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Spiro Khoury [a,b], Vanessa Soubeyre [a], Stéphanie Cabaret [a,b], Stéphane Grégoire [a], Esther Mézière [a,b], Elodie Masson [a], Xavier Grosmaitre [a], Lionel Bretillon [a], Olivier Berdeaux [a,b], Niyazi Acar [a], Anne Marie Le Bon [a,*]

[a] Centre des Sciences du Goût et de l'Alimentation, CNRS, INRAE, Institut Agro, Université Bourgogne Franche-Comté, F-21000 Dijon, France
[b] INRAE, PROBE Research Infrastructure, ChemoSens facility, F-21000 Dijon, France

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ABSTRACT

The olfactory mucosa (OM) and olfactory bulb (OB) are neuronal tissues that contribute to the early processing of olfactory information. They contain significant amounts of n-3 and n-6 polyunsaturated fatty acids (PUFAs), which are crucial for neuronal tissue development. In this study, we evaluated the impact of feeding mice diets that are either deficient in α-linolenic acid (ALA) or supplemented with n-3 long-chain PUFAs from gestation to adolescence on the phospholipid and ganglioside composition of these tissues. Both diets modified the levels of some phospholipid classes, notably the phosphatidylserine and phosphatidylethanolamine levels. In addition, the low-ALA diet enriched n-6 PUFAs in the main phospholipid classes of both tissues, while the diet supplemented with n-3 PUFAs enhanced the n-3 PUFA-containing phospholipid species level, mainly in OM. The diets also modulated the levels and profiles of several ganglioside classes in OM and OB. These modifications may have repercussions on the olfactory sensitivity.

1. Introduction

Polyunsaturated fatty acids (PUFAs) from the n-6 and n-3 families are essential for the development and normal growth of mammals. In addition to energy production through β-oxidation, PUFAs from these two series play crucial roles in the structure and function of cell membranes and the regulation of numerous signaling pathways. Linoleic acid (LA; 18:2n-6) and α-linolenic acid (ALA; 18:3n-3) are precursors for the long-chain n-6 and n-3 PUFAs, respectively. They cannot be synthesized by mammals and must be provided by the diet. Conversion of LA and ALA to longer and more desaturated PUFAs is achieved *via* a series of desaturation and elongation processes that occur in the endoplasmic reticulum and one step of β-oxidation within peroxisomes. From LA, these metabolic pathways produce many derivatives, including arachidonic acid (AA; 20:4n-6) and n-6 docosapentaenoic acid (n-6 DPA; 22:5n-6). For the n-3 family, conversion of ALA notably results in the formation of eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). Because n-3 PUFAs and n-6 PUFAs share the same metabolic pathways, there is competition between both series for their desaturation and elongation. The n-6 and n-3 PUFAs also compete for oxygenase enzymes that convert them into important bioactive compounds such as eicosanoids and specialized pro-resolving mediators [1, 2]. Oxidized compounds derived from n-6 and n-3 PUFAs have very similar molecular structures but markedly different biologic effects.

Abbreviations: AA, arachidonic acid; ACN, acetonitrile; ALA, α-linolenic acid; CAD, charged aerosol detector; CHCl3, chloroform; CH3OH, methanol; CON, control; DHA, docosahexaenoic acid; DMA, dimethyl acetal; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; ESI, electrospray ionization; FA, fatty acid; GG, ganglioside; H-ESI, heated electrospray ionization; HIGH, supplemented in long-chain n-3 PUFAs; HILIC, hydrophilic interaction liquid chromatography; HPLC, high performance liquid chromatography; LA, linoleic acid; LOW, deficient in α-linolenic acid; LPC, lysophosphatidylcholine; MS, mass spectrometry; MUFA, monounsaturated fatty acid; OB, olfactory bulb; OEC, olfactory ensheathing cell; OM, olfactory mucosa; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, phospholipid; PlsE, plasmenyl-ethanolamine; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; SM, sphingomyelin.

* Corresponding author.
E-mail address: anne-marie.le-bon@inrae.fr (A.M. Le Bon).

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Generally, n–6 PUFA derivatives promote inflammation, whereas n–3 PUFA derivatives have both anti-inflammatory and pro-resolving properties.

N-3 and n-6 PUFAs are particularly important for neuronal tissue development during gestation and childhood [3–5]. AA and DHA accumulate at high levels in the mammalian brain from the fetal stage to weaning [3,5-8]. AA, DHA and their derivatives are involved in the regulation of several developmental processes, such as cell differentiation, proliferation, neurogenesis, synaptogenesis and myelinization. The retina also requires high amounts of DHA for optimal visual development. DHA helps prevent cell apoptosis and oxidative damage, contributes to the phototransduction cellular mechanisms and helps develop and maintain photoreceptor membranes [9].

Because of their important physiological roles and their opposite and competitive properties, particular attention has been given to the dietary n-6:n-3 PUFA ratios over recent decades. The current Western diets are very rich in n-6 PUFAs and relatively deficient in n-3 PUFAs, which results in an average ratio of 15:1 [10]. This imbalance between n-6 and n-3 PUFA levels in conjunction with genetic factors has been associated with the prevalence of neurodevelopmental and metabolic diseases [11]. Animal studies have shown that rodents deficient in n-3 PUFAs during perinatal development have neuropathological, neurochemical and behavioral characteristics that reflect mood disorders [5], and n-3 PUFA supplementation can correct these alterations [12]. A lower visual acuity and irreversible abnormalities in electroretinograms were also reported in rhesus monkeys that were fed a diet low in ALA during the prenatal period [13].

Surprisingly, the impact of diets with imbalanced n-6:n-3 ratios on the physiology of olfactory tissues has received little attention thus far. The olfactory system consists of two closely interacting neuronal tissues: the olfactory mucosa (OM), which is located in the nasal cavity, and the olfactory bulb (OB), which is the first central relay before integration of olfactory information by higher centers of the brain. We have shown that like the brain and retina, mammal OM and OB contain high amounts of PUFAs. N-3 and n-6 PUFAs account for 13% and 23% of total fatty acids (FAs) in rodent OM, respectively [14]. In rat OB, the levels of n-3 and n-6 PUFA families are within the same range (20% and 16%, respectively) [15].

In a recent study, we assessed the effects of feeding mice during the perinatal and postweaning periods with diets containing an adequate level of fat (5%) but either deficient in ALA or supplemented in n-3 long chain PUFAs on OM physiology [16]. Both diets were found to significantly modify the FA composition and expression of various genes in the OM of 8-week-old male offspring. In addition, a decrease in electrophysiological responses of OM to odorant stimuli was observed in mice exposed to the ALA-deficient diet. To characterize the impact of these diets on the lipid composition of olfactory tissues, in the present study, we analyzed the molecular species profiles of phospholipids (PLs) and gangliosides (GGs) in OM and OB from male offspring using liquid chromatography coupled with electrospray ionization source-mass spectrometry (LC-ESI-MS). These lipid groups are important components of cell membranes. The length and unsaturation degree of esterified FAs into PLs are determinants of membrane biochemical and biophysical properties. In addition, PLs are important sources for various signaling molecules [17]. Regarding GGs, these glycosphingolipids contribute to axon stability and regeneration, synaptic plasticity, and cellular differentiation in neuronal tissues [18,19].

2. Methods

2.1. Chemicals

Chloroform ($CHCl_3$), methanol (CH_3OH), ammonium acetate, acetonitrile (ACN) and water (H_2O) of liquid chromatography–mass spectrometry grade were purchased from Fisher Scientific (Illkirch, France). Commercially available GG standards from natural sources (bovine or human) were obtained from Matreya LLC (State College, PA, USA) (Table S1). Commercially available PL standards were purchased from Avanti Polar Lipids INC - Coger (Paris, France) (Table S1). Other chemical reagents were obtained from Merck (St Quentin Fallavier, France).

