OCL 2016, 23(1) D106

DOI: 10.1051/ocl/2015060

OCL Oilseeds & fats Crops and Lipids

Available online at:
www.ocl-journal.org

RESEARCH ARTICLE – DOSSIER OPEN ACCESS

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# Early life exposure to polyunsaturated fatty acids and psychomotor development in children from the EDEN mother-child cohort

Jonathan Y. Bernard[1,*], Martine Armand[2], Anne Forhan[1], Maria De Agostini[1], Marie-Aline Charles[1] and Barbara Heude[1], on behalf of the EDEN mother-child cohort study group[**]

[1] Inserm, UMR1153 Epidemiology and Biostatistics Sorbonne Paris Cité Centre (CRESS), Developmental Origins of Health and Disease (ORCHAD) Team, 94807 Villejuif, France; Descartes University, France
[2] Aix-Marseille Université, CNRS, CRMBM UMR 7339, 13385 Marseille, France

Received 9 October 2015 – Accepted 14 October 2015

**Abstract –** Epidemiological studies have reported that breastfed children have improved psychomotor development compared to never breastfed children. Human studies suggest that polyunsaturated fatty acids (PUFA), especially long chain PUFA (LC-PUFA) which are highly contained in breast milk, could explain this link, since they are needed for pre- and postnatal brain development. Our aim was to study the relationships between several measures of pre- and postnatal exposures to PUFA and child's psychomotor development at 2 and 3 years in the EDEN cohort. We evaluated breastfeeding duration, colostrum PUFA levels and maternal dietary PUFA intake during pregnancy, that we related with three scores of psychomotor development, after taking into account potential confounders. Breastfeeding duration was positively associated with psychomotor development. No relationship was found with both pre- and postnatal exposure to LC-PUFA. However, the maternal dietary omega-6/omega-3 ratio was negatively associated with psychomotor development, mainly driven by intake in linoleic acid (LA). Among breastfed children, linoleic acid levels were negatively associated with psychomotor development. Furthermore, children exposed to the highest colostrum LA levels tended to score closer to never breastfed children than to children exposed to the lowest colostrums LA levels. Taken together, these results do not provide evidence in favour of a positive role of pre- and postnatal exposure to LC-PUFA on later psychomotor development, but highlight a potential negative role of being exposed in early life to high LA levels. From a public health perspective, this work reiterates the need to promote breastfeeding duration, and to monitor the balance of PUFA intake during pregnancy and lactation periods.

**Keywords:** Cohort studies / maternal nutrition / breast milk / polyunsaturated fatty acids / psychomotor development

**Résumé – Exposition précoce aux acides gras polyinsaturés et développement psychomoteur des enfants de la cohorte mère-enfant EDEN.** Nombre d'études épidémiologiques ont montré que les enfants allaités au sein bénéficiaient d'un meilleur développement psychomoteur que les enfants non allaités. Des études suggèrent une implication des acides gras polyinsaturés (AGPI) oméga-6 et oméga-3, notamment ceux à longue chaine (AGPI-LC) que l'on trouve en quantité dans le lait maternel, et qui sont nécessaires au développement du cerveau du fœtus et du bébé. Notre objectif était d'étudier les relations entre plusieurs mesures d'expositions pré- et postnatales aux AGPI, et le développement psychomoteur à deux et trois ans des enfants de la cohorte EDEN. Ainsi nous avons évalué la durée d'allaitement maternel, la teneur du colostrum en AGPI, et les apports nutritionnels maternels pendant la grossesse, qui ont été ensuite mis en relation, après prise en compte de facteurs de confusion potentiels, à trois scores de développement psychomoteur évalué par questionnaire. La durée d'allaitement maternel était positivement associée aux trois scores de développement

* Correspondence: jonathan.bernard@inserm.fr
** The EDEN mother-child cohort study group: Isabella Annesi-Maesano, Jonathan Y. Bernard, Jeremie Botton, Marie-Aline Charles, Patricia Dargent-Molina, Blandine de Lauzon-Guillain, Pierre Ducimetiere, Maria De Agostini, Bernard Foliguet, Anne Forhan, Xavier Fritel, Alice Germa, Valerie Goua, Regis Hankard, Barbara Heude, Monique Kaminski, Béatrice Larroque, Nathalie Lelong, Johanna Lepeule, Guillaume Magnin, Laetitia Marchand, Cathy Nabet, FabricePierre, Remy Slama, Marie-Josephe Saurel-Cubizolles, Michel Schweitzer, Olivier Thiebaugeorges.

