Final Report

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1. **The associations of plasma fatty acid patterns during pregnancy with respiratory and allergy outcomes at school age. The Generation R Study.**

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ABSTRACT

Background Fatty acids during pregnancy might have an effect on the fetal lung and immune system development, and subsequently can influence respiratory and allergy outcomes in childhood.

Objective To examine the associations of patterns of plasma fatty acids during pregnancy with lung function, asthma and allergy outcomes at 10 years age.

Method This study among 4,400 children and their mothers was performed in a population-based prospective cohort study. We measured maternal plasma glycerophospholipid concentrations of 22 fatty acids in the second trimester of pregnancyby gas chromatography. We used 3 fatty acids patterns, 'high n-6 polyunsaturated fatty acid(PUFA)' pattern, a 'monounsaturated (MUFA) and saturated (SFA)' pattern, and a 'high n-3PUFA' pattern, as previously identified by principal components analysis. At the age of 10 years, lung function was assessed by spirometry, current asthma and physician-diagnosed inhalant allergy by ISAAC-questionnaire, and inhalant allergic sensitization by skin prick tests. We used multivariate linear and logistic regression models to examine associations.

Results A higher 'high n-6 PUFA' pattern only was associated with a higher Forced Expiratory Volume in 1 second/FVC (FEV_1/FVC) and a higher Forced Expiratory Flow after exhaling 75% of FVC(FEF_{75}) (Z-score difference(95% CI 0.03 (0.00, 0.07) and 0.04 (0.01, 0.07), respectively, per SD increase in the fatty acid pattern).We did not find associations of maternal fatty acids patterns with current asthma, inhalant allergic sensitization or inhalant allergy.

Conclusion Our results suggest that a maternal high n-6 PUFA pattern may beneficially influence lung function at school age, but not asthma and allergy.

INTRODUCTION

Maternal diet during pregnancy is associated with respiratory and allergy outcomes in childhood(1-3).Fatty acids play an important role among many dietary nutrients(3), as they can pass through the placenta(4), and subsequently might have an effect on the fetal lung and immune system development(5, 6).Our and other previous studies mainly used polyunsaturated fatty acids (PUFAs) to determine fatty acid status(7-12), and suggested that maternal n-3 PUFAs during pregnancy may have a protective effect against allergic disorders of the child(13, 14).A randomized control trial showed that maternal supplementation with n-3 long chain PUFA during the third trimester of pregnancy reduced the risk of persistent wheeze or asthma with 7% in the first 5 years of life of the child, but had no effect on allergic sensitization(15).The role of n-6 PUFAs, saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) on the development of respiratory and allergy outcomes are less studied. It has been suggested that n-6PUFAshave pro-inflammatory properties and their mediators may promote the production of IgE and airway constriction, which might lead to a higher risk of asthma and allergies in later life(5, 6). SFA can directly stimulate inflammatory mediators(16, 17). However, a cohort study found that higher intake of SFAs during pregnancy was associated with a decreased risk of asthma in the offspring at the age of 5 years(18). MUFAs have an anti-inflammatory capacity(19), but their role in asthma and allergic disease is still unknown. Previous studies on the associations of fatty acid status during pregnancy with respiratory and allergy outcomes in childhood mainly focused on specific individual fatty acids or groups and showed inconsistent results(7-12). By examining only individual fatty acids, synergistic or additive effects may be missed, which can be overcome by using fatty acid patterns(20).Furthermore, these studies were performed at a young childhood age.

Therefore, our aim was to examine among 4,400 children and their mothers participating in a population-based cohort study the associations of maternal plasma fatty acids patterns during pregnancy with lung function, asthma and allergy outcomes at 10 years age.

METHODS

Design and cohort This study was embedded in the Generation R Study, a population-based prospective cohort study from early fetal life onwards in Rotterdam, the Netherlands(21).The study has been approved by the Medical Ethical Committee of the Erasmus MC, University Medical Centre in Rotterdam, the Netherlands (MEC-2012-165-NL40020.078.12). Written informed consent was obtained from the parents or legal representatives of all participating children. A total of 8,661 women were enrolled before 25 weeks of gestation. Complete information on fatty acid profiles was available for 6,997 women. Of this group 6,923 women gave birth to singleton live-born children of whom 4,400 had data on lung function, asthma, inhalant allergic sensitization or inhalant allergy at the age of 10 years (Figure S-1).

Maternal plasma fatty acid patterns Maternal venous samples for fatty acid composition analysis in plasma glycerophospholipids were drawn in the second trimester of pregnancy at a median gestational age of 20.5 weeks (95% range 16.5-24.9)(22-24). The samples were centrifuged in the regional laboratory, stored at - 80°C and transported to the Division of Metabolic Diseases and Nutritional Medicine, Dr. von Hauner Children's Hospital, Ludwig-Maximilians-University of Munich, Germany. They were analysed using gas chromatography(25).We had information available on the concentrations of 22 individual fatty acids. A principal component analysis on the weight percentage of these 22 fatty acids was performed among all women in our study with information on fatty acid profiles (n=6,997), as previously described (20). Factor loadings, which describe how strongly each individual fatty acid contributes to each fatty acid pattern, were calculated and are presented in Table S-1. On the basis of high factor loadings ($\geq|0.20|$) for the respective fatty acids (Table S-1), three patterns were defined: 1) 'highn-6 PUFA' pattern; 2) 'MUFA and SFA' pattern; and 3) 'high n-3PUFA' pattern. Each woman had an individual score on each of the fatty acid patterns.

School age lung function, asthma and allergy outcomes Lung function was assessed by spirometry, which was performed at a median age of 9.7 years (95% range 8.5-12.0 years)according to the American Thoracic Society and European Respiratory Society (ATS/ERS) guidelines(26).Spirometry measurements included Forced Expiratory Volume in 1 second (FEV_1), Forced Vital Capacity (FVC), FEV_1/FVC and Forced Expiratory Flow after exhaling 75% of FVC (FEF_{75}), and were converted into sex-, height-, age-, and ethnicity-adjusted z-scores according to the Global Lung Initiative reference data(27).Additionally, we included 284 spirometry curves that did not meet the reproducibility criteria, but with at least one adequate curve according to the ATS/ERS criteria. Information on ever asthma (no; yes), wheezing in the previous 12 months (no, yes),and on physician-diagnosed inhalant allergy (no; yes) to pollen (hay fever), house dust mite, cat or dog was obtained from questionnaires based on the International Study on Asthma and Allergy in Childhood (ISAAC) Questionnaire(28).Information on asthma medication use in the past 12 months was obtained during the research centre visit. Current asthma (no, yes) was defined as everphysician-diagnosed asthma with either wheezing or the use of inhalant medication in the previous 12 months. Inhalant allergic sensitization (no; yes) to house dust mite, five-grass mixture, birch, cat or dog (ALK-Abelló B.V., Almere, the Netherlands) was measured by skin prick tests using the scanned area method(29). We used two positive controls (histamine dihydrochloride 10 mg/mL) and one negative control (sodium chloride 9 mg/mL). Skin responses were considered positive if the area of the wheal was ≥40% of that of the histamine response (i.e. histamine equivalent prick index area ≥ 0.40)(29). Contra-indications for a skin prick test were eczema on the volar surface of the left forearm, the use of oral prednisone ≥ 10 mg daily, antihistamine intake <72h prior to the test or use of corticosteroid ointment ≤ 48h prior to the test.

