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Maternal n-3 polyunsaturated fatty acid dietary supply modulates microglia lipid content in the offspring

Charlotte Rey[a,b,c], Agnès Nadjar[a,b], Florent Joffre[c], Camille Amadieu[a,b], Agnès Aubert[a,b], Carole Vaysse[c], Véronique Pallet[a,b], Sophie Layé[a,b], Corinne Joffre[a,b,*]

[a] INRA, Nutrition et Neurobiologie Intégrée, UMR 1286, Bordeaux 33076, France
[b] Bordeaux University, Nutrition et Neurobiologie Intégrée, UMR 1286, Bordeaux 33076, France
[c] ITERG, Institut des corps gras, Canéjan 33610, France

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ABSTRACT

The brain is highly enriched in long chain polyunsaturated fatty acids (LC-PUFAs) that are esterified into phospholipids, the major components of cell membranes. They accumulate during the perinatal period when the brain is rapidly developing. Hence, the levels of LC-PUFAs in the brains of the offspring greatly depend on maternal dietary intake. Perinatal n-3 PUFA consumption has been suggested to modulate the activity of microglial cells, the brain's innate immune cells which contribute to the shaping of neuronal network during development. However, the impact of maternal n-3 PUFA intake on microglial lipid composition in the offspring has never been studied.

To investigate the impact of maternal dietary n-3 PUFA supply on microglia lipid composition, pregnant mice were fed with n-3 PUFA deficient, n-3 PUFA balanced or n-3 PUFA supplemented diets during gestation and lactation. At weaning, microglia were isolated from the pup's brains to analyze their fatty acid composition and phospholipid class levels.

We here report that post-natal microglial cells displayed a distinctive lipid profile as they contained high levels of eicosapentaenoic acid (EPA), more EPA than docosahexaenoic acid (DHA) and large amount of phosphatidylinositol (PI) / phosphatidylserine (PS). Maternal n-3 PUFA supply increased DHA levels and decreased n-6 docosapentaenoic acid (DPA) levels whereas the PI/PS membrane content was inversely correlated to the quantity of PUFAs in the diet. These results raise the possibility of modulating microglial lipid profile and their subsequent activity in the developing brain.

1. Introduction

The central nervous system (CNS) is highly enriched in long-chain polyunsaturated fatty acids (LC-PUFAs), in particular in arachidonic acid (AA, 20:4 n-6) and docosahexaenoic acid (DHA, 22:6 n-3) [1–3]. These LC-PUFAs are esterified into phospholipids, the major components of cell membranes. DHA is preferentially incorporated in phosphatidylethanolamine (PE), and phosphatidylserine (PS), while AA is more present in phosphatidylinositol (PI), phosphatidylcholine (PC) and PE [1,4,5]. Membranes are also rich in cholesterol, a lipid that confers rigidity to their structure.

Humans can synthesize AA and DHA from precursors that are mainly found in vegetables, linoleic acid (LA) and alpha-linolenic acid (ALA), respectively. However, the conversion efficiency is very low (<1%) [6,7], so that DHA and AA must also be provided through the diet. Hence, the dietary intake of PUFAs and particularly of LC-PUFAs is crucial to maintain a proper balance between n-6 and n-3 PUFA species. Accordingly, low n-3 PUFA dietary intake induces a decrease in brain DHA levels [2,8–10] and increases in brain AA and n-6 docosapentaenoic acid (n-6 DPA, 22:5n-6), the molecular equivalent of DHA [9,10]. Conversely, ingestion of fish oil rich in n-3 LC-PUFAs (eicosapentaenoic acid (EPA) and DHA) results in partial replacement of AA by EPA and DHA in cell membranes [11].

Most LC-PUFAs accumulate during brain development, at a period

Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; CNS, central nervous system; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; FAME, fatty acid methyl esters; LA, linoleic acid; LC-PUFA, long chain polyunsaturated fatty acid; MSHFBA, N-methyl-N-trimethylsilyl-heptafluorobutyramide; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PCA, principal component analysis; PI, phosphatidylinositol; PS, phosphatidylserine; PUFAs, polyunsaturated fatty acids; RvE1, resolvin E1; SEM, standard error of the mean; SM, sphingomyelin

* Corresponding author at: INRA, Nutrition et Neurobiologie Intégrée, UMR 1286, Bordeaux 33076, France.
E-mail address: corinne.joffre@inra.fr (C. Joffre).

