Received Date : 12-Dec-2015

Revised Date : 08-Mar-2016

Accepted Date : 04-Apr-2016

Article type : Original Article: Airway Diseases

Vitamin D supplementation during pregnancy and infancy reduces aeroallergen sensitisation: a randomised controlled trial

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This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/all.12909](https://doi.org/10.1111/all.12909)

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Keywords

Vitamin D, 25-hydroxyvitamin D; pregnancy; infant; supplementation; asthma.

Word count

3379 words

Abstract

Background

Vitamin D has immune modulating effects. We determined whether vitamin D supplementation during pregnancy and infancy prevents aeroallergen sensitisation and primary care respiratory illness presentations.

Methods

A randomised, double-blind, placebo-controlled parallel-group trial. We assigned pregnant women, from 27 weeks gestation to birth, and then their infants, from birth to six months, to placebo or one of two dosages of daily oral vitamin D. Woman/infant pairs were randomised to: placebo/placebo, 1000IU/400IU, or 2000IU/800IU.

When the children were 18 months old we measured specific serum IgE antibodies and identified acute primary care visits described by the doctor to be due to a cold, otitis media, an upper respiratory infection, croup, asthma, bronchitis, bronchiolitis, a wheezy lower respiratory infection or fever and cough.

Results

Specific IgE was measured on 185/260 (71%) enrolled children.

The proportion of children sensitised differed by study group for four mite antigens:

Dermatophagoides farinae (Der-f1, Der-f2) and *Dermatophagoides pteronyssinus* (Der-p1, Der-p2).

With results presented for placebo, lower-dose, and higher-dose vitamin D, respectively (all $P < 0.05$):

Der-f1 (18%, 10%, 2%), Der-f2 (14%, 3%, 2%), Der-p1 (19%, 14%, 3%), and Der-p2 (12%, 2%, 3%).

There were study group differences in the proportion of children with primary care visits described by the doctor as being for asthma (11%, 0%, 4%, $P = 0.002$), but not for the other respiratory diagnoses.

Conclusions

Vitamin D supplementation during pregnancy and infancy reduces the proportion of children sensitised to mites at age 18 months. Preliminary data indicate a possible effect on primary care visits where asthma is diagnosed.

Introduction

Atopy is defined as a tendency to become sensitised and produce IgE antibodies in response to ordinary exposure to allergens, usually proteins (1). Early life events, including those before birth, influence the development of atopy. Those at the atopic response extreme already have elevated specific IgE at birth (2).

T-helper lymphocytes (Th) play a central role in atopic development (3, 4). Th lymphocytes differentiate into subgroups in response to the maturity of the cells presenting antigens to them and the cytokines to which they are exposed (5). Th2 lymphocytes are the Th subset principally involved in the development of atopy (6).

Vitamin D receptors are present on many immune cell types, with vitamin D having effects on cells within both the innate and adaptive components of the immune system. The biologically active form of vitamin D, 1,25-dihydroxyvitamin D ($1,25-(OH)_2D$), causes macrophages and monocytes to proliferate and modulates their cytokine production (7). Under the influence of $1,25-(OH)_2D$ dendritic cells reduce expression of costimulatory molecules, with inhibition of interleukin 12-induced Th1 lymphocyte skewing. In the presence of $1,25-(OH)_2D$, dendritic cells become more tolerogenic, and promote preferential expansion of regulatory T lymphocytes (5, 8). Regulatory T lymphocytes decrease production of pro-inflammatory cytokines such as interferon gamma, and increase production of immune-modulatory cytokines interleukin 10 and transforming growth factor beta (8).

The relationship between vitamin D status and atopy is non-linear (9). In comparison with children with a cord blood 25-hydroxyvitamin D ($25(OH)D$) concentration of 50 to <75 nmol/L, both children with low (<50 nmol/L) or high (>100 nmol/L) cord blood $25(OH)D$ concentration have an increased odds of detectable inhalant allergen-specific IgE and skin prick test positivity in the first 5 years of life (10).

