Enrichment of infant formula with long-chain polyunsaturated fatty acids and risk of infection and allergy in the nationwide ELFE birth cohort

Moufidath Adjibade, Camille Davisse-Paturet, Jonathan Y Bernard, Karine Adel-Patient, Amandine Divaret-Chauveau, Sandrine Lioret, Marie-Aline Charles, Blandine de Lauzon-Guillain

Enrichment of infant formula with long-chain polyunsaturated fatty acids and risk of infection and allergy in the nationwide ELFE birth cohort

Running title: LCPUFA-enriched formula and infection or allergy

Moufidath Adjibade[1], Camille Davisse-Paturet[1], Jonathan Y. Bernard[1,2], Karine Adel-Patient[3], Amandine Divaret-Chauveau[4,5], Sandrine Lioret[1], Marie-Aline Charles[1,6], Blandine de Lauzon-Guillain[1]

[1] Université de Paris, CRESS, Inserm, INRAE, F-75004 Paris, France

[2] Singapore Institute for Clinical Sciences (SICS), Agency for Science, Technology and Research (A*STAR), Singapore

[3] Université Paris-Saclay, CEA, INRAE, DMTS, 91191 Gif-sur-Yvette, France

[4] EA3450, Université de Lorraine, 54500 Vandoeuvre-lès-Nancy, France

[5] Unité d'allergologie pédiatrique, Hôpital d'Enfants, CHRU de Nancy, Vandoeuvre-lès-Nancy, France

[6] Unité mixte Inserm-Ined-EFS Elfe, Ined, F-75020 Paris, France

Acknowledgments:
We thank the scientific coordinators (B Geay, H Léridon, C Bois, JL Lanoé, X Thierry, C Zaros), IT and data managers, statisticians (M Cheminat, C Ricourt, A Candea, S de Visme), administrative and family communication staff, study technicians (C Guevel, M Zoubiri, L G L Gravier, I, Milan, R Popa) of the ELFE coordination team, as well as the families that gave their time for the study.

Conflict of interest:
Amandine Divaret-Chauveau has been invited to speak at conferences organized by laboratories such as Nutricia, Sodilac, Mead Johnson, outside of the submitted work. Karine Adel-Patient has been invited to speak at a conference organized by Stallergènes, outside of the submitted work. None of the other authors declare any conflicts of interest.

Author's contributions:
M-A C, JYB, SL and BL-G were responsible for the development of the design and protocol of the study; MA performed the statistical analysis and wrote the paper; BLG provided

methodological guidance; MA, CD-P, JYB, KA-P, AD-C, SL, M-AC and BL-G were involved in interpreting the results and editing the manuscript for important intellectual content. All authors read and approved the final manuscript.

Financial support:
This study is funded by an ANR grant (InfaDiet project, ANR-19-CE36-0008).

The ELFE survey is a joint project between the French Institute for Demographic Studies (INED) and the French National Institute of Health and Medical Research (INSERM), in partnership with the French blood transfusion service (Etablissement français du sang [EFS]), Santé publique France, the National Institute for Statistics and Economic Studies (INSEE), the Direction générale de la santé (DGS; part of the Ministry of Health and Social Affairs), the Direction générale de la prévention des risques (DGPR; Ministry for the Environment), the Direction de la recherche, des études, de l'évaluation et des statistiques (DREES, Ministry of Health and Social Affairs), the Département des études, de la prospective et des statistiques (DEPS; Ministry of Culture), and the Caisse nationale des allocations familiales (CNAF), with the support of the Ministry of Higher Education and Research and the Institut national de la jeunesse et de l'éducation populaire (INJEP). Via the RECONAI platform, it receives a government grant managed by the National Research Agency under the "Investissements d'avenir" programme (ANR-11-EQPX-0038).

The funders had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript.

Corresponding author:
Blandine de Lauzon-Guillain
Inserm CRESS – Team 6 EAROH
16 avenue Paul Vaillant Couturier, 94800 Villejuif Cedex, France
Tel: +33 1 45 59 50 19
E-mail: blandine.delauzon@inserm.fr

Abbreviations:

ARA: arachidonic acid

DHA: docosahexaenoic acid

EPA: eicosapentaenoic acid

LCPUFA: long-chain polyunsaturated fatty acids

LRTI: lower respiratory tract infection

n-3: omega-3

PANDiet: Probability of Adequate Nutrient intake based Diet quality index

RCT: randomized controlled trials

URTI: upper respiratory tract infection

Manuscript word count: 3895

Abstract

Background: The new European regulations require the enrichment of formulas with docosahexaenoic acid (DHA) because of the positive effects of long-chain polyunsaturated fatty acids (LCPUFAs) on neurodevelopment and visual acuity. In this observational study, we aimed to evaluate whether the consumption of LCPUFA-enriched formula was associated with the risk of infection and allergy in early childhood.