Covariates We obtained information on maternal age (years), gestational age at fatty acid measurement (weeks), pre-pregnancy body mass index, educational level (lower, higher), parity (nullipara, multipara), history of asthma or atopy (no, yes), smoking during pregnancy

(no, yes), pet keeping (no, yes), folic acid supplementationin early pregnancy(no, yes)and total daily energy intake in early pregnancy (kcal) from questionnaires during pregnancy. Maternal psychological distress (no, yes) was defined by the global severity index (GSI). Child's sex, gestational age at birth (weeks) and birthweight (grams) were obtained from midwife and hospital registries or questionnaires at birth. Child's ethnic background was based on the country of birth of both parents. Postal questionnaires at age 6 and 12 months provided information about breastfeeding.

Statistical analysis We compared the characteristics of children included and non-included in the study using student's t-test, Mann-Whitney U test and chi-square test. We used linear and logistic regression models to examine the associations of maternal fatty acid patterns with respiratory and allergy outcomes at the age of 10 years.Fatty acid pattern scores were examined as continuous variables. In model 1 (basic model)we studied the crude associations of fatty acid patterns with respiratory and allergy outcomes. In model 2 (confounder model) we adjusted for maternal age, gestational age at fatty acid measurement, pre-pregnancy body mass index, educational level, parity, history of asthma or atopy, smoking during pregnancy, pet keeping, folic acid supplementation, total daily energy intake, psychological distress, child's sex and ethnic background. Confounders were included in our model based on the literature(30), if they were associated with both the determinant and the outcome, or if they changed the effect estimates with ≥10%. We considered the confounder model as main model. Model 3 (final model) comprised the confounder model and was additionally adjusted for the intermediates gestational age at birth, birthweight and breastfeeding. Intermediates were included in our model if they were hypothesized to be within the causal pathway. Missing data for covariates were <20%, except for folic acid supplementation in early pregnancy (22.5%). To reduce bias and imprecision, we imputed missing data of the covariates with multiple imputations (m=10) using chained equations and we report the pooled effect estimates or Odds Ratios with their 95% confidence intervals (95% CI). Statistical analyses were performed using SPSS version

24.0 for Windows (IBM Corp., Armonk, NY, USA) and R version 3.5.0 (R Foundation, Vienna, Austria.

RESULTS

Subject characteristics Characteristics of mothers and children included in the analyses are shown in Table 1.The prevalence of current asthma was 5.7% (n=202), of inhalant allergic sensitization 32.7% (n=1,007) and of physician-diagnosed inhalant allergy 12.7% (n=311). Children not included most prominently had mothers with a younger age, a lower education level, were less often nulliparous,had less often folic acid supplementation, had a lower total daily energy intake, and were born at a later gestational age at birth, with a lower birth weight, and were more often from a non-European ethnicity (Table S-2).

Maternal fatty acid patterns, respiratory and allergy outcomes In the basic model, we observed that a higher 'high n-6 PUFA' pattern was associated with a higher FEV_1/FVC and FEF_{75}(Z-score difference (95% CI) 0.04 (0.01, 0.07) and 0.05 (0.02, 0.08), respectively, per SD increase in the fatty acid pattern). A higher 'MUFA an SFA' pattern was associated with a alower FEV_1/FVC and FEF_{75} (Z-score difference (95% CI)-0.04 (-0.07, -0.01) and-0.04 (-0.07, -0.01), respectively, per SD increase). A higher 'high n-3 PUFA' pattern was associated with a lower FEV_1, FEV_1/FVC and FEF_{75}(-0.04 (-0.07, -0.01), -0.04 (-0.07,-0.01) and -0.04 (-0.07,-0.02) per SD increase).After adjusting for confounders, only the associations of a 'high n-6 PUFA' pattern with higherFEV_1/FVC and FEF_{75}remained (Z-score difference (95% CI) 0.03 (0.00, 0.07) and 0.04 (0.01, 0.07) per SD increase in the fatty acid pattern).Also after additionally adjusting for intermediates, the associations of a higher 'high n-6 PUFA' pattern with a higher FEV_1/FVC (Z-score difference0.04 (0.00, 0.07), per SD) and a higher FEF_{75} (0.04 (0.01, 0.07)) at school age remained (Figure 1, Table S-3).

In all models, we did not observe any associations of maternal fatty acids patterns with current asthma, inhalant allergic sensitization and inhalant allergy.

DISCUSSION

In this population-based prospective cohort studies, we observed that a pattern characterized by high levels of maternal n-6 PUFA was associated with a higher FEV_1/FVC and FEF_{75} in the children at school age. Patterns characterized by maternal MUFA and SFA or high levels of n-3 PUFA were not consistently associated with any lung function measure of the child. We did not observe any associations of maternal fatty acid patterns with asthma and allergy outcomes in children at school age.

Comparison with previous studies We found that a higher maternal 'high n-6 PUFA' pattern was associated with a higher FEV_1/FVC and FEF_{75}, not FEV_1or FVC, in children at school age. These results are not in line with a previous population-based cohort study among 865 subjects age 6 years that measured lung function by spirometry. The study found no associations of any PUFAs including total n-3 PUFAs, total n-6 PUFAs, and total n-3: n-6 ratio PUFAs with FEV_1(8). The difference in results may be explained by the smaller sample size and measurement of fatty acids in a different period of pregnancy, namely late pregnancy. We previously observed no association of fatty acid levels during pregnancy with childhood airway resistance measured by Rint(9).Thus, the role of maternal fatty acids in different periods of pregnancy on lung function in children remains not fully clear.
We did not observe any associations of maternal fatty acid patterns with current asthma in the children. We previously showed that a higher maternal total PUFA and total n-6 PUFA levels were associated with a decreased risk of current asthma at the age of 6 years (9).Another cohort study found that high intake of SFA was associated with a decreased risk of asthma in the offspring at the age of 5 years(18). Our findings do not support the hypothesis that a higher n-3 PUFA might protect against asthma in childhood(31), which was also shown by a randomized control trial that showed that supplementation with n-3 long chain PUFA during the third trimester of pregnancy reduced the risk of persistent wheeze or asthma with 7% in the first 5 years of life(15). Additionally to previous studies, we now used fatty acids patterns to determine maternal fatty acid status during pregnancy which method

may have influenced our results. By examining only individual fatty acids, synergistic or additive effects may be missed. Thus, future studies should focus on fatty acids patterns during pregnancy with asthma in the offspring.