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of intense cell division, synaptogenesis, white matter expansion, etc. [12–14]. In humans, this accumulation starts at the beginning of the third trimester of gestation until 2 years of age, corresponding to 7–21 postnatal days (P7-P21) in rodents [15–18]. Maternal LC-PUFAs are provided to the offspring via placental exchange during gestation and via lactation after birth, emphasizing the importance of maternal dietary habits. Consequently, brain LC-PUFA levels in the offspring greatly depend on maternal PUFA intake [19,20]. Dietary n-3 PUFA deficiency, from the first day of gestation to P21, induces a decrease in offspring's brain DHA [21]. Conversely, n-3 LC-PUFA dietary enrichment during gestation and lactation increases brain DHA levels [22,23]. Adequate DHA supply during the perinatal period is essential for optimal CNS development and function, as n-3 PUFA intake promotes neurogenesis, neuroplasticity and protects against oxidative stress and neuroimmune dysregulation [20,24–29]. In contrast, dietary n-3 PUFA deficiency impairs neuronal plasticity [21,30–33].

The effects of maternal dietary intake on brain cells lipid composition have been poorly addressed. Two studies described the effect of maternal dietary n-3 PUFA supplementation on glial cells as a whole. They found an increase in DHA in PE and PS in neonatal glia [22,23]. Bourre and colleagues showed that perinatal n-3 PUFA deficiency decreases DHA levels in neurons, astrocytes and oligodendrocytes in the offspring [34,35].

However, nothing is known about the effects of n-3 PUFA dietary supply on microglial lipid profile. Microglia are the brain's innate immune cells and play a major role in development by regulating synaptic pruning and synaptogenesis and by phagocytosing apoptotic bodies [36–39]. Hence, microglia are a crucial partner for the shaping of neuronal networks during the perinatal period. *In vitro* studies showed that application of DHA increases DHA and PS levels in microglial cells [25,40,41]. *In vivo*, we previously showed that maternal dietary n-3 PUFA deficiency alters microglia motility and promotes pro-inflammatory cytokine production at P21 [21]. However, while it impacts on physiology during development, the consequences of maternal dietary n-3 PUFA supply on postnatal microglial lipid composition remains to be elucidated.

Pregnant mice were fed with differential dietary n-3 PUFA supply and microglial lipid composition in the offspring was assessed at weaning. The impact of maternal ALA deficient diet ("n-3 PUFA deficient diet"), ALA balanced diet ("n-3 PUFA balanced diet") and n-3 LC-PUFA enriched diet ("n-3 LC-PUFA supplemented diet") was evaluated by fatty acid and phospholipid compositions in microglial membranes.

2. Experimental procedures

2.1. Animals

Animal husbandry and experimental procedures were in accordance with the EU Directive 2010/63/EU for animal experiments and approved by the national ethical committee for care and use of animals (approval ID A13169). All the experiments were performed on wild type CD1 mice obtained from Janvier Labs (Le Genest-Saint-Isle, France). Mice were maintained under standard housing conditions on corncob litter in a temperature (23 ± 1 °C) and humidity (40%) controlled animal room with a 12 h light/dark cycle (07–19 h), with *ad libitum* access to food and water.

After mating with males fed with a standard diet, female pregnant mice were fed with a diet containing 5% fat in the form of sunflower oil (rich in LA, n-3 PUFA deficient diet) or a mixture of different oils containing ALA (n-3 PUFA balanced diet) or a mixture containing fish oil (rich in n-3 LC-PUFAs, n-3 LC-PUFA supplemented diet) from the first day of gestation until weaning [21,42,43] (Table 1). Diets are isocaloric and varied only by their lipid composition. The dose of n-3 LC-PUFA used (EPA + DHA) corresponds to 40 mg/d in mice. Extrapolating to human [44], it represents 3.8 g/d (60 kg). This dose is in the range of those administered to pregnant women in previous studies [45]. At P21, male offspring mice were quickly anesthetized by isoflurane inhalation and euthanized by decapitation. Brains were rapidly dissected out to isolate microglia.

