September 2004: 340–347

Micronutrients and the Risk of Type 1 Diabetes: Vitamin D, Vitamin E, and Nicotinamide

Elina Hyppönen, Ph.D., M.Sc., M.P.H.

Evidence from animal experiments and human observational studies suggests that some dietary micronutrients may protect against the development of type 1 diabetes. The most promising data so far have been obtained for a beneficial role of vitamin D. Beneficial effects of vitamin E (or other antioxidants) in diabetes development remain hypothetical. Despite plausible theoretical background evidence from animal experiments and supportive data from pilot studies, randomized, controlled trials using nicotinamide have not provided any evidence for a beneficial effect.

Key words: type 1 diabetes, vitamin D, vitamin E, nicotinamide

doi: 10.1301/nr.2004.sept.340–347

Introduction

Type 1 diabetes is an autoimmune disease caused by progressive destruction of insulin-secreting β-cells.[1] Pancreatic β-cell destruction may actually begin several years before type 1 diabetes is diagnosed. It is generally accepted that a genetic predisposition and exposure to environmental agents contribute to the development of the disease. Scientific understanding of the etiology of type 1 diabetes has improved markedly during the past decades, although it is still not complete.

Chronic inflammatory infiltrate (i.e., insulitis), which consists of CD8 and CD4 T-cells, B lymphocytes, macrophages, and natural killer cells, is found in the islets of Langerhans near the time of onset of type 1 diabetes, as well as in patients with long-standing disease.[2] Currently it is believed that β-cell destruction is T-cell dependent, and that the disease results from Th1- rather than Th2-associated cytokine deviation.[3] It has also been suggested that after autoimmune activation, free radicals produced by macrophages and T-cells may contribute to β-cell dysfunction and death.[4,5]

Several stages in the suggested pathogenesis leading to type 1 diabetes could be affected by nutrition. Early exposure to cow's milk,[6] intake of dietary nitrites and nitrosoamines,[7] rapid childhood growth, and excessive weight gain[8] have all been suggested to increase the risk of the disease. By contrast, intake of vitamin D,[9,10] vitamin E,[11] and nicotinamide[12] are among the few nutritional factors that have been suggested to have a beneficial, protective role in type 1 diabetes development. Finding a way to reduce the occurrence of this serious chronic disease—one that requires continuous high-cost treatment—by dietary micronutrients is an exciting prospect. This paper summarizes the current evidence for the role of vitamin D, vitamin E, and nicotinamide in type 1 diabetes development.

Vitamin D

In recent years, vitamin D has been suggested to have a beneficial effect for a wide range of conditions in addition to its well-established role in bone health.[13] Mechanisms for vitamin D action are varied; it is essential in the regulation of calcium metabolism, has a modulating effect on the immune system,[14] and induces insulin secretion.[15,16]

Vitamin D_3 (cholecalciferol) can be synthesized in the skin from a precursor molecule, 7-dehydrocholesterol, after exposure to UVB radiation. Exposure to sunlight is the most important source of vitamin D because only a few foods contain significant amounts of the vitamin. Vitamin D does not have significant biologic activity, and before exerting its functional properties it must undergo one or two hydroxylation processes. The first hydroxylation in the liver converts vitamin D to $25(OH)D_3$, the storage form that also reflects vitamin D status (and intake) during the past month. Recent evidence indicates that $25(OH)D_3$ may also act as a hormone.[17] Further hydroxylation to the main hormonally active form, $1,25(OH)_2D_3$, in the kidneys is tightly regulated. It is currently recognized that $1,25(OH)_2D_3$ hydroxylation can occur in other tissues, although the

Dr. Hyppönen is with the Centre for Paediatric Epidemiology and Biostatistics, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, United Kingdom.

physiologic function of this extra renal production is not well understood.[13] The primary toxicity of vitamin D is related to hypercalcemia, which results from $1,25(OH)_2D_3$-stimulated intestinal calcium absorption and bone demineralization. Toxicity is not believed to result from prolonged sun exposure, because D_3 synthesis in the skin from the provitamin is regulated, nor is it believed to result from a high-vitamin D diet because a normal diet contains only small amounts of vitamin D.

In vitro studies and animal experiments have shown that $1,25(OH)_2D_3$ has important influences on T-cell activity, which may have profound effects on the autoimmune process that leads to β-cell destruction. In vitro, $1,25(OH)_2D_3$ inhibits T-cell proliferation and decreases the production of Th1 cytokines IL-2 and INF-γ.[14] β-cell destruction in type 1 diabetes has been suggested to be mediated by up-regulation of Th1 type cytokines; vitamin D might therefore decrease diabetes risk by suppressing the production of these cytokines.[18] Vitamin D is believed to affect regulatory T-cell activity and dendritic cell maturation, which might explain its beneficial effects on diabetes development. Although vitamin D is known to be important for normal insulin secretion,[15] it is not clear whether the direct effects that $1,25(OH)_2D_3$ has on β-cells are related to the mechanism by which vitamin D might reduce the risk of developing type 1 diabetes.