Methods: Analyses involved data from 8,389 formula-fed infants from the ELFE birth cohort. Formula enrichment was identified from the list of ingredients of the formula consumed at 2 months. Infections (gastro-intestinal, lower respiratory tract [LRTI], upper respiratory tract) and allergies (wheezing, itchy rash, asthma medication, food allergy) from age 2 months to 5.5 years were reported by parents during follow-up surveys. Multivariable logistic regression models were used to assess associations between the consumption of LCPUFA-enriched formula and the risk of infection and allergy.

Results: Among formula-fed infants at 2 months, 36% consumed formula enriched with DHA and arachidonic acid (ARA), and 11% consumed formula additionally enriched with eicosapentaenoic acid (EPA). Enriched formula consumption was not associated with infection or allergy, except for an association between consumption of DHA/ARA/EPA-enriched formula and lower use of asthma medications. Furthermore, as compared with non-DHA/ARA/EPA-enriched formula, consumption of formula with high EPA content (≥3.2 mg/100 kcal) was related to lower risk of LRTI and lower use of asthma medications.

Conclusion: This study suggests that consumption of DHA/ARA/EPA-enriched formula (especially those with high EPA content) is associated with a lower risk of LRTI and lower use of asthma medications.

Keywords: allergies, infant formula, infections, LCPUFA-enrichment

INTRODUCTION

Allergic diseases and infections are the leading causes of morbidity in childhood. The prevalence of infections has decreased in recent years [1-3], but that of allergic diseases and asthma (both considered a major public health issue) has increased over the last decades [1, 4, 5]. In addition to genetic predisposition, several modifiable factors are associated with risk of developing allergic diseases, including low microbial exposure, high exposure to air pollutants or cigarette smoke and inadequate early feeding practices [1, 6, 7]. With regard to early feeding, the protective effect of breastfeeding on infections is well established [8], but its potential effect on allergic diseases is more controversial [8-12].

Breastmilk supplies all essential bioactive factors and nutrients, including long-chain polyunsaturated fatty acids (LCPUFAs), which have an important role in immune system development and regulation and in neurodevelopment, including neurotransmission, visual function and cognitive development [13-15]. LCPUFA content in breastmilk varies according to the lactation period and maternal diet, with an average content of 0.37% of total fatty acids for docosahexaenoic acid (DHA), 0.55% of total fatty acids for arachidonic acid (ARA) and 0.10% of total fatty acids for eicosapentaenoic acid (EPA) [16-18]. Several studies have examined the association between omega-3 (n-3) LCPUFAs level in breastmilk and risk of allergic diseases during infancy, but the findings remained inconsistent [19, 20]. Several randomized controlled trials (RCTs) have also assessed the effect of prenatal or postnatal n-3 LCPUFA supplementation on allergy development, but the available systematic reviews on this topic found that evidence remains insufficient to recommend supplementation with n-3 LCPUFAs during pregnancy, lactation and early life to prevent the development of allergic diseases in childhood [19-23].

Exclusive breastfeeding is recommended for the first 6 months of life or at least 4 months [24], but several studies have shown a high level of non- compliance with these recommendations [8, 25, 26]. For non-breastfed or partially breastfed infants, a wide range of infant formula is available, especially in France [27]. From scientific evidence suggesting beneficial effects of DHA on cognitive abilities [28-30] and to meet the nutritional needs of healthy infants, a European regulation (EU 2016/127) required that from February 2020, all infant and follow-on formula had to be enriched in DHA (20-50 mg/100 kcal, equivalent to 0.5-1% of total fatty acids). The European Academy of Pediatrics further suggested that DHA enrichment had to be combined with ARA enrichment [31].

To our knowledge, only 4 studies of small sample sizes have specifically investigated the association between DHA/ARA enrichment of formula and infection or allergy in childhood [32-35]. These studies support a beneficial effect of this enrichment on the incidence of diarrhea and some allergic and respiratory diseases [32-35]. However, no study has examined the potential effect of EPA enrichment of infant formula on infection and allergy in childhood.

The purpose of this observational study was to evaluate among formula-fed infants the associations between the enrichment of infant formula with LCPUFAs (DHA, ARA and EPA) and the risk of infection and allergy in early childhood in a large French nationwide birth cohort. The infant formula consumed in the present study were those available on the market in France before the enforcement of the new European regulation on DHA enrichment of formula.

We hypothesized that (1) consumption of LCPUFA-enriched formula at 2 months of age is associated with a lower risk of infection and allergy up to 5.5 years; (2) the observed risk reductions is greater for high LCPUFA contents in the consumed formula.

MATERIALS AND METHODS

Study population

Our analyses were based on data from the ELFE (Étude longitudinale française depuis l'enfance) multidisciplinary nationwide birth cohort including children born in France in 2011 [36]. This study was designed to assess the impact of environmental exposures and socioeconomic and familial factors on children's development, behaviours and health. The design and protocol of the study have been described in detail elsewhere [37]. Briefly, the study enrolled singletons or twins born after 33 weeks' gestation, from mothers aged 18 years or older who were able to read French, Arabic, Turkish or English and were not planning to move outside of metropolitan France in the next 3 years.