Table 1
Fatty acid composition of the dietary lipids (% wt of total fatty acids).

diets	n-3 PUFA deficient diet	n-3 PUFA balanced diet	n-3 LC-PUFA supplemented diet
16:0	9.3	21.9	19.6
18:0	5.4	3.6	4.1
Other SFA	2.4	1.8	7.6
Total SFA	**17.1**	**27.3**	**31.3**
18:1n-9	35.8	56.4	24.6
18:1n-7	0.8	1.1	2.9
Other MUFA	0.4	0.7	9.1
Total MUFA	**37.5**	**58.2**	**36.6**
18:2n-6 (LA)	45.3	12.9	12.9
Other n-6 PUFA	n.d.	n.d.	0.6
Total n-6 PUFA	**45.3**	**12.9**	**14.0**
18:3n-3 (ALA)	0.1	1.6	1.1
20:5 n-3 (EPA)	n.d.	n.d.	10.6
22:5 n-3 (n-3 DPA)	n.d.	n.d.	0.9
22:6 n-3 (DHA)	n.d.	n.d.	5.6
Total n-3 PUFA	**0.1**	**1.6**	**18.1**
Total PUFA	**45.4**	**14.5**	**32.1**

FAs, fatty acids; PUFAs, polyunsaturated fatty acids; LA: linoleic acid; ALA: α-linolenic acid; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; MUFA: monounsaturated fatty acid; SFA: saturated fatty acid; n.d., not detected (under the limit for the detection by gas chromatography, < 0.05%).

2.2. Isolation of microglial cells

Microglia cells were isolated from the whole brain as previously described [46,47]. Briefly, brains were homogenized in Hanks' Balanced Salt Solution (HBSS, pH 7.4), homogenates were centrifuged at 600 g and cells were resuspended in 70% isotonic Percoll (GE-Healthcare, Aulnay-sous-Bois, France). A discontinuous Percoll density gradient was set up (70%, 50%, 35% and 0%). Gradients were centrifuged at 2000 g for 25 min. Microglia cells collected at the interphase between the 70% and 50% Percoll layers were washed, re-suspended in PBS and stored at −80 °C until lipid analyses. Ten pup brains from 5 dams were pooled for each replicate and 4–6 replicates were carried out to perform lipidomic analyses. In total, microglial cells were isolated from 60 mice in n-3 PUFA deficient group, 40 mice in n-3 balanced group and 40 mice in n-3 LC-PUFA supplemented group.

2.3. Lipidomic analysis

Lipids were extracted according to the Folch's method [48]. Total lipids were stored at −20 °C in chloroform:methanol (2:1).

Phospholipid classes were separated by HPLC coupled to an evaporative light-scattering detector [49,50]. The different classes of phospholipids were separated using a silica column (Spherisorb silice, 10 cm, 4.6 mm, 3 µm; WATERS, Merck) and identified using commercial standards (Avanti polar Lipids, USA). Results were expressed as % of total phospholipids.

Total fatty acids were transmethylated according to the method of Morrison and Smith [51]. Fatty acid methyl esters (FAMEs) were analyzed on a FOCUS GC gas chromatograph (Thermo Electron Corporation) equipped with a splitless injector and a flame ionization detector. Separation of FAMEs was performed with a BPX70-fused silica capillary column (50 m length × 0.25 mm internal diameter, 0.22 µm film thickness; SGE, Courtaboeuf, France). Hydrogen was used as a carrier

gas (inlet pressure, 100 kPa). The injector and detector temperatures were maintained at 250 and 280 °C, respectively. The oven was held at 70 °C for 2 min, increased to 150 °C at a rate of 15 °C/min with a 1-min hold, increased to 190 °C at a rate of 1.5 °C/min with a 27-min hold and increased to 230 °C at a rate of 20 °C/min and then held at this temperature for 25 min. FAMEs were identified by comparison with commercial standards.

Cholesterol was analyzed by gas chromatography after silylation with n-methyl imidazole/MSHFBA (1/20) according to the method NF EN ISO 12228 modified for small amount of lipids. Especially the saponification step was carried out by adapting the quantities of reagents and the thin layer chromatography step was suppressed. This purification was essential for the analysis of the phytosterols to avoid coelution with vitamin E but without interest for the quantification of cholesterol. The quantification of cholesterol was realized using 5β-cholestanol (Sigma Aldrich) as internal standard. Cholesterol analysis was performed with a ZB5-fused silica capillary column (30 m length × 0.25 mm internal diameter, 0.25 µm film thickness; SGE, Courtaboeuf, France). Hydrogen was used as carrier gas (constant flow, 1.5 mL/min). The injector and detector temperatures were maintained at 300 °C and 340 °C, respectively. The oven was set at 240 °C, increased to 255 °C at a rate of 4 °C/min with a 31-min hold, increased to 320 °C at a rate of 50 °C/min and then held at this temperature for 1 min. Cholesterol and 5β-cholestanol were identified by comparison with commercial standards.

