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Vitamin D supplement in early childhood and risk for Type I (insulin-dependent) diabetes mellitus

The EURODIAB Substudy 2 Study Group

Summary The initiation of the immunopathogenetic process that can lead to Type I (insulin-dependent) diabetes mellitus in childhood probably occurs early in life. Studies in vitro have shown that vitamin D3 is immunosuppressive or immunomodulating and studies in experimental models of autoimmunity, including one for autoimmune diabetes, have shown vitamin D to be protective. Seven centres in Europe with access to population-based and validated case registers of insulin-dependent diabetes patients participated in a case-control study focusing on early exposures and risk of Type I diabetes. Altogether data from 820 patients and 2335 control subjects corresponding to 85 % of eligible patients and 76 % of eligible control subjects were analysed. Questions focused on perinatal events and early eating habits including vitamin D supplementation. The frequency of vitamin D supplementation in different countries varied from 47 to 97 % among control subjects. Vitamin D supplementation was associated with a decreased risk of Type I diabetes without indication of heterogeneity. The Mantel-Haenszel combined odds ratio was 0.67 (95 % confidence limits: 0.53, 0.86). Adjustment for the possible confounders: a low birth weight, a short duration of breast feeding, old maternal age and study centre in logistic regression analysis did not affect the significant protective effect of vitamin D. In conclusion, this large multicentre trial covering many different European settings consistently showed a protective effect of vitamin D supplementation in infancy. The findings indicate that activated vitamin D might contribute to immune modulation and thereby protect or arrest an ongoing immune process initiated in susceptible people by early environmental exposures. [Diabetologia (1999) 42: 51–54]

Keywords Type I diabetes, childhood, prevention, vitamin D, case-control study

Type I (insulin-dependent) diabetes mellitus is thought to be the consequence of an autoimmune destruction of the insulin producing beta cell as a result of interactions between different susceptibility genes and environmental exposures [1, 2].

It has been shown that 1,25 dihydroxyvitamin D prevents experimental autoimmune disease such as experimental encephalo-myelitis [3], autoimmune thyroiditis [4] or experimental rat nephritis [5]. Studies in the nonobese diabetic (NOD) mouse, a model for human autoimmune (Type I) diabetes, showed that treatment with the active form of vitamin D or its analogues prevented the development of insulitis [6–8]. Epidemiological studies showed that there is a north-south gradient with higher incidence rates of type I diabetes in northern than in southern latitudes in global comparisons [9] within Europe [10] as well as in a genetically homogeneous country [11]. An ecological study within Sweden, covering a large range of mean monthly sunshine hours, showed a correlation between mean incidence within counties and mean monthly sunshine hours [12].

In a large multicentre and population-based case-control study on early risk factors of childhood onset Type I diabetes we have collected data on vitamin D

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Corresponding author: G. Dahlquist, Department of Paediatrics, University Hospital, SE-901 85 Umeå, Sweden
Abbreviations: OR, Odds ratio; CI, confidence interval; NOD, nonobese diabetic.

Table 1. Data collection

Centre	Register years	Number of patients	Response rate (%)	Number of control subjects	Response rate (%)	Selection of control subjects	Data collection	Validation of Vit D health care records (booklets)
Austria	1989–1994	117	88.9	477	79.7	[a] Schools	Q	No
Bucharest	1989–1994	111	73.9	342	81.0	[a] Health service register	I	Partly
Bulgaria	1991–1994	176	72.7	562	78.6	[a] Schools and Polyclinics	I	Partly
Latvia	1989–1994	143	98.6	410	79.0	Population register	I	Partly
Lithuania	1989–1994	124	94.4	369	72.9	[a] Polyclinics	Q	Yes
Luxembourg	1989–1995	59	100.0	188	94.7	[a] Preschools and schools	I	Partly
N. Ireland	1990–1992	240	78.8	723	64.3	General Practitioner register	Q	Partly

Vit = Vitamin, Q = Questionnaire, I = Interviews, [a] two stage random sampling

prophylaxis to test the hypothesis that vitamin D offers protection from human autoimmune diabetes.