We observed no associations of fatty acid patterns with inhalant allergy and inhalant allergic sensitization. These findings are in line with some previous cohort studies(7, 8, 11). However, a recent randomized controlled trial with 6 year follow up showed that n-3 long chain PUFA supplementation during pregnancy was associated with a decrease risk of house dust mite sensitization at 6 years age(32). Also, a combined meta-analysis of three randomized controlled trials showed that children who were born from mother included in the n–3 long chain PUFA group during pregnancy had a lower risk of inhalant allergic sensitization(33). Thus, the effect of maternal fatty acids during pregnancy in the development of allergy remains inconclusive.

Interpretation of the results A higher maternal'high n-6 PUFA' pattern was associated with a higher FEV_1/FVC and FEF_{75} at school age. These results could be partly explain based on the evidence of recent human and animal studies showing that prostaglandin E2 may reduce bronchoconstriction through increasing smooth muscles relaxation and inhibit the recruitment of inflammatory cells and mediators, and potentially to a better lung function(34, 35).We did not observe associations of a 'high n-3 PUFA' pattern and 'MUFA and SFA' pattern with lung function, asthma and allergy at school age. Different types of fatty acids pass through the placenta in different ways. n-3 PUFA are preferentially transported to the fetus through the placenta, while the others are less. Consequently, the maternal fatty acid concentrations may not reflected very well the concentrations of fatty acids that the fetus was exposed to(36), which could explain the lack of part of the associations. Furthermore, MUFA and SFA may reach to the fetus in a small amount, so they may be less important for the child's health. This could explain the null findings for 'MUFA and SFA' pattern.

Strengths and limitations Our study was embedded in a large population-

based cohort study. We had detailed information available on maternal fatty acid levels during pregnancy and potential confounders that might influence the association of fatty acids during pregnancy with respiratory and allergy outcomes. The most important strength of our study is the application of principal component analyses (PCA) for discovering fatty acids patterns, taking into account the correlation of individual fatty acids(20). Our study has some limitations. First, selection bias due to loss to follow-up could have occurred towards if results would have been different in those included versus non-included. Furthermore, we measured fatty acids in plasma glycerophospholipids from single blood sample in mid-pregnancy. Their concentrations reflect dietary intake and fatty acid metabolism on only a short period (2-8 weeks), Thus only for short term in the second trimester of pregnancy, and not during the whole pregnancy(37). Information on asthma and physician-diagnosed inhalant allergy was obtained from questionnaires based on the International Study on Asthma and Allergy in Childhood (ISAAC) Questionnaire. Despite this, information bias cannot be excluded. Last, we cannot exclude residual confounders, such as maternal fatty acid status in other periods of pregnancy, child's PUFA intake and breastmilk fatty acid composition.

Conclusion Our study showed that a maternal fatty acid pattern characterized by high levels of n-6 PUFAs was associated with a higher FEV_1/FVC and FEF_{75}, but not with asthma and allergy. We did not find any consistent associations of other fatty acid patterns with respiratory or allergy outcomes. Further studies and randomized controlled trials are needed to understand the role and mechanism of fatty acids during pregnancy in the development of asthma and allergy in the offspring.

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Table 1. Characteristics of mothers and children included in the analysis.

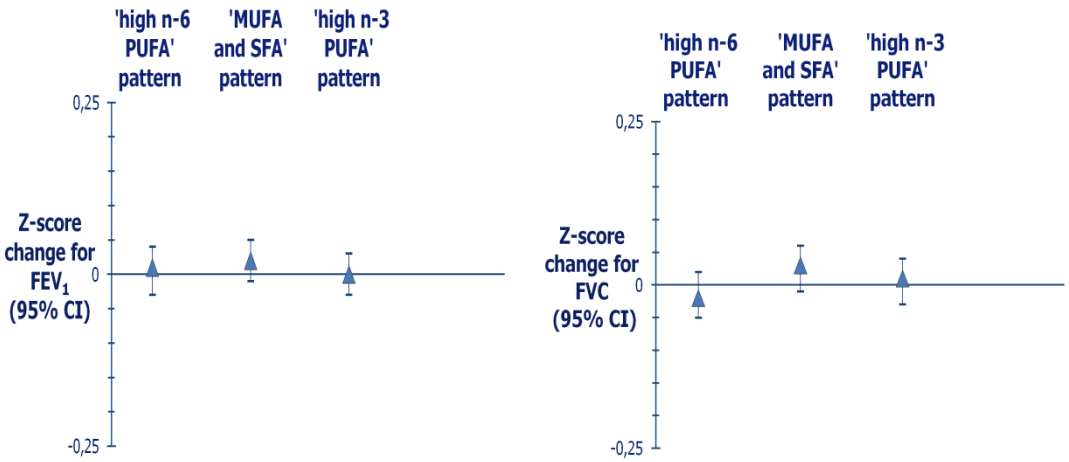
	n = 4,400
Maternal characteristics	
Age, years	30.7 (4.8)
Gestational age at fatty acid measurement	20.7 (1.2)
Pre-pregnancy body mass index(kg/m^2)[1]	22.6. (18.0-34.5)
Educational level, higher (%)	48.9 (2,150)
Parity, nullipara (%)	58.2 (2,560)
History of asthma or atopy, yes (%)	37.5 (1,651)
Smoking during pregnancy, yes (%)	25.5 (1,120)
Pet keeping, yes (%)	34.9 (1,535)
Folic acid supplementation in early pregnancy, yes (%)	77.5 (3,411)
Total daily energy intake in 1st trimester of pregnancy (kcal)	2041 (553)
Psychiatric symptoms, yes (%)	9.6 (422)
SD scores for fatty acid patterns	
‘high n-6 PUFA‘ pattern	-0.05 (1.00)
‘MUFA and SFA’ pattern	0.05 (0.97)
‘high n-3 PUFA’ pattern	0.08 (0.99)
Child characteristics	
Female sex (%)	50.3 (2,211)
Gestational age at birth (weeks)[1]	40.1 (35.7- 42.4)
Birth weight (grams)	3,445 (553)
Ethnic background (%)	
European	67.0 (2,947)
Non-European	33.0 (1,453)
Ever breastfeeding, yes (%)	88.2 (3,882)
FEV_1 (z- score)	0.17 (0.98)
FVC (z-score)	0.20 (0.93)
FEV_1/FVC (z-score)	-0.09 (0.96)
FEF_{75} (z-score)	0.04 (0.92)
Current asthma at age 10 years, yes (%)	5.7 (202)
Inhalant allergic sensitization at age 10 years, yes (%)	32.7 (1,007)

