review article

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REVIEW ARTICLE

Vitamin D and increasing incidence of type 1 diabetes—evidence for an association?

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There has been an important shift in the views about the actions of vitamin D during the past decade. In addition to its well-established role in the regulation of calcium metabolism, vitamin D deficiency has been associated with the risk of several extra-skeletal diseases, including type 1 diabetes among other chronic conditions. It is notable that $1,25(OH)_2D$ is known to regulate the expression of over 200 different genes, including the ones related to apoptosis and immune modulation. Increased vitamin D intake is currently considered as one of the most promising candidates for the prevention of type 1 diabetes, and it has been suggested that changes in vitamin D intake during the past decades have contributed to the recent trends in the incidence of the disease. This study reviews the evidence for the role of vitamin D in type 1 diabetes development, demonstrating that support has been obtained from various lines of investigation and that the possible biological mechanisms are plausible. However, much of the evidence has been obtained from animal experiments or observational studies in humans and there is an urgent need for well-designed, randomized, controlled trials to show whether the observed associations are indeed causal.
Keywords: animal experiments, epidemiology, genetic studies, type 1 diabetes, vitamin D

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Introduction

Despite initially being named as a 'vitamin', vitamin D is more correctly a hormone precursor that is mostly obtained through sunlight-induced synthesis in the skin. It can be obtained through diet, but in the absence of artificial fortification, food is generally a very poor source. Vitamin D content is naturally high only in oily fish. Before exerting its metabolic effects, vitamin D undergoes two successive hydroxylations. The first hydroxylation converts vitamin D to 25-hydroxyvitamin D [25(OH)D, which provides an indicator of vitamin D status] [1] and the second to the main active hormonal form, 1,25-dihydroxyvitamin D [$1,25(OH)_2D$, calcitriol]. The classical actions of hormonal vitamin D are related to the regulation of calcium metabolism and its importance for bone health is well known. However, evidence is accumulating for a role of vitamin D deficiency in the development of several extra-skeletal diseases, including cardiovascular disease, various types of cancer and type 1 diabetes, among other chronic conditions [2]. The wide-ranging health effects of vitamin D are supported by the presence of vitamin D receptors (VDR) in a range of organs and tissues of the body, including monocytes and pancreatic beta cells [3]. It is notable that $1,25(OH)_2D$ is known to regulate the expression of over 200 different genes, including the ones related to apoptosis and immune modulation[2].

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Increasing vitamin D intake is currently considered to be one of the most promising candidates for the prevention of type 1 diabetes [4], and it has been suggested that changes in vitamin D intake during the past decades may have contributed to the recent trends in the incidence of the disease [5]. As described in the following, evidence to support an association between vitamin D and type 1 diabetes has been obtained from various lines of investigation, and several stages in the pathogenic process leading to the progressive autoimmune destruction of the pancreatic beta cells could potentially be affected by vitamin D administration.

Evidence From Ecological Correlations

In many countries, vitamin D supplementation is recommended to infants and young children with an aim to prevent rickets, a disease resulting from extreme clinical deficiency [6]. The incidence of type 1 diabetes has been increasing in the industrialized countries, including the UK [7–9], where since the World War II, there has been a gradual decrease in the vitamin D fortification of foods and a break down of a systematic vitamin D/cod liver oil supplementation strategy [10]. In Finland, vitamin D supplementation to infants has been reduced to one tenth of the recommended dose in the 1940–1950s [11], and the incidence of type 1 diabetes has increased fourfold since the first nation-wide surveys in the early 1950s [12]. As seen in Figure 1, the recommended dosage of vitamin D has decreased gradually, which together with the observed decrease in the compliance to supplementation recommendations [5,13] could be speculated to have contributed to

