

Effects of prolonged breastfeeding and colostrum fatty acids on allergic manifestations and infections in infancy

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Clinical & Experimental Allergy

Summary

Background In developed countries World Health Organization recommendation of 6 months' exclusive breastfeeding is under debate.

Objective We assessed the impact of predominant breastfeeding (PBF) duration and colostrum long-chain polyunsaturated fatty acids (LC-PUFAs) profile on the risk of allergic manifestations (wheezing and atopic eczema) and infections [low respiratory tract infections (LRTIs) and gastroenteritis] in infancy.

Methods Information on child feeding practices was obtained from 580 infants of a pregnancy cohort. Presence of infant's health outcomes was documented through questionnaires at 6 and 14 months of age. The LC-PUFAs were measured in colostrum. Adjusted odds ratios (adjOR) and 95% confidence intervals (CI) were estimated using multivariable logistic regression models.

Results In comparison with never breastfeeding, PBF for 4–6 months was associated with lower risk of wheezing (adjOR = 0.53; 95% CI, 0.32, 0.89), LRTIs (adjOR = 0.51; 95% CI, 0.31, 0.83) and atopic eczema (adjOR = 0.58; 95% CI, 0.32, 1.04) between months 7 and 14 of life. Results of a risk period-specific analysis (restricted to infants at risk for outcome onset after 6 months of age), showed no indication for reverse causation (results were not very different compared with an overall analysis). Predominantly breastfeeding for 4–6 months was associated with lower risk of gastroenteritis during the first 6 months of life (adjOR = 0.34; 95% CI, 0.18, 0.64). Among breastfed infants higher doses of arachidonic acid (AA), docosahexaenoic acid, and total n-3 in were associated with a decreased risk of gastroenteritis, but no association was found for allergic manifestations or LRTI.

Conclusions and Clinical Relevance Promotion of PBF for 4–6 months could reduce the burden of allergic manifestations and infections in infancy. Beneficial effects of breastfeeding on gastroenteritis were explained in part by exposure to higher doses of n-3 and AA received from colostrum. No significant effects from fatty acid dose were found on risk of allergic manifestations or LRTIs.

Keywords breastfeeding, eczema, gastroenteritis, long-chain polyunsaturated fatty acids, lower respiratory tract infections, wheeze

Abbreviations AdjOR, adjusted odds ratio; AA, arachidonic acid; ALA, α -linolenic acid; CI, confidence interval; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; INMA, Infancia y Medio Ambiente; LA, linoleic acid; LC-PUFA, long-chain polyunsaturated fatty acid; LRTI, lower respiratory tract infection; MAR, missing at random; OR, odds ratio; PBF, predominant breastfeeding; SPT, Skin prick test.

Submitted 02 June 2011; revised 12 December 2011; accepted 04 January 2012

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Cite this as: E. Morales, R. García-Esteban, M. Guxens, S. Guerra, M. Mendez, C. Moltó-Puigmartí, M. C. Lopez-Sabater and J. Sunyer, *Clinical & Experimental Allergy*, 2012 (42) 918–928.

Introduction

In developed countries, the World Health Organization (WHO) recommendation of 6 months' exclusive breastfeeding is under debate [1–3]. Efficacy of greater duration and exclusivity of breastfeeding on reducing common infection rate (respiratory and gastrointestinal tract infections) is accepted [4–8]; but evidence on asthma and allergic diseases is conflicting [9–18], and potential risks are described (iron deficiency anaemia and subsequent adverse neurodevelopment outcomes) [19]. Consequently, a recent expert review for the European Food Safety Authority concluded that for infants across the EU complementary foods may be introduced safely between 4 and 6 months [20].

The biological mechanisms underpinning the effects of breast milk on infant health outcomes are diverse. Immunomodulatory and anti-inflammatory factors present in human milk could protect infants against infections and allergic manifestations [21, 22]. Specifically, the profile in human milk of long-chain polyunsaturated fatty acids (LC-PUFAs) has been studied in relation to asthma, atopy, and eczema in childhood showing controversial findings in part due to the limited size of these studies [23–27].

The elucidation of the benefits and risks of prolongation and exclusively breastfeeding, as well as, the biological mechanisms involved would be of great public health impact and will help guide new policies and recommendations. We aimed to assess the effects of predominant breastfeeding (PBF) duration and colostrum LC-PUFA profile on the risk of allergic manifestations (wheezing and atopic eczema) and infections [lower tract respiratory infections (LRTIs) and gastroenteritis] in infants.

Methods

Study population and design

Data for this report come from a population-based pregnancy cohort set up in Sabadell (Spain) as part of the *Infancia y Medio Ambiente (INMA)* Project [28]. Criteria for inclusion of the mothers were: 1) to be resident in the study area, 2) to be at least 16 years old, 3) to have a singleton pregnancy, 4) to not have followed any programme of assisted reproduction, 5) to wish to deliver in the reference hospital, and 6) to have no communication problems. A total of 657 women who fulfilled the inclusion criteria were enrolled between June 2004 and July 2006 during their visits to the public health centre in the first trimester of pregnancy, and 622 (95%) were followed until the child's birth. Complete information about breastfeeding practices was available for 580 (88%) participants considered to be

eligible for the present study. Between the 580 children included and the original 622, no differences in characteristics—including gestational age, birth weight, siblings at birth, maternal smoking during pregnancy, maternal atopy status or child's gender—were found ($P > 0.05$). However, children not included had lower parental social class and lower maternal education ($P < 0.05$). Informed consent was obtained from all participants and the study was approved by the Clinical Research Ethical Committee of the Municipal Institute of Health Care and the Hospital of Sabadell.

Information on parental characteristics including education, socioeconomic background (using the International Standard Classification of Occupations code), marital status, maternal health and obstetric history, siblings at birth, medication use, maternal alcohol and smoking habits, anthropometric measures, environmental exposures, time-activity patterns and dietary intake was obtained by questionnaire at the first and third trimester of pregnancy [28]. In serum extracted during the first trimester of pregnancy levels of total IgE and specific IgE [dust mite (Der p 1), cat allergen (Fel d 1) and mixed grass pollen] were determined by using electrochemical luminescence. Maternal atopy was defined as a positive value to any of the allergens (levels ≥ 0.35 kU/L). Levels of organochlorines, including dichlorodiphenyldichloroethylene, were analysed in maternal serum extracted during the first trimester of pregnancy, using methods described elsewhere [29].

Outcomes

Information about the outcomes of interest (wheezing, physician-confirmed diagnosis of LRTIs, atopic eczema, and gastroenteritis) was obtained from parents through two questionnaires completed when the child was 6 and 14 months old. Wheezing was defined as a positive answer to the question 'Has ever your child experienced whistling or wheezing from the chest, but not noisy breathing from the nose since birth to six months, and from 7 to 14 months?' Occurrence of an LRTI was defined as a positive answer to the question 'Has a doctor ever told you that your son/daughter has had a chest infection since birth to six months, and from 7 to 14 months?' A second question referred to a specific question on doctor diagnosis of each bronchitis, bronchiolitis or pneumonia. Agreement between the two questions was almost perfect. Atopic eczema was defined as a positive response to the question 'Has your child ever experienced atopic eczema since birth to six months, and from 7 to 14 months?' Gastroenteritis was defined as a positive answer to the question 'Has your child ever had any episode of diarrhea and vomiting since birth to six months, and from 7 to 14 months?'

Recurrent outcomes were defined as positive answer both at 6 and 14 months interviews.

Breastfeeding assessment and measurement of colostrum fatty acids

Detailed information about child feeding was completed by interviewer-administered questionnaires with mothers at 6 and 14 months. Dates of food introduction and types of foods introduced, date of formula-fed introduction, and monthly average of daily number of formula-feeds and breastfeeds were reported. PBF was defined as infant received breast milk only and allowed supplementation of non milk liquids [water or water-based drinks (sweetened and flavoured water, teas, infusions, etc.)], fruit juice, oral rehydration salts solution, drops and syrup forms of vitamins, minerals and medicines according to the WHO's definition [30]. Children were grouped into the following five breastfeeding categories: 1) children who were never breastfed (used as the reference group), 2) very short-term predominantly breastfeeding (< 2 months), 3) short-term predominantly breastfeeding (2–4 months), 4) long-term predominantly breastfeeding (4–6 months) and 5) very long-term predominantly breastfeeding (> 6 months).

Colostrum samples were collected between the first 48 and 96 h post-partum at the hospital by an experienced nurse in a random subsample ($n = 352$). Parents of children with colostrum sample had lower education level ($P < 0.05$). Children of mothers providing colostrum sample were predominantly breastfed for longer duration and had lower prevalence of atopic eczema between 7 and 14 months of life and gastroenteritis in the first 6 months of life ($P < 0.05$). No differences were found in the rest of variables. Samples were collected in the morning and at the end of the feeding in sterile polypropylene tubes by mechanical expression of one breast using a breast pump and transported to the laboratory in ice boxes in less than 2 h after collection. Samples were stored at -80°C until analysed. Fatty acid methyl esters were prepared with sodium methylate and methanolic boron trifluoride and extracted into hexane following the method developed by Moltó-Puigmartí *et al.* [31]. Then, they were separated and quantified by fast-gas chromatography with flame ionization detection, according to the same method. Fatty acid levels were expressed as proportions of each individual fatty acid with respect to the total fatty acids identified in the samples (wt% of total fatty acids). To assess evidence for the effects of fatty acid profile in colostrum on children health outcomes, the following fatty acids were selected as exposure variables: linoleic acid (LA; 18:2n-6), α -linolenic acid (ALA; 18:3n-3), arachidonic acid (AA; 20:4n-6), eicosapentaenoic acid (EPA; 20:5n-3), docosahexaenoic acid (DHA; 22:6n-3); total n-6

series [the sum of LA; γ -linolenic acid (18:3n-6), Dihomo- γ -linolenic acid (20:3n-6), AA (20:4n-6), Adrenic acid (22:4n-6), Docosapentaenoic acid (22:5n-6)]; and total n-3 series [the sum of ALA (18:3n-3), EPA (20:5n-3), Docosapentaenoic acid (DPA) (22:5n-3) and DHA (22:6n-3)].

