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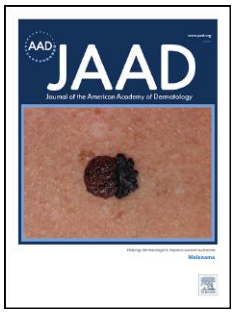

Effect of prenatal high-dose vitamin D on childhood atopic dermatitis is modified by maternal cotinine metabolome: A secondary analysis of a randomized clinical trial

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Title

Effect of prenatal high-dose vitamin D on childhood atopic dermatitis is modified by maternal cotinine metabolome: A secondary analysis of a randomized clinical trial

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Ethics: The study was approved by the Danish Ethics Committee (H-B-2008-093) and the Danish Data Protection Agency (2015-41-3696). Both parents gave written informed consent before enrollment.

Data sharing: Data will be available upon request.

CAPSULE SUMMARY

Environmental factors are speculated to influence the risk of childhood atopic dermatitis. We found a significant effect modification of the maternal tobacco exposure blood metabolome on the effect of prenatal high-dose vitamin D on offspring risk of atopic dermatitis

The findings suggest a beneficial effect of prenatal high-dose vitamin D on atopic dermatitis until school-age among mothers with a high tobacco exposure blood metabolome score suggesting a possible targeted prevention strategy against smoking mothers.

KEYWORDS

Atopic dermatitis; vitamin D; primary prevention; environment; tobacco exposure; metabolomics

ABSTRACT

Background: Tobacco exposure has been shown to modulate the effect of vitamin D on the risk of atopic diseases. However, randomized clinical trials investigating potential effect modification between tobacco exposure and vitamin D supplementation on atopic disease risk are lacking.

Objective: We sought to investigate the potential effect modification from maternal tobacco exposure on the effect of prenatal high-dose vitamin D supplementation on risk of child atopic dermatitis (AD), asthma and allergic rhinitis (AR).

Methods: A post hoc analysis in the double-blinded $COPSAC_{2010}$ randomized clinical trial (RCT) (NCT00856947) including 581 mother-child pairs randomized to 2800 IU/day (high-dose) vs 400 IU/d (standard-dose) from pregnancy week 24. Maternal blood metabolomic profiling was performed at inclusion reflecting maternal tobacco exposure using a supervised sparse partial least square model.

Results: We found a significant effect modification from the maternal cotinine metabolome score ($p_{interaction}<0.01$) where high-dose vitamin D reduced AD until age 6 years in offspring from mothers with highest (4^{th} quartile) cotinine metabolome score: crude; HR=0.46 (0.23-0.93), p=0.03, and adjusted for sex, birth season, socioeconomic circumstances, living environment, air pollution, maternal diet, vitamin D levels and fish oil intervention; HR=0.36 (0.15-0.85), p=0.02. Similarly, significant effect modification was demonstrated on risk of asthma ($p_{interaction}=0.03$) until age 6 years but not AR ($p_{interaction}=0.08$) at age 6 years.

Conclusions: This exploratory study of prespecified RCT outcomes demonstrates effect modifications of the maternal tobacco exposure metabolome on the primary preventive effect of prenatal high-dose vitamin D on offspring atopic disease risk suggesting a potential personalized prevention strategy targeting mothers exposed to tobacco smoking.

INTRODUCTION

Atopic dermatitis (AD) is one of the most common diseases in childhood affecting 13-20% of the pediatric population with a continuous increase in prevalence[1,2,3]. AD is a chronic inflammatory skin disease characterized by itching, skin lesions and periods of exacerbations and remission and is associated with other atopic diseases including asthma and allergy[4,5]. However, there is still no cure for the disease, which highlights the need for early preventive strategies[6].

The pathophysiology of AD is complex involving genetic predisposition, immune dysregulation and environmental exposures such as allergens and diet[3,7]. Maternal smoking during pregnancy and around birth has been suggested as a strong risk factor for offspring risk of AD as well as for childhood asthma and allergy[8,9]. In contrast, vitamin D is hypothesized to hold a protective role against AD development and disease severity as well as development of asthma/wheeze due to the effects on mechanisms involved in disease progression[10,11]. Studies have proposed an effect of vitamin D on AD[10,11,12]. Our previous findings have shown protective effects on asthma/wheeze but interestingly not AD for the whole population[13,14]. Further, a study from the Vitamin D Antenatal Asthma Reduction Trial (VDAART) demonstrated interaction between maternal tobacco exposure and prenatal vitamin D status on offspring atopic diseases[15]. We therefore hypothesize that maternal tobacco exposure could modify the effect of prenatal vitamin D intervention on risk of atopic diseases.