psychomoteur. Ni les apports nutritionnels en AGPI-LC pendant la grossesse, ni les teneurs en AGPI-LC du colostrum n'étaient associés au développement psychomoteur. En revanche, le rapport oméga-6/oméga-3 dans l'alimentation maternelle était négativement associé au développement psychomoteur, notamment chez les enfants non allaités ; cette relation s'expliquait essentiellement par des apports nutritionnels élevés en acide linoléique (LA). Chez les enfants allaités, la teneur du colostrum en LA était négativement associée aud éveloppement psychomoteur. De plus, les enfants exposés via l'allaitement à des quantités élevés de LA avaient des scores de développement psychomoteur plus proches de ceux des enfants non allaités au sein que de ceux des enfants exposés à du colostrum contenant de plus bas taux de LA. Mis en commun, ces résultats ne suggèrent pas un rôle positive des AGPI-LC sur le développement psychomoteur des enfants, mais met en évidence un rôle négatif potentiel d'une exposition précoce à des taux élevés de LA. Dans une perspective de santé publique, ces travaux soutiennent la nécessité de promouvoir l'allaitement maternel, et de surveiller l'équilibre des apports nutritionnels en AGPI des femmes enceintes et allaitantes.

**Mots clés :** Étude de cohortes / nutrition maternelle / lait maternel / acides gras polyinsaturés / développement psychomoteur

## 1 Introduction

The two series of polyunsaturated fatty acids (PUFA), the omega-3 (n-3) and omega-6 (n-6) PUFA, are essential fatty acids which must be provided by the diet and play important biological roles in metabolism, membrane structure and cell signalling, especially in nervous system (German, 2011). Animal studies have greatly participated to improve our understanding of PUFA-related mechanisms (Innis, 2000). It is now clear that dietary deficiency in n-3 PUFA may affect neural system functions in rodents (Bourre *et al.*, 1989), but also behaviour and cognition in non-human primates (Hsieh and Brenna, 2009, Pifferi, 2014). Nevertheless, metabolic pathways and nutrient requirements differ across species, limiting to fully extend inputs from animal studies to humans (Innis, 2000).

Studies in humans have shown that PUFA, and particularly the long-chain PUFA (LC-PUFA) arachidonic acid (AA, n-6 LCPUFA) and docosahexaenoic acid (DHA, n-3 LCPUFA), rapidly accumulates into foetus brain during the last trimester of pregnancy (Clandinin *et al.*, 1980a), a phenomenon continuing over the first two years after birth (Clandinin *et al.*, 1980b). A scientific consensus has since emerged on the requirements of LC-PUFA during pregnancy, lactation and infancy, now clearly identified as a critical period for brain development (Koletzko *et al.*, 2008), especially in preterm infants (Lapillonne *et al.*, 2013). Despite evidences on the link between LC-PUFA and human brain development, most randomized controlled trials have failed to prove the benefit of LC-PUFA on child's psychomotor development. Indeed, recent meta-analyses and systematic reviews have concluded to no clear benefit on child's psychomotor development of supplementing both term and preterm infants with formulas enriched in LC-PUFA (Schulzke *et al.*, 2011; Simmer *et al.*, 2011). Trials supplementing pregnant or lactating women with fish-oil rich in LC-PUFA have not resulted in more evidence regarding similar outcomes in children (Delgado-Noguera *et al.*, 2010; Gould *et al.*, 2013).

Nevertheless, many observational studies have found a positive association between breastfeeding and psychomotor development (Anderson *et al.*, 1999; Brion *et al.*, 2011), few studies highlighting a dose-effect relationship when considering duration and intensity of breastfeeding (Belfort *et al.*, 2013; Bernard *et al.*, 2013a). Whether this link is causal remains controversial because of sociodemographic differences between breastfeeding and non-breastfeeding mothers (Der *et al.*, 2006). Yet, such findings may also be attributed to LC-PUFA contents in milk, which are much higher in humans than among other mammals species (Zou *et al.*, 2013), despite variability across human populations (Brenna *et al.*, 2007). To date, few large observational studies have investigated associations of breast milk PUFA contents with child's psychomotor development: Guxens *et al.* (2011)showed a negative association between the total n-6/n-3 ratio in colostrum and mental development, but only in infants who were breastfed the longest time.

Observational studies having focused on prenatal period, have assessed foetal exposure to PUFA either with PUFA levels measured in cord blood, either by using maternal fish and seafood consumption during pregnancy as a proxy. One study using cord blood data found a positive association between DHA levels and motor development (Bakker *et al.*, 2009), while two other studies found no relationship with cognitive development (Bakker *et al.*, 2003; Ghys *et al.*, 2002). Regarding maternal fish consumption, findings from existing studies are more consistent by showing positive associations with child's psychomotor development (Daniels *et al.*, 2004; Hibbeln *et al.*, 2007; Oken *et al.*, 2008). However, this approach by food group has the limitation not to take other dietary sources of n-3 and n-6 PUFA into account. Indeed, there are growing evidence that not only n-3 LCPUFA matters, but also the dietary balance between n-6 and n-3 PUFA, because of the endogenous metabolic competition between these two series (Lands, 2015; Simopoulos, 2011a). Furthermore, this balance has dramatically changed worldwide over the last century, and this could have multiple implications for health in general, and for the developing brain in particular (Simopoulos, 2011b). There is thus a need for studying in a more comprehensive way, the roles of n-6 and n-3 PUFA and LCPUFA in early human life.

