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Declarations

Ethical approval

The ELFE study received approvals from the Advisory Committee for the Processing of Information for Health Research (Comité Consultatif sur le Traitement des Informations pour la Recherche en Santé: CCTIRS) and National Data Protection Authority (Commission National Informatique et Libertés: CNIL) and the National Council for Statistical Information (CNIS).

Consent to participate

Participating mothers had to provide written consent for their own and their child's participation. Fathers gave signed consent for the child's participation if present at inclusion or were informed about their rights to oppose it.

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Conflict of interest/Competing interests

The authors declare no competing financial interest.

Availability of data and material

The data underlying the findings cannot be made freely available for ethical and legal restrictions imposed because this study includes a substantial number of variables that together could be used to re-identify the participants based on a few key characteristics and then used to access other personal data. Therefore, the French ethics authority strictly forbids making these data freely available. However, they can be obtained upon request from the ELFE principal investigator. Readers may contact marie-aline.charles@inserm.fr to request the data. The code book and analytic code will be made available upon request pending application and approval.

Abstract

Purpose: For decades, consistent associations between breastfeeding and children's neurodevelopement have been attributed to breastmilk content in long-chain polyunsaturated fatty acids (LC-PUFAs). However, the beneficial effect of LC-PUFA enrichment of infant formula on neurodevelopment remains controversial. This study examined the association of LC-PUFA enrichment of infant formulas with neurodevelopment up to age 3.5 years.

Methods: Analyses were based on 9,372 children from the French nationwide ELFE birth cohort. Monthly from 2 to 10 months, parents declared their infant's feeding mode, including breastfeeding and the name of the infant formula, which allowed for identifying formulas enriched in arachidonic (ARA), eicosapentaenoic (EPA) and/or docosahexaenoic (DHA) acids. Neurodevelopment was assessed at age 1 and 3.5 years with the Child Development Inventory (CDI-1 and CDI-3.5); at 2 years with the MacArthur-Bates Communicative Development Inventories (MB-2); and at 3.5 years with the Picture Similarities subtest of the British Ability Scale (BAS-3.5). Associations were assessed by linear regression adjusted for any breastfeeding duration and main confounding factors, including socioeconomic characteristics.

Results: One third of formula-fed infants consumed LC-PUFA–enriched formulas. Most of these formulas were enriched in both DHA and ARA, and about 10% of infants consumed formula further enriched in EPA. LC-PUFA enrichment of infant formula was not associated with neurodevelopmental scores at age 1 (CDI-1, -0.16 [-0.39, 0.07]), age 2 (MB-2, 0.78 [-0.33, 1.89]), or age 3.5 (CDI-3.5, -0.05 [-0.27, 0.17]; BAS-3.5, -0.93 [-2.85, 0.98]).

Conclusion: In the ELFE study, LC-PUFA enrichment of infant formula was not associated with neurodevelopmental scores up to 3.5 years.

Keywords. Long-chain polyunsaturated fatty acids, infant formula, neurodevelopment, birth cohort

Introduction

Breast milk is considered the most appropriate food for the infant's optimal development, not only because its nutritional composition varies according to the child's needs [1] but also because of its benefits for later health and development [1], including language and cognition [2-4]. More specifically, breastfed children have higher cognitive scores than never-breastfed children: numerous studies found better language and motor development among breastfed than non-breastfed children, with a dose–effect relationship for both any and exclusive breastfeeding duration [2-7]. If causal, these consistent associations between breastfeeding and neurodevelopment are thought to be explained in part by the long-chain polyunsaturated fatty acids (LC-PUFAs) contained in breast milk [8,9]. This hypothesis is supported by studies of premature newborn babies, in which two major LC-PUFAs, arachidonic acid (ARA) and docosahexaenoic acid (DHA), were discovered as crucial for retinal and neuronal cell development [9,10].

According to these elements, in 2016, the European Food Safety Authority (EFSA) registered LC-PUFAs as essential components to be mandatorily added to all infant and follow-on formulas from 2020, amending previous EFSA regulation [11]. Yet, this last recommendation is still debated in the scientific literature because to date, there remains no conclusive evidence of beneficial health effects for infants [12] and DHA enrichment may need to be combined with AHA enrichment [12]. Some experts have also warned that fatty-acids–enriched infant formulas may increase metabolic disorders and overweight prevalence among infants [13].