Because of the stigma around smoking, pregnant women are not being honest when interviewed[16]. Therefore, we measured blood levels of cotinine as well. We previously showed that environmental exposures could have effects on the blood metabolome differing from individual to individual[17], hence, we utilize individual blood metabolome profiles based on cotinine levels to capture systemic

101 effects of tobacco exposure. Here, we investigate whether maternal tobacco exposure and the
102 related blood metabolome profile during pregnancy could modify the effects of prenatal vitamin D
103 supplementation on risk of AD, asthma and allergic rhinitis in a double-blinded randomized clinical
104 trial (RCT).

METHODS

The COPSAC$_{2010}$ vitamin D RCT

The COPSAC$_{2010}$ cohort has previously been described in detail including baseline characteristics and successful randomization of the pregnancy double-blinded RCT[18,19,20,21]. The vitamin D intervention consisted of 2800 IU/day of vitamin D compared with 400 IU/day according to the Danish recommended intake. 623 pregnant women in pregnancy week 24 were randomized. The children were followed longitudinally from birth until age 6 years with 12 scheduled visits including acute care visits of symptoms of asthma, allergy or eczema[19,21].

Atopic dermatitis, asthma and allergic rhinitis

The predefined outcomes of AD was diagnosed prospectively according to the criteria of Hanifin and Rajka including typical morphology and localization of skin lesions based upon clinical examination in the COPSAC clinic as previously described[22]. Asthma and allergic rhinitis by age 6 years were diagnosed in the COPSAC clinic according to previously validated algorithms[19].

Maternal blood metabolomics

Maternal blood samples were collected at inclusion in pregnancy for untargeted metabolomic profiling of the mothers. Profiling was performed by Metabolon (NC, USA) using HD4 liquid chromatography-mass spectrometry (LC-MS)/MS platform as previously described including our protocol for pre-processing of data[23,24]. See our previous publication for more details[26].

Maternal tobacco exposure

Prenatal tobacco exposure was defined as maternal smoking during the first trimester of pregnancy (yes/no) i.e. before the intervention and was determined by parental interviews at enrolment.

Cotinine levels were measured as part of the metabolomic profiling by Metabolon at pregnancy week 24 and were categorized into two groups (detectable vs not detectable levels).

Maternal systemic low-grade inflammation

Using the same blood samples as for metabolomics measurements high-sensitivity C-reactive protein (hs-CRP) levels were determined by a high-sensitivity electrochemiluminescence assay from MesoScale[25].

Statistical analysis

We created a maternal metabolome score reflecting prenatal tobacco exposure based on detectable blood cotinine levels (yes/no) using a supervised sparse partial least square (sPLS) model. This was achieved by selecting the optimal set of metabolites through 10-fold cross-validation repeated 10 times based on the Area Under the Curve (AUC) as previously detailed[26]. The final sPLS model was chosen for its highest median AUC value and lowest variance across cross-validation rounds. A predicted cotinine metabolome score was calculated for each individual by multiplying the original data matrix by the loadings derived from the final model. Thereafter, we analyzed potential effect modification from this maternal cotinine metabolome score on the effect of prenatal high-dose vitamin D on the risk of AD and asthma in children until age 6 years by using Cox proportional hazard regression models and on the risk of allergic rhinitis at age 6 years using logistic regression. We then performed stratified analyses of the effect of high-dose vitamin D dividing the cotinine metabolome score into quartiles analyzing the effect on risk of AD, asthma and allergic rhinitis in each group. In sensitivity analyses, we adjusted for socio-economic circumstances (household income, maternal age and maternal level of education), sex, maternal 25-hydroxyvitamin D levels, birth season, living environment (urban vs rural)[17], air pollution (fine particulate matter <2.5 μm), maternal diet based on a food frequency questionnaire at pregnancy week 24[27] and the embedded

prenatal fish-oil trial[19,28]. Finally, we investigated the relationship between the cotinine metabolome score and cotinine levels vs systemic low-grade inflammation (hs-CRP). An enrichment analysis was performed for top 50 metabolites contributing to the sPLS derived cotinine-based maternal metabolome model. We applied a false discovery rate (FDR) of 5% to account for multiple testing. All analyses were performed with R (v4.0.3). P-values less than 0.05 were considered statistically significant.