In this context, we hypothesized that breastfeeding duration is positively associated to child's psychomotor development, that higher AA and DHA levels in colostrum may explain this postnatal association, and finally that higher prenatal exposure to AA and DHA through maternal dietary intake during pregnancy may also be beneficial for child's psychomotor development. Thus, we investigated the associations of pre- and postnatal exposures to PUFA, with child's psychomotor

development, by using prospective data from a large mother-child cohort. This article presents a synthesis of the three main results of this study, previously published in peer-review journals (Bernard *et al.*, 2013a, 2013b, 2015), more extensively detailed in a Ph.D. thesis (Bernard, 2013), and finally presented in March 2015 during the Lipids and Brain III Conference held in Paris, France (Heude and Bernard, 2015).

## 2 Methods

### 2.1 Study design

The EDEN study is a French mother-offspring cohort aiming at investigating the roles of pre- and postnatal determinants of child developmentand health. Recruitment of pregnant women started in 2003 in the university hospitals of Poitiers and Nancy and ended in 2006. All women presenting to their first antenatal visit before 24 weeks of amenorrhea were invited to participate in the cohort. Exclusion criteria were multiple pregnancies, known diabetes prior to pregnancy, illiteracy, and intention to move outside the region in the next 3 years. A total of 2002 women were enrolled. More details on the study protocol are available (Heude *et al.*, 2015). Informed written consents concerning the parents were obtained at enrolment, and that for the child was acquired after birth. The study was approved by the ethics research committee (Comite Consultatif de protection des personnes dans la recherche biomedicale) of the Bicetre Hospital, and by the Data Protection Authority (Commission Nationale de l'Informatique et des Libertés).

### 2.2 Assessment of exposure to PUFA

#### 2.2.1 Dietary intake during pregnancy

Maternal dietary intake during the last trimester of pregnancy was evaluated within few days after delivery using a food frequency questionnaire (FFQ) combined to a portion-size picture booklet. The FFQ was adapted from the one used in the Fleurbaix-Laventie Ville Santé study, by adding items concerning seafood consumption and other foods rich in vitamin A and B9, and in n-3 PUFA (Lauzon *et al.*, 2004; Deschamps *et al.*, 2009). Using a food composition database (SU.VI.MAX, 2006), we estimated maternal dietary intakes in LA, AA, $\alpha$-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and DHA, and we calculated total dietary intakes in n-6 and n-3 PUFA. We derived dietary ratios between n-6 and n-3 series: total n-6/n-3, LA/ALA and AA/DHA. More details on this method have been published elsewhere (Bernard *et al.*, 2013b; Drouillet *et al.*, 2009).

#### 2.2.2 Breastfeeding

Infant's feeding modes from birth to maternity discharge were obtained from medical records. In the questionnaire mailed at 4 months, parents reported infant's consumption of breast milk, infant formulas, cow's milk, water and other fluids, and solids over periods defined as following: first week, second to fourth weeks, second month, third month and fourth month. In questionnaire mailed at 8 months, 12 months and 24 months, mothers answered the question: "Do you still breastfeed your infant?" and use of infant formulas was also recorded. Mothers who had stopped breastfeeding reported the date of full weaning. Duration of 'any breastfeeding' (including partial and exclusive breastfeeding, in months) was derived from the date of birth and the date of full weaning with an accuracy to the day. Duration of 'exclusive breastfeeding'(in months) was estimated from answers about infant's feeding mode. We defined 'exclusive breastfeeding' as not receiving formulas, as few infants (5%) received other liquids or food in addition to breast milk. Infants ($n$ = 166) who received formulas for medical reasons during their stay at maternity unit, and who were exclusively breastfed after discharge were considered as exclusively breastfed. Infant's 'ever breastfed' were defined as having been initiated to breast milk at some time or other. It is important to note that infant formulas sold in France at that time were rarely supplemented in LC-PUFA. More details on breastfeeding practices in the EDEN study have been published (Bonet *et al.*, 2013).