2.4. Statistical analysis

2.4.1. Bivariate statistical analysis

Data were expressed as means ± SEM (standard error of the mean). Analyses were carried out using the non-parametric Kruskal–Wallis test. Significant values between two sample means were evaluated using Mann–Whitney test. A $p < 0.05$ was considered statistically significant.

2.4.2. Principal component analysis (PCA)

PCA was used to assess the fatty acid pattern from dietary groups. The PCA is a dimension-reduction technique that summarizes data into principal components (PC) that maximize the variance of the data considered. These PCs are uncorrelated linear combinations of the initial variables which can be interpreted as pattern. PCA generates factor loadings that reflect the correlation of each variable with the PC and a principal component score for each individual. We selected the number of components using the Cattell criterion. All data were expressed as means ± SEM. Statistical analyses were performed using R version 3.3.0 (FactoMineR package).

3. Results

3.1. Maternal dietary n-3 PUFA intake alters phospholipid class distribution in microglia

We investigated the impact of maternal n-3 PUFA supply on phospholipid class distribution within microglial membranes (expressed in % of total phospholipids). PC and PI/PS were the major lipid classes represented (PC: 29.3–42.2%, PI/PS: 34.1–52.6%), while PE and SM were present in low amounts (PE: 14.1–18.5% and SM: 3.9–5.2%) (Table 2).

Differential maternal n-3 PUFA dietary supply significantly impacted PI/PS and PC proportions (PI/PS $p < 0.01$; PC $p < 0.01$) (Table 2). N-3 LC-PUFA supplementation induced a significant decrease in PI/PS contents as compared to n-3 PUFA deficient diets (34.1–52.6%, $p < 0.05$) and increase in PC content as compared to the n-3 PUFA deficient diet (42.2–29.3%, $p < 0.01$). Diets tended to change PE and SM contents but it did not reach significance (PE $p = 0.09$; SM $p = 0.10$).

Table 2
Phospholipid class levels in microglial cells isolated from P21 mice brains (expressed as % of total phospholipids). Values are expressed as means ± SEM. Values with different superscript letters (a, b, c) are significantly different ($p < 0.05$).

	n-3 PUFA deficient diet ($n = 5$)	n-3 PUFA balanced diet ($n = 4$)	n-3 PUFA supplemented diet ($n = 4$)	Statistical significance
PE	14.1 ± 1.57	15.3 ± 0.65	18.5 ± 0.92	$p = 0.09$
PI/PS	52.6 ± 4.21[a]	44.0 ± 2.11[ab]	34.1 ± 1.05[b]	$\mathbf{p < 0.01}$
PC	29.3 ± 2.55[a]	35.9 ± 1.87[ab]	42.2 ± 0.74[b]	$\mathbf{p < 0.01}$
SM	3.9 ± 0.38	4.8 ± 0.29	5.2 ± 0.48	$p = 0.10$

PE: phosphatidylethanolamine; PI: phosphatidylinositol; PS: phosphatidylserine; PC: phosphatidylcholine; SM: sphingomyelin.

3.2. Maternal dietary n-3 PUFA intake affects microglial fatty acid composition

Fatty acid composition of microglial cells was characterized by high levels of saturated fatty acids (39.2–50.7%) and monounsaturated fatty acids (31.4–37.1%) (Table 3). PUFAs represented 16.9–22.0% of the total fatty acids (Table 3). The main n-3 PUFAs were EPA (2.5–5.0%) and ALA (1.3–1.8%). DHA was present at low level (0.6–2.1%). N-6 PUFAs were mainly represented by LA (8.0–10.4%) and AA (1.0–1.6%).

Fatty acid composition was next analyzed in microglia of P21 pups from mothers fed with n-3 PUFA deficient, n-3 PUFA balanced, n-3 LC-PUFA supplemented diets. Dietary n-3 PUFA deficiency increased n-6 DPA levels as compared to n-3 PUFA balanced diet and n-3 LC-PUFA supplemented diet (0.0–0.6% $p < 0.01$) and decreased DHA and DHA/AA ratio as compared to n-3 LC-PUFA supplemented diet (DHA: 0.6–2.1% $p < 0.05$, DHA/AA: 0.5–1.3 $p < 0.05$). Dietary n-3 PUFA deficiency also increased 20:1 content as compared to the n-3 LC-PUFA supplementation (0.7–1.7% $p < 0.05$) (Table 3).