Prevention of atopy could therefore theoretically be achieved by maintaining normal vitamin D status during the life phase when atopy develops. We recently completed a randomised, double-blind, placebo-controlled parallel-group trial of vitamin D supplementation during pregnancy and infancy in New Zealand (NZ) (11). In NZ there is a sun-avoidance public health policy, the diet has a low vitamin D content and vitamin D supplementation is used infrequently (12, 13). Hence vitamin D deficiency is prevalent with 57% of newborns having cord blood $25(OH)D$ concentrations <50 nmol/L (14). Both asthma and atopy are highly prevalent in NZ with 25% of six-to-seven year olds having reported asthma (15), and 35% of 11-to-12 year olds being atopic (16). Our objective was to

98 determine whether vitamin D supplementation during pregnancy and infancy prevents aeroallergen
99 sensitisation and atopic respiratory illness presentations to primary care.

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Methods

Trial design

We conducted a previously described randomised, double-blind, placebo-controlled, parallel-group study in Auckland, NZ (latitude 36°S) (11, 17). The objective of this study was to determine the vitamin D dose necessary to achieve 25(OH)D concentration ≥ 50 nmol/L during infancy(11). We randomly assigned pregnant mothers (from enrolment at 27 weeks gestation to birth) and their infants (from birth to age 6 months), to one of three groups: daily placebo; lower-dose; or higher-dose oral vitamin D (Figure 1). Ethical approval was from the Ministry of Health ethics committee (NTX09/11/101). We obtained written informed consent from all participating women. Registration was with the Australian NZ Clinical Trials Registry (ACTRN12610000483055).

Participants

We recruited women from an urban primary care maternity clinic from April 2010 to July 2011. We excluded women taking vitamin D supplementation >200 IU/day, and those with any serious pregnancy complication (11, 17).

Interventions

Pregnant women took one drop of placebo or 1000 IU or 2000 IU of vitamin D per day (enrolment to birth) and infants took placebo or 400 IU or 800 IU of vitamin D per day (birth to age 6 months). Reported adherence was 95% during pregnancy and 86% during infancy (11).

Outcomes

The findings presented here are secondary analyses of sensitisation to airborne allergens and acute primary healthcare visits for respiratory illnesses. Measurement of aeroallergen sensitisation and healthcare visits required additional funding obtained when the children were, on average, 18 months old.

Sample size

Sample size calculations were based upon the primary study protocol objective of achieving a serum 25(OH)D concentration ≥ 50 nmol/L (11).

Randomisation and blinding

Randomisation was performed by the study biostatistician (AS) (11). Study participants and research staff remained unaware of treatment allocations throughout the recruitment, enrolment, treatment and data acquisition phases.

Data collection

We interviewed enrolled women during pregnancy and when their infant was 2, 4, 6, and 18 months old. For this study, we collected data describing demographics, adherence, mother's food consumption, infant feeding, sunlight exposure, primary care visits for asthma, and drugs prescribed (11, 17). The food frequency questionnaire (FFQ) used contained 12 food items that captured the major dietary sources of vitamin D in the NZ food supply (18).

At the assessment completed when the children were 18 months old, we obtained a capillary blood sample from each child and measured serum 25(OH)D concentration (11, 17). We determined aeroallergen sensitisation by skin prick testing (SPT) to house dust mite, cat and grass pollens (Supplementary Table 1)(19); and semi-quantitative measurement (ImmunoCAP, Pharmacia, Uppsala, Sweden) of specific serum IgE antibodies to antigens in the following groups: animal; polcalcin; grass, tree, and weed pollens; mould; mites; and cockroach (Supplementary Table 1) (20). We defined a positive SPT response as a wheal diameter ≥ 3 mm larger than the negative control (21). We categorised IgE responses as undetectable (ISAC Standardized Units (ISU) 0-0.3), low (ISU >0.3 -1.0), moderate/high (ISU >1 -15) and very high (ISU >15).

We reviewed the primary care records for the doctor diagnosis assigned at each acute respiratory illness visit. In NZ each patient's primary care file includes a free text record completed by the doctor at each visit and stored in an electronic practice management system. Each patient's records are identified by their National Health Index number, a unique identifier assigned to each person in NZ at their first contact with the healthcare system. We read each child's primary care record and assigned each respiratory illness visit to one of nine diagnostic categories based upon the doctor's written record. These categories were a cold, otitis media, an upper respiratory infection, croup, asthma, bronchitis, bronchiolitis, a wheezy lower respiratory infection or fever and cough. We determined whether salbutamol or prednisone were prescribed at each visit.