Physician-diagnosed inhalant allergy at age 10 years, yes (%)	12.7 (311)

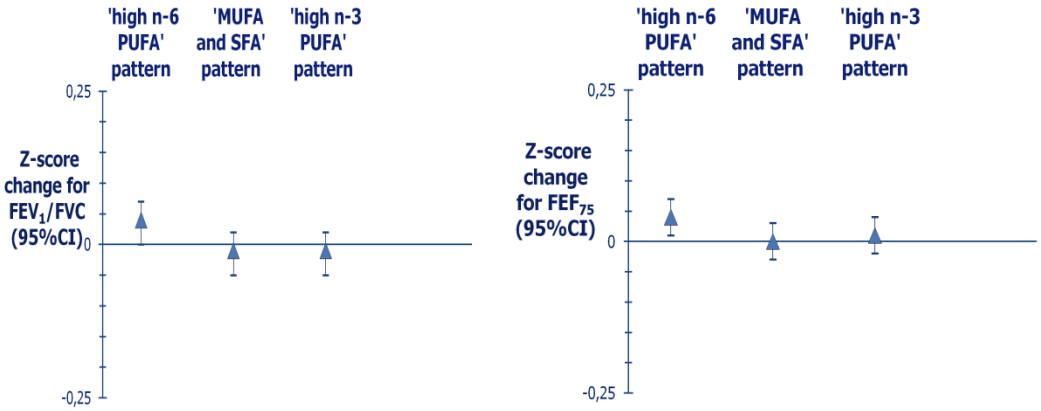
Values are means (SD), medians (2.5-97.5th percentile) or valid percentages (absolute numbers), based on imputed data. Missing data on Forced Expiratory Flow in 1 second (FEV_1) (n=673), Forced Vital Capacity (FVC) (n=673), FEV_1/FVC ratio (n=673), Forced Expiratory Flow after exhaling 75% of FVC (FEF75) (n=673), current asthma (n=874), inhalant allergic sensitization (n=1,322), physician-diagnosed inhalant allergy (n=1,947) were not imputed.

Figure 1. Associations of maternal fatty acid patterns with respiratory and allergy outcomes in children aged 10 years

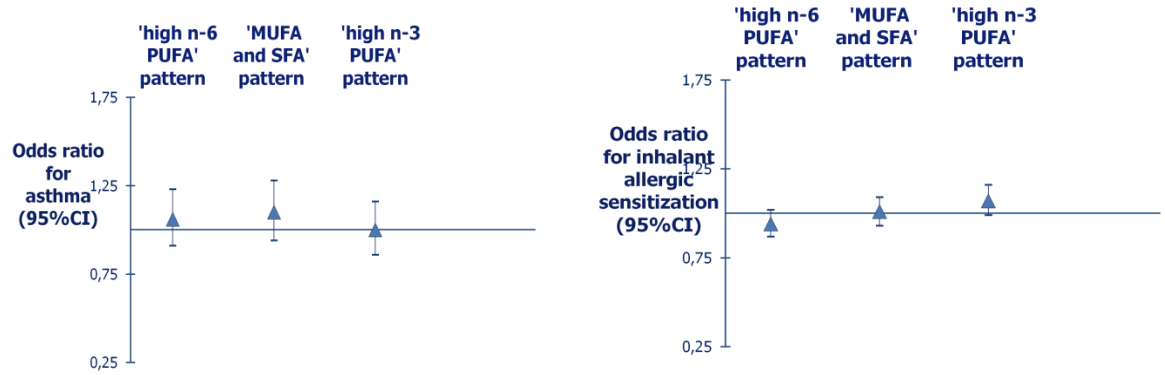
a. Maternal fatty acids patterns and FEV_1 b.Maternal fatty acids patterns and FVC



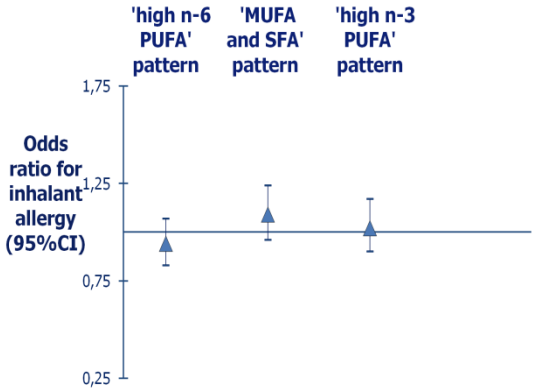
c. Maternal fatty acids patterns and FEV_1/FVCd.Maternal fatty acids patterns and FEF_{75}



e.Maternal fatty acids patterns and asthma f. Maternal fatty acids patterns and inhalant sensitiz



g. Maternal fatty acids patterns and inhalant allergy



Values are Z-score changes in lung function measurements (FEV_1, FVC, FEV1/FVC, $FEF_{75)}$ or odds ratios (OR) for current asthma and inhalant allergic sensitization and inhalant allergy with 95% confidence interval (95% CI), derived from linear or logistic regression models per standard deviation score (SD) increase of fatty acid patterns during pregnancy. Forced Expiratory Flow in 1 second (FEV_1), Forced Vital Capacity (FVC), Forced Expiratory Flow after exhaling 75% of FVC (FEF_{75}).). Fatty acids are classified into patterns: 'High n-6 PUFA' pattern, 'MUFA and SFA' pattern, 'High n-3 PUFA' pattern. Model 3 (final model) was adjusted for the confounders maternal age, gestational age at fatty acid measurement, pre-pregnancy body mass index, educational level, parity, history of asthma or atopy, smoking, pet keeping, folic acid supplement use and total daily energy intake in the first trimester of pregnancy, psychological distress and child's sex and ethnicity, and additionally adjusted for child's gestational age at birth, birth weight and breastfeeding.

Supplementary material

1. The associations of plasma fatty acid patterns during pregnancy with respiratory and allergy outcomes at school age. The Generation R Study.

Table S-1. Factor loadings of the individual fatty acids in fatty acid patterns.