To further explore the global effect of the diets on PUFA composition of microglia cells, we used PCA to produce visual summary of observed variation in PUFA composition in the three experimental groups. Two main principal components were extracted explaining together 61.27% of overall variance: the 1st component (“pattern 1”) and the 2nd component (“pattern 2”) explained 35.26% and 26.01% of the total variance, respectively. The 1st component comprised strong positive loadings with LA, total n-6 PUFA, total PUFA and n-6/n-3 PUFA and negative score for EPA (Fig. 1A). The 2nd component comprised positive score for EPA, n-3 DPA, DHA, total n-3 PUFA and total PUFA and negative score for n-6 DPA (Fig. 1A).

Based on PCA visualization of individuals (Fig. 1B), n-3 LC-PUFA group showed a positive average for the 2nd component whereas n-3 PUFA deficient group showed a negative average for the 2nd component. The PCA revealed a greater differentiation between n-3 LC-PUFA supplemented group and the two other groups: n-3 PUFA deficient and n-3 PUFA balanced groups (Fig. 1B). The 2nd component revealed significant diet effects ($p < 0.05$). The fatty acid composition of the n-3 PUFA balanced and n-3 PUFA deficient groups were comparable and differed from that of the n-3 LC-PUFA supplemented group (Fig. 1B).

3.3. Maternal dietary n-3 PUFA intake had no effect on microglial cholesterol levels

Microglial cells contained 8.0–9.8 µg/mg protein of cholesterol (Table 4). No significant differences were observed between all dietary groups.

Table 3
Fatty acid composition of lipids in microglial cells isolated from P21 mice brains (expressed as % of total fatty acids, except n-6 PUFA/n-3 PUFA and DHA/AA). Values are expressed as means ± SEM. Values with different superscript letters (a, b, c) are significantly different ($p < 0.05$).

	n-3 PUFA deficient diet ($n = 6$)	n-3 PUFA balanced diet ($n = 4$)	n-3 LC-PUFA supplemented diet ($n = 4$)	Statistical significance
16:0	25.5 ± 2.76	26.3 ± 3.33	18.5 ± 1.95	0.23
18:0	14.6 ± 2.00	18.7 ± 2.32	16.6 ± 3.23	0.38
Other SFA	5.1 ± 0.82	5.7 ± 0.44	4.1 ± 0.55	0.38
Total SFA	**45.2 ± 4.23**	**50.7 ± 5.32**	**39.2 ± 3.62**	0.20
16:1	1.8 ± 0.41	1.6 ± 0.16	3.6 ± 0.74	0.08
18:1 n-9	30.6 ± 2.67	24.7 ± 4.60	27.9 ± 3.12	0.44
18:1 n-7	1.5 ± 0.19	1.6 ± 0.44	2.1 ± 0.21	0.24
20: 1	1.7 ± 0.31[a]	1.8 ± 0.57[ab]	0.7 ± 0.24[b]	$p < 0.05$
Other MUFA	1.5 ± 0.18	1.7 ± 0.24	2.4 ± 1.04	0.76
Total MUFA	**37.1 ± 2.15**	**31.4 ± 4.79**	**36.7 ± 4.23**	0.43
18:2 n-6 (LA)	8.3 ± 2.38	8.0 ± 2.00	10.4 ± 3.49	0.86
20:4 n-6 (AA)	1.5 ± 0.45	1.6 ± 0.49	1.0 ± 0.34	0.76
22:4 n-6	0.0 ± 0.05	0.0 ± 0.00	0.1 ± 0.06	0.60
22:5 n-6 (n-6 DPA)	0.6 ± 0.16[a]	0.0 ± 0.00[b]	0.0 ± 0.00[b]	$p < 0.01$
Other n-6 PUFA	1.0 ± 0.17	1.4 ± 0.64	3.0 ± 1.56	0.59
Total n-6 PUFA	**11.4 ± 2.31**	**11.0 ± 0.92**	**14.5 ± 4.17**	0.92
18:3 n-3 (ALA)	1.8 ± 0.53	1.3 ± 0.66	1.4 ± 0.35	0.69
20:5 n-3 (EPA)	2.5 ± 0.63	3.7 ± 1.48	5.0 ± 1.97	0.69
22:5 n-3 (n-3 DPA)	0.1 ± 0.03	0.1 ± 0.01	0.2 ± 0.08	0.16
22:6 n-3 (DHA)	0.6 ± 0.16[a]	0.8 ± 0.13[ab]	2.1 ± 0.49[b]	$p < 0.05$
Total n-3 PUFA	**5.1 ± 0.82**	**5.9 ± 1.48**	**8.8 ± 1.58**	0.23
Total n-9 PUFA	0.5 ± 0.14	0.5 ± 0.11	0.3 ± 0.13	0.61
Total PUFA	**16.9 ± 2.54**	**17.5 ± 0.74**	**22.0 ± 2.01**	0.19
DMA	**0.6 ± 0.24**	**0.5 ± 0.20**	**0.5 ± 0.11**	0.97
n-6 /n-3 PUFA	**2.6 ± 0.57**	**2.4 ± 0.68**	**1.8 ± 0.67**	0.47
DHA/AA	**0.5 ± 0.10[a]**	**0.5 ± 0.08[ab]**	**1.3 ± 0.28[b]**	$p < 0.05$