RESULTS

Of the 581 children, 572 (98%) had information on maternal metabolomics at pregnancy week 24 including measurements of cotinine and AD during the first 6 years of life and were included in the time to first event analyses. At age 6 years, 553 (95%) children completed the clinical visit. There were no differences in baseline characteristics between the mother-child pairs in each intervention group[19].

Maternal tobacco exposure and risk of AD, asthma and allergic rhinitis age 0-6 years

Of the 572 mothers with cotinine levels measured, 59 (10%) had a detectable cotinine level. There was a significant relationship between mothers answering "yes" to being a smoker in the first trimester (n=45, 8%) and their cotinine levels at week 24.

There was no association between answering "yes" to being a smoker or having a detectable cotinine level and risk of AD, asthma or allergic rhinitis age 0-6 years ($p>0.05$) and no significant interaction with the vitamin D intervention for AD ($p_{interaction}=0.15$ and $p_{interaction}=0.47$), asthma ($p_{interaction}=0.64$ and $p_{interaction}=0.86$) or allergic rhinitis ($p_{interaction}=0.99$ and $p_{interaction}=0.57$).

Given the trend of effect modification on vitamin D intervention on AD, we analyzed the effect of vitamin D supplementation on risk of AD among mothers having a detectable cotinine level at week 24: HR; 0.70 (0.28-1.78), p=0.46 and mothers not having a detectable cotinine level: 1.00 (0.73-1.38), p=0.99.

Maternal cotinine blood metabolome score

The cotinine-based maternal metabolome score at week 24 showed a cross-validated median repeat AUC of 0.72 (range of 0.71-0.73) and was strongly associated with cotinine levels (**Figure 1A**). The top 50 metabolites contributing to the score with the highest loadings are displayed in **Figure**

1B, which showed highest positive loadings from xenobiotics and highest negative loadings from co-factors, vitamins and lipids including beta-cryptoxanthin and hydroxy-CMPF. The metabolite pathways are illustrated in the Sankey plot and enrichment analysis in **Figure 2** illustrating metabolite pathways, which passed the 5% FDR multiple test correction.

There was no direct association between the maternal cotinine metabolome score reflecting tobacco exposure and risk of AD, asthma or allergic rhinitis.

Maternal cotinine metabolome score and effect of prenatal high-dose vitamin D on risk of AD, asthma and allergic rhinitis age 0-6 years

We investigated potential effect modification of the maternal cotinine metabolome score reflecting tobacco exposure on the effect of prenatal high-dose vitamin D supplementation on risk of AD age 0-6 years and found a significant interaction between these ($p_{interaction}<0.01$). A similar interaction was observed after adjustment for all covariates described[28]. Then, we investigated the effect of high-dose vitamin D on risk of AD age 0-6 years (age at onset) stratified by quartiles of the maternal cotinine metabolome score and found a significant beneficial effect of the supplement among children whose mothers had the highest (4^{th} quartile) score (n=143): HR; 0.46 (0.23-0.93), p=0.03 (**Figure 3**). The results were even stronger after covariate adjustments showing a larger reduced risk in the 4^{th} quartile: 0.36 (0.15-0.85), p=0.02. This finding was further supported by an effect of vitamin D supplementation on risk of having an AD diagnosis (yes/no) during the first 6 years of life in the same group of children, i.e., born to mothers with the highest metabolome score: OR; 0.43 (0.19-0.95), p=0.04 (**Figure 4**).

Thereafter, we similarly investigated effect modification of the metabolome score on the effect of prenatal high-dose vitamin D on asthma risk age 0-6 years using Cox regression ($p_{interaction}=0.03$)

and allergic rhinitis at age 6 years using logistic regression ($p_{interaction}$=0.08). We found no significant effects on asthma or allergic rhinitis in the stratified analyses (p>0.05).

Maternal cotinine metabolome score and systemic low-grade inflammation

Finally, we investigated the relationship between the maternal metabolome score based on cotinine levels vs hs-CRP levels (pg/mL) at the same timepoint at week 24 in pregnancy and found a significant positive association: beta-estimate; 0.49 (0.32-0.66), p=1.8e-10. In contrast, there was no association between maternal cotinine levels (detectable vs not detectable) and hs-CRP levels at week 24; 0.04 (-0.25-0.32), p=0.79.

