Vitamin D and diabetes: the odd couple

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Abstract

The beneficial effects of vitamin D supplementation for a number of health-related issues, including the prevention of diabetes, are a topic of intense discussion. Data from epidemiological studies suggest a correlation between vitamin D deficiency and higher prevalence of both type 1- and type 2–diabetes. In animal models, vitamin D deficiency predisposes to diabetes, whereas vitamin D supplementation prevents disease. Still, well-designed clinical intervention studies are lacking. Here, we discuss evidence available on a role for vitamin D in diabetes and propose that vitamin D deficiency should be avoided especially in all at-risk people. This should be possible by implementing global guidelines and by focusing on daily dietary supplementation with small doses of vitamin D.

Vitamin D and type 2 diabetes

A physiological role for vitamin D in maintaining normal glucose metabolism is proposed, as the vitamin D receptor (VDR) is found in all insulin-responsive tissues as well as in the pancreatic beta-cells. Thus, deficiency in vitamin D (Box 1) is one of the environmental factors suspected of contributing to the growing epidemic of T2D (Box 2). As discussed below, data from epidemiological observations link vitamin D deficiency to glucose intolerance or T2D – either through a direct action via VDR or indirectly via the effects of vitamin D deficiency on calcium levels. Basic and translational research data also suggest that correcting vitamin D deficiency improves both beta-cell function and insulin sensitivity. Unfortunately, well-designed adequately-powered clinical trials that unequivocally support or refute a therapeutic role for vitamin D in T2D are still lacking.

Association studies and epidemiology

Genetic polymorphisms could help identify groups that are more susceptible to vitamin D deficiency and to developing T2D. Contrasting results have been published on the importance of allelic variations of vitamin D-related genes in the occurrence of insulin resistance and T2D. Despite reported correlations between VDR gene polymorphisms, especially the *FokI* single nucleotide polymorphism (SNP) [1], and insulin secretory responses to glucose [2, 3] and insulin resistance [4], case-control studies have failed to show differences in frequencies of VDR polymorphisms between T2D patients and healthy controls [5, 6]. Most associations so far have been found in Asian populations and additional studies in other populations are required.

As elegantly reviewed by Mezza et al. [7], many association studies link vitamin D deficiency to an increased risk for the development of T2D. In addition, the European Prospective

Investigation into Cancer (EPIC)-Norfolk study [8] and a new meta-analysis of prospective studies demonstrated a significant association between low 25(OH)D concentrations and risk of T2D, irrespective of gender, study duration, sample size, diabetes diagnostic criteria, or type of 25(OH)D assay used [9]. Epidemiological studies show correlations between low serum 25(OH)D concentrations and increased insulin resistance, as well as impaired beta-cell function (reviewed in [10]). The large cross-sectional National Health and Nutrition Examination Survey (NHANES) study showed an inverse correlation between serum 25(OH)D concentration and T2D incidence and insulin resistance [11]. The Medical Research Council Ely Study 1990-2000 also reported an inverse relationship between vitamin D status and glycemia [12]. A recent study in non-obese Asian patients, confirmed this inverse correlation between vitamin D status and T2D risk, insulin resistance and beta-cell function [13]. Clear correlations are also observed between vitamin D deficiency and gestational diabetes, considered a precursor of T2D, as confirmed in a recent systematic review [14].

However, some reports failed to find an association between serum concentrations of 25(OH)D and T2D risk, in particular when correcting for body mass index (BMI) and obesity [15]. The latter are major confounders, as excess body fat is the single most important contributor to the development of T2D and most T2D patients have an increased BMI. Serum 25(OH)D concentration on the other hand is inversely correlated with body fat content and BMI thus introducing an important confounder when interpreting data linking vitamin D deficiency and T2D [16]. Explanations for the low concentrations of serum 25(OH)D in obese individuals can be: 1) inadequate dietary input and reduced cutaneous synthesis linked to unhealthy lifestyle, 2) negative feedback from high concentrations of $1,25(OH)_2D$ and parathyroid hormone (PTH) on hepatic 25(OH)D synthesis, or 3) a larger vitamin D storage capacity (sequestration) in body tissue, particularly fat. However, volumetric dilution could account for the variability in serum 25(OH)D concentration attributable to obesity: once

concentrations of circulating 25(OH)D are adjusted for body size, the difference between obese and lean subjects disappears [17].

Taken together, strong evidence from epidemiology suggests that vitamin D insufficiency predisposes to beta-cell dysfunction, insulin resistance and T2D.

Translational research

Impaired glucose tolerance due to beta-cell dysfunction was one of the first extra-skeletal effects of vitamin D deficiency described in experimental animal models [18], a defect restored by vitamin D supplementation [19] (Figure 1). A few intervention studies in rodent models of T2D support the hypothesis that vitamin D treatment improves glucose homeostasis and T2D [20]. Mechanistically, circulating $1,25(OH)_2D$ can bind directly to VDR in beta-cells and $1,25(OH)_2D$ directly stimulates the expression of insulin receptor and promotes insulin-mediated glucose transport *in vitro* (reviewed in [21]). Furthermore, exposure of pancreatic islets to $1,25(OH)_2D$ affects the expression of genes involved in cellular growth, cytoskeletal organization, intracellular trafficking, formation of intercellular junctions, and insulin secretion [22]. $1,25(OH)_2D$ may also reduce the low grade chronic inflammation present in obesity and T2D, as $1,25(OH)_2D$ decreases the production of inflammatory products like cytokines by activated macrophages and inflammation impairs both beta-cell function and insulin sensitivity [23]. Finally, vitamin D deficiency leads to increased activity of the renin angiotensin system (RAS) [24], known to impair beta-cell function and peripheral insulin sensitivity, and $1,25(OH)_2D$ suppresses the RAS [25].

Caution is needed however, when interpreting *in vivo* studies, as vitamin D deficiency also affects serum calcium levels and calcium is a crucial ion in insulin secretion and action [26]. Hypo- or hyper-calcemia impair the proper function of pancreatic islets and affect insulin sensitivity of target tissues. The alteration of calcium levels by vitamin D deficiency as well as administration of high doses of vitamin D or $1,25(OH)_2D$ creates a major confounder in

data interpretation. Experimental data show that VDR knock-out (KO) mice have completely normal glucose tolerance and beta-cell function when calcium levels are kept normal using high lactose and high calcium diets [27].

