



HAL
open science

LC-PUFA enrichment in infant formula and neurodevelopment up to age 3.5 years in the French nationwide ELFE birth cohort

Pauline Martinot, Moufidath Adjibade, Marion Taine, Camille Davaisse-Paturet, Sandrine Lioret, Marie-Aline Charles, Blandine de Lauzon-Guillain, Jonathan Y Bernard

► To cite this version:

Pauline Martinot, Moufidath Adjibade, Marion Taine, Camille Davaisse-Paturet, Sandrine Lioret, et al.. LC-PUFA enrichment in infant formula and neurodevelopment up to age 3.5 years in the French nationwide ELFE birth cohort. *European Journal of Nutrition*, 2022, 61 (6), pp.2979-2991. 10.1007/s00394-022-02863-6 . hal-03694022

HAL Id: hal-03694022

<https://hal.inrae.fr/hal-03694022>

Submitted on 13 Jun 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **LC-PUFA enrichment in infant formula and neurodevelopment up to age**
2 **3.5 years in the French nationwide ELFE birth cohort**

3
4 Pauline Martinot^a, Moufidath Adjibade^a, Marion Taine^a, Camille Davaisse-Paturet^a, Sandrine
5 Lioret^a, Marie-Aline Charles^{a,b}, Blandine de Lauzon-Guillain^{a*}, Jonathan Y. Bernard^{a,c*}

6
7 ^a Université de Paris, Centre for Research in Epidemiology and StatisticS (CRESS), Inserm,
8 INRAE, F-75004 Paris, France

9 ^b Unité mixte Inserm-Ined-EFS ELFE, Ined, F-75020 Paris, France

10 ^c Singapore Institute for Clinical Sciences (SICS), Agency for Science, Technology and
11 Research (A*STAR), Singapore, Singapore

12

13 *Blandine de Lauzon-Guillain and Jonathan Bernard contributed equally to this work.

14

15 **Corresponding author :**

16 Blandine de Lauzon-Guillain,

17 INSERM CRESS —Eq6 EAROH,

18 16 avenue Paul Vaillant Couturier,

19 94807 Villejuif Cedex, France.

20 Tel.: +33-145-595-019.

21 E- mail: blandine.delauzon@inserm.fr

22

23 **Declarations**

24 **Ethical approval**

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

30 **Consent to participate**

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

34 **Funding source**

35 The present study is part of the InfaDiet project, funded by an ANR grant (InfaDiet project,
36 grant no.: ANR-19-CE36-0008).

37 The ELFE study is a joint project between the French Institute for Demographic Studies
38 (INED) and the National Institute of Health and Medical Research (INSERM), in partnership
39 with the French blood transfusion service (Etablissement français du sang [EFS]), Santé
40 publique France, the National Institute for Statistics and Economic Studies (INSEE), the
41 Direction générale de la santé (DGS, part of the Ministry of Health and Social Affairs), the
42 Direction générale de la prévention des risques (DGPR, Ministry for the Environment), the
43 Direction de la recherche, des études, de l'évaluation et des statistiques (DREES, Ministry of
44 Health and Social Affairs), the Département des études, de la prospective et des statistiques
45 (DEPS, Ministry of Culture), and the Caisse nationale des allocations familiales (CNAF),
46 with the support of the Ministry of Higher Education and Research and the Institut national de

47 la jeunesse et de l'éducation populaire (INJEP). Via the RECONAI platform, it receives a
48 government grant managed by the National Research Agency under the "*Investissements*
49 *d'avenir*" programme (ANR-11-EQPX-0038).

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

52 **Conflict of interest/Competing interests**

53 The authors declare no competing financial interest.

54 **Availability of data and material**

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

63 **Abstract**

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

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

79 **Results:** One third of formula-fed infants consumed LC-PUFA-enriched formulas. Most of
80 these formulas were enriched in both DHA and ARA, and about 10% of infants consumed
81 formula further enriched in EPA. LC-PUFA enrichment of infant formula was not associated
82 with neurodevelopmental scores at age 1 (CDI-1, -0.16 [-0.39, 0.07]), age 2 (MB-2, 0.78 [-
83 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]).

