INVESTIGATIVE REPORT

Does Vitamin D Intake During Infancy Promote the Development of Atopic Allergy?

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The active metabolite of vitamin D, 1,25-(OH)$_2$D$_3$, has immunomodulatory properties in addition to its more established action on bone and calcium metabolism. Recently vitamin D has been proposed as one of several environmental factors responsible for the increase in atopic diseases during the last decades. The objective of this study was to determine whether the estimated dose of dietary vitamin D$_3$ during the first year of life is associated with atopic diseases up to the age of 6 years. In a prospective birth cohort study 123 six-year-old children were investigated for the cumulative incidence of atopic dermatitis, allergic rhinitis or asthma by means of a postal questionnaire. Their vitamin D$_3$ intake during infancy was recorded in a previous study and the relationship between lower or higher vitamin D$_3$ intake and atopic illness later in childhood was assessed. Atopic manifestations were more prevalent in the group with higher intake of vitamin D$_3$. Although small, this study supports previous investigations suggesting a role of vitamin D intake during infancy in the development of atopic allergy later in childhood. If these findings are confirmed in prospective controlled clinical trials, prevention through modified vitamin D$_3$ supplementation in infancy could be discussed to reduce the burden of atopic illnesses. *Key words: prospective survey; atopic dermatitis; allergic rhinitis; asthma.*

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The incidence of allergic diseases has been increasing in the westernized world during the last three decades (1). The most likely cause is various changes in environmental exposures. One common hypothesis is related to improved hygiene, with a reduced spectrum of environmental trigger factors of microbial origin confronting the immune system early in life (2). This means a sustained imbalance in the regulation of the immune system between T$_H$1 and T$_H$2 cytokine responses, continuing the T$_H$2 polarization that exists *in utero* (3). Recently a vitamin hypothesis has been put forward, based partly on the coincidence between the "allergic epidemic" and vitamin D supplementation to reduce the risk of rickets (4, 5). In experimental short-term studies in animals, pharmacological doses of the active metabolite of vitamin D, 1,25-(OH)$_2$D$_3$, up-regulate T$_H$2-type immune responses in an asthma model (6), whereas T$_H$1-type responses in various autoimmune disease models are down-regulated (7–9). Putative mechanisms involved have been further elucidated, showing that active ligands of the vitamin D receptor induce expression of thymic stromal lymphopoietin (TSLP) in murine epidermal keratinocytes. This cytokine plays a key role in initiating a skin and systemic atopic dermatitis-like phenotype (10).

Epidemiological studies of humans given dietary vitamin D supplementation in infancy, lend support to the findings of the animal studies, suggesting both increased risk of atopy and allergic rhinitis (11) and reduced risk of type 1 diabetes (12) later in life. In this paper we present some data on the cumulative incidence of allergic diseases in 6-year-old children in relation to their average daily intake of vitamin D$_3$ during the first year of life.

SUBJECTS AND METHODS

In 1998, the families of the first 206 babies, born consecutively from January to March at the Department of Obstetrics at the University Hospital in Umeå, were approached with a questionnaire regarding the diet of their infants. This survey was detailed, with 56 questions on breastfeeding, food items, frequency and volumes consumed, including vitamin supplements. The intake of vitamin D$_3$ from regular diet and supplements was calculated based on the content of vitamin D$_3$ per 100 ml: breast milk 0.02 µg, infant formula 1.3 µg, milk cereal drinks 1.1–1.4 µg, standard milk 0.02 µg, lower fat milk products 0.38 µg, and industry manufactured porridge 2.6 µg. The content of vitamin D$_3$ in other food items was estimated according to information provided by the manufacturers and the National Food Administration. In addition, all children were prescribed vitamin A and D supplements containing 1000 IU vitamin A (retinol palmitate) and 400 IU (= 10 µg) vitamin D$_3$ daily from 6 weeks to 24 months of age. The vitamins were dissolved in 5 drops of sunflower seed oil. The individual vitamin D$_3$ intake was assessed at 5, 7 and 10 months of age and the intake of every child at these time-points was calculated (13).