DISCUSSION

In this study, we demonstrated significant effect modification by the maternal blood cotinine metabolome profile on the effect of prenatal high-dose vitamin D vs placebo on time to AD and asthma diagnosis during the offsprings' first 6 years of life. We found a beneficial effect of the supplement in children of mothers who had the highest blood metabolome score reflecting tobacco exposure. Finally, we found a significant effect modification from the maternal cotinine metabolome score on risk of asthma.

Strengths and limitations

This study is strengthened by the RCT study design with successful randomization procedure[19] mitigating the risk of residual confounding[29]. Further, the $COPSAC_{2010}$ cohort is well known for its high follow-up rate, and we here report an unparalleled 95% clinical follow-up at age 6 years with 12 scheduled clinical visits from birth throughout early childhood, which allows for thorough registration of symptoms and close monitoring of AD, asthma and allergic rhinitis. COPSAC physicians were responsible for the clinical diagnostic procedure at the COPSAC research unit ensuring diagnostic accuracy and consistency. Finally, the population is representing the Danish background population by including an unselected group of pregnant women allowing for generalization of our findings.

The main limitation is the relatively few mothers reporting being actively smoking or exposed to smoking with a detectable cotinine level in the blood, which reduced the statistical power for the effect modification analyses. However, we used the information from cotinine blood measurements to create a cotinine-based metabolome score reflecting tobacco exposure for each pregnant women, which was used for the effect modification analysis and which we hypothesized was a more precise measure of how tobacco exposure affected the individual mothers.

Interpretation

Here, we found a protective effect of vitamin D supplementation among a specific group of children whose mothers metabolome reflected a high tobacco exposure during pregnancy compared with the null finding we and others have previously reported[19,14,30]. The reported effect modification and significant effect of the high-dose vitamin D intervention on AD in the children born to mothers whose metabolome were most similar to cotinine reflecting maternal tobacco exposure, suggests a possible targeted intervention strategy that should be confirmed in trials among a high-risk group of smoking mothers, since we were limited by a low prevalence of 8% of mothers smoking compared to other populations reporting between 14-40% dependent on socio-economic group[16]. This has previously been done with prenatal vitamin C supplementation among a population of pregnant smokers (n=251) showing reduction in asthma symptoms with supplementation[33] and a similar sample size may be required to demonstrate effects of prenatal vitamin D supplementation as well, which is more than 5 times larger than our sample size of pregnant smokers. However, we demonstrated effect modification from the cotinine metabolome score reflecting environmental tobacco exposure in the whole metabolome and interestingly, the results were unchanged after adjustment for several socioeconomic and environmental exposures.

By using mechanistic data layers, it allows for a deeper understand of the interplay between prenatal tobacco exposure and high-dose vitamin D intervention. The effect modification was not observed for maternal reported tobacco smoking or blood cotinine levels underscoring the utility of blood metabolomics profiling to capture individual adverse effects of environmental tobacco exposure. We have previously used this approach with success to understand the effect of living environment on common infection risk[17] and the role of infant gut virome on preschool asthma[34]. The added strength of utilizing this metabolome score compared with smoking habits from interviews or a single cotinine measure alone is that we are hereby able to characterize the whole metabolomic

profile reflecting the individual adverse effects of environmental tobacco exposure, which can then be applied on all individuals in the study population. This is even more important when being reduced in sample size due to a low number of smoking mothers as our initial analyses indicated an effect of vitamin D among mothers with a detectable cotinine level although not statistically significant. Interestingly, our cotinine metabolome score was also highly associated with systemic low-grade inflammation, which was not the case when analyzing cotinine as a single metabolite measure, which came as a surprise given that a relationship between smoking and increased inflammation previously has been reported[35,36]. It is likely that other environmental factors, which we were not able to identify with our data, are also reflected in the cotinine metabolome score as this is driven by several metabolites from different pathways as demonstrated in our enrichment analysis. However, it does not seem to reflect maternal diet, socioeconomic status, living environment or air pollution.