Statistical analysis

To increase efficiency and minimize selection bias we used multivariate multiple imputation to impute missing variables for participants considered to be eligible (children having information on breastfeeding practices, $n = 580$), including exposures (colostrum's LC-PUFA), covariables, outcomes, and potential predictors of missing data in the imputation equations (Table S1). Multiple imputation of missing values using chained equations [32] was carried out to account for the 580 children. One hundred completed data sets were generated and analysed separately, and the results were combined using Rubin's rules [33]. Distributions in imputed datasets were similar to those observed (Table S2), which indicates that the missing at random (MAR) assumption holds true and the imputation model used to generate imputations enables to preserve the associations among all variables in the data set. Analyses were also repeated restricting to those with complete data on all variables used in any analyses—that is, 'complete case' analysis. Analysis based on complete cases may suffer more chance variation, and under the MAR assumption multiple imputation increases efficiency and reduces biases that may arise in complete cases analyses [34]. Thus, multiple imputation results are presented in the main article and the complete case analysis results are presented in the supplementary information file (Tables S3 and S4).

In addition, to explore reverse causation (i.e. influence of disease onset on exposure), we compared the results for an overall analysis with risk period-specific analyses considering exposure only before the risk period (7–14 months) and restricting the analysis to infants at risk (i.e. excluding infants with onset of outcomes before 7 months of age) [17].

In separate multivariable logistic regression models for each LC-PUFA of interest we assessed the relationship between an estimated fatty acid dose with infant's health outcomes among breast-fed children ($n = 449$). Fatty acid dose groups were calculated by using a categorization of LC-PUFA's concentration and duration of breastfeeding calculated to reflect short or long duration of feeding (divided at the median in weeks) and low (lower tertile) vs. medium-high (medium and high tertiles) LC-PUFA concentration [35]. All variables significantly related with outcomes in the bivariate models ($P < 0.2$) were included in the multivariable model, and

only retained if they had at least marginally significant association ($P < 0.1$) or changed the OR more than 5%. Results are presented as odds ratios (OR) and 95% confident intervals. Significance was accepted at P less than 0.05. All statistical analyses were performed using the STATA 10.1 statistical software (Stata Corporation, College Station, TX, USA).

Results

Baseline characteristics

Overall 131 (22.6%) infants were never breastfed, 75 (12.9%) were predominantly breastfed for less than 2 months, 97 (16.7%) for 2–4 months, 215 (37.1%) for 4–6 months and 62 (10.7) for more than 6 months. Overall 12.7% of the children recorded recurrent wheezing episodes and 12.4% recurrent LRTIs in the first 14 months of life. 7.2% of the children developed recurrent atopic eczema, and 12.4% had recurrent gastroenteritis episodes (Table 1). Longer PBF duration was related to higher maternal education level and higher social class (Table 1). Children of obese and smoker mothers tended to be less likely long-term breastfed. Infants never breastfed recorded more likely recurrent health outcomes in comparison with children predominantly breastfed (Table 1).

Breastfeeding practices and infant's health outcomes

After adjustment for main confounders, PBF for 2–4 months was associated with lower risk of recurrent wheezing (adjOR = 0.29; 95% CI 0.10, 0.83) and LRTIs (adjOR = 0.33; 95% CI 0.13, 0.86). Predominant breastfeeding for 4–6 months was related to a significant decreased risk of wheezing between months 7 and 14 (adjOR = 0.53; 95% CI 0.32, 0.89) and for recurrent wheezing (adjOR = 0.52; 95% CI 0.27, 0.99) (Fig. 1). Similarly, children PBF for 4–6 months presented a significant lower risk of being diagnosed for LRTIs between months 7 and 14 (adjOR = 0.51; 95% CI 0.31, 0.83) and for recurrent LRTIs (adjOR = 0.48; 95% CI 0.24, 0.96). Predominant breastfeeding for 4–6 months tended to protect against atopic eczema during 7–14 months of age (adjOR = 0.58; 95% CI 0.32, 1.04) and against recurrent episodes (adjOR = 0.44; 95% CI 0.19, 1.04). Also, children predominantly breastfed for 4–6 months showed a reduced risk of having gastroenteritis during the first 6 months (adjOR = 0.34; 95% CI 0.18–0.64) and recurrent gastroenteritis (adjOR = 0.37; 95% CI 0.17–0.77) (Fig. 1). Associations did not differ according to maternal atopic status (Table S5).

The results of the overall analysis showed a lower risk of wheezing and LRTI in the first 14 months of life associated with PBF for 4–6 months (Table 2). A

tendency toward a lower risk of atopic eczema was found for PBF for 4–6 months, but this was not statistically significant. For the risk period of 7–14 months, we considered only cases with onset after 6 months. The results again showed a lower risk of wheezing and LRTI developing between 7 and 14 months of life associated with PBF for 4–6 months, and a tendency towards a lower risk of atopic eczema, which was not statistically significant (Table 2). Although estimations for gastroenteritis in the overall analysis showed a tendency towards a lower risk, the risk period-specific analysis (7–14 months) showed null results, similar to results showed in Fig. 1.

Fatty acids in colostrum and infant's health outcomes

Among mothers breastfeeding colostrum samples were provided by 283 women. The concentrations of n-6 and n-3 fatty acids in colostrum were in agreement with previously published data. The means of total n-6 and total n-3 fatty acid were 15.3 (SD = 2.8) and 1.45 (SD = 0.41), respectively. The n-6/n-3 ratio was 11.3 (Table 3). No significant differences were found between atopic and non-atopic mothers in the level of individual fatty acids, and total n-6 or total n-3. LC-PUFA concentrations in colostrum of mothers breastfeeding did not differ among participants with complete information and with data with missing data imputed (Table S2).

In multivariable analyses the risk of gastroenteritis in the first 6 months of life was significantly decreased with increasing AA (adjOR = 0.38; 95% CI 0.16–0.92), DHA (adjOR = 0.25; 95% CI 0.11–0.57), and total n-3 (adjOR = 0.28; 95% CI 0.12–0.66) doses (long breastfeeding and medium-high LC-PUFA level compared with short breastfeeding and low LC-PUFA level) (Table 4). In addition, a reduced risk of recurrent gastroenteritis was found with increasing DHA and total n-3 doses (Table 4). By contrast, there were no significant associations between LC-PUFA dose and wheezing (Table S6), LRTIs (Table S7) or atopic eczema (Table S8).

Discussion

In this pregnancy cohort study, we found that PBF for 4–6 months conferred protection against allergic manifestations (wheezing and atopic eczema) and LRTIs between months 7 and 14 of life and recurrent episodes. Results of overall analysis and risk period (7–14 months)-specific analysis were not very different, which indicated no reverse causation. In addition, PBF for 4–6 months was associated with lower risk of gastroenteritis during the first 6 months of life and recurrent episodes. No effects were observed for

Table 1. Distribution of infant and parental characteristics according to breastfeeding practices. The INMA Sabadell cohort (*n* = 580 eligible participants)