2.2. Experimental diets

The diets were prepared by the Experimental Foods Preparation Unit (INRAE, Jouy-en-Josas, France). They were formulated according to AIN-93 G standards with 5% fat [20]. The composition of the diets was as follows (g/kg): fat, 50; casein, 200; corn starch, 418; sucrose, 100; maltodextrin, 132; cellulose, 50; mineral mix, 35; vitamin mix, 10; L-cystine, 3; choline bitartrate, 2.5. The formulations of the mineral and vitamin mixes are detailed in Simon *et al.* [21]. Commercial high-oleic sunflower, sunflower, palm, rapeseed and fish oils were mixed in various proportions to prepare lipid blends that were incorporated into different diets. Compared to the control (CON) diet, the low n-3 PUFA (LOW) diet contained less ALA, whereas the high n-3 PUFA (HIGH) diet contained n-3 long-chain PUFAs (EPA, n-3 DPA (22:5n-3) and DHA) (Table 1). Therefore, the CON, LOW and HIGH diets had n-6:n-3 ratios of ~6, 117 and 3, respectively. All diets were stored at +4 °C until given to the animals.

2.3. Animals

The experimental procedure was conducted in accordance with the guidelines of the European Community for the use and care of laboratory animals (2010/63/EU). It was approved by the local Ethics Committee (Comité d'Ethique de l'Expérimentation Animale Grand Campus Dijon) and the French Ministry for Research and Higher Education (reference

Table 1
Fatty acid composition of the experimental diets (% of total fatty acids). CON, control diet; LOW, low n-3 PUFA diet; HIGH, high n-3 PUFA diet. AA, arachidonic acid, ALA, α-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids.

	Experimental diets		
Fatty acids	**CON**	**LOW**	**HIGH**
12:0	0.15	0.15	0.14
14:0	0.53	0.60	1.32
15:0			0.12
16:0	13.32	16.00	9.44
17:0			0.14
18:0	4.62	4.65	4.31
20:0	0.40	0.28	0.45
22:0	0.43	0.58	0.50
24:0	0.16	0.21	0.15
Total SFAs	19.59	22.44	16.55
16:1n-9			0.10
16:1n-7	0.20	0.17	1.19
18:1 t	3.85	3.39	3.22
18:1n-9	54.32	55.81	51.74
18:1n-7	2.09	0.99	2.32
20:1n-9	0.52	0.17	0.66
22:1n-9	0.11		0.13
24:1n-9			0.10
Total MUFAs	61.08	60.52	59.43
18:2n-6 (LA)	16.49	16.91	17.62
20:4n-6 (AA)			0.14
Total n-6 PUFAs	16.49	16.91	17.76
18:3n-3 (ALA)	2.84	0.15	3.03
20:5n-3 (EPA)			1.97
22:5n-3 (n-3 DPA)			0.19
22:6n-3 (DHA)			1.08
Total n-3 PUFAs	2.84	0.15	6.27
Total PUFAs	19.33	17.05	24.03
n-6:n-3 ratio	5.80	116.73	2.83

APAFIS#23807-2020021909594039).

Twelve-week-old male and nulliparous female C57BL/6 mice were obtained from colonies established in the animal quarters of our laboratory (center des Sciences du Goût et de l'Alimentation, Dijon, France). They were maintained on a 12:12-h light-dark cycle and under constant temperature conditions (20–22 °C). Female mice were placed for 5 days with males for mating and fed ad libitum with one of the three experimental diets until the weaning of their litters. Then, weaned male pups were fed the same diets for 5 weeks. At 8 weeks of age, the young mice were deeply anesthetized by an intraperitoneal injection of ketamine and xylazine (150 mg/kg and 10 mg/kg bodyweight, respectively) and sacrificed by decapitation. The OM and OB were quickly dissected and snap-frozen in liquid nitrogen. Olfactory tissues were stored at −80 °C until further processing.

2.4. Lipid extraction

Total lipids from olfactory tissues were extracted with the monophasic extraction method as described by Khoury et al. [22]. Briefly, lipids were extracted from olfactory samples homogenized in H_2O overnight at 4 °C with 10 vol of $CHCl_3/CH_3OH$ (1:1, v/v). Then, the residual pellets obtained after centrifugation (1500 g, 5 min) were re-extracted with 3 mL $CHCl_3/CH_3OH$ (1:1, v/v), 3 mL $CHCl_3/CH_3OH$ (2:1, v/v) and 3 mL $CHCl_3/CH_3OH/H_2O$ (48:35:10, v/v/v). The four lipid extracts were combined, evaporated to dryness under a stream of nitrogen, redissolved in $CHCl_3/CH_3OH$ (1:1, v/v) and stored at −20 °C until further analyses.

2.5. Fatty acid analysis by gas chromatography

FAs were analyzed as described by Le Bon et al. [14]. Briefly, FAs were trans-methylated using boron trifluoride in methanol. Then, FA methyl esters were extracted with hexane and analyzed by gas chromatography coupled to flame ionization detection.

2.6. Separation and quantification of phospholipid classes by liquid chromatography coupled to charged aerosol detection (Corona-CAD)

The phosphorus content of the total lipid extracts was determined according to the method developed by Bartlett and Lewis [23], as previously described [24]. Then, the lipid extracts were diluted to the appropriate concentration of 500 μg/mL PLs in $CHCl_3/CH_3OH$ 1:1 (v/v) for analysis. PL classes (phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylcholine (PC), sphingomyelin (SM), and lysophosphatidylcholine (LPC)) were separated by high-performance liquid chromatography (HPLC) under hydrophilic interaction liquid chromatography (HILIC) conditions according to their increasing polarities. PL classes were detected and quantified using a Corona™ Ultra RS Charged Aerosol Detector™ (CCAD, Thermo Scientific, USA). For more details about the analysis method, see Le Bon et al. [14].

2.7. Structural analysis of phospholipids by liquid chromatography coupled to mass spectrometry

2.7.1. Characterization of phospholipid species

PL classes were separated by HPLC under HILIC conditions, and PL molecular species were characterized by coupling the chromatographic system to an Orbitrap Fusion™ Tribrid mass spectrometer (Thermo Scientific, USA) equipped with an EASY-Max NGTM ion source (heated electrospray ionization H-ESI). Positive and negative ions were alternatively monitored by a polarity switching approach coupled with MS/MS experiments. PL species were characterized based on high-accuracy MS data and MS/MS experiments using the LipidSearch™ software and LIPID MAPS™ database. The Orbitrap mass spectrometer was controlled by Xcalibur™ 4.1 software. The HILIC-(ESI-MS and MS/MS) conditions were completely described in our recent paper [25].

2.7.2. Quantification of phospholipid species

The HPLC system under HILIC conditions was coupled to a triple quadrupole mass spectrometer (Thermo Finnigan TSQ Quantum) equipped with a standard electrospray ionization source to quantify the PL molecular species. Specific acquisition methods in positive and negative ion modes were optimized and used according to the studied compounds. PC and SM species were quantified in the positive ion mode by precursor ion scanning of *m/z* 184 amu, which corresponds to the choline head group. PE species lose their ethanolamine phosphate head group as a neutral fragment of 141 Da. Therefore, the neutral loss scanning of 141 Da in the positive ion mode was used to selectively detect and quantify PE. Similarly, PS species lose their serine-phosphate head group as a neutral fragment of 185 Da. Therefore, neutral loss scanning of 185 Da in the positive ion mode was used to quantify PS compounds. The PI species showed in the negative ion mode a fragment at *m/z* 241 amu, which was identified as inositol phosphate minus one molecule of H_2O. This fragment was used for the precursor ion scanning to quantify these compounds. Plasmenyl-ethanolamine (PlsE) was quantified in the negative ionization mode by multiple reaction monitoring (MRM) of the parent/fragment transition for each selected plasmalogen. The amount of PL molecular species in each PL class was expressed relative to the total lipids in this class. The data were processed using the Xcalibur software. In addition, corrections were applied to the data for isotopic overlap. More details about the electrospray source parameters and mass spectrometer methods are available in Le Bon et al. [14].

2.8. Separation, characterization and quantification of gangliosides

GGs were separated from total lipid extracts by HPLC, under specific HILIC conditions, using a silica Kinetex column (150 mm x 2.1 mm inner diameter, 2.6 μm, Phenomenex, USA). The mobile phase composition and other parameters of the chromatographic method were described by Khoury *et al.* [22]. The injection volume was 10 μL. Then, GGs were analyzed by a triple quadrupole mass spectrometer (QqQ-MS) equipped with a heated electrospray ionization source. For MS experiments, GGs were analyzed in selected reaction monitoring in the negative ion mode. Sialic acid with *m/z* 290 was a characteristic and abundant fragment for all GG species in the negative ion mode. The proportion of each GG species in a specific GG class was calculated as the ratio of its peak area to the sum of all detected peak areas in this class, and every GG class was separately considered. For more details about the MS method, see our recent papers [14,22,26].