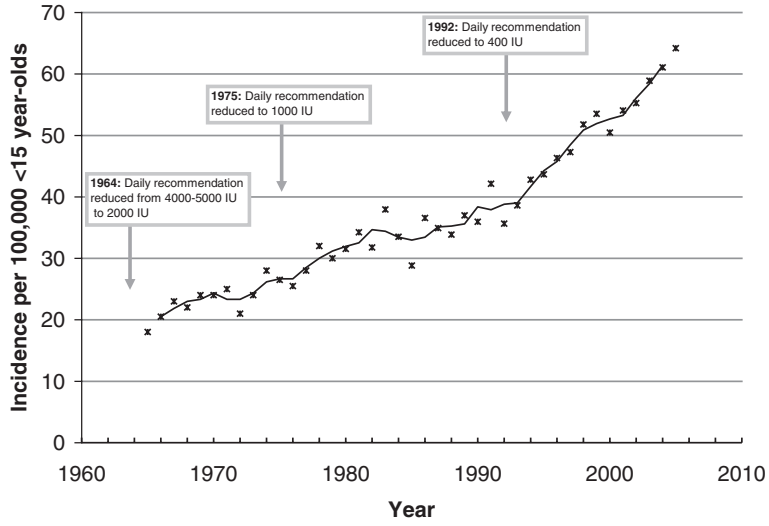

Figure 1. Increase in the incidence of type 1 diabetes [72,73] and changes in the vitamin D supplementation recommendations [5,11] in Finland.