			Breastfeeding practices					<i>P</i> -value
	N with data	All	Never BF	< 2 months	2–4 months	4–6 months	> 6 months	
			<i>n</i> = 131	PBF <i>n</i> = 75	PBF <i>n</i> = 97	PBF <i>n</i> = 215	PBF <i>n</i> = 62	
Child characteristics								
Male gender	580	51.9	55.0	49.3	50.5	51.6	51.6	0.942
Siblings at birth, yes	577	43.3	50.4	33.3	40.0	41.1	53.2	0.060
Preterm (<37weeks)	554	2.7	4.0	1.4	2.1	2.9	1.7	0.796
Birth weight, mean (SD)	553	3251.6 (417.7)	3285.6 (462.5)	3232.8 (393.5)	3232.0 (432.3)	3226.9 (383.9)	3321.6 (439.1)	0.435
Day care attendance, yes	551	31.2	36.4	28.6	22.8	32.7	31.6	0.292
Maternal characteristics								
Age at delivery, mean (SD), years	553	31.6 (4.2)	32.2 (4.7)	30.4 (3.9)	31.5 (4.5)	31.5 (3.9)	32.5 (4.1)	0.009
Education level, primary or less	577	27.0	37.2	26.7	24.7	22.9	24.2	0.016
Social class, non manual	575	67.7	57.2	71.6	72.6	70.4	67.7	0.016
BMI prior pregnancy (kg/m ²)	580							
20–24.99	336	57.9	58.8	56.0	63.9	57.2	51.6	0.001
< 20	86	14.8	12.2	6.7	17.5	19.1	11.3	
25–29.99	111	19.1	14.5	22.7	16.5	19.1	29.0	
≥ 30	47	8.1	14.5	14.7	2.1	4.7	8.1	
Smoker in pregnancy, yes	572	15.6	22.1	16.2	11.7	15.0	8.3	0.098
Postnatal smoker, yes	552	25.7	28.9	24.3	26.1	28.0	12.1	0.139
Allergic asthma	553	8.3	12.1	5.6	9.7	7.3	5.1	0.361
Atopic eczema	553	12.5	16.1	11.1	8.6	12.7	11.9	0.567
Allergic rhinitis	553	16.5	19.3	16.7	17.2	13.7	18.6	0.704
Atopy*, yes	557	28.7	30.4	28.8	37.0	25.4	24.1	0.291
Paternal characteristics								
Education level, primary or less	548	35.4	43.0	32.4	33.3	34.3	30.5	0.378
Social class, manual	508	58.7	66.1	55.2	61.5	57.6	47.3	0.188
Smoker, yes	548	40.1	39.8	34.3	37.0	46.7	29.3	0.094
Allergic asthma	553	6.7	4.0	5.6	4.3	8.3	11.9	0.217
Atopic eczema	553	7.8	8.9	5.6	8.6	8.3	5.1	0.833
Allergic rhinitis	553	14.3	12.1	11.1	18.3	12.7	22.0	0.230
Outcomes								
Wheezing								
In months 1–6	568	19.5	26.9	12.2	11.6	18.9	28.1	0.009
In months 7–14	550	28.9	36.7	40.0	29.3	21.4	25.9	0.008
Recurrent	543	12.7	22.7	8.7	5.6	11.5	12.3	<0.001
LRTIs								
In months 1–6	575	20.0	23.7	17.3	13.4	18.7	31.0	0.069
In months 7–14	548	31.9	41.7	35.7	27.2	25.4	38.6	0.018
Recurrent	548	12.4	18.3	11.4	7.6	9.6	19.3	0.090
Atopic eczema								
In months 1–6	546	13.6	17.2	11.3	14.1	11.8	14.0	0.686
In months 7–14	543	16.6	23.7	14.5	16.7	13.9	14.0	0.203
Recurrent	513	7.2	12.8	4.6	5.9	4.9	10.2	0.278
Gastroenteritis								
In months 1–6	545	16.7	24.8	25.4	18.5	9.9	10.0	0.001
In months 7–14	548	62.2	63.6	58.6	62.0	60.5	70.7	0.639
Recurrent	517	12.4	19.4	15.2	11.5	8.7	10.0	0.266

BF, Breastfeeding; PBF, predominant breastfeeding; BMI, body mass index. Values are percentages for categorical variables and mean (SD) for continuous variables.

*IgE levels ≥ 0.35 kU/L to any of dust mite (Der p 1), cat allergen (Fel d 1) and mixed grass pollen.

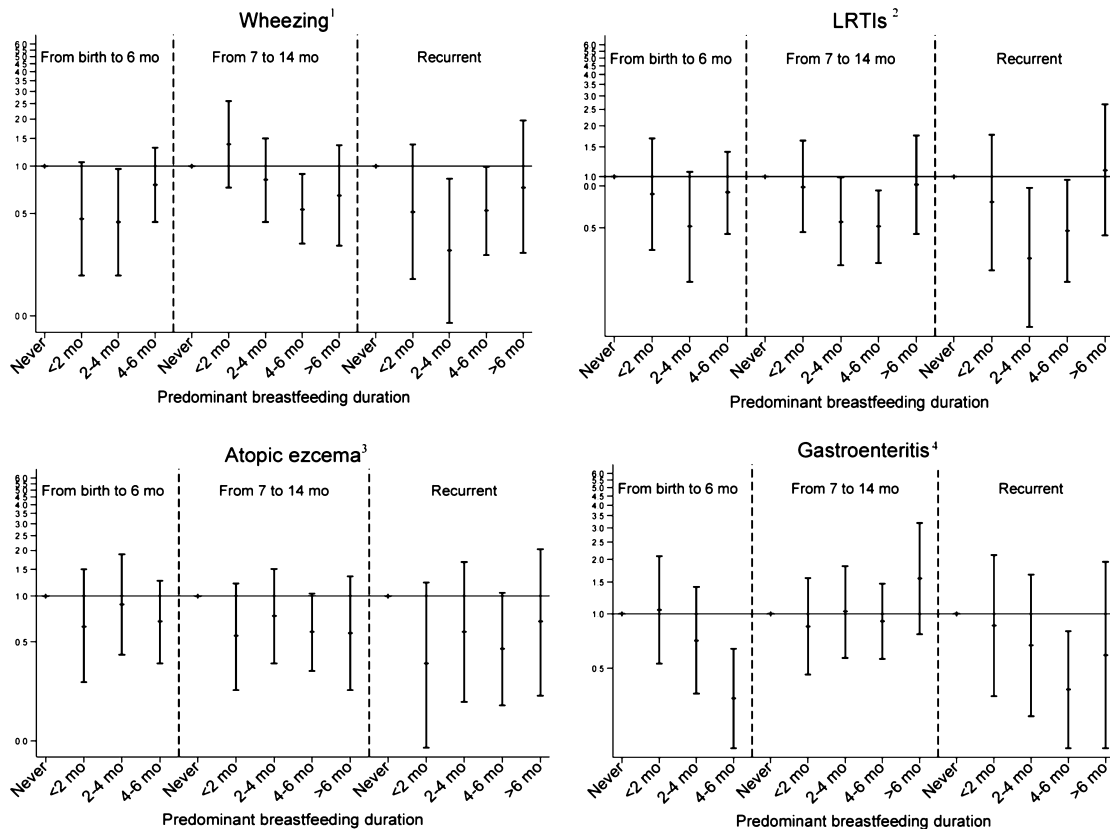


Fig. 1. Associations between breastfeeding duration and offspring outcomes among eligible participants with missing data imputed by multivariate multiple imputation ($n = 580$). Lines represent odds ratios (OR) and 95% confidence interval (CI). ¹All models adjusted for child gender, maternal social class, siblings at birth, maternal smoking in pregnancy, and DDE levels in cord blood. ²All models adjusted for child gender, maternal social class, and siblings at birth. ³All models adjusted for child gender, maternal atopy [positive skin prick test (SPT)], and maternal pre-pregnancy body mass index. ⁴All models adjusted for siblings at birth, maternal smoking in pregnancy, maternal social class, and day care attendance.

children predominantly breastfed for more than six months. Among breast-fed children, exposure to higher doses of AA, DHA, and total n-3 was associated with a reduced risk of gastroenteritis, but no significant associations were found on risk of allergic manifestations or LRTIs.

The strengths of the present study include the measurement of LC-PUFAs in colostrum, its prospective nature, and the population-based design. Information on child feeding was completed by interviewer-administered questionnaires with mothers at 6 and 14 months which reduces potential retrospective recall bias about feeding history [36]. We used the standard definition for PBF practices adopted by WHO which takes into account the introduction of solids. Moreover, models were adjusted for a large number of potential confounders minimizing the possibility of residual confounding. In addition, we used multiple imputation procedure for accounting of missing data over time, a common problem in prospective studies resulting in biased results. Overall 88% of our initial population was included in the present study, which minimizes the selection bias. Distributions in imputed datasets were

similar to those observed supporting the assumption that the missing data were MAR. Under this assumption multiple imputations approach results are considered valid.

However, the study also has some limitations. Information on offspring health outcomes was obtained from parental report through interviewer-administered questionnaires, which could result in misclassification. An important concern is the risk of disease-related modification of exposure or reverse causality. In this case symptoms of allergic diseases might occur already during breastfeeding and may encourage the mother to continue to breastfeed. Reverse causation can be avoided by excluding infants with onset of disease during lactation. This is equivalent to a risk period-specific analysis restricted to infants at risk (avoiding overlap between exposure and disease outcome). In the present study, the results for wheezing, LRTIs and atopic eczema of such a risk period-specific analysis were not substantially different compared with the results of the overall analysis. In our opinion, we have demonstrated that our main findings (PBF for 4–6 months does reduce the risk of wheezing, LRTI and atopic eczema in

Table 2. Overall analysis of predominant breastfeeding and risk of infant's outcomes in the first 14 months and period-specific analysis* of predominant breastfeeding and risk of infant's outcomes between 7 and 14 months