The mechanisms behind the reported effect modification in this trial is unclear and can only be speculated. It may be related to the immunomodulatory, anti-inflammatory and antioxidant effects of vitamin D[37] since tobacco exposure is known for increasing inflammation and produce oxidative stress[38]. In support of this theory, the metabolite that contributed to the model with the highest negative loading was the antioxidant beta-cryptoxanthin suggesting an inverse relationship between this antioxidant and tobacco exposure. In addition, we reported a positive association between the maternal cotinine metabolome score and low-grade inflammation, but we did not find levels of hs-CRP to associate with AD, asthma or allergic rhinitis risk in the offspring. Others have demonstrated that tobacco exposure decreases the conversion of inactive to active vitamin D in human epithelial cells suggesting a reduction in activity of the vitamin from smoking[39].

In conclusion, this study of prespecified atopic outcomes of the high-dose vitamin D RCT in COPSAC$_{2010}$ demonstrates effect modification of a maternal tobacco exposure blood metabolome

278 profile on the effect of prenatal vitamin D supplementation on AD risk that may hold potential for a
279 personalized prevention strategy.

280 REFERENCES

281 1. Wheeler KE, Chu DK, Schneider L. Updated Guidelines for Atopic Dermatitis—
282 AAAAI/ACAAI Joint Task Force. *JAMA Pediatr*. 2024;178(10):961-962.
283 doi:10.1001/jamapediatrics.2024.1395

284 2. Brandt EB, Sivaprasad U. Th2 Cytokines and Atopic Dermatitis. *J Clin Cell Immunol*.
285 2011;2(3):110. doi:10.4172/2155-9899.1000110

286 3. Kim G, Bae JH. Vitamin D and atopic dermatitis: A systematic review and meta-analysis.
287 *Nutrition*. 2016;32(9):913-920. doi:10.1016/j.nut.2016.01.023

288 4. Langan SM, Irvine AD, Weidinger S. Atopic dermatitis. *The Lancet*. 2020;396(10247):345-360.
289 doi:10.1016/S0140-6736(20)31286-1

290 5. Paller AS, Simpson EL, Siegfried EC, et al. Dupilumab in children aged 6 months to younger
291 than 6 years with uncontrolled atopic dermatitis: a randomised, double-blind, placebo-
292 controlled, phase 3 trial. *The Lancet*. 2022;400(10356):908-919. doi:10.1016/S0140-
293 6736(22)01539-2

294 6. AAAAI/ACAAI JTF Atopic Dermatitis Guideline Panel, Chu DK, Schneider L, et al. Atopic
295 dermatitis (eczema) guidelines: 2023 American Academy of Allergy, Asthma and
296 Immunology/American College of Allergy, Asthma and Immunology Joint Task Force on
297 Practice Parameters GRADE- and Institute of Medicine-based recommendations. *Ann Allergy*
298 *Asthma Immunol Off Publ Am Coll Allergy Asthma Immunol*. 2024;132(3):274-312.
299 doi:10.1016/j.anai.2023.11.009

300 7. Weidinger S, Beck LA, Bieber T, Kabashima K, Irvine AD. Atopic dermatitis. *Nat Rev Dis*
301 *Primer*. 2018;4(1):1-20. doi:10.1038/s41572-018-0001-z

302 8. Schäfer T, Dirschedl P, Kunz B, Ring J, Uberla K. Maternal smoking during pregnancy and
303 lactation increases the risk for atopic eczema in the offspring. *J Am Acad Dermatol*.
304 1997;36(4):550-556. doi:10.1016/s0190-9622(97)70242-1

305 9. Ruan Q, Jiang Y, Shi Y. Maternal smoking around birth and its influence on offspring allergic
306 diseases: A mendelian randomization study. *World Allergy Organ J*. 2024;17(2):100875.
307 doi:10.1016/j.waojou.2024.100875

308 10. Kim G, Bae JH. Vitamin D and atopic dermatitis: A systematic review and meta-analysis.
309 *Nutrition*. 2016;32(9):913-920. doi:10.1016/j.nut.2016.01.023

310 11. Kim MJ, Kim SN, Lee YW, Choe YB, Ahn KJ. Vitamin D Status and Efficacy of Vitamin D
311 Supplementation in Atopic Dermatitis: A Systematic Review and Meta-Analysis. *Nutrients*.
312 2016;8(12):789. doi:10.3390/nu8120789

