Original Investigation

Effect of Vitamin D_3 Supplementation During Pregnancy on Risk of Persistent Wheeze in the Offspring
A Randomized Clinical Trial

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IMPORTANCE Observational studies have suggested that increased dietary vitamin D intake during pregnancy may protect against wheezing in the offspring, but the preventive effect of vitamin D supplementation to pregnant women is unknown.

OBJECTIVE To determine whether supplementation of vitamin D_3 during the third trimester of pregnancy reduces the risk of persistent wheeze in the offspring.

DESIGN, SETTING, AND PARTICIPANTS A double-blind, single-center, randomized clinical trial conducted within the Copenhagen Prospective Studies on Asthma in Childhood 2010 cohort. Enrollment began March 2009 with a goal of 708 participants, but due to delayed ethical approval, only 623 women were recruited at 24 weeks of pregnancy. Follow-up of the children (N = 581) was completed when the youngest child reached age 3 years in March 2014.

INTERVENTIONS Vitamin D_3 (2400 IU/d; n = 315) or matching placebo tablets (n = 308) from pregnancy week 24 to 1 week postpartum. All women received 400 IU/d of vitamin D_3 as part of usual pregnancy care.

MAIN OUTCOMES AND MEASURES Age at onset of persistent wheeze in the first 3 years of life. Secondary outcomes included number of episodes of troublesome lung symptoms, asthma, respiratory tract infections, and neonatal airway immunology. Adverse events were assessed.

RESULTS Of the 581 children, persistent wheeze was diagnosed during the first 3 years of life in 47 children (16%) in the vitamin D_3 group and 57 children (20%) in the control group. Vitamin D_3 supplementation was not associated with the risk of persistent wheeze, but the number of episodes of troublesome lung symptoms was reduced, and the airway immune profile was up-regulated (principal component analysis, $P = .04$). There was no effect on additional end points. Intrauterine death was observed in 1 fetus (<1%) in the vitamin D_3 group vs 3 fetuses (1%) in the control group and congenital malformations in 17 neonates (5%) in the vitamin D_3 group vs 23 neonates (8%) in the control group.

End Point	No. (%) Vitamin D_3	No. (%) Control	Estimate (95% CI)	*P* Value
Persistent wheeze	47 (16)	57 (20)	Hazard ratio (HR), 0.76 (0.52-1.12)	.16
Episodes of troublesome lung symptoms, mean (95% CI)	5.9 (5.2-6.6)	7.2 (6.4-8.1)	Incidence risk ratio (IRR), 0.83 (0.71-0.97)	.02
Asthma at 3 y	32 (12)	47 (14)	Odds ratio, 0.82 (0.50-1.36)	.45
Respiratory tract infections				
Upper, annual mean (95% CI)	5.2 (4.8-5.5)	5.3 (4.9-5.6)	IRR, 0.99 (0.90-1.09)	.84
Lower	94 (32)	95 (33)	HR, 0.96 (0.72-1.27)	.76

CONCLUSIONS AND RELEVANCE The use of 2800 IU/d of vitamin D_3 during the third trimester of pregnancy compared with 400 IU/d did not result in a statistically significant reduced risk of persistent wheeze in the offspring through age 3 years. However, interpretation of the study is limited by a wide CI that includes a clinically important protective effect.

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Asthma often begins in early childhood and is the most common chronic childhood disorder.[1] The incidence has increased during the last half-century in westernized societies, presumably related to a changing lifestyle or environment inducing immune deregulation in early life and subsequent chronic inflammation.[2] In parallel, vitamin D deficiency has also become a common health problem in westernized societies, possibly caused by a more sedentary indoor lifestyle and decreased intake of vitamin D containing foods.[3] Vitamin D possesses a range of immune regulatory properties, and it has been speculated that vitamin D deficiency during pregnancy may affect fetal immune programming and contribute to asthma pathogenesis.[4,5]

This hypothesis is supported by a recent observational study in the Copenhagen Prospective Studies on Asthma in Childhood 2000 ($COPSAC_{2000}$) high-risk birth cohort showing an association between low cord blood vitamin D levels and an increased risk of childhood wheezing.[6] Results of other studies have been ambiguous, as some showed an association between decreased maternal dietary vitamin D intake[7,8] or decreased cord blood vitamin D levels[6,9,10] and increased risk of wheezy disorders, whereas others reported no such association.[11,12]

Therefore, we conducted the COPSAC 2010 ($COPSAC_{2010}$) Vitamin D RCT, a double-blind RCT of vitamin D_3 supplementation during pregnancy in the 2010 population-based COPSAC mother-child cohort, to assess the potential effect on risk of persistent wheeze during the first 3 years of life.[13]

Methods

The trial protocol and statistical analysis plan are available in Supplement 1.[14]

The $COPSAC_{2010}$ study was approved by the local ethics committee with a separate approval for the vitamin D_3 during pregnancy RCT, by the Danish Data Protection Agency, and by the Danish Health and Medicines Authority. Written and oral informed consent were obtained at enrollment of participants.