	Never BF	< 2 months PBF	2–4 months PBF	4–6 months PBF	> 6 months PBF
Overall analysis (<i>n</i> = 580)					
Wheezing					
Crude	1.0 (ref.)	1.06 (0.59–1.93)	0.75 (0.43–1.30)	0.57 (0.36–0.91)	1.01 (0.53–1.92)
Adjusted [†]	1.0 (ref.)	1.37 (0.73–2.57)	0.92 (0.51–1.66)	0.65 (0.40–1.06)	1.15 (0.58–2.28)
LRTI					
Crude	1.0 (ref.)	0.79 (0.44–1.43)	0.55 (0.31–0.96)**	0.59 (0.37–0.93)**	1.16 (0.61–2.19)
Adjusted [‡]	1.0 (ref.)	0.89 (0.48–1.66)	0.56 (0.31–1.01)	0.62 (0.39–0.99)**	1.07 (0.55–2.08)
Atopic eczema					
Crude	1.0 (ref.)	0.69 (0.34–1.37)	0.81 (0.44–1.50)	0.67 (0.40–1.12)	0.64 (0.29–1.43)
Adjusted [§]	1.0 (ref.)	0.67 (0.33–1.36)	0.90 (0.48–1.69)	0.72 (0.42–1.22)	0.64 (0.28–1.44)
Gastroenteritis					
Crude	1.0 (ref.)	0.98 (0.52–1.84)	1.00 (0.55–1.81)	0.74 (0.46–1.19)	1.10 (0.54–2.21)
Adjusted [¶]	1.0 (ref.)	1.00 (0.52–1.92)	1.09 (0.59–2.02)	0.75 (0.46–1.23)	1.21 (0.59–2.49)
Period-specific analysis*					
Wheezing (<i>n</i> = 467)					
Crude	1.0 (ref.)	2.13 (1.01–4.50)	1.37 (0.67–2.83)	0.56 (0.27–1.12)	0.86 (0.33–2.29)
Adjusted [†]	1.0 (ref.)	2.50 (1.15–5.43)	1.53 (0.72–3.24)	0.59 (0.28–1.20)	0.96 (0.35–2.63)
LRTI (<i>n</i> = 464)					
Crude	1.0 (ref.)	0.92 (0.45–1.90)	0.65 (0.33–1.30)	0.54 (0.30–0.97)**	0.91 (0.39–2.08)
Adjusted [‡]	1.0 (ref.)	0.99 (0.47–2.07)	0.67 (0.33–1.36)	0.55 (0.30–0.99)**	0.87 (0.37–2.03)
Atopic eczema (<i>n</i> = 503)					
Crude	1.0 (ref.)	0.77 (0.28–2.11)	0.84 (0.34–2.09)	0.70 (0.33–1.50)	0.44 (0.12–1.64)
Adjusted [§]	1.0 (ref.)	0.75 (0.27–2.08)	0.95 (0.37–2.44)	0.74 (0.34–1.63)	0.42 (0.11–1.57)
Gastroenteritis (<i>n</i> = 480)					
Crude	1.0 (ref.)	0.95 (0.47–1.90)	1.14 (0.60–2.15)	0.96 (0.58–1.61)	1.48 (0.71–3.08)
Adjusted [¶]	1.0 (ref.)	0.98 (0.48–2.01)	1.26 (0.65–2.43)	1.01 (0.59–1.72)	1.67 (0.78–3.56)

*The incidence of infant's outcomes in the risk period of 7–14 months was analysed (i.e. excluding infants with onset of outcomes before 7 months of age).

[†]Adjusted for child gender, maternal social class, siblings at birth, maternal smoking in pregnancy, and DDE levels in cord blood.

[‡]Adjusted for child gender, maternal social class, and siblings at birth.

[§]Adjusted for child gender, maternal atopy [positive skin prick test (SPT)], and maternal pre-pregnancy body mass index.

[¶]Adjusted for siblings at birth, maternal smoking in pregnancy, maternal social class, and day care attendance.

***P*-value < 0.05.

BF, Breastfeeding; LRTI, lower respiratory tract infection; PBF, predominant breastfeeding.

Table 3. LC-PUFA concentrations (wt% of total fatty acids, mean, SD) in colostrum among breastfed infants (*n* = 283)

	All <i>N</i> = 283	Non-atopic mothers <i>n</i> = 205	Atopic mothers* <i>n</i> = 74	<i>P</i> -value
n-6				
Linoleic acid (LA 18:2n-6)	12.58 (2.62)	12.75 (2.57)	12.13 (2.76)	0.052
γ-linolenic acid (18:3n-6)	0.034 (0.024)	0.036 (0.025)	0.031 (0.019)	0.079
Dihomo-γ-linolenic acid (20:3-n6)	0.83 (0.26)	0.84 (0.26)	0.81 (0.26)	0.262
Arachidonic acid (AA) (20:4n-6)	1.07 (0.29)	1.07 (0.29)	1.08 (0.28)	0.528
Adrenic acid (22:4-n6)	0.63 (0.22)	0.63 (0.22)	0.65 (0.22)	0.474
Docosapentaenoic (22:5-n6)	0.16 (0.05)	0.16 (0.06)	0.16 (0.05)	0.834
Total n-6 series	15.31 (2.80)	15.49 (2.72)	14.86 (3.05)	0.039
n-3				
α-Linolenic acid (ALA) (18:3 n3)	0.35 (0.09)	0.35 (0.09)	0.35 (0.08)	0.798
Eicosapentanoic (EPA) (20:5-n3)	0.055 (0.02)	0.055 (0.02)	0.056 (0.022)	0.642
Docosapentanoic (DPA) (22:5-n3)	0.40 (0.13)	0.40 (0.12)	0.42 (0.16)	0.840
Docosahexaenoic acid (DHA) (22:6n-3)	0.63 (0.26)	0.63 (0.25)	0.65 (0.28)	0.841
Total n-3 series	1.45 (0.41)	1.44 (0.39)	1.48 (0.45)	0.948

*IgE levels ≥ 0.35 kU/L to any of dust mite (Der p 1), cat allergen (Fel d 1) and mixed grass pollen.

Table 4. Associations (Odds ratios and 95% confidence intervals) between LC-PUFA dose groups[†] and risk of gastroenteritis (*n* = 449)

	Birth to 6 months	7 to 14 months	Recurrent
LA dose group			
Short PBF/low LA	1.00	1.00	1.00
Short PBF/medium-high LA	0.95 (0.37–2.42)	0.86 (0.39–1.89)	0.86 (0.24–3.06)
Long PBF/low LA	0.38 (0.12–1.18)	0.91 (0.40–2.08)	0.46 (0.11–1.88)
Long PBF/medium-high LA	0.59 (0.23–1.52)	0.92 (0.44–1.91)	0.71 (0.21–2.36)
AA dose group			
Short PBF/low AA	1.00	1.00	1.00
Short PBF/medium-high AA	0.76 (0.32–1.82)	0.76 (0.36–1.59)	0.63 (0.18–2.14)
Long PBF/low AA	0.57 (0.21–1.55)	0.88 (0.40–1.92)	0.67 (0.19–2.41)
Long PBF/medium-high AA	0.38 (0.16–0.92)*	0.83 (0.42–1.63)	0.41 (0.13–1.33)
Total n-6 dose group			
Short PBF/low total n-6	1.00	1.00	1.00
Short PBF/medium-high total n-6	1.04 (0.37–2.91)	0.86 (0.39–1.92)	0.98 (0.25–3.81)
Long PBF/low total n-6	0.56 (0.18–1.70)	1.01 (0.44–2.31)	0.73 (0.18–3.01)
Long PBF/medium-high total n-6	0.54 (0.19–1.54)	0.87 (0.41–1.83)	0.65 (0.18–2.38)
ALA dose group			
Short PBF/low ALA	1.00	1.00	1.00
Short PBF/medium-high ALA	1.21 (0.42–3.45)	1.04 (0.46–2.35)	1.25 (0.32–4.85)
Long PBF/low ALA	0.51 (0.14–1.83)	1.06 (0.44–2.57)	0.67 (0.14–3.20)
Long PBF/medium-high ALA	0.65 (0.23–1.81)	1.04 (0.48–2.25)	0.87 (0.24–3.14)
EPA dose group			
Short PBF/low EPA	1.00	1.00	1.00
Short PBF/medium-high EPA	0.77 (0.26–2.32)	0.75 (0.30–1.89)	0.62 (0.14–2.75)
Long PBF/low EPA	0.91 (0.27–3.07)	0.86 (0.29–2.55)	0.96 (0.19–4.82)
Long PBF/medium-high EPA	0.34 (0.12–1.01)	0.80 (0.34–1.91)	0.36 (0.08–1.52)
DHA dose group			
Short PBF/low DHA	1.00	1.00	1.00
Short PBF/medium-high DHA	0.41 (0.17–0.98)*	0.81 (0.41–1.62)	0.39 (0.12–1.23)
Long PBF/low DHA	0.47 (0.19–1.17)	1.09 (0.50–2.35)	0.66 (0.20–2.11)
Long PBF/medium-high DHA	0.25 (0.11–0.57)**	0.82 (0.44–1.56)	0.28 (0.09–0.81)*
Total n-3 dose group			
Short PBF/low total n-3	1.00	1.00	1.00
Short PBF/medium-high total n-3	0.42 (0.17–1.04)	0.62 (0.30–1.28)	0.27 (0.08–0.91)*
Long PBF/low total n-3	0.38 (0.14–0.99)*	0.85 (0.39–1.86)	0.41 (0.12–1.35)
Long PBF/medium-high total n-3	0.28 (0.12–0.66)**	0.70 (0.36–1.36)	0.25 (0.08–0.76)*

Children never breastfed (*n* = 131) were excluded from this analysis.

PBF, predominant breastfeeding.

[†]Dose groups were calculated by categorizing short or long duration of predominant breastfeeding (divided at the median in weeks) and low (lower tertile) vs. medium-high (medium and high tertiles) LC-PUFA concentration.

