation between monthly hours of sunshine and the incidence of diabetes. A seasonal pattern of disease onset is well described in type 1 diabetes. Dietary vitamin D supplementation is often recommended in pregnant women and in children to prevent vitamin D deficiency. Besides the study of Stene et al., who demonstrated that cod liver oil taken during the first year of life could reduce the risk for childhood-onset type 1 diabetes, the EURODIAB group suggested an association between vitamin D supplementation in infancy and a decreased risk for type 1 diabetes in a multi-centre case-control study. Moreover, Hypponen et al. reported that intake of 2,000 IU of vitamin D during the first year of life diminished the risk of developing type 1 diabetes. This study also showed that suspected rickets was associated with a higher incidence of childhood diabetes. Moreover, the Diabetes Autoimmunity Study in the Young (DAISY) revealed that dietary vitamin D intake by women during pregnancy was correlated with diminished islet autoantibodies in their children. Protection against type 1 diabetes mellitus by vitamin D supplements may be due to a combination of immune effects and β-cell protection. In NOD mice, there was no clear evidence that regular neonatal and early life supplements of vitamin D provided protection against type 1 diabetes. However, in NOD mice, clear preservation of insulin content of β-cells was observed.

Vitamin D and its metabolites: effects on the immune system in type 1 diabetes

Based on studies in animal models of type 1 diabetes, and supported by studies in humans, type 1 diabetes can
be considered an autoimmune disease, in which the body’s own immune system plays a central role in the destruction of the β-cell. Almost all the cells in this immune system (monocytes/macrophages, T lymphocytes, B lymphocytes, NK cells, dendritic cells) play a role.

To date, most prevention studies have been carried out in the NOD mouse and can be divided into several major categories: pure immune suppression, immune modulation, antigen-(specific) tolerance induction and β-cell protection. Results in NOD mice with many of these treatments are promising, but many obstacles to human applications still exist. Studies involving long-term immune suppression are inconceivable as a strategy for the prevention of a chronic disease striking mainly children. Moreover, the preliminary results with these drugs in recent-onset diabetic patients are disappointing. At this moment, interest is focused mainly on immune modulation and β-cell protection, two characteristics of 1,25(OH)2D3 and many of its newer analogues.

As 1,25(OH)2D3 can be produced by monocytes/macrophages/dendritic cells,44 and since receptors are present in several immune cells, a physiological role for this substance as a cytokine-like molecule or messenger between cells of the immune system is probable, and therapeutic possibilities in the prevention of this autoimmune disease are to be expected. Moreover, as described above, β-cell protective effects of 1,25(OH)2D3 against several inflammatory agents involved in β-cell destruction have been observed.

Prevention of type 1 diabetes in NOD mice by 1,25(OH)2D3 and its analogues

Chronic administration of pharmacological doses of 1,25(OH)2D3 can reduce the incidence of both insulitis and diabetes in NOD mice.9,10 Although treatment was globally well tolerated, hypercalcaemia and bone decalcification were seen. However, a major finding was the correction of a well-known defect of the NOD mouse: the absence of suppressor-cell function.45 Adorini et al. demonstrated that the regulator cell induced by 1,25(OH)2D3 or its analogues is most likely a CD4+CD25+cell.46 Doubts remain, however, as to whether the restoration of suppressor cells is the main mechanism involved in protection against diabetes by 1,25(OH)2D3, since protection against insulitis was also seen, a fact that points towards interference with the induction of autoimmunity itself. The basis of protection by 1,25(OH)2D3 seems to be more a reshaping of the immune repertoire, with elimination of effector cells, but the direct β-cell protective effects of 1,25(OH)2D3 may also play a major role in disease prevention (figure 2).47,48 The reshaping of the immune system involves more specifically a shift in the production of T-cell cytokines from predominantly Th1 (IL2, IFN-γ) in control mice to Th2 (IL4, IL10) in 1,25(OH)2D3- or analogue-treated mice. Moreover, this shift appears to be antigen-specific and most probably is due to a direct interference of 1,25(OH)2D3 or its analogues with the antigen-presenting dendritic cells.49,50 Indeed, 1,25(OH)2D3 induces a reshaping of the dendritic cells towards tolerogenic cells. We even demonstrated that dendritic cells generated in the presence of 1,25(OH)2D3 or an analogue can redirect already committed T-cell clones derived from a type 1 diabetic patient towards non-proliferation.51,52 In the NOD mouse, the reshaping of the immune system already occurs centrally, in the thymus, where treatment with 1,25(OH)2D3 restores the sensitivity of T lymphocytes to apoptosis-inducing signals, thus allowing better elimination of autoimmune effector cells.50,53
Several small intervention trials in newly-diagnosed type 1 diabetic patients have been performed without any clear conclusions. However, great caution is to be observed with trials of this kind since the doses administered are either very low or on the borderline of toxicity and, as the NOD model suggests, only a delay in disease progression is to be expected.