In conclusion, *in vitro* studies suggest a direct role for the vitamin D system in beta-cell function and insulin sensitivity, and data from animal models imply a direct relationship between vitamin D deficiency and T2D.

Clinical trials

To date, there is still a lack of large scale, blinded trials demonstrating the beneficial effect of vitamin D supplementation on the prevention of T2D (measured by improved beta-cell function or improved insulin sensitivity). Older, small scale trials [28] have suggested that high doses of vitamin D reverse T2D in vitamin D deficient populations such as dialysis patients, and more recent small scale trials show a positive effect of vitamin D supplements on insulin sensitivity, both in healthy and vitamin D-deficient individuals [29, 30]. Studies focusing on pre-diabetes or gestational diabetes in young individuals demonstrate improved insulin sensitivity by vitamin D supplementation [31]. In contrast, a recent double-blind, randomized, placebo-controlled trial demonstrated that high-dose vitamin D supplements (11,200 IU per day for 2 weeks, followed by 5,600 IU per day for 10 weeks) could not reduce insulin resistance, blood pressure, inflammation or glycosylated hemoglobin (HbA1c) in patients with established T2D [32]. Hope is now set on the more than 25 registered trials investigating the impact of vitamin D supplements on the prevention of insulin resistance, T2D or gestational diabetes (www.clinicaltrials.gov).

Vitamin D and type 1 diabetes

Vitamin D (Box 1) might be a candidate influencing T1D incidence (Box 3), based on results from association studies, epidemiology, and from basic and translational studies. For instance, increased prevalence of T1D with increased latitude suggests a link with lower availability of sunshine and thus less potential synthesis of vitamin D in the skin (see below). In addition, VDR is present in beta-cells as well as many immune cells and high doses of $1,25(OH)_2D$ are able to alter T1D presentation in animal models. However, clinical trials have so far failed to substantiate the therapeutic potential of vitamin D in T1D prevention and intervention (see below). It is important to note here that several randomized controlled trials using vitamin D have been unsatisfactory, possibly due to using too low doses or too large intermittent doses, a too short treatment period, looking at people who already have dysglycemia, etc.

Association studies- Epidemiology

In recent years, many epidemiological and genetic studies have attempted to link vitamin D deficiency or defects in the vitamin D system to the increased prevalence of autoimmune diseases.

The hypothesis that vitamin D deficiency plays a role in T1D is supported by reports that the incidence of T1D follows a clustered seasonal pattern. For example, the US-based SEARCH study concluded that spring births were associated with increased likelihood of T1D, but possibly not in all US regions [33]. Similarly, significantly more children with diabetes were born during the Spring-Summer than in Autumn-Winter in Greece during the period 1978-2008 [34]. Seasonal variation was also found in clinical onset of T1D. In Sardinia and Finland, two areas with the highest incidence of T1D worldwide, a significant seasonal pattern was found for two age-groups (0-9 and 10-14 years)[35]. In Germany, Greece and in Denmark, seasonal variation was also observed with cases increasing during the cold months [34, 36, 37]. A large, global and standardized study based on incidence data from the World

Health Organization Diabetes Mondiale (WHO DiaMond) Project (0-14 years old children, over the period 1990-1999) confirmed that seasonal variation in T1D incidence rates in children under 15 years of age is a real phenomenon [38].

Studies focusing on latitude and UVB irradiance have revealed an inverse correlation between the monthly hours of available sunshine and the incidence of T1D [39-41]. Where initial data showed an inverse association of T1D prevalence and latitudinal gradients in the Northern Hemisphere [42], later studies, for example in Australia and in Newfoundland and Labrador, suggested that actual exposure to UVB radiation may be geospatially associated with the incidence of T1D, rather than the degree of latitude itself [40, 41]. Analysis of data from the Diamond project indeed showed an association between low UVB irradiance and high incidence rates of T1D after controlling for per capita health expenditure [39]. Strikingly, incidence rates of T1D approached zero in regions worldwide with high UVB irradiance [39].

Because vitamin D is predominantly synthesized by UVB exposure of the skin, studies have assessed and revealed a worldwide association between vitamin D deficiency and the onset of T1D. North Indian [43], Italian [44], Swedish [45], and British [46] children or young adults with newly diagnosed T1D had lower concentrations of serum 25(OH)D than healthy controls. An increased prevalence of vitamin D deficiency in children and adolescents with T1D compared with non-diabetic individuals was also observed in Swiss [47], American [48], Australian [49], and Qatari [50] populations. Interestingly, vitamin D supplementation in early childhood has been shown to reduce the risk for T1D [51].

Also genetic studies have searched for a link between the vitamin D system and T1D. SNPs in three key genes of enzymes involved in vitamin D metabolism, namely CYP27B1, DHCR7, and CYP2R1, affect concentrations of serum 25(OH)D [52] and are associated with susceptibility to T1D [46, 53]. SNPs in CYP24A1 (encoding for the vitamin D-catabolizing enzyme) are associated with lower vitamin D status [52] but not with susceptibility to T1D

[46, 53]. A link between SNPs in the DBP gene and T1D has also been demonstrated [54]. The search for an association between VDR genetic polymorphisms and T1D has yielded conflicting. Several small studies have linked VDR gene variations to T1D and a recent meta-analysis suggests that at least one of four known polymorphisms in the VDR gene is associated with a higher risk for T1D in Asians [55]. However, in contrast, an earlier meta-analysis [56] and the largest association studies [57, 58] found no association between VDR polymorphisms and T1D.

Thus, it can be concluded that epidemiology points towards a role for the vitamin D system in the onset of T1D, but data are conflicting.

Translational research

The awareness of a role for vitamin D in the regulation of immune responses was triggered by the discovery that VDR is expressed in almost all immune cells of the innate and adaptive immune system. $1,25(OH)_2D$ or its analogs can effectively inhibit dendritic cell differentiation and immune activation by inhibiting the surface expression of MHC class II-complexed antigen, co-stimulatory molecules and the production of the proinflammatory cytokines interleukin-12 and -23 (IL-12, IL-23) [59] (Figure 2). This causes an indirect shift in T cell polarization from a T helper (Th)1/Th17 to a Th2 phenotype and favors regulatory T cell (Treg) development [60]. In addition, $1,25(OH)_2D$ can also act directly on T cells [61, 62], reducing Th1/Th9/Th17 responses [63], increasing Th2 responses and promoting a Treg profile in T cells from healthy individuals [62].