**P*-value < 0.05;

***P*-value < 0.01.

All models adjusted for siblings at birth, maternal smoking in pregnancy, maternal social class, and day care attendance.

the first 14 months of life) are unlikely to be biased by reverse causation. However, we cannot rule out that reverse causation could exist for results in the first 6 months and for recurrent outcomes, and thus, these results should be interpreted with caution. Also parental history of allergy has been reported to influence breastfeeding practices; however, results did not differ according to maternal atopic status or educational level in our study. Another weak point is that colostrum samples were obtained in a subsample of participants, which could result in biased analyses; however, multiple imputation of missing values allows taking into

account the entire sample in the analyses. In addition, results from association between estimated doses of PUFAs and infant outcomes are based on multiple hypothesis tests and sample size on estimated dose groups may have been relatively small in some of the sub-analyses. Although this may have affected the precision of the estimates, it is unlikely that it has introduced a type I error (i.e. spurious results) because of the high statistical significance of some reported associations. Moreover, fatty acids in colostrum are markers of maternal diet (e.g. fish intake) and related nutrients (e.g. fat soluble vitamins) that might also

influence the risk of the assessed outcomes. Furthermore, duration of colostrum feeding generally only lasts up to 3–4 days, so this brief exposition could have unlikely long-term effects on infants that could explain failed results and taking into account that composition of mature breast milk differs from colostrum composition. Finally, beneficial effects on allergic manifestations and LRTIs of prolonged breastfeeding could rely in part on other biological components than LC-PUFAs present in human milk that stimulate the development of the infant's immune system [37]. Fatty acid composition is influenced by genetic variants in enzymes involved in LC-PUFA metabolism [38, 39]. Additional studies using genetic variants in LC-PUFA-metabolizing enzymes could be a way to prove causality between breast milk fatty acid composition and health outcomes in infancy, although recent studies have shown inconclusive results for eczema [40].

In our study, although PBF for 2–4 months showed some positive effects on reducing the risk of wheezing and LRTI episodes, the greatest benefits were observed among children predominantly breastfed for 4–6 months who additionally reported less likely atopic eczema and gastroenteritis. However, no significant associations were observed for more than 6 months of PBF. Our results are in accordance with some previous studies suggesting that prolonged and exclusively breastfeeding protect against asthma, allergic diseases, and atopy [9–11]. By contrast, several studies have failed to confirm this [16] or even have reported breastfeeding to be associated with an increased risk of these outcomes [12–15]. Furthermore, different results have been reported according to ages at which outcomes are assessed, with some studies reported protection from prolonged and exclusive breastfeeding before age 2 years and opposite effects beyond 6 years [14, 41]. Although other authors have reported reduced risk of atopy and asthma in breast-fed children followed up until 5–6 years [9, 42]. Whether or not breastfeeding protective effects found in our study prolong beyond 14 months of life in our cohort remain to be determined in following studies.

Exclusive breastfeeding for 6 months has no apparent growth deficits but can raise some clinical concerns including iron deficiency anaemia, higher incidence of food allergies, and coeliac disease [3]. However, introduction of solid food before 3–4 months may be associated with increased fatness and wheezing later in life [43]. Recommendation of exclusive breastfeeding for 6 months has been revised recently, and an expert review concluded that for infants across the EU complementary foods may be introduced safely between 4 and 6 months. Our results support the recommendation of not extending PBF beyond 6 months of age. However, the point when breastfeeding should cease as a sole

source of nutrition needs more evidence and should take into account potential risks and benefits for the infant.

In our study, we found beneficial effects of exposure to higher doses of AA and n-3 fatty acids (EPA and DHA) on the risk of gastroenteritis, but no significant associations were found for allergic manifestations or LRTIs. Although some studies have reported protective effect of n-3 levels in breast milk on allergic manifestations and asthma [24, 27], other have failed to find significant associations between LC-PUFA profile in breast milk and allergic manifestations [17]. Inconsistency across studies could be explained by reduced sample size and selection bias (i.e. breast milk samples are provided for participants with more favourable socioeconomic and life-style factors). Moreover, other studies have been carried out on selected populations at higher risk of developing allergic manifestations (i.e. children with a family history of allergies). Also the use of different reference groups, outcome definitions, age at assessment, and the absence of definitive laboratory measures for some outcomes favour inconsistent results among studies and limit the conclusions. However, it is well established that breast milk promotes gastrointestinal maturation and reduces incidence and severity of infections [4, 6, 21, 44]. Moreover, LC-PUFAs, present in breast milk, are known to affect the gastrointestinal development by regulation of epithelial structure/function [21, 45, 46]. Specific PUFA of the n-6 (GLA, DGLA, AA) and n-3 series (EPA, DHA) may support natural resistance by enhancing intestinal epithelial barrier integrity *in vitro* contributing to the protection against infections and allergic disease [46]. In addition, AA and DHA have been shown to reduce the development of necrotizing enterocolitis in neonatal rats, possibly by improving barrier function [46, 47].

In conclusion, PBF for 4–6 months of life conferred highest benefits by reducing the burden of allergic manifestations, respiratory infections, and gastroenteritis in infants. Beneficial effects of breastfeeding on gastroenteritis were explained in part by exposure to higher doses of n-3 and AA received from colostrum. No significant effects from fatty acid dose were found on risk of allergic manifestations or LRTIs.

Acknowledgements

The authors are grateful to Silvia Fochs, Anna Sánchez, Maribel López, and Nuria Pey for their assistance in contacting the families and administering the questionnaires. Moreover, the authors would particularly like to thank all the participants for their generous collaboration. A full roster of the INMA Sabadell Study Investigators can be found at <http://www.proyectoinma.org/cohorts/sabadell/membres-sabadell.html>. This study was

funded by grants from the Spanish Ministry of Health (FIS-PI041436), Instituto de Salud Carlos III (Red INMA G03/176 and CB06/02/0041), the Generalitat de Catalunya-CIRIT 1999SGR 00241, and the EU sixth framework project NEWGENERIS FP6-2003-Food-3-A-016320. The authors acknowledge the Spanish Ministry of Education and Science for the predoctoral grant to C. Moltó-Puigmartí and the Spanish Ministry of Science and Innovation for their financial support (projects AGL2008-04124/ALI and AGL2009-09730/ALI).

Conflict of interest: All authors declare no conflict of interest.

Author contributions: Dr Sunyer had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. EM, RGE, MG, MM, and JS designed the study. EM and RGE did the statistical analysis. EM drafted the manuscript. CMP and MCLS performed the laboratory measurement of LC-PUFAs. All authors did a critical revision of the manuscript for important intellectual content.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Details of the imputation modelling. The INMA Sabadell cohort.

Table S2. Distributions of imputed characteristics in the imputation datasets and in observed data (i.e. without imputation). The INMA Sabadell cohort (*N* eligible = 580).

Table S3. Associations [OR (95% CI)] between breastfeeding practises and health outcomes in infants. Complete case analysis. The INMA Sabadell cohort.

Table S4. Associations [OR (95% CI)] between fatty acid concentrations in colostrum and risk of offspring outcomes among breastfed infants. Complete case analysis. Figures are highest vs. lowest tertile OR and 95% confidence intervals. The INMA Sabadell cohort.

Table S5. Associations [OR (95% CI)] between breastfeeding practises and health outcomes in infants by

maternal atopic status with missing data imputed by multivariate multiple imputations. The INMA Sabadell cohort (*n* = 580).

Table S6. Associations (Odds ratios and 95% confidence intervals) between LC-PUFA dose groups§ and risk of wheezing (*n* = 449).

Table S7. Associations (Odds ratios and 95% confidence interval) between LC-PUFA dose groups§ and risk of lower respiratory tract infections (*n* = 449).

Table S8. Associations (Odds ratios and 95% confidence interval) between LC-PUFA dose groups§ and risk of atopic eczema (*n* = 449).

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Supporting Information for

Effects of prolonged breastfeeding and colostrum fatty acids on allergic manifestations and infections in infancy

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Table S8. Associations (Odds ratios and 95% confidence interval) between LC-PUFA dose groups§ and risk of atopic eczema (n=449).

Table S1. Details of the imputation modelling. The INMA Sabadell cohort.

Software used and key setting: STATA 10.1 software (Stata Corporation, College Station, Texas, USA) – Ice command (with 10 cycles)
Number of imputed datasets created: 100
Variables included in the imputation procedure: <u>Variables used in the main analyses (outcome, exposure and potential confounders)::</u> wheezing 1-6 months, wheezing 7-14 mo, LRTI 1-6 mo, LRTI 7-14 mo, atopic eczema 1-6 mo, atopic eczema 7-14 mo, gastroenteritis 1-6 mo, gastroenteritis 7-14 mo, duration of breastfeeding (weeks), age formula fed started, age foods introduction started, LA (18:2n-6), γ-linolénic acid (18:3n-6), Dihomo- γ-linolenic acid (20:3-n6), AA (20:4n-6), Adrenic acid (22:4-n6), Docosapentaenoic (22:5-n6), ALA (18:3-n3), EPA (20:5-n3), DPA (22:5-n3), DHA (22:6n-3), total n-3, total n-6, child gender, siblings at birth, Maternal pre-pregnancy BMI, maternal social class, maternal education, maternal pre-natal and post-natal smoking (yes/no; cig/day), paternal post-natal smoking (yes/no; cig/day), day care attendance, p,p'-DDE levels in cord blood, specific IgE to cat allergen (Fel d 1), specific IgE to dust mite (Der p 1), specific IgE to mixed grass pollen, C-reactive protein levels in pregnancy. <u>Variables only used for imputation models:</u> otitis 1-6 mo, otitis 7-14mo, child's gestational age, birth weight, type of delivery, Apgar score at 5 min, parity, maternal pre-pregnancy weight and height, maternal and paternal age at delivery and birth country, paternal social class and education, maternal ethnic group, gas cooking during pregnancy, paternal and others pre- and post- natal smoking, maternity and paternity leave, main childminder, maternal and paternal mental health and attachment (Condon's questionnaire) scores at child's age 14 mo, child's Bayley mental and psicomotor scores at age 14 mo (adjusted for age at examination, quality of the test and psychologist), sum PCBs at 1st trimester maternal serum samples, maternal total IgE, maternal alcohol intake during pregnancy, and maternal intake (FFQ questionnaire; median or tertiles except if * specified, which means as a continuous variable) of: total energy* (calories), total fat *, full-cream milk, skimmed milk, cream, yoghurt, fresh cheese, creamy cheese, eggs, liver, sausages, chicken, meat, fatty fish, lean fish, seafood*, other fish, vegetables, spinach, cabbage, fruits, olives, nuts, cereals, wholemeal bread, pizza, olive oil and other vegetable oil, margarine, butter, pastries, biscuits and biscuits with chocolate, powder chocolate, croquettes, fish croquettes, mayonnaise, tomato sauce, and PUFA gene variants (rs12207094 rs174464 rs174468 rs174537 rs174570 rs174602).