Animal Experiments

The effect of vitamin D on the development of type 1 diabetes was first tested using a non-obese diabetic (NOD) mouse, which is a spontaneously developing animal model for autoimmune diabetes. Previously, $1,25(OH)_2D_3$ was observed to have beneficial effects in other autoimmune diseases, including experimental encephalitis and thyroiditis.[19–21] In the early experiments on type 1 diabetes, Mathieu et al.[22,23] observed that administration of $1,25(OH)_2D_3$ to NOD mice (5 μg/kg i.p. every other day from weaning) reduced the occurrence of insulitis and progression to diabetes. The incidence of insulitis in the treated mice was reduced from 75% to 41% by day 100[22]; in the treated mice, only 8% progressed to overt disease by day 200 compared with 56% in the control group.[23] More recent observations in the NOD mouse suggest that complete diabetes prevention may be achieved using sufficiently large doses of $1,25(OH)_2D_3$. Zella et al.[24] gave NOD mice 50 ng of $1,25(OH)_2D_3$ orally every day from weaning, and reported that none of the treated mice developed diabetes by day 200. Animals that were not given vitamin D were also used in an experiment comparing the effects of adequate vitamin D intake (ensured by housing animals under fluorescent light) with vitamin D deprivation (no intake either from diet or UVB radiation) on diabetes risk. In this experiment, adequate vitamin D intake provided complete protection to the male mice (0% developed diabetes in the vitamin D-sufficient vs. 44% in the vitamin D-deficient group) but not in the female animals (44% vs. 88%, respectively).[24]

In the experiments described above, diabetes prevention with pharmacologic doses of $1,25(OH)_2D_3$ was accompanied by increases in serum calcium levels in the treated animals. Because hypercalcemia may have very serious consequences, there is great research interest in determining whether an equally beneficial effect on diabetes could be obtained by using non-hypercalcemic structural analogs of $1,25(OH)_2D_3$. More than 2000 different vitamin D analogs have been synthesized to date.[25] Findings have been very promising, and some vitamin D analogs have been found to be as effective as $1,25(OH)_2D_3$ in preventing diabetes in the NOD mouse; however, complete protection from disease progression has not yet been achieved. For example, administration of either KH1060[26] or TX527[27] from weaning was reported to be equally effective in reducing diabetes incidence as $1,25(OH)_2D_3$ in the earlier studies.[23] The effect of both of these analogs was observed to be dose dependent, and protection from diabetes was obtained without material increases in serum calcium levels.

It has been suggested that prevention from progression to diabetes may be achieved by vitamin D analogs even after the initiation of autoimmune attack. In an experiment using MC1288 after established insulitis, with or without cyclosporin A (a known immunosupressant), MC1288 alone was not effective, whereas the combination treatment led to a marked reduction in diabetes incidence.[28] The authors reported that the treatment was well tolerated with no signs of generalized immunosuppression or major side effects on calcium metabolism. A study using yet another vitamin D analog found that administration of RO-26-2198 reduced the progression to diabetes in the NOD mouse, with the longest treatment giving the best results.[29]

Studies in Humans

To date, there are few data on the effect of vitamin D intake or status on diabetes risk in humans, and studies have been restricted to looking at either very early exposure (i.e., vitamin D supplementation in the uterus or during infancy) or vitamin D status and supplementation after diabetes diagnosis. The first report on the association between vitamin D supplementation in infancy and diabetes risk came from the EURODIAB study that combined data from seven European countries.[9] Pooled analysis of this multinational case-control study suggested a 33% reduction in the subsequent risk of developing type 1 diabetes if the child had received vitamin D supplementation during the first year of life.[9] In a Norwegian case-control study published a year later, offspring born to mothers who had used cod liver oil

supplements during pregnancy had a reduced risk of type 1 diabetes, whereas findings on the effect of vitamin D supplementation in infancy were inconclusive (the suggested effect ranged from a nearly 90% reduction in diabetes risk to a twofold increase).[30] In a recent, larger Norwegian case-control study, infants who had received cod liver oil had a reduced risk of type 1 diabetes, while vitamin D supplementation in infancy or maternal vitamin D or cod liver oil supplementation were not significantly associated with diabetes risk.[31] In our own study on the 1966 North Finland Birth Cohort, there was a remarkably consistent association between several indicators of vitamin D intake and status with risk of type 1 diabetes, which was robust to adjustment for a wide range of neonatal, anthropometric, and social indicators.[10] Risk of type 1 diabetes by age 31 years was reduced by more than 80% if vitamin D supplementation during the first year was regular (compared with no supplementation). Furthermore, among children who had received vitamin D supplementation regularly, a further 80% risk reduction was seen if the dose given had been at least on the level of the contemporary recommendation of 2000 IU (50 μg) per day.[10] Infants suspected of having had rickets during the first year of life had a threefold risk of developing diabetes compared with others.

There is some evidence that the levels of active vitamin D (i.e., circulating $1,25(OH)_2D_3$) may be decreased at time of diagnosis in diabetic individuals.[32,33] Furthermore, administration of $1,25(OH)_2D_3$ to children with newly diagnosed diabetes was observed to increase the length of remission phase in a recent experiment.[34] In this study, children who received 0.25 μg $1,25(OH)_2D_3$ every other day in addition to insulin treatment (which was started in all individuals) had increased C-peptide secretion compared with those receiving nicotinamide.[34] These preliminary data suggest that even late administration of the active hormone ($1,25(OH)_2D_3$) may improve residual insulin secretion; however, it is uncertain if this could have beneficial long-term consequences.