All participating mothers provided written consent for themselves and their child. Fathers also signed the consent form for their child's participation when present at inclusion or were informed about their rights to oppose it. The ELFE study was approved by the Advisory Committee for the Treatment of Health Research Information (Comité Consultatif sur le Traitement de l'Information en matière de Recherche en Santé [CCTIRS]), the National Agency Regulating Data Protection (Commission National de l'Informatique et des Libertés [CNIL]) and the National Council on Statistical Information (Conseil National de l'information Statistique [CNIS]).

Data collection

Infant feeding

Infant feeding was collected at age 2 months by phone interview. For infants consuming infant formula, parents were asked to report the brand and product names of the mainly used formula. All formulas used in the ELFE study were then listed and classified as enriched in LCPUFAs (including DHA, ARA and EPA) or not according to the ingredient list and, when available, the nutrient composition. When these data were not available, we used data from another question that directly reported whether the formula was enriched or not with LCPUFAs. Parents were also asked to report the age at infant formula introduction, which was used to derive the duration of predominant breastfeeding. Infants who received both breastmilk and infant formula were classified based on the formula consumed.

The enrichment level of the LCPUFA-enriched formula identified in this study ranged from 9.0 to 20.3 mg/100 kcal for DHA and 2.1 to 3.3 mg/100 kcal for EPA. When nutrient composition was available, the level of DHA and EPA enrichment was classified as low or high according to the median calculated in the study population (10 mg/100 kcal, equivalent to 0.19% of total fatty acids for DHA; and 3.2 mg/100 kcal, equivalent to 0.06% of total fatty acids for EPA). The median value was used because only 1 infant who consumed LCPUFA-enriched formula had consumed a formula with a DHA content at the minimum dose now recommended by the European regulation (20 mg/100 kcal) and only 75 (3%) infants the dose used in most RCTs (17 mg/100 kcal). Linoleic acid and alpha-linolenic acid contents were also available and modeled as continuous variables.

Infection and allergy

Data on infection and allergy were collected by phone interviews at the 2-month and, 1-, 2-, 3.5- and 5.5-year follow-ups, each referring to events that occurred since the last follow-up. To define incident cases of infection and allergy from age 2 months to 5.5 years, only cases occurring after the 2-month follow-up were considered.

In this observational study, infections were assessed by using 3 indicators: ever gastrointestinal infection, ever lower respiratory tract infection (LRTI) and ever upper respiratory tract infection (URTI), which were defined as follows:

- Ever gastrointestinal infection: parental report of at least one hospitalization for gastroenteritis/dehydration or one emergency consultation for diarrhea/vomiting/dehydration;

- Ever LRTI: parental report of at least one bronchiolitis event or one hospitalization for bronchitis, bronchiolitis or pneumopathy;
- Ever URTI: parental report of at least 3 otitis events from birth, or at least 3 laryngitis events from birth or at least 3 angina events from birth, or one hospitalization URTI or one emergency consultation for otalgia.

With regard to allergies, we considered the following indicators to enhance international comparison with other birth cohorts from the LifeCycle consortium [38]: ever wheezing, ever itchy rash, ever food allergy and ever use of asthma medications. Children were classified as with ever wheezing or ever itchy rash from age 2 months to 5.5 years if parents reported at least one episode. Children were classified as with ever food allergy from age 2 months to 5.5 years if parents reported at least once medical advice to avoid certain foods due to an allergy. 'Ever use of asthma medications' refers to at least one parental report of use of inhaled corticosteroids or bronchodilators at home or the hospital during the first 3.5 years of life (these data were not collected at the 5.5-year follow-up).

Infant and familial characteristics

Maternal and household characteristics were collected at the maternity and 2-month interviews. Birth characteristics were also collected from the medical records. Maternal and household characteristics considered in this study were defined as: age (< 25, 25–29, 30–34, ≥ 35 years), city size (rural, urban), region of residence (Paris region, North, East, Paris Basin–East, Paris Basin–West, West, South-West, South-East, Mediterranean), education level (up to lower secondary, upper secondary, intermediate, 3-year university degree, at least 5-year university degree), employment status in early pregnancy (employed, unemployed, out of the labor force [student/housewife/retired/handicapped pension]), pre-pregnancy body mass index (<18.5, 18.5–24.9, 25–29.9, ≥30 kg/m²), maternal allergy history (including eczema, asthma, or hay fever; yes/no), smoking status (never smoker, smoker only before pregnancy, smoker only in early pregnancy, smoker throughout pregnancy), diet quality during pregnancy (measured by using the Probability of Adequate Nutrient intake based Diet quality index [PANDiet] score, which reflects nutrient-based reference guidelines adapted for pregnancy [39]), n-3 fatty acids supplementation during pregnancy (yes/no) and household income per consumption unit (≤€750, €751–1111, €1112–1500, €1501–1944, €1945–2500, >€2500 /month).