Statistical methods

Analyses were performed on an intention to treat basis using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) and R version 3.1.3 (R Foundation for Statistical Computing, Vienna, Austria). We described maternal and child characteristics, including those specifically relevant to vitamin D status. We compared maternal and child characteristics between study groups and also between groups based upon whether they had serum obtained at age 18 months. We used the chi-square and Fisher's exact tests to compare proportions, and the Wilcoxon rank sum test to compare continuous data. For the comparison of the semi-quantitative specific IgE responses, we combined the moderate/high and very high categories. For antigens to which measureable serum IgE antibody

164 responses were present in more than 5% of the children, the proportion in each study group with IgE
165 antibodies present were compared using relative risks (RR) and 95% confidence intervals (CI). The
166 number needed to treat (NNT) to prevent sensitisation was calculated as $1/(\text{absolute risk reduction})$.

Results

Characteristics of study participants (Table 1)

Enrolment of the pregnant women was evenly distributed by season. Fifty-nine (23%) of the mothers and 28 (11%) of the fathers had a history of doctor-diagnosed asthma. Median maternal daily dietary vitamin D intake during the month prior to study enrolment was 2.9 µg/day. While virtually all (95%) enrolled children were breastfed at birth, 47% had commenced infant milk formula prior to age six months.

As previously described, at enrolment mean serum 25(OH)D (63 nmol/L) did not differ between study groups ($P=0.19$). At 36 weeks gestation and in cord blood samples mean serum 25(OH)D was higher in both the lower- and higher-dose vitamin D groups than in the placebo group. In the infants at two, four and six months of age mean serum 25(OH)D differed between the three study groups with the higher-dose vitamin D group having the highest and the placebo group the lowest 25(OH)D concentrations (11). At age 18 months, 12 months after completion of the intervention, mean serum 25(OH)D (61 nmol/L) did not differ between study groups ($P=0.30$).

Comparisons between those in whom atopy was versus was not measured at age 18 months

At age 18 months serum was obtained from 186 (75%) children of the 260 women enrolled (Figure 1). In comparison with those in whom specific IgE concentration was measured, the mothers of children in whom it was not were older (28 vs. 25 years, $P<0.001$) but did not otherwise differ with respect to season of enrolment, demographics, health, vitamin D intake or sunlight exposure. Nor were there differences in cord blood 25(OH)D concentration or infant feeding (Supplementary Table 2).

Skin prick test results and serum IgE concentration comparisons between placebo, lower- and higher-dose vitamin D supplementation groups

Skin prick test (SPT) results for the three aeroallergens (HDM, cat, grass pollen) were available for 184 of the 186 children from whom serum was obtained at age 18 months. The proportion with SPT positivity varied between study groups for HDM ($P=0.03$) but not for cat ($P=0.74$) or grass pollen ($P=0.99$). In comparison with children randomised to the placebo group, a smaller portion of those randomised to the higher-dose (0% vs. 9%, $P=0.03$), but not the lower-dose (3% vs. 9%, $P=0.28$) vitamin D group were SPT positive to HDM (Table 2).

With the exception of mites (26/186, 14%) fewer than 5% of children had a measureable serum specific IgE response to each antigen group: animals (6/186, 3%); grass pollen (0/186, 0%); tree

pollen (0/186, 0%); weed pollen (1/186, 1%); mould (2/186, 1%); and cockroach (0/186, 0%)

Therefore we only proceeded with between group comparisons for the mite antigen group.

The proportion of children with measureable IgE antibodies to the *D. farinae* antigens and *D. pteronyssinus* antigens varied by study group (Table 3). In comparison with the placebo group, a smaller proportion of children in the lower-dose vitamin D group had measurable IgE antibodies to Der-f2 ($P=0.03$) and Der-p2 ($P=0.03$); and a smaller proportion of children in the higher-dose vitamin D group had measurable IgE antibodies to Der-f1 ($P=0.003$), Der-f2 ($P=0.02$) and Der-p1 ($P=0.009$).