A major obstacle to human application of 1,25(OH)2D3 is its important effects on Ca2+ and bone metabolism. New structural analogues of 1,25(OH)2D3 with less effects on Ca2+ metabolism, but conserved or even more pronounced immunological effects, have been developed, especially through side-chain and, more recently, through CD-ring modifications.54-56 Several of the most promising of these analogues coming from different chemical laboratories have been tested in the NOD mouse. The mechanism of protection against insulitis and diabetes appears to be similar to that of 1,25(OH)2D3. Effects of the analogues on the dendritic-cell phenotype, regulator-cell induction and β-cell protection have been described.24,51,57 In the search for the optimal analogue, a combination of β-cell protection, immune modulation and low calcaemic effects is being pursued. To date there are several promising analogues but, before embarking on long-term interventions in individuals at high-risk for type 1 diabetes, long-term safety data will have to be gathered. A critical question for the applicability of analogues in the human situation is also whether these analogues of 1,25(OH)2D3 can arrest progression to clinically overt diabetes if administered when active β-cell destruction is already present, which is the situation in pre-diabetic subjects in whom immune intervention is considered. We demonstrated that some of these analogues, when combined with a short induction course of a classical immunosuppressant such as cyclosporine A, can arrest the progression of the disease when administered after autoimmune diabetes has already started.58 This approach of combining a short induction course of a classical immunosuppressant with non-hypercalcaemic analogues of 1,25(OH)2D3 is very promising and might open new perspectives in the prevention of autoimmune diabetes also in humans. Some analogues of 1,25(OH)2D3 have even been tested for their capacity to prevent disease recurrence after islet transplantation in spontaneously diabetic NOD mice. The most spectacular results were obtained when combining analogues of vitamin D with classical immune suppressants such as cyclosporine A or mycophenolate mofetil, or even with newer recombinant cytokines.59-61

**Clinical perspectives**

Clear effects of 1,25(OH)2D3 and its analogues on the different major players in the pathogenesis of diabetes mellitus, both type 1 and 2 diabetes, have been described. A modest stimulation of insulin synthesis and insulin secretion by 1,25(OH)2D3 is observed in vitro as well as in vivo. This positive effect is not only observed upon repletion of 1,25(OH)2D3 in the vitamin D-deficient state, but can also be observed in the vitamin D-replete state. Moreover, a direct β-cell protection by 1,25(OH)2D3 and its analogues against metabolic and inflammatory stress has been demonstrated. On the other hand, major effects on the immune system, involved in the pathogenesis of type 1 diabetes have been described in vitro as well as in vivo, and prevention of type 1 diabetes and its recurrence after islet transplantation can be achieved by 1,25(OH)2D3 and its analogues (alone or in combination with other immune modulators).

A major problem with using 1,25(OH)2D3 or the currently available analogues in prevention or cure of diabetes are their hypercalcaemic and bone remodelling effects when administered in the doses needed for immune or β-cell protective effects. Future applications of this therapy in human diabetes are conceivable, since through chemical alterations of the 1,25(OH)2D3 molecule, even better analogues, with an optimal dissociation between calcaemic and immune modulator effects can be synthesised.

A place for these analogues in the treatment (prevention or cure) of diabetes can be conceived first of all as β-cell protective and especially immune stimulating agents, added to the current treatment modalities of type 2 diabetes. Furthermore, these substances could play a major role in prevention strategies for type 1 diabetes in humans, because of their ideal profile as β-cell protective and especially immune active drugs. However, before applying these drugs in humans, more information should be gathered not only on their mechanism of action, but especially on the safety of these products in long-term use.

In conclusion, solid evidence exists that vitamin D deficiency is detrimental to β-cell function and leads to glucose intolerance in animal models and in humans through calcium dependent mechanisms. Vitamin D deficiency predisposes to type 2 and probably also to type 1 diabetes in animal models and in humans. Interventional studies with pharmacological doses of 1,25(OH)2D3 have only been performed in animal models and demonstrate
prevention of type 1 diabetes by 1,25(OH)2D3 through immune modulation. Important lessons should be drawn from the data on vitamin D deficiency where, in animals and humans, vitamin D deficiency early in life in genetically at risk individuals is linked to an increased diabetes incidence later in life. The only practical conclusion from the studies on vitamin D and diabetes at this time is that avoiding vitamin D deficiency is a priority not only for calcium and bone issues, but also for diabetes prevention.

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