#### 2.2.3 Composition of colostrum in fatty acids

About 5 ml of colostrum were collected in mothers who breastfed their child during the first week after delivery. Analysis of fatty acid composition was performed by direct transmethylation and fast gas chromatography. More details are available elsewhere (Bernard *et al.*, 2015). Fatty acids were expressed as proportion of total milk fat (wt% of total fatty acids).Twelve well represented PUFA were identified: linoleic acid (LA, 18:2 n-6), $\gamma$-linolenic acid (18:3 n-6), dihomo-$\gamma$-linolenic acid (20:3 n-6), arachidonic acid (AA, 20:4 n-6), adrenic acid (22:4 n-6), n-6 docosapentaenoic or osbond acid (22:5 n-6), $\alpha$-linolenic acid (ALA, 18:3 n-3), stearidonic acid (18:4 n-3), eicosatetraenoic acid (20:4 n-3), eicosapentaenoic acid (EPA, 20:5 n-3), n-3 docosapentaenoic or clupanodonic acid (22:5 n-3), and docosahexaenoic acid (DHA, 22:6 n-3). We calculated the total levels in n-6 PUFA and in n-3 PUFA and derived ratios between PUFA: total n-6/n-3 ratio, LA/ALA and AA/DHA.

### 2.3 Assessment of child cognitive development

In parental questionnaire mailed at two years of age, child's motor development was assessed using 22 motor-related items from the French Psychomotor Developmental Scale for Early Childhood of Brunet-Lézine (Josse, 1997). Items were summed to obtain a score of motor development (Motor-2) comprised between 0 and 22. Still in two-year parental questionnaire, child's language ability was evaluated using the short form of the MacArthur Communicative Development Inventory (CDI-2) (Fenson *et al.*, 1993), adapted in French by Kern (2003). Parents reported from a list of 100 words, those that the child was able to spontaneously pronounce. CDI-2 score ranged between 0 and 100. At three years,

child's cognition was investigated with the second French edition of the Ages and Stages Questionnaire (ASQ-3) (Squires *et al.*, 1999). ASQ-3is a parent-reported assessment which includes five domains of development (communication, gross motor, fine motor, problem solving and personal-social). For each of the 30 questions, a child scored 10 points when parents reported that the child's ability was acquired, 5 points when the ability was occasionally observed, and 0 point otherwise. ASQ-3 score ranged between 0 and 300 points.

### 2.4 Population selection

A total of 1907 children was born and included in the cohort. We excluded from the subsequent analysis the infants born before 33 weeks of gestation ($n$ = 12), since early preterm infants are more at-risk of later developmental delay than infants born at term or late preterm. Among the mother-child pairs having either dietary fatty acids data, either colostrum fatty acid data, 1367 children had at least one available psychomotor assessment at 2 or 3 years of age (1260 for Motor-2, 1277 for CDI-2 and 1157 for ASQ-3).

### 2.5 Statistical analyses

Main characteristics of the population, average dietary PUFA intake and colostrum PUFA levels, and psychomotor development scores were described by means ± standard deviations and percentages. Multivariable linear regression was performed to evaluate whether child's psychomotor scores were associated with three proxies of early PUFA exposures:

1. Proxy of duration of postnatal exposure: breastfeeding status, among all children, and breastfeeding durations, among breastfed children.
2. Proxy of prenatal exposure: maternal dietary PUFA intake during pregnancy, among all children, then separately according to breastfeeding status (ever vs. never breastfed).
3. Proxy of qualitative postnatal exposure: colostrum PUFA levels, among breastfed children.

All models were adjusted for the following covariates: study centre, child's sex, exact age at child's assessment, gestation length, maternal age, primiparity, pre-pregnancy maternal body mass index, smoking status and alcohol consumption during pregnancy, parental education, household income, main caregiver of the child at two years, and frequency of mother-child activities. Models explaining ASQ-3 were additionally adjusted for duration of preschool attendance at three years. Models on maternal dietary intake were additionally adjusted for total energy intake during pregnancy. Models on colostrum PUFA levels were additionally adjusted for day of colostrum collection after delivery, and exclusive breastfeeding duration.

As these results have been previously published, we here only report the main findings to be put into perspective. In a last analysis, we compared adjusted psychomotor scores in never breastfed children and in ever breastfed children as two groups separated by the median (9.7% of total fatty acids): the lowest LA levels *vs.* the highest LA levels.

All statistical analyses were performed with a two-sided alpha risk of 5%, and conducted using SAS 9.3 software (SAS Institute, Cary, NC).

## 3 Results

Main characteristics of the 1367 mother-child pairs are reported in Table 1. At inclusion, pregnant women were on average 29.4 ± 4.7 years old, and 46.6% were primiparous. Almost 7% were obese before pregnancy, and 22.5 smoked during pregnancy. Children were born on average at 39.3 ± 1.5 weeks of gestation, 52.5% were boys, and 76.5% were ever breastfed. Daily total n-6 PUFA and DHA intakes of pregnant women were respectively 9.64 ± 4.4 and 0.17 ± 0.11 g/d. In colostrum, total n-6 PUFA and DHA represented respectively 12.2 ± 1.8 and 0.64 ± 0.19% of total fatty acids.