AA: arachidonic acid; ALA: α-linolenic acid DHA: docosahexaenoic acid; DPA: docosapentaenoic acic; DMA: dimethylacetal; EPA: eicosapentaenoic acid; LA: linoleic acid; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids. Other SFA: 10:0, 14:0, 15:0, 17:0, 20:0, 22:0, 24:0; other MUFA: 17:1, 18:1 trans, 22:1, 24:1; Other n-6 PUFA: 18:3 n-6, 20:2 n-6, 20:3 n-6.

A

	Loadings[a]	
	Pattern 1	**Pattern 2**
18:2 n-6 (LA)	0,477[b]	0,029
20:4 n-6 (AA)	-0,266	-0,150
22:4 n-6	0,062	-0,033
22:5 n-6 (n-6 DPA)	-0,069	-0,361[b]
Toal n-6 PUFA	0,444[b]	0,046
18:3 n-3 (ALA)	0,253	0,037
20:5 n-3 (EPA)	-0,339[b]	0,369[b]
22:5 n-3 (n-3 DPA)	0,202	0,317[b]
22:6 n-3 (DHA)	0,102	0,404[b]
Total n-3 PUFA	-0,170	0,492[b]
Total PUFA	0,318[b]	0,333[b]
n-6 / n-3 PUFA	0,367[b]	-0,298

[a] Loading coefficients represent the actual correlations between each variable and the principal component score from PCA.
[b] Considered the dominant variables in the pattern. Fatty acid pattern interpretation is based on the strongest loading coefficients (>|0.30|) within each pattern.