2.9. Statistical analyses

The values are expressed as the mean ± standard error of the mean (s.e.m.). Statistical analyses were performed using XLStat (Addinsoft). The nonparametric Mann–Whitney *U* test was used to compare the control group and experimental groups. Differences were considered significant at $p<0.05$. Heatmaps were generated using XLStat (Addinsoft).

3. Results

3.1. Olfactory mucosa

3.1.1. Phospholipid classes and molecular species

Analysis of the PL classes by HPLC—Corona-CAD showed that the PL profile in OM was affected by the experimental diets (Fig. 1A). Compared to the CON diet, the LOW diet significantly reduced the PS level (- 23%), while the HIGH diet significantly modified the levels of PI (- 6%), PE (+ 3%) and PS (- 16%) in OM.

Structural analyses using high-resolution MS were performed to

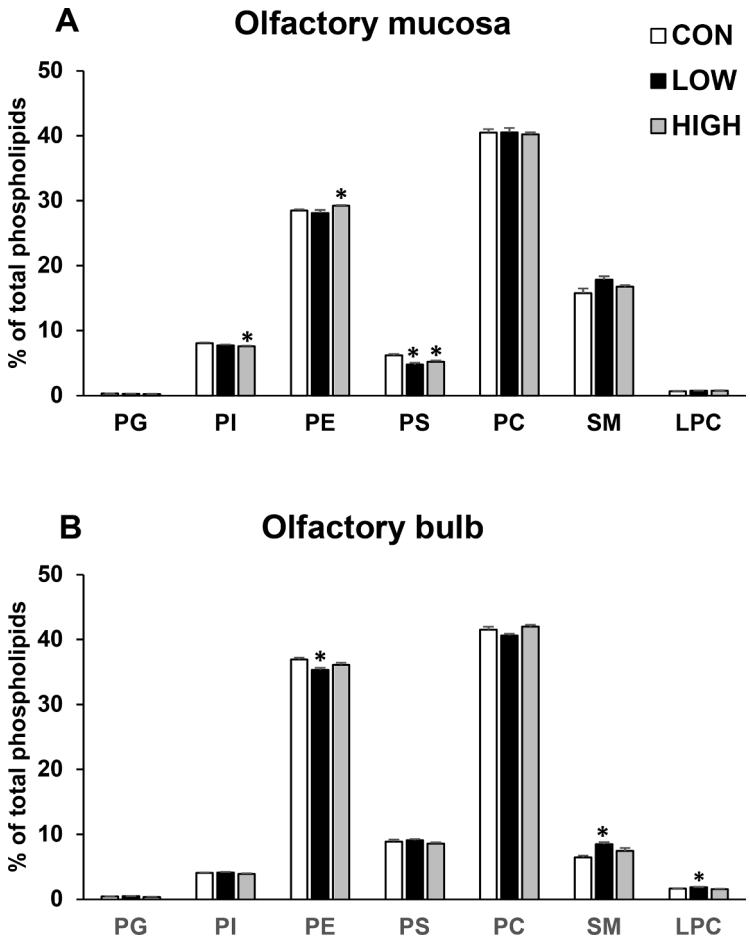

Fig. 1. Phospholipid classes in the mouse olfactory mucosa (OM) and olfactory bulb (OB). The data are expressed as the mean ± S.E.M. ($n = 5$ mice/group). * The values are significantly different from the CON group (Mann–Whitney U test, $p < 0.05$). PG, phosphatidylglycerol; PI, phosphatidylinositol; PE, phosphatidylethanolamine; PS, phosphatidylserine; PC, phosphatidylcholine; SM, sphingomyelin; LPC, lysophosphatidylcholine.

assess the impact of the n-3 PUFA unbalanced diets on the molecular species profiles of each PL class in OM. Compared to the CON group, significant increases in n-6 PUFA-containing species (including PC 16:0/20:4, PC 18:1/20:4 and PC 18:0/20:4) and significant decreases in n-3 PUFA-containing species (notably PC 16:0/22:6, PC 18:1/22:6 and PC 18:0/22:6) were observed in the PC class from the LOW group (Fig. 2A; Supplementary Table S2). In contrast, the HIGH diet significantly decreased the levels of PC species containing 20:4 and 22:4 PUFAs, whereas it led to a significant increase in PC16:0/20:5. The levels of the main molecular species of PC, i.e., PC 16:0/18:1 and PC 16:0/16:0, were not modified by the diets.

The concentrations of numerous PE and PlsE species, including the most represented ones, were modified by both diets (Fig. 2B; Supplementary Table S3). The LOW diet induced significant increases in several n-6 PUFA-containing PE/PlsE species (including PE 18:1/20:4, PE 18:0/20:4, PE 18:0/22:5; PE 18:0/22:4 and PE 16:0p/20:4) and significant decreases in n-3 PUFA-containing species (notably PE 16:0/22:6, PE 18:0/22:6 and PE 16:0p/22:6) in offspring OM. The HIGH diet was associated with opposite effects; a significant reduction in n-6 PUFA-containing PE/PlsE species and an increase in n-3 PUFA-containing PE/PlsE species (PE 16:0/20:5, PE 16:0p/22:6 and PE 18:1p:22:6) were observed.

Regarding the PI class, most of the main species were significantly affected by both diets (Fig. 2C; Supplementary Table S4). The LOW diet produced a significant increase in PI 18:0/20:4, PI 18:0/22:4, PI 16:0/16:0 and PI 18:0/16:0, and a decrease in PI 16:0/22:6 and PI 18:0/22:6. The HIGH diet significantly reduced the level of PI 18:0/20:4 and increased the amounts of n-3 PUFA-containing PI species (PI 16:0/22:6, PI 18:0/20:5 and PI 18:0/22:6).

In the PS class, the LOW diet significantly affected the levels of several species (Fig. 2D; Supplementary Table S5); an increase in n-6 PUFA-containing species (PS 16:0/22:5, PS 18:0/20:4 and PS 18:0/22:4) and a decrease in n-3 PUFA-containing species (PS 16:0/22:6 and PS 18:0/22:6) were observed. In contrast, the HIGH diet only decreased the n-6 PUFA-containing PS species (PS 16:0/20:4, PS 16:0/22:5 and PS 18:0/20:4). The levels of n-3 PUFA-containing PS species in OM were not affected by the HIGH diet.

Regarding the SM class (Fig. 2E; Supplementary Table S6), an increase in SM (d34:1) and a decrease in SM (d42:1) were significantly induced by the LOW diet, while the HIGH diet significantly reduced the level of SM (d36:2).

Finally, analysis of the LPC species showed a significant reduction in LPC 20:4 in OM from the LOW group (Fig. 2F; Supplementary Table S7).

3.1.2. Ganglioside classes and molecular species

Consumption of the LOW and HIGH diets was associated with significant modifications in the profiles of GG classes in OM (Fig. 3A). Compared to the CON group, the LOW diet provoked a significant increase in the GD1a class (x 2.5). The HIGH diet also caused a substantial increase in the GD1a class (x 3.9). In addition, significant increases in GM4 and GD1b (x 2.2 and x 14.4, respectively) were observed in OM. The results showed a trend for a lower level of GM3 class in OM from the HIGH group (- 27%; $p = 0.06$).