Ever breastfed children scored higher for all psychomotor scores than never breastfed children (results not shown, see Bernard *et al.* (2013a)). Among ever breastfed children, both exclusive and any breastfeeding durations were positively and significantly associated with psychomotor scores (results not shown).

Associations between maternal dietary PUFA intake during pregnancy and child's psychomotor scores are presented in Table 2. Among all children, total n-6/n-3 ratio was negatively associated with Motor-2 (−0.06 ± 0.03, $P$ = 0.04) and ASQ-3 (−0.89 ± 0.36, $P$ = 0.01) but not with CDI-2 ($P$ = 0.4). However, when focusing on never breastfed children only, CDI-2 score was negatively associated with total n6/n3 ratio (−2.1 ± 0.7, $P$ = 0.002). Same patterns of associations were observed with LA intake and with LA/ALA ratio. Overall, the strength of associations was higher in never breastfed than in ever breastfed children for the three psychomotor scores.

Associations between colostrums PUFA levels and child's psychomotor scores are shown in Table 3. Total n-6 levels were negatively and significantly associated with Motor-2 (−0.10 ± 0.05, $P$ = 0.04) and ASQ-3 (−1.06 ± 0.55, $P$ = 0.05) scores, but not with CDI-2 ($P$ = 0.2). These associations were mostly driven by colostrum LA levels, as indicated by Figure 1, comparing psychomotor scores of never breastfed children to scores of two groups of breastfed children: those fed with the lowest colostrum LA levels, and those fed with the highest LA levels (separated according to the median in LA levels). The group 'lowest LA levels' scored significantly higher than the group 'highest LA levels' on Motor-2 ($P$ < 0.01), CDI-2 ($P$ < 0.05) and ASQ-3 ($P$ < 0.01). They also scored higher than the group 'never breastfed' on Motor-2 ($P$ < 0.001) and ASQ-3 ($P$ < 0.001), but not on CDI-2. Last, no difference on Motor-2 and CDI-2 scores was observed between the groups 'highest LA levels and 'never breastfed'.

## 4 Discussion

As hypothesized, we found a positive association between breastfeeding duration and child's psychomotor scores in the children of the EDEN mother-child cohort. However, we found no association of prenatal nor postnatal exposure to the LC-PUFA AA and DHA, with psychomotor development. Instead,

**Table 1.** Characteristics of the EDEN study population.

| | *n* | *M* ± SD or % |
|---|---|---|
| Study centre, % enrolled in Poitiers | 1367 | 48.5 |
| Mothers | 1367 | |
| Age, y | | 29.4 ± 4.7 |
| Parental education, y | | 13.7 ± 2.3 |
| Prepregnancy Body Mass Index, kg/m$^2$ | | 23.1 ± 4.3 |
| Prepregnancy obesity, % | | 7.32 |
| Smoking status, % | | 22.5 |
| Alcohol consumption, % | | 44.1 |
| Primiparous, % | | 46.6 |
| Infants | 1367 | |
| Sex, % boys | | 52.5 |
| Gestational age at birth, wk | | 39.3 ± 1.5 |
| Late preterm birth, % | | 4.8 |
| Ever breastfed, % | | 76.5 |
| Any breastfeeding duration, mo | 1046 | 4.7 ± 4.5 |
| Maternal dietaty intake | 1278 | |
| n6 PUFA, $g/d$ | | 9.64 ± 4.4 |
| LA, g/d | | 9.49 ± 4.36 |
| AA, g/d | | 0.15 ± 0.08 |
| n-3 PUFA, g/d | | 1.15 ± 0.45 |
| ALA, g/d | | 0.86 ± 0.34 |
| EPA, g/d | | 0.08 ± 0.06 |
| DHA, g/d | | 0.17 ± 0.11 |
| Total n6/n3 ratio | | 8.51 ± 2.36 |
| LA/ALA ratio | | 11.06 ± 2.77 |
| AA/DHA ratio | | 1.09 ± 0.53 |
| Colostrum PUFA levels, % total fatty acids | 745 | |
| n6 PUFA, % | | 12.15 ± 1.84 |
| LA, % | | 9.82 ± 1.78 |
| AA, % | | 0.86 ± 0.15 |
| n-3 PUFA, % | | 2.05 ± 0.41 |
| ALA, % | | 0.65 ± 0.22 |
| EPA, % | | 0.06 ± 0.03 |
| DHA, % | | 0.64 ± 0.19 |
| Total n6/n3 ratio | | 6.13 ± 1.48 |
| LA/ALA ratio | | 16.43 ± 5.39 |
| AA/DHA ratio | | 1.42 ± 0.37 |
| Psychomotor development scores | | |
| Motor-2 | 1260 | 16.2 ± 2.3 |
| CDI-2 | 1277 | 60.7 ± 29.5 |
| ASQ-3 | 1157 | 270.5 ± 29.3 |