Six years later a follow-up survey was performed regarding the cumulative incidence of clinical symptoms of allergy by means of a validated postal questionnaire. This survey used relevant questions from the international ISAAC study (14), adapted as described (15). The questionnaire also included questions on past or present allergic disease of the parents. It was sent to the 175

Table I. *Number and percentage of subjects with different manifestations of atopic illnesses in children with "low" (n = 61) or "high" (n = 62) daily intake of vitamin D_3 during infancy*

	Vitamin D intake		
	Low dose (≤ 13.0 µg) n (%)	High dose (> 13.1 µg) n (%)	*p*-value
AD	5 (8)	18 (29)	0.005
AR	7 (11)	11 (18)	0.445
AA	2 (3)	6 (10)	0.273
AD or AR or AA	12 (20)	25 (40)	0.018

AD: atopic dermatitis; AR: allergic rhinitis; AA: allergic asthma.

families who had participated fully in the previous diet survey, and answers were received from 123 families after one reminder. The children were ranked according to their individual daily intake of vitamin D_3 (arithmetic mean of the intake at 5, 7 and 10 months). Subsequently, the cohort ($n=123$) was divided in half; one half with "lower" ranks, range 0.6–13.0 µg ($n=61$) and the other half with "higher" ranks, range 13.2–25.1 µg ($n=62$), of daily vitamin D_3 intake. The study was approved by the regional ethics committee in Lund and performed according to the Declaration of Helsinki after obtaining written informed consent.

Differences in the proportions of allergic diseases between the "lower" and "higher" vitamin D_3 intake groups were assessed with Fisher's exact test (two-tailed). Risk assessments were performed with uni- and multi-variate logistic regression analysis with SPSS software, version 14.0. Interaction according to an additive model was calculated with the synergy index (S), as suggested by Rothman (16). The 95% confidence interval (CI) for S was calculated as suggested by Hosmer & Lemeshow (17). Interaction according to a multiplicative model was analysed in SPSS, version 14.0, with a standard procedure available in logistic regression analysis.

RESULTS

The questionnaires revealed that 37 of the 123 children had had atopic dermatitis (AD) or allergic rhinitis or asthma during the period from infancy to 6 years of age. AD was reported by 23, allergic rhinitis by 18 and asthma by 8 children. The combination of AD and rhinitis was found in 5, rhinitis and asthma in 2, AD and asthma in one and finally AD, rhinitis and asthma in 2 children. In the group of children with a high intake of vitamin D_3, both AD and the combination of AD, or rhinitis or asthma were more common than in the low intake group (Table I). When the average vitamin D_3 intake was used as a continuous predictor in logistic

regression analysis with adjustment for family history of atopic illnesses, the relative risk (odds ratio; OR) was 1.18 (95% CI 1.05–1.32), indicating that OR of having AD increased by 18% for each unit (1 µg) of daily vitamin D_3 dose. As the children were growing from 5 to 10 months of age and hence also their daily intake of food and vitamin D_3 increased, we expressed their daily vitamin intake per kilogram body weight and used it as a risk indicator for development of atopic illness in logistic regression analyses. In Table II the OR for AD is approximately 2.4 in all age groups, suggesting that a high intake at 5 months of age could have influenced the development of AD later in life with no further risk added by continuous high vitamin D_3 intake. Table II further suggests that high vitamin D_3 intake in infancy does not increase the risk of atopic respiratory illness up to the age of 6 years.

Since family history is a major risk factor in atopy, the parents were asked about present or past AD, allergic rhinitis or asthma. Out of the 123 children, 41 had no family history of allergic disease, while 55 had one parent and 27 both parents with atopic illness. There was no significant difference with regard to heredity between the groups of low and high intake of vitamin D_3, thus these risk factors were not confounding factors to each other. In multivariate logistic regression analysis, the relative risk (OR) of developing AD until the age of 6 years for those with family history of atopic illness was 2.2 (95% CI 0.7–6.6) and 4.7 (95% CI 1.6–13.8) for those with a high vitamin D_3 intake, when both factors were entered together. Adjusted for average vitamin D_3 intake, family history of atopy in the mother (OR 2.53; 95% CI 0.92–6.9) seemed to be more important than atopy in the father (OR 1.32; 95% CI 0.5–3.52), while atopy in both parents gave an OR of 3.06 (95% CI 0.78–12.0).