Study Design

This double-blind, placebo-controlled study identified pregnant women in Denmark by reviewing monthly the lists of reimbursements to general physicians for first pregnancy visits. The identified women received a written invitation to contact the COPSAC clinic by telephone, and they were given detailed verbal information and screened for eligibility. Thereafter, detailed information was sent and the first visit to the clinic was planned within pregnancy weeks 22 through 26. Exclusion criteria were gestational age above week 26; any endocrine, cardiovascular, or nephrological disorders; or vitamin D_3 (cholecalciferol) intake more than 600 IU/d.[13]

The offspring were recruited to the $COPSAC_{2010}$ birth cohort and followed up by the study pediatricians with scheduled visits at 1 week, 1, 3, 6, 12, 18, 24, 30, and 36 months, and with acute visits for any respiratory or skin-related symptoms. The symptom burden between visits was captured with daily diary cards monitoring: (1) significant troublesome lung symptoms including components of cough, wheeze, and dyspnea; (2) skin symptoms; and (3) respiratory tract infections. The study pediatricians acted as general practitioners for the cohort and were solely responsible for diagnosis and treatment of persistent wheeze, asthma, allergy, and eczema adhering to predefined algorithms and blinded to the intervention.[13]

Study Intervention

Women were randomized 1:1 to a daily dose of 2400 IU vitamin D_3 supplementation or matching placebo tablets (Camette A/S) from pregnancy week 24 to 1 week postpartum. In addition, all women were instructed to continue supplementation of 400 IU of vitamin D_3 during pregnancy as recommended by the Danish National Board of Health; thus, the study is a dose comparison of 2800 IU/d vs 400 IU/d of vitamin D_3 supplementation. Women were randomized using a computer-generated list of random numbers, supplied by an external investigator who had no further involvement in the RCT. The intervention code was unblinded when the youngest child reached age 3 years or in case of a medical emergency.

The mother's serum vitamin D level was measured[15,16] at time of randomization corresponding to pregnancy week 24 and at the first visit after birth (ie, 1 week postpartum) (Supplement 2), when the women stopped the supplement. This allowed assessment of adherence to the treatment plan, which was further complemented by counting returned tablets.

All the women also participated in a concomitant factorial designed, double-blind RCT of 2.4 g per day of long-chain n-3 polyunsaturated fatty acids (PUFAs) during pregnancy (ClinicalTrials.gov: NCT00798226).

Primary End Point

Persistent wheeze was diagnosed according to a previously validated quantitative algorithm[17,18] requiring all of the following: (1) recurrent wheeze (verified diary recordings of ≥5 episodes of troublesome lung symptoms [cough, wheeze, and/or dyspnea] lasting ≥3 days within 6 months), (2) typical symptoms of asthma (eg, exercise-induced symptoms, prolonged nocturnal cough, or persistent cough outside common cold), (3) need for intermittent bronchodilator, and (4) response to a 3-month trial of inhaled corticosteroids and relapse upon cessation.[17] Risk of persistent wheeze analyzed by age-at-onset analysis (Cox proportional hazards regression model) from birth to age 3 years was the primary end point.