Analysis of GG molecular species in each GG class revealed that the

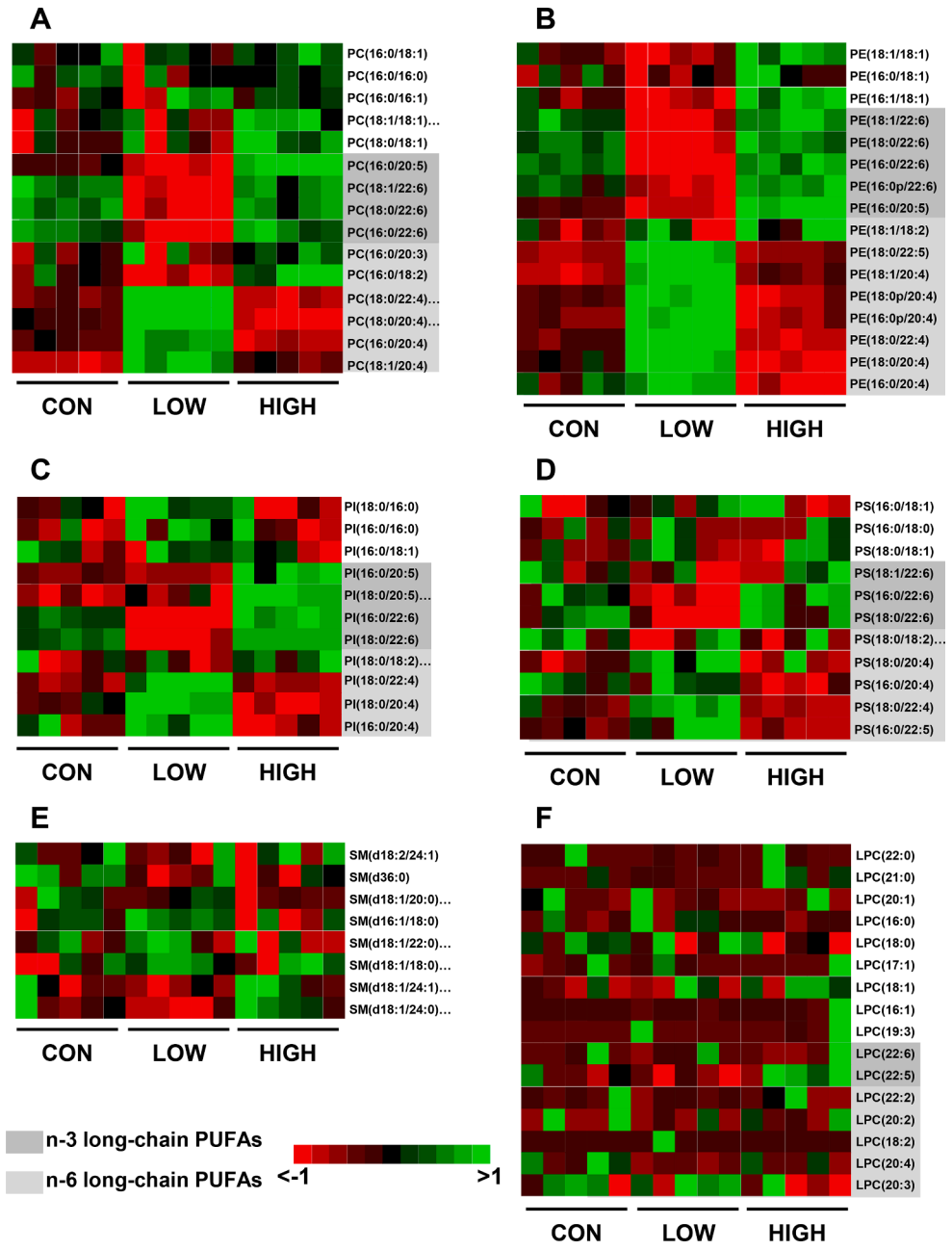

Fig. 2. Heatmaps of phospholipid molecular species in the mouse olfactory mucosa (OM) as a function of the dietary interventions (CON, LOW and HIGH diets). PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin; LPC lysophosphatidylcholine. A variability threshold (interquartile range < 0.25) was applied prior to heatmap analyses. All raw data can be found in Supplementary Tables S2–S7.

HIGH diet induced many changes in this lipid group, while the LOW diet had limited effects (Fig. 4). Concerning the GM3 class, the most important GG class in OM, the HIGH diet provoked a significant reduction in three species (34:1, 36:1 and 38:1), while a significant increase in 42:1 was observed. The LOW diet significantly enhanced the 42:2 level in this GG class. Concerning the GM4 class, the HIGH diet significantly decreased the levels of 41:0, 43:1, 43:0 and 44:1 and increased the level of 40:1. In addition, the HIGH diet significantly reduced the level of 36:4 in the GT1b class, and the LOW diet significantly reduced 36:1 in the GD1b class.

3.2. Olfactory bulb

3.2.1. Fatty acid composition of total lipids

The LOW diet and HIGH diet did not affect the levels of total saturated FAs (SFAs), monounsaturated FAs (MUFAs) and PUFAs in the offspring OB (Table 2). However, compared to the CON group, the percentages of n-6 and n-3 PUFAs were significantly modified in the OB of young mice exposed to unbalanced diets. An important increase in n-6 PUFAs (+ 50%) and a strong reduction in n-3 PUFAs (- 46%) were observed in the LOW group. Therefore, the n-6:n-3 ratio was significantly enhanced (x 2.8). The OB from the LOW mice had significantly higher levels of AA (+ 14%), 22:4n-6 (+ 35%) and n-6 DPA (x 11.8) than the OB of the CON mice. Conversely, the levels of EPA, n-3 DPA and DHA strongly decreased (−38%, −65% and −46%, respectively). Compared to the CON group, the BO unsaturation index was significantly reduced in the LOW group (−7%) (Table 3). The HIGH diet led to the opposite effects (Table 2). Compared to the CON group, the level of total n-6 PUFAs in the OB of the HIGH group decreased by −17%. A slight but nonsignificant increase in total n-3 PUFAs was also observed. These

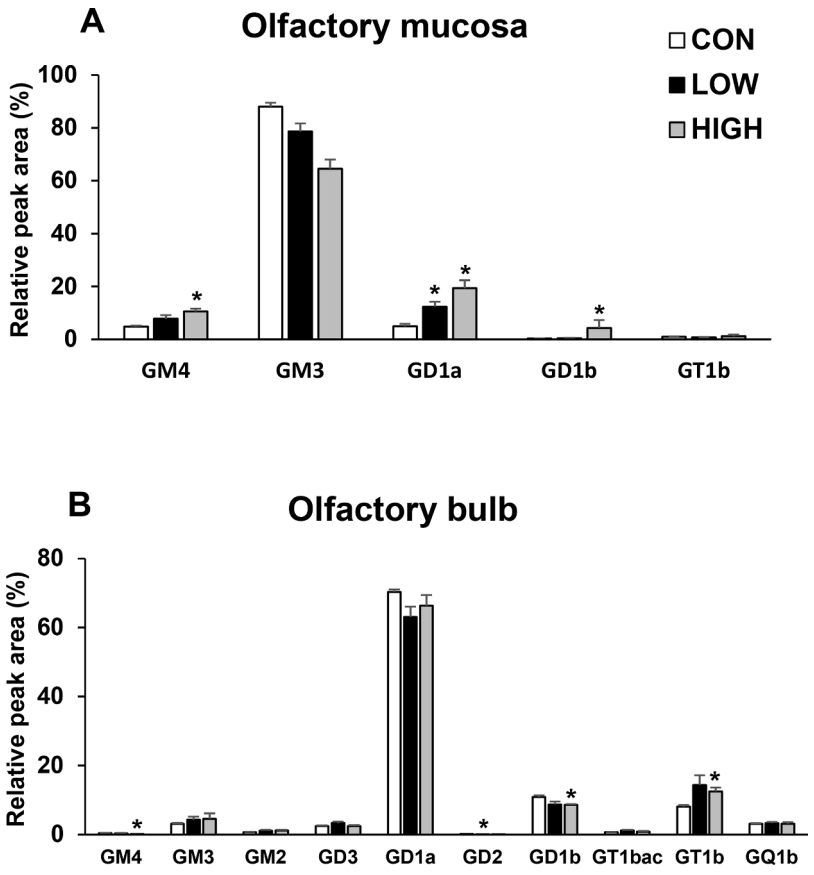

Fig. 3. Ganglioside classes in the mouse olfactory mucosa (OM) and olfactory bulb (OB). The data are expressed as the mean ± S.E.M. ($n = 5$ mice/group). * The values are significantly different from the CON group (Mann–Whitney U test, $p < 0.05$).

modifications significantly reduced the n-6:n-3 ratio (- 22%). The main modifications in the OB of the HIGH group included a decrease in 22:5n-6 (- 70%) and 22:4n-6 (- 30%) and an increase in EPA and n-3 DPA (x 2.4 and x 1.94, respectively). The BO unsaturation index was not affected by the HIGH diet (Table 3).