In NOD mice, a rodent model for T1D, chronic administration of $1,25(OH)_2D$ inhibits insulitis and delays diabetes onset [64]. These effects might stem from the effects of $1,25(OH)_2D$ on immune cells, as NOD mice treated with $1,25(OH)_2D$ display a Th1 to Th2 shift in the pancreas and pancreas-draining lymph nodes [65], have decreased Th1 cell infiltration in the pancreas and increased $CD4^+CD25^+$ Tregs in the pancreatic lymph nodes

189 [66]. A major issue with the doses needed to achieve this protection are the hypercalcemia
190 and bone-effects (decalcification in the presence of calcium-poor diet) observed. Structural
191 analogues of vitamin D that share the immune effects, but have greatly reduced effects on
192 calcium and bone compared to $1,25(OH)_2D$, can avoid these side-effects [62].

193 Direct effects of $1,25(OH)_2D$ on the beta-cells are also documented (see above, section on
194 T2D), but data on protection against cytokine-impaired insulin release [22] and cytokine-
195 mediated beta-cell death [67] have been inconsistent, likely because of different experimental
196 conditions and models used. Moreover, $1,25(OH)_2D$ may improve beta-cell function because
197 it can limit chemokine expression [67], partly normalize the gene expression of MHC class I
198 molecules, and decrease the density of MHC class I proteins of beta-cells. This can effectively
199 reduce T cell infiltration and activation in the islets [67], consequently decreasing the pro-
200 inflammatory cytokines in the micro-environment.

201

202 *Clinical trials*

203 Several retrospective studies have suggested that supplementation with regular vitamin D in
204 early life lowers the risk of T1D onset. For instance, a birth-cohort study in Finland in 1966
205 showed an almost 80% reduction in the risk for T1D development by the intake of the daily
206 recommended (at that time) dose of 2,000 IU of vitamin D during the first year of life [51].
207 This was confirmed in 1999 by the EURODIAB study, which reported a 33% reduction in
208 T1D in children receiving (unspecified doses of) vitamin D supplements in early childhood
209 [68]. Meta-analysis of data from four case-control studies and one cohort-study showed that
210 the T1D prevalence was 29% lower in infants receiving vitamin D supplements [69]. Overall,
211 these studies suggest that vitamin D-mediated diabetes protection may be dose-dependent.
212 However, some studies did not find a correlation between T1D prevention and vitamin D
213 supplementation. In Norway, intake of cod-liver oil by children under 1 y resulted in a (not

statistically significant) tendency of lowered T1D incidence [70] and the ABIS study in Sweden found that the use of vitamin D-containing supplements during pregnancy was associated with reduced development of autoantibodies to GAD or IA-2A in the offspring at 1 year, but not at 2.5 years [71]. Despite the fact that some studies failed to show an association between reduced T1D risk and vitamin D supplementation during infancy, none of them were associated with an increased risk.

Doses of 2,000 IU/d regular vitamin D were regarded as high 10 years ago, and they still are, based on toxicity and side-effect thresholds. However, it is suggested that the currently recommended daily allowance (RDA) of 600 IU/d (age up to 70 years) might be inadequate to achieve the intended effects on immune cells or beta-cells. For instance, in an experimental system, Cheng et al. found that ten times the concentrations of $1,25(OH)_2D$ normally seen in the circulation were needed for optimal effects on insulin secretory responses to glucose [24]. For that reason, several recent studies evaluate the effects of much higher doses of regular vitamin D or $1,25(OH)_2D$ (see below).

So, what about intervention studies in recent-onset T1D patients? Large scale studies are again lacking. Some small studies gave a hint that safe doses of vitamin D may possibly dampen beta-cell loss allowing better glucose control after T1D onset, but small sample sizes, short duration of follow-up and lack of control groups constitute major limitations of the reported studies. Confirmation at a much larger scale is therefore needed. For instance, the IMDIAB XI and XIII studies showed that administration of 0.25 µg $1,25(OH)_2D$ daily or on alternate days was safe but nevertheless failed to reduce loss of beta-cell function [72], even in patients with high C-peptide at diagnosis [73]. Again, the use of too low doses or too short regimens of regular vitamin D or $1,25(OH)_2D$ administration might be responsible for this failure. In addition, in many studies, it is unclear whether the administered vitamin D

supplements restored existing deficiencies or augmented circulating vitamin D in already sufficient individuals.

Several trials in T1D patients are registered at www.clinicaltrials.gov. A study at the universities of Graz and Vienna in Austria will provide data on how ultra-high monthly bolus vitamin D supplementation affects Tregs and metabolic outcome in T1D patients (NCT01390480). This study should confirm the increased Treg levels observed in vitamin D-supplemented healthy individuals [74] and form a direct immunological basis for using vitamin D supplementation in T1D. An alternative approach is to use vitamin D in combination treatment. A completed unpublished study (NCT00873925) combined vitamin D with omega-3 and umbilical cord blood (UCB, used because it contains highly functional populations of Tregs), despite earlier failure of transfusion of autologous UCB to preserve C-peptide in young children with T1D [75]. The DIABGAD study will test the efficacy of alum-formulated GAD65 (GAD-Alum, Diamyd), in combination with vitamin D and ibuprofen (NCT01785108), notwithstanding earlier failure of GAD-Alum treatment to significantly reduce the loss of stimulated C-peptide or improve clinical outcomes in T1D patients [76]. As a reminder, GAD-alum vaccination aims at intervening in the autoimmune destructive process by modulating the immune system in a discrete, antigen-specific (GAD is a major auto-antigen in T1D) manner to prevent the destruction of beta-cells.

One of the major obstacles for translating the successful animal data to humans is the need for supra-physiological doses to modulate immune responses, as these elicit hypercalcemia, hypercalciuria and kidney stones. The use of structural analogs of $1,25(OH)_2D$ with reduced calcemic effects but similar immunoregulatory activity might overcome this issue, but no trials are registered to investigate effects of vitamin D analogues on T1D prevention or reversal.