The infant's characteristics included: child's sex, gestational age (in weeks), caesarean section (yes/no), birth weight, type of health professional for the first visit after delivery (paediatrician, other child doctor, general practitioner, other including emergency), age at introduction of infant formula (< 1 month, ≥1 month), presence of older siblings (no sibling, one sibling, at least 2 siblings) and family history of allergy (neither parent nor sibling with allergy; at least one parent or sibling with allergy including eczema, asthma, or hay fever). Birth weight categories (small, adequate or large for gestational age) were defined according to the French Audipog reference curves [40].

Selection of the study sample

In the present study, we randomly selected one twin out of two (n=287) to avoid family clustering and excluded infants without data at the 2-month follow-up (*n*=1,696). Among the 11,244 infants who were not exclusively breastfed at the 2-month follow-up (eligible infants for this study), we excluded infants with insufficient detail on infant formula consumed at 2 months (*n*=667), those who had a medical diagnosis of cow's milk protein allergy reported at 2 months (*n*=193), and those with missing data for allergy or infection outcomes (n=1,995). Finally, our study sample included 8,389 children (**Figure 1**).

Statistical analyses

Infants included in this study were compared to excluded eligible infants using chi-square test or Student *t* test as appropriate. Characteristics of the study population were presented according to LCPUFA enrichment of infant formula at the 2-month follow-up.

To provide national statistics on LCPUFA enrichment among formula-fed infants, data were weighted to account for inclusion procedure and biases related to non-consent [41]. Weighting also included calibration on margins from the state register's statistical data and the 2010 French National Perinatal study [42] on the following variables: age, region, marital status, migration status, level of education, and primiparity. This weighting was calculated for the subsample that completed the 2-month interview.

The associations between consumption of LCPUFA-enriched formula at age 2 months and the risk of infection or allergy were assessed with logistic regression models adjusted for potential confounders identified from the literature and validated with the directed acyclic graph method [43]. All analyses were adjusted for maternal characteristics (age, education level, city size, region of residence, employment status in early pregnancy, pre-pregnancy body mass index, smoking status, diet quality during pregnancy and n-3 PUFA supplementation during

pregnancy), household income, family history of allergy, infant characteristics (sex, gestational age, caesarean section, birth weight category, type of health professional for the first visit after delivery, presence of older siblings and age at infant formula introduction) and variables related to study design (recruitment wave and maternity size). Missing covariates data were handled by using the Hot Deck method, which consists of replacing the missing value by that for respondents with the same characteristics [44].

In this study, interactions between consumption of LCPUFA-enriched formula and family or maternal history of allergy were not significant for any of the modelled outcomes. Therefore, we evaluated the associations in the whole sample.

Sensitivity analyses

To evaluate the potential presence of bias and test the robustness of our findings, several sensitivity analyses were performed. First, because infants consuming both breastmilk and infant formula may also benefit from LCPUFAs contained in breastmilk, analyses were conducted on the subsample of infants exclusively formula-fed at age 2 months (n=6,251). Then, to limit the potential effect of the reverse causation bias, analyses were conducted on the subsample of infants who were fed the same formula during their first 2 months of life (n=4,429). Our analyses were also repeated among infants without any infection or allergic disease before age 2 months (n=6,490).

Because our previous findings showed that the consumption of partially hydrolyzed formulas at age 2 months was associated with increased risk of wheezing and food allergy [45], we conducted a sensitivity analysis excluding all infants who consumed an infant formula with hydrolyzed proteins at age 2 months (n=364).

We also performed weighted analyses to assess the generalization of our results, using the weighting presented above.

In addition, instead of the Hot Deck method, we handled missing covariates data by using multiple imputations [46, 47]. We assumed that data were missing completely at random conditionally to the set of variables included in the analysis and generated 5 independent datasets with the fully conditional specification method (MI procedure, FCS statement, NIMPUTE option). We then calculated pooled effect estimates by using the SAS MIANALYSE procedure. Further details are available in **Supplementary table 1**.

All statistical analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC).

RESULTS

Sample characteristics

Of the 8,389 children formula-fed at 2 months of age that we included in this study, 4,282 were boys. As compared with eligible infants who were excluded from the analysis (n=2,855), those who were included were more often born with adequate weight for gestational age (80.1% vs. 78.4%; p=0.003), to an older mother (mean age 31± 4.9 vs. 29.4 ± 5.6 years; p<0.0001), with a higher education level (18.3% vs. 9.2% with a Master's degree, p<0.0001) and a higher income (1,663 ± 977 vs. 1,356 ± 1,219 euros per consumption unit; p<0.0001) and were more likely to stop smoking before pregnancy (24.7% vs. 18.5%; p<0.0001).

At age 2 months, 36.8% (CI=35.5-38.1) of formula-fed infants consumed DHA-enriched and 36.0% (CI=34.7-37.2) ARA-enriched formula. Since almost all of the DHA-enriched formulas were also enriched in ARA, we examined the effect of both DHA and ARA enrichment. Moreover, 11.3% (CI=10.4-12.2) of infants consumed a formula enriched in both DHA/ARA and EPA.

Table 1 presents the main characteristics of the study sample according to the type of formula consumed at age 2 months.

Enrichment of the formula consumed at age 2 months and risk of infection and allergy up to age 5.5 years

Consumption of DHA/ARA-enriched formula at 2 months of age was not associated with the occurrence of infection or allergy up to age 5.5 years (**Tables 2 and 3)**. As compared with non-DHA/ARA/EPA-enriched formula, the consumption of DHA/ARA/EPA-enriched formula was associated with a lower use of asthma medications up to age 3.5 years. We found no significant association between DHA/ARA/EPA enrichment and the occurrence of infections such as gastrointestinal infection, LRTI, URTI, nor with wheezing, itchy rash and food allergy up to age 5.5 years.

As compared with non-DHA/ARA-enriched formula, the consumption of formula with low DHA content (<10 mg/100 kcal) was associated with a higher risk of LRTI and wheezing, whereas an opposite trend was observed with consumption of formula with high DHA content (**Tables 2 and 3)**. We found no significant association between DHA enrichment level and the other outcomes.

As compared with non-DHA/ARA/EPA-enriched formula, the consumption of formula with high EPA content was associated with a lower risk of LRTI and a lower use of asthma

medications. We found no significant association between EPA enrichment level and the occurrence of gastrointestinal infection, URTI, wheezing, itchy rash or food allergy up to age 5.5 years.

We found no significant association for formula content of linoleic acid and alpha-linolenic acid in any model (**data not shown**).

Sensitivity analyses

When restricting the analyses to exclusively formula-fed infants at age 2 months, the consumption of formula with high EPA content was also associated with a lower risk of LRTI, URTI and a lower use of asthma medications (**Tables 2 and 3**).

In sensitivity analyses conducted to deal with the potential indication bias (including only infants without any change in formula up to age 2 months or without any infection or allergy up to age 2 months [**Supplementary tables 2 and 3**]), the negative association between EPA enrichment and asthma medications or LRTI remained similar. In addition, as compared with non-DHA/ARA-enriched formula, the consumption of formula with high DHA content (≥10 mg/100 kcal) was associated with a lower use of asthma medications (**Supplementary table 3**). In addition, among infants without any infection or allergy up to age 2 months, as compared with non-DHA/ARA/EPA-enriched formula, the consumption of DHA/ARA/EPA-enriched formula was also associated with a lower risk of food allergy.

Similar associations were observed for weighted analyses, analyses using multiple imputation to deal with missing data (**Supplementary tables 4 and 5**) and analyses among infants who did not consume infant formula with hydrolyzed proteins (**data not shown**).

DISCUSSION

In this observational study, one quarter of formula-fed infants consumed a formula enriched in DHA/ARA and just over one tenth consumed a formula additionally enriched in EPA. In the full sample, consumption of infant formula with low DHA content (<10 mg/100 kcal) was associated with a higher risk of LRTI and wheezing up to age 5.5 years, whereas an opposite trend was observed for high DHA content. The consumption of formula with high EPA content (≥3.2 mg/100 kcal) was associated with a lower risk of LRTI and use of asthma medications up to age 5.5 years. No significant association was found between LCPUFA enrichment and occurrence of gastrointestinal infection, URTI, itchy rash or food allergy up to

age 5.5 years. Among exclusively formula-fed infants, the consumption of formula with high EPA content was associated with a lower risk of URTI.

Few studies have evaluated the association between the consumption of DHA/ARA-enriched formula and the risk of infection or allergy in childhood [32-35], and none has evaluated the effect of additional enrichment in EPA, which makes comparing our results difficult. In addition, the outcomes definition was quite heterogeneous across the studies, which suggests caution in comparing results.

Available studies suggest that consumption of DHA/ARA-enriched formula (17 mg /100 kcal for DHA and 34 mg /100 kcal for ARA) was associated with a lower incidence or delayed onset of bronchitis/bronchiolitis [34, 35]. A similar trend was observed in the present study among infants who consumed formula with high DHA content (10-20 mg/100 kcal), but the association did not reach statistical significance (OR=0.88; 95% CI=0.76-1.02 for LRTI). Moreover, an additional enrichment of formula with high EPA content was associated with a lower risk of LRTI. As infant formula with high EPA content also had high DHA content in this study (mean DHA enrichment level=14.7 mg/100 kcal), the observed findings suggest that formula with DHA content close to the recommendations would have a better effect when it is additionally enriched with EPA.