3.2.2. *Phospholipid classes and molecular species*

PC and PE were the most abundant PL classes in the offspring OB. The PL class profile was significantly modulated by the LOW diet (Fig. 1B). Compared to the CON diet, the LOW diet enhanced the levels of SM (+ 31%) and LPC (+ 13%) and slightly reduced the PE level (- 4%). The HIGH diet did not change the distribution of PL classes.

Structural analyses show that compared to the CON diet, the LOW diet significantly increased the n-6 PUFA-containing PC species (including PC 16:0/20:4, PC 18:1/20:4, PC 18:0/20:4 and/or PC 16:0/22:4) and decreased the DHA-containing species (notably PC 16:0/22:6 and PC 18:0/22:6) in offspring OB (Fig. 5A; Supplementary Table S8). The HIGH diet significantly reduced the levels of n-6 PUFA-containing species (PC 16:0/20:4 and PC 18:0/20:4 and/or PC 16:0/22:4) and had no impact on the levels of n-3 PUFA-containing species. As observed in the OM, the levels of the main PC molecular species detected in the OB (PC 16:0/16:0 and PC 16:0/18:1) were not affected by the experimental diets.

In the PE/PlsE class (Fig. 5B; Supplementary Table S9), the LOW diet induced a significant increase in many n-6 PUFA-containing species (notably PE 18:1/20:4, PE 18:0/20:4, PE 18:0/22:5 and PE 18:0/22:4) and decreased the levels of species containing DHA (PE 16:0/22:6, PE 18:1/22:6 and PE 18:0/22:6) in OM. The HIGH diet led to a significant reduction in several n-6 PUFA-containing species, but this diet did not change the levels of n-3 PUFA-containing species.

Regarding the PI class, both diets were associated with few changes (Fig. 5C; Supplementary Table S10). The LOW diet significantly reduced the levels of two DHA-containing species (PI 16:0/22:6 and PI 18:0/22:6), while the HIGH diet slightly reduced the PI 16:0/20:4 level:

In the PS class (Fig. 5D; Supplementary Table S11), the levels of n-6-containing species (PS 16:0/22:5, PS 18:0/20:4 and PS 18:0/22:4) increased in the OB from the LOW group. The HIGH diet reduced PS 18:1/22:6 and increased PS 18:0/18:1.

In the SM class, a significant decrease in two minor species (SM (d36:2) and SM (d42:3)) was induced by the LOW diet (Fig. 5E; Supplementary Table S12). The HIGH diet provoked a significant increase in SM (d36:1), which is the major SM species in OB, and a decrease in two minor SM species (SM (d42:3) and SM (d42:1)).

Finally, the levels of two minor LPC species (LPC 20:1, LPC 22:2) significantly decreased in the OB from the LOW group, while the level of LPC 22:0 increased due to this diet (Fig. 5F; Supplementary Table S13).

3.2.3. *Ganglioside classes and molecular species*

Unlike OM, GD1a is the most prominent GG class in OB. This class was unaffected by the diets, which were associated with modifications on other GG classes. The LOW diet significantly reduced the GD2 level (−36%) compared to the CON group. The HIGH diet elicited a significant increase in GT1b class (x 1.5) and a decrease in GD1b and GM4 classes (- 20% and - 56%, respectively) (Fig. 3B).

Analysis of GG molecular species shows that both diets significantly changed the profiles of different GG classes (Fig. 6). Even if the GD1a class was not impacted by the diets, modifications were observed in the distribution of certain molecular species in this class. The LOW diet increased the 36:1 level in GD1a and GT1b and decreased the 38:1 level in GD1a and the 38:2 and 40:4 levels in GT1b. Concerning the HIGH diet, a significant increase in 36:1 and 36:0 was observed in the GT1b and GQ1b classes, respectively. The HIGH diet also decreased many

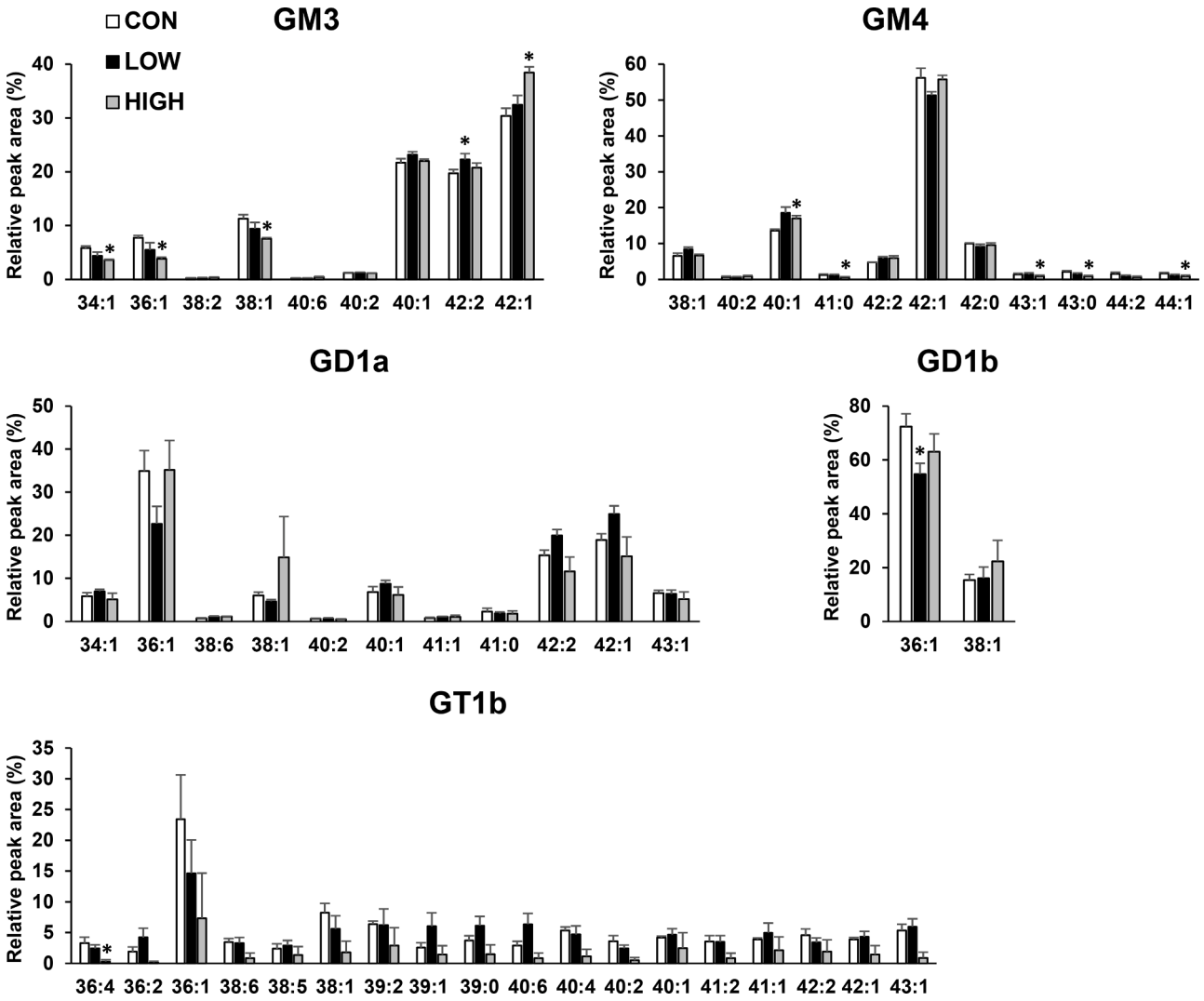

Fig. 4. Molecular species composition of the ganglioside classes in the mouse olfactory mucosa (OM). The percentage of each ceramide species is expressed relative to the sum of all detected species in its specific ganglioside class, and every ganglioside class was separately considered. The values are means ± sems ($n = 5$ mice/group). * The values are significantly different from the CON group (Mann–Whitney U test, $p < 0.05$).

species in several GG classes (42:1 in GM3, 41:0 in GM4, 36:2 and 41:1 in GD1a, 38:2 and 40:4 in GT1b, 39:1 in GQ1b). Interestingly, both diets induced similar effects in the GT1b class (increase in 36:1 and decrease in 38:0 and 40:4).