B

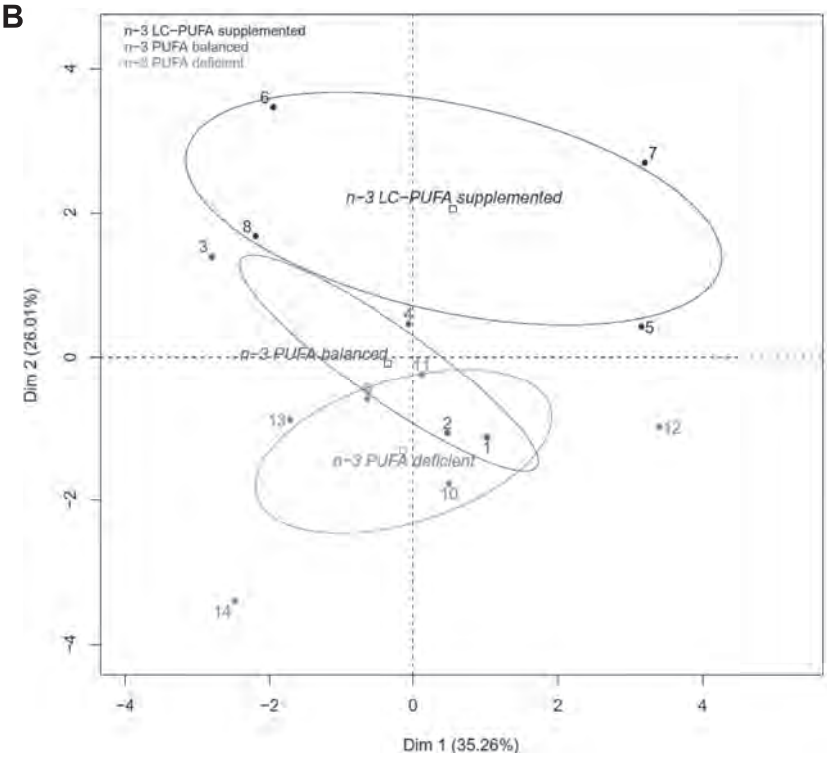

Fig. 1. Principal component analysis (PCA). (A): Correlation between each variable and the principal component score from PCA. (B): Individuals map of PCA analysis. Individual variables are represented as circle.

Table 4
Cholesterol content in microglial cells isolated from P21 mice brains (expressed as µg/mg protein). Values are expressed as means ± SEM.

	n-3 PUFA deficient diet (n = 6)	n-3 PUFA balanced diet (n = 4)	n-3 LC-PUFA supplemented diet (n = 4)	Statistical significance
Cholesterol	9.8 ± 1.69	8.0 ± 0.55	8.5 ± 2.40	p = 0.74

4. Discussion

The present study is the first to describe how modulating dietary n-3 PUFA intake affects microglial lipid profile in the developing brain.

Many *in vitro* studies have investigated the ability of macrophages, lymphocytes and microglial cells to incorporate fatty acids [25,52,53]. None of them were performed *in situ*. We here found that at P21, microglial cells displayed a distinctive fatty acid composition, as they contained measurable amounts of EPA, with higher proportion of EPA than DHA. This result differs from previous data obtained *in vitro* on BV2 microglial cell line in which DHA is the main measurable n-3 PUFAs while EPA levels are low [25,40,54]. Many studies have questioned the relevance of studying microglia *in vitro* as the transcriptomic signature of these cells in culture is significantly different from freshly isolated microglia [55–58]. Our results also support this statement and allow extending it to fatty acid profile.

The fatty acid composition of acutely isolated microglia differed from neurons, astrocytes and oligodendrocytes lipid profiles. In all these cells, DHA is the predominant form of n-3 PUFAs [34,35,59] while EPA was the major n-3 PUFA in P21 isolated microglia. Finally, microglial fatty acid composition also differed from whole structure fatty acid composition. Hippocampus from P21 mice contained higher quantities of DHA than EPA [21]. EPA is considered to be 250–300 times lower than DHA because of its rapid β-oxidation in the brain [60]. Our results thus raise the questions of β-oxidation processes within microglia and of how EPA modulates microglial function as a structural component and signaling molecule. During brain development, one of the main functions of microglia is to phagocytose non-functional and/or immature synapses [36,61]. Because EPA is higher than DHA, it should be of interest to compare the role of both fatty acids in this function. EPA is the precursor of several oxidative products called oxylipins, which play various biological roles [62]. Most notably, EPA derived-resolvin E1 (RvE1) is of interest in the resolution of inflammation [63]. We and others have previously shown that RvE1 inhibits LPS-induced microgliosis and pro-inflammatory cytokine release *in vitro* [64–66]. RvE1 also significantly alters the inflammatory profile and activation of microglia in mice [67] and inhibits spinal cord microglial activation following peripheral nerve injury [64,66]. EPA-enriched microglia could synthesize higher amounts of RvE1 at P21, hence promoting pro-resolution-like mechanisms. Fatty acid composition of microglia should also be measured at adulthood to evaluate if the specific high level of EPA is maintained.

In this study, we showed that n-3 LC-PUFA dietary intake affects the fatty acid composition in offspring's microglia cells as compared to n-3 PUFA deficient and balanced diets. DHA level increased following dietary n-3 LC-PUFA supplementation while n-6 DPA content decreased. Our results are in line with previous reports on other brain cells. Maternal n-3 PUFA supplementation during gestation and lactation induces an increase in DHA in glial cells of pups at P14 [22,23]. Conversely, maternal n-3 PUFA deficiency decreases DHA levels in neurons, oligodendrocytes and total hippocampus that is compensated by an increase in n-6 DPA [21,34,35]. Interestingly, our results show that n-3 PUFA deficient diet increases n-6 DPA level as compared to n-3 PUFA balanced diet but does not impact DHA levels in this group. Our data suggest that microglia DHA content was only impacted by maternal increases in dietary n-3 LC-PUFA in the offspring. N-3 PUFA balanced and deficient diets displayed similar DHA content suggesting that 1) ALA supply is not sufficient to modulate DHA membrane level and 2) microglia cells seem to be protected from n-3 PUFA deficiency for DHA level.