Semi-quantitative IgE responses varied between study groups to the mite antigens Der-f1 ($P=0.01$), Der-f2 ($P=0.004$), Der-p1 ($P=0.02$) and Der-p2 ($P=0.001$) (Figure 2). In comparison with the placebo group, the semi-quantitative IgE responses were lower in the lower-dose vitamin D group to the Der-f2 ($P=0.03$) and Der-p2 ($P=0.03$) antigens; and in the higher-dose vitamin D group to the Der-f1 ($P=0.002$), Der-f2 ($P=0.009$), Der-p1 ($P=0.01$) and Der-p2 ($P=0.004$) antigens.

Measureable IgE antibodies to any mite antigen were present in 13 (20%) of the children in the placebo group, 9 (14%) of the lower-dose and 4 (7%) of the higher-dose vitamin D groups. In comparison with the placebo group, the risk of mite antigen sensitisation was reduced in the higher-dose (RR=0.34, 95% CI 0.12-0.94) but not the lower-dose (RR=0.71, 95% CI 0.33-0.1.52) vitamin D groups. The NNT for the higher-dose vitamin D group was 8 (95% CI 4 to 109) and for the lower-dose vitamin D group was 18 (95% CI -13 to 6).

Comparison of primary care visits and of salbutamol and prednisone prescribing between placebo, lower- and higher-dose vitamin D supplementation groups (Table 4)

The primary care visit audit occurred at a median age of 18 (IQR, 14–21) months (placebo, 18; lower-dose vitamin D, 18; higher-dose vitamin D, 17 months; $P=0.59$). The proportion of children with a primary care visit where asthma was recorded as being present, but not where any of the other acute respiratory illness diagnoses were recorded, varied by study group ($P=0.002$), as did the number of primary care visits per child where asthma was recorded as being present ($P=0.004$).

The proportion of children who, at any acute primary care respiratory visit, were prescribed salbutamol did not differ by study group: placebo 32%, lower-dose vitamin D 30%, higher-dose vitamin D 28%, $P=0.81$), but the proportion who were prescribed prednisone did: placebo 27%, lower-dose vitamin D 14%, higher-dose vitamin D 15%, $P=0.04$). The proportion of children with a primary care visit recorded as being due to asthma at which salbutamol ($P=0.02$) or prednisone ($P=0.02$) was prescribed varied by study group. The number of primary care visits per child where

asthma was recorded and salbutamol ($P=0.004$) or prednisone ($P=0.03$) was prescribed also varied by study group.

Of the 26 (14%) children with serum specific IgE responses to a mite antigen, 13 (50%) were in the placebo group, nine (35%) in the lower-dose and four (15%) in the higher-dose vitamin D group. Of these, one child in the placebo group, but none in either of the vitamin D supplementation groups, made any primary care visits described by the doctor as being for asthma. No association was evident between the presence of HDM sensitisation and having a primary care visit described by the doctor as being for asthma ($P=0.79$).

Discussion

Statement of principal findings

In this post-hoc analysis of a randomised, placebo-controlled trial of vitamin D supplementation during the third trimester of pregnancy and the first six months of infancy we showed that vitamin D supplementation resulted in a decrease in the proportion of children sensitised to HDM at age 18 months. The number of mite antigens to which the children were sensitised and the magnitude of IgE responses to these mite antigens were both lower in the vitamin D supplemented groups.

In comparison with the placebo group the proportion of children with any primary care visits for which asthma was recorded as being present and the number of such primary care asthma visits per child were smaller in the vitamin D supplemented groups; as was the proportion of these primary care asthma visits and the number of visits per child for which salbutamol or prednisone were prescribed.

Strengths and weaknesses of the study

Vitamin D was given from 27 weeks gestation and continued until the infants were six months old. Atopic sensitisation can start *in utero* (2). Children of atopic parents who have evidence at birth of sensitisation to mites are at increased risk of developing allergic symptoms by age one year (22). Interventions that seek to prevent atopy sensitisation are therefore likely to need to begin before birth. As immune responses to allergens can be detected as early as 22 weeks gestation, vitamin D supplementation may have been more effective if started earlier in pregnancy (23).

We used a daily dose of vitamin D and thus avoided the wide variations in serum 25(OH)D concentration seen when large intermittent vitamin D doses are used and which are believed to cause immune responses that are not beneficial to the host (24, 25). We maintained study blinding until the children were 18 months old and at this age measured atopic sensitisation and acute primary care visits.