we found negative relationships with either then-6/n-3 ratio, either total n-6 PUFA, principally driven by LA levels, and this was consistent with prenatal and postnatal assessments. Importantly, children exposed to colostrum with the highest LA levels tended to have psychomotor scores closer to the never breastfed children than to the children breastfed with lower LA levels.

The reliability of these results is supported by strengths inherent to the EDEN cohort. Its prospective design starting from mid-pregnancy enabled to accurately measure several pathways of exposure to PUFA in early life, which increases the consistency of our findings. The amount of data collected in the cohort allowed to control for many potential confounders, although residual confounding may remain. The sample size finally allowed this analysis to be powerful enough to highlight modest differences in child's psychomotor scores, as assessed at an early age. Nonetheless, this study has limitations. First, psychomotor assessments were parent-reported and not assessed by psychologists. Although these tools are good predictors of standardized psychological tests, it may have introduced observer bias. Second, no measure of maternal intelligence was available in the cohort. Yet, this proxy of both genetic and environmental inheritances has been previously described as a potential confounder (Jacobson and Jacobson, 2002). Last, the EDEN study was not representative of the general population, participants having a higher socioeconomic status, which limits its external validity.

The observed association between breastfeeding and child's psychomotor development was reported by many previous articles (Anderson *et al.*, 1999), while whether the link

**Table 2.** Associations of dietary LA intake, n-6/n-3 and LA/ALA ratios during pregnancy with child's psychomotor development at 2 and 3 years in the EDEN study, according to child's breastfeeding status.

| | Motor-2 | | CDI-2 | | ASQ-3 | |
|---|---|---|---|---|---|---|
| | $\beta \pm$ SE | *P* | $\beta \pm$ SE | *P* | $\beta \pm$ SE | *P* |
| All children | *n* = 1203 | | *n* = 1215 | | *n* = 1061 | |
| LA, *g*/*d* | −0.03 ± 0.02 | 0.11 | −0.04 ± 0.26 | 0.9 | −0.47 ± 0.28 | 0.10 |
| Total n6/n3 ratio | −0.06 ± 0.03 | 0.04 | −0.26 ± 0.34 | 0.4 | −0.89 ± 0.36 | 0.01 |
| LA/ALA ratio | −0.04 ± 0.02 | 0.07 | −0.31 ± 0.29 | 0.3 | −0.85 ± 0.31 | 0.005 |
| Breastfed children | *n* = 896 | | *n* = 905 | | *n* = 790 | |
| LA, *g*/*d* | −0.02 ± 0.02 | 0.4 | 0.24 ± 0.30 | 0.4 | −0.22 ± 0.31 | 0.5 |
| Total n6/n3 ratio | −0.05 ± 0.03 | 0.2 | 0.38 ± 0.34 | 0.3 | −0.65 ± 0.40 | 0.11 |
| LA/ALA ratio | −0.03 ± 0.03 | 0.2 | 0.33 ± 0.34 | 0.3 | −0.57 ± 0.35 | 0.10 |
| Never breastfed children | *n* = 307 | | *n* = 310 | | *n* = 271 | |
| LA, *g*/*d* | −0.09 ± 0.05 | 0.07 | −1.23 ± 0.58 | 0.03 | −1.38 ± 0.70 | 0.05 |
| Total n6/n3 ratio | −0.08 ± 0.06 | 0.2 | −2.07 ± 0.65 | 0.002 | −1.33 ± 0.79 | 0.09 |
| LA/ALA ratio | −0.06 ± 0.05 | 0.2 | −1.89 ± 0.54 | 0.0005 | −1.38 ± 0.64 | 0.03 |

Models were adjusted for study centre, child's sex and age at assessment, duration of gestation, maternal age, primiparity, maternal pre-pregnancy obesity, maternal total energy intake, smoking status, alcohol consumption, parental education level, household income, child's caregiver at 2 years, frequency of mother-child activities. Models with ASQ were additionally adjusted for duration of preschool attendance at 3 years. SE, standard error; LA, linoleic acid; ALA, alpha-linolenic acid; Motor-2, motor development at 2 years; CDI-2, communicative development inventory at 2 years; ASQ-3, ages and stages questionnaire at 3 years.