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

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

88 **Introduction**

89 Breast milk is considered the most appropriate food for the infant's optimal development, not
90 only because its nutritional composition varies according to the child's needs [1] but also
91 because of its benefits for later health and development [1], including language and cognition
92 [2-4]. More specifically, breastfed children have higher cognitive scores than never-breastfed
93 children: numerous studies found better language and motor development among breastfed
94 than non-breastfed children, with a dose-effect relationship for both any and exclusive
95 breastfeeding duration [2-7]. If causal, these consistent associations between breastfeeding
96 and neurodevelopment are thought to be explained in part by the long-chain polyunsaturated
97 fatty acids (LC-PUFAs) contained in breast milk [8,9]. This hypothesis is supported by
98 studies of premature newborn babies, in which two major LC-PUFAs, arachidonic acid
99 (ARA) and docosahexaenoic acid (DHA), were discovered as crucial for retinal and neuronal
100 cell development [9,10].

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

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

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

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

122

123 **Materials and Methods**

124 *Study design and population*

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

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

138 ***Infant feeding methods***

139 Data on infant feeding were collected during face-to-face interviews during the maternity
140 stay, by phone interview at the 2-month and 1-year interviews, and by Internet/paper
141 questionnaire each month from 3 to 10 months after delivery. At every time point up to 10
142 months, parents reported their infant's feeding method (breast or formula milk) as well as the
143 name and brand of the infant formula used when relevant [18]. From these monthly data, LC-
144 PUFA-enriched infant formulas were identified, and infants were classified as receiving
145 breast milk only, regular infant formula, or LC-PUFA-enriched infant formula. Infants
146 consuming both breast milk and infant formula at a given month were classified according to
147 the formula used (regular or LC-PUFA-enriched). Any breastfeeding duration was also
148 calculated as previously described [19] and classified as never, <1 month, 1 to 3 months, 3 to
149 6 months, ≥ 6 months.

150 ***Children's neurodevelopment***

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

153 At the 1- and 3.5-year follow-ups, an adapted version of the Child Development Inventory
154 (CDI-1 and CDI-3.5) was used to assess the child's overall development by the parental report
155 [20,21]. The CDI-1 includes 6 domains of development (social, self-help, gross motor skills,
156 fine motor skills, expressive language, language comprehension), and the CDI-3.5 includes
157 two additional domains (characters and numbers). For each item, the child earns 1 point when
158 the ability is acquired and 0 when it is not. Scores for all items were summed for an overall
159 CDI score ranging from 0 to 50 at 1 year and from 17 to 62 at 3.5 years.

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

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

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

169 *Family and children characteristics*

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

176 Parental demographic and socioeconomic characteristics studied were maternal age at
177 delivery (18–24, 25–29, 30–34, ≥ 35 years); number of older children in the household (no
178 sibling, one sibling, at least two siblings); maternal migration status (“majority population”,
179 which included women born with a French nationality from two French parents [within or
180 outside of France]; “descendants of migrants” including women born in France with at least
181 one non-French parent; and “migrants” including women not born in France and without
182 French citizenship at birth); maternal education level (up to lower secondary, upper
183 secondary, high school graduate, 3-year university degree, at least 5-year university degree);
184 employment status during pregnancy (employed, unemployed, not in the labour force; e.g.
185 housewife, student, disabled, retired); and monthly household income per consumption unit
186 (<€750, €751–1,111, €1,112–1,500, €1,501–1,944, €1,945–2,500, \geq €2,500). From the postal

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

190 Maternal health characteristics included self-reported height and pre-pregnancy weight used
191 to calculate pre-pregnancy body mass index (BMI) (<18.5, 18.5–24.9, 25.0–29.9,
192 ≥ 30.0 kg/m²) and maternal smoking status (never smoker, smoker only before pregnancy,
193 smoker only in early pregnancy, smoker throughout pregnancy).