The electronic primary care records we audited to describe primary care asthma and other respiratory illness visits were not coded. Hence they were not subject to the under-estimations, by approximately 25%, of primary care asthma visits in preschool aged children that result when only coded primary care data are used (26). As the description of primary care visits was not one of the primary objectives of the trial, we would expect the care received by the children in this study to reflect usual practice.

There is considerable variability between doctors in their assignment of specific diagnoses to episodes of acute respiratory illness in young children (27). To an extent such variability is site

specific with, for example, presentation to some emergency departments being associated with an increased likelihood of a diagnosis of asthma being made in young children presenting with acute lower respiratory tract illness symptoms (27). That the children enrolled were seen at 126 different primary practices meant that any such institutional bias will have been addressed adequately by randomisation.

Strengths and weaknesses in relation to other studies, discussing particularly any differences in results

Although no studies to date have reported the effect of vitamin D supplementation during pregnancy and early infancy on asthma during childhood, placebo controlled clinical trials have shown efficacy of vitamin D supplementation in children with asthma. A meta-analysis, published in 2015 (28), that included data from five clinical trials which used a vitamin D doses ranging from 500 to 2000 IU/day in children aged 5 to 18 years, showed a statistically significant reduction in asthma exacerbations (29-33). One further trial published since this meta-analysis, which enrolled 130 individuals aged 10 to 50 years and randomly assigned them vitamin D or placebo, showed a significant improvement in lung function after 24 weeks of the intervention (34).

This was a post-hoc analysis performed on additional data, measurement of SPT and serum IgE responses to aeroallergens at age 18 months. We collected data specifically for this analysis and prior to study unblinding. Thus, we did not initiate these analyses following an inspection of already collected data, which is the principle methodological weakness of a post-hoc approach (35).

Meaning of the study: possible mechanisms and implications for clinicians or policymakers

Based upon specific serum IgE measurement the proportion of children sensitised to mites (14%) was greater than for all other aeroallergens tests (all <5%). In children, reported rates of mite antigen sensitisation range from 5% to 28% with population based estimates from NZ being at the higher end of this range (36-38). Internationally, mite antigen sensitisation is one of the most prevalent forms of aeroallergen sensitisation (38). The differences between study groups were seen to the antigens of the house dust mites *D. farinae* and *D. pteronyssinus*, but not to those of the storage mites *Blomia* and *Lepidoglyphus* which, while major sensitising aeroallergen in some countries, are not in NZ (39, 40).

Sensitisation to mites is associated with an increased risk of childhood asthma as shown in cohort studies where asthma prevalence in children was measured at an older age than in this study, for example seven and 11 years (41, 42). That no association was evident is likely a reflection of the younger age at which we estimated asthma prevalence and the difficulty in diagnosing asthma in the first two years of life.

Our demonstration that vitamin D supplementation during pregnancy and infancy prevents HDM sensitisation is consistent with vitamin D having immune modulatory functions. Vitamin D promotes the induction of regulatory T-cells which in turn can prevent the pathological immune responses to antigens that lead to clinical allergic disease (8). In animal models, exposure to adequate vitamin D prior to sensitisation prevents cellular airway responses to aeroallergens (43).

Unanswered questions and future research

As rates of aeroallergen antigen sensitisation increase with increasing age, between-group differences may emerge for sensitisation to the other aeroallergens tested and in clinical manifestations of allergic disease (44). Longer-term follow-up is also important to ensure that vitamin D supplementation does not place some individuals at increased risk of atopic sensitisation and allergic disease. In birth cohort studies from the United States(10) and Germany(45), both high (≥ 100 nmol/L) and low (< 50 nmol/L) cord blood 25(OH)D concentrations were associated with an increased risk of aeroallergen sensitisation at age five years (10), and higher maternal 25(OH)D concentration during late pregnancy was associated with an increased risk of food allergen sensitisation at age two years (45).

Children who become atopic are at increased risk of asthma and of having more severe and persistent asthma (46, 47). We do not know if the differences in mite sensitisation and primary care asthma visits that we observed to age 18 months, as a result of vitamin D supplementation during late pregnancy and early infancy, are a temporary phenomenon or one that has lifelong consequences in terms of an individual's risk of developing asthma. The diagnosis of asthma is very insecure at age 18 months. Follow-up to an age when the diagnosis of asthma can be made with greater precision is necessary to determine the true effect of pregnancy and early infancy interventions on asthma prevalence (48). Our findings do indicate though the potential for daily supplemental vitamin D to prevent asthma in populations where both vitamin D deficiency and asthma are prevalent. Studies with more robust asthma case definitions need to follow these preliminary findings.