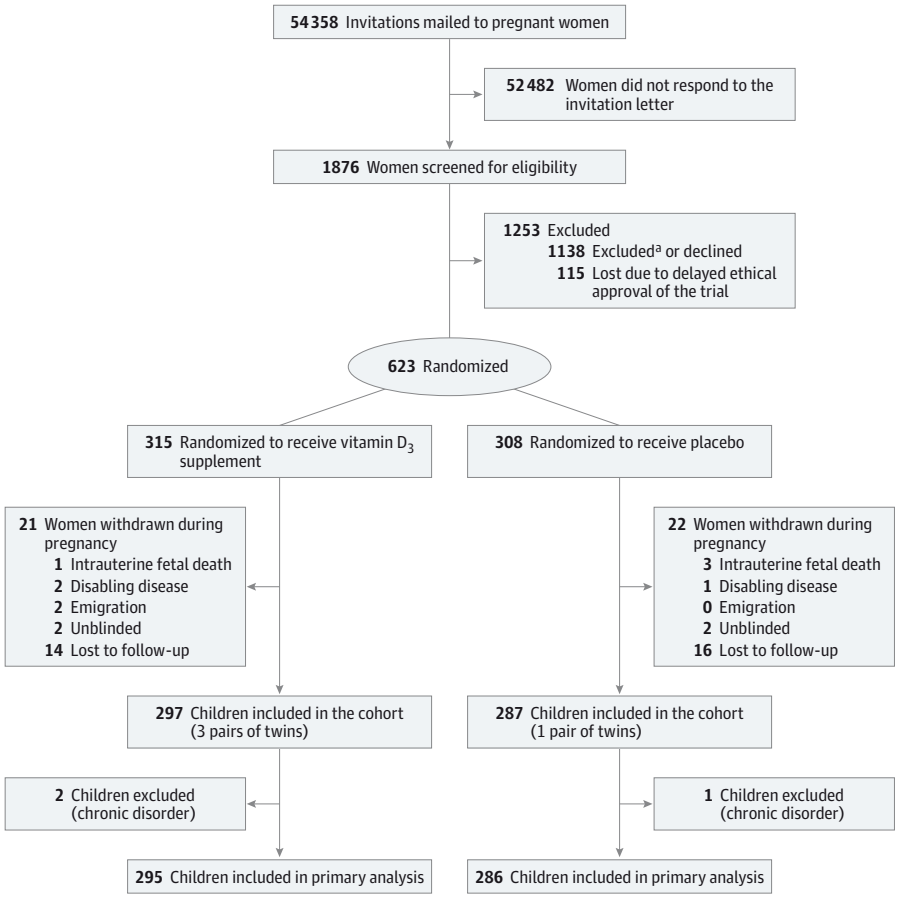
Secondary End Points

Asthma was diagnosed in children fulfilling the persistent wheeze criteria at age 3 years.

Episodes of troublesome lung symptoms included the number of episodes of troublesome lung symptoms lasting 3 or more consecutive days in the first 3 years of life.

Upper respiratory tract infections included episodes of common cold, acute tonsillitis, croup, and acute otitis media until age 3 years.[13]

Figure 1. Flow of Participants Through the $COPSAC_{2010}$ Vitamin D RCT



COPSAC indicates Copenhagen Prospective Studies on Asthma in Childhood; RCT, randomized clinical trial.

[a] Exclusion criterions were gestational age above week 26, any endocrine, cardiovascular, or nephrological disorders, or vitamin D intake more than 600 IU per day.

Lower respiratory tract infections included pneumonia and bronchiolitis. Pneumonia was diagnosed in children with significant cough, tachypnea, fever, and abnormal lung stethoscopy, whereas bronchiolitis was defined as cough, tachypnea, chest retractions, auscultative widespread crepitation, or rhonchi in an infant below 1 year.[19-21]

Airway immunology was assessed at age 1 month by measuring unstimulated levels of 20 cytokines and chemokines in airway mucosal lining fluid sampled by a nasosorption technique as previously detailed[22-24] (Supplement 2). The 20 cytokines and chemokines include interleukin (IL)-12p70, CXCL10 (interferon gamma-induced protein [IP]-10), interferon (IFN)-γ, tumor necrosis factor (TNF)-α, CCL4 (macrophage inflammatory protein [MIP]-1β), CCL2 (monocyte chemoattractant protein [MCP]-1), CCL13 (MCP-4), IL-4, IL-5, IL-13, CCL11 (eotaxin-1), CCL26 (eotaxin-3), CCL17 (thymus- and activation-regulated chemokine [TARC]), CCL22 (macrophage-derived chemokine [MDC]), IL-17, IL-1β, CXCL8 (IL-8), transforming growth factor (TGF)-β1, IL-10, and IL-2.

Systemic low-grade inflammation was determined by measuring serum levels of high-sensitivity C-reactive protein, IL-6, TNF-α, and CXCL8 (also known as IL-8) at age 6 months.[25]

Allergic sensitization was diagnosed at 6 and 18 months by any skin prick test (ALK-Abelló) result 2 mm or larger or specific immunoglobulin E (IgE) level of 0.35 kU_A/L or higher against raw milk, pasteurized eggs, dogs, or cats (ImmunoCAP; Thermo Fischer Scientific).[13]

Eczema at age 0 through 3 years was diagnosed according to the criteria of Hanifin and Rajka[26] including typical morphology and localization of skin lesions.[27,28]

Safety

Parents were routinely interviewed about the mother's medical history during pregnancy and the health of the child(ren) at all scheduled and unscheduled visits to the research unit. All diagnoses were registered online in the dedicated COPSAC database.

Study Power

The prespecified sample size calculation found that 708 participants (354 in each group) would be required to obtain 80% power to detect a difference between the treatment groups (2-tailed α = .05) based on a 12% expected frequency of persistent wheeze in the control group (estimated from the 16.5% observed in the $COPSAC_{2000}$ high-risk cohort) and an effect of 0.5 in the vitamin D_3 group.

Table 1. Baseline Characteristics of Mothers, Children, and Births in the $COPSAC_{2010}$ Vitamin D RCT

	No. (%)		
		Randomization	
	All (n = 581)	Vitamin D_3 (n = 295 [51%])	Control (n = 286 [49%])
Mothers			
Socioeconomics			
Maternal			
Age at birth, mean (SD), y	32.3 (4.3)	32.5 (4.4)	32.0 (4.3)
Asthma[a]	152 (26)	79 (27)	73 (26)
Maternal educational level[b]			
Low	45 (8)	20 (7)	25 (9)
Medium	375 (65)	186 (63)	189 (66)
High	160 (27)	88 (30)	72 (25)
Household annual income[c]			
Low	54 (9)	27 (9)	27 (9)
Medium	304 (53)	151 (51)	153 (54)
High	222 (38)	116 (40)	106 (37)
Pregnancy			
Smoking	46 (8)	20 (7)	26 (9)
Cat or dog in the home during pregnancy	202 (35)	92 (31)	110 (38)
Antibiotic usage during pregnancy	205 (35)	103 (35)	102 (37)
Participation in the long-chain n-3 PUFA RCT	581 (100)	295 (51)	286 (49)
Serum vitamin D level, mean (SD), ng/mL	31 (10)	31 (10)	31 (10)
Children			
Male	298 (51)	155 (53)	143 (50)
Age at unblinding, mean (SD), y	4.1 (0.5)	4.1 (0.5)	4.1 (0.5)
White	555 (96)	283 (96)	272 (95)
Season of birth			
Winter	209 (36)	106 (36)	103 (36)
Spring	106 (18)	53 (18)	53 (18)
Summer	120 (21)	63 (21)	57 (20)
Fall	146 (25)	73 (25)	73 (26)
Births			
Term birth >37 wk	561 (97)	284 (96)	277 (97)
Primiparity	263 (45)	122 (41)	141 (49)
Intrapartum antibiotics	183 (32)	97 (33)	86 (30)
Antibiotics to the neonate	14 (2)	5 (2)	9 (3)
Apgar score at 5 min <10	26 (5)	14 (5)	12 (4)
Neonate hospitalized after birth	56 (10)	29 (10)	27 (9)
Section			
Cesarean	128 (22)	69 (23)	59 (20)
Emergency	71 (12)	39 (13)	32 (11)
Elective	57 (10)	30 (10)	27 (9)

Abbreviations: COPSAC, Copenhagen Prospective Studies on Asthma in Childhood; PUFA, polyunsaturated fatty acids; RCT, randomized clinical trial.

SI Conversion: to convert vitamin D to nmol/L, multiply by 2.496.

[a] History of physician-diagnosed asthma.

[b] Low (primary school, secondary school, or college graduate), medium (tradesman or bachelor's degree), high (master's degree).

[c] Low (below €50 000), medium (€50 000-€110 000), high (above €110 000); €1 = $1.07.

Statistical Analysis

The effect of vitamin D_3 supplementation on the primary end point, age at onset of persistent wheeze, as well as lower respiratory infections and eczema, was analyzed by Cox proportional hazards regression, for which *P* values correspond to Wald tests. The children were retained in the model from birth until age of diagnosis, drop out, or age at their last clinic visit before the RCT was unblinded.

The effect of vitamin D_3 supplementation on the cross-sectional end points of asthma and allergic sensitization was analyzed by logistic regression, whereas the effect on number of episodes of troublesome lung symptoms and upper respiratory tract infections was analyzed by a generalized estimating equation Poisson regression model taking account of repeated participant measurements.

The effect on airway immunology in the vitamin D_3 group vs control group was analyzed and visualized by a principal component analysis (PCA)[29] capturing the overall immunological trends in the data and their relation to the intervention analyzed by Wilcoxon rank sum test. Initially, the mediator levels were log-transformed. Prior to the PCA the variables were scaled to unit variance.

The primary analysis of age at onset of persistent wheeze was an intention-to-treat analysis, which is presented crude and adjusted for sex, birth season, maternal vitamin D level at randomization, and participation in the PUFA RCT. A 2-sided *P* value of .05 for significance was used in all types of analyses, which were conducted using SAS (SAS Institute), version 9.3, and MATLAB R2014a (Natick). No imputation was performed for missing data.

Additional methodological details are outlined in Supplement 2 and the $COPSAC_{2010}$ design paper.[13]

Results

Of 1876 pregnant Danish women screened for eligibility to the $COPSAC_{2010}$ mother-child cohort, 1138 were not included or declined to participate. We randomized 623 of the 738 eligible women from March 4, 2009, to November 17, 2010, as the ethical approval of the vitamin D trial was delayed during enrollment of the first 115 eligible women into the $COPSAC_{2010}$ cohort. In addition, 43 women were withdrawn before childbirth. We unblinded 8 randomizations during pregnancy (including 4 intrauterine deaths) and 3 children were excluded due to chronic disorders, leaving 581 children for the primary analysis (**Figure 1**). The clinical follow-up rate of the children was 94% at age 3 years.

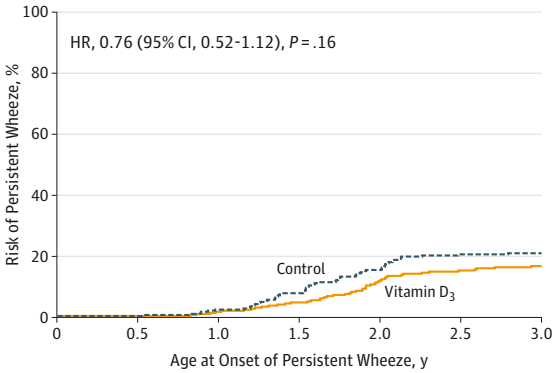
At randomization, 52% of the women had sufficient vitamin D (25-hydroxyvitamin D) levels (>30 ng/mL; to convert to nmol/L, multiply by 2.496), 34% had insufficient levels (20-30 ng/mL) and 14% had deficient levels (<20 ng/mL). Baseline characteristics of the participating mother-child pairs are outlined in **Table 1** showing no clinically important differences in maternal serum vitamin D levels at randomization (mean [SD] level: 31 ng/mL [10] for the vitamin D_3 group vs 31 ng/mL [10] for the control group) or season of birth (eg, winter birth: 36% for the vitamin D_3 group vs 36% for the control group).