Vitamin D Receptor (VDR) Genotype

Vitamin D receptors are essential for insulin secretion,[35] and allelic variants of the VDR gene have been associated with type 1[36–42] and type 2 diabetes.[43,44] Associations between VDR genotype and type 1 diabetes have been reported by investigators in several countries in Europe[37,40,41] and Asia,[36,38,39,42] suggesting that the vitamin D system may be involved in determining genetic susceptibility of developing type 1 diabetes. Most studies have assessed four major polymorphic sites: FokI has been shown to result in an alternative transcription initiation site,[45] whereas TaqI, BsmI, and ApaI sites are presented at the non-coding sequences of the VDR gene. However, there are discrepancies in the reported VDR risk alleles/genotypes between and even within the populations. For example, the b allele of the BsmI has been suggested to confer both increased[36,46] and reduced[37,38,47] risk of type 1 diabetes. FokI has not been determined in all studies, but within a Japanese population the F allele was found to be more common in diabetic patients,[39,42] whereas studies from Germany found no association.[37,46] In a recent Finnish study, diabetes risk was significantly associated both with BsmI and FokI sites, but the associations were not present after correction for multiple testing.[48] This heterogeneity in the association between VDR genotype and disease susceptibility could indicate that the investigated risk markers may not have a direct etiologic role in diabetes development. Rather, these associations could reflect a linkage disequilibrium between these markers and a second disease cause variant in the VDR locus or closely situated gene.

Vitamin E

Oxidative stress is believed to contribute to the β-cell destruction seen with type 1 diabetes,[4,5] and the diabetogenic effect of two toxins that have been used widely to induce diabetes in experimental animals, streptozotocin and alloxan, is believed to be at least partly mediated by their actions as pro-oxidant agents. Therefore, there is theoretical background as to why antioxidant vitamins such as vitamin E (α-tocopherol) could provide some protection against type 1 diabetes. Furthermore, there is some evidence that diabetes risk may be increased by dietary consumption of nitrites,[7] which are believed to become diabetogenic after conversion to N-nitroso compounds in the digestive system.[49] N-nitroso compounds are structurally closely related to streptozotocin,[50] and their formation from dietary nitrites can be prevented by administration of α-tocopherol or other antioxidant vitamins.[51]

Animal Experiments

Evidence that progression to overt type 1 diabetes can be prevented by the administration of vitamin E has been obtained in chemically induced and spontaneously developing animal models of type 1 diabetes. Administration of vitamin E prior to streptozotocin or alloxan injection preserved insulin levels in the blood and pancreas of the treated rats at the same level as control animals, whereas rats that were treated with either toxin alone had increased glucose and reduced insulin responses to a glucose tolerance test.[52] There was some damage in the pancreatic islets of all animals treated with either of the diabetogenic toxins, but this was observed to be less severe in rats receiving vitamin E. Similar findings were subsequently reported for a vitamin E derivative (U-83836-E) in mice with diabetes induced by the

administration of multiple low doses of streptozotocin.[53] The authors hypothesized that the inhibition of lipoperoxidation by U-83836-E counteracted diabetes induced by low-dose streptozotocin. However, the diabetogenic action of alloxan is believed to be due to its ability to form hydroxyl free radicals. This could explain the difference in the effect between α-tocopherol and ubiquinone in an experiment on alloxan-treated mice in which administration of α-tocopherol inhibited 30% of alloxan-induced hyperglycemia, whereas ubiquinone reduced the occurrence of hyperglycemia by more than 90%.[54] Ubiquinone is known to be most potent in blocking hydroxyradicals, while vitamin E specifically scavenges lipid peroxyl radicals. Therefore, it seems plausible that the relative beneficial effect of different free radical scavengers in toxin-induced animal models of type 1 diabetes may depend upon the properties of the diabetogenic toxin.

Early studies on animal models of spontaneously developing type 1 diabetes suggested that vitamin E metabolism may be altered in diabetes-prone animals.[55,56] In the first study investigating the effect of vitamin E supplementation on diabetes risk, 11% of rats that were fed diets containing high doses of vitamin E (1000 IU/kg diet) developed diabetes, compared with 24% of animals fed diets containing only traces of vitamin E.[57] In another experiment, vitamin E supplementation together with other free radical scavengers caused a 20% decrease in diabetes incidence in the supplemented rats compared with control animals.[58] Studies in the NOD mouse also suggest that administration of vitamin E may delay or decrease diabetes onset. In one small study, none of the mice fed diets high in vitamin E (1000 IU/kg diet) developed diabetes, whereas all in the control group progressed to the disease.[59] However, in this study, all of the animals developed insulitis (suggesting ongoing β-cell destruction) by the age of 52 weeks. In a subsequent study, occurrence of insulitis or diabetes incidence was not affected by vitamin E supplementation, whereas there was a significant difference in the age at diabetes onset in the supplemented animals.[60] The lack of effect on insulitis, despite some decreases in diabetes incidence, suggests that vitamin E may not be able to interfere with the autoimmune process underlying the β-cell destruction despite having some beneficial effect at the β-cell level (e.g., by reducing cytotoxicity mediated by cytokines and their products). Interestingly, mice who have depleted vitamin E stores also seem to be protected from diabetes.[59] However, adequate vitamin E status is required for maintaining normal immune responses,[61] and in this experiment vitamin E-deficient mice had delayed growth and demonstrated responses indicative of secondary immunodeficiency.[59]