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

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

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

208 *Sample selection*

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

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

216 *Statistical analysis*

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

219 To provide representative descriptive statistics of births in 2011 in Metropolitan France in
220 terms of the consumption of LC-PUFA–enriched formulas from age 2 to 10 months, the data
221 were weighted to take into account the inclusion procedure and biases related to non-consent
222 [26]. Weighting also included calibration on margins from the state register's statistical data
223 and the 2010 French National Perinatal study on the following variables: age, region, marital
224 status, migration status, level of education, and primiparity [27]. For descriptive statistics, this
225 weighting was calculated for the subsample that completed the 2-month interview and for the
226 subsample that completed the questionnaire on infant diet, at least once from 3 to 10 months.
227 For the main analyses, the weighting was calculated for the subsample included in the
228 multiple imputation analysis (as described below) and for the complete-case subsample.

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

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

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

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

261 *Sensitivity analyses*

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

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

273

274 **Results**

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

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

280 *LC-PUFA-enriched formula's consumption*

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

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

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

290 Three distinct longitudinal trajectories of LC-PUFA-enriched formula consumption were
291 identified (**Figure 3**: non-consumers [65%], regular consumers until age 6 months only
292 [28%], regular consumers throughout the first year [7%]).

293 *LC-PUFA-enriched formula consumption and neurodevelopment*

294 In unadjusted analyses (**Table 2**), 2-month LC-PUFA-enriched formula consumption was
295 related to higher MB-2 score at 2 years but not other neurodevelopmental scores from age 1 to
296 3.5 years. Neither the 2-month LC-PUFA enrichment category nor the 2- to 10-month
297 trajectories were related to neurodevelopmental scores from age 1 to 3.5 years. Similar
298 associations were found in the sensitivity analyses based on complete cases (**Supplementary**
299 **table 1**), except that consumption of DHA/ARA/EPA-enriched formula at 2 months was
300 related to lower CDI scores at 1 year but not other neurodevelopmental scores from 2 to 3.5
301 years.

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

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

312 **Discussion**

313 In the nationwide ELFE birth cohort, one third of formula-fed infants consumed an LC-
314 PUFA-enriched formula. Most formulas were enriched in both DHA and ARA, and about
315 10% of infants consumed a formula further enriched in EPA. In our study, we did not find any
316 association between DHA/ARA-enriched formula consumption in infancy and
317 neurodevelopmental scores up to 3.5 years. We even found a negative association between
318 EPA enrichment and neurodevelopmental score at 1 year, but this association was not
319 significant after excluding infants who received different formula types before age 2 months,
320 which suggests a reverse causation bias.

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

329 Some meta-analyses tried to differentiate the potential influence of LC-PUFA
330 supplementation by gestational age and highlighted the benefit of such supplementation
331 among pre-term infants only [9]. In the present study, we did not highlight any moderating
332 effect of gestational age on the association between LC-PUFA enrichment and
333 neurodevelopmental scores. In a recent review on the effects of LC-PUFA enrichment of
334 infant formula on cognitive function among children aged 2.5 years or older, no beneficial
335 effect of such enrichment was found neither among pre-term infants, nor among term-infants
336 [16]. Another issue could be the dose of LC-PUFA enrichment that is rarely taken into

337 account in meta-analyses. In the RCT showing the strongest beneficial effect of LC-PUFA
338 enrichment, the association between DHA enrichment and neurodevelopmental outcomes did
339 not appear to be linear [32]. In the ELFE study, the DHA content in LC-PUFA–enriched
340 infant formulas was quite low (mean 11 mg/100 kcal) and none reached the lower limit
341 mentioned in the new European regulations (20 mg/100 kcal). The European Academy of
342 Paediatrics suggested that DHA enrichment must be combined with ARA enrichment [12],
343 and this was the case for most enriched formulas in the ELFE study. Finally, we cannot
344 exclude that the association between breastfeeding and neurodevelopment was explained by
345 aspects of breastfeeding other than LC-PUFA composition, such as mother–child interaction.
346 Even if the evidence of the benefit of LC-PUFA enrichment of infant formula on the child’s
347 neurodevelopmental scores remains controversial, the LC-PUFA supplementation during this
348 period seemed to affect biological markers, with higher LC-PUFA levels in erythrocytes
349 among supplemented children, which suggests that enrichment at a high level has potential
350 biological effects [33,34].