Concluding remarks and future perspectives

Vitamin D deficiency is a threat to health in general, with a suggestive link to the two most common forms of diabetes, T1D and T2D. Receptors for vitamin D are found in all tissues and organs involved in the diseases - from the common player that is the beta-cell, to the immune system and the insulin target organs, muscle, adipose tissue and liver. Several enzymes responsible for 25-hydroxylation of vitamin D and even the CYP27B1-hydroxylase, responsible for the final activation of 25(OH)D to 1,25$(OH)_2$D, are also widely expressed in human tissues involved in the pathogenesis of both T1D and T2D, suggesting the possibility of local production and function of 1,25$(OH)_2$D . Association studies and epidemiology data convincingly point to a link between vitamin D deficiency, glucose intolerance and T2D, and also suggest that vitamin D deficiency is associated with a higher risk for T1D in genetically susceptible individuals. Basic science and animal models of the diseases point towards the potential of the vitamin D system not only being one of the contributors to the changing behavior of the diseases worldwide, but also being of therapeutic use, with supplements of vitamin D or of its most active form, 1,25$(OH)_2$D, altering the course of the disease. However, clinical trial data demonstrating a therapeutic role for vitamin D or its metabolites and analogues are lacking. These trials are not easy to conduct, because vitamin D is present in many ‘fortified’ foods and the hype over vitamin D has resulted in increased consumption in recent years (via over-the-counter supplements). Trials are nevertheless desperately needed to confirm or refute a therapeutic potential of this compound (see Outstanding questions box). One should not forget that vitamin D is a fat-soluble vitamin and that toxicity is observed when chronically supra-physiological doses are administered. Until satisfactory randomized controlled trial data is available, the only sound advice that can be given to people at risk for T1D and T2D, is to avoid vitamin D deficiency. This can easily be achieved by following the recent guidelines from the Institutes of Medicine (IOM, www.iom.edu). If all individuals

achieved recent IOM guideline intakes of vitamin D [77], and deficiency were to be virtually eliminated, then, if vitamin D does have a role in protection against T1D and T2D, this alone might lead to reductions in the future incidence and prevalence of T1D and T2D, respectively.

References

1 Li, L., *et al.* (2013) Vitamin D receptor gene polymorphisms and type 2 diabetes: a meta-analysis. *Archives of medical research* 44, 235-241

2 Hitman, G.A., *et al.* (1998) Vitamin D receptor gene polymorphisms influence insulin secretion in Bangladeshi Asians. *Diabetes* 47, 688-690

3 Ogunkolade, B.W., *et al.* (2002) Vitamin D receptor (VDR) mRNA and VDR protein levels in relation to vitamin D status, insulin secretory capacity, and VDR genotype in Bangladeshi Asians. *Diabetes* 51, 2294-2300

4 Jain, R., *et al.* (2012) Association of vitamin D receptor gene polymorphisms with insulin resistance and response to vitamin D. *Metabolism* 61, 293-301

5 Malecki, M.T., *et al.* (2003) Vitamin D receptor gene polymorphisms and association with type 2 diabetes mellitus in a Polish population. *Exp Clin Endocrinol Diabetes* 111, 505-509

6 Dilmec, F., *et al.* (2010) Detection of VDR gene ApaI and TaqI polymorphisms in patients with type 2 diabetes mellitus using PCR-RFLP method in a Turkish population. *J Diabetes Complications* 24, 186-191

7 Mezza, T., *et al.* (2012) Vitamin D deficiency: a new risk factor for type 2 diabetes? *Annals of nutrition & metabolism* 61, 337-348

8 Forouhi, N.G., *et al.* (2012) Circulating 25-hydroxyvitamin D concentration and the risk of type 2 diabetes: results from the European Prospective Investigation into Cancer (EPIC)-Norfolk cohort and updated meta-analysis of prospective studies. *Diabetologia* 55, 2173-2182

9 Song, Y., *et al.* (2013) Blood 25-hydroxy vitamin D levels and incident type 2 diabetes: a meta-analysis of prospective studies. *Diabetes Care* 36, 1422-1428

10 Khan, H., *et al.* (2013) Vitamin D, type 2 diabetes and other metabolic outcomes: a systematic review and meta-analysis of prospective studies. *The Proceedings of the Nutrition Society* 72, 89-97

11 Scragg, R., *et al.* (2004) Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. *Diabetes Care* 27, 2813-2818

12 Forouhi, N.G., *et al.* (2007) Incidence of Type 2 diabetes in England and its association with baseline impaired fasting glucose: the Ely study 1990-2000. *Diabet Med* 24, 200-207

13 Lim, S., *et al.* (2013) Association of vitamin D deficiency with incidence of type 2 diabetes in high-risk Asian subjects. *Am J Clin Nutr* 97, 524-530

14 Aghajafari, F., *et al.* (2013) Association between maternal serum 25-hydroxyvitamin D level and pregnancy and neonatal outcomes: systematic review and meta-analysis of observational studies. *BMJ* 346, f1169

15 Grimnes, G., *et al.* (2010) Baseline serum 25-hydroxyvitamin D concentrations in the Tromso Study 1994-95 and risk of developing type 2 diabetes mellitus during 11 years of follow-up. *Diabet Med* 27, 1107-1115

16 Vimaleswaran, K.S., *et al.* (2013) Causal relationship between obesity and vitamin D status: bi-directional Mendelian randomization analysis of multiple cohorts. *PLoS medicine* 10, e1001383

17 Drincic, A.T., *et al.* (2012) Volumetric dilution, rather than sequestration best explains the low vitamin D status of obesity. *Obesity (Silver Spring)* 20, 1444-1448

18 Norman, A.W., *et al.* (1980) Vitamin D deficiency inhibits pancreatic secretion of insulin. *Science* 209, 823-825

19 Kadowaki, S. and Norman, A.W. (1984) Dietary vitamin D is essential for normal insulin secretion from the perfused rat pancreas. *J Clin Invest* 73, 759-766

20 de Souza Santos, R. and Vianna, L.M. (2005) Effect of cholecalciferol supplementation on blood glucose in an experimental model of type 2 diabetes mellitus in spontaneously hypertensive rats and Wistar rats. *Clinica chimica acta; international journal of clinical chemistry* 358, 146-150

21 Wolden-Kirk, H., *et al.* (2011) Vitamin D and diabetes: its importance for beta cell and immune function. *Mol Cell Endocrinol* 347, 106-120