Fatty acids		Wt% of total fatty acids[a]	Fatty acid patterns		
			'High n-6 PUFA' pattern	'MUFA and SFA' pattern	'High n-3 PUFA' pattern
Saturated fatty acids					
Myristic acid	14:0	0.64	**0.23**	**0.30**	0.06
Palmitic acid	16:0	30.67	0.06	**0.88**	0.14
Margaric acid	17:0	0.36	-0.03	**-0.31**	0.03
Stearic acid	18:0	11.50	0.08	**-0.84**	-0.02
Monounsaturated fatty acids (cis)					
Pentadecanoic acid	15:1n-5	0.06	0.01	0.16	0.14
Palmitoleic acid	16:1n-7	0.68	**0.43**	**0.63**	0.16
Oleic acid	18:1n-9	10.29	0.19	**0.29**	**0.21**
Vaccenic acid	18:1n-7	1.46	0.00	**0.28**	0.08
Eicosenoic acid	20:1n-9	0.19	**-0.25**	**-0.31**	-0.09
n-3					
α-Linolenic acid (ALA)	18:3n-3	0.30	-0.13	-0.03	0.09
Eicosatrienoic acid	20:3n-3	0.10	0.13	-0.07	0.16
Eicosapentaenoic acid (EPA)	20:5n-3	0.44	**-0.33**	0.06	**0.69**
Docosapentaenoic acid (DPA)	22:5n-3	0.70	0.09	-0.02	**0.59**

Docosahexaenoic acid (DHA)	22:6n-3	4.67	**-0.39**	0.06	**0.67**
n-6					
Linoleic acid (LA)	18:2n-6	22.37	**-0.45**	**-0.45**	**-0.67**
ɣ-linolenic acid (GLA)	18:3n-6	0.08	**0.53**	0.10	0.19
Eicosadienoic acid	20:2n-6	0.52	-0.06	-0.04	**-0.69**
Dihomo-ɣ-linolenic acid (DGLA)	20:3n-6	3.68	**0.44**	**0.42**	--0.19
Arachidonic acid (ARA)	20:4n-6	9.73	**0.40**	-0.15	**0.30**
Adrenic acid	22:4n-6	0.42	**0.85**	0.03	-0.08
Osbond acid	22:5n-6	0.47	**0.82**	0.04	-0.17
n-9					
Mead acid	20:3n-9	0.12	**0.53**	0.12	**0.37**
Explained variance (%)			14.4	12.7	12.2

Factor loadings ≥ | 0.20 are presented in bold font.

Abbreviations: MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

[a] Median percentage in all 6,997 study participants with information available on fatty acid concentrations.

Table S-2. Comparison of maternal and child's characteristics between participants and not participants in the analysis.

	Participants n= 4,400	**Non-participants** n= 2,523	**p-value for difference**
Maternal characteristics			
Age, years	30.7 (4.8)	28.0 (5.5)	<0.001
Gestational age at fatty acid measurement	20.7 (1.2)	20.7 (1.3)	N.S.
Pre-pregnancy body mass index(kg/m$^{2)1}$	22.6 (18.1-34.4)	22.8 (17.6-36.2)	0.013
Educational level, higher (%)	50.5 (2,115)	26.9 (586)	<0.001
Parity, nulliparous (%)	58.5 (2,558)	51.8 (1,286)	<0.001
History of asthma or atopy, yes (%)	37.2 (1,445)	39.1 (63)	N.S.
Smoking during pregnancy, yes (%)	24.5 (968)	23.3 (40)	0.034
Pet keeping, yes (%)	33.5 (1,276)	24.5 (39)	0.024
Folic acid supplementation in early pregnancy, yes (%)	78.7 (2,684)	57.6 (1021)	<0.001
Total daily energy intake in 1st trimester of pregnancy (kcal)	2,058 (548)	2,001 (606)	0.001
Psychiatric symptoms, yes (%)	8.4 (310)	9.3 (14)	N.S.
Child characteristics			
Female sex (%)	50.3 (2,211)	48.3 (1,217)	N.S.
Gestational age at birth (weeks)[1]	39.9 (35.7-42.4)	40.0 (35.0-42.3)	<0.001
Birth weight (grams)	3,446 (553)	3,370 (574)	<0.001
Ethnic background (%)			
European	68.0 (2,946)	47.8 (1,094)	<0.001
Non-European	32.0 (1,385)	52.2 (52.2)	
Ever breastfeeding, yes (%)	92.9 (3,335)	92.5 (147)	N.S.

Values are means (SD), medians[1] (2.5-97.5th percentile) or valid percentages (absolute numbers) based on observed data. P-value for difference was calculated using Student's t-test for continuous normally distributed variables, Mann-Whitney U test for continuous not normally distributed variables, and chi-square test for categorical variables. N.S. (non-significant)

Table S-3. Association of maternal fatty acid patterns with respiratory and allergy outcomes in children aged 10 years

Fatty acid pattern (per SD)	FEV_1 Z-score change (95% CI) n = 3,727	FVC Z-score change (95% CI) n = 3,727	FEV_1/FVC Z-score change (95% CI) n = 3,727	FEF_{75} Z-score change (95% CI) n = 3,727	Current asthma OR (95% CI) n = 3,526	Inhalant allergic sensitization OR (95% CI) n = 3,078	Inhalant allergy OR (95% CI) n = 2,453
Model 1: Basic model							
'high n-6 PUFA' pattern	0.01	-0.02	**0.04**	**0.05**	1.09	0.95	0.96
	(-0.02, 0.04)	(-0.05, 0.01)	**(0.01, 0.07)***	**(0.02, 0.08)****	(0.95, 1.26)	(0.88, 1.02)	(0.85, 1.08)
'MUFA and SFA' pattern	-0.01	0.01	**-0.04**	**-0.04**	1.06	0.98	1.03
	(-0.04, 0.03)	(-0.02, 0.05)	**(-0.07, -0.01)***	**(-0.07, -0.01)***	(0.92, 1.23)	(0.91, 1.06)	(0.91, 1.17)
'high n-3 PUFA' pattern	**-0.04**	-0.02	**-0.04**	**-0.04**	0.94	1.03	0.94
	(-0.07, -0.01)*	(-0.05, 0.01)	**(-0.07, -0.01)***	**(-0.07, -0.02)****	(0.82, 1.09)	(0.95, 1.11)	(0.83, 1.06)
Model 2: Confounder model							
'high n-6 PUFA' pattern	0.01	-0.02	**0.03**	**0.04**	1.05	0.94	0.94
	(-0.03, 0.04)	(-0.05, 0.01)	**(0.00, 0.07)***	**(0.01, 0.07)****	(0.90, 1.22)	(0.87, 1.02)	(0.83, 1.07)
'MUFA and SFA' pattern	0.02	0.03	-0.02	-0.00	1.12	1.01	1.09
	(-0.01, 0.05)	(-0.00, 0.06)	(-0.05, 0.02)	(-0.03, 0.03)	(0.96, 1.30)	(0.93, 1.09)	(0.96,1.24)
'high n-3 PUFA' pattern	0.00	0.01	-0.01	0.01	1.00	1.07	1.02
	(-0.03, 0.03)	(-0.02, 0.04)	(-0.04, 0.02)	(-0.02, 0.04)	(0.86, 1.17)	(0.99, 1.16)	(0.90, 1.16)
Model 3: Final model							
'high n-6 PUFA' pattern	0.01	-0.02	**0.04**	**0.04**	1.06	0.94	0.94
	(-0.03, 0.04)	(-0.05, 0.02)	**(0.00, 0.07)***	**(0.01, 0.07)****	(0.91, 1.23)	(0.87, 1.02)	(0.83, 1.07)
'MUFA and SFA' pattern	0.02	0.03	-0.01	0.00	1.10	1.01	1.09
	(-0.01, 0.05)	(-0.01, 0.06)	(-0.05, 0.02)	(-0.03, 0.03)	(0.94, 1.28)	(0.93, 1.09)	(0.96, 1.24)
'high n-3 PUFA' pattern	-0.00	0.01	-0.01	0.01	1.00	1.07	1.02
	(-0.03, 0.03)	(-0.03, 0.04)	(-0.05, 0.02)	(-0.02, 0.04)	(0.86, 1.16)	(0.99, 1.16)	(0.90, 1.17)