313 12. El-Heis S, D'Angelo S, Curtis EM, et al. Maternal antenatal vitamin D supplementation and
314 offspring risk of atopic eczema in the first 4 years of life: evidence from a randomized
315 controlled trial. *Br J Dermatol*. 2022;187(5):659-666. doi:10.1111/bjd.21721

13. Wolsk HM, Chawes BL, Litonjua AA, et al. Prenatal vitamin D supplementation reduces risk of asthma/recurrent wheeze in early childhood: A combined analysis of two randomized controlled trials. *PloS One*. 2017;12(10):e0186657. doi:10.1371/journal.pone.0186657

14. Brustad N, Wang T, Chen L, et al. Prenatal high-dose vitamin D supplementation and childhood atopic dermatitis until age 6 years: A secondary analysis of a randomized clinical trial. *Br J Dermatol*. Published online May 30, 2025:ljaf208. doi:10.1093/bjd/ljaf208

15. Knihtilä HM, Huang M, Prince N, et al. Maternal vitamin D status modifies the effects of early life tobacco exposure on child lung function. *J Allergy Clin Immunol*. 2023;151(2):556-564. doi:10.1016/j.jaci.2022.10.030

16. Grant A, Morgan M, Gallagher D, Mannay D. Smoking during pregnancy, stigma and secrets: Visual methods exploration in the UK. *Women Birth J Aust Coll Midwives*. 2020;33(1):70-76. doi:10.1016/j.wombi.2018.11.012

17. Brustad N, Thorsen J, Pedersen CET, et al. Urban metabolic and airway immune profiles increase the risk of infections in early childhood. *Thorax*. Published online August 7, 2024:thorax-2024-221460. doi:10.1136/thorax-2024-221460

18. Bisgaard H, Vissing NH, Carson CG, et al. Deep phenotyping of the unselected COPSAC2010 birth cohort study. *Clin Exp Allergy J Br Soc Allergy Clin Immunol*. 2013;43(12):1384-1394. doi:10.1111/cea.12213

19. Chawes BL, Bønnelykke K, Stokholm J, et al. Effect of Vitamin D3 Supplementation During Pregnancy on Risk of Persistent Wheeze in the Offspring: A Randomized Clinical Trial. *JAMA*. 2016;315(4):353—361. doi:10.1001/jama.2015.18318

20. Brustad N, Bisgaard H, Chawes BL. Maternal High-Dose Vitamin D Supplementation and Offspring Bone Mineralization Until Age 6 Years-Reply. *JAMA Pediatr*. 2021;175(1):104. doi:10.1001/jamapediatrics.2020.2017

21. Brustad N. High-Dose Vitamin D Supplementation During Pregnancy and Asthma in Offspring at the Age of 6 Years. *JAMA J Am Med Assoc*. 2019;321(10):1003-1005.

22. Thorsteinsdottir S, Stokholm J, Thyssen JP, et al. Genetic, Clinical, and Environmental Factors Associated With Persistent Atopic Dermatitis in Childhood. *JAMA Dermatol*. 2019;155(1):50-57. doi:10.1001/jamadermatol.2018.4061

23. Rago D, Rasmussen MA, Lee-Sarwar KA, et al. Fish-oil supplementation in pregnancy, child metabolomics and asthma risk. *EBioMedicine*. 2019;46:399-410. doi:10.1016/j.ebiom.2019.07.057

24. Kim M, Brustad N, Ali M, et al. Maternal vitamin D-related metabolome and offspring risk of asthma outcomes. *J Allergy Clin Immunol*. 2023;152(6):1646-1657.e11. doi:10.1016/j.jaci.2023.06.030

25. Brustad N, Fink NR, Stokholm J, et al. Associations of 25 Hydroxyvitamin D and High Sensitivity C-reactive Protein Levels in Early Life. *Nutrients*. 2022;14(1):15. doi:10.3390/nu14010015

26. Leal Rodríguez C, Shah SA, Rasmussen MA, et al. The infant gut virome is associated with preschool asthma risk independently of bacteria. *Nat Med*. 2024;30(1):138-148. doi:10.1038/s41591-023-02685-x

27. Brustad N, Olarini A, Kim M, et al. Diet-associated vertically transferred metabolites and risk of asthma, allergy, eczema, and infections in early childhood. *Pediatr Allergy Immunol Off Publ Eur Soc Pediatr Allergy Immunol*. 2023;34(2):e13917. doi:10.1111/pai.13917