Subjects and methods

Seven centres with population-based childhood-onset diabetes registers operating since 1989 and validated by the standards of EURODIAB ACE [10] participated in the study: Austria, Latvia, Lithuania, Luxembourg, Romania, Bulgaria, Northern Ireland (UK). Altogether 970 patients with onset of diabetes before the age of 15 and 3071 population-based control subjects selected within four age strata with similar age distribution to the patients were invited to join the study. Overall 820 out of 970 (85%) patients and 2335 out of 3071 (76%) control subjects participated. Among participants 746 (91%) of the patients and 2188 (94%) of the control subjects answered the questions on vitamin D prophylaxis. Population-based control subjects were selected from different sources, e.g. population registers, general practitioners' lists (of all children in the area) or school rolls. The study base was temporally and geographically well defined for each country and the procedure for selection of control subjects was designed in collaboration with the study coordinators in the individual centres according to local circumstances to properly represent all children in the study area. Details of case-control ascertainment and non-response rates in different countries are shown in Table 1. To maintain uniform standards between centres, local study leaders received detailed written instructions for: completion of the record sheet, selection of control subjects, providing patient information and obtaining consent, preparation of interview schedules and completion of the record sheet. In addition, two workshops were organised and all centres were visited by the coordinators. A set of core variables focusing on perinatal events, eating habits during the first year of life including vitamin D supplements, vaccinations and early growth was defined and data were collected either by standardised questionnaires or interviews after appropriate training had been given. Those centres which interviewed were instructed to ensure that each interviewer handled a similar proportion of the patients and control subjects. Core variables were transferred from the interview schedule or questionnaire to a standardised record sheet and the data were computerized centrally. In six centres booklets completed by health-care professionals were available in which data on, e.g. vaccinations, growth, nutritional advice were recorded. These booklets were used as a second source of information, but vitamin D information was only partially recorded in the booklets of five centres. In one centre (Lithuania) it was possible to validate questionnaire data on D vitamin prophylaxis against the data in the booklet. The overall concordance was 93% for patients and 90% for control subjects but in both groups there was a high frequency of vitamin D prophylaxis given in that country. Each local study centre leader obtained permission from a research ethics committee where one existed.

Statistical analysis. For each centre exact 95% confidence limits were calculated for the odds ratio (OR). The Mantel-Haenszel method was used to pool results across centres and to provide a test for heterogeneity. To adjust for confounders logistic regression analysis was used.

Results

The Mantel-Haenszel pooled odds ratio was decreased ($p < 0.001$; Table 2). The odds ratio for the Bulgarian centre appears different, but this centre contributed little to the pooled analysis because of its very high rate of vitamin D supplementation. The test for heterogeneity did not detect a statistically significant difference in the odds ratios between the centres.

To assess if the protective effect of vitamin D supplementation was different for diabetes onset in different age groups, Mantel-Haenszel pooled odds ratios were calculated for age at onset before 5 years (OR = 0.83, 95% confidence interval (CI) 0.50 to 1.40), between 5 and 9 years (OR = 0.81, 95% CI 0.55 to 1.20) and between 10 and 14 years

Table 2. Odds ratio (OR) and 95 % confidence limits for developing diabetes before the age of 15 when exposed to vitamin D supplements in early infancy relative to children who were not

Study centre	Number given vitamin D/Total (%)		Odds ratio	95 % confidence limits
	patients	Control subjects		
Austria	89/95 (94 %)	319/346 (92 %)	1.26	(0.49, 3.83)
Bulgaria	124/126 (98 %)	416/430 (97 %)	2.09	(0.47, 19.1)
Latvia	99/109 (91 %)	261/293 (89 %)	1.21	(0.56, 2.87)
Lithuania	107/111 (96 %)	243/250 (97 %)	0.77	(0.19, 3.67)
Luxembourg	31/55 (56 %)	133/176 (76 %)	0.40	(0.20, 0.80)
Romania	43/82 (52 %)	172/276 (62 %)	0.67	(0.39, 1.13)
Northern Ireland	55/168 (33 %)	196/419 (47 %)	0.55	(0.37, 0.82)
	Mantel-Haenszel pooled estimate		0.67	(0.53, 0.85)

Mantel-Haenszel test for heterogeneity $\chi^2 = 10.07$, $df = 6$, $p = 0.12$

(OR = 0.47, 95 % CI 0.33 to 0.68). Although the effect was most obvious in the oldest onset patients, a test of heterogeneity of odds ratios in the three age groups was not significant ($p = 0.14$).

Logistic regression analysis was also done to adjust for possible confounders: duration of breast feeding less than 3 months, maternal age over 35 years, birth weight less than 2500 g, and study centre. There was little change in the adjusted odds ratio (OR = 0.65, 95 % CI 0.52 to 0.83).

Results were also analysed according to the reported duration of vitamin D supplementation. Relative to those who received no supplement, those who received supplements for 1 year or less (OR = 0.69, 95 % CI 0.52 to 0.93) and those who received supplements for longer than 1 year (OR = 0.64, 95 % CI 0.47 to 0.89) showed a similar reduction in risk.