Statement of contribution by each named author

Cameron Grant conceived and designed the study, developed the data collection instruments, analysed and interpreted the data and completed the first and final drafts of the manuscript.

Julian Crane developed the data collection instruments, reviewed and revised the manuscript and approved the final manuscript as submitted.

Edwin A. Mitchell conceived and designed the study, developed the data collection instruments, reviewed and revised the manuscript and approved the final manuscript as submitted.

Jan Sinclair developed the data collection instruments, reviewed and revised the manuscript and approved the final manuscript as submitted.

Alistair Stewart conceived and designed the study, analysed and interpreted the data, reviewed and revised the manuscript and approved the final manuscript as submitted.

Tania Milne developed the recruitment and retention strategy for the study, designed the data collection instruments, coordinated the collection of the data and approved the final manuscript as submitted.

Joshua Knight analysed and interpreted the data, reviewed and revised the manuscript and approved the final manuscript as submitted.

Catherine Gilchrist interpreted the data, participated in the construction of the first draft, reviewed and revised the manuscript and approved the final manuscript as submitted.

Carlos A. Camargo Jr. conceived and designed the study, developed the data collection instruments, analysed and interpreted the data, reviewed and revised the manuscript and approved the final manuscript as submitted.

Statement of conflicts of interest for each named author

The authors have no conflicts of interests to declare

Acknowledgements

The authors acknowledge the following people who helped to create the Pregnancy and Infancy Vitamin D Trial within which this study was completed: Alec Ekeroma, Suhina Kaur, Judy Rowden, Robert Scragg, Adrian Trenholme, Ellen Waymouth, Clare Wall

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488 **Figure Legends**

489 **Figure 1.** Flow diagram of enrolment, randomisation, attrition, and measurement of 25-
490 hydroxyvitamin D (25(OH)D) and allergen sensitisation.

491 **Figure 2.** Quantification of serum specific IgE response to mite antigens by study group: placebo,
492 lower-dose vitamin D, higher-dose vitamin D.

493

Table 1. Demographics and clinical characteristics of enrolled pregnant women and their children and health history of the women and their partners by study group assignment to placebo, lower-dose, or higher-dose vitamin D supplementation from 27 weeks gestation to age 6 months.

Variable	Study group			P-value
	Placebo (mother placebo/infant placebo) n ₁ = 87	Lower-dose vitamin D (mother 1000 IU daily/infant 400 IU daily) n ₂ = 87	Higher-dose vitamin D (mother 2000 IU daily/infant 800 IU daily) n ₃ = 86	
Season of enrolment*, n (%)				0.99
Summer	20 (23)	21 (24)	18 (21)	
Autumn	25 (29)	22 (25)	24 (28)	
Winter	23 (26)	23 (26)	18 (21)	
Spring	19 (22)	21 (24)	22 (26)	
Maternal demographics				
Age in years, median (IQR)	28 (23,33)	27 (23, 31)	26 (22, 33)	0.72
Ethnicity, n (%)				0.98
NZ European	15 (17)	12 (14)	13 (15)	
Māori	21 (24)	23 (26)	19 (22)	
Pacific	36 (41)	39 (45)	38 (44)	
Other	15 (17)	13 (15)	16 (19)	
Education, n (%)				0.37
Primary	10 (12)	17 (20)	12 (14)	
Secondary	23 (26)	28 (32)	23 (27)	
Tertiary	54 (62)	42 (48)	51 (59)	
Body mass index in kg/m ² , median (IQR)	33 (26, 38)	32 (27, 39)	31 (26, 38)	0.51
Mother was a cigarette smoker pre-pregnancy (n=258), n (%)	32 (37)	42 (49)	35 (41)	0.26
Number of people in house, median (IQR)	4 (3, 6)	4 (3, 7)	4 (4, 7)	0.74
Maternal health				
Doctor diagnosed asthma, n (%)	22 (25)	20 (23)	17 (20)	0.68