In order to illustrate possible effect modification we made a stratified risk analysis of having AD at the age of 6 years with respect to family history of any atopic illnesses and the average dose of vitamin D_3 intake (Fig. 1). It was not possible to make more detailed strata for family history of different atopic illnesses due to the small sample size. These results indicated that a high vitamin D_3 intake is a risk factor of developing AD independently of family history. Family history doubled the OR, high vitamin D_3 intake increased the OR 4.5 times and the combination approximately 10-fold. Fig. 1

Table II. *Risk of having atopic dermatitis, atopic respiratory illness or any atopic illness at the age of 6 years expressed as odds ratio (OR) with 95% confidence interval (95% CI) from logistic regression analysis. OR is adjusted for family history of atopic illness*

| Risk indicator	Atopic dermatitis OR (95% CI)	Atopic respiratory illness OR (95% CI)	Any atopic illness OR (95% CI)
Vitamin D dose per kg at 5 months (continuous variable)	2.43 (1.29–4.57)	0.82 (0.42–1.59)	1.63 (0.95–2.17)
Vitamin D dose per kg at 7 months (continuous variable)	2.31 (1.20–4.45)	0.84 (0.43–1.63)	1.44 (0.83–2.50)
Vitamin D dose per kg at 10 months (continuous variable)	2.44 (1.0–5.95)	1.06 (0.44–2.57)	1.67 (0.79–3.54)
Average vitamin D dose per kg (continuous variable)	3.63 (1.49–8.87)	0.84 (0.37–1.90)	1.87 (0.91–3.82)
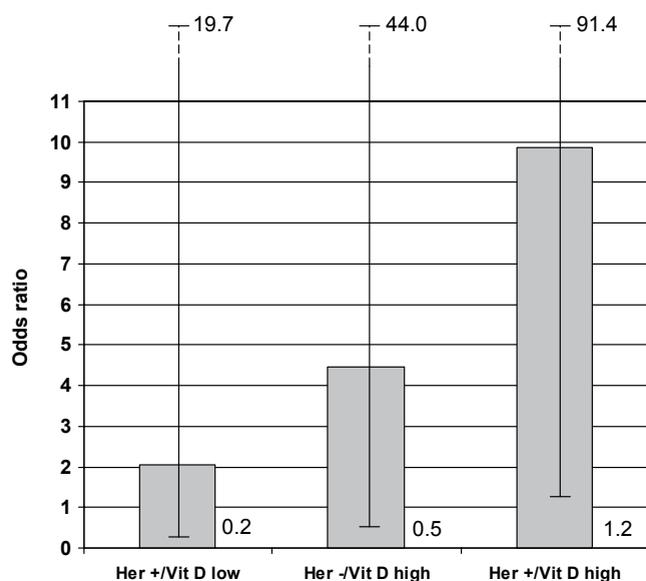
Fig. 1. Risk of having atopic dermatitis at the age of 6 years in relation to the risk factors family history of atopy and intake of vitamin D_3 in infancy expressed as odds ratio (OR) (95% confidence interval). Subjects with no family history and low vitamin D_3 intake constitute the reference category (OR=1). Her: heritage.

also suggested that there was an interaction between family history and vitamin D_3 intake. We therefore analysed possible interaction in both an additive and a multiplicative model. The synergy index was 1.96, indicating an interaction in an additive scale, however not significant (95% CI 0.42–9.01). In the multiplicative model no interaction was found ($p=0.86$).

DISCUSSION

We have previously estimated the average daily dietary intake of vitamin D_3, including natural food, fortified complementary food and vitamin supplements, in Swedish infants and found it to be above the recommended daily allowance of 10 μg (13). Considering that the transformation rate of dietary vitamin D_3 to the active hormone is less well known, that the relative contribution of endogenous synthesis of vitamin D_3 is difficult to estimate, and that the therapeutic window and timetable for various metabolic effects of vitamin D_3 is hardly studied at all in children, we investigated the association between the dietary intake in infancy from 5 to 10 months and the cumulative incidence of atopic manifestations later in childhood.

Although we have a limited number of individuals in this study and no control group unexposed to vitamin D_3 supplementation, this is to some extent compensated by the rather strict estimate of the individual intake of vitamin D_3. There is a significant association between a high intake of vitamin D_3 during infancy and AD later in childhood. Earlier studies have shown that allergic sensitization to peanuts from vitamin D preparations

containing peanut oil has been described, with a 9-fold increased risk of sensitization during combined exposure to vitamin D and peanut oil (18). The risk increased in children receiving the oily vitamin D preparation in the neonatal period (19). In addition breastfeeding for the first 4 months has been shown to reduce the risk of childhood eczema at the age of 4 years (20). Breastfeeding usually implies a low vitamin D_3 intake, while replacement with infant formula and milk cereal drinks, fortified with vitamin D_3, gives a considerably higher intake.