4. Discussion

In the present study, we evaluated the impact of feeding mice with n-3 PUFA unbalanced diets during the key periods of development (gestation, lactation, childhood and adolescence) on the PL and GG molecular profiles of male offspring olfactory tissues. Unbalanced diets contained an adequate LA amount (1.83% of energy) [27] and were either deficient in ALA (n-6:n-3 ratio= 116.7) or supplemented in n-3 long chain PUFAs (n-6:n-3 ratio= 2.8). The effects of these diets were compared to those of a control diet with an n-6:n-3 ratio of 5.8.

Our results show that consuming both diets significantly modified the levels of some PL classes in OM and OB. In particular, the ALA-deficient diet decreased the PS and PE levels in OM and OB, respectively. This finding is consistent with observations made by Hamilton et al. [28], who reported a significant reduction in the PS class in neuronal tissues (including OB) from rats fed an n-3 PUFA-deficient diet for 2 generations. In mammalian tissues, PE and PS are metabolically related [29]. PS can be synthesized from pre-existing molecules of PE *via* a base-exchange reaction between the polar head-group of PE (ethanolamine) and L-serine. As a consequence, the observed decrease in PE in OB from the LOW group can decrease the PS level in the long term. In addition, PS precursors containing long chain n-6 PUFAs are not as good substrates as DHA-containing precursors for the PS synthesis by brain microsomes [30]. This particularity would be responsible for the observed decrease in PS levels in neuronal tissues under n-3 PUFA deficiency conditions [28,31,32]. In the present study, we observed that the LOW diet induces significant increases in n-6 PUFA-containing species in PE and PC, which are the precursors of PS; our finding is consistent with this hypothesis. The qualitative and quantitative modifications induced by the LOW diet in the PS class may negatively affect the olfactory tissue physiology. Indeed, PS participates in key signaling pathways in neuronal survival and differentiation [33]. PS also modulates the properties of numerous synaptic receptors and proteins in neuronal membranes [33]. We observed that the HIGH diet decreased both PS and PI levels and enhanced the PE level in OM. These effects are not fully consistent with a previous study showing that gerbils fed EPA or DHA (by oral gavage) had significantly increased brain levels of PE, PS and PI compared with the control group [34]. These divergences may be due to differences in analyzed tissue, experimental model, age of the animals and/or administration method of n-3 PUFAs in this study.

The PL structural analysis by LC–MS shows that most changes

Table 2
Fatty acid composition of the olfactory bulb (% of total fatty acids). Data are expressed as the mean ± s.e.m. ($n = 5$ mice/group). * The values are significantly different from the CON group (Mann–Whitney U test, $p < 0.05$). DMAs, dimethyl acetals; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids.

Fatty acids	CON			LOW			HIGH		
14:0	0.12	±	0.01	0.16	±	0.02	0.15	±	0.01
15:0	0.05	±	0.00	0.05	±	0.00	0.05	±	0.00
dma16:0	3.45	±	0.08	3.43	±	0.03	3.52	±	0.07
16:0	23.77	±	0.46	25.02	±	0.61	24.76	±	0.64
16:1n-9	0.20	±	0.01	0.24*	±	0.01	0.21	±	0.01
16:1n-7	0.62	±	0.02	0.69*	±	0.02	0.71*	±	0.01
17:0	0.12	±	0.00	0.12	±	0.00	0.12	±	0.00
dma18:0	2.32	±	0.09	2.06*	±	0.06	2.14	±	0.06
dma18:1n-9	0.84	±	0.03	0.78	±	0.02	0.78	±	0.01
dma18:1n-7	0.75	±	0.02	0.77	±	0.02	0.71	±	0.02
18:0	19.75	±	0.20	19.77	±	0.17	20.17	±	0.30
18:1t	0.03	±	0.00	0.04*	±	0.00	0.03	±	0.00
18:1n-9	13.78	±	0.14	13.12*	±	0.15	13.85	±	0.09
18:1n-7	4.07	±	0.07	4.50*	±	0.08	3.96	±	0.05
18:2n-6	0.54	±	0.02	0.50	±	0.02	0.64*	±	0.02
20:0	0.25	±	0.01	0.22*	±	0.01	0.24	±	0.01
18:3n-6	0.03	±	0.00	0.03	±	0.00	0.02	±	0.00
20:1n-9	0.70	±	0.01	0.63*	±	0.02	0.68	±	0.03
18:3n-3	0.22	±	0.00	0.22	±	0.01	0.22	±	0.01
20:2n-6	0.10	±	0.00	0.10	±	0.00	0.10	±	0.00
20:3n-9	0.16	±	0.01	0.17	±	0.00	0.19*	±	0.01
22:0	0.19	±	0.01	0.14*	±	0.00	0.16	±	0.01
20:3n-6	0.41	±	0.01	0.26*	±	0.01	0.52*	±	0.01
22:1n-9	0.60	±	0.11	0.44	±	0.11	0.65	±	0.11
20:4n-6	8.96	±	0.19	10.25*	±	0.15	7.48*	±	0.10
20:5n-3	0.13	±	0.01	0.08*	±	0.00	0.31*	±	0.02
24:0	0.21	±	0.02	0.16*	±	0.00	0.18	±	0.01
24:1n-9	0.32	±	0.02	0.26	±	0.01	0.28	±	0.02
22:4n-6	1.77	±	0.04	2.38*	±	0.07	1.25*	±	0.04
22:5n-6	0.45	±	0.01	5.31*	±	0.18	0.14*	±	0.01
22:5n-3	0.17	±	0.00	0.06*	±	0.00	0.33*	±	0.01
22:6n-3	14.90	±	0.31	8.03*	±	0.19	15.44	±	0.42
SFAs	44.46	±	0.43	45.65	±	0.73	45.85	±	0.69
MUFAs	20.32	±	0.20	19.93	±	0.27	20.37	±	0.20
PUFAs	27.85	±	0.47	27.37	±	0.39	26.64	±	0.53
DMAs	7.36	±	0.08	7.05*	±	0.08	7.15	±	0.04
Total n-3 PUFAs	15.43	±	0.31	8.39*	±	0.19	16.30	±	0.42
Total n-6 PUFAs	12.27	±	0.23	18.81*	±	0.38	10.15*	±	0.12
n-6:n-3 ratio	0.80	±	0.02	2.25*	±	0.07	0.62*	±	0.01

Table 3
Contribution of long-chain n-6 PUFAs to the reciprocal replacement of DHA in olfactory tissues. The data are expressed as the mean ± s.e.m. ($n = 5$ mice/group). *: The values are significantly different from the CON group (Mann–Whitney U test; $p < 0.05$). [a] Original data were published in Soubeyre et al. [16]. [b] Sum of the percent unsaturated fatty acids multiplied by their number of double bonds.

Olfactory mucosa[a]	**CON**			**LOW**			**HIGH**		
DHA + 22:5n-6	14.1	±	0.54	**10.25**	±	0.93*	14.30	±	1.09
DHA + 22:5n-6 + 22:4n-6	14.94	±	0.60	**11.88**	±	1.01*	14.77	±	1.13
DHA + 22:5n-6 + 22:4n-6 + AA	27.14	±	1.33	28.40	±	1.30	**23.69**	±	1.51*
Unsaturation index[b]	170.71	±	3.51	159.96	±	2.16	165.80	±	4.07
Olfactory bulb									
DHA + 22:5n-6	15.35	±	0.71	**13.33**	±	0.49*	15.58	±	0.93
DHA + 22:5n-6 + 22:4n-6	17.12	±	0.73	**15.71**	±	0.60*	16.83	±	1.00
DHA + 22:5n-6 + 22:4n-6 + AA	26.08	±	1.03	25.96	±	0.87	24.30	±	1.21
Unsaturation index	161.75	±	2.33	**150.61**	±	**2.11***	157.63	±	3.08

induced by the LOW and HIGH diets in the olfactory PL classes of 8-week-old mice were similar to those observed at 3 weeks of age [35]. Thus, modifications that occur during early development persist in young adult mice. The main differences between both stages were related to some PS species (PS 16:0/22:6(n-3) and PS 18:0/20:4(n-6)), which were significantly modulated in the LOW group at 8 weeks of age. In most olfactory PL classes, the LOW diet led to the incorporation of n-6 PUFAs to the detriment of n-3 PUFAs. These observations are consistent with the concept of reciprocal replacement, which claims that long chain n-6 PUFAs such as n-6 DPA can replace DHA in nervous system tissues [36,37]. Because n-6 DPA has an identical structure to DHA except for the absence of a double bond at the n-3 position, this FA contributes to maintaining an optimal amount of highly unsaturated FA. However, n-6 DPA does not completely replace DHA in the rat brain cortex and retina during early developmental periods [38,39]. In the cortex, the complete reciprocal replacement of DHA with n-6 DPA occurs after postnatal day 20, while in the retina, this phenomenon was observed after postnatal day 50. Before this developmental stage, the reciprocal replacement of DHA was complete in the retina when both n-6 DPA and 22:4n-6 were summed with DHA. Analyses of total FAs in the OM [16] and OB (present study) show that the reciprocal replacement of DHA with n-6 DPA in mouse olfactory tissues was not complete at the age of 8 weeks (i.e., 56 days) (Table 3). In addition to n-6 DPA, 22:4n-6 and AA contribute to the reciprocal replacement of DHA in