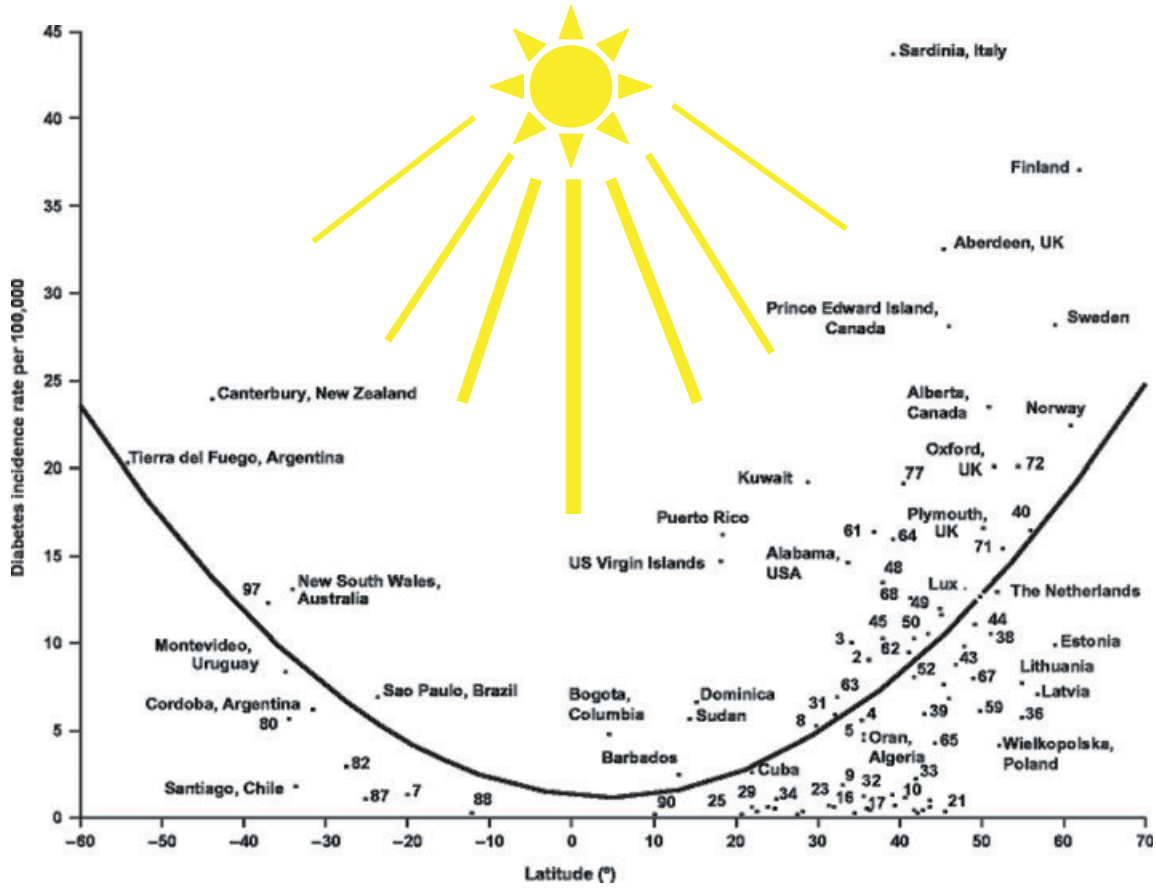

Figure 2. Figure adapted with kind permission from Springer Science+Business Media: Diabetologia "The association between ultraviolet B irradiance, vitamin D status and incidence rates of type 1 diabetes in 51 regions worldwide" 51, 2008, p.1393. Mohr et al.

the increase in the incidence of type 1 diabetes in Finland during the past decades. In addition, the observed pattern of geographical variation in the incidence of type 1 diabetes is fairly striking as recently reported (Figure 2), and it has been suggested that the increase in type 1 diabetes with the increase in distance from the equator could reflect variations in ultraviolet radiation-induced vitamin D synthesis in the skin [14]. Another piece of ecological data which is sometimes used to

support an association between vitamin D and type 1 diabetes is the seasonal patterns in the timing of onset of type 1 diabetes, where the highest rates have typically been reported during the winter and the lowest during the summer months [15–18]. These observed seasonal patterns may suggest the presence of a season-dependent precipitating factor, such as a low vitamin D status or a viral infection, or a combination of the two. Some studies have reported seasonality in the month of birth of the diabetic populations [19,20]; however, the observed patterns are not consistent across studies [21,22]. Taken together, the available ecological data appear attractive in its support for an association between vitamin D and type 1 diabetes. However, ecological studies are typically very open to interpretation and this type of information is mainly used in helping to create hypotheses rather than in providing evidence for them.

Experiments in the Non-obese Diabetic Mice

The hypothesis that vitamin D could protect from type 1 diabetes was first tested in the early 1990s using the non-obese diabetic (NOD) mice. In the early experiments, administration of the active hormonal metabolite (calcitriol) led to marked reductions in the progression to both insulitis (41 vs. 75%) and active diabetes (8 vs. 56%, in treated vs. control mice, respectively) [23,24]. Subsequent experiments with the NOD mouse suggest that by using sufficiently large doses of calcitriol, even a complete protection from progression to diabetes may be achieved [25]. However, diabetes prevention in these experiments, typically using pharmacological doses of calcitriol, has led to increases in serum calcium levels in the treated animals. Hypercalcaemia resulting from excessive intake is the main mechanism for vitamin D toxicity [26]; hence, the research interest has been naturally directed to examine whether corresponding reductions in diabetes risk can be achieved using non-hypercalcaemic structural analogues of calcitriol. There are over 2000 vitamin D analogues [27]. The findings are promising, and some vitamin D analogues have been found to be as effective as calcitriol in preventing diabetes in the NOD mouse [28,29]. The effect of the analogues has typically been dependent on the dosage used, with diabetes protection achieved without increases in serum calcium levels.

Some attempts have been made through animal experiments for evaluating the critical period when calcitriol/analogue administration may exert their influence on diabetes risk. NOD mice that were vitamin D deficient *in utero* and in early life (up to day 100) had earlier disease onset and higher incidence of diabetes compared with controls in one study [30]. In this study, vitamin D deficiency was achieved by housing the animals under a UVB-free light and feeding them a diet lacking vitamin D. The more aggressive disease pattern in the vitamin D deficient group continued after restoration of normal vitamin D status and metabolism, suggesting that early life influences may contribute to the disease progression in the NOD mouse. However, in one experiment where vitamin D was administered to NOD mice during pregnancy and early life, no alteration in disease incidence or progression was found [31]. This could be explained by the lack of effectiveness of vitamin D *per se* in the NOD mouse, because in all the studies, benefits on diabetes risk have been observed only with the use of active calcitriol or related analogues [32].