**Table 3.** Associations of total n-6, LA levels, n-6/n-3 and LA/ALA ratios in colostrum with child's psychomotor development at 2 and 3 years among breastfed children of the EDEN study.

| | Motor-2 *n* = 579 | | CDI-2 *n* = 585 | | ASQ-3 *n* = 519 | |
|---|---|---|---|---|---|---|
| PUFA, % total fatty acids | $\beta \pm$ SE | *P* | $\beta \pm$ SE | *P* | $\beta \pm$ SE | *P* |
| n-6 PUFA, % | –0.10 ± 0.05 | 0.04 | –0.80 ± 0.62 | 0.2 | –1.06 ± 0.55 | 0.05 |
| LA, % | –0.09 ± 0.05 | 0.06 | –0.89 ± 0.64 | 0.2 | –1.05 ± 0.57 | 0.07 |
| Total n-6/n-3 ratio | –0.04 ± 0.06 | 0.5 | –1.24 ± 0.78 | 0.1 | –0.70 ± 0.69 | 0.3 |
| LA/ALA ratio | –0.03 ± 0.02 | 0.10 | –0.15 ± 0.23 | 0.5 | 0.04 ± 0.20 | 0.8 |

Models were adjusted for study centre, day of colostrum collection after delivery, child's sex and age at assessment, duration of gestation, maternal age, primiparity, maternal pre-pregnancy body mass index, smoking status, alcohol consumption, parental education level, household income, child's caregiver at 2 years, frequency of mother-child activities, any breastfeeding duration. Models with ASQ were additionally adjusted for duration of preschool attendance at 3 years. SE, standard error; LA, linoleic acid; ALA, alpha-linolenic acid; Motor-2, motor development at 2 years; CDI-2, communicative development inventory at 2 years; ASQ-3, ages and stages questionnaire at 3 years.

is causal is still in debate (Der *et al.*, 2006). In our study, we showed a dose-effect relationship, which is one criteria for arguing in favour of causation (Hill, 1965). Using data from a large cluster-randomized controlled trial, Kramer *et al.* (2008) have provided further evidence on causation by reporting higher cognitive abilities in children born in maternities which had experienced a promotion for breastfeeding. Whereas it remains difficult to definitively conclude on the causal nature of this link (Walfisch *et al.*, 2013), we have undertaken to test whether AA and DHA contained in breast milk could explain or mediate the associationsbetween breastfeeding and child's psychomotor development. Using data of colostrum fatty acid content (exhibiting relatively high levels of DHA and AA), we found no evidence of such a benefit of LC-PUFA in breast milk. Conversely, we found negative associations between colostrum total n-6 fatty acids, especially in LA, and child's outcomes, which is in accordance with a previous study (Sabel *et al.*, 2012). Previously, Guxens *et al.* (2011) had found that the association between the total n-6/n-3 PUFA ratio in breast milk and infant's mental development was moderated by the cumulative intensity of breastfeeding. In our study, we did not report this interaction. Maternal dietary LA intake during pregnancy was also associated with poorer psychomotor development among never breastfed children. This could be explained by the metabolic competition between n-6 and n-3 series for the same desaturases and elongases. Indeed, studies have reported that elevated n-6 PUFA intake may decrease the metabolic activity of the n-3 family, which might in turn decrease the synthesis and the accretion of DHA into the developing brain (Novak *et al.*, 2008; Simopoulos, 2011b). Recently, studies have investigated the roles of single nucleotide peptides located on genes involved in the metabolism of PUFA, especially the FADS cluster (Lattka *et al.*, 2010). As an example, a study has reported that the association between breastfeeding and child cognition was moderated by genetic variant in FADS2 gene (Caspi *et al.*, 2007).