Treatment of non-normally distributed variables: prediction matching for number of cigarettes (mother during pregnancy; and parents and relative at child's age 14 mo), seafood intake, parental mental health and attachment scores, duration of breastfeeding, age of formula fed and foods introduction, maternal DDE and PCBs levels at serum 1st trimester samples, LC-PUFAs, total IgE and C-reactive protein levels.
Treatment of binary/categorical variables: logistic, ordinal, and multinomial models
Statistical interactions included in imputation models: none
No missing in the imputation sample: child's gender, gestational age, maternal pre-pregnancy weight, height and BMI, and paternal social class.

Table S2. Distributions of imputed characteristics in the imputation datasets and in observed data (i.e. without imputation). The INMA Sabadell cohort (N eligible= 580).

Imputed variable		% data	Distribution	
		imputed	Mean (SE) for continuous variables	
			% for categorical variables	
			In:	
			Imputed datasets	Observed (with no missing) dataset
Wheezing 1-6 mo (yes)		2.1	19.7	19.5
Wheezing 7-14 mo (yes)		5.2	29.0	28.9
Recurrent wheezing (yes)		6.4	12.6	12.7
LRTI 1-6 mo (yes)		0.9	20.0	20.0
LRTI 7-14 mo (yes)		5.5	32.0	31.9
Recurrent LRTI (yes)		5.5	12.3	12.4
Atopic eczema 1-6 mo (yes)		5.9	13.8	13.5
Atopic eczema 14 mo (yes)		6.4	16.6	16.6
Recurrent atopic eczema (yes)		11.6	7.2	7.2
Gastroenteritis 1-6 mo (yes)		6.0	16.9	16.7
Gastroenteritis 14 mo (yes)		5.5	62.1	62.2
Recurrent gastroenteritis (yes)		10.9	12.6	12.4
Siblings at birth	0	0.5	56.7	56.7
	1		37.1	37.1
	2		6.2	6.2
Maternal social class	Non manual	0.9	67.7	67.7
	Manual		21.0	21.0
	Unknown		11.3	11.3

Maternal education level	Primary or less	0.5	27.1	27.0
	Secondary		42.5	42.5
	High		30.4	30.5
Maternal atopy (yes)		4.0	29.5	28.7
Maternal smoking in pregnancy		1.4	15.4	15.6
Maternal postnatal smoking		4.8	26.2	25.7
p,p'-DDE levels in cord blood (ng/g lipid)		3.3	231.6 (35.6)	228.0 (35.0)
LA (18:2n-6)		42.2	12.6 (0.13)	12.6 (0.14)
AA (20:4n-6)		42.2	1.08 (0.01)	1.08 (0.02)
Total n-6		42.2	15.3 (0.15)	15.3 (0.15)
ALA (18:3 n3)		42.2	0.35 (0.01)	0.35 (0.005)
EPA (20:5-n3)		42.2	0.05 (0.001)	0.06 (0.001)
DHA (22:6n-3)		42.2	0.63 (0.01)	0.64 (0.01)
Total n-3		42.2	1.43 (0.02)	1.44 (0.02)
C-reactive protein in pregnancy (mg/L)		4.5	5.65 (0.30)	5.65 (0.30)
Predominant breastfeeding duration (weeks)		1.2	12.3 (0.39)	12.3 (0.39)
Day care attendance first year (yes)		5.0	30.7	31.2

	Breastfeeding practices				
	Never BF	<2 mo PBF	2-4 mo PBF	4-6 mo PBF	> 6 mo PBF
WHEEZE					
≤ 6 mo					
Crude (n=568)	1	0.38 (0.17-0.83)	0.36 (0.17-0.74)	0.63 (0.37-1.06)	NA
Adjusted ¹ (n=541)	1	0.49 (0.21-1.12)	0.51 (0.23-1.12)	0.76 (0.44-1.33)	NA
7-14 mo					
Crude (n=550)	1	1.15 (0.63-2.11)	0.72 (0.40-1.28)	0.47 (0.29-0.77)	0.60 (0.30-1.21)
Adjusted ¹ (n=525)	1	1.52 (0.79-2.90)	0.82 (0.43-1.57)	0.51 (0.30-0.87)	0.69 (0.33-1.45)
Recurrent					
Crude (n=543)	1	0.40 (0.15-1.05)	0.22 (0.08-0.61)	0.42 (0.22-0.77)	0.53 (0.21-1.35)
Adjusted ¹ (n=518)	1	0.56 (0.20-1.55)	0.31 (0.11-0.91)	0.48 (0.24-0.94)	0.73 (0.27-1.97)
LRTI					
≤ 6 mo					
Crude (n=575)	1	0.68 (0.33-1.39)	0.50 (0.25-1.01)	0.74 (0.44-1.26)	NA
Adjusted ² (n=569)	1	0.80 (0.38-1.69)	0.54 (0.25-1.13)	0.81 (0.47-1.41)	NA
7-14 mo					
Crude (n=548)	1	0.78 (0.42-1.43)	0.52 (0.29-0.94)	0.48 (0.29-0.77)	0.88 (0.46-1.68)
Adjusted ² (n=543)	1	0.85 (0.45-1.59)	0.49 (0.26-0.90)	0.49 (0.30-0.80)	0.83 (0.43-1.60)
Recurrent					
Crude (n=548)	1	0.55 (0.22-1.35)	0.32 (0.13-0.81)	0.41 (0.21-0.81)	1.07 (0.46-2.49)
Adjusted ² (n=543)	1	0.64 (0.25-1.62)	0.31 (0.12-0.82)	0.43 (0.21-0.86)	0.94 (0.39-2.29)
ATOPIC ECZEMA					
≤ 6 mo					
Crude (n=546)	1	0.61 (0.26-1.46)	0.79 (0.37-1.68)	0.65 (0.34-1.21)	NA
Adjusted ³ (n=533)	1	0.59 (0.24-1.42)	0.71 (0.32-1.59)	0.69 (0.36-1.32)	NA
7-14 mo					
Crude (n=543)	1	0.54 (0.25-1.20)	0.64 (0.32-1.29)	0.52 (0.29-0.92)	0.52 (0.22-1.24)

Adjusted ³ (n=529)	1	0.60 (0.26-1.36)	0.79 (0.38-1.68)	0.61 (0.32-1.13)	0.60 (0.25-1.46)
Recurrent					
Crude (n=513)	1	0.32 (0.09-1.18)	0.42 (0.14-1.24)	0.34 (0.14-0.79)	0.70 (0.24-2.10)
Adjusted ³ (n=501)	1	0.33 (0.09-1.22)	0.38 (0.12-1.26)	0.40 (0.16-0.96)	0.72 (0.23-2.20)
GASTROENTERITIS					
≤ 6 mo					
Crude (n=545)	1	1.03 (0.52-2.02)	0.69 (0.35-1.34)	0.33 (0.18-0.62)	NA
Adjusted ⁴ (n=512)	1	1.10 (0.54-2.23)	0.74 (0.36-1.52)	0.35 (0.19-0.66)	NA
7-14 mo					
Crude (n=548)	1	0.81 (0.44-1.49)	0.93 (0.53-1.64)	0.88 (0.55-1.40)	1.38 (0.70-2.72)
Adjusted ⁴ (n=538)	1	0.83 (0.44-1.55)	0.95 (0.53-1.72)	0.90 (0.55-1.46)	1.44 (0.71-2.91)
Recurrent					
Crude (n=517)	1	0.74 (0.29-1.85)	0.60 (0.24-1.48)	0.36 (0.17-0.77)	0.51 (0.16-1.58)
Adjusted ⁴ (n=508)	1	0.79 (0.30-2.02)	0.60 (0.23-1.56)	0.39 (0.18-0.83)	0.59 (0.18-1.90)

Table S3. Associations (OR (95% CI)) between breastfeeding practises and health outcomes in infants. Complete case analysis. The INMA Sabadell cohort.

PBF: Predominant breastfeeding; NA: Not applicable.

¹Adjusted for child gender, maternal social class, siblings at birth, maternal smoking in pregnancy, and p,p'-DDE levels in cord blood.

²Adjusted for child gender, maternal social class, and siblings at birth.

³Adjusted for child gender, maternal atopy (positive SPT), and maternal pre-pregnancy body mass index.

⁴Adjusted for siblings at birth, maternal smoking in pregnancy, maternal social class and day care attendance.

Table S4. Associations (OR (95% CI)) between fatty acid concentrations in colostrum and risk of offspring outcomes among breastfed infants. Complete case analysis. Figures are highest vs. lowest tertile odds ratios and 95% confidence intervals. The INMA Sabadell cohort.