One meta-analysis of randomised controlled trials (RCTs) found that LC-PUFA supplementation improved cognitive scores of infants [14]; however, another more recent meta-analysis found this effect in pre-term but not full-term infants [9,10]. None of these meta-analyses highlighted a beneficial effect of LC-PUFA supplementation on cognitive

abilities later in childhood [9,15]. A Cochrane review included more specifically RCTs with LC-PUFA enrichment of infant formula among full-term infants but did not highlight any effect of this enrichment on neurodevelopment (language, memory, visual-spatial abilities, attention) at an early age or on visual acuity development [15]. More recently, a systematic review of RCTs did not highlight any beneficial effect of LC-PUFA enrichment of infant formula on cognitive function among children aged 2.5 years or older [16].

In this context, this study examined, under real conditions of use, the associations between LC-PUFA enrichment of infant formula and children's neurodevelopment up to age 3.5 years in a large birth cohort.

Materials and Methods

Study design and population

The present analysis was based on data from the Étude Longitudinale Française depuis l'Enfance (ELFE) study, a nationwide birth cohort that included 18,329 children born in 2011 in a random sample of 349 maternity units in metropolitan France [17]. Inclusion took place during 25 selected days spread over four waves (one per season) of 4 to 8 days each. Inclusion criteria were children born after 33 weeks of gestation to mothers aged 18 years or older and who were not planning to move outside of metropolitan France during the following 3 years.

Participating mothers had to provide written consent for their own and their child's participation. Fathers gave signed consent for the child's participation if present at inclusion or were informed about their rights to oppose it. The ELFE study received approvals from the Advisory Committee for the Treatment of Information on Health Research (Comité Consultatif sur le Traitement des Informations pour la Recherche en Santé), the National Agency Regulating Data Protection (Commission Nationale Informatique et Libertés), and the National Statistics Council (Conseil National de l'Information Statistique).

Infant feeding methods

Data on infant feeding were collected during face-to-face interviews during the maternity stay, by phone interview at the 2-month and 1-year interviews, and by Internet/paper questionnaire each month from 3 to 10 months after delivery. At every time point up to 10 months, parents reported their infant's feeding method (breast or formula milk) as well as the name and brand of the infant formula used when relevant [18]. From these monthly data, LC-PUFA–enriched infant formulas were identified, and infants were classified as receiving breast milk only, regular infant formula, or LC-PUFA–enriched infant formula. Infants consuming both breast milk and infant formula at a given month were classified according to the formula used (regular or LC-PUFA–enriched). Any breastfeeding duration was also calculated as previously described [19] and classified as never, <1 month, 1 to 3 months, 3 to 6 months, ≥6 months.

Children's neurodevelopment

Children's neurodevelopment was assessed during the 1-, 2- and 3.5-year phone interviews and the face-to-face interview at the 3.5-year home visit.

At the 1- and 3.5-year follow-ups, an adapted version of the Child Development Inventory (CDI-1 and CDI-3.5) was used to assess the child's overall development by the parental report [20,21]. The CDI-1 includes 6 domains of development (social, self-help, gross motor skills, fine motor skills, expressive language, language comprehension), and the CDI-3.5 includes two additional domains (characters and numbers). For each item, the child earns 1 point when the ability is acquired and 0 when it is not. Scores for all items were summed for an overall CDI score ranging from 0 to 50 at 1 year and from 17 to 62 at 3.5 years.

At the 2-year follow-up, the child's language development was assessed by using the short French version of the MacArthur-Bates Communicative Development Inventory (MB-2) [22]:

from a list of 100 words, parents reported those used spontaneously by their child. The score ranged from 0 to 100.

At the 3.5-year follow-up, a trained investigator directly administered the Picture Similarities subscale of the British Ability Scale (BAS-3.5) to the child during the home visit [23]. The subscale assesses fundamental aspects of pictorial reasoning abilities. The BAS-3.5 is scored by complex manual scoring, most scales scored with a multi-point system. In the present study, we used percentiles of the score adjusted for the child's exact age.

Family and children characteristics

Mothers were interviewed in the maternity ward for medical information about their pregnancy and their newborn, their demographic and socioeconomic and lifestyle-related characteristics, and their eating habits during pregnancy. Information was complemented with obstetric and paediatric medical records. At 2 months post-partum, mothers and fathers were interviewed by phone, and more details on demographic and socioeconomic characteristics were collected.