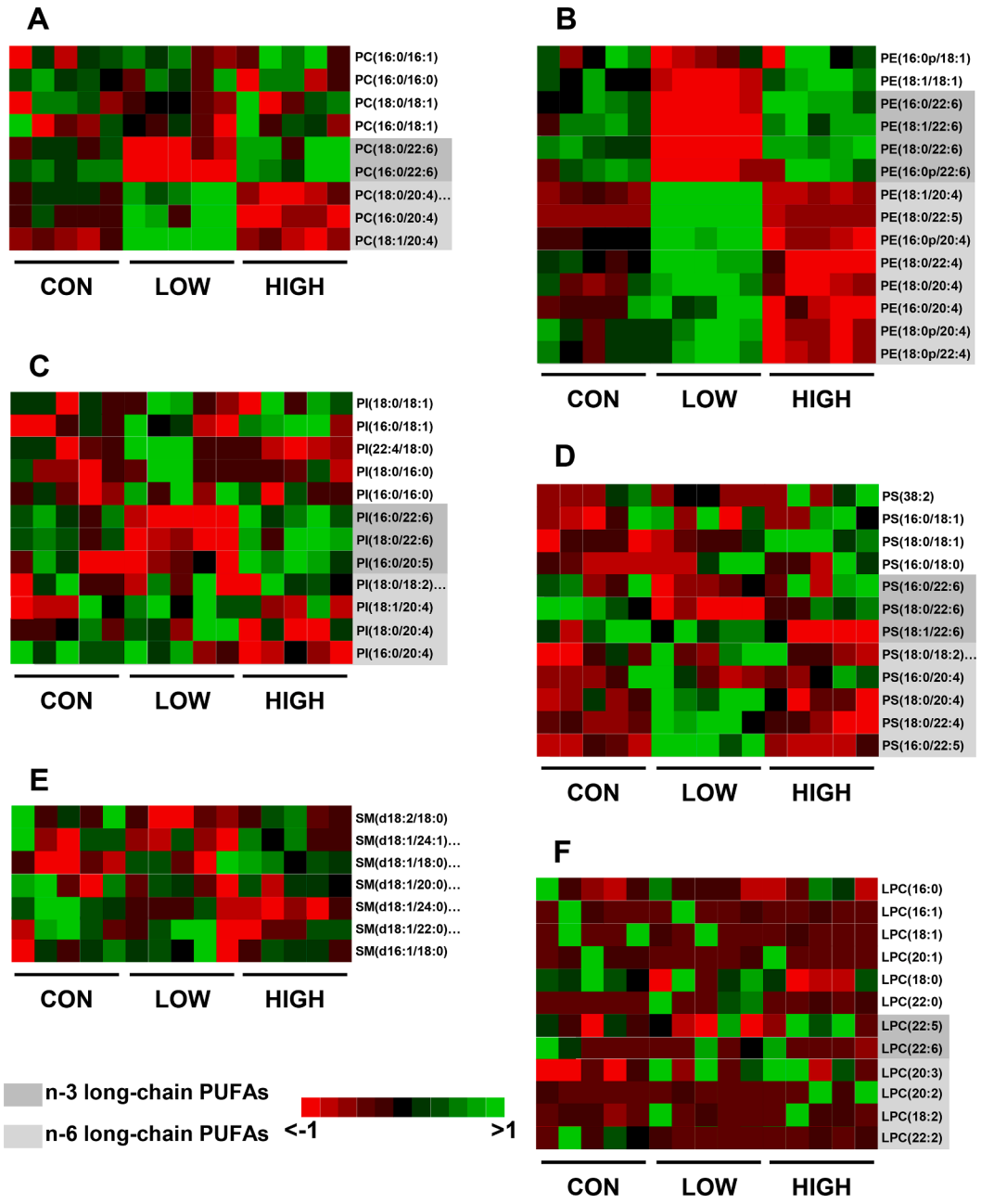

Fig. 5. Heatmaps of phospholipid molecular species in the mouse olfactory bulb (OB) as a function of the dietary interventions (CON, LOW and HIGH diets). PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin; LPC lysophosphatidylcholine. A variability threshold (interquartile range < 0.25) was applied prior to the heatmap analyses. All raw data can be found in Supplementary Tables S8-S13.

olfactory tissues at this stage of development, which is comparable to events that occur in the retina [39]. Furthermore, FA analyses revealed that the LOW diet significantly reduced the unsaturation index in OB (Table 3). This index also tends to decrease in OM from the LOW group. These findings indicate that although long chain n-6 PUFAs balanced the reduction in DHA levels, an overall deficiency in the total number of unsaturated bonds remained in the olfactory tissues of mice fed the LOW diet. The OM and OB unsaturation indexes of the HIGH group are similar to the CON group, which implies that the number of double bonds was not affected by the HIGH diet. However, in our previous study [16], we showed that the HIGH diet significantly reduced the AA, 22:4n-6 and 22:5n-6 levels in OM. Meanwhile, the HIGH diet enhanced the n-3 PUFA level (especially EPA and n-3 DPA) in OM [16]. Therefore, the enrichment in n-3 PUFA counterbalances the loss of n-6 PUFAs induced by the HIGH diet, which allows the maintenance of an adequate level of double bonds in OM membranes.

Our data show that in OM, the HIGH diet significantly promoted the incorporation of EPA or DHA in several PC, PE, PlsE and PI species. In OB, we observed that the HIGH diet had less impact on the n-3 PUFA-containing species levels. This discrepancy may be due to a differential distribution of n-3 PUFAs in these tissues. One possible explanation is that the absorption of long-chain n-3 PUFAs by OB is less efficient because the blood–brain barrier regulates FA transport into the brain. The endothelium that constitutes the blood–brain barrier contains a specific transporter, Mfsd2a, which preferentially transports DHA in the form of LPC [40]. Consistent with this observation, recent in vivo studies reported a greater increase in brain DHA in rodents supplemented with DHA-LPC than in those supplemented with free DHA, PL-DHA or DHA-triacylglycerol [41,42]. In our study, fish oil containing EPA and DHA in the form of triacylglycerol was employed to prepare the HIGH