Studies in Humans

Very little is known about the association between vitamin E or other antioxidants and the risk of type 1 diabetes in humans. However, some indication that serum α-tocopherol levels may be lower in individuals developing diabetes compared with others has been obtained from a Finnish case-control study nested within a 21-year follow-up.[11] In this study, compared with participants in the lowest third of serum α-tocopherol, diabetes risk was reduced by 79% and 88% for men in the middle and highest third, respectively. However, because the study was small, with only 19 affected participants, and because all of the diabetic individuals in the study were relatively old at time of diagnosis (range 21 to 46 years), these findings remain suggestive and need to be replicated in larger studies.

Nicotinamide

Niacin or vitamin B_3 consists of nicotinic acid and nicotinamide, two separate but chemically very similar components. Like other water-soluble vitamins, niacin is well tolerated, even in relatively high doses, and to date nicotinamide is the most extensively studied micronutrient in diabetes prevention in humans. Several mechanisms have been suggested through which nicotinamide could prevent β-cell destruction. Nicotinamide is a precursor of nicotinamide adenine dinucleotide (NAD^+), which is a coenzyme involved in several energy transfer processes within the cell. NAD^+ is required during DNA repair by poly (ADP) ribose polymerase (PARP). Excessive activation of PARP levels leads to depletion of NAD^+ and programmed cell death through energy starvation. Administration of nicotinamide could affect this pathway by preventing NAD^+ depletion.[62] Furthermore, nicotinamide may influence diabetes development through inhibition of MHC class II expression[63,64] or by preventing free radical-induced β-cell damage (as nicotinamide is known to act as a weak free radical scavenger).[65]

Animal Experiments

The first suggestion that pancreatic β-cells may be protected by the administration of nicotinamide was obtained more than 50 years ago from the animal experiments on chemically induced diabetes. In 1947, Lazarow[66] observed that the onset of diabetes in alloxan-treated rats could be prevented by nicotinamide; two decades later, a similar effect was reported for diabetes induced by streptozotocin.[67] Studies on the effects of nicotinamide in the NOD mouse suggested that large-dose nicotinamide injections started before the onset of diabetes may be able to prevent the occurrence of glycosuria, and that injections even after the onset of glycosuria had a significant therapeutic effect on diabetes.[68]

Oral administration of nicotinamide was also observed to have beneficial effects, with less severe or delayed progression to insulitis and disease onset.[69,70] However, in the other spontaneous model of type 1 diabetes, the BB rat, nicotinamide did not show marked effects on disease progression.[71,72]

Studies in Humans

Nicotinamide is well tolerated even in large doses,[73] which has allowed its effect to be extensively tested in human intervention trials on type 1 diabetes. The effect of nicotinamide on diabetes prevention was first investigated in newly diagnosed patients with type 1 diabetes in trials aiming at increasing the length of the remission phase. Meta-analysis of nine randomized trials (five of which were placebo controlled) suggested increased insulin secretion in children receiving nicotinamide.[74] However, there was no clinical improvement and the required insulin dose or levels of glycosylated hemoglobin did not differ between children receiving nicotinamide and those who did not.

Early findings from intervention trials of nicotinamide before the diagnosis of diabetes were promising.[75,76] In a pilot study on high-risk children (islet cell autoantibody-positive, first-degree relatives of type 1 diabetic patients), only one out of 14 children treated with nicotinamide developed diabetes during the 5-year follow-up, whereas all eight control children progressed to overt disease.[75] Further evidence for a beneficial effect was obtained from another larger trial, which included screening more than 30,000 New Zealand schoolchildren and providing nicotinamide supplementation to 173 of them who had increased diabetes risk.[76] In this study, nicotinamide was associated with a 60% reduction in diabetes incidence in the treated children compared with the control population. However, the effect of nicotinamide on diabetes incidence was not significant in the intention-to-treat analysis, which included data from those children who refused to participate in the study.

Subsequent randomized, placebo-controlled efforts to prevent type 1 diabetes among family members of diabetic patients have not been as successful as these early human experiments. The first randomized, placebo-controlled trial, "The Deutche Nicotinamide Intervention Study" (DENIS), was terminated according to the study protocol when the second interim analysis showed no preventive effect.[77] In this relatively small study of autoantibody-positive, first-degree relatives of type 1 diabetic patients ($n = 55$), there was no difference in the proportion of children developing diabetes by treatment group, and first-phase insulin response was even somewhat lower in the group that had received nicotinamide than in the other subjects.