Animal experiments also suggest that it may be possible to reduce the disease progression to diabetes by the administration of vitamin D analogues even after the initiation of autoimmune attack. In an experiment using a vitamin D analogue after established insulitis with or without treatment with an immunosuppressant (cyclosporin A), the analogue alone was not effective, whereas the combination treatment led to a reduction in diabetes incidence [33]. Further support was obtained from a study using yet another vitamin D analogue, where analogue administration to adult NOD mice reduced the progression to diabetes, arresting Th1 infiltration and insulitis, with the longest treatment giving the best results [34].

Overall, experiments in the NOD mouse have provided fairly consistent support for a potentially beneficial role of vitamin D in diabetes prevention. There are some exceptions, most important of which is the unaltered disease expression in VDR knockout NOD mouse [35]. Neither the onset of insulitis nor the progression to diabetes was altered in the animals lacking vitamin D receptor, despite the observation of intensified defects in both innate and adaptive immunity [35]. However, there are changes in VDR expression in rodents over the lifespan; during weaning these animals do not express VDR in their small intestine, but this only develops after weaning has been completed [36]. It is not known whether the VDR is expressed in the immune cells or in the islet cells in foetal and neonatal mice. Given the likely differences in VDR expression and vitamin D metabolism between mice and humans, and the previous observations of different effects produced by the lack of receptor compared with that of a missing ligand (in this context, vitamin D deficiency) [35], these studies cannot be used to discount a possible influence of vitamin D deficiency on the development of diabetes either in mice or humans.

Observational Studies in Humans

The studies published on humans have so far been restricted to looking at either very early exposure (i.e. vitamin D supplementation in uterus/during infancy) or vitamin D status and supplementation at or after diabetes diagnosis. The first study on infant supplementation was from the EURODIAB project, suggesting a 33% reduction (OR 0.67) in the subsequent risk of developing type 1 diabetes by vitamin D supplementation during the first year of life [37]. The pooled effect estimate was very similar (OR 0.71) in a more recent meta-analyses, which combined the EURODIAB study with the three other case–control studies of infant vitamin D supplementation and type 1 diabetes published to date [38]. In our own study using data from the 1966 Northern Finland Birth Cohort, the association between vitamin D supplementation and subsequent risk of type 1 diabetes was markedly consistent across indicators of intake and status, showing clear evidence for a dose–response effect [5]. In this study, children who had received vitamin D supplementation regularly had 88% lower risk of type 1 diabetes compared to those receiving no supplementation. Conversely, children who were suspected of having had rickets during the first year

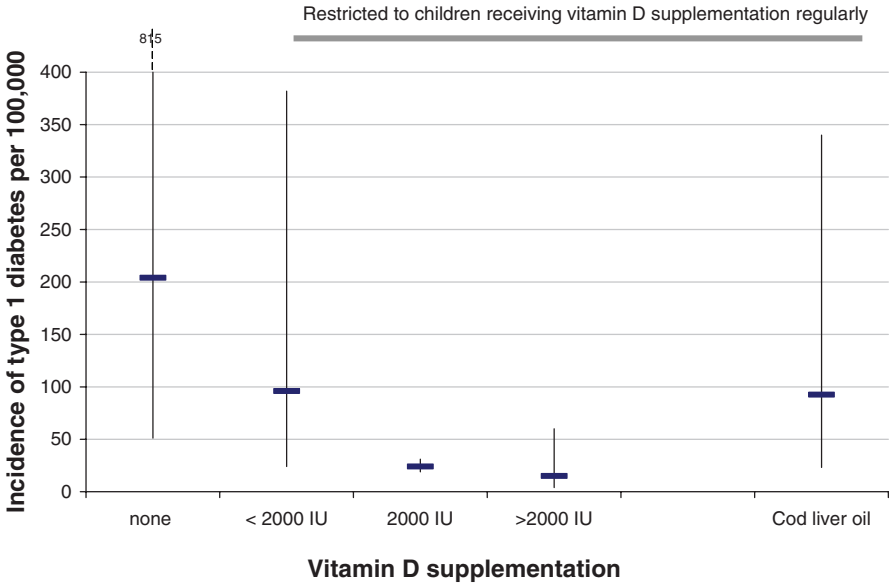

Figure 3. Incidence of type 1 diabetes by dose and type of infant vitamin D supplementation in the Northern Finland Birth Cohort 1966 [5]. The 95% confidence intervals for the incidence are represented by error bars.

had a threefold increase in their risk of diabetes. However, perhaps the most striking finding was the strong reduction in the incidence of type 1 diabetes obtained by increasing the doses of vitamin D supplementation observed in the sub-group of children who had all received vitamin D supplementation regularly ($n = 9087$), where a further 86% risk reduction was observed if the dose given to the infant was at least 2000 IU (50 μg) per day (Figure 3), the level of the contemporary recommendation [5,11].