Parental demographic and socioeconomic characteristics studied were maternal age at delivery (18–24, 25–29, 30–34, ≥35 years); number of older children in the household (no sibling, one sibling, at least two siblings); maternal migration status ("majority population", which included women born with a French nationality from two French parents [within or outside of France]; "descendants of migrants" including women born in France with at least one non-French parent; and "migrants" including women not born in France and without French citizenship at birth); maternal education level (up to lower secondary, upper secondary, high school graduate, 3-year university degree, at least 5-year university degree); employment status during pregnancy (employed, unemployed, not in the labour force; e.g. housewife, student, disabled, retired); and monthly household income per consumption unit (<€750, €751–1,111, €1,112–1,500, €1,501–1,944, €1,945–2,500, ≥€2,500). From the postal

code of residence, the region of residence (Paris region, north, east, Paris Basin – East, Paris Basin – West, west, southwest, southeast, Mediterranean) and city size (rural area, urban area) were determined.

Maternal health characteristics included self-reported height and pre-pregnancy weight used to calculate pre-pregnancy body mass index (BMI) (<18.5, 18.5–24.9, 25.0–29.9, ≥30.0 kg/m²) and maternal smoking status (never smoker, smoker only before pregnancy, smoker only in early pregnancy, smoker throughout pregnancy).

During the maternity stay, mothers completed a semi-quantitative food frequency questionnaire (FFQ) on their diet during the last 3 months of their pregnancy [24]. In the present analyses, we used the frequency of fish consumption (continuous variable) as well as the consumption of dietary supplementation with LC-PUFAs during pregnancy (binary variable).

Newborn characteristics collected from the medical record were child sex, gestational age and birth weight. Birth weight categories (small, adequate or large for gestational age) were defined according to the French Audipog reference curves [25]. The 2-month questionnaire collected the type of physician consulted for the first visit after delivery (paediatrician, other child doctor, general practitioner, other including emergency).

At the 1-year interview, the mother indicated the frequency (never or seldom, sometimes, often, always) of some activities with their child: playing, reading books, drawing, speaking, tickling/massage. The modal value of these activities was used to estimate a maternal stimulation score.

Sample selection

Parents who withdrew their consent within the first year and requested deletion of their data (n=57) were excluded from the study. In the present analysis, we randomly selected one twin out of two (n=287) to avoid family clustering. We excluded children not followed at the 2-

month interview (n=1,696) and those exclusively breastfed at 2 months (n=5,045), without sufficient data on the infant formula used at 2 months (n=667), or without any data on neurodevelopment from 1 to 3.5 years (n=1,205). The main analyses involved 9,372 children and complete-case analyses 7,040 children **(Figure 1).**

Statistical analysis

Differences between excluded and included children were assessed with Student *t* test and chi-square test for continuous and categorical variables, respectively.

To provide representative descriptive statistics of births in 2011 in Metropolitan France in terms of the consumption of LC-PUFA–enriched formulas from age 2 to 10 months, the data were weighted to take into account the inclusion procedure and biases related to non-consent [26]. Weighting also included calibration on margins from the state register's statistical data and the 2010 French National Perinatal study on the following variables: age, region, marital status, migration status, level of education, and primiparity [27]. For descriptive statistics, this weighting was calculated for the subsample that completed the 2-month interview and for the subsample that completed the questionnaire on infant diet, at least once from 3 to 10 months. For the main analyses, the weighting was calculated for the subsample included in the multiple imputation analysis (as described below) and for the complete-case subsample.

To deal with missing data, multiple imputations were performed. We assumed data were missing at random and generated five independent data sets using the fully conditional specification method (MI procedure) and the calculated estimates of pooled effects (MIANALYSE procedure; SAS software). Categorical variables were imputed by using a multinomial model, with logistic regression for ordinal and binary variables and with linear regression for continuous variables.

Unadjusted and adjusted associations between consumption of LC-PUFA–enriched formula and child neurodevelopment were examined by simple and multivariable linear regression,

respectively. The following potential confounding factors were identified from the literature, then selected by using the directed acyclic graph method [28,29]: family characteristics (maternal age, education level, migration status, employment during pregnancy, smoking status, pre-pregnancy BMI, household income, number of older children, rural/urban area and region of residence), maternal diet during pregnancy (fish intake, consumption of dietary supplements with LC-PUFA), infant characteristics (sex, gestational age, birth weight category, type of physician consulted after discharge), 2-month breastfeeding status, maternal stimulation and variables related to study design (maternity size and recruitment wave). Models were also adjusted for the child's exact age at neurodevelopmental assessment, except for analyses with BAS-3.5 as the outcome because it is already age-adjusted. The interaction between gestational age and LC-PUFA enrichment was tested for each neurodevelopmental score in the adjusted model. Because we found no evidence favouring an interaction (all p-values for interaction term > 0.25), gestational age was considered a confounding factor and not an effect modifier.