The "European Nicotinamide Diabetes Intervention Trial" (ENDIT) was an impressive multinational undertaking aimed at diabetes prevention, which included centers from 18 European countries, the United States, and Canada.[62] In this double-blind trial designed to detect a 35% to 40% reduction in diabetes incidence during the 5-year follow-up, more than 30,000 first-degree relatives of type 1 diabetes patients were screened for islet cell autoantibodies; 274 autoantibody-positive individuals were randomized to the treatment group and 275 participants were randomized to receive placebo. Unfortunately, this carefully designed study also failed to provide any evidence for a beneficial effect of nicotinamide on diabetes prevention.[78]

Effects on Insulin Sensitivity

With the intervention studies using therapeutic doses of nicotinamide, some concern has arisen over increased insulin resistance in the treated individuals. Nicotinic acid is structurally very similar to nicotinamide and known to induce insulin resistance. Although nicotinamide has been shown not to affect insulin sensitivity in healthy subjects,[79] there is some evidence that high doses may increase insulin resistance in subjects at high risk of developing diabetes[80] and in newly diagnosed diabetic patients.[81] It has been proposed that the increased C-peptide secretion observed for newly diagnosed patients as a response to treatment with nicotinamide may be caused by insulin resistance.[80]

Conclusion

This paper summarizes the available evidence for a role of micronutrients in the etiology of type 1 diabetes. Evidence suggesting that progression to type 1 diabetes might be prevented by micronutrients has been largely obtained from animal experiments, but there are some confirmatory data from human studies. The most promising data so far have been obtained for a beneficial role of vitamin D in type 1 diabetes, while the protective effect of antioxidant vitamins remains hypothetical. Intervention studies with nicotinamide have demonstrated that a plausible theoretical background, evidence for a preventive role from animal models, and even supportive findings from observational studies in humans do not guarantee successful prevention, especially when β-cell destruction is already ongoing.

To date, more than 125 therapies have been reported to prevent or slow the progression to type 1 diabetes in the NOD mouse, which suggests that the pathologic events leading to β-cell destruction are likely to be very complex.[82] It is also noteworthy that from the genetic background, the NOD mouse more closely resembles a case study in human type 1 diabetes than natural hereditary variations in diabetic populations.[82] Reported associations between VDR genotype and type 1 diabetes risk from several populations may suggest that the effects of

vitamin D intake or status in diabetic individuals is modified by their genetic background.

More research is required to evaluate the effect of vitamin D on diabetes risk after infancy and establish how vitamin D interacts in its role as a genetic and environmental risk factor for type 1 diabetes.

Acknowledgements

I thank Dr. Tessa Parsons for useful comments on an earlier version. This review is based on an invited presentation given at the 63rd Scientific Sessions of the American Diabetes Association, June 13, 2003. Financial support was obtained from the BUPA Foundation.