Adherence to the intervention, defined as mothers taking more than 80% of the prescribed tablets, was 74%. The intervention resulted in a significant increase in maternal serum vitamin D level in the treatment group (mean [SD] at randomization vs postpartum: vitamin D_3 group, 31 ng/mL [10] at randomization vs 43 ng/mL [14] postpartum; control group, 31 ng/mL [10] at randomization vs 29 ng/mL [13] postpartum; mean between-group difference, 13 ng/mL [95% CI, 11-16], $P < .001$) (eFigure in Supplement 2). Correspondingly, the percentage of women with sufficient levels of vitamin D (>30 ng/mL) after the intervention was 81% in the vitamin D_3 group compared with 44% in the control group (mean difference, 37% [95% CI, 30%-45%], $P < .001$).

Vitamin D_3 Supplementation and Risk of Persistent Wheeze

During the first 3 years of life, persistent wheeze was diagnosed in 104 (18%) of the 581 children, with 47 affected children (16%) in the vitamin D_3 group vs 57 affected children (20%) in the control group. The intention-to-treat analysis of age at onset of persistent wheeze did not show a significant effect on risk of persistent wheeze from vitamin D_3 supplementation during pregnancy (hazard ratio [HR], 0.76 [95% CI, 0.52-1.12], $P = .16$) (**Figure 2**).

Figure 2. Effect of Vitamin D_3 Supplementation on Risk of Persistent Wheeze in Children in the $COPSAC_{2010}$ Vitamin D RCT



No. at risk				
Vitamin D_3	295	287	253	198
Control	286	275	231	184

COPSAC indicates Copenhagen Prospective Studies on Asthma in Childhood; HR, hazard ratio; RCT, randomized clinical trial. *P* values were evaluated using the Wald test.

Sex, season of birth, maternal vitamin D_3 level at randomization, and the long-chain n-3 PUFA RCT did not interact with the supplementation effect ($P > .05$ for all interaction analyses). Adjusting the primary analysis for these variables did not modify the results (HR, 0.75 [95% CI, 0.51-1.10], $P = .14$) (**Table 2**). An analysis of the full 3 to 5 years follow-up at the time of unblinding of the study showed similar results (HR, 0.78 [95% CI, 0.54-1.13], $P = .20$).

A post hoc analysis of the effect of vitamin D_3 levels after the intervention showed a reduced risk of persistent wheeze per 4 ng/mL increase in maternal serum vitamin D level after intervention (HR, 0.94 [95% CI, 0.89-0.99], $P = .03$). Accordingly, the risk of persistent wheeze was increased in children born to mothers with postinterventional vitamin D_3 levels in the lowest vs middle and upper tertiles (**Figure 3**). These findings are consistent with the effect estimate for persistent wheeze of 0.76 (approximately 20% reduced risk) associated with the intervention, which on average resulted in a 13 ng/mL higher level of vitamin D after the intervention. Adjusting the analysis for sex, season of birth, maternal smoking during pregnancy, and vitamin D_3 level at randomization did not modify the result (HR, 0.93 [95% CI, 0.88-0.99], $P = .02$).

Vitamin D_3 Supplementation and Secondary End Points

Episodes of Troublesome Lung Symptoms

Vitamin D_3 supplementation during pregnancy resulted in significantly fewer episodes of troublesome lung symptoms during the first 3 years of life in the vitamin D_3 group vs the control group (mean episodes, 5.9 in the vitamin D_3 group vs 7.2 in the control group; incidence risk ratio [IRR], 0.83 [95% CI, 0.71-0.97], $P = .02$ (Table 2).

Table 2. Primary and Secondary End Point Analyses in the $COPSAC_{2010}$ Vitamin D RCT

