Dietary n-3 long chain PUFA supplementation promotes a pro-resolving oxylipin profile in the brain

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Abstract

The brain is highly enriched in long chain polyunsaturated fatty acids (LC-PUFAs) that display immunomodulatory properties in the brain. At the periphery, the modulation of inflammation by LC-PUFAs occurs through lipid mediators called oxylipins which have anti-inflammatory and pro-resolving activities when derived from n-3 LC-PUFAs and pro-inflammatory activities when derived from n-6 LC-PUFAs. However, whether a diet rich in LC-PUFAs modulates oxylipins and neuroinflammation in the brain has been poorly investigated. In this study, the effect of a dietary n-3 LC-PUFA supplementation on oxylipin profile and neuroinflammation in the brain was analyzed. Mice were given diets deficient or supplemented in n-3 LC-PUFAs for a 2-month period starting at post-natal day 21, followed by a peripheral administration of lipopolysaccharide (LPS) at adulthood. We first showed that dietary n-3 LC-PUFA supplementation induced n-3 LC-PUFA enrichment in the hippocampus and subsequently an increase in n-3 PUFA-derived oxylipins and a decrease in n-6 PUFA-derived oxylipins. In response to LPS, n-3 LC-PUFA deficient mice presented a pro-inflammatory oxylipin profile whereas n-3 LC-PUFA supplemented mice displayed an anti-inflammatory oxylipin profile in the hippocampus. Accordingly, the expression of cyclooxygenase-2 and 5-lipoxygenase, the enzymes implicated in pro- and anti-inflammatory oxylipin synthesis, was induced by LPS in both diets. In addition, LPS-induced pro-inflammatory cytokine increase was reduced by dietary n-3 LC-PUFA supplementation. These results indicate that brain n-3 LC-PUFAs increase by dietary means and promote the synthesis of anti-inflammatory derived bioactive oxylipins. As neuroinflammation plays a key role in all brain injuries and many neurodegenerative disorders, the present data suggest that dietary habits may be an important regulator of brain cytokine production in these contexts.

Highlights

1. Dietary n-3 LC-PUFA supplementation modulates hippocampal oxylipins profile.
2. Dietary n-3 LC-PUFA supplementation decreases hippocampal pro-inflammatory cytokines induced by LPS.

3. Oxylipin profile depending on dietary intake could orchestrate inflammatory response to LPS.

Key words

Oxylipin, neuroinflammation, n-3 PUFA, DHA, EPA, 18-HEPE, TxB2, EET

Abbreviations

5-oxoETE: 5-oxo-eicosatetraenoic, 18-HEPE: hydroxy-eicosapentaenoic acid, AA: arachidonic acid, ALA: α-linolenic acid, COX: cyclooxygenase, CNS: central nervous system; CYP450: cytochrome P450, DHA: docosahexaenoic acid, DPA: docosapentaenoic acid, EET: epoxy-eicosatrienoic acid, EPA: eicosapentaenoic acid, FA: fatty acid, HDoHE: hydroxy-docosahexaenoic acid, HETE: hydroxy-eicosatetraenoic acid, HODE: hydroxy-octadecadienoic acid, IL: interleukine, LA: linoleic acid, LC-PUFA: long chain polyunsaturated fatty acid, LOX: lipoxygenase, LPS: lipopolysaccharide, PCA: principal component analysis, PG: prostaglandin, PUFAs: polyunsaturated fatty acids, Rv: resolvin, SEM: standard error of the mean, TNF: tumor necrosis factor, TxB2: thromboxane B2

1. Introduction

The brain is highly enriched in long chain polyunsaturated fatty acids (LC-PUFAs) that are essential bioactive compounds with a large range of physiological roles such as cerebral plasticity, cell survