**Associations between maternal vitamin D levels during pregnancy and allergic outcomes in the offspring in the first 5 years of life.**

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**Running title:** Maternal Vitamin D and allergic outcomes

**Keywords** : maternal vitamin D, eczema, allergic outcomes, family history of allergy

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**To the Editor,**

There is increasing evidence to support the Developmental Origins of Health and Disease (DOHaD) concept that fetal life and early life exposures are important determinants of fetal immune regulation and the development of disorders in later life, including allergies (1). Among the early life exposures, maternal vitamin D [25(OH)D] status during pregnancy, in particular 25(OH)D insufficiency (25(OH)D concentration of 50-75nmol/l) and/or 25(OH)D deficiency (25(OH)D concentration <50nmol/l) has been associated with allergic outcomes such as eczema, asthma and rhinitis in their offspring (2).

Vitamin D in humans is largely derived from the synthesis of 7-dehydrocholesterol in the skin from UVB solar radiation or ingestion via the gastrointestinal tract through food and supplements. It is converted to 25-hydroxy vitamin D in the liver and undergoes hydroxylation in the kidney, by the enzyme 25-hydroxyvitamin D-1 α-hydroxylase, into 1, 25-dihydroxyvitamin D – the active form of Vitamin D(3). Vitamin D receptors have an ubiquitous expression and can be found on keratinocytes and immune cells such as dendritic cells and natural killer cells which are able to modulate cytokine responses, which in turn influence the development of allergic outcomes (4). It has also been postulated that increasing sedentary indoor lifestyle and decreasing exposure to sunlight may be responsible for the prevalence of Vitamin D deficiency seen in developed countries today. In addition, other factors such as maternal vitamin D intake, age and body mass index also affect maternal vitamin D status during pregnancy.

Predictive markers of allergic diseases are evident even in early life. Differing levels of cord blood 25-hydroxyvitamin D3 levels have been associated with differences in allergen triggered cytokine responses and clinical eczema at 6 and 12 months of age (5), raising the question of whether factors much earlier in fetal life may modulate this risk. Current studies regarding the relationship between maternal vitamin D status, or antenatal Vitamin D supplementation, and offspring allergic outcomes have demonstrated conflicting results – whilst some data suggests a negative association, others have shown no significant link or even a positive relationship between maternal 25(OH)D levels and atopic outcomes. One study suggested a beneficial effect of prenatal 25(OH)D consumption on offspring allergic outcomes such as asthma (2), however others such as the World Health Organization Guidelines for Allergic Disease Prevention (GLAD-P) study did not find sufficient evidence to support recommending prenatal 25(OH)D supplementation for the prevention of allergic diseases in offspring (6).

Our study aimed to examine possible associations between maternal 25(OH)D levels and specific allergic outcomes in their offspring up to 5 years of age in the prospective mother-offspring cohort study Growing Up in Singapore Towards Healthy Outcomes (GUSTO). Evidence indicates that maternal serum and fetal cord blood 25(OH)D levels are closely correlated and assessment of maternal 25(OH)D status in the third trimester would thus be a good reflection of the infant’s 25(OH)D status at birth and hence its potential influences on immunomodulation and infant allergic outcomes.

The methodology of the GUSTO study has been described previously(7). Briefly, we recruited healthy pregnant mothers who agreed to enroll their offspring for future follow-up. Interviewers gathered information on demographics, family history of allergy, social data and lifestyle factors. Definitions of allergic outcomes were standardized in the questionnaires administered at 3, 6, 9, 12, 15 18, 24, 36, 48 and 60 months to ensure consistency during interviews and home visits. Ethics approval was obtained from the Domain Specific Review Board of Singapore National Healthcare Group and the Centralised Institutional Review Board of SingHealth. Conduct of this study was based on the guidelines in the Declaration of Helsinki.

 Parental report of physician-diagnosed eczema was based on a positive answer to the written question: “Has your child ever been diagnosed with eczema?” “Wheezing” was based on positive answers to the written questions “Has your child ever wheezed?” and “Has your child ever been prescribed with nebulizer/inhaler?”, while “rhinitis” was based on a positive response to the question “Has your child ever had sneezing, running nose, blocked or congested nose, snoring or noisy breathing during sleep or when awake that has lasted for 2 or more weeks duration?” Study team members called the subjects who reported rhinitis to collect information on the number of episodes of rhinitis and the duration of each episode. A case prior to 18 months required a single episode that lasted for at least 4 weeks or two or more episodes each lasting at least 2 weeks. New cases of rhinitis after 18 months were defined by one or more episodes lasting at least 2 weeks.

For allergic outcomes (eczema, rhinitis, wheeze and use of nebulizer) by 18 months, the allergic outcome was classified as absent when the answers for the visits and/or at 18 month were “no.” For allergic outcomes (eczema, rhinitis, wheeze and use of nebulizer) by 36 months, the allergic outcome was classified as absent when the answers for the visits were “no” for the first 18 months and subsequent time points. For allergic outcomes (eczema, rhinitis, wheeze and use of nebulizer) by 60 months, the allergic outcome was classified as absent when the answers for the visits were “no” for the first 18 months and at least 75% of subsequent timepoints. Family history of allergy was defined as positive if the mother, father or an older sibling ever had eczema, asthma or allergic rhinitis.

Allergen sensitization was determined by skin prick testing. Skin prick testing (SPT) to inhalant allergens (house dust mites *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae,* and *Blomia tropicalis*) and food allergens (egg, peanut and cow’s milk) was carried out at 18, 36 and 60 months. At 60 months, skin prick testing was also carried out to shrimp and crab allergens. All of the allergens for skin prick testing were obtained from Greer Laboratories (Lenoir, NC, USA), except for *B. tropicalis*, which was obtained from our laboratory. All tests were interpreted as positive if the wheal was at least 3 mm, and a child was considered as SPT-positive if any one or more of the individual tests was positive with a positive reaction to the positive control (histamine) and a negative reaction to the negative control (saline).

Fasting blood samples were obtained from subjects at 26 weeks pregnancy and isotope-dilution liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS) was used to analyse plasma 25(OH)D levels as 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3 (8). The detection limit was <4nmol/l for both metabolites. Women were categorized as having 25(OH)D sufficiency (25(OH)D ≥ 75nmol/l), insufficiency (25(OH)D concentration of 50-75nmol/l) and/or 25(OH)D deficiency (25(OH)D concentration below 50nmol/l).

Comparison between demographic variables and maternal 25(OH)D status was performed using Pearson’s Chi Square tests and the strength of associations between maternal 25(OH)D status and allergen sensitization, eczema, rhinitis and wheeze were assessed using multivariable logistic regression (adjusting for sex, ethnicity, maternal education levels, maternal age, maternal BMI during pregnancy, family history of allergy, total energy intake during pregnancy and vitamin D supplementation.), using a p value of < 0.05 as significant; we report adjusted odds ratios with exact 95% confidence intervals.

After exclusion of participants who dropped out of the study in early pregnancy, 925 (85.3%) had maternal 25(OH)D data. Out of these 925 participants, 557 (60.2%) had adequate 25(OH)D levels ≥75 nmol/l, 246 (26.6%) had insufficient 25(OH)D levels between 50nmol/l and 75nmol/l while 122 (13.2%) had deficiency in 25(OH)D levels ≤50nmol/l. There were differences in ethnicity, maternal age, maternal education levels, maternal BMI during pregnancy, total energy intake and vitamin D supplementation among the groups. There were more participants of Malay and Indian ethnicity and maternal BMI during pregnancy was higher in the 25(OH)D deficient group (Table 1). In addition, there were more participants with lower maternal education levels and vitamin D supplementation in the 25(OH)D deficient group. The mean maternal age and total energy intake were also lower in the 25(OH)D deficient group. We did not observe any significant associations between maternal 25(OH)D levels and allergic outcomes in the first 5 years of life (Table 2 and 3).

In our study, we found no significant associations between maternal vitamin D levels with allergic outcomes in the first 5 years of life. This is in line with current evidence provided by other cohort studies such as the Southampton Women’s Survey which involved the measurement of serum 25-hydroxyvitamin D at 34 weeks gestation from the mothers of 860 full term children and found no link between maternal vitamin D levels and asthma, wheeze or atopy at age of 6 years (9). Levels of maternal and cord vitamin D levels are known to be correlated, further evidence is provided by the EDEN birth cohort which involved a follow up of 239 newborns to age of 5 years that showed no associations between cord serum vitamin D and allergic rhinitis (10). Besides this, supporting evidence is provided by a randomized controlled trial involving180 pregnant women at 27 weeks gestation who were randomized to receive either no vitamin D, 800 IU ergocalciferol or single oral bolus of 200000 IU cholecalciferol and no differences in atopy, eczema and lung function between the supplemented groups and control was observed in the offspring at the age of 3 years (11).

However, there are other studies such as that in Finland which involved 1669 children with HLA-DQB1-conferred susceptibility for type 1 diabetes that have reported an inverse association between higher maternal intake of vitamin D during pregnancy and rhinitis in children by age of 5 years (2).

Vitamin D in humans is largely derived from the cutaneous synthesis of 7-dehydrocholesterol in the skin by UVB solar radiation. Seasonal variability in serum Vitamin D levels has been shown to be paralleled by changes in peripheral blood adaptive immunity,(12) which may in turn influence allergic predisposition in populations which experience seasonal solar variability.(13) However, as Singapore is a tropical country with no seasonal sunlight variability, the majority of the mothers in our study would have been exposed to constant levels of UVB radiation throughout the year, thus this and the low numbers of vitamin D deficient mothers in our cohort could account for the lack of any observed associations between maternal vitamin D deficiency and allergic outcomes in their offspring in this study.

The strengths of our study are the regular collection of data at multiple time points which enable us to pick up atopic outcomes in a timely manner. In addition, antenatal 25(OH)D levels were objectively measured from maternal serum levels, compared to other studies which used maternal dietary intake of 25(OH)D as a surrogate measure of 25(OH)D status. However, we acknowledge that there are limitations to our study. Parental reporting of allergic outcomes may be subjected to recall and reporting bias. A single measurement of serum maternal vitamin D levels may not be an accurate representation of the vitamin D levels throughout pregnancy or the vitamin D status of their offspring between birth and the time at which outcomes were assessed. Varying vitamin D levels in early childhood with rapid growth and dietary changes may further impact upon the risk of developing allergic disorders in later childhood. Data on pre-pregnancy maternal vitamin D levels and the doses of antenatal vitamin D supplements consumed by individual mothers during pregnancy were also not available in our cohort, which limited our ability to perform secondary analyses based on these factors. The Vitamin D Antenatal Asthma Reduction Trial (VDAART)(14) and the Copenhagen Prospective Studies on Asthma in Childhood 2010 (COPSAC2010) clinical trial(15) separately showed trends towards a reduced risk of asthma or persistent wheezing in offspring of mothers receiving antenatal Vitamin D3 supplementation, but which did not reach statistical significance due to underpowering. Combined secondary analyses from both cohorts, however, later demonstrated a significant protective effect of antenatal vitamin D supplementation against asthma/recurrent wheeze in the offspring, an effect which was more pronounced in women with higher 25(OH)D levels (>30ng/ml) at trial entry.(16, 17) Multiple measurements of vitamin D levels during pregnancy and in the first few years of life alongside detailed capture of maternal and offspring vitamin D supplementation in future studies would allow for more detailed subgroup analyses.

In conclusion, we found no associations between maternal 25(OH)D deficiency and allergic outcomes in the first 5 years of life.

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**Competing interests**

Chong YS has received reimbursement for speaking at conferences sponsored by Abbott Nutrition, Nestle, and Danone. Godfrey KM has received reimbursement for speaking at conferences sponsored by Nestle and Shek LP has received reimbursement for speaking at conferences sponsored by Danone and Nestle and consulting for Mead Johnson and Nestle.

Godfrey KM, Chong YS are part of an academic consortium that has received research funding from Abbot Nutrition, Nestle and Danone. Shek LP has received research funding from Danone.

**Funding**

This research is supported by the Singapore National Research Foundation under its Translational and Clinical Research (TCR) Flagship Programme and administered by the Singapore Ministry of Health’s National Medical Research Council (NMRC), Singapore- NMRC/TCR/004-NUS/2008; NMRC/TCR/012-NUHS/2014. This work is also supported by the National Medical Research Council, NMRC/CSA/022/2010. Additional funding is provided by the Singapore Institute for Clinical Sciences, Agency for Science Technology and Research (A\*STAR), Singapore. KMG is funded by the NIHR through the NIHR Southampton Biomedical Research Centre. The funders are not involved in the design and conduct of the study, data analysis and preparation of manuscript.

**Acknowledgements:**

The co-authors acknowledge the contribution of the rest of the GUSTO study group which includes Pang weiwei, Lee Yung Seng, Pratibha Agarwal, Dennis Bier, Arijit Biswas, Shirong Cai, Jerry Kok Yen Chan, Cornelia Yin Ing Chee, Helen Y. H Chen, Audrey Chia, Amutha Chinnadurai, Chai Kiat Chng, Mary Foong-Fong Chong, Shang Chee Chong, Mei Chien Chua, Chun Ming Ding, Eric Andrew Finkelstein, Doris Fok, Marielle Fortier, Yam Thiam Daniel Goh, Joshua J. Gooley, Wee Meng Han, Mark Hanson, Christiani Jeyakumar Henry, Joanna D Holbrook, Chin-Ying Hsu, Hazel Inskip, Jeevesh Kapur, Birit Leutscher-Broekman, Sok Bee Lim, Seong Feei Loh, Yen-Ling Low, Iliana Magiati, Lourdes Mary Daniel, Michael Meaney, Susan Morton, Cheryl Ngo, Krishnamoorthy Niduvaje, Anqi Qiu, Boon Long Quah, Victor Samuel Rajadurai, Mary Rauff, Jenny L. Richmond, Anne Rifkin-Graboi, Allan Sheppard, Borys Shuter, Leher Singh, Wing Chee So, Walter Stunkel, Lin Lin Su, Kok Hian Tan, Soek Hui Tan, Rob M. van Dam, Sudhakar K. Venkatesh, Inez Bik Yun Wong, P. C. Wong, George Seow Heong Yeo.

Table 1 : Comparison of demographic variables across the 25(OH)D groups

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **N(%)** |  |  |  |
|  | **25(OH)D sufficiency (≥75nmol/l)****N = 557**  | **25(OH)D insufficiency (≥50nmol/l and <75nmol/l))****N = 246** | **25(OH)D deficiency (<50nmol/l)****N = 122** | **p-value** |
| Sex |  |  |  |  |
| Male  | 303 (54.4) | 116 (47.2) | 65 (53.3) | 0.2 |
| Female | 254 (45.6) | 130 (52.8) | 57 (46.7) |  |
|  |  |  |  |  |
| Ethnicity |  |  |  |  |
| Chinese | 390 (70.0) | 96 (39.0) | 26 (21.3) | <0.01 |
| Malay  | 91 (16.3) | 92 (37.4) | 60 (49.2) |  |
| Indian | 76 (13.6) | 58 (23.6) | 36 (29.5) |  |
|  |  |  |  |  |
| Maternal education levels <12 years | 213 (38.7) | 101 (41.6) | 61 (51.3) | 0.04 |
| Maternal education levels ≥ 12 years | 337 (61.3) | 142 (58.4) | 58 (48.7) |  |
|  |  |  |  |  |
| Maternal age (mean±standard deviation) | 31.3 ± 5.0 | 29.7 ± 5.0 | 29.0 ± 5.3 | <0.01 |
|  |  |  |  |  |
| Maternal BMI during pregnancy ( mean ± standard deviation) | 23.2 ± 4.3 | 24.0 ± 4.9 | 24.6 ± 5.0  | <0.01 |
|  |  |  |  |  |
| Total energy intake during pregnancy ( mean ± standard deviation)  | 1888.2 ± 555.8  | 1839.9 ± 549.4 | 1738.7 ± 608.8 | 0.03 |
|  |  |  |  |  |
| No vitamin D supplementations during pregnancy | 69 (13.3) | 41 (19.0) | 35 (35.7) | <0.01 |
| Taken vitamin D supplementations during pregnancy  | 449 (86.7) | 175 (81.0) | 63 (64.3) |  |
|  |  |  |  |  |
| Do not smoke during pregnancy | 537 (96.9) | 242 (98.8) | 117 (96.7) | 0.3 |
| Smoke during pregnancy | 17 (3.1) | 3 (1.2) | 4 (3.3) |  |

Table 2 : Numbers of subjects with allergic outcomes across the 25(OH)D groups

|  |  |  |  |
| --- | --- | --- | --- |
|   | **25(OH)D sufficiency** **(≥ 75nmol/l)** | **25(OH)D insufficiency** **(≥50 nmol/l and <75 nmol/l)** | **25(OH)D deficiency** **(<50 nmol/l)** |
| **Outcomes by month 18**  | **N(%)** | **N(%)** | **N(%)** |
| Allergen sensitization  | 59 (13.5) | 24 (12.5) | 16 (17.6) |
| Eczema | 103 (21.1) | 37 (17.5) | 19 (19.4) |
| Rhinitis | 72 (18.0) | 36 (21.8) | 14 (21.2) |
| Wheeze and use nebulizer  | 56 (14.3) | 26 (15.9) | 11 (15.5) |
|   |   |   |   |
| **Outcomes by month 36**  |   |   |   |
| Allergen sensitization | 110 (25.1) | 36 (18.4) | 24 (17.0) |
| Eczema | 119 (26.6) | 47 (24.2) | 23 (27.4) |
| Rhinitis | 157 (40.7) | 68 (43.6) | 33 (50.8) |
| Wheeze and use nebulizer | 91 (24.7)  | 34 (22.4) | 13 (21.7) |
|   |   |   |   |
| **Outcomes by month 60** |   |   |   |
| Allergen sensitization | 157 (38.2) | 55 (31.4) | 24 (30.0) |
| Eczema | 126 (27.6) | 48 (24.9) | 25 (30.1) |
| Rhinitis | 154 (41.6) | 66 (44.3) | 27 (46.6) |
| Wheeze and use nebulizer | 108 (29.3) | 46 (30.3) | 20 (31.2) |

Table 3 : Associations between maternal 25(OH)D levels and allergic outcomes (odds ratio and 95% confidence intervals)

|  |  |  |  |
| --- | --- | --- | --- |
|   | **25(OH)D sufficiency** **(≥ 75nmol/l )****OR (95% CI)\*** | **25(OH)D insufficiency** **(≥50 nmol/l and <75 nmol/l)****OR (95% CI)\*** | **25(OH)D deficiency** **(<50 nmol/l)****OR (95% CI)\*** |
| **Outcomes by month 18**  |   |   |   |
| Allergen sensitization  | Ref | 0.7 (0.3-1.3) | 1.2 (0.5-2.6) |
| Eczema | Ref | 0.9 (0.5-1.6) | 1.4 (0.7-2.9) |
| Rhinitis | Ref | 0.9 (0.5-1.6) | 0.9 (0.4-2.1) |
| Wheeze and use nebulizer  | Ref | 0.7 (0.3-1.5) | 0.7 (0.3-1.8) |
|   |   |   |   |
| **Outcomes by month 36**  |   |   |   |
| Allergen sensitization | Ref | 0.6 (0.3-1.1) | 1.2 (0.6-2.5) |
| Eczema | Ref | 1.0 ( 0.6-1.8) | 1.5 (0.8-3.1) |
| Rhinitis | Ref | 1.1 ( 0.7-2.0) | 1.3 (0.6-2.8) |
| Wheeze and use nebulizer | Ref | 0.6 (0.3-1.2) | 0.5 (0.2-1.3) |
|   |   |   |   |
| **Outcomes by month 60** |   |   |   |
| Allergen sensitization | Ref | 0.7 (0.4-1.1) | 0.9 (0.4-1.8) |
| Eczema | Ref | 1.1 (0.6-1.8) | 1.6 (0.8-3.2) |
| Rhinitis | Ref | 1.2 (0.7-2.1) | 1.3 (0.6-2.7) |
| Wheeze and use nebulizer | Ref | 1.0 (0.6-1.7) | 0.9 (0.4-1.9) |

\*Adjusted for sex, ethnicity, maternal education levels, maternal age, maternal BMI during pregnancy, family history of allergy, total energy intake during pregnancy and vitamin D supplementation.

References

1. Waterland, R. A., and K. B. Michels. 2007. Epigenetic epidemiology of the developmental origins hypothesis. *Annu. Rev. Nutr.* 27: 363-388.

2. Erkkola, M., M. Kaila, B. Nwaru, C. Kronberg‐Kippilä, S. Ahonen, J. Nevalainen, R. Veijola, J. Pekkanen, J. Ilonen, and O. Simell. 2009. Maternal vitamin D intake during pregnancy is inversely associated with asthma and allergic rhinitis in 5‐year‐old children. *Clinical & Experimental Allergy* 39: 875-882.

3. Bikle, D. D. 2014 Vitamin D Metabolism, Mechanism of Action, and Clinical Applications. *Chemistry and Biology* 21(3): 319-329.

4. Barragan, M., M. Good, and J. Kolls. 2015. Regulation of dendritic cell function by vitamin D. *Nutrients* 7: 8127-8151.

5. Jones, A., N. D'Vaz, S. Meldrum, D. Palmer, G. Zhang, and S. L. Prescott. 2015. 25‐hydroxyvitamin D3 status is associated with developing adaptive and innate immune responses in the first 6 months of life. *Clinical & Experimental Allergy* 45: 220-231.

6. Cuello-Garcia, C. A., A. Fiocchi, R. Pawankar, J. J. Yepes-Nuñez, G. P. Morgano, Y. Zhang, K. Ahn, S. Al-Hammadi, A. Agarwal, and S. Gandhi. 2016. World Allergy Organization-McMaster University guidelines for allergic disease prevention (GLAD-P): prebiotics. *World Allergy Organization Journal* 9: 10.

7. Soh, S. E., S. S. M. Lee, S. W. Hoon, M. Y. Tan, A. Goh, B. W. Lee, L. P.-C. Shek, O. H. Teoh, K. Kwek, and S. M. Saw. 2012. The methodology of the GUSTO cohort study: a novel approach in studying pediatric allergy. *Asia Pacific Allergy* 2: 144-148.

8. Maunsell, Z., D. J. Wright, and S. J. Rainbow. 2005. Routine isotope-dilution liquid chromatography–tandem mass spectrometry assay for simultaneous measurement of the 25-hydroxy metabolites of vitamins D2 and D3. *Clinical chemistry* 51: 1683-1690.

9. Pike, K. C., H. M. Inskip, S. Robinson, J. S. Lucas, C. Cooper, N. C. Harvey, K. M. Godfrey, G. Roberts, and S. W. s. S. S. Group. 2012. Maternal late-pregnancy serum 25-hydroxyvitamin D in relation to childhood wheeze and atopic outcomes. *Thorax* 67: 950-956.

10. Baïz, N., P. Dargent-Molina, J. D. Wark, J.-C. Souberbielle, I. Annesi-Maesano, and E. M.-C. C. S. Group. 2014. Cord serum 25-hydroxyvitamin D and risk of early childhood transient wheezing and atopic dermatitis. *Journal of Allergy and Clinical Immunology* 133: 147-153.

11. Goldring, S. T., C. J. Griffiths, A. R. Martineau, S. Robinson, C. Yu, S. Poulton, J. C. Kirkby, J. Stocks, R. Hooper, and S. O. Shaheen. 2013. Prenatal vitamin d supplementation and child respiratory health: a randomised controlled trial. *PloS one* 8: e66627.

12. Khoo, A. L., H. J. Koenen, L. Y. Chai, F. C. Sweep, M. G. Netea, A. J. van der Ven, and I. Joosten. 2012. Seasonal variation in vitamin D(3) levels is paralleled by changes in the peripheral blood human T cell compartment. *PloS one* 7: e29250.

13. Klingberg, E., G. Olerod, J. Konar, M. Petzold, and O. Hammarsten. 2015. Seasonal variations in serum 25-hydroxy vitamin D levels in a Swedish cohort. *Endocrine* 49: 800-808.

14. Litonjua, A. A., V. J. Carey, N. Laranjo, B. J. Harshfield, T. F. McElrath, G. T. O'Connor, M. Sandel, R. E. Iverson, Jr., A. Lee-Paritz, R. C. Strunk, L. B. Bacharier, G. A. Macones, R. S. Zeiger, M. Schatz, B. W. Hollis, E. Hornsby, C. Hawrylowicz, A. C. Wu, and S. T. Weiss. 2016. Effect of Prenatal Supplementation With Vitamin D on Asthma or Recurrent Wheezing in Offspring by Age 3 Years: The VDAART Randomized Clinical Trial. *Jama* 315: 362-370.

15. Chawes, B. L., K. Bonnelykke, J. Stokholm, N. H. Vissing, E. Bjarnadottir, A. M. Schoos, H. M. Wolsk, T. M. Pedersen, R. K. Vinding, S. Thorsteinsdottir, L. Arianto, H. W. Hallas, L. Heickendorff, S. Brix, M. A. Rasmussen, and H. Bisgaard. 2016. Effect of Vitamin D3 Supplementation During Pregnancy on Risk of Persistent Wheeze in the Offspring: A Randomized Clinical Trial. *Jama* 315: 353-361.

16. Wolsk, H. M., B. J. Harshfield, N. Laranjo, V. J. Carey, G. O'Connor, M. Sandel, R. C. Strunk, L. B. Bacharier, R. S. Zeiger, M. Schatz, B. W. Hollis, S. T. Weiss, and A. A. Litonjua. 2017. Vitamin D supplementation in pregnancy, prenatal 25(OH)D levels, race, and subsequent asthma or recurrent wheeze in offspring: Secondary analyses from the Vitamin D Antenatal Asthma Reduction Trial. *The Journal of allergy and clinical immunology* 140: 1423-1429.e1425.

17. Wolsk, H. M., B. L. Chawes, A. A. Litonjua, B. W. Hollis, J. Waage, J. Stokholm, K. Bonnelykke, H. Bisgaard, and S. T. Weiss. 2017. Prenatal vitamin D supplementation reduces risk of asthma/recurrent wheeze in early childhood: A combined analysis of two randomized controlled trials. *PloS one* 12: e0186657.