Regarding URTI, we found no significant association with the consumption of DHA/ARA-enriched formula. Currently available studies showed inconsistent findings, with one RCT which highlights a protective effect of DHA/ARA enrichment on risk and delayed onset of URTI [33], and another that showed no significant association with URTI incidence [35].

Although a previous observational study [34] found that DHA/ARA enrichment of infant formula was associated with a lower risk and delayed onset of diarrhea requiring medical attention, we were not able to highlight any association between this enrichment and gastrointestinal infections. This lack of association could be due to the cases definition (diarrhea requiring medical attention vs. hospitalization for gastroenteritis/dehydration or emergency consultation for diarrhea/vomiting/dehydration) and the type of outcome investigated (gastrointestinal infections [yes/no] and not age at first episode).

Finally, in agreement with the DIAMOND study [32], the consumption of DHA/ARA-enriched formula was not associated with the risk of itchy rash. However, a protective effect of DHA/ARA enrichment was found for allergic illness or skin allergic illnesses in children without a maternal history of allergy [32]. One observational study also found that consumption

of DHA/ARA-enriched formula was associated with a lower risk of having an increased number of eczema episodes, but not with its incidence [34].

Although the literature focused mainly on DHA, our findings suggest that EPA could also play an important role. Indeed, DHA and EPA are both n-3 LCPUFAs and are important for growth, development and many aspects of health including cell function and inflammation [48-52].

The new European regulation now requires the enrichment of all infant and follow-on formula in DHA, whereas the enrichment in ARA and EPA remains optional. The European Academy of Pediatrics further suggested that DHA enrichment had to be combined with ARA enrichment [31]. Our results suggest that EPA enrichment could also be of great interest, as even with sufficient ALA in infant formula, the conversion capacity of ALA to EPA is very limited [51]. However, further well-designed studies are needed to examine the potential benefits of such enrichment.

The ELFE cohort is a nationwide study of birth in 2011 in metropolitan France (excluding very preterm babies). The detailed data on infant diet allowed for examining precisely, under real conditions of use, the effect of LCPUFA enrichment on infection and allergy in childhood. We have to acknowledge that health data were reported by parents and not validated by medical records, which could lead to a potential measurement bias. However, the items used were derived from international ones [4] to limit this bias. Moreover, the prospective design and the availability of repeated data on health-related outcomes in childhood limited memory bias. The very large sample and the collection of detailed socio-demographic or economic data ensured large statistical power and favoured control for potential confounders. Furthermore, the interpretation of our findings should be done with caution as an arbitrary cut-off point (median LCPUFA content of formula) was used to classify infant formula consumed in this study as "low LCPUFA content" or "high LCPUFA content". Indeed, this methodological approach was used since very few infants consumed infant formula with the minimum dose used on the available RCTs (17 mg /100 kcal for DHA, n=75 infants) or that recommended by the European regulation (20 mg /100 kcal for DHA, n=1 infant). Further studies are needed to confirm the present results with less arbitrary cut-offs. We also performed several sensitivity analyses to examine the robustness of our results and to address selection and reverse causation bias. As findings remained stable across these sensitivity analyses, these potential biases, if any, should have a limited impact on our findings.

In conclusion, this observational study under real life conditions suggests that LCPUFA-enriched formula, especially those with additional enrichment with high EPA level was associated with a lower risk of LRTI, URTI and use of asthma medications. However, URTI, findings were inconsistent across analyses. Replication in other observational studies and larger well-designed RCTs using the recommended levels are needed to confirm these results.

References

1. Pawankar R., Canonica G. W., ST Holgate S. T., Lockey R. F., M. B. *The WAO White Book on Allergy (Update. 2013)*. United States: World Allergy Organization; 2013.

2. Collaborators. GLRI. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Infect Dis.* Nov 2018;18(11):1191-1210.

3. Collaborators. GLRI. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Infect Dis.* Nov 2018;18(11):1211-1228.

4. Asher MI, Montefort S, Björkstén B, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *Lancet.* Aug 26 2006;368(9537):733-743.

5. Nwaru BI, Hickstein L, Panesar SS, Roberts G, Muraro A, Sheikh A. Prevalence of common food allergies in Europe: a systematic review and meta-analysis. *Allergy.* Aug 2014;69(8):992-1007.

6. Beasley R, Semprini A, Mitchell EA. Risk factors for asthma: is prevention possible? *Lancet.* Sep 12 2015;386(9998):1075-1085.

7. Halken S. Prevention of allergic disease in childhood: clinical and epidemiological aspects of primary and secondary allergy prevention. *Pediatr Allergy Immunol.* Jun 2004;15 Suppl 16:4-5, 9-32.

8. Victora CG, Bahl R, Barros AJ, et al. Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. *Lancet.* Jan 30 2016;387(10017):475-490.