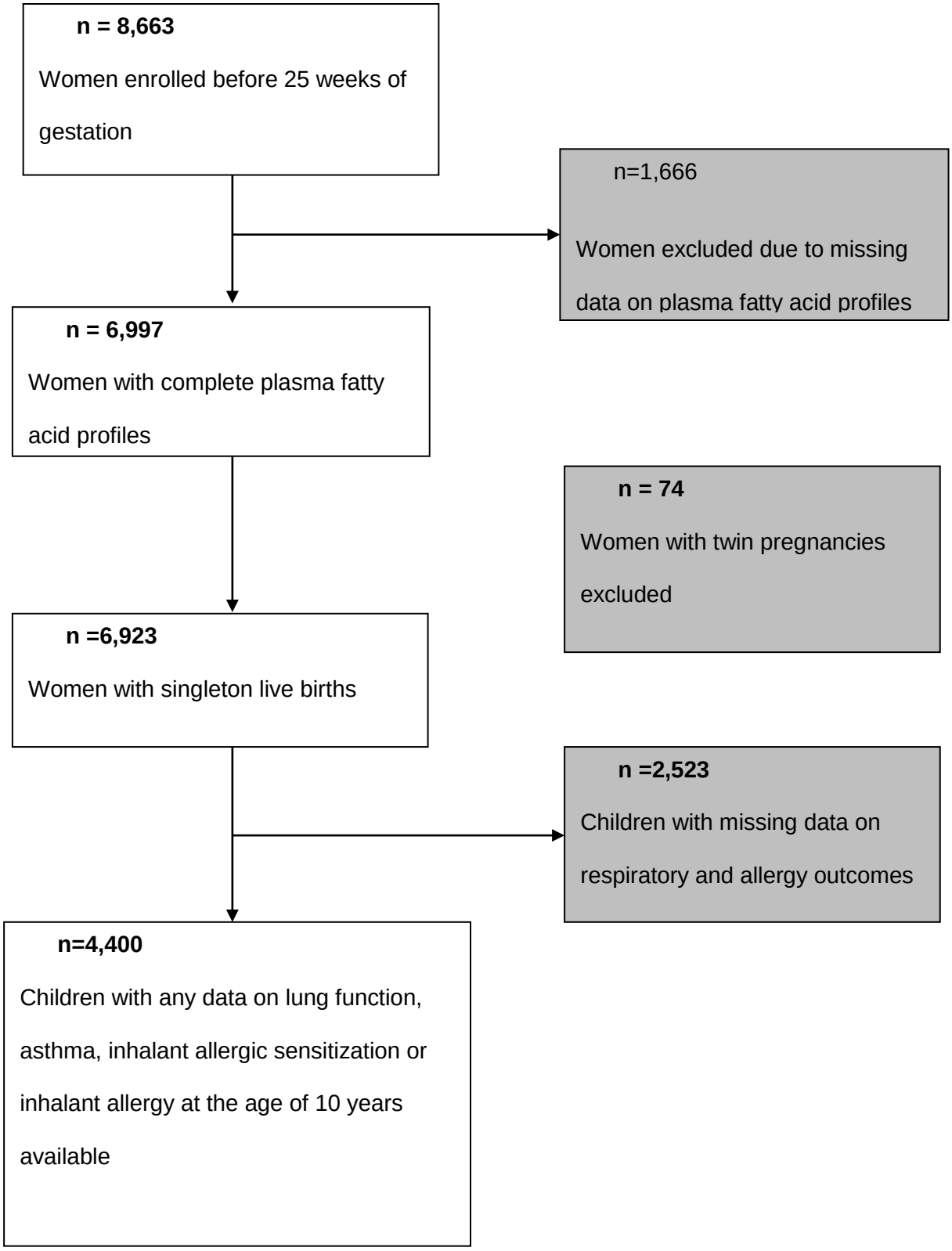
Values are Z-score changes in lung function measurements (FEV_1, FVC, FEV1/FVC, $FEF_{75)}$ or odds ratios (OR) for current asthma and inhalant allergic sensitization and inhalant allergy with 95% confidence interval (95% CI), derived from linear or logistic regression models per standard deviation score (SD) increase of fatty acid patterns during pregnancy. “n” represents number of total group. Bold indicates *p-value <0.05 and **p-value <0.01. Forced Expiratory Flow in 1 second (FEV_1), Forced Vital Capacity (FVC), Forced Expiratory Flow after exhaling 75% of FVC (FEF_{75}).). Fatty acids are classified into patterns: ‘High n6 PUFA’ pattern, ‘MUFA and SFA’ pattern, ‘High n-3 PUFA’ pattern. Model 1 (basic model) was not adjusted for confounders. Model 2 (confounder model) was adjusted for the confounders maternal age, gestational age at fatty acid measurement, pre-pregnancy body mass index, educational level, parity, history of asthma or atopy, smoking, pet keeping, folic acid supplement use and total daily calorie intake in the first trimester of pregnancy, psychological distress, child’s sex and ethnicity. Model 3 (final model) comprised the confounder model and was additionally adjusted for child’s gestational age at birth, birth weight, breastfeeding.