During brain development, microglia acts as guidepost cells to monitor, guide neurons and control neural circuits formation. Microglia eliminate redundant neurons and phagocytose dendritic spines to promote healthy brain development [36,68]. Dietary n-3 PUFA deficiency during perinatal period alters microglia phenotype and motility and neuronal plasticity-related gene expression at P21, which contributes to microglia activation, neuroinflammation and brain neuronal network alterations [21]. In addition, a maternal immune activation-triggered neuroinflammatory events in the developing brain of offspring has been linked to neurodevelopmental disorders such as autism spectrum disorder [21,69].

Microglial phenotype modification could be explained by direct effect on microglial fatty acid composition, through changes in n-6 DPA and/or by specific response to dietary lipids. Indeed, exposure to saturated fatty acids induces the accumulation of microglia and astrocytes in the hypothalamus, yet only microglia are activated [70]. Moreover, maternal exposure to a high-fat diet induces an increase in hippocampal microglial activation both at birth and at adulthood [71,72]. Finally, microglia express a wide range of key lipid metabolism-related genes as well as lipoprotein lipase and lipid-sensitive receptors such as CD36, receptors for endocannabinoids, prostaglandins, phospholipids [73–76]. It was suggested that in lipoprotein lipase knock-down mice, microglia presented morphological changes in hippocampus and cortex and altered phagocytic activity [73]. These results highlight the influence of lipid metabolism on microglial activity. It should be of interest to further investigate this point since microglia likely represent a unique CNS lipid sensor that initiates inflammation in response to lipids [64]. In contrast to creating n-3 PUFA deficiency, clinical trials conducted on n-3 PUFA supplementation during the perinatal period shows favorable effects on cognition [77,78]. However, results are conflicting since other studies also shows that early-life n-3 PUFA supplementation has no effect or negative effect on cognition and behavior of the offspring [45,79–82]. These discrepancies could be due to the source and dose of n-3 PUFAs, the duration of n-3 PUFA intake or the sufficient n-3 PUFA status of women at the beginning of trials that supports adequate infant neurodevelopment. According to our results, the beneficial effects of n-3 LC-PUFA supplementation may be due to increases in microglial DHA that could allow for an optimal development of neuronal network.

Acutely isolated microglial cells were composed of a large amount of PI/PS and PC. These classes of phospholipids classically contain higher amount of monounsaturated fatty acids (MUFAs) [22] in line with high level of MUFA observed in microglia cells. Thus, microglia displayed a specific phospholipid profile, since total brain phospholipids are mostly PE and PC [83]. PI and PS are key signaling molecules. PS is known to have anti-inflammatory effects on microglia by modulating the microglia phenotype and reducing pro-inflammatory mediator production and release [84,85]. PI is involved in the phosphorylated PI signaling cascade involved in many cellular functions such as cell signaling, membrane trafficking and survival [86]. PI through PIP2 signaling pathway, mediates microglial immune response [87]. These PI and PS signaling pathways could be highly activated in microglial cells to maintain healthy brain development involving maturation of synapses and microglial proliferation [36,88].

Microglial PI/PS content is inversely correlated to n-3 LC-PUFA supply. This result was unexpected since PS is a DHA carrier [4,22]. Moreover, Murthy et al. previously showed that the proportion of PS is lower in the hippocampus of n-3 PUFA deficient rats [89]. This discrepancy could be explained by fatty acid remodeling in the microglia phospholipids that could be fundamental for normal development [90]. The decrease in PI/PS was compensated by an increase in PC and a

tendency to PE increase. PE is one of the most DHA-enriched class of phospholipids. Increased levels of PE is in agreement with previous studies showing that maternal n-3 LC-PUFA supplementation increases DHA in PE in P14-old pups [22,23], supporting the hypothesis of DHA remodeling in microglial cell phospholipids.

In summary, our findings define the specific lipid composition of microglial cells in the developing brain. Maternal dietary n-3 PUFAs given from the first day of gestation until weaning modified fatty acid contents in microglial cells and changed the distribution of phospholipid classes. The consequences on microglial function during development and the cognitive and behavioral impact on offspring still need to be addressed.

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Declaration of interest

None.

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