Our results are compatible with animal studies, where the active vitamin D hormone, $1,25\text{-}(OH)_2D_3$, promoted an immune response with interleukin (IL)-4- and IL-13-driven IgE production in mice genetically non-biased for T_H2-type responses (6). Furthermore, prospective studies in a large human birth cohort showed that vitamin D supplementation during the first year of life was associated with higher prevalence of atopy and allergic rhinitis at the age of 31 years (11). This cohort was supplemented with 2000 IU of vitamin D per day, a dose probably higher than the dose received by our children. Likewise, register studies reveal that early infant multivitamin supplementation was associated with increased risk of food allergy and asthma (21). Maternal vitamin D status during pregnancy has also been shown to be of importance, since mothers with high $25\text{-}(OH)D_3$ concentrations in serum in late pregnancy delivered children with increased risk of eczema at 9 months and asthma at 9 years compared with mothers with low concentrations (22). Taken together, it is possible that the vitamin D_3 intake during infancy could be one of several environmental factors potentially involved in the "allergic epidemic".

When different atopic manifestations were compared it seemed that AD was comparatively more prevalent than allergic rhinitis and asthma. This finding could be explained by the fact that the investigated children were only 6 years of age, when mucosal allergy with asthma and allergic rhinitis still may not have developed. Although there was a trend for respiratory allergy to be associated with high intake of vitamin D_3, the association did not reach statistical significance. It cannot be excluded that families with allergic parents were more interested in participating in the study, and that there could be a selection bias in returning the questionnaires. This possible bias influences the cumulative incidence figure, but does not affect the associations shown, given that there is no association between vitamin D_3 intake and proneness to respond in the survey. We cannot exclude an interaction between the effect of family history of atopy and that of vitamin D_3 intake. The precision of our interaction analyses was low due to a small sample size.

More recent investigations lend strong support to the vitamin D hypothesis. Thus it has been demonstrated

that epithelial cells in skin and airways upon proper stimulation express and produce TSLP. This is a cytokine which induce myeloid dendritic cells to prime naïve T helper cells to produce the proallergic cytokines IL-4, IL-5, IL-13 and tumour necrosis factor (TNF)-α, while down-regulating IL-10 and interferon (IFN)-γ, thus constituting a subgroup of inflammatory T_H2 cells. TSLP is also highly expressed in keratinocytes from patients with AD and promotes the activation and migration of Langerhans' cells (23). Therefore TSLP is considered a master switch for allergic inflammation (24). TSLP may be induced in murine keratinocytes by selective ablation of retinoid X receptors α and β, but also by topical application of physiologically active vitamin D receptor ligands, which will result in an AD-like skin condition (10). Furthermore, this is a skin inflammation independent of T and B cells, possibly explaining why a proportion of patients with severe AD may have normal serum IgE levels. Here we have a pathway through which vitamin D and retinoic acid could be involved in AD and other atopic diseases.

However, active metabolites of vitamin D may also induce expression of antimicrobial peptides in human keratinocytes, monocytes and neutrophils with corresponding secretion of antimicrobial activity against human pathogens (25, 26). Since there is an association between both clinical and subclinical vitamin D deficiency and severe lower respiratory tract infections in young children (27, 28), adequate vitamin D intake may reduce the burden of infections, thereby diminishing the stimulation of T_H1-mediated immune responses. In this respect the hygiene hypothesis and the vitamin hypothesis may work synergistically.

However, the role of vitamin D and its metabolites on the pre- and post-natal maturation of epithelial and lymphoid tissues involved in the development of allergic manifestations in the skin and airways is still controversial, suggesting both enhancing and protective mechanisms (5, 29). If breastfeeding with a comparatively modest transfer of vitamin D_3 from mother to child is considered physiological, then early supplementation with vitamin D before weaning may jeopardize the development of a balanced T_H1/T_H2 immune response. Careful controlled clinical trials, addressing the vitamin D status through serum 25-(OH)D_3 levels, together with timing and dosing of vitamin D_3 supplementation, are needed to elucidate when and how vitamin D is involved in the allergic epidemic in relation to other perinatal medical and public health risk factors.

ACKNOWLEDGEMENTS

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