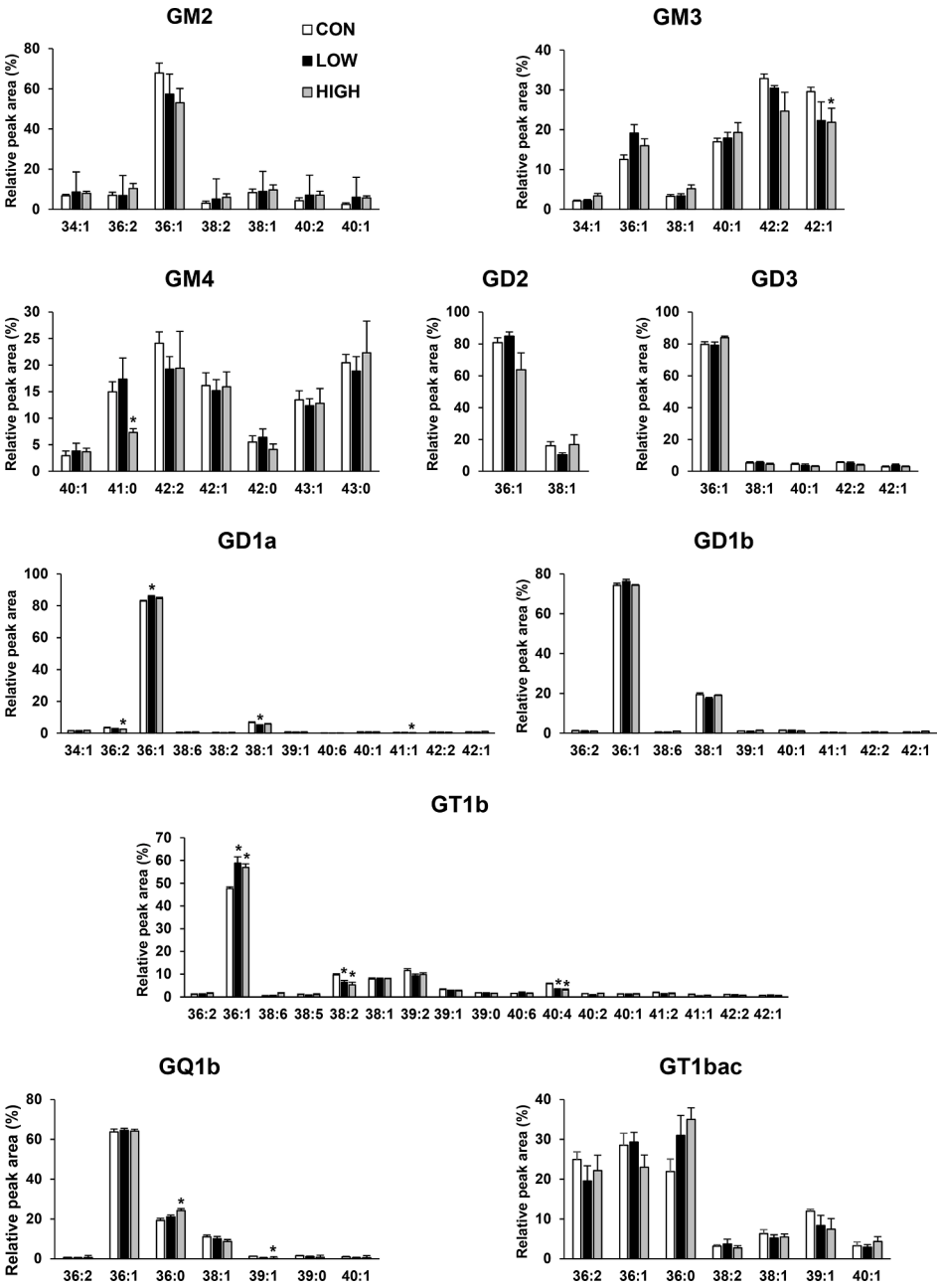

Fig. 6. Molecular species composition of the ganglioside classes in the mouse olfactory bulb (OB). The percentage of each ceramide species is expressed relative to the sum of all detected species in its specific ganglioside class, and every ganglioside class was separately considered. The values are means ± sems ($n = 5$ mice/group). * The values are significantly different from the CON group (Mann–Whitney U test, $p < 0.05$).

diet. This form is probably not the most effective for enriching barrier-protected tissues with n-3 PUFAs, as shown by others [43,44].

In the present study, we also analyzed the impact of diets unbalanced in n-3 PUFAs on the GG composition in the offspring OM and OB. GGs are sialic acid-containing glycosphingolipids. The oligosaccharide chains of GGs are based on a combination of glucose, galactose and *N*-acetylgalactosamine. One to six sialic residues can be linked to galactose. This results in a great diversity of GG classes [45]. GGs are ubiquitously expressed in mammalian tissues and particularly abundant in the brain and nervous system, where they participate in the regulation of many cellular functions, including neural differentiation and maturation and signal transduction [19,46-48]. The oligosaccharide chain of GGs is known to modulate GG interactions with extracellular environment and functional membrane components to give each GG class its specific roles in cell signaling [49]. Modifications of the dietary n-6:n-3 ratio affect the GG level in rodent brain [50]. In addition, a diet supplementation with AA and DHA for 2 weeks increases the proportion of GD3 in rat retina [51]. In the present study, we observed that dietary n-3 intake also influenced the GG composition of olfactory tissues. We found that the consumption of both LOW and HIGH diets significantly enhanced the GD1a level in offspring OM. The HIGH diet was also associated with significant increases in GD1b and GM4 levels in this tissue. In the mammalian brain, GD1a and GD1b are involved in the regulation of neuronal development and differentiation [52,53]. This finding suggests that diets unbalanced in n-3 PUFAs may affect the development of OSNs localized in OM. The HIGH diet and, to a lesser extent, the LOW diet also modulated the levels of some GG classes in OB. Notably, the HIGH diet decreased GD1b and increased GT1b. Similar to GD1a and GD1b, GT1b contributes to the regulation of neuron growth [52]. The changes in GG pattern in OM and OB may result from a dysfunction of enzymes that are

responsible for their synthesis, i.e., GG synthases [54].

The physicochemical properties of GGs depend on the structure of their polar head group and ceramide moiety. The latter, which is responsible for anchoring GGs into the membrane, mainly contains SFAs associated with sphingosine, but MUFAs have also been identified. The high occurrence of SFAs in GGs strongly favors the nanodomain formation in membranes [46]. In the present study, LC–MS analyses of GG molecular species show that in OM, the HIGH diet enhanced the levels of ceramides 42:1 and 40:1 in the GM3 and GM4 classes, respectively. In OB, both diets increased ceramide 36:1 level in the GD1a and GT1b classes. While the significance of such changes is unknown, one can hypothesize that they may modify the membrane organization with repercussions on cellular functions. In the olfactory system, GGs have been shown to play a significant role in the migration and behavior of olfactory ensheathing cells (OECs) [55]. OECs are specialized glial cells that wrap the unmyelinated olfactory axons in the peripheral and central olfactory systems [56]. These cells are believed to play a role in the continuous production of olfactory neurons [57]. GGs have been detected in OEC lipid rafts, which are membrane domains involved in a wide range of important cellular processes such as signal transduction, apoptosis, cell adhesion and migration [55,58]. Contrary to PLs, GGs do not contain PUFAs. Hence, the effects of n-3 PUFA modulation on the FA content of GGs would be indirect, as suggested in a study showing an impact of the membrane PL composition on the ceramide-specificity of GM3 synthase [59].

In conclusion, the present study demonstrates that consumption of diets either deficient or supplemented in n-3 PUFAs during critical stages of development significantly affects the levels and composition of PLs and GGs in male mouse olfactory tissues. These modifications may have significant repercussions on membrane properties, peripheral neuronal signaling and cellular homeostasis. In the long term, an n-3 PUFA-deficient diet may impair olfactory function. Indeed, we observed a relationship between high levels of 20:4n-6 in human nasal tissues and olfactory deficiency [25]. Meanwhile, there is evidence that n-3 PUFA supplementation can improve the olfactory scores in patients who suffer from post-operative or post-covid olfactory dysfunctions [60,61]. Further studies are required to elucidate the mechanisms that regulate the incorporation of PUFAs into the OM and OB and to understand their functions in these tissues.

Supplementary materials

Supplementary material associated with this article can be found in the online version.

Author contributions

Spiro Khoury: Investigation, Data curation, Visualization, Writing - Original Draft. Vanessa Soubeyre: Investigation. Stéphane Grégoire: Investigation. Stéphanie Cabaret: Investigation. Esther Mézière: Investigation. Elodie Masson: Writing - Review & Editing. Xavier Grosmaitre: Conceptualization; Funding acquisition, Resources. Lionel Bretillon: Conceptualization, Funding acquisition, Resources. Olivier Berdeaux: Conceptualization, Project administration, Funding acquisition, Resources, Writing - Original Draft. Niyazi Acar: Conceptualization, Project administration, Funding acquisition, Resources, Writing - Original Draft. Anne Marie Le Bon: Conceptualization; Supervision, Funding acquisition, Resources, Formal analysis, Writing - Original Draft. All authors read and approved the final manuscript.

Declarations of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.plefa.2023.102556.

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