1. Atkinson MA, Maclaren NK. The pathogenesis of insulin-dependent diabetes mellitus. *N Engl J Med.* 1994;331:1428–1436.
2. Foulis AK, McGill M, Farquharson MA. Insulitis in type 1 (insulin-dependent) diabetes mellitus in man—macrophages, lymphocytes, and interferon-gamma containing cells. *J Pathol.* 1991;165:97–103.
3. Roep BO. The role of T-cells in the pathogenesis of Type 1 diabetes: from cause to cure. *Diabetologia.* 2003;46:305–321.
4. Eizirik DL, Darville MI. beta-cell apoptosis and defense mechanisms: lessons from type 1 diabetes. *Diabetes.* 2001;50:S64–S69.
5. Mandrup-Poulsen T, Corbett JA, McDaniel ML, Nerup J. What are the types and cellular sources of free radicals in the pathogenesis of type 1 (insulin-dependent) diabetes mellitus? *Diabetologia.* 1993; 36:470–471.
6. Gerstein HC, VanderMeulen J. The relationship between cow's milk exposure and type 1 diabetes. *Diabet Med.* 1996;13:23–29.
7. Dahlquist GG, Blom LG, Persson LA, Sandstrom AI, Wall SG. Dietary factors and the risk of developing insulin dependent diabetes in childhood. *BMJ.* 1990;300:1302–1306.
8. Hyppönen E, Virtanen SM, Kenward MG, Knip M, Åkerblom HK. Obesity, increased linear growth, and risk of type 1 diabetes in children. *Diabetes Care.* 2000;23:1755–1760.
9. The EURODIAB Substudy 2 Study Group. Vitamin D supplement in early childhood and risk for Type I (insulin-dependent) diabetes mellitus. *Diabetologia.* 1999;42:51–54.
10. Hyppönen E, Läärä E, Reunanen A, Järvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. *Lancet.* 2001;358: 1500–1503.
11. Knekt P, Reunanen A, Marniemi J, Leino A, Aromaa A. Low vitamin E status is a potential risk factor for insulin-dependent diabetes mellitus. *J Intern Med.* 1999;245:99–102.
12. Gale EA. Nicotinamide: potential for the prevention of type 1 diabetes? *Horm Metab Res.* 1996;28:361–364.
13. Holick MF. Vitamin D: a millenium perspective. *J Cell Biochem.* 2003;88:296–307.
14. Lemire J. 1,25-Dihydroxyvitamin D3—a hormone with immunomodulatory properties. *Z Rheumatol.* 2000;59:24–27.
15. Norman AW, Frankel JB, Heldt AM, Grodsky GM. Vitamin D deficiency inhibits pancreatic secretion of insulin. *Science.* 1980;209:823–825.
16. Cade C, Norman AW. Rapid normalization/stimulation by 1,25-dihydroxyvitamin D3 of insulin secretion and glucose tolerance in the vitamin D-deficient rat. *Endocrinology.* 1987;120:1490–1497.
17. Lou YR, Laaksi I, Syvälä H, et al. 25-Hydroxyvitamin D3 is an active hormone in human primary prostatic stromal cells. *FASEB J.* 2004;18:332–334.
18. Zella JB, DeLuca HF. Vitamin D and autoimmune diabetes. *J Cell Biochem.* 2003;88:216–222.
19. Koizumi T, Nakao Y, Matsui T, et al. Effects of corticosteroid and 1,24R-dihydroxy-vitamin D3 administration on lymphoproliferation and autoimmune disease in MRL/MP-lpr/lpr mice. *Int Arch Allergy Appl Immunol.* 1985;77:396–404.
20. Fournier C, Gepner P, Sadouk M, Charreire J. In vivo beneficial effects of cyclosporin A and 1,25-dihydroxyvitamin D3 on the induction of experimental autoimmune thyroiditis. *Clin Immunol Immunopathol.* 1990;54:53–63.
21. Lemire JM, Archer DC. 1,25-dihydroxyvitamin D3 prevents the in vivo induction of murine experimental autoimmune encephalomyelitis. *J Clin Invest.* 1991;87:1103–1107.
22. Mathieu C, Laureys J, Sobis H, Vandeputte M, Waer M, Bouillon R. 1,25-Dihydroxyvitamin D3 prevents insulitis in NOD mice. *Diabetes.* 1992;41:1491–1495.
23. Mathieu C, Waer M, Laureys J, Rutgeerts O, Bouillon R. Prevention of autoimmune diabetes in NOD mice by 1,25 dihydroxyvitamin D3. *Diabetologia.* 1994;37:552–558.
24. Zella JB, McCary LC, DeLuca HF. Oral administration of 1,25-dihydroxyvitamin D3 completely protects NOD mice from insulin-dependent diabetes mellitus. *Arch Biochem Biophys.* 2003;417:77–80.
25. Guyton KZ, Kensler TW, Posner GH. Vitamin D and vitamin D analogs as cancer chemopreventive agents. *Nutr Rev.* 2003;61:227–238.
26. Mathieu C, Waer M, Casteels K, Laureys J, Bouillon R. Prevention of type I diabetes in NOD mice by nonhypercalcemic doses of a new structural analog of 1,25-dihydroxyvitamin D3, KH1060. *Endocrinology.* 1995;136:866–872.
27. Van Etten E, Decallonne B, Verlinden L, Verstuyf A, Bouillon R, Mathieu C. Analogs of 1alpha,25-dihydroxyvitamin D3 as pluripotent immunomodulators. *J Cell Biochem.* 2003;88:223–226.
28. Casteels K, Waer M, Bouillon R, Allewaert K, Laureys J, Mathieu C. Prevention of type I diabetes by late intervention with nonhypercalcemic analogues of vitamin D3 in combination with cyclosporin A. *Transplant Proc.* 1996;28:3095.
29. Gregori S, Giarratana N, Smiroldo S, Uskokovic M, Adorini L. A 1alpha,25-dihydroxyvitamin D(3) analog enhances regulatory T-cells and arrests autoimmune diabetes in NOD mice. *Diabetes.* 2002;51: 1367–1374.
30. Stene LC, Ulriksen J, Magnus P, Joner G. Use of cod liver oil during pregnancy associated with lower risk of Type I diabetes in the offspring. *Diabetologia.* 2000;43:1093–1098.