9. Horta BL, Victora CG. *Short-term effects of breastfeeding: a systematic review of the benefits of breastfeeding on diarrhoea and pneumonia mortality*. Geneva: World Health Organization; 2013.

10. Gungor D, Nadaud P, LaPergola CC, et al. Infant milk-feeding practices and food allergies, allergic rhinitis, atopic dermatitis, and asthma throughout the life span: a systematic review. *Am J Clin Nutr.* Mar 1 2019;109(Suppl_7):772S-799S.

11. Tromp I, Kiefte-de Jong J, Raat H, et al. Breastfeeding and the risk of respiratory tract infections after infancy: The Generation R Study. *PLoS One.* 2017;12(2):e0172763.

12. Munblit D, Peroni DG, Boix-Amoros A, et al. Human Milk and Allergic Diseases: An Unsolved Puzzle. *Nutrients.* Aug 17 2017;9(8).

13. Gould JF, Smithers LG, Makrides M. The effect of maternal omega-3 (n-3) LCPUFA supplementation during pregnancy on early childhood cognitive and visual development: a systematic review and meta-analysis of randomized controlled trials. *Am J Clin Nutr.* Mar 2013;97(3):531-544.

14. Janssen CI, Kiliaan AJ. Long-chain polyunsaturated fatty acids (LCPUFA) from genesis to senescence: the influence of LCPUFA on neural development, aging, and neurodegeneration. *Prog Lipid Res.* Jan 2014;53:1-17.

15. Richard C, Lewis ED, Field CJ. Evidence for the essentiality of arachidonic and docosahexaenoic acid in the postnatal maternal and infant diet for the development of the infant's immune system early in life. *Applied Physiology, Nutrition, and Metabolism.* 2016;41(5):461-475.

16. Koletzko B. Human Milk Lipids. *Annals of Nutrition and Metabolism.* 2016;69(Suppl. 2):27-40.

17. Bobiński R, Bobińska J. Fatty acids of human milk - a review. *Int J Vitam Nutr Res.* Apr 21 2020:1-12.

18. Fu Y, Liu X, Zhou B, Jiang AC, Chai L. An updated review of worldwide levels of docosahexaenoic and arachidonic acid in human breast milk by region. *Public Health Nutr.* Oct 2016;19(15):2675-2687.

19. Venter C, Meyer RW, Nwaru BI, et al. EAACI position paper: Influence of dietary fatty acids on asthma, food allergy, and atopic dermatitis. *Allergy.* Aug 2019;74(8):1429-1444.

20. Waidyatillake NT, Dharmage SC, Allen KJ, et al. Association of breast milk fatty acids with allergic disease outcomes-A systematic review. *Allergy.* Feb 2018;73(2):295-312.

21. Gunaratne AW, Makrides M, Collins CT. Maternal prenatal and/or postnatal n-3 long chain polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood. *Cochrane Database Syst Rev.* Jul 22 2015(7):CD010085.

22. Schindler T, Sinn JK, Osborn DA. Polyunsaturated fatty acid supplementation in infancy for the prevention of allergy. *Cochrane Database Syst Rev.* Oct 28 2016;10:CD010112.

23. Vandenplas Y, Meyer R, Chouraqui JP, et al. The role of milk feeds and other dietary supplementary interventions in preventing allergic disease in infants: Fact or fiction? *Clin Nutr.* Oct 20 2020.

24. WHO. *Feeding and nutrition of infants and young children, guidelines for the WHO European region, with emphasis on the former Soviet countries*. Geneva; 2003.

25. Schiess S, Grote V, Scaglioni S, et al. Introduction of complementary feeding in 5 European countries. *J Pediatr Gastroenterol Nutr.* Jan 2010;50(1):92-98.

26. Cai X, Wardlaw T, Brown DW. Global trends in exclusive breastfeeding. *Int Breastfeed J.* Sep 28 2012;7(1):12.

27. de Lauzon-Guillain B, Davisse-Paturet C, Lioret S, et al. Use of infant formula in the ELFE study: The association with social and health-related factors. *Matern Child Nutr.* Jan 2018;14(1).

28. Drover JR, Hoffman DR, Castañeda YS, et al. Cognitive function in 18-month-old term infants of the DIAMOND study: a randomized, controlled clinical trial with multiple dietary levels of docosahexaenoic acid. *Early Hum Dev.* Mar 2011;87(3):223-230.

29. Lepping RJ, Honea RA, Martin LE, et al. Long-chain polyunsaturated fatty acid supplementation in the first year of life affects brain function, structure, and metabolism at age nine years. *Dev Psychobiol.* Jan 2019;61(1):5-16.

30. Liao K, McCandliss BD, Carlson SE, et al. Event-related potential differences in children supplemented with long-chain polyunsaturated fatty acids during infancy. *Dev Sci.* Sep 2017;20(5).