	Vitamin D_3 Group		Control Group		Difference, % (95% CI)	Estimate (95% CI)	*P* Value
	Total, No.	Cases, No. (%)	Total, No.	Cases, No. (%)			
Primary End Point							
Persistent wheeze, 0-3 y	295	47 (16)	286	57 (20)	4 (−2 to 10)	HR, 0.76 (0.52 to 1.12)	.16
Persistent wheeze, 0-3 y, adjusted[a]	292	47 (16)	285	57 (20)	4 (−2 to 10)	aHR, 0.75 (0.51 to 1.10)	.14
Secondary End Points							
Episodes of troublesome lung symptoms, 0-3 y[b]	287	5.9 (5.2 to 6.6)	278	7.2 (6.4 to 8.1)	1.3 (0.2 to 2.4)	IRR, 0.83 (0.71 to 0.97)	.02
Asthma, 3 y	278	32 (12)	271	47 (14)	2 (−3 to 8)	OR, 0.82 (0.50 to 1.36)	.45
Upper respiratory tract infections, 0-3 y[c]	295	5.2 (4.8 to 5.5)	284	5.3 (4.9 to 5.6)	0.1 (−0.6 to 0.4)	IRR, 0.99 (0.90 to 1.09)	.84
Lower respiratory tract infections, 0-3 y	292	94 (32)	284	95 (33)	1 (−1 to 6)	HR, 0.96 (0.72 to 1.27)	.76
Eczema, 0-3 y	295	68 (23)	286	72 (25)	2 (−5 to 9)	HR, 0.90 (0.65 to 1.26)	.55
Allergic Sensitization, 0-3 y[d]							
Skin prick test	294	24 (8)	283	19 (7)	−1 (−6 to 3)	OR, 1.24 (0.66 to 2.31)	.51
Specific IgE	289	34 (12)	278	22 (8)	−4 (−9 to 2)	OR, 1.55 (0.89 to 2.73)	.13

Abbreviations: aHR, adjusted hazard ratio; COPSAC indicates Copenhagen Prospective Studies on Asthma in Childhood; HR, hazard ratio; IgE, immunoglobulin E; IRR, incidence risk ratio; OR, odds ratio; RCT, randomized clinical trial.

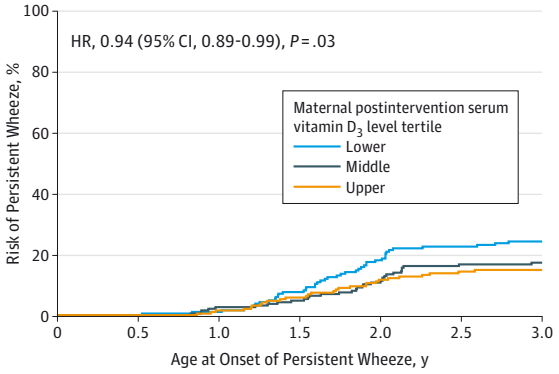
[a] Adjusted for sex, birth season, the long-chain n-3 polyunsaturated fatty acids randomized clinical trial, and maternal vitamin D at randomization.

[b] Reported as mean No. of episodes (95% CI) at age 0 to 3 y.

[c] Reported as mean No. of episodes per year (95% CI) at age 0 to 3 y.

[d] Positive result for allergic sensitization at age 6 months or 18 months against dogs, cats, milk, or eggs.

Figure 3. Effect of Maternal Serum Vitamin D_3 Level at 1 Week Postpartum (n = 573) on Risk of Persistent Wheeze in Children in the $COPSAC_{2010}$ Vitamin D RCT



No. at risk by maternal serum vitamin D_3 level tertile

Lower	190	182	147	108
Middle	191	184	165	129
Upper	192	189	165	141

COPSAC indicates Copenhagen Prospective Studies on Asthma in Childhood; HR, hazard ratio; RCT, randomized clinical trial. To convert vitamin D to nmol/L, multiply by 2.496. The HR describes risk of persistent wheeze per 4 ng/mL increase in maternal serum vitamin D level after intervention. Mean (range) of tertiles: lower tertile, 20 ng/mL (6-29); middle tertile, 35 ng/mL (29-41); upper tertile, 53 ng/mL (42-103). *P* values were evaluated using the Wald test.

Asthma

Asthma at age 3 years was diagnosed in 69 children (13%) of 549, with 32 children (12%) in the vitamin D_3 group and 47 children (14%) in the control group (odds ratio [OR], 0.82 [95% CI, 0.50-1.36], P = .45).

Airway Immunology

The purely data-driven PCA showed a uniform up-regulated mediator pattern in principal component 1, which explained 54% of the variation in the data and significantly separated children in the intervention groups (P = .04) (**Figure 4** and eTable 1 in Supplement 2).

Systemic Low-Grade Inflammation

Children in the vitamin D_3 group vs control group did not show a significant difference in levels of high-sensitivity C-reactive protein at age 6 months (median [interquartile range], 1.10 mg/L [0.56-4.23] vs 1.45 mg/L [0.51-4.90], P = .09). There were also no significant differences in levels of IL-6, TNF-α, or CXCL8 between children in the vitamin D_3 group vs control group (eTable 2 in Supplement 2).