Cod Liver Oil vs. Vitamin D Supplementation

In Norway, among some other countries, vitamin D supplementation is provided in the form of cod liver oil, which in addition to vitamin D also has vitamin A and omega-3 fatty acids. It has been suggested that it is the omega-3 fatty acids in the cod liver oil that reduce the progression of type 1 diabetes-related autoimmunity [39]. Although there are some studies where associations are observed for cod liver oil but not vitamin D supplementation *per se*, differences in vitamin D dosages and bioavailability between products prevent reliable comparisons between the groups. In one Norwegian case–control study, offspring of mothers who had used cod liver oil supplements during pregnancy had a reduced risk of type 1 diabetes, with inconclusive findings on the effect of vitamin D supplementation during pregnancy or in infancy (estimated effect ranged from a nearly 90% reduction to a twofold increase in diabetes risk) [40]. In another somewhat larger case–control study, infants who had received cod liver oil had a reduced risk of type 1 diabetes, although vitamin D supplementation in infancy or maternal vitamin D supplements or cod liver oil were not significantly associated with diabetes risk [41]. There is also one study where omega-3 fatty acid intake during childhood was associated with reduced development of islet cell autoantibodies and vitamin D intake had no independent association [39]. However, in an earlier report on the same study, seroconversion rates were reported to be reduced for offspring to mothers with higher compared to lower vitamin D intakes [42] . It is also possible that the apparent discrepancy between these findings reflects either an early critical window for the influence of vitamin D on diabetes-related autoimmunity (pregnancy/infancy, not childhood) or instability in the effect estimation caused by small numbers. As seen in Figure 3, in the Northern Finland Birth Cohort 1966, it was clearly vitamin D supplementation and not cod liver oil which accounted for the observed benefits on type 1 diabetes risk [5], and the incidence of diabetes in children who received cod liver oil ($n = 86$) was higher compared with that in those who received the supplementation as vitamin D droplets (78.15 per 100 000 vs. 24.66 100 000 person years at risk). When infants receiving cod liver oil regularly were compared with those who were given the recommended dose of vitamin D (2000 IU/day), the data obtained were suggestive of an increased risk of type 1 diabetes for the cod liver oil users (hazard ratio 3.9, $p = 0.06$). These data probably reflect a higher concentration of vitamin D in droplets compared with that of cod liver oil (rather than harmful effects of cod liver oil); however, no support is provided for a superior influence on type 1 diabetes risk for supplements containing omega-3 fatty acids compared with supplements containing vitamin D only.

Comparisons After the Onset of Clinical Disease

There is evidence to suggest that nutritional vitamin D status (measured by the circulating concentrations of 25-hydroxyvitamin D) [43,44], and in some studies calcitriol concentrations [45,46] also, are lower in newly diagnosed type 1 diabetes patients compared to population controls. In a study investigating the effect of administration of calcitriol ($1,25(OH)_2D_3$) (compared to nicotinamide) to children with

newly diagnosed diabetes, there appeared to be some short-term benefits with calcitriol treatment (0.25 μg every other day) compared to nicotinamide on the required insulin dose at 3 and 6 months [47]. However, C-peptide or HbA1c concentrations (reflecting residual insulin secretion and glucose homeostasis) did not differ between the calcitriol and nicotinamide groups at 1 year, suggesting that long-term benefits for calcitriol supplementation with treatment initiated after the diagnosis of diabetes are probably limited.