31. Stene LC, Joner G; Norwegian Childhood Diabetes Study Group. Use of cod liver oil during the first year of life is associated with lower risk of childhood-onset type 1 diabetes: a large, population-based, case-control study. *Am J Clin Nutr.* 2003;78:1128–1134.
32. Pozzilli P, Cherubini V, Pinelli A, Valenti L, Suraci C, IMDIAB Group. Low levels of 1,25-dihydroxyvitamin D3 in patients with recent onset type 1 diabetes living in a Mediterranean country. *Diabetes.* 2002; 51:A289.
33. Baumgartl HJ, Standl E, Schmidt-Gayk H, Kolb HJ, Janka HU, Ziegler AG. Changes of vitamin D3 serum concentrations at the onset of immune-mediated type 1 (insulin-dependent) diabetes mellitus. *Diabet Res.* 1991;16:145–148.
34. Pozzilli P, Crino A, Constantino A, et al. Vitamin D supplementation in patients with recent onset type 1 diabetes: IMDIAB XI Trial. *Diabetes.* 2003;52:A44.
35. Zeitz U, Weber K, Soegiarto DW, Wolf E, Balling R, Erben RG. Impaired insulin secretory capacity in mice lacking a functional vitamin D receptor. *FASEB J.* 2003;17:509–511.
36. McDermott MF, Ramachandran A, Ogunkolade BW, et al. Allelic variation in the vitamin D receptor influences susceptibility to IDDM in Indian Asians. *Diabetologia.* 1997;40:971–975.
37. Pani MA, Knapp M, Donner H, et al. Vitamin D receptor allele combinations influence genetic susceptibility to type 1 diabetes in Germans. *Diabetes.* 2000;49:504–507.
38. Chang TJ, Lei HH, Yeh JI, et al. Vitamin D receptor gene polymorphisms influence susceptibility to type 1 diabetes mellitus in the Taiwanese population. *Clin Endocrinol.* 2000;52:575–580.
39. Ban Y, Taniyama M, Yanagawa T, et al. Vitamin D receptor initiation codon polymorphism influences genetic susceptibility to type 1 diabetes mellitus in the Japanese population. *BMC Med Genet.* 2001; 2:7.
40. Gyorffy B, Vasarhelyi B, Krikovszky D, et al. Gender-specific association of vitamin D receptor polymorphism combinations with type 1 diabetes mellitus. *Eur J Endocrinol.* 2002;147:803–808.
41. Skrabic V, Zemunik T, Situm M, Terzic J. Vitamin D receptor polymorphism and susceptibility to type 1 diabetes in the Dalmatian population. *Diabet Res Clin Pract.* 2003;59:31–35.
42. Yokota I, Satomura S, Kitamura S, et al. Association between vitamin D receptor genotype and age of onset in juvenile Japanese patients with type 1 diabetes. *Diabetes Care.* 2002;25:1244.
43. Hitman GA, Mannan N, McDermott MF, et al. Vitamin D receptor gene polymorphisms influence insulin secretion in Bangladeshi Asians. *Diabetes.* 1998; 47:688–690.
44. Ortlepp JR, Lauscher J, Hoffmann R, Hanrath P, Joost HG. The vitamin D receptor gene variant is associated with the prevalence of type 2 diabetes mellitus and coronary artery disease. *Diabet Med.* 2001;18:842–845.
45. Gross C, Krishnan AV, Malloy PJ, Eccleshall TR, Zhao XY, Feldman D. The vitamin D receptor gene start codon polymorphism: a functional analysis of FokI variants. *J Bone Miner Res.* 1998;13:1691–1699.
46. Fassbender WJ, Goertz B, Weismuller K, et al. VDR gene polymorphisms are overrepresented in German patients with type 1 diabetes compared to healthy controls without effect on biochemical parameters of bone metabolism. *Horm Metab Res.* 2002;34:330–337.
47. Motohashi Y, Yamada S, Yanagawa T, et al. Vitamin D receptor gene polymorphism affects onset pattern of type 1 diabetes. *J Clin Endocrinol Metab.* 2003;88:3137–3140.
48. Turpeinen H, Hermann R, Vaara S, et al. Vitamin D receptor polymorphisms: no association with type 1 diabetes in the Finnish population. *Eur J Endocrinol.* 2003;149:591–596.
49. Mirvish SS. Formation of N-nitroso compounds: chemistry, kinetics, and in vivo occurrence. *Toxicol Appl Pharmacol.* 1975;31:325–351.
50. Like AA, Rossini AA. Streptozotocin-induced pancreatic insulitis: new model of diabetes mellitus. *Science.* 1976;193:415–417.
51. Mirvish SS. Effects of vitamins C and E on N-nitroso compound formation, carcinogenesis, and cancer. *Cancer.* 1986;58:1842–1850.
52. Slonim AE, Surber ML, Page DL, Sharp RA, Burr IM. Modification of chemically induced diabetes in rats by vitamin E. Supplementation minimizes and depletion enhances development of diabetes. *J Clin Invest.* 1983;71:1282–1288.
53. Papaccio G, Baccari GC, Frascatore S, Sellitti S, Pisanti FA. The vitamin-E derivative U-83836-E in the low-dose streptozocin-treated mouse: effects on diabetes development. *Diabet Res Clin Pract.* 1995;30:163–171.
54. Stetinova V, Grossmann V. Effects of known and potential antioxidants on animal models of pathological processes (diabetes, gastric lesions, allergic bronchospasm). *Exp Toxicol Pathol.* 2000;52:473–479.
55. Behrens WA, Scott FW, Madere R, Trick KD. Increased plasma and tissue levels of vitamin E in the spontaneously diabetic BB rat. *Life Sci.* 1984;35: 199–206.
56. Behrens WA, Madere R. Vitamin C and vitamin E status in the spontaneously diabetic BB rat before the onset of diabetes. *Metabolism.* 1991;40:72–76.
57. Behrens WA, Scott FW, Madere R, Trick K, Hanna K. Effect of dietary vitamin E on the vitamin E status in the BB rat during development and after the onset of diabetes. *Ann Nutr Metab.* 1986;30:157–165.
58. Murthy VK, Shipp JC, Hanson C, Shipp DM. Delayed onset and decreased incidence of diabetes in BB rats fed free radical scavengers. *Diabetes Res Clin Pract.* 1992;18:11–16.
59. Hayward AR, Shriber M, Sokol R. Vitamin E supplementation reduces the incidence of diabetes but not insulitis in NOD mice. *J Lab Clin Med.* 1992;119: 503–507.
60. Beales PE, Williams AJ, Albertini MC, Pozzilli P. Vitamin E delays diabetes onset in the non-obese diabetic mouse. *Horm Metab Res.* 1994;26:450–452.
61. Meydani M. Vitamin E. *Lancet.* 1995;345:170–175.