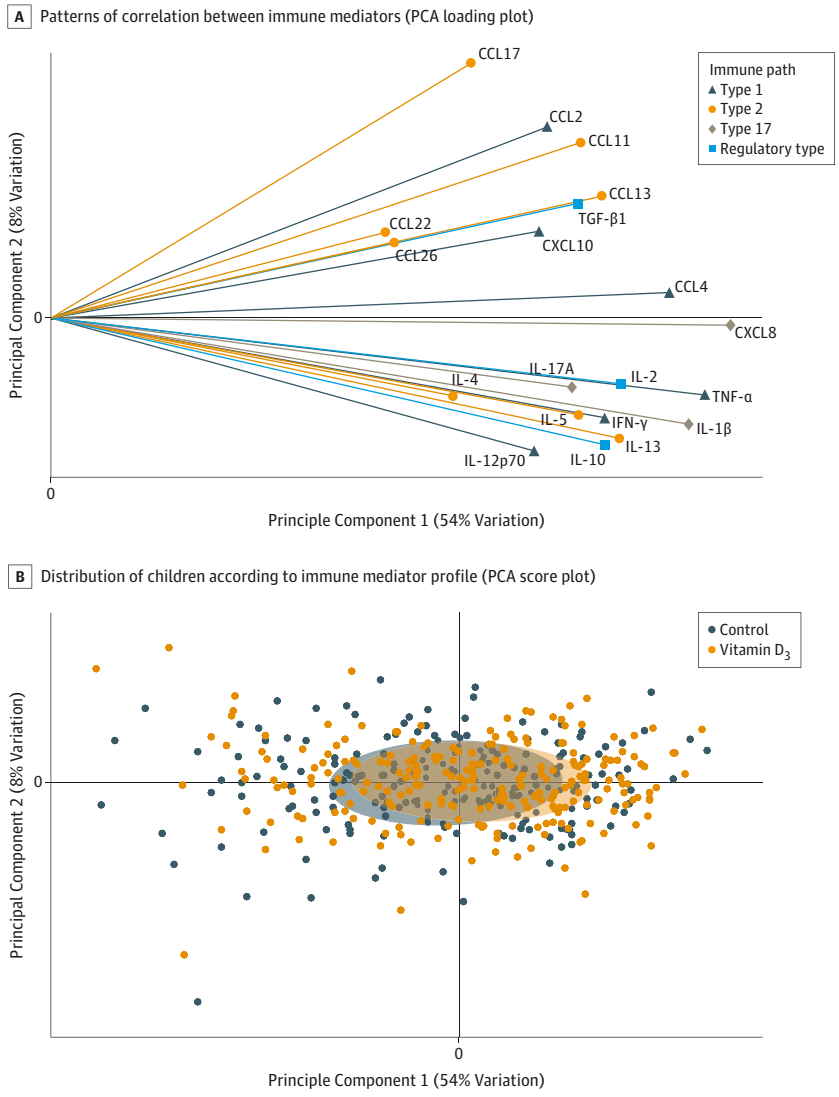
Respiratory Tract Infections

Vitamin D_3 supplementation did not modify the number of upper respiratory tract infections (5.2/y in the vitamin D_3 group vs 5.3/y in the control group; IRR, 0.99 [95% CI, 0.90-1.09], P = .84) or lower respiratory tract infections at age 0 to 3 years (94 children [32%] in the vitamin D_3 group vs 95 children [33%] in the control group; HR, 0.96 [95% CI, 0.72-1.27], P = .76).

Allergic Sensitization

The risk of allergic sensitization was not significantly affected by the vitamin D_3 supplement assessed by either skin prick test (OR, 1.24 [95% CI, 0.66-2.31], P = .51) or specific IgE level (OR, 1.55 [95% CI, 0.89-2.73], P = .13).

Figure 4. Effect of Vitamin D_3 Supplementation on the Neonatal Airway Immune Profile in the $COPSAC_{2010}$ Vitamin D RCT



COPSAC indicates Copenhagen Prospective Studies on Asthma in Childhood; IFN, interferon; IL, interleukin; PCA, principal component analysis; RCT, randomized clinical trial; TGF, transforming growth factor; TNF, tumor necrosis factor. Panel A, 0/0 is the base for interpreting how well a particular mediator is explained. Increasing length of the arrows from 0/0 corresponds to an increased proportion of the mediator being explained by the model. Principal component 1 reflects the overall immune activity regardless of individual mediators, accounting for 54% of the variation in data. Principal component 2 reflects a pattern related to pertubation of CCL17, CCL2, CCL11, CCL13, and TGF-1β vs IL-12p70, IL-10, IL-13, IL-5, IFN-γ, IL-1β, TNF-α, IL-4, IL-2, and IL-17α, accounting for additionally 8% of the variation. Thus, children with loading values above 0 on principal component 2 have an immune profile skewed toward CCL17, CCL2, CCL11, CCL13, and TGF-1β, whereas children with loading values below 0 have an immune profile skewed toward IL-12p70, IL-10, IL-13, IL-5, IFN-γ, IL-1β, TNF-α, IL-4, IL-2, and IL-17α. Panel B, each point corresponds to a child, and 2 points closely positioned indicate a similar profile. The ellipses reflect the 2-dimensional distribution of children in the vitamin D_3 group (orange) vs control group (blue), for which 0/0 is the multivariate average of the distribution. Principal component 1 reflects a significant discrepancy between the groups (P = .04), pointing at generally higher airway immune activity in children from mothers supplemented with vitamin D_3 during pregnancy.

Eczema

Eczema development was unaffected by the intervention (68 [23%] for the vitamin D_3 group vs 72 [25%] for the control group; HR, 0.90 [95% CI, 0.65-1.26], P = .55).