22 Wolden-Kirk, H*., et al.* (2013) Unraveling the effects of 1,25(OH)2D3 on global gene expression in pancreatic islets. *J Steroid Biochem Mol Biol* 136, 68-79
23 Baeke, F*., et al.* (2010) Vitamin D insufficiency: implications for the immune system. *Pediatr Nephrol* 25, 1597-1606
24 Cheng, Q*., et al.* (2013) Modulation of hypovitaminosis D-induced islet dysfunction and insulin resistance through direct suppression of the pancreatic islet renin-angiotensin system in mice. *Diabetologia* 56, 553-562
25 Cheng, Q*., et al.* (2011) A novel role for vitamin D: modulation of expression and function of the local renin-angiotensin system in mouse pancreatic islets. *Diabetologia* 54, 2077-2081
26 Hagstrom, E*., et al.* (2007) Serum calcium is independently associated with insulin sensitivity measured with euglycaemic-hyperinsulinaemic clamp in a community-based cohort. *Diabetologia* 50, 317-324
27 Gysemans, C*., et al.* (2008) Unaltered diabetes presentation in NOD mice lacking the vitamin D receptor. *Diabetes* 57, 269-275
28 Boucher, B.J. (2005) Reduced cardiovascular mortality in oral 1alpha-hydroxy vitamin D3 users in a haemodialysis population; do CRP and MMP markers of inflammation reflect this finding? *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 20, 846; author reply 846
29 Nagpal, J*., et al.* (2009) A double-blind, randomized, placebo-controlled trial of the short-term effect of vitamin D3 supplementation on insulin sensitivity in apparently healthy, middle-aged, centrally obese men. *Diabet Med* 26, 19-27
30 von Hurst, P.R*., et al.* (2010) Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient - a randomised, placebo-controlled trial. *The British journal of nutrition* 103, 549-555
31 Mozaffari-Khosravi, H*., et al.* (2012) Effects of a single post-partum injection of a high dose of vitamin D on glucose tolerance and insulin resistance in mothers with first-time gestational diabetes mellitus. *Diabet Med* 29, 36-42
32 Kampmann, U*., et al.* (2012) Lack of effects of high dose colecalciferol (D3) on insulin sensitivity and metabolic markers in type 2 diabetic patients: a double-blind, randomised, placebo-controlled trial. *Endocrine Abstracts* 29, P214
33 Kahn, H.S*., et al.* (2009) Association of type 1 diabetes with month of birth among U.S. youth: The SEARCH for Diabetes in Youth Study. *Diabetes Care* 32, 2010-2015
34 Kalliora, M.I*., et al.* (2011) Seasonal variation of type 1 diabetes mellitus diagnosis in Greek children. *Hormones (Athens)* 10, 67-71
35 Karvonen, M*., et al.* (1998) Comparison of the seasonal pattern in the clinical onset of IDDM in Finland and Sardinia. *Diabetes Care* 21, 1101-1109
36 Neu, A*., et al.* (1997) Incidence of IDDM in German children aged 0-14 years. A 6-year population-based study (1987-1993). *Diabetes Care* 20, 530-533
37 Svensson, J*., et al.* (2009) Long-term trends in the incidence of type 1 diabetes in Denmark: the seasonal variation changes over time. *Pediatr Diabetes* 10, 248-254
38 Moltchanova, E.V*., et al.* (2009) Seasonal variation of diagnosis of Type 1 diabetes mellitus in children worldwide. *Diabet Med* 26, 673-678
39 Mohr, S.B*., et al.* (2008) The association between ultraviolet B irradiance, vitamin D status and incidence rates of type 1 diabetes in 51 regions worldwide. *Diabetologia* 51, 1391-1398
40 Sloka, S*., et al.* (2010) The geospatial relation between UV solar radiation and type 1 diabetes in Newfoundland. *Acta Diabetol* 47, 73-78
41 Staples, J.A*., et al.* (2003) Ecologic analysis of some immune-related disorders, including type 1 diabetes, in Australia: latitude, regional ultraviolet radiation, and disease prevalence. *Environ Health Perspect* 111, 518-523
42 Karvonen, M*., et al.* (1993) A review of the recent epidemiological data on the worldwide incidence of type 1 (insulin-dependent) diabetes mellitus. World Health Organization DIAMOND Project Group. *Diabetologia* 36, 883-892

43 Borkar, V.V*., et al.* (2010) Low levels of vitamin D in North Indian children with newly diagnosed type 1 diabetes. *Pediatr Diabetes* 11, 345-350
44 Pozzilli, P*., et al.* (2005) Low levels of 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 in patients with newly diagnosed type 1 diabetes. *Horm Metab Res* 37, 680-683
45 Littorin, B*., et al.* (2006) Lower levels of plasma 25-hydroxyvitamin D among young adults at diagnosis of autoimmune type 1 diabetes compared with control subjects: results from the nationwide Diabetes Incidence Study in Sweden (DISS). *Diabetologia* 49, 2847-2852
46 Cooper, J.D*., et al.* (2011) Inherited variation in vitamin D genes is associated with predisposition to autoimmune disease type 1 diabetes. *Diabetes* 60, 1624-1631
47 Janner, M*., et al.* (2010) High prevalence of vitamin D deficiency in children and adolescents with type 1 diabetes. *Swiss medical weekly* 140, w13091
48 Svoren, B.M*., et al.* (2009) Significant vitamin D deficiency in youth with type 1 diabetes mellitus. *J Pediatr* 154, 132-134
49 Greer, R.M*., et al.* (2007) Australian children and adolescents with type 1 diabetes have low vitamin D levels. *The Medical journal of Australia* 187, 59-60
50 Bener, A*., et al.* (2009) High prevalence of vitamin D deficiency in type 1 diabetes mellitus and healthy children. *Acta Diabetol* 46, 183-189
51 Hypponen, E*., et al.* (2001) Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. *Lancet* 358, 1500-1503
52 Wang, T.J*., et al.* (2010) Common genetic determinants of vitamin D insufficiency: a genome-wide association study. *Lancet* 376, 180-188
53 Bailey, R*., et al.* (2007) Association of the vitamin D metabolism gene CYP27B1 with type 1 diabetes. *Diabetes* 56, 2616-2621
54 Ongagna, J.C*., et al.* (2005) Vitamin D-binding protein gene polymorphism association with IA-2 autoantibodies in type 1 diabetes. *Clinical biochemistry* 38, 415-419
55 Zhang, J*., et al.* (2012) Polymorphisms in the vitamin D receptor gene and type 1 diabetes mellitus risk: an update by meta-analysis. *Mol Cell Endocrinol* 355, 135-142
56 Guo, S.W*., et al.* (2006) Meta-analysis of vitamin D receptor polymorphisms and type 1 diabetes: a HuGE review of genetic association studies. *Am J Epidemiol* 164, 711-724
57 Nejentsev, S*., et al.* (2004) Analysis of the vitamin D receptor gene sequence variants in type 1 diabetes. *Diabetes* 53, 2709-2712
58 Kahles, H*., et al.* (2009) Association analyses of the vitamin D receptor gene in 1654 families with type I diabetes. *Genes Immun* 10 Suppl 1, S60-63
59 Ferreira, G.B*., et al.* (2009) Proteome analysis demonstrates profound alterations in human dendritic cell nature by TX527, an analogue of vitamin D. *Proteomics* 9, 3752-3764
60 Jeffery, L.E*., et al.* (2012) Availability of 25-hydroxyvitamin D(3) to APCs controls the balance between regulatory and inflammatory T cell responses. *J Immunol* 189, 5155-5164
61 Jeffery, L.E*., et al.* (2009) 1,25-Dihydroxyvitamin D3 and IL-2 combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3. *J Immunol* 183, 5458-5467
62 Baeke, F*., et al.* (2011) The vitamin D analog, TX527, promotes a human CD4+CD25highCD127low regulatory T cell profile and induces a migratory signature specific for homing to sites of inflammation. *J Immunol* 186, 132-142
63 Palmer, M.T*., et al.* (2011) Lineage-specific effects of 1,25-dihydroxyvitamin D(3) on the development of effector CD4 T cells. *J Biol Chem* 286, 997-1004
64 Mathieu, C*., et al.* (1995) Prevention of type I diabetes in NOD mice by nonhypercalcemic doses of a new structural analog of 1,25-dihydroxyvitamin D3, KH1060. *Endocrinology* 136, 866-872
65 Overbergh, L*., et al.* (2000) 1alpha,25-dihydroxyvitamin D3 induces an autoantigen-specific T-helper 1/T-helper 2 immune shift in NOD mice immunized with GAD65 (p524-543). *Diabetes* 49, 1301-1307
66 Gregori, S*., et al.* (2002) A 1alpha,25-dihydroxyvitamin D(3) analog enhances regulatory T-cells and arrests autoimmune diabetes in NOD mice. *Diabetes* 51, 1367-1374