62. European Nicotinamide Diabetes Intervention Trial Group. Intervening before the onset of type 1 diabetes: baseline data from the European Nicotinamide Diabetes Intervention Trial (ENDIT). *Diabetologia.* 2003;46:339–346.
63. Yamada K, Miyajima E, Nonaka K. Inhibition of cytokine-induced MHC class II but not class I molecule expression on mouse islet cells by niacinamide and 3-aminobenzamide. *Diabetes.* 1990;39: 1125–1130.
64. Kim KA, Kim S, Chang I, et al. IFN gamma/TNF alpha synergism in MHC class II induction: effect of nicotinamide on MHC class II expression but not on islet-cell apoptosis. *Diabetologia.* 2002;45:385–393.
65. Hoorens A, Pipeleers D. Nicotinamide protects human beta cells against chemically-induced necrosis, but not against cytokine-induced apoptosis. *Diabetologia.* 1999;42:55–59.
66. Lazarow A. Protection against alloxan diabetes. *Anat Rec.* 1947;97:353–358.
67. Dulin WE, Wyse BM. Reversal of streptozotocin diabetes with nicotinamide. *Proc Soc Exp Biol Med.* 1969;130:992–994.
68. Yamada K, Nonaka K, Hanafusa T, Miyazaki A, Toyoshima H, Tarui S. Preventive and therapeutic effects of large-dose nicotinamide injections on diabetes associated with insulitis. An observation in nonobese diabetic (NOD) mice. *Diabetes.* 1982;31: 749–753.
69. Reddy S, Bibby NJ, Elliott RB. Early nicotinamide treatment in the NOD mouse: effects on diabetes and insulitis suppression and autoantibody levels. *Diabetes Res.* 1990;15:95–102.
70. Kim JY, Chi JK, Kim EJ, Park SY, Kim YW, Lee SK. Inhibition of diabetes in non-obese diabetic mice by nicotinamide treatment for 5 weeks at the early age. *J Korean Med Sci.* 1997;12:293–297.
71. Hermitte L, Vialettes B, Atlef N, et al. High dose nicotinamide fails to prevent diabetes in BB rats. *Autoimmunity.* 1989;5:79–86.
72. Flechner I, Maruta K, Burkart V, Kawai K, Kolb H, Kiesel U. Effects of radical scavengers on the development of experimental diabetes. *Diabetes Res.* 1990;13:67–73.
73. Knip M, Douek IF, Moore WP, et al. Safety of high-dose nicotinamide: a review. *Diabetologia.* 2000;43: 1337–1345.
74. Pozzilli P, Browne PD, Kolb H. Meta-analysis of nicotinamide treatment in patients with recent-onset IDDM. The Nicotinamide Trialists. *Diabetes Care.* 1996;19:1357–1363.
75. Elliott RB, Chase HP. Prevention or delay of type 1 (insulin-dependent) diabetes mellitus in children using nicotinamide. *Diabetologia.* 1991;34:362–365.
76. Elliott RB, Pilcher CC, Fergusson DM, Stewart AW. A population based strategy to prevent insulin-dependent diabetes using nicotinamide. *J Pediatr Endocrinol Metab.* 1996;9:501–509.
77. Lampeter EF, Klinghammer A, Scherbaum WA, et al. The Deutsche Nicotinamide Intervention Study: an attempt to prevent type 1 diabetes. DENIS Group. *Diabetes.* 1998;47:980–984.
78. Gale EA, Bingley PJ, Emmett CL, Collier T; European Nicotinamide Diabetes Intervention Trial (ENDIT) Group. European Nicotinamide Intervention Trial (ENDIT): a randomised controlled trial of intervention before the onset of type 1 diabetes. *Lancet*. 2004;363:925–931.
79. Bingley PJ, Caldas G, Bonfanti R, Gale EA. Nicotinamide and insulin secretion in normal subjects. *Diabetologia.* 1993;36:675–677.
80. Greenbaum CJ, Kahn SE, Palmer JP. Nicotinamide's effects on glucose metabolism in subjects at risk for IDDM. *Diabetes.* 1996;45:1631–1634.
81. Visalli N, Cavallo MG, Signore A, et al. A multicentre randomized trial of two different doses of nicotinamide in patients with recent-onset type 1 diabetes (the IMDIAB VI). *Diabetes Metab Res Rev.* 1999;15:181–185.
82. Atkinson MA, Leiter EH. The NOD mouse model of type 1 diabetes: as good as it gets? *Nat Med.* 1999;5:601–604.