Doctor diagnosed dermatitis, n (%)	6 (7)	11 (13)	5 (6)	0.22
Doctor diagnosed hay fever, n (%)	16 (18)	23 (26)	19 (22)	0.44
Doctor diagnosed heart disease, n (%)	2 (2)	0 (0)	1 (1)	0.55 [†]
Doctor diagnosed diabetes, n (%)	2 (2)	3 (3)	0 (0)	0.38 [†]
Doctor diagnosed hypertension, n (%)	7 (8)	6 (7)	0 (0)	0.02 [†]
Paternal health				
Doctor diagnosed asthma, n (%)	11 (13)	8 (10)	9 (11)	0.55
Doctor diagnosed dermatitis, n (%)	4 (5)	1 (1)	2 (2)	0.41 [†]
Doctor diagnosed hay fever, n (%)	7 (8)	14 (17)	12 (14)	0.26
Pregnancy health				
First pregnancy, n (%)	67 (77)	66 (76)	58 (67)	0.30
Gestation at enrolment, median weeks (IQR)	27 (26, 29)	28 (26, 29)	27 (26, 29)	0.79
Current cigarette smoker, n (%)	18 (21)	17 (19)	14 (16)	0.74
Dietary sources of vitamin D during pregnancy				
Dietary vitamin D intake during pregnancy in µg/day, median (IQR)	3.1(1.7, 4.1)	2.5(1.6, 3.9)	3.2(1.8, 4.2)	0.34
Vitamin D supplement use at 36 weeks gestation, [‡] n (%)	9 (11)	9 (11)	7 (10)	0.85
Sunlight exposure during pregnancy				
Hours/day outdoors past four weeks, median weeks (IQR)	0.5 (0.3, 1.6)	1.0 (0.5, 2.0)	1.0 (0.5, 1.8)	0.08
Avoids sun exposure 10:00-16:00 hours in summer, n (%)	51 (59)	53 (61)	61 (71)	0.23
Sunscreen with an SPF15 or stronger if outside in summer, n (%)	32 (37)	36 (41)	40 (47)	0.39
Child Demographics				
Male gender, n (%)	44 (52)	41 (49)	36 (44)	0.63
Gestation, in weeks, median (IQR)	39 (38, 40)	39 (39, 40)	39 (38, 41)	0.51
Birth weight, in kilograms, median (IQR)	3.47(3.20, 3.77)	3.49(3.16, 3.87)	3.54(3.23, 3.96)	0.61
Child Characteristics				
Any breastfeeding, n (%)	74 (94)	70 (92)	69 (99)	0.17
Received infant formula, n (%)	51 (79)	49 (78)	44 (75)	0.86

Started infant formula prior to age six months, n (%)	30 (46)	31 (50)	26 (45)	0.84
Used multivitamin containing vitamin D from age 6 to 18 months (n = 185), [§] n (%)	2 (3)	1 (2)	1 (2)	0.99 [†]
Hours per day spent outdoors on average over past two months, median (IQR)				
At age 2 months	1.3 (0.6, 2.8)	1.2 (0.6, 3.5)	1.8 (0.6, 3.5)	0.18
At age 4 months	1.2 (0.6, 2.8)	2.0 (0.6, 3.5)	1.8 (0.6, 3.5)	0.39
At age 6 months	2.0 (0.8, 3.9)	1.8 (0.9, 3.5)	2.8 (1.2, 3.8)	0.55
At age 18 months	5.3 (2.7, 10.9)	5.3 (2.8, 10.6)	5.4 (2.8, 10.6)	0.96

* Summer (December to February), Autumn (March to May), Winter (June to August), Spring (September to November)

† Fisher's exact test

‡ All vitamin D supplements used were at a dose of ≤ 200 IU per day

§ Vitadol-C (Nutricia, NZ Limited, Auckland, NZ) which contains 466 IU of vitamin D per daily recommended dose

Table 2. Positive skin prick test results at age 18 months in children randomly assigned to placebo, lower-dose, or higher-dose vitamin D supplementation from 27 weeks gestation to age 6 months.