Discussion

In our study we show that the intake of vitamin D supplement given to prevent rickets in early childhood could also contribute to a decrease in risk for childhood-onset insulin-dependent diabetes. Our data are based mainly on questionnaire and interview responses and therefore could lack sensitivity and specificity. If these factors affect patients and control subjects equally our estimate of the magnitude of the true association would be conservative. To take account of differences between study centres in demography, mode of delivery of vitamin D prophylaxis as well as data collection procedures (despite strong efforts to harmonise data collection), we always calculated odds ratios adjusted for centre effects and estimated statistically the degree of heterogeneity between centres.

One form of systematic disease-dependent bias which could affect our results is the possibility that mothers of patients were less diligent in recalling vitamin D supplementation than mothers of control subjects, but we consider this to be unlikely. Another possible cause of bias is that interviewers asked control mothers more assiduously about vitamin D supplementation, but against this it must be stressed that centres were instructed that each interviewer should take care of a similar proportion of the patients and control subjects. Further there was no statistically significant heterogeneity between different centres, some of which used questionnaires and other interviews, to support such a bias. It is also unlikely that mothers or interviewers were aware of the possibility that vitamin D supplement might protect against diabetes. The association could be confounded by variables such as breast feeding and low birth weight as both factors have been shown to decrease the risk for diabetes [13, 14] and could also be associated with the giving of vitamin D supplements. Adjustment for these confounders and also for maternal age had little effect on the odds ratio. We found no evidence that the duration of vitamin D supplementation was relevant but our data on duration is perhaps too crude for useful analysis.

EURODIAB substudy 2 focuses on the hypothesis that non-genetic risk factors which can trigger the events subsequently leading to childhood-onset diabetes are operating early in life. There is a large body of evidence that in most cases of insulin-dependent diabetes the beta-cell destructive process is in progress over several years. Signs of humoral or cellular autoimmune activity or both have been observed many years before disease onset in follow-up studies of twins and other first degree relatives of patients [15, 16]. The mechanism of destruction of the beta cell is still under debate but it is clear that the immune system is heavily involved with macrophages and T-cells, together with cytokines, having a major role [17, 18]. A series of experiments in the NOD mice focusing on primary prevention of diabetes and using 1.25 $(OH)_2D_3$ or its analogues [6–8] showed that it was important to start treatment early in life for protection against destruction of beta cells. The active form of vitamin D has earlier been shown to be a powerful immunosuppressive substance which might suppress lymphocyte proliferation and cytokine production in vitro [19]. The long-term protection by early and short-term supplementation of activated vitamin D in the NOD mouse suggested that tolerance to the beta cells was induced and an improved sensitivity to apoptosis, leading to a better elimination of

effector cells could be an important immunomodulating mechanism by which vitamin D protects against insulitis [20].

In conclusion, in a large multicentre case-control study focusing on early risk factors for childhood-onset insulin-dependent diabetes, we found that vitamin D supplementation in infancy decreased the risk in a fairly consistent manner over the different study centres. This finding was unaltered by adjustment for potential confounders. A relative lack of activated vitamin D could contribute to the risk of developing diabetes in childhood.

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The EURODIAB Substudy 2 study group

Study co-ordinators: Gisela Dahlquist, Department of Paediatrics, Umeå University, Umeå, Sweden

Chris Patterson, Department of Epidemiology and Public Health, University of Belfast, Northern Ireland, UK

Gyula Soltesz, Department of Pediatrics, University of Pecs, Hungary

Study centre leaders: Austria: E. Schober and U. Schneider, Department of Paediatrics, University of Vienna, Vienna, Austria.

Bulgaria (western): S. Goranova, D. Stovanova and M. Ivanova, Children's Diabetic Centre, Sofia, Bulgaria.

Lithuania: B. Urbonaite and A. Pundziute-Lyckå, Department of Paediatrics, University of Kaunas, Kaunas, Lithuania.

Latvia: G. Brigis and I. Rubietza, Department of Public Health and Epidemiology, Medical Academy of Latvia, Riga, Latvia.

Luxembourg: C. de Beaufort, Clinique Pédiatrique, Luxembourg

Romania: A.-M. Konnerth and K. Ionescu-Tirgoviste, Department of Nutrition and Metabolic Disease, University of Bucharest, Romania.

United Kingdom (Leeds): P. McKinney, Institute of Epidemiology and Health Services Research, University of Leeds, Leeds, UK.

United Kingdom (Northern Ireland): C. Patterson and R. Greenlees, Department of Epidemiology and Public Health, Queen's University of Belfast and David Hadden, Metabolic Unit, Royal Victoria Hospital, Belfast, UK.

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