Genetic Association Studies

There are several genes which are known to affect vitamin D activation and/or metabolism [48]. Some evidence for vitamin D related genetic susceptibility for type 1 diabetes has been found; however, for most polymorphisms there are only a handful of studies and replication is needed. For example, variations in 25-hydroxylase (CYP2R1) [49,50], 1-alpha hydroxylase (CYP27B1) [51–53], and vitamin D binding protein (Gc-globulin) [54–57] have been studied in relation to type 1 diabetes, but for any of the sites there is no robust evidence for an association with replication. VDR polymorphisms have been related to insulin secretion capacity in humans [58] and several studies have investigated the association between VDR and type 1 diabetes. However, results from these studies have also been inconclusive and in a meta-analysis including information on all association and family studies published from 1997 to 2005 for the four most common variations (ApaI, BsmI, FokI and TaqI), no support for an association with type 1 diabetes was found [59]. Interestingly, meta-analyses on the same VDR polymorphisms and bone mineral density also failed to identify associations [60]. Vitamin D status is known to influence bone mineral density and the risk of osteoporosis [2], hence these data may suggest that these common VDR variations may not be on their own sufficient to detect variations in VDR activity or function. Indeed, of the four major polymorphic sites, FokI has been shown to result in an alternative transcription initiation site [61], whereas TaqI, BsmI and ApaI sites are presented at the non-coding regions. It has also been suggested that environmental ultraviolet radiation (UVR) exposure may influence the association between VDR genotype and type 1 diabetes risk. Support for this has been obtained from observed geographical correlations in the odds ratios for VDR genotype—type 1 diabetes association, where the absolute association between VDR alleles and type 1 diabetes strengthened with increasing amount of winter time UVR [62]. It is also possible that the influence of variations in the VDR (or indeed, any other) locus is affected by other genes as well as by the environment, which would make it more difficult to identify disease associations given the high power requirements and other methodological challenges in investigating gene × gene interactions[63].

Mechanisms

The autoimmune destruction of the pancreatic beta cells in type 1 diabetes occurs in a T-cell dependent process [64,65], and disruption in the polarization between T helper type 1 (Th1) and Th2 type responses towards Th1 up-regulation is believed to be central to the pathogenesis [4,66]. This provides one of the commonly proposed mechanisms to explain the potential role of vitamin D in type 1 diabetes, as the overall net result of the complex $1,25(OH)_2D$ immunomodulatory action in the T-cells leads to the blockage of the induction of Th1 cytokines (most notably INF-γ) [67,68]. Another potential mechanism arises from the proposed direct impact of $1,25(OH)_2D$ on barrier integrity, where locally produced $1,25(OH)_2D_3$ could protect beta cells by helping to maintain barrier exclusion of pathogens [69]. This appears attractive in the light of the proposed disease promoting effects of viruses (notable enteroviruses) and bacteria both through the aggravation of immunological processes and the induction of direct damage to the pancreas [70]; $1,25(OH)_2D$ has influences on barrier function which together with the established immunodulatory effects [67,68], would provide a two-tier mechanism of defense should the barrier become breached [69].

Conclusions

Despite strong interest in the topic, there are no published randomized controlled trials using vitamin D to prevent type 1 diabetes, which hinders firm conclusions about the possible causality for the observed associations. However, the available evidence for an association between vitamin D and type 1 diabetes is fairly consistent; it arises from several lines of investigation, and there are biologically plausible explanations for the potential benefits. It can be argued, that given the complex, multifactorial nature of the disease [65,71], it is unlikely that vitamin D deficiency would be the only or main cause for type 1 diabetes. As discussed above, higher vitamin D intakes may operate at least in part through offsetting some of the increased type 1 diabetes risk as a result of viral or bacterial infections (at an early age), which is one intriguing possibility. However, globally millions of children are affected by vitamin D deficiency [2] and given the very high prevalence of this potential risk factor, even fairly modest effects could have important implications for the prevention of type 1 diabetes if the association is real. More studies, including clinical trials, are clearly required to establish the role of vitamin D in type 1 diabetes and to test whether by using supplementation with vitamin D or vitamin D analogues, this serious disease could be safely prevented. However, vitamin D deficiency is a known avoidable health hazard, and it is important to take action to reduce the prevalence of it even while waiting for further evidence to accumulate for any specific health outcome.

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