	Wheezing ¹		
	Birth to 6 mo	7 to 14 mo	Recurrent
	N=272	N=269	N=197
n-6			
LA (18:2n-6)	0.39 (0.17-0.90)	1.32 (0.67-2.60)	0.49 (0.16-1.53)
AA (20:4n-6)	1.19 (0.51-2.76)	1.36 (0.69-2.65)	1.34 (0.44-4.06)
Total n-6 series	0.40 (0.17-0.93)	1.14 (0.57-2.27)	0.44 (0.14-1.37)
n-3			
ALA (18:3 n3)	1.54 (0.62-3.78)	0.82 (0.40-1.69)	1.57 (0.42-5.84)
EPA (20:5-n3)	2.24 (0.76-6.55)	0.58 (0.27-1.24)	0.56 (0.15-2.08)
DHA (22:6n-3)	0.91 (0.41-2.03)	0.94 (0.48-1.86)	0.91 (0.31-2.67)
Total n-3 series	1.35 (0.58-3.13)	0.72 (0.36-1.44)	1.08 (0.37-3.18)
	LRTIs ²		
	Birth to 6 mo	7 to 14 mo	Recurrent
	N=280	N=274	N=201
n-6			
LA (18:2n-6)	0.49 (0.22-1.05)	1.27 (0.67-2.41)	0.61 (0.23-1.66)
AA (20:4n-6)	0.87 (0.41-1.84)	1.35 (0.71-2.59)	1.35 (0.48-3.76)
Total n-6 series	0.40 (0.18-0.88)	1.13 (0.59-2.14)	0.43 (0.15-1.25)
n-3			
ALA (18:3 n3)	1.18 (0.53-2.63)	0.59 (0.30-1.12)	0.97 (0.35-2.66)
EPA (20:5-n3)	1.17 (0.48-2.84)	0.84 (0.41-1.71)	1.17 (0.34-4.03)
DHA (22:6n-3)	0.63 (0.29-1.36)	0.94 (0.49-1.80)	0.62 (0.23-1.67)
Total n-3 series	0.68 (0.31-1.48)	0.80 (0.41-1.54)	0.54 (0.19-1.50)
	Atopic eczema ³		
	Birth to 6 mo	7 to 14 mo	Recurrent
	N=280	N=274	N=201

	Birth to 6 mo	7 to 14 mo	Recurrent
	N=269	N=266	N=224
n-6			
LA (18:2n-6)	0.39 (0.15-1.01)	0.45 (0.17-1.16)	0.30 (0.07-1.34)
AA (20:4n-6)	0.84 (0.32-2.23)	0.44 (0.17-1.15)	0.30 (0.06-1.41)
Total n-6 series	0.58 (0.23-1.47)	0.54 (0.22-1.33)	0.60 (0.17-2.14)
n-3			
ALA (18:3 n3)	0.40 (0.15-1.10)	0.52 (0.20-1.32)	0.55 (0.15-1.97)
EPA (20:5-n3)	1.08 (0.31-3.71)	1.76 (0.46-6.72)	0.93 (0.16-5.55)
DHA (22:6n-3)	0.90 (0.33-2.43)	0.46 (0.17-1.22)	0.35 (0.08-1.57)
Total n-3 series	0.91 (0.32-2.57)	0.40 (0.14-1.15)	0.54 (0.11-2.64)
Gastroenteritis⁴			
	Birth to 6 mo	7 to 14 mo	Recurrent
	N=265	N=273	N=120
n-6			
LA (18:2n-6)	1.91 (0.75-4.91)	1.08 (0.57-2.05)	2.12 (0.69-6.50)
AA (20:4n-6)	0.27 (0.09-0.79)	0.60 (0.32-1.13)	0.20 (0.06-0.70)
Total n-6 series	1.18 (0.48-2.93)	0.81 (0.43-1.54)	1.01 (0.34-3.01)
n-3			
ALA (18:3 n3)	1.38 (0.52-3.65)	0.66 (0.34-1.26)	1.01 (0.31-3.26)
EPA (20:5-n3)	0.43 (0.17-1.06)	0.80 (0.38-1.67)	0.38 (0.13-1.12)
DHA (22:6n-3)	0.19 (0.06-0.53)	0.78 (0.41-1.48)	0.16 (0.05-0.58)
Total n-3 series	0.21 (0.07-0.62)	0.62 (0.32-1.20)	0.16 (0.05-0.59)

¹Adjusted for child gender, maternal social class, siblings at birth, maternal smoking in pregnancy, and DDE levels in cord blood.

²Adjusted for child gender, maternal social class, and siblings at birth.

³Adjusted for child gender, maternal atopy (positive SPT), and maternal pre-pregnancy body mass index.

⁴Adjusted for siblings at birth, maternal smoking in pregnancy, maternal social class and day care attendance.

Breastfeeding practices					
	Never BF	< 2mo PBF	2-4 mo PBF	4-6 mo PBF	> 6 mo PBF
Non-atopic (n=408)					
Recurrent wheezing ¹	1	0.69 (0.22-2.20)	0.26 (0.05-1.22)	0.55 (0.24-1.25)	0.83 (0.23-3.00)
Recurrent LRTIs ²	1	0.83 (0.26-2.63)	0.35 (0.10-1.22)	0.54 (0.23-1.29)	0.92 (0.28-2.98)
Recurrent atopic eczema ³	1	0.22 (0.03-1.48)	0.94 (0.28-3.20)	0.30 (0.09-0.92)	0.82 (0.22-3.02)
Gastroenteritis in mo 1-6 ⁴	1	1.10 (0.47-2.59)	0.78 (0.32-1.90)	0.44 (0.21-0.92)	NA
Atopic* (n=172)					
Recurrent wheezing ¹	1	0.26 (0.03-2.43)	0.36 (0.07-1.68)	0.44 (0.14-1.39)	0.61 (0.11-3.27)
Recurrent LRTIs ²	1	0.67 (0.13-3.28)	0.38 (0.08-1.80)	0.41 (0.12-1.41)	1.90 (0.40-9.12)
Recurrent atopic eczema ³	1	0.94 (0.14-6.18)	0.26 (0.03-2.64)	1.17 (0.26-5.36)	0.53 (0.05-5.40)
Gastroenteritis in mo 1-6 ⁴	1	0.92 (0.28-3.08)	0.52 (0.17-1.57)	0.18 (0.05-0.64)	NA

Table S5. Associations (OR (95% CI)) between breastfeeding practises and health outcomes in infants by maternal atopic status with missing data imputed by multivariate multiple imputations. The INMA Sabadell cohort (n=580).

*IgE levels ≥ 0.35 kU/l to any of dust mite (Der p 1), cat allergen (Fel d 1) and mixed grass pollen.

PBF: predominant breastfeeding; NA: Not applicable.

¹Adjusted for child gender, maternal social class, siblings at birth, maternal smoking in pregnancy and DDE levels in cord blood.

²Adjusted for child gender, maternal social class and siblings at birth.

³Adjusted for child gender, maternal atopy (positive SPT) and maternal pre-pregnancy body mass index.

⁴Adjusted for siblings at birth, maternal smoking in pregnancy, maternal social class and day care attendance.

Table S6. Associations (Odds ratios and 95% confidence intervals) between LC-PUFA dose groups^s and risk of wheezing (n=449).

	Birth to 6 mo	7 to 14 mo	Recurrent
LA dose group			
Short BF/low LA	1.00	1.00	1.00
Short BF/medium-high LA	0.73 (0.24-2.26)	0.85 (0.38-1.91)	0.75 (0.15-3.78)
Long BF/low LA	1.97 (0.72-5.40)	0.44 (0.18-1.07)	1.42 (0.34-5.90)
Long BF/medium-high LA	1.22 (0.46-3.23)	0.61 (0.29-1.31)	1.09 (0.28-4.25)
AA dose group			
Short BF/low AA	1.00	1.00	1.00
Short BF/medium-high AA	1.18 (0.39-3.57)	1.20 (0.53-2.73)	1.37 (0.27-6.87)
Long BF/low AA	1.75 (0.58-5.31)	0.74 (0.30-1.78)	1.78 (0.36-8.75)
Long BF/medium-high AA	2.21 (0.81-5.98)	0.68 (0.31-1.46)	1.87 (0.44-8.00)
Total n-6 dose group			
Short BF/low total n-6	1.00	1.00	1.00
Short BF/medium-high total n-6	0.64 (0.21-1.96)	0.88 (0.38-2.03)	0.66 (0.14-3.20)
Long BF/low total n-6	1.74 (0.63-4.79)	0.51 (0.21-1.23)	1.36 (0.33-5.63)
Long BF/medium-high total n-6	1.14 (0.43-3.04)	0.59 (0.27-1.29)	0.96 (0.24-3.75)
ALA dose group			
Short BF/low ALA	1.00	1.00	1.00
Short BF/medium-high ALA	1.57 (0.41-6.06)	0.86 (0.38-1.95)	1.30 (0.22-7.75)
Long BF/low ALA	2.42 (0.63-9.35)	0.51 (0.20-1.29)	1.34 (0.21-8.74)
Long BF/medium-high ALA	2.68 (0.75-9.55)	0.57 (0.26-1.24)	1.98 (0.38-10.33)
EPA dose group			

Short BF/low EPA	1.00	1.00	1.00
Short BF/medium-high EPA	1.90 (0.46-7.88)	0.86 (0.36-2.04)	1.14 (0.23-5.57)
Long BF/low EPA	1.72 (0.33-8.92)	0.70 (0.24-2.03)	1.51 (0.26-8.95)
Long BF/medium-high EPA	3.44 (0.90-13.19)	0.51 (0.22-1.19)	1.64 (0.37-7.15)
DHA dose group			
Short BF/low DHA	1.00	1.00	1.00
Short BF/medium-high DHA	1.02 (0.36-2.93)	1.10 (0.51-2.40)	0.98 (0.24-4.08)
Long BF/low DHA	1.82 (0.66-5.01)	0.75 (0.32-1.73)	1.60 (0.42-6.14)
Long BF/medium-high DHA	1.87 (0.76-4.61)	0.62 (0.30-1.25)	1.39 (0.42-4.56)
Total n-3 dose group			
Short BF/low total n-3	1.00	1.00	1.00
Short BF/medium-high total n-3	1.44 (0.46-4.56)	0.76 (0.36-1.63)	0.88 (0.21-3.58)
Long BF/low total n-3	1.85 (0.58-5.86)	0.51 (0.22-1.16)	1.19 (0.30-4.62)
Long BF /medium-high total n-3	2.62 (0.95-7.23)	0.52 (0.26-1.04)	1.42 (0.43-4.68)

Children never breastfeed (n=131) were excluded from this analysis.