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

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

367 The ELFE cohort is a nationwide birth cohort study conducted from 2011 onward in
368 metropolitan France. Its prospective design limits memory bias for both exposure and
369 outcome assessment. Data collection also allowed for a detailed assessment of infant diet:
370 from the name and brand of all infant formulas consumed by infants from the ELFE study, we
371 were able to identify specific types of LC-PUFAs (DHA, ARA and EPA) contained in infant
372 formula and the monthly assessment allowed for characterising longitudinal trajectories of
373 such enrichment. About 80% of infants consuming LC-PUFA-enriched formula were
374 exposed to these formulas for up to 6 months, whereas about 20% were exposed to them
375 throughout the first year. However, we were not able to calculate the precise daily intake of
376 each LC-PUFA. Moreover, infants consuming both breast milk and infant formula were
377 classified according to the formula used, leading to a potential underestimation of LC-PUFA
378 intake for these infants. The sensitivity analysis excluding ever-breastfed infant suggested that
379 this potential classification bias had a minor influence on our findings. To assess
380 neurodevelopmental outcomes, parents completed validated instruments to allow for
381 international comparisons. Even though using parental questionnaires may have introduced
382 biases, including social desirability bias and imprecision, our results with parental
383 questionnaires were similar to those obtained with the Picture Similarities test from the BAS-
384 3.5, which was administered by a trained investigator in a face-to-face interview with the
385 child. The very large sample and the collection of detailed socio-demographic or economic

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

394 **Conclusions**

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

402 **Acknowledgments**

403 We thank the scientific coordinators (B Geay, H L  ridon, C Bois, MN Dufourg, JL Lano  , X
404 Thierry, C Zaros), IT and data managers, statisticians (T Simeon, A Candea, S de Visme),
405 administrative and family communication staff, and study technicians (C Guevel, M Zoubiri,
406 L Gravier, I, Milan, R Popa) of the ELFE coordination team as well as the families that gave
407 their time for the study.