Safety

Intrauterine death was observed in 1 fetus (<1%) in the vitamin D_3 group vs 3 fetuses (1%) in the control group, any congenital malformations in 17 neonates (5%) in the vitamin D_3 group vs 23 neonates (8%) in the control group, and child hospitalization after birth in 32 children (11%) in the vitamin D_3 group vs 28 children (10%) in the control group (eTable 3 in Supplement 2).

Discussion

Maternal supplementation with 2800 IU/d vs 400 IU/d of vitamin D_3 during the third trimester of pregnancy did not result in a reduced risk of persistent wheeze through age 3 years in the offspring. However, a clinically important protective effect cannot be excluded as the lower limit of the HR CI was 0.52. The possibility of a protective effect is further supported by secondary end point analyses showing a significant reduction in number of episodes of troublesome lung symptoms, an up-regulated neonatal airway immune profile. However, the development of asthma, upper and lower respiratory tract infections, allergic sensitization, and eczema was unaffected by the vitamin D_3 supplementation.

The main limitation of the study is a reduced statistical power to detect an effect on the primary end point of persistent wheeze. In addition, the vitamin D_3 supplementation dose may have been too low[30,31] as suggested by the significant decreased risk of persistent wheeze per increase in maternal serum vitamin D level at cessation of the trial (Figure 3). The normal level of vitamin D in a mother during pregnancy for optimalimmune and lung development is unknown and might be as high as 40-60 ng/mL.

Also, we may have begun supplementation too late, while only 81% of the women had serum vitamin D_3 above 30 ng/mL after the intervention. Initiating vitamin D_3 supplementation at earlier pregnancy stages may be beneficial, as recent data in humans suggest that vitamin D affects fetal lung development as early as the start of the second trimester.[32] However, our data suggesting no effect of early (pre-intervention) levels of vitamin D argue against this hypothesis. Finally, the study did not include postnatal supplementation of the children, which could have induced an additive effect if the effects of maternal supplementation declined postnatally.

The primary strength of the study is the single-center design with standardized diagnoses performed solely by the experienced COPSAC research pediatricians. Other multicenter studies have used various clinicians with different training and experience, which may introduce diagnostic heterogeneity. The longitudinal clinical assessments at both scheduled and unscheduled visits to the research center accompanied by daily diary recordings of troublesome lung symptoms to capture disease burden between visits provided a highly specific primary end point with strong data on age at onset, which is a major advantage compared with studies using retrospective or cross-sectional unspecific community-based diagnoses.[7-9] This approach is important as diagnosis of wheezy disorders in young children is heterogeneous outside research settings due to lack of objective tests and nonstandardized diagnostic procedures.[2] Our close clinical surveillance resulted in a high follow-up rate of the children during the 3-year double-blinded period with more than 94% completing the year 3 visit.

Vitamin D levels are associated with lifestyle factors such as diet, sun exposure, physical activity, and tobacco smoke exposure, which confer a risk of residual confounding in observational studies.[33] Our placebo-controlled study design with unbiased randomization mitigated such confounding and allowed us to examine the isolated effect of vitamin D_3 supplementation. In addition, the mothers had good adherence to the intervention with 74% taking more than 80% of the intervention tablets with no differences between intervention groups.

Vitamin D deficiency may lead to wheezy disorders by interfering with fetal lung cell maturation during pregnancy and subsequent lung function development.[34] An alternative mechanism founded on alterations of the airway microbiome by induction of the antimicrobial cathelicidin in bronchial epithelial cells has also been proposed.[35] It is a common belief that vitamin D possesses a range of immune regulatory properties, which are important for immune constitution in early life.[5] Our finding of a significantly up-regulated airway immune profile at age 1 month in the vitamin D_3 supplemented group supports this hypothesis and the interrelationship between the relative up-regulations of immune mediators related to type 1, type 2, type 17, and regulatory immune paths may protect against development of wheeze.

Vitamin D immune-modulatory mechanisms have been suggested to increase the frequency of respiratory tract infections,[12] leading to virus-induced wheezing.[36] Such a pathway is not supported by our results showing no effect of vitamin D_3 supplementation on either upper or lower respiratory tract infections.

Effective preventive strategies to alleviate the large burden of childhood wheezing and related disorders represent a major unmet clinical need. This RCT of vitamin D_3 supplementation during pregnancy did not show a statistically significant effect on the primary end point of persistent wheeze, although a clinically important protective effect cannot be excluded, and a protective effect is suggested by the observed effect on airway immunology and symptomatic episodes. Therefore, further studies with a larger sample size, higher dose, and potentially earlier intervention during pregnancy and postnatal supplementation should be performed to establish the potential benefits of vitamin D_3 supplementation to pregnant women to reduce occurrence of wheezy disorders in the offspring.

Conclusions

The use of 2800 IU/d of vitamin D_3 during the third trimester of pregnancy compared with 400 IU/d did not result in a statistically significant reduced risk of persistent wheeze in the offspring through age 3 years. However, interpretation of the study is limited by a wide CI that includes a clinically important protective effect.

ARTICLE INFORMATION

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