Table S- 4. Association of fatty acid patterns with ever asthma

	Ever asthma
Fatty acid pattern (per SD)	**OR (95% CI) n = 3,526**
Model 1: Basic model	
'high n-6 PUFA' pattern	**1.13 (1.02, 1.27)***
'MUFA and SFA' pattern	1.06 (0.94, 1.19)
'high n-3 PUFA' pattern	**0.89 (0.80, 1.00)***
Model 2: Confounder model	
'high n-6 PUFA' pattern	1.09 (0.97, 1.22)
'MUFA and SFA' pattern	1,11 (0.98, 1.25)
'high n-3 PUFA' pattern	0.94 (0.83, 1.06)
Model 3: Final model	
'high n-6 PUFA' pattern	1.09 (0.96, 1.22)
'MUFA and SFA' pattern	1.09 (0.97, 1.23)
'high n-3 PUFA' pattern	0.94(0.83, 1.06)

Values are odds ratios (OR) for ever asthma with 95% confidence interval (95% CI), derived from logistic regression models per standard deviation score (SDS) increase of fatty acid patterns during pregnancy. "n" represents number of total group. Bold indicates *p-value <0.05 and **p-value <0.01. Fatty acids are classified into patterns: 'High n6 PUFA' pattern, 'MUFA and SFA' pattern, 'High n-3 PUFA' pattern. Model 1 (basic model) was not adjusted for confounders. Model 2 (confounder model) was adjusted for the confounders: maternal age, gestational age at fatty acid measurement, pre-pregnancy body mass index, educational level, parity, history of asthma or atopy, smoking, pet keeping, folic acid supplement use and total daily calorie intake in the first trimester of pregnancy, psychological distress, child's sex and ethnicity. Model 3 (final model) comprised the confounder model and was additionally adjusted for child's gestational age at birth, birth weight, breastfeeding.

Figure S-1. Flow chart of participants included for analysis



2. 25-Hydroxyvitamin D levels during pregnancy and lung volumes in childhood. The Generation R Study.

Sources of financial support

The Generation R Study is made possible by financial support from the Erasmus Medical Centre, Rotterdam, the Erasmus University Rotterdam and The Netherlands Organization for Health Research and Development. MD received an EAACI Research Fellowship for this project. The study sponsors had no role in the study design, data analysis, interpretation of data, or writing of this report.

ABSTRACT

Background Maternal 25-hydroxyvitamin D concentrations during pregnancy seems associated with the risk asthma of the child. The underlying mechanism might be smaller lung volumes.

Objective To examine the associations of maternal 25-hydroxyvitamin D concentrations during pregnancy with lung volumes measured by MRI scans and lung function measured by spirometry of the children at school age

Methods This study among 518 children and their mothers was performed in a population-based prospective cohort study in a population-based prospective cohort study. Maternal venous blood samples in mid-gestation and umbilical cord blood samples at birth were used to determine 25-hydroxyvitamin D concentrations. At age 10 years, lung function was measured by spirometry, and spirometry-guided lung volumes were measured from MRI scans obtained according to standardized protocols using a 3.0 Tesla GE 750 MR scanner. Adjusted multivariate linear regression models were applied.

Results The median 25-hydroxyvitamin D concentration in mid-gestation was 66.4 nmol/L (95% range 19.0-124.7). Mean right lung volume Z-score (SD) was 0.02 (0.86), left lung volume was 0.01 (0.87), and total lung volume 0.02 (0.86). No associations were found for maternal 25-hydroxyvitamin D concentration during pregnancy with lung volumes.

Conclusion The relation of maternal 25-hydroxyvitamin D concentrations during pregnancy with the risk asthma of the child is not explained by altered lung volumes. Further studies on other potential underlying mechanisms are needed.

Introduction

Vitamin D during pregnancy plays an important role in the development of childhood respiratory disease through its effects on fetal lung development and immunomodulation(1). Vitamin D deficiency might influence lung structure and function through its effect on the pulmonary surfactant release(2) and on gene expression(3). Studies in animals showed that prenatal 25-hydroxyvitamin D deficiency is associated with decreased lung volumes and lung function(4, 5). A recent meta-analysis of observational studies did not find any consistent association of maternal 25-hydroxyvitamin D levels during pregnancy with lung function from age 6 months to 8 years(6). We previously observed that higher maternal 25-hydroxyvitamin D concentrations in mid-pregnancy, but not at birth, were associated with a lower Forced Expiratory Volume in 1 second/Forced Vital Capacity (FEV_1/FVC) and Forced Expiratory Flow after exhaling 75% of FVC (FEF_{75}), but not with asthma(7). The underlying mechanism might be smaller lung volumes. So far, no human studies have assessed lung volumes measured by magnetic resolution imaging (MRI).

Therefore, we examined in a population-based prospective cohort study among 518 children and their mothers the associations of maternal 25-hydroxyvitamin D concentrations during pregnancy with lung volumes measured by MRI scans and lung function measured by spirometry of the children at school age.

Method

This study was embedded in the Generation R Study, a population-based prospective cohort study from early fetal life onwards in Rotterdam, the Netherlands(8). The study was approved by the Medical Ethical Committee of the Erasmus University Medical Centre in Rotterdam, the Netherlands (MEC-2012-165-NL40020.078.12). Written informed consent was obtained from all parents or legal guardians. We used data from a randomly selected subgroup of children with a Dutch ethnic background(8), and included a total of 518 singleton children for the current

analyses (Figure 1). Maternal venous blood samples in mid-gestation (median 20.4 (95% range 18.5-22.8)) were used to determine 25-hydroxyvitamin D concentrations, as previously described in detail(9, 10) . At the age of 10 years, lung volumes were measured from MRI scans, which were obtained according to standardized protocols using a 3.0 Tesla GE 750 MR scanner (GE Healthcare, Milwaukee, WI, USA). All scans were spirometry-guided to ascertain collaboration and full inspiration by the participant(11). We selected the children who only had good quality MRI scans. Right lung volume, left lung volume and the total lung volume were measured from inspiratory scans and were converted into height- adjusted Z-scores to enable comparison of the effect sizes. We calculated the average of lung volumes of two inspiratory scans. FEV_1, FVC , FEV_1/FVC were measured by spirometry and were converted into sex-, height-, age-, and ethnicity-adjusted Z-scores according to the Global Lung Initiative reference data(12).

We obtained information on covariates maternal age, body mass index at enrolment , educational level, and smoking during pregnancy from questionnaires during pregnancy. Child's sex, gestational age at birth and birthweight were obtained from midwife and hospital registries or questionnaires at birth. We used multivariate linear regression models to examine the associations of maternal 25-hydroxyvitamin D concentrations in mid-pregnancy with lung volumes and lung function of the children at school age. Model 1 (Basic model) was adjusted for child's sex and age. Model 2 (Main model) was additionally adjusted for the confounders maternal age, body mass index at enrolment, educational level, and smoking during pregnancy. Model 3 (Intermediate model) comprised model 2, and was additionally adjusted for gestational age at birth and birth weight. Confounders were included in our model based on literature.