408 **References**

- 409 1. Victora CG, Bahl R, Barros AJ, Franca GV, Horton S, Krasevec J, Murch S, Sankar
410 MJ, Walker N, Rollins NC, Lancet Breastfeeding Series G (2016) Breastfeeding in the 21st
411 century: epidemiology, mechanisms, and lifelong effect. *Lancet* 387 (10017):475-490.
412 doi:10.1016/S0140-6736(15)01024-7
- 413 2. Horta BL, de Sousa BA, de Mola CL (2018) Breastfeeding and neurodevelopmental
414 outcomes. *Curr Opin Clin Nutr Metab Care* 21 (3):174-178.
415 doi:10.1097/MCO.0000000000000453
- 416 3. Kramer MS, Aboud F, Mironova E, Vanilovich I, Platt RW, Matush L, Igumnov S,
417 Fombonne E, Bogdanovich N, Ducruet T, Collet JP, Chalmers B, Hodnett E, Davidovsky S,
418 Skugarevsky O, Trofimovich O, Kozlova L, Shapiro S, Promotion of Breastfeeding
419 Intervention Trial Study G (2008) Breastfeeding and child cognitive development: new
420 evidence from a large randomized trial. *Arch Gen Psychiatry* 65 (5):578-584.
421 doi:10.1001/archpsyc.65.5.578
- 422 4. Mortensen EL, Michaelsen KF, Sanders SA, Reinisch JM (2002) The association
423 between duration of breastfeeding and adult intelligence. *JAMA* 287 (18):2365-2371.
424 doi:10.1001/jama.287.18.2365
- 425 5. Belfort MB, Rifas-Shiman SL, Kleinman KP, Guthrie LB, Bellinger DC, Taveras EM,
426 Gillman MW, Oken E (2013) Infant feeding and childhood cognition at ages 3 and 7 years:
427 Effects of breastfeeding duration and exclusivity. *JAMA Pediatr* 167 (9):836-844.
428 doi:10.1001/jamapediatrics.2013.455
- 429 6. Bernard JY, Armand M, Peyre H, Garcia C, Forhan A, De Agostini M, Charles MA,
430 Heude B, Eden Mother-Child Cohort Study Group (2017) Breastfeeding, Polyunsaturated
431 Fatty Acid Levels in Colostrum and Child Intelligence Quotient at Age 5-6 Years. *J Pediatr*
432 183:43-50 e43. doi:10.1016/j.jpeds.2016.12.039
- 433 7. Tozzi AE, Bisiacchi P, Tarantino V, Chiarotti F, D'Elia L, De Mei B, Romano M,
434 Gesualdo F, Salmaso S (2012) Effect of duration of breastfeeding on neuropsychological
435 development at 10 to 12 years of age in a cohort of healthy children. *Dev Med Child Neurol*
436 54 (9):843-848. doi:10.1111/j.1469-8749.2012.04319.x
- 437 8. Sanchez-Hernandez S, Esteban-Munoz A, Gimenez-Martinez R, Aguilar-Cordero MJ,
438 Miralles-Buraglia B, Olalla-Herrera M (2019) A Comparison of Changes in the Fatty Acid
439 Profile of Human Milk of Spanish Lactating Women during the First Month of Lactation
440 Using Gas Chromatography-Mass Spectrometry. A Comparison with Infant Formulas.
441 *Nutrients* 11 (12). doi:10.3390/nu11123055
- 442 9. Shulkin M, Pimpin L, Bellinger D, Kranz S, Fawzi W, Duggan C, Mozaffarian D
443 (2018) n-3 Fatty Acid Supplementation in Mothers, Preterm Infants, and Term Infants and
444 Childhood Psychomotor and Visual Development: A Systematic Review and Meta-Analysis.
445 *J Nutr* 148 (3):409-418. doi:10.1093/jn/nxx031
- 446 10. Moon K, Rao SC, Schulzke SM, Patole SK, Simmer K (2016) Longchain
447 polyunsaturated fatty acid supplementation in preterm infants. *Cochrane Database Syst Rev*
448 12:CD000375. doi:10.1002/14651858.CD000375.pub5
- 449 11. European Food Safety Authority (2014) Scientific Opinion on the essential
450 composition of infant and follow-on formulae. *EFSA J* 12 (7). doi:10.2903/j.efsa.2014.3760
- 451 12. Koletzko B, Bergmann K, Brenna JT, Calder PC, Campoy C, Clandinin MT, Colombo
452 J, Daly M, Decsi T, Demmelmair H, Domellof M, FidlerMis N, Gonzalez-Casanova I, van
453 Goudoever JB, Hadjipanayis A, Hernell O, Lapillonne A, Mader S, Martin CR, Matthaus V,
454 Ramakrishan U, Smuts CM, Strain SJJ, Tanjung C, Tounian P, Carlson SE (2020) Should
455 formula for infants provide arachidonic acid along with DHA? A position paper of the
456 European Academy of Paediatrics and the Child Health Foundation. *Am J Clin Nutr* 111
457 (1):10-16. doi:10.1093/ajcn/nqz252