67 Gysemans, C.A.*, et al.* (2005) 1,25-Dihydroxyvitamin D3 modulates expression of chemokines and cytokines in pancreatic islets: implications for prevention of diabetes in nonobese diabetic mice. *Endocrinology* 146, 1956-1964
68 EURODIAB (1999) Vitamin D supplement in early childhood and risk for Type I (insulin-dependent) diabetes mellitus. The EURODIAB Substudy 2 Study Group. *Diabetologia* 42, 51-54
69 Zipitis, C.S. and Akobeng, A.K. (2008) Vitamin D supplementation in early childhood and risk of type 1 diabetes: a systematic review and meta-analysis. *Arch Dis Child* 93, 512-517
70 Stene, L.C.*, et al.* (2000) Use of cod liver oil during pregnancy associated with lower risk of Type I diabetes in the offspring. *Diabetologia* 43, 1093-1098
71 Brekke, H.K. and Ludvigsson, J. (2007) Vitamin D supplementation and diabetes-related autoimmunity in the ABIS study. *Pediatr Diabetes* 8, 11-14
72 Walter, M.*, et al.* (2010) No effect of the 1alpha,25-dihydroxyvitamin D3 on beta-cell residual function and insulin requirement in adults with new-onset type 1 diabetes. *Diabetes Care* 33, 1443-1448
73 Bizzarri, C.*, et al.* (2010) No protective effect of calcitriol on beta-cell function in recent-onset type 1 diabetes: the IMDIAB XIII trial. *Diabetes Care* 33, 1962-1963
74 Prietl, B.*, et al.* (2010) Vitamin D supplementation and regulatory T cells in apparently healthy subjects: vitamin D treatment for autoimmune diseases? *Isr Med Assoc J* 12, 136-139
75 Haller, M.J.*, et al.* (2009) Autologous umbilical cord blood transfusion in very young children with type 1 diabetes. *Diabetes Care* 32, 2041-2046
76 Ludvigsson, J.*, et al.* (2012) GAD65 antigen therapy in recently diagnosed type 1 diabetes mellitus. *N Engl J Med* 366, 433-442
77 Ross, A.C.*, et al.* (2011) The 2011 Dietary Reference Intakes for Calcium and Vitamin D: what dietetics practitioners need to know. *J Am Diet Assoc* 111, 524-527
78 Bland, R.*, et al.* (2004) Expression of 25-hydroxyvitamin D3-1alpha-hydroxylase in pancreatic islets. *J Steroid Biochem Mol Biol* 89-90, 121-125
79 Jones, G.*, et al.* (1998) Current understanding of the molecular actions of vitamin D. *Physiol Rev* 78, 1193-1231
80 Berends, L.M. and Ozanne, S.E. (2012) Early determinants of type-2 diabetes. *Best Pract Res Clin Endocrinol Metab* 26, 569-580
81 DeFronzo, R.A. (2010) Current issues in the treatment of type 2 diabetes. Overview of newer agents: where treatment is going. *The American journal of medicine* 123, S38-48
82 Van Belle, T.L.*, et al.* (2011) Type 1 diabetes: etiology, immunology, and therapeutic strategies. *Physiol Rev* 91, 79-118
83 Bluestone, J.A.*, et al.* (2010) Genetics, pathogenesis and clinical interventions in type 1 diabetes. *Nature* 464, 1293-1300
84 Patterson, C.C.*, et al.* (2009) Incidence trends for childhood type 1 diabetes in Europe during 1989-2003 and predicted new cases 2005-20: a multicentre prospective registration study. *Lancet* 373, 2027-2033
85 Atkinson, M.A. and Chervonsky, A. (2012) Does the gut microbiota have a role in type 1 diabetes? Early evidence from humans and animal models of the disease. *Diabetologia* 55, 2868-2877
86 Orban, T.*, et al.* (2009) Pancreatic islet autoantibodies as predictors of type 1 diabetes in the Diabetes Prevention Trial-Type 1. *Diabetes Care* 32, 2269-2274