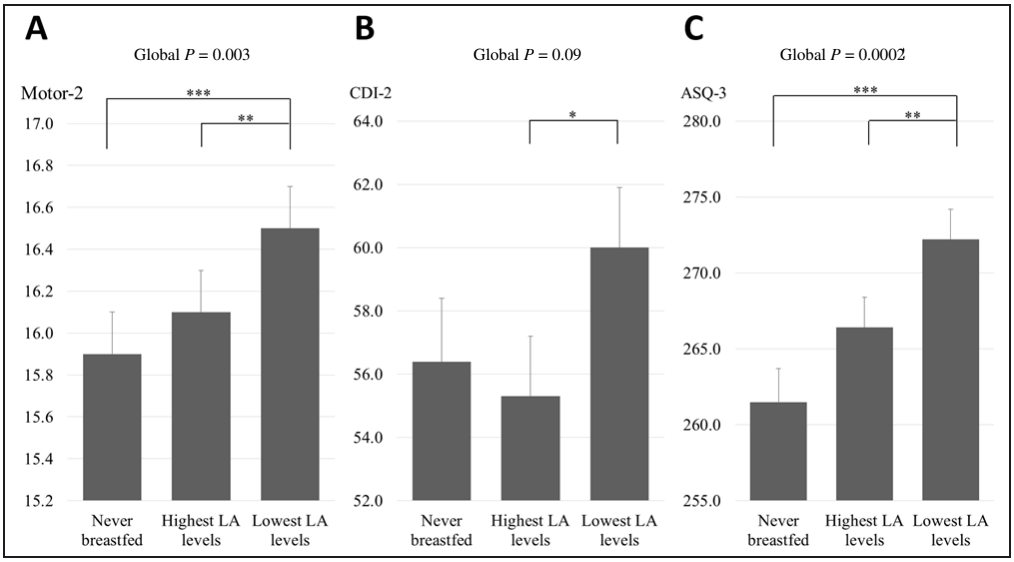


**Fig. 1.** Values are adjusted mean ± SEM. Panels A, B and C respectively represent Motor-2, CDI-2 and ASQ-3 scores. P-values thresholds are as follow: $*P \leqslant 0.05$, $**P \leqslant 0.01$, $***P \leqslant 0.001$. The lowest LA levels are comprised between 5.4 and 9.7% of total fatty acids; the highest LA levels are comprised between 9.7 and 15.9% of total fatty acids. Models were adjusted for study centre, child's sex and age at assessment, duration of gestation, maternal age, primiparity, maternal pre-pregnancy body mass index, smoking status, alcohol consumption, parental education level, household income, child's caregivers at 2 years, exclusive breastfeeding duration. Abbreviations: LA, linoleic acid; Motor-2, motor development at 2 years; CDI-2, communicative development inventory at 2 years; ASQ-3, ages and stages questionnaire at 3 years; SEM, standard error of the mean.

The input from gene-environment studies are promising to better understand how pre- and postnatal exposure to PUFA may affect brain development and psychomotor development.

## 5 Conclusion

We aimed at investigating the link between several measures of exposure to PUFA in early life and child's psychomotor development at 2 and 3 years in a French prospective mother-child cohort. In our study, we confirmed positive association between breastfeeding duration and psychomotor development, but we found no evidence supporting the hypothesis of a beneficial effect on psychomotor development of both prenatal and postnatal exposure to the LC-PUFA AA and DHA. However, we highlighted negative associations involving total n-6 levels, driven by LA levels, the precursor. This suggests that in early life, an exposure to a too high level of LA, through maternal diet during pregnancy and through breastfeeding, may have adverse effects on child's psychomotor development. Although this finding needs replication later in childhood and in other large cohorts, it supports current nutritional recommendation of balancing dietary n-6 and n-3, especially during pregnancy and lactation. This also calls for research evaluating potential effects of LA levels found in infant formulas, and highlights the importance of carefully selecting the lipid ingredients used. Finally, this study supports the policies promoting initiation and continuation of breastfeeding.

*Acknowledgements.* We are grateful to the participating families, the midwife research assistants (Lorraine Douhaud, Sophie Bedel, Brigitte Lortholary, Sophie Gabriel, Muriel Rogeon, and Monique Malinbaum) for data collection, and the data entry operators (Patricia Lavoine, Josiane Sahuquillo and Ginette Debotte). We thank Cyrielle Garcia for milk analysis, and Sophie Kern for providing the French version of the MacArthur Communicative Development Inventory.

*Sources of funding.* The EDEN mother-child cohort study was supported by: Fondation pour la Recherche Medicale (FRM), French Ministry of Research IFR and Cohort Program, INSERM Nutrition Research Program, French Ministry of Health Perinatality Program, French Agency for Environment Security (AFFSET), French National Institute for Population Health Surveillance (INVS), Paris-Sud University, French National Institute for Health Education (INPES), Nestle, Mutuelle Generale de l'Education Nationale (MGEN), French Speaking Association for the Study of Diabetes and Metabolism (Alfediam), National Agency for Research (ANR non thematic program), Groupes Lipides Nutrition (GNL), and National Institute for Research in Public Health (IRESP TGIR Cohorte Sante 2008 Program). Biological analyses of colostrum samples were funded by the PremUp foundation (foundation for scientific cooperation in connection with pregnancy and prematurity). Study sponsors were not involved in study design, data collection, or data analyses.

*Disclosure.* Authors have no conflicts of interest to declare.

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**Cite this article as:** Jonathan Y. Bernard, Martine Armand, Anne Forhan, Maria De Agostini, Marie-Aline Charles, Barbara Heude, on behalf of the EDEN mother-child cohort study group. Early life exposure to polyunsaturated fatty acids and psychomotor development in children from the EDEN mother-child cohort. OCL 2016, 23(1) D106.