BF: breastfeeding.

[§]Dose groups were calculated by categorizing short or long duration of feeding (divided at the median in weeks) and low (lower tertile) versus medium-high (medium and high tertiles) LC-PUFA concentration.

All models adjusted for child gender, maternal social class, siblings at birth, maternal smoking in pregnancy, and DDE levels in cord blood.

Table S7. Associations (Odds ratios and 95% confidence interval) between LC-PUFA dose groups[§] and risk of lower respiratory tract infections (n=449).

	Birth to 6 mo	7 to 14 mo	Recurrent
LA dose group			
Short BF/low LA	1.00	1.00	1.00
Short BF/medium-high LA	0.78 (0.30-2.09)	1.16 (0.51-2.64)	0.80 (0.23-2.82)
Long BF/low LA	1.67 (0.65-4.27)	1.15 (0.48-2.74)	1.60 (0.48-5.30)
Long BF/medium-high LA	1.13 (0.46-2.75)	1.03 (0.48-2.21)	1.17 (0.38-3.62)
AA dose group			
Short BF/low AA	1.00	1.00	1.00
Short BF/medium-high AA	0.89 (0.34-2.33)	1.89 (0.81-4.39)	1.64 (0.41-6.47)
Long BF/low AA	1.41 (0.54-3.72)	1.58 (0.66-3.78)	2.05 (0.51-8.27)
Long BF/medium-high AA	1.46 (0.61-3.45)	1.49 (0.67-3.31)	2.21 (0.62-7.81)
Total n-6 dose group			
Short BF/low total n-6	1.00	1.00	1.00
Short BF/medium-high total n-6	0.76 (0.28-2.05)	1.17 (0.52-2.61)	0.81 (0.23-2.86)
Long BF/low total n-6	1.65 (0.63-4.30)	1.17 (0.50-2.71)	1.63 (0.49-5.58)
Long BF/medium-high total n-6	1.08 (0.43-2.71)	1.03 (0.48-2.25)	1.14 (0.36-3.63)
ALA dose group			
Short BF/low ALA	1.00	1.00	1.00
Short BF/medium-high ALA	1.29 (0.43-3.87)	0.63 (0.28-1.43)	0.91 (0.23-3.63)
Long BF/low ALA	1.80 (0.57-5.69)	0.75 (0.32-1.79)	1.25 (0.29-5.35)
Long BF/medium-high ALA	1.93 (0.69-5.40)	0.67 (0.31-1.43)	1.49 (0.42-5.34)

EPA dose group			
Short BF/low EPA	1.00	1.00	1.00
Short BF/medium-high EPA	1.40 (0.40-4.92)	0.89 (0.36-2.23)	2.14 (0.27-17.16)
Long BF/low EPA	1.96 (0.48-8.04)	0.94 (0.31-2.82)	2.72 (0.28-26.85)
Long BF/medium-high EPA	2.07 (0.64-6.73)	0.87 (0.37-2.06)	2.98 (0.41-21.77)
DHA dose group			
Short BF/low DHA	1.00	1.00	1.00
Short BF/medium-high DHA	0.77 (0.32-1.89)	1.32 (0.62-2.83)	0.82 (0.26-2.58)
Long BF/low DHA	1.55 (0.62-3.88)	1.37 (0.61-3.06)	1.67 (0.53-5.21)
Long BF/medium-high DHA	1.23 (0.56-2.70)	1.08 (0.53-2.19)	1.24 (0.46-3.36)
Total n-3 dose group			
Short BF/low total n-3	1.00	1.00	1.00
Short BF/medium-high total n-3	0.68 (0.27-1.73)	0.92 (0.44-1.92)	0.54 (0.17-1.71)
Long BF/low total n-3	1.14 (0.45-2.90)	0.95 (0.43-2.09)	1.02 (0.33-3.17)
Long BF /medium-high total n-3	1.26 (0.57-2.82)	0.90 (0.46-1.78)	1.06 (0.40-2.82)

Children never breastfeed (n=131) were excluded from this analysis.

BF: breastfeeding.

[§]Dose groups were calculated by categorizing short or long duration of feeding (divided at the median in weeks) and low (lower tertile) versus medium-high (medium and high tertiles) LC-PUFA concentration.

All models adjusted for child gender, maternal social class, and siblings at birth.

Table S8. Associations (Odds ratios and 95% confidence interval) between LC-PUFA dose groups[§] and risk of atopic eczema (n=449).

	Birth to 6 mo	7 to 14 mo	Recurrent
LA dose group			
Short BF/low LA	1.00	1.00	1.00
Short BF/medium-high LA	1.03 (0.34-3.08)	1.00 (0.34-2.90)	1.25 (0.24-6.44)
Long BF/low LA	1.70 (0.59-4.91)	1.30 (0.45-3.75)	2.20 (0.43-11.3)
Long BF/medium-high LA	0.61 (0.20-1.89)	0.89 (0.32-2.47)	1.00 (0.19-5.25)
AA dose group			
Short BF/low AA	1.00	1.00	1.00
Short BF/medium-high AA	0.65 (0.24-1.75)	0.45 (0.17-1.15)	0.31 (0.07-1.41)
Long BF/low AA	0.66 (0.22-1.97)	0.58 (0.21-1.58)	0.63 (0.15-2.63)
Long BF/medium-high AA	0.76 (0.32-1.82)	0.66 (0.29-1.48)	0.60 (0.18-1.97)
Total n-6 dose group			
Short BF/low total n-6	1.00	1.00	1.00
Short BF/medium-high total n-6	1.02 (0.34-3.04)	1.05 (0.36-3.05)	1.20 (0.23-6.13)
Long BF/low total n-6	1.50 (0.51-4.42)	1.43 (0.49-4.17)	2.19 (0.44-11.04)
Long BF/medium-high total n-6	0.70 (0.24-2.05)	0.89 (0.31-2.55)	0.92 (0.17-4.89)
ALA dose group			
Short BF/low ALA	1.00	1.00	1.00
Short BF/medium-high ALA	0.82 (0.27-2.49)	0.83 (0.29-2.43)	0.78 (0.16-3.87)
Long BF/low ALA	1.15 (0.37-3.58)	1.22 (0.40-3.72)	1.46 (0.28-7.56)
Long BF/medium-high ALA	0.70 (0.24-2.03)	0.78 (0.29-2.13)	0.80 (0.17-3.78)

EPA dose group			
Short BF/low EPA	1.00	1.00	1.00
Short BF/medium-high EPA	1.03 (0.29-3.64)	0.84 (0.24-2.88)	0.72 (0.13-3.91)
Long BF/low EPA	0.76 (0.14-4.19)	NA	NA
Long BF/medium-high EPA	1.03 (0.32-3.32)	1.01 (0.32-3.16)	1.04 (0.22-4.82)
DHA dose group			
Short BF/low DHA	1.00	1.00	1.00
Short BF/medium-high DHA	0.83 (0.31-2.21)	0.53 (0.21-1.35)	0.44 (0.10-1.91)
Long BF/low DHA	0.77 (0.24-2.41)	0.69 (0.25-1.94)	0.90 (0.21-3.76)
Long BF/medium-high DHA	0.89 (0.36-2.16)	0.73 (0.33-1.61)	0.69 (0.20-2.32)
Total n-3 dose group			
Short BF/low total n-3	1.00	1.00	1.00
Short BF/medium-high total n-3	1.06 (0.37-3.04)	0.68 (0.26-1.74)	0.94 (0.20-4.49)
Long BF/low total n-3	1.01 (0.33-3.10)	0.84 (0.31-2.28)	1.36 (0.27-6.78)
Long BF /medium-high total n-3	0.99 (0.37-2.65)	0.80 (0.34-1.87)	1.03 (0.24-4.48)

Children never breastfeed (n=131) were excluded from this analysis.

NA: Not applicable; BF: breastfeeding.

[§]Dose groups were calculated by categorizing short or long duration of feeding (divided at the median in weeks) and low (lower tertile) versus medium-high (medium and high tertiles) LC-PUFA concentration.

All models adjusted for child gender, maternal atopy (positive SPT), and maternal pre-pregnancy body mass index.