Results

Maternal and child characteristics are shown in Table 1. The median 25-hydroxyvitamin D concentration in mid-gestation was 66.4 nmol/L (95% range 19.0-124.7). Mean FEV_1 Z-score

(SD) was 0.05 (0.90), FVC was 0.11 (0.84), and FEV_1/FVC was −0.14 (0.93). Mean right lung volume Z-score (SD) was 0.02 (0.86), left lung volume was 0.01 (0.87), and total lung volume 0.02 (0.86). We tested the correlation between lung volumes and lung function measures and showed that they were statistically correlated (sig. (2-tailed) < 0.05) (Table 2).

In the basic model, we did not observe any associations of 25-hydroxyvitamin D concentrations in mid-gestation with lung volumes and spirometry lung function measures (Table 3). Also, in our main and intermediate model the results remained the same.

Discussion

In this population-based prospective cohort studies, we did not observe associations of maternal 25-hydroxyvitamin D levels in mid-pregnancy with lung volumes of the children at school age. This suggest that smaller lung volumes do not underlie the previous observed associations of maternal 25-hydroxyvitamin D levels in mid-pregnancy with lower lung function of the children. However, in this subgroup we also did not observe associations of maternal 25-hydroxyvitamin D levels in mid-pregnancy with lower lung function of the children. The latter results are in line with two previous cohort studies, which reported no association of maternal 25-hydroxyvitamin D levels in late-pregnancy with any lung function measures(13, 14). We previously found in a larger sample size that a higher maternal 25- hydroxyvitamin D concentration in mid-pregnancy were associated with a lower FEV_1/FVC and FEF_{75}(7). The difference in results may be explained by the current smaller sample size and participation of only children with Dutch ethnic background. Furthermore, our findings are in variance with studies in animals which showed that 25-hydoxyvitamin D deficiency was associated with lower lung volumes and lung function (4, 5). Also, a recent meta-analysis pooled 4 cohort studies and found no associations of maternal 25-hydroxyvitamin D concentrations during pregnancy with FEV_1 and FVC(6). Thus, based on our and previous studies, the role of maternal 25-hydroxyvitamin D during pregnancy

on respiratory morbidity, and the potential underlying role smaller lung volumes of the child remains unclear.

Conclusion

Our results suggest that 25-hydroxyvitamin D concentrations during pregnancy do not influence lung volumes and lung function in children at school age. Further larger sample-sized studies and randomized control trials are needed to explore the role of maternal vitamin D during pregnancy on lung volumes and lung function in the children, and the role of child's vitamin D status.

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Table 1. Characteristics of mothers and children included in the analysis.

	n=518
Maternal characteristics	
Age, years	32.0 (3.8)
Missing (%)	-
Body mass index at enrolment (kg/m^2)[1]	23.3 (18.8-36.4)
Missing (%)	-
Educational level, higher (%)	64.9 (336)
Missing (%)	0.8 (4)
Smoking during pregnancy, yes (%)	20.4 (96)
Missing (%)	9.3 (48)
25-Hydroxyvitamin D concentration in mid-gestation (nmol/L)[1]	66.4 (19.0-124.7)
Child characteristics	
Gender, female (%)	50.8 (263)
Missing (%)	-
Gestational age at birth (weeks)[1]	40.3 (36.9-42.4)
Missing (%)	-
Birth weight (grams)[1]	3560(2380-4490)
Missing (%)	-
Age at the lung MRI visit (years)[1]	9.98 (9.4-11.6)
Missing (%)	-
Right lung volume (Z- score)	0.02 (0.86)
Left lung volume (Z- score)	0.01 (0.87)
Total lung volume (Z- score)	0.02 (0.86)
FEV_1 (Z- score)	0.05 (0.90)
FVC (Z- score)	0.11 (0.84)
FEV_1/FVC (Z-score)	-0.14 (0.93)

Values are means (SD), medians[1] (2.5-97.5th percentile) or valid percentages (absolute numbers), based on observed data.

2 . Correlation between lung volumes and lung function variables (n = 518)

	Right lung volume (Z- score)	**Left lung volume (Z- score)**	**Total lung volume (Z- score)**	**FEV_1 (Z-score)**	**FVC (Z-score)**	**FEV_1/FVC (Z-score)**
Right lung volume (Z- score)	1	0.92**	0.98**	0.41**	0.50**	-0.12**
Left lung volume (Z- score)	0.92**	1	0.98**	0.43**	0.53**	-0.13**
Total lung volume (Z- score)	0.98**	0.98**	1	0.43**	0.53**	-0.13**
FEV_1 (Z-score)	0.41**	0.43**	0.43**	1	0.81**	0.35**
FVC (Z-score)	0.50**	0.53**	0.53**	0.81**	1	-0.26**
FEV_1/FVC (Z-score)	-0.12**	-0.13**	-0.13**	0.35**	-0.26**	1

Values are Pearson correlation coefficients.
** Correlation is significant at the 0.01 level (2-tailed).

Table 3. Associations of 25-hydroxyvitamin D concentrations in mid-gestation with lung volumes and lung function at age 10 years.

25-hydroxyvitamin D concentrations (per 10 nmol/L)	**Right lung volume Z-score change (95% CI) n=518**	**Left lung volume Z-score change (95% CI) n=518**	**Total lung volume Z-score change (95% CI) n=518**	**FEV_1 Z-score change (95% CI) n=518**	**FEV_1 Z-score change (95% CI) n=518**	**FEV_1/FVC Z-score change (95% CI) n=518**
Model 1 (Basic model)	-0.01 (-0.03, 0.02)	-0.01 (-0.04, 0.01)	-0.01(-0.03, 0.02)	-0.02 (-0.04, 0.01)	-0.02 (-0.04, 0.01)	0.00 (-0.03, 0.03)
Model 2 (Main model)	-0.01 (-0.03, 0.02)	-0.01 (-0.04, 0.02)	-0.01 (-0.03, 0.02)	-0.01 (-0.04, 0.02)	-0.01 (-0.04, 0.02)	0.00 (-0.03, 0.03)
Model 3 (Intermediate model)	-0.01 (-0.03, 0.02)	-0.01 (-0.03, 0.02)	-0.01 (-0.03, 0.02)	-0.01 (-0.04, 0.02)	-0.01 (-0.04, 0.02)	0.00 (-0.03, 0.04)

Values are derived from linear regression models and reflect change in Z-scores with 95% confidence interval (95% CI) per 10 nmol/L increase in the 25-hydroxyvitamin D concentration. Forced Expiratory Flow in 1 second (FEV_1), Forced Vital Capacity (FVC). Model 1 (Basic model) was adjusted for child's sex and age. Model 2 (Main model) was additionally adjusted for the confounders maternal age, body mass index at enrolment, educational level, smoking during pregnancy. Model 3 (Intermediate model) comprised model 2 and was additionally adjusted for gestational age at birth and birth weight. Bold indicates p-value < 0.05.

Figure 1. Flow chart of participants included for analysis