31. Koletzko B, Bergmann K, Brenna JT, et al. Should formula for infants provide arachidonic acid along with DHA? A position paper of the European Academy of Paediatrics and the Child Health Foundation. *Am J Clin Nutr.* Jan 1 2020;111(1):10-16.

32. Foiles AM, Kerling EH, Wick JA, Scalabrin DM, Colombo J, Carlson SE. Formula with long-chain polyunsaturated fatty acids reduces incidence of allergy in early childhood. *Pediatr Allergy Immunol.* Mar 2016;27(2):156-161.

33. Birch EE, Khoury JC, Berseth CL, et al. The impact of early nutrition on incidence of allergic manifestations and common respiratory illnesses in children. *J Pediatr.* Jun 2010;156(6):902-906.e901.

34. Lapillonne A, Pastor N, Zhuang W, Scalabrin DM. Infants fed formula with added long chain polyunsaturated fatty acids have reduced incidence of respiratory illnesses and diarrhea during the first year of life. *BMC Pediatr.* Jul 2 2014;14:168.

35. Pastor N, Soler B, Mitmesser SH, Ferguson P, Lifschitz C. Infants fed docosahexaenoic acid- and arachidonic acid-supplemented formula have decreased incidence of bronchiolitis/bronchitis the first year of life. *Clin Pediatr (Phila).* Nov 2006;45(9):850-855.

36. Charles MA, Thierry X, Lanoe JL, et al. Cohort Profile: The French national cohort of children (ELFE): birth to 5 years. *Int J Epidemiol.* Apr 1 2020;49(2):368-369j.

37. Vandentorren S, Bois C, Pirus C, et al. Rationales, design and recruitment for the Elfe longitudinal study. *BMC Pediatr.* Sep 21 2009;9:58.

38. Jaddoe VWV, Felix JF, Andersen AN, et al. The LifeCycle Project-EU Child Cohort Network: a federated analysis infrastructure and harmonized data of more than 250,000 children and parents. *Eur J Epidemiol.* Jul 2020;35(7):709-724.

39. Bianchi CM, Mariotti F, Verger EO, Huneau JF. Pregnancy Requires Major Changes in the Quality of the Diet for Nutritional Adequacy: Simulations in the French and the United States Populations. *PLoS One.* 2016;11(3):e0149858.

40. Mamelle N, Munoz F, Grandjean H. [Fetal growth from the AUDIPOG study. I. Establishment of reference curves]. *J Gynecol Obstet Biol Reprod (Paris).* 1996;25(1):61-70.

41. Juillard H. Weighting of Elfe survey data at time 0. https ://pandora.vjf.inserm.fr/publi c/2015; 2015. Accessed.

42. Blondel B, Lelong N, Kermarrec M, Goffinet F. Trends in perinatal health in France from 1995 to 2010. Results from the French National Perinatal Surveys. *J Gynecol Obstet Biol Reprod (Paris).* Jun 2012;41(4):e1-e15.

43. Shrier I, Platt RW. Reducing bias through directed acyclic graphs. *BMC Med Res Methodol.* Oct 30 2008;8:70.

44. Andridge RR, Little RJ. The Use of Sample Weights in Hot Deck Imputation. *J Off Stat.* 2009;25(1):21-36.

45. Davisse-Paturet C, Raherison C, Adel-Patient K, et al. Use of partially hydrolysed formula in infancy and incidence of eczema, respiratory symptoms or food allergies in toddlers from the ELFE cohort. *Pediatr Allergy Immunol.* Sep 2019;30(6):614-623.

46. Sterne JA, White IR, Carlin JB, et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. *Bmj.* Jun 29 2009;338:b2393.

47. van Buuren S. Multiple imputation of discrete and continuous data by fully conditional specification. *Stat Methods Med Res.* Jun 2007;16(3):219-242.

48. Shek LP, Chong MF, Lim JY, Soh SE, Chong YS. Role of dietary long-chain polyunsaturated fatty acids in infant allergies and respiratory diseases. *Clin Dev Immunol.* 2012;2012:730568.

49. Hageman JH, Hooyenga P, Diersen-Schade DA, Scalabrin DM, Wichers HJ, Birch EE. The impact of dietary long-chain polyunsaturated fatty acids on respiratory illness in infants and children. *Curr Allergy Asthma Rep.* Dec 2012;12(6):564-573.

50. Quin C, Gibson DL. Dietary Fatty Acids and Host-Microbial Crosstalk in Neonatal Enteric Infection. *Nutrients.* Sep 3 2019;11(9).

51. Swanson D, Block R, Mousa SA. Omega-3 fatty acids EPA and DHA: health benefits throughout life. *Adv Nutr.* Jan 2012;3(1):1-7.

52. Hadders-Algra M. The role of long-chain polyunsaturated fatty acids (LCPUFA) in growth and development. *Adv Exp Med Biol.* 2005;569:80-94.