Supplementary Online Content

Chawes BL, Bønnelykke K, Stokholm J, et al. Effect of vitamin D_3 supplementation during pregnancy on risk of persistent wheeze in the offspring: a randomized clinical trial. *JAMA*. doi:10.1001/jama.2015.18318.

This supplementary material has been provided by the authors to give readers additional information about their work.

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eMethods.

Maternal serum Vitamin D levels:
The venous blood samples obtained before and after the intervention were centrifuged for 10 min at 4300 rpm to separate serum, and thereafter frozen at -80^{o}C until analysis. The serum samples were transported on dry ice for duplicate analyses for 25-hydroxyvitamin D_2 (25(OH)-Vitamin D2) and 25(OH)-Vitamin D_3 at the Dept. of Clinical Biochemistry, Aarhus University Hospital, Denmark. Serum 25-hydroxyvitamin D levels were analyzed by isotope dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS)[1,2]. Calibrators traceable to NIST SRM 972 (Chromsystems, DE) were used. Mean coefficients of variation (CV) for 25(OH)-Vitamin D_3 were 6.4% and 9.1% at levels of 66.5 and 21.1 nmol/L and for 25(OH)-Vitamin D_2 the CV values were 8.8% and 9.4% at levels of 41.2 and 25.3 nmol/L. The average of the combined 25(OH)-Vitamin D values was calculated and used in the analysis. If both 25(OH)-Vitamin D_2 and 25(OH)-Vitamin D_3 were under the detection level, the combined value was defined as equal to 10 nmol/L.

Neonatal airway immunology assessment:
Unstimulated airway mucosal lining fluid was sampled at age one month with 3 x 15-mm strips of filter paper (Accuwik Ultra, fibrous hydroxylatedpolyester sheets, cat. no. SPR0730; Pall Life Sciences, Portsmouth, Hampshire, UK). The strips were inserted bilaterally into the nasal cavity against the anterior part of the inferior turbinate, where it was left for absorption for 2 minutes. After removal, the filter papers were immediately frozen to -80°C and stored until analysis. The samples were analyzed in two batches and after thawing, the mucosal lining fluid was analyzed for levels of IL-12p70, CXCL10, IFN-γ, TNF-α, CCL4, CCL2, CCL13, IL-4, IL-5, IL-13, CCL11, CCL26, CCL17, CCL22, IL-17, IL-1β, CXCL8, TGF-β1 (transforming growth factor), IL-10, and IL-2, as previously described in details[3–5]. The lower limit of detection was set as the mean signal from blanks +3 standard deviations (SD).

eReferences.

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3. Chawes, B. L. K. *et al.* A novel method for assessing unchallenged levels of mediators in nasal epithelial lining fluid. *J. Allergy Clin. Immunol* **125,** 1387–1389.e3 (2010).
4. Følsgaard, N. V. *et al.* Neonatal Cytokine Profile in the Airway Mucosal Lining Fluid Is Skewed by Maternal Atopy. *Am. J. Respir. Crit. Care Med.* **185,** 275 –280 (2012).
5. Følsgaard, N. V. *et al.* Pathogenic bacteria colonizing the airways in asymptomatic neonates stimulates topical inflammatory mediator release. *Am. J. Respir. Crit. Care Med.* **187,** 589–595 (2013).

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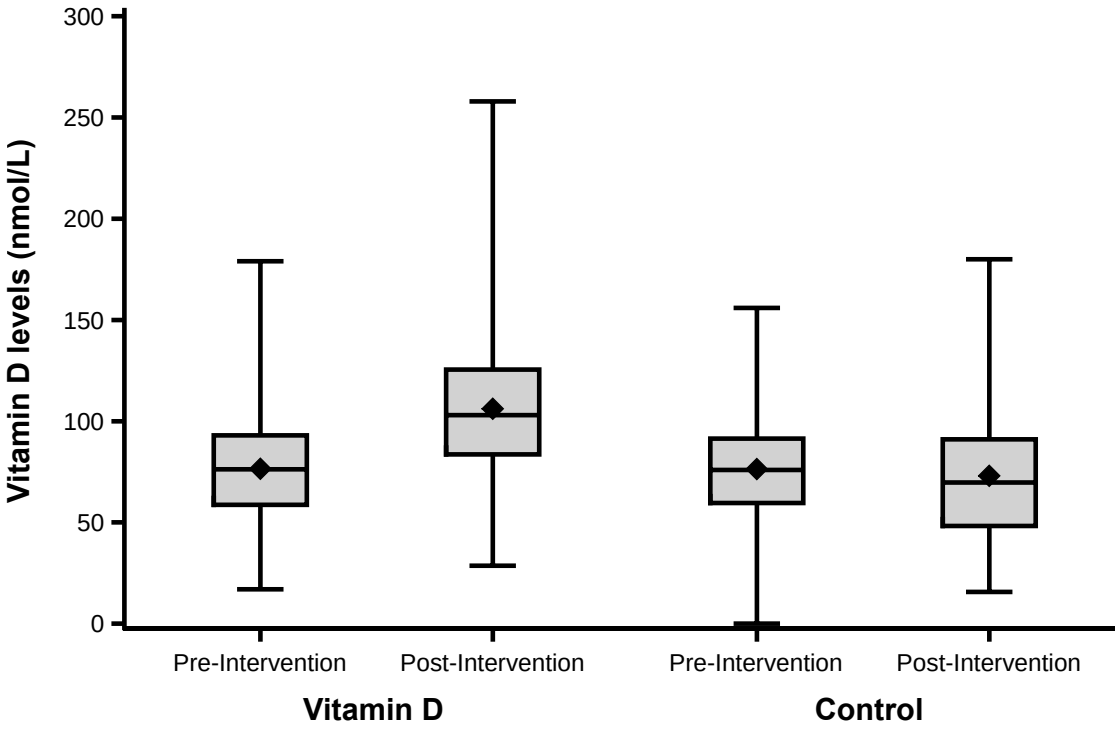
eFigure 1. Pre- and post-intervention levels of maternal serum Vitamin D among women receiving vitamin D_3 and control.

Diamond data marker = mean, extremes of the box = interquartile range, middle line in the box = median, error bars = minimum and maximum.

Pre-intervention N=577, vitamin D_3 N=292, control N=285
Post-intervention N=573, vitamin D_3 N=292, control N=281



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eTable 1. Levels of 20 unstimulated airway immune mediators measured in the children at age 1 month in the vitamin D_3 vs. control group.

N corresponds to the number of children, where nasal lining fluid was available for assessment of immune mediators.

	Randomization	
	Vitamin D_3 (N=289)	**Control (N=276)**
Mediator	**Median (IQR), pg/mL**	
IL-12p70	5.33 (2.33 – 10.65)	4.22 (2.00 – 9.01)
CXCL10	489.39 (214.95 – 1505.00)	483.17(192.84 – 1791.68)
IFN-g	5.33 (2.05 – 15.11)	4.17 (1.83 – 12.32)
TNFα	37.95 (12.08 – 99.37)	23.22 (9.09 – 78.84)
CCL4	206.67 (59.74 – 603.32)	130.83 (49.66 – 459.01)
CCL2	147.82 (72.89 – 269.23)	135.71 (82.62 – 248.31)
CCL13	16.75 (12.17 – 25.17)	17.03 (10.65 – 25.47)
IL-4	2.51 (0.87 – 4.91)	2.07 (0.68 – 5.12)
IL-5	3.33 (1.15 – 6.93)	2.82 (1.06 – 6.13)
IL-13	17.79 (9.39 – 31.92)	14.59 (5.99 – 26.81)
CCL11	91.23 (52.59 – 154.58)	79.06 (47.55 – 130.94)
CCL26	81.74 (22.12 – 148.19)	55.74 (21.80 – 141.84)
CCL17	15.81 (10.32 – 25.25)	14.81 (8.34 – 23.32)
CCL22	71.47 (46.17 – 168.68)	62.15 (40.24 – 125.37)
IL-17	1.68 (0.56 – 5.64)	1.74 (0.43 – 5.27)
IL-1β	113.58 (24.66 – 611.76)	87.06 (21.14 – 443.72)
CXCL8	4816.36 (1545.43 – 6871.50)	3650.20 (1049.20 – 6181.31)
TGF-β1	54.78 (40.30 – 76.26)	52.58 (36.07 – 72.14)
IL-10	22.91 (10.44 – 50.04)	19.76 (7.79 – 44.12)
IL-2	22.36 (10.23 – 43.68)	16.90 (7.76 – 36.01)

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eTable 2. Levels of hs-CRP, IL-6, TNF-α and CXCL8 measured in the children at age 6 months in the vitamin D_3 vs. control group.

N corresponds to the number of children, where blood was available for assessment of systemic inflammation markers.

	Randomization		
	Vitamin D_3 (N=286)	**Control (N=274)**	**p-value***
Mediator	**Median (IQR)**		
hs-CRP, mg/L	1.10 (0.56-4.23)	1.45 (0.51-4.90)	0.09
IL-6, ng/L	0.19 (0.12-0.34)	0.19 (0.12-0.35)	0.96
TNF-α, ng/L	3.43 (2.49-4.60)	3.15 (2.21-4.37)	0.12
CXCL8, ng/L	8.48 (7.34-8.84)	8.20 (6.96-7.23)	0.06

*Non-parametric Wilcoxon rank sum test.

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eTable 3. Safety assessment in relation to the vitamin D_3 RCT during pregnancy.

	Randomization	
Adverse Events	**Vitamin D_3**	**Control**
Mother events: women available for analysis, N (%)	**315 (51)**	**308 (49)**
Death, N (%)	0 (0)	0 (0)
Intrauterine death, N (%)	1 (0)	3 (1)
Gestational diabetes, N (%)	5 (2)	9 (3)
Preeclampsia, N (%)	16 (5)	12 (4)
Days hospitalized after birth, mean (SD)	2.7 (2.3)	2.7 (3.0)
Mother hospitalized >5 days, N (%)	26 (9)	24 (8)
Emergency caesarean section, N (%)	44 (14)	35 (12)
Antibiotics in third pregnancy trimester, N (%)	52 (17)	57 (19)
Infection in third pregnancy trimester, N (%)	82 (27)	97 (34)
Infant events: children available for analysis, N (%)	**297 (51)**	**287 (49)**
Death, N (%)	0 (0)	0 (0)
Prematurity		
Gestational age: <28 weeks, N (%)	0 (0)	0 (0)
Gestational age: 28 to <32 weeks, N (%)	2 (1)	0 (0)
Gestational age: 32 to <37 weeks, N (%)	11 (3)	11 (3)
Child Hospitalized after Birth, N (%)	32 (11)	28 (10)
Any Congenital Malformation, N (%)	17 (5)	23 (8)

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