Positive response to skin prick test antigen*	Study group				<i>P</i> -value
	All three study groups n = 184	Placebo (mother placebo/infant placebo) n ₁ = 65	Lower-dose vitamin D (mother 1000 IU daily/infant 400 IU daily) n ₂ = 61	Higher-dose vitamin D (mother 2000 IU daily/infant 800 IU daily) n ₃ = 58	
House dust mite, n (%)	8 (4)	6 (9)	2 (3)	0 (0)	0·03
Cat, n (%)	5 (3)	1 (2)	2 (3)	2 (3)	0·74
Grass pollen, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0·99

* Positive response defined as a wheal diameter at least 3 mm greater than the negative control (21).

Table 3. Presence of measureable serum IgE responses to mite antigens and number of children with a measureable response to *Dermatophagoides farinae* and *D. pteronyssinus* antigens at age 18 months in children randomly assigned to placebo, lower-dose, or higher-dose vitamin D supplementation from 27 weeks gestation to age six months.

Mite antigens to which specific IgE response was measurable	Study group				P-value
	All three study groups n = 186	Placebo (mother placebo/infant placebo) n ₁ = 65	Lower-dose vitamin D (mother 1000 IU daily/infant 400 IU daily) n ₂ = 63	Higher-dose vitamin D (mother 2000 IU daily/infant 800 IU daily) n ₃ = 58	
<i>Dermatophagoides farinae</i> , n (%)					
Der-f1	19 (10)	12 (18)	6 (10)	1 (2)	0·009
Der-f2	12 (6)	9 (14)	2 (3)	1 (2)	0·02
<i>Dermatophagoides pteronyssinus</i> , n (%)					
Der-p1	23 (12)	12 (18)	9 (14)	2 (3)	0·04
Der-p2	11 (6)	8 (12)	1 (2)	2 (3)	0·04
<i>Blomia Blo t 5</i> , n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0·99
<i>Lepidoglyphus</i> Lep d 2, n (%)	1 (1)	1 (2)	0 (0)	0 (0)	0·99

Table 4. Number of children with an acute primary care respiratory illness visit up to age 18 months by doctor diagnosis of acute respiratory illness type for children randomly assigned to placebo, lower-dose, or higher-dose vitamin D supplementation from 27 weeks gestation to age 6 months

Illness diagnosis at primary care visit, n (%)	Study group				P-value
	All three study groups n = 236	Placebo (mother placebo/infant placebo) n ₁ = 80	Lower-dose vitamin D (mother 1000 IU daily/infant 400 IU daily) n ₂ = 80	Higher-dose vitamin D (mother 2000 IU daily/infant 800 IU daily) n ₃ = 76	
Cold or influenza	99 (42)	34 (42)	32 (40)	33 (43)	0·90
Otitis media	77 (33)	28 (35)	24 (30)	25 (33)	0·80
Upper respiratory infection	167 (71)	61 (76)	54 (68)	52 (68)	0·41
Croup	68 (29)	21 (26)	27 (34)	20 (26)	0·49
Bronchitis	15 (6)	7 (9)	3 (4)	5 (7)	0·43
Asthma	12 (5)	9 (11)	0 (0)	3 (4)	0·002
Bronchiolitis	61 (26)	22 (27)	22 (27)	18 (24)	0·82
Wheezy lower respiratory infection	56 (24)	19 (24)	17 (21)	20 (26)	0·76
Fever and cough	14 (6)	6 (7)	5 (6)	3 (4)	0·64
Number of primary care asthma visits					0·004
0	224 (95)	71 (89)	80(100)	73 (96)	
1	8 (3)	5 (6)	0 (0)	3 (4)	
2 or more	4 (2)	4 (5)	0 (0)	0 (0)	

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Figure 1. Flow diagram of enrollment, randomisation, distribution, and measurement of 25-hydroxyvitamin D (25(OH)D) and plunger sophistication

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Figure 2. Quantification of serum specific IgE response to mite antigens by study group: placebo, lower-dose vitamin D, higher-dose vitamin D.

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Title:

Vitamin D supplementation during pregnancy and infancy reduces aeroallergen sensitization: a randomized controlled trial

Date:

2016-09-01

Citation:

Grant, C. C., Crane, J., Mitchell, E. A., Sinclair, J., Stewart, A., Milne, T., Knight, J., Gilchrist, C. & Camargo, C. A. (2016). Vitamin D supplementation during pregnancy and infancy reduces aeroallergen sensitization: a randomized controlled trial. *ALLERGY*, 71 (9), pp.1325-1334. <https://doi.org/10.1111/all.12909>.

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