28. Chen L, Brustad N, Luo Y, et al. Prenatal Fish Oil Supplementation, Maternal COX1 Genotype, and Childhood Atopic Dermatitis: A Secondary Analysis of a Randomized Clinical Trial. *JAMA Dermatol*. 2024;160(10):1082-1090. doi:10.1001/jamadermatol.2024.2849

29. Sedgwick P. Randomised controlled trials: understanding confounding. *BMJ*. Published online September 25, 2015:h5119. doi:10.1136/bmj.h5119

30. Litonjua AA, Carey VJ, Laranjo N, et al. Six-Year Follow-up of a Trial of Antenatal Vitamin D for Asthma Reduction. *N Engl J Med*. 2020;382(6):525-533. doi:10.1056/NEJMoa1906137

31. Blighe K, Chawes BL, Kelly RS, et al. Vitamin D prenatal programming of childhood metabolomics profiles at age 3 y. *Am J Clin Nutr*. 2017;106(4):1092-1099. doi:10.3945/ajcn.117.158220

32. Sunde RB, Thorsen J, Pedersen CET, et al. Prenatal tobacco exposure and risk of asthma and allergy outcomes in childhood. *Eur Respir J*. 2022;59(2):2100453. doi:10.1183/13993003.00453-2021

33. McEvoy CT, Shorey-Kendrick LE, Milner K, et al. Effect of Vitamin C Supplementation for Pregnant Smokers on Offspring Airway Function and Wheeze at Age 5 Years: Follow-up of a Randomized Clinical Trial. *JAMA Pediatr*. 2023;177(1):16-24. doi:10.1001/jamapediatrics.2022.4401

34. Leal Rodríguez C, Shah SA, Rasmussen MA, et al. The infant gut virome is associated with preschool asthma risk independently of bacteria. *Nat Med*. Published online December 15, 2023:1-11. doi:10.1038/s41591-023-02685-x

35. Yasue H, Hirai N, Mizuno Y, et al. Low-grade inflammation, thrombogenicity, and atherogenic lipid profile in cigarette smokers. *Circ J Off J Jpn Circ Soc*. 2006;70(1):8-13. doi:10.1253/circj.70.8

36. Elisia I, Lam V, Cho B, et al. The effect of smoking on chronic inflammation, immune function and blood cell composition. *Sci Rep*. 2020;10(1):19480. doi:10.1038/s41598-020-76556-7

37. Baeke F, Takiishi T, Korf H, Gysemans C, Mathieu C. Vitamin D: modulator of the immune system. *Curr Opin Pharmacol*. 2010;10(4):482-496. doi:10.1016/j.coph.2010.04.001

38. Caliri AW, Tommasi S, Besaratinia A. Relationships among smoking, oxidative stress, inflammation, macromolecular damage, and cancer. *Mutat Res Mutat Res*. 2021;787:108365. doi:10.1016/j.mrrev.2021.108365

39. Hansdottir S, Monick MM, Lovan N, Powers LS, Hunninghake GW. Smoking Disrupts Vitamin D Metabolism In The Lungs. In: *A36. INFLAMMATION AND THE AIRWAY EPITHELIUM*. American Thoracic Society International Conference Abstracts. American Thoracic Society; 2010:A1425-A1425. doi:10.1164/ajrccm-conference.2010.181.1_MeetingAbstracts.A1425

FIGURE LEGENDS

FIGURE 1

Sparse partial least squares (sPLS) model predicting cotinine exposure during pregnancy from week 24 metabolomic profiles. A) Overview of top 50 metabolites with corresponding loadings. B) The cross-validated prediction with AUC and p-value, which were strongly associated with detectable cotinine levels.

FIGURE 2

Sankey plot and enrichment analysis of top 50 metabolites contributing to the sPLS derived cotinine-based maternal metabolome model. fdr=false discovery rate.

FIGURE 3

Effect of high-dose vitamin D vs placebo on risk of atopic dermatitis (AD) until age 6 years in a time to first event (age at onset) analysis. Estimates, 95% CI and p-values from a Cox proportional hazard regression model.

FIGURE 4

Effect of high-dose vitamin D vs placebo on risk of atopic dermatitis (AD) between age 0-6 years (yes/no) in each quartile of the maternal cotinine metabolomics score “fingerprint”. Estimates from a logistic regression model.