- 458 13. Crawford MA, Wang Y, Forsyth S, Brenna JT (2015) The European Food Safety
459 Authority recommendation for polyunsaturated fatty acid composition of infant formula
460 overrules breast milk, puts infants at risk, and should be revised. *Prostaglandins Leukot
461 Essent Fatty Acids* 102-103:1-3. doi:10.1016/j.plefa.2015.07.005
- 462 14. Jiao J, Li Q, Chu J, Zeng W, Yang M, Zhu S (2014) Effect of n-3 PUFA
463 supplementation on cognitive function throughout the life span from infancy to old age: a
464 systematic review and meta-analysis of randomized controlled trials. *Am J Clin Nutr* 100
465 (6):1422-1436. doi:10.3945/ajcn.114.095315
- 466 15. Jasani B, Simmer K, Patole SK, Rao SC (2017) Long chain polyunsaturated fatty acid
467 supplementation in infants born at term. *Cochrane Database Syst Rev* 3:CD000376.
468 doi:10.1002/14651858.CD000376.pub4
- 469 16. Verfuenden ML, Dib S, Jerrim J, Fewtrell M, Gilbert RE (2020) Effect of long-chain
470 polyunsaturated fatty acids in infant formula on long-term cognitive function in childhood: A
471 systematic review and meta-analysis of randomised controlled trials. *PLoS One* 15
472 (11):e0241800. doi:10.1371/journal.pone.0241800
- 473 17. Charles MA, Thierry X, Lanoe JL, Bois C, Dufourg MN, Popa R, Cheminat M, Zaros
474 C, Geay B (2020) Cohort Profile: The French national cohort of children (ELFE): birth to 5
475 years. *Int J Epidemiol* 49 (2):368-369j. doi:10.1093/ije/dyz227
- 476 18. de Lauzon-Guillain B, Davisse-Paturet C, Lioret S, Ksiazek E, Bois C, Dufourg MN,
477 Bournez M, Nicklaus S, Wagner S, Charles MA (2018) Use of infant formula in the ELFE
478 study: The association with social and health-related factors. *Matern Child Nutr* 14 (1).
479 doi:10.1111/mcn.12477
- 480 19. Wagner S, Kersuzan C, Gojard S, Tichit C, Nicklaus S, Thierry X, Charles MA, Lioret
481 S, de Lauzon-Guillain B (2019) Breastfeeding initiation and duration in France: The
482 importance of intergenerational and previous maternal breastfeeding experiences - results
483 from the nationwide ELFE study. *Midwifery* 69:67-75. doi:10.1016/j.midw.2018.10.020
- 484 20. Duyme M, Zorman M, Tervo R, Capron C (2011) French norms and validation of the
485 Child Development Inventory (CDI): Inventaire du Développement de l'Enfant (IDE). *Clin
486 Pediatr (Phila)* 50 (7):636-647. doi:10.1177/0009922811398390
- 487 21. Ireton H, Glascoe FP (1995) Assessing children's development using parents' reports.
488 The Child Development Inventory. *Clin Pediatr (Phila)* 34 (5):248-255.
489 doi:10.1177/000992289503400504
- 490 22. Kern S, Langue J, Zesiger P, Bovet F (2010) Adaptations françaises des versions
491 courtes des inventaires du développement communicatif de MacArthur-Bates. *ANAE
492 Approche neuropsychologique des apprentissages chez l'enfant* 22 (107-108):217-228
- 493 23. Elliott CD, Smith P, McCulloch K (1996) British Ability Scales Second Edition (BAS
494 II): Administration and Scoring Manual. NFER-Nelson, London
- 495 24. Kadawathagedara M, Ahluwalia N, Dufourg MN, Forhan A, Charles MA, Lioret S, de
496 Lauzon-Guillain B (2021) Diet during pregnancy: Influence of social characteristics and
497 migration in the ELFE cohort. *Matern Child Nutr*:e13140. doi:10.1111/mcn.13140
- 498 25. Mamelle N, Munoz F, Grandjean H (1996) [Fetal growth from the AUDIPOG study. I.
499 Establishment of reference curves]. *J Gynécologie Obstétrique Biol Reprod* 25 (1):61-70
- 500 26. Juillard H (2015) Weighting of Elfe survey data at time 0.
501 pandora.vjf.inserm.fr/public/
- 502 27. Blondel B, Lelong N, Kermarrec M, Goffinet F, National Coordination Group of the
503 National Perinatal Surveys (2012) Trends in perinatal health in France from 1995 to 2010.
504 Results from the French National Perinatal Surveys. *J Gynecol Obstet Biol Reprod (Paris)* 41
505 (4):e1-e15. doi:10.1016/j.jgyn.2012.04.014
- 506 28. Ferguson KD, McCann M, Katikireddi SV, Thomson H, Green MJ, Smith DJ, Lewsey
507 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, Peoples 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