LC-PUFA enrichment of infant formula was examined first as a binary variable at the 2-month follow-up (enriched/not enriched), then as a three-category variable according to LC-PUFA types in the infant formula (not enriched, enriched with DHA/ARA only, enriched with DHA/ARA and EPA). Finally, to account for the temporal evolution of LC-PUFA–enriched formula consumption between 2 and 10 months, we identified groups of children with similar longitudinal patterns of consumption by using the Nagin method for group-based trajectory modelling [30] with the TRAJ procedure from SAS software. These trajectories were modeled among children who consumed infant formulas during at least 2 time points from 2 to 10 months. Analyses based on these trajectories were adjusted for any breastfeeding duration instead of 2-month breastfeeding status.

Sensitivity analyses

Sensitivity analyses were conducted on the subsample of never-breastfed infants. Moreover, to deal with the issue of change in infant formula from birth to the 2-month interview, we conducted a sensitivity analysis on the subsample of children without any change in infant formula up to 2 months. To deal with selection and attrition bias, we conducted a sensitivity analysis using weighted data, according to the weighting method described previously. This weighting was calculated for the subsamples with data for both infant formula used at the 2-month interview and neurodevelopmental outcomes. Finally, because the main analyses used multiple imputation to deal with missing data, all analyses were replicated with the complete-case sample.

All analyses involved using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). $P<0.05$ was considered statistically significant.

Results

As compared with children excluded from the analysis, included children were more frequently the firstborn of the household (46.5% vs 42.9%), born to older mothers (mean [SD] age 30.9 [4.8] vs 30.7 [5.2] years), who were employed during pregnancy (73.7% vs 66.7%) and had a higher education level (40.8% vs. 35.2% with at least a 3-year university degree).

The characteristics of included children are in **Table 1.**

LC-PUFA–enriched formula's consumption

The weighted prevalence of formula-fed infants consuming DHA-enriched formula was very stable for the 2- to 6-month infant formula period (35% to 38%) and decreased to about 12% for the 7- to 10-month follow-on formula period (**Figure 2**).

For most infants, DHA enrichment was combined with ARA enrichment and therefore we were not able to define two distinct groups for these enrichments. About one third of infants consuming DHA/ARA–enriched formulas also received EPA. The weighted prevalence of

formula-fed infants consuming EPA-enriched formula was very stable for the 2- to 6-month infant formula period (9% to 11%) and decreased to about 4% for the 7- to 10-month follow-on period.

Three distinct longitudinal trajectories of LC-PUFA–enriched formula consumption were identified (**Figure 3**: non-consumers [65%], regular consumers until age 6 months only [28%], regular consumers throughout the first year [7%]).

LC-PUFA–enriched formula consumption and neurodevelopment

In unadjusted analyses (**Table 2)**, 2-month LC-PUFA–enriched formula consumption was related to higher MB-2 score at 2 years but not other neurodevelopmental scores from age 1 to 3.5 years. Neither the 2-month LC-PUFA enrichment category nor the 2- to 10-month trajectories were related to neurodevelopmental scores from age 1 to 3.5 years. Similar associations were found in the sensitivity analyses based on complete cases (**Supplementary table 1**), except that consumption of DHA/ARA/EPA–enriched formula at 2 months was related to lower CDI scores at 1 year but not other neurodevelopmental scores from 2 to 3.5 years.

In adjusted analyses, the positive association between 2-month LC-PUFA–enriched formula consumption and MB-2 score was no longer significant (**Table 2**), except among infants consuming the same formula from birth or from breastfeeding cessation (**Table 3**). Neither the 2-month LC-PUFA enrichment category nor the 2- to 10-month trajectories were related to neurodevelopmental scores from 1 to 3.5 years (**Table 2**). On complete-case analysis, the negative association between consumption of 2-month DHA/ARA/EPA–enriched formula and CDI-1 score was still significant after adjustment for potential confounders (**Supplementary table 1**), except among infants consuming the same formula from birth or from breastfeeding cessation (**Supplementary table 2**).

Weighted analyses (**Table 2**) showed similar findings.

Discussion

In the nationwide ELFE birth cohort, one third of formula-fed infants consumed an LC-PUFA–enriched formula. Most formulas were enriched in both DHA and ARA, and about 10% of infants consumed a formula further enriched in EPA. In our study, we did not find any association between DHA/ARA–enriched formula consumption in infancy and neurodevelopmental scores up to 3.5 years. We even found a negative association between EPA enrichment and neurodevelopmental score at 1 year, but this association was not significant after excluding infants who received different formula types before age 2 months, which suggests a reverse causation bias.

In line with our results, the latest Cochrane review did not highlight a significant effect of LC-PUFA enrichment of infant formulas on children's neurodevelopment (language and memory) in full-term infants [15], whereas consistent associations between breastfeeding and neurodevelopmental scores were reported [2,31]. However, meta-analyses examining breastfeeding were mainly based on observational single studies instead of RCTs, and all studies were compared together regardless of child age and the tool used to assess neurodevelopmental outcomes; in contrast, the Cochrane review summarized findings of studies performed at similar ages and using similar tools [15].

Some meta-analyses tried to differentiate the potential influence of LC-PUFA supplementation by gestational age and highlighted the benefit of such supplementation among pre-term infants only [9]. In the present study, we did not highlight any moderating effect of gestational age on the association between LC-PUFA enrichment and neurodevelopmental scores. In a recent review on the effects of LC-PUFA enrichment of infant formula on cognitive function among children aged 2.5 years or older, no beneficial effect of such enrichment was found neither among pre-term infants, nor among term-infants [16]. Another issue could be the dose of LC-PUFA enrichment that is rarely taken into

account in meta-analyses. In the RCT showing the strongest beneficial effect of LC-PUFA enrichment, the association between DHA enrichment and neurodevelopmental outcomes did not appear to be linear [32]. In the ELFE study, the DHA content in LC-PUFA–enriched infant formulas was quite low (mean 11 mg/100 kcal) and none reached the lower limit mentioned in the new European regulations (20 mg/100 kcal). The European Academy of Paediatrics suggested that DHA enrichment must be combined with ARA enrichment [12], and this was the case for most enriched formulas in the ELFE study. Finally, we cannot exclude that the association between breastfeeding and neurodevelopment was explained by aspects of breastfeeding other than LC-PUFA composition, such as mother–child interaction.

Even if the evidence of the benefit of LC-PUFA enrichment of infant formula on the child's neurodevelopmental scores remains controversial, the LC-PUFA supplementation during this period seemed to affect biological markers, with higher LC-PUFA levels in erythrocytes among supplemented children, which suggests that enrichment at a high level has potential biological effects [33,34].

The ELFE study found an early but transitory negative association between EPA enrichment and neurodevelopmental scores. Similar transitory and unfavourable effects of LC-PUFA enrichment were highlighted for DHA on the early neurodevelopmental score in some RCTs, but the unfavourable effects did not persist at older ages, as in the present study [32,35]. Despite efforts to account for potential confounders, we cannot exclude that this association is due to differences in other components of the diet not accounted for in the present study or to differences in the home/childcare environment [36-38]. A recent review concluded that the long-term effect of LC-PUFA enrichment of infant formula on cognition is highly uncertain and included potential large benefits but also large harm [16], underlying the need of more robust evidence excluding long-term harm .

Finally, cognitive research into child neurodevelopment provided us with insights into periods of greater neuroplasticity and development. Indeed, rather than fatty acids provided in the first months after birth, as implemented in our study, fatty acid intake would be of crucial importance during the third trimester of pregnancy, when a surge of cerebral synaptogenesis and photoreceptor development occurs [39,40]. This observation may explain why meta-analyses were more conclusive for supplementation among pre-term infants [9].

The ELFE cohort is a nationwide birth cohort study conducted from 2011 onward in metropolitan France. Its prospective design limits memory bias for both exposure and outcome assessment. Data collection also allowed for a detailed assessment of infant diet: from the name and brand of all infant formulas consumed by infants from the ELFE study, we were able to identify specific types of LC-PUFAs (DHA, ARA and EPA) contained in infant formula and the monthly assessment allowed for characterising longitudinal trajectories of such enrichment. About 80% of infants consuming LC-PUFA–enriched formula were exposed to these formulas for up to 6 months, whereas about 20% were exposed to them throughout the first year. However, we were not able to calculate the precise daily intake of each LC-PUFA. Moreover, infants consuming both breast milk and infant formula were classified according to the formula used, leading to a potential underestimation of LC-PUFA intake for these infants. The sensitivity analysis excluding ever-breastfed infant suggested that this potential classification bias had a minor influence on our findings. To assess neurodevelopmental outcomes, parents completed validated instruments to allow for international comparisons. Even though using parental questionnaires may have introduced biases, including social desirability bias and imprecision, our results with parental questionnaires were similar to those obtained with the Picture Similarities test from the BAS-3.5, which was administered by a trained investigator in a face-to-face interview with the child. The very large sample and the collection of detailed socio-demographic or economic

data ensure good statistical power and favour control for potential confounders, even if residual confounding may not be excluded. Despite the number of variables considered in our models, the variance of neurodevelopmental scores explained by our models remains quite low (R-square ranging from 5% for BAS-3.5 to 16% for CDI-1). Finally, the sample considered for the present analysis was based on a higher rate of privileged families than the initial ELFE sample, which could limit the generalization of our results. However, sensitivity analysis based on weighted data, accounting for selection and attrition biases, provided similar findings, which suggests that these biases had limited impact on our results.

Conclusions

In a nationwide birth cohort conducted in France in 2011, one third of formula-fed infants consumed a formula enriched in LC-PUFAs. Most of these infants benefitted from enrichment in both DHA and ARA and a minority also in EPA. We were not able to highlight any positive association between this enrichment and child neurodevelopment up to 3.5 years at the rather low doses of formula enrichment in the French market in 2012-2013. Further studies are warranted to determine the potential effects of LC-PUFA enrichment on other health outcomes.

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References

1. Victora CG, Bahl R, Barros AJ, Franca GV, Horton S, Krasevec J, Murch S, Sankar MJ, Walker N, Rollins NC, Lancet Breastfeeding Series G (2016) Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. Lancet 387 (10017):475-490. doi:10.1016/S0140-6736(15)01024-7
2. Horta BL, de Sousa BA, de Mola CL (2018) Breastfeeding and neurodevelopmental outcomes. Curr Opin Clin Nutr Metab Care 21 (3):174-178. doi:10.1097/MCO.0000000000000453
3. Kramer MS, Aboud F, Mironova E, Vanilovich I, Platt RW, Matush L, Igumnov S, Fombonne E, Bogdanovich N, Ducruet T, Collet JP, Chalmers B, Hodnett E, Davidovsky S, Skugarevsky O, Trofimovich O, Kozlova L, Shapiro S, Promotion of Breastfeeding Intervention Trial Study G (2008) Breastfeeding and child cognitive development: new evidence from a large randomized trial. Arch Gen Psychiatry 65 (5):578-584. doi:10.1001/archpsyc.65.5.578
4. Mortensen EL, Michaelsen KF, Sanders SA, Reinisch JM (2002) The association between duration of breastfeeding and adult intelligence. JAMA 287 (18):2365-2371. doi:10.1001/jama.287.18.2365
5. Belfort MB, Rifas-Shiman SL, Kleinman KP, Guthrie LB, Bellinger DC, Taveras EM, Gillman MW, Oken E (2013) Infant feeding and childhood cognition at ages 3 and 7 years: Effects of breastfeeding duration and exclusivity. JAMA Pediatr 167 (9):836-844. doi:10.1001/jamapediatrics.2013.455
6. Bernard JY, Armand M, Peyre H, Garcia C, Forhan A, De Agostini M, Charles MA, Heude B, Eden Mother-Child Cohort Study Group (2017) Breastfeeding, Polyunsaturated Fatty Acid Levels in Colostrum and Child Intelligence Quotient at Age 5-6 Years. J Pediatr 183:43-50 e43. doi:10.1016/j.jpeds.2016.12.039
7. Tozzi AE, Bisiacchi P, Tarantino V, Chiarotti F, D'Elia L, De Mei B, Romano M, Gesualdo F, Salmaso S (2012) Effect of duration of breastfeeding on neuropsychological development at 10 to 12 years of age in a cohort of healthy children. Dev Med Child Neurol 54 (9):843-848. doi:10.1111/j.1469-8749.2012.04319.x
8. Sanchez-Hernandez S, Esteban-Munoz A, Gimenez-Martinez R, Aguilar-Cordero MJ, Miralles-Buraglia B, Olalla-Herrera M (2019) A Comparison of Changes in the Fatty Acid Profile of Human Milk of Spanish Lactating Women during the First Month of Lactation Using Gas Chromatography-Mass Spectrometry. A Comparison with Infant Formulas. Nutrients 11 (12). doi:10.3390/nu11123055
9. Shulkin M, Pimpin L, Bellinger D, Kranz S, Fawzi W, Duggan C, Mozaffarian D (2018) n-3 Fatty Acid Supplementation in Mothers, Preterm Infants, and Term Infants and Childhood Psychomotor and Visual Development: A Systematic Review and Meta-Analysis. J Nutr 148 (3):409-418. doi:10.1093/jn/nxx031
10. Moon K, Rao SC, Schulzke SM, Patole SK, Simmer K (2016) Longchain polyunsaturated fatty acid supplementation in preterm infants. Cochrane Database Syst Rev 12:CD000375. doi:10.1002/14651858.CD000375.pub5
11. European Food Safety Authority (2014) Scientific Opinion on the essential composition of infant and follow-on formulae. EFSA J 12 (7). doi:10.2903/j.efsa.2014.3760
12. Koletzko B, Bergmann K, Brenna JT, Calder PC, Campoy C, Clandinin MT, Colombo J, Daly M, Decsi T, Demmelmair H, Domellof M, FidlerMis N, Gonzalez-Casanova I, van Goudoever JB, Hadjipanayis A, Hernell O, Lapillonne A, Mader S, Martin CR, Matthaus V, Ramakrishan U, Smuts CM, Strain SJJ, Tanjung C, Tounian P, Carlson SE (2020) 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 111 (1):10-16. doi:10.1093/ajcn/nqz252

13. Crawford MA, Wang Y, Forsyth S, Brenna JT (2015) The European Food Safety Authority recommendation for polyunsaturated fatty acid composition of infant formula overrules breast milk, puts infants at risk, and should be revised. Prostaglandins Leukot Essent Fatty Acids 102-103:1-3. doi:10.1016/j.plefa.2015.07.005
14. Jiao J, Li Q, Chu J, Zeng W, Yang M, Zhu S (2014) Effect of n-3 PUFA supplementation on cognitive function throughout the life span from infancy to old age: a systematic review and meta-analysis of randomized controlled trials. Am J Clin Nutr 100 (6):1422-1436. doi:10.3945/ajcn.114.095315
15. Jasani B, Simmer K, Patole SK, Rao SC (2017) Long chain polyunsaturated fatty acid supplementation in infants born at term. Cochrane Database Syst Rev 3:CD000376. doi:10.1002/14651858.CD000376.pub4
16. Verfuerden ML, Dib S, Jerrim J, Fewtrell M, Gilbert RE (2020) Effect of long-chain polyunsaturated fatty acids in infant formula on long-term cognitive function in childhood: A systematic review and meta-analysis of randomised controlled trials. PLoS One 15 (11):e0241800. doi:10.1371/journal.pone.0241800
17. Charles MA, Thierry X, Lanoe JL, Bois C, Dufourg MN, Popa R, Cheminat M, Zaros C, Geay B (2020) Cohort Profile: The French national cohort of children (ELFE): birth to 5 years. Int J Epidemiol 49 (2):368-369j. doi:10.1093/ije/dyz227
18. de Lauzon-Guillain B, Davisse-Paturet C, Lioret S, Ksiazek E, Bois C, Dufourg MN, Bournez M, Nicklaus S, Wagner S, Charles MA (2018) Use of infant formula in the ELFE study: The association with social and health-related factors. Matern Child Nutr 14 (1). doi:10.1111/mcn.12477
19. Wagner S, Kersuzan C, Gojard S, Tichit C, Nicklaus S, Thierry X, Charles MA, Lioret S, de Lauzon-Guillain B (2019) Breastfeeding initiation and duration in France: The importance of intergenerational and previous maternal breastfeeding experiences - results from the nationwide ELFE study. Midwifery 69:67-75. doi:10.1016/j.midw.2018.10.020
20. Duyme M, Zorman M, Tervo R, Capron C (2011) French norms and validation of the Child Development Inventory (CDI): Inventaire du Developpement de l'Enfant (IDE). Clin Pediatr (Phila) 50 (7):636-647. doi:10.1177/0009922811398390
21. Ireton H, Glascoe FP (1995) Assessing children's development using parents' reports. The Child Development Inventory. Clin Pediatr (Phila) 34 (5):248-255. doi:10.1177/000992289503400504
22. Kern S, Langue J, Zesiger P, Bovet F (2010) Adaptations françaises des versions courtes des inventaires du développement communicatif de MacArthur-Bates. ANAE Approche neuropsychologique des apprentissages chez l'enfant 22 (107-108):217-228
23. Elliott CD, Smith P, McCulloch K (1996) British Ability Scales Second Edition (BAS II): Administration and Scoring Manual. NFER-Nelson, London
24. Kadawathagedara M, Ahluwalia N, Dufourg MN, Forhan A, Charles MA, Lioret S, de Lauzon-Guillain B (2021) Diet during pregnancy: Influence of social characteristics and migration in the ELFE cohort. Matern Child Nutr:e13140. doi:10.1111/mcn.13140
25. Mamelle N, Munoz F, Grandjean H (1996) [Fetal growth from the AUDIPOG study. I. Establishment of reference curves]. J Gynécologie Obstétrique Biol Reprod 25 (1):61-70
26. Juillard H (2015) Weighting of Elfe survey data at time 0. pandora.vjf.inserm.fr/public/
27. Blondel B, Lelong N, Kermarrec M, Goffinet F, National Coordination Group of the National Perinatal Surveys (2012) Trends in perinatal health in France from 1995 to 2010. Results from the French National Perinatal Surveys. J Gynecol Obstet Biol Reprod (Paris) 41 (4):e1-e15. doi:10.1016/j.jgyn.2012.04.014
28. Ferguson KD, McCann M, Katikireddi SV, Thomson H, Green MJ, Smith DJ, Lewsey JD (2020) Evidence synthesis for constructing directed acyclic graphs (ESC-DAGs): a novel

508 and systematic method for building directed acyclic graphs. Int J Epidemiol 49 (1):322-329.
509 doi:10.1093/ije/dyz150
510 29. Shrier I, Platt RW (2008) Reducing bias through directed acyclic graphs. BMC Med
511 Res Methodol 8:70. doi:10.1186/1471-2288-8-70
512 30. Nagin DS, Odgers CL (2010) Group-based trajectory modeling in clinical research.
513 Annu Rev Clin Psychol 6:109-138. doi:10.1146/annurev.clinpsy.121208.131413
514 31. Horta BL, Loret de Mola C, Victora CG (2015) Breastfeeding and intelligence: a
515 systematic review and meta-analysis. Acta Paediatr 104 (467):14-19. doi:10.1111/apa.13139
516 32. Drover JR, Felius J, Hoffman DR, Castaneda YS, Garfield S, Wheaton DH, Birch EE
517 (2012) A randomized trial of DHA intake during infancy: school readiness and receptive
518 vocabulary at 2-3.5 years of age. Early Hum Dev 88 (11):885-891.
519 doi:10.1016/j.earlhumdev.2012.07.007
520 33. Carlson SE, Ford AJ, Werkman SH, Peeples JM, Koo WW (1996) Visual acuity and
521 fatty acid status of term infants fed human milk and formulas with and without
522 docosahexaenoate and arachidonate from egg yolk lecithin. Pediatr Res 39 (5):882-888.
523 doi:10.1203/00006450-199605000-00024
524 34. Voigt RG, Jensen CL, Fraley JK, Rozelle JC, Brown FR, 3rd, Heird WC (2002)
525 Relationship between omega3 long-chain polyunsaturated fatty acid status during early
526 infancy and neurodevelopmental status at 1 year of age. J Hum Nutr Diet 15 (2):111-120.
527 doi:10.1046/j.1365-277x.2002.00341.x
528 35. de Jong C, Kikkert HK, Fidler V, Hadders-Algra M (2012) Effects of long-chain
529 polyunsaturated fatty acid supplementation of infant formula on cognition and behaviour at 9
530 years of age. Dev Med Child Neurol 54 (12):1102-1108. doi:10.1111/j.1469-
531 8749.2012.04444.x
532 36. Garwolinska D, Namiesnik J, Kot-Wasik A, Hewelt-Belka W (2018) Chemistry of
533 Human Breast Milk-A Comprehensive Review of the Composition and Role of Milk
534 Metabolites in Child Development. J Agric Food Chem 66 (45):11881-11896.
535 doi:10.1021/acs.jafc.8b04031
536 37. Innis SM (2011) Dietary triacylglycerol structure and its role in infant nutrition. Adv
537 Nutr 2 (3):275-283. doi:10.3945/an.111.000448
538 38. Lind T, Johansson U, Ohlund I, Lindberg L, Lonnerdal B, Tennefors C, Hernell O
539 (2019) Study protocol: optimized complementary feeding study (OTIS): a randomized
540 controlled trial of the impact of a protein-reduced complementary diet based on Nordic foods.
541 BMC Public Health 19 (1):134. doi:10.1186/s12889-019-6466-1
542 39. Jacobson JL, Jacobson SW, Muckle G, Kaplan-Estrin M, Ayotte P, Dewailly E (2008)
543 Beneficial effects of a polyunsaturated fatty acid on infant development: evidence from the
544 inuit of arctic Quebec. J Pediatr 152 (3):356-364. doi:10.1016/j.jpeds.2007.07.008
545 40. Middleton P, Gomersall JC, Gould JF, Shepherd E, Olsen SF, Makrides M (2018)
546 Omega-3 fatty acid addition during pregnancy. Cochrane Database Syst Rev 11:CD003402.
547 doi:10.1002/14651858.CD003402.pub3
548