Box 1: Vitamin D metabolism

In humans, vitamin D is mainly derived from endogenous vitamin D production as a result of skin exposure to ultraviolet B (UVB) light and only a minor portion is obtained via food intake of fortified milk and dairy products, eggs and wild oily sea fish [21]. Vitamin D is a fat-soluble precursor of the major circulating 25-hydroxyvitamin D (25(OH)D) metabolite which is transformed – predominantly in the kidney – by a single enzyme 1α-hydroxylase (1α(OH)ase; CYP27B1) into the most active hormonal metabolite 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), known to stimulate bone mineralization through its capacity to stimulate intestinal calcium absorption. Many immune cells also contain the machinery for the two-step conversion of vitamin D to $1,25(OH)_2D$ [23]. Moreover, $1,25(OH)_2D$ can be produced locally in the pancreas from the main circulating form, 25(OH)D, because 1α-hydroxylase is present in islets [78]. 25(OH)D itself also has some biological activity, but $1,25(OH)_2D$ has about 1000 times the affinity for the vitamin D receptor (VDR) than 25(OH)D. All vitamin D metabolites are transported by the vitamin D-binding protein (DBP) that has a different affinity for the individual metabolites [79].Geographic distribution and seasonality, skin pigmentation, age, and lifestyle may predispose certain people to a higher risk of developing vitamin D insufficiency (defined as 25(OH)D concentrations 20 to 30 ng/mL or 50 to 75 nmol/L), or deficiency (25(OH)D concentration <20 ng/mL or <50 nmol/L).

Box 2. Type 2 diabetes.

The last decades have confronted us with a steep rise in the prevalence of type-2diabetes (T2D), with estimates for 2030 of more than 550 million people with diabetes worldwide (www.idf.org). The epidemic is noticeable in all continents, with the highest prevalence at present in the US. The most dramatic growth is forecasted to happen in new economies, with

a near-doubling of prevalence predicted in Asia, Africa and Latin-America in the next decade. Contributors to the rise in T2D include a number of environmental factors such as increased sedentarism that drives the increasing occurrence of overweight and obesity, but also increasing age predisposes to T2D [80]. A major hallmark of T2D is insulin resistance, namely the inability of cells to respond to the normal actions of insulin to control glucose homeostasis. Insulin resistance is observed in organs such as liver, fat tissue and muscle, but also contributes to a progressive failure of the pancreatic islet beta-cells to produce insulin. Clinically, T2D is treated with drugs that reduce insulin resistance (e.g. metformin) and/or increase insulin secretion by the beta-cell (e.g. sulphonylurea). Recently, therapies suppressing glucagon secretion in pancreatic islet alpha-cells, modulating appetite, or decreasing gastric emptying (e.g. incretin-based therapies) or affecting glucose-reabsorption in the kidney (e.g. SGLT2 blockade) have been added to the arsenal of glucose lowering therapies (reviewed in [81]).

Box 3. Type 1 diabetes (T1D).

Type 1 diabetes (T1D) is one of the most common chronic diseases of childhood and is an autoimmune disorder that results in the destruction of the insulin-producing beta-cell in the pancreas, rendering the patient dependent on insulin administration for survival (reviewed in [82, 83]. The prevalence of T1D ranges from less than 5 in every 100,000 individuals in eastern countries to as many as 40 in every 100,000 individuals in European and other western countries [84]. In contrast to T2D, T1D is not increasing dramatically in prevalence, but shifts towards onset at younger ages [84]. The peak incidence of disease onset is between 6 and 15 years of age, and a second peak occurs later in adolescence. Thus, when studying only children and adolescents, the numbers increase dramatically. Environmental factors, such

as viral infections, changes in gut microbiota and dietary components, are considered culprits for this alteration in clinical presentation [82, 85].

Preclinical and clinical studies have provided insight into the cells, antigens, and mechanisms involved. For instance, B cells have been documented as antigen-presenting cells and producers of auto-antibodies, T cells as producers of proinflammatory mediators and executioners of beta-cells killing, and regulatory T cells (Tregs) as cells counteracting the autoimmune attacks by effector cells [82, 83]. Important for clinical diagnosis, more than 90% of individuals with T1D test positive for at least one auto-antibody. Moreover, the presence of auto-antibodies identifies relatives of patients who are at a high risk for the disease. Typical targets of these auto-antibodies include antigens such as glutamic acid decarboxylase (GAD65), I-A2, insulin, islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), zinc transporter 8 (ZNT8) and islet cell auto-antigen (ICA)[86].

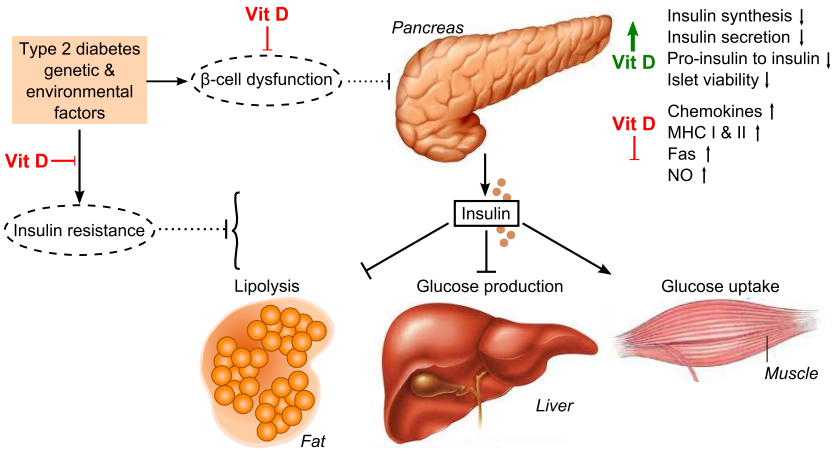
Outstanding Questions box:

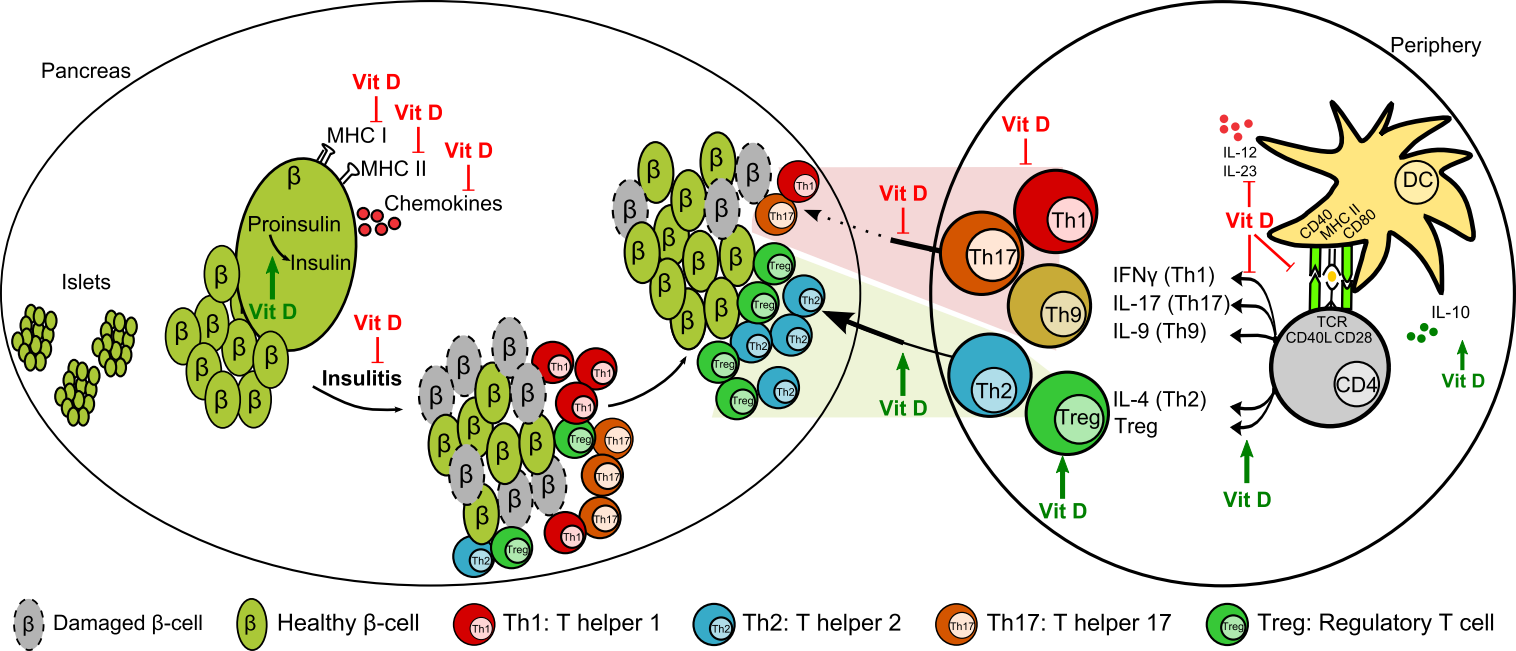
- Will eradication of vitamin D deficiency affect type 1 or type 2 diabetes presentation?
- Can supplements of vitamin D, over and above the doses advised for bone health, prevent type 1 or type 2 diabetes?
- Can structural analogues of vitamin D or $1,25(OH)_2D$ that affect the immune system without causing hypercalcemia, prevent or reverse type 1 or type 2 diabetes in humans?
- Can *ex vivo* treatment of immune cells offer a way to safely exploit the immune modulatory properties of vitamin D?

Figure Legends

Figure 1. Effect of vitamin D on pathophysiology in type 2 diabetes. Type 2 diabetes is 'a dual disease' characterized by increased insulin resistance followed by beta-cell dysfunction. As a consequence, insulin can no longer block lipolysis in fat tissue, glucose production in the liver and stimulate glucose uptake in the muscle. Biologically active vitamin D, $1,25(OH)_2D$ (depicted as Vit D), can reduce beta-cell dysfunction by restoring impaired insulin production and islet viability and by dampening T2D-associated inflammation in the pancreas. Vitamin D can also counteract increased insulin resistance. MHC I: Major Histocompatibility Complex 1; MHC II: Major Histocompatibility Complex 2; NO: nitric oxide.

Figure 2. Effect of vitamin D on immune and beta-cell-related events in type 1 diabetes pathogenesis. Beta-cells and various immune cells express VDR, the receptor for $1,25(OH)_2D$ (depicted as Vit D). $1,25(OH)_2D$ inhibits the expression of MHC class II and co-stimulatory molecules (such as CD40, CD80, CD86) on the cell surface of antigen-presenting cells (APC), including DCs, and inhibits the release of pro-inflammatory cytokines, such as IL-12 and IL-23. In this way, $1,25(OH)_2D$ indirectly shifts $CD4^+$ T cell polarization from a pro-inflammatory Th1/Th17 to an anti-inflammatory Th2/Treg phenotype. $1,25(OH)_2D$ also directly modulates T cell responses, by inhibiting inflammatory Th1- and Th17 cytokines and upregulation of Th2 cytokines. In addition, $1,25(OH)_2D$ reduces pancreatic infiltration by Th1 and Th17 cells. $1,25(OH)_2D$ also limits surface expression of MHC class I and II on beta-cells, as well as chemokine release by beta-cells, leading to reduced pancreatic recruitment and/or retention of T cells. β: beta-cell; Vit D: $1,25(OH)_2D$; MHC I: Major Histocompatibility Complex 1; MHC II: Major Histocompatibility Complex 2; IFNγ: Interferon-gamma; IL: Interleukin; Treg: Regulatory T cell; DC: dendritic cell; CD: Cluster of Differentiation.


Vit D
Type 2 diabetes genetic & environmental factors
β-cell dysfunction
Pancreas
Vit D
Insulin synthesis ↓
Insulin secretion ↓
Pro-insulin to insulin ↓
Islet viability ↓
Vit D
Chemokines ↑
MHC I & II ↑
Fas ↑
NO ↑
Vit D
Insulin resistance
Insulin
Lipolysis
Glucose production
Glucose uptake
Fat
Liver
Muscle


Pancreas
Islets
Vit D
MHC I
MHC II
Chemokines
Proinsulin
Insulin
Insulitis
Periphery
Th1
Th17
Th9
Th2
Treg
IFNγ (Th1)
IL-17 (Th17)
IL-9 (Th9)
IL-4 (Th2)
Treg
IL-12
IL-23
DC
CD40
MHC II
CD80
TCR
CD40L CD28
CD4
IL-10
β
Damaged β-cell
Healthy β-cell
Th1: T helper 1
Th2: T helper 2
Th17: T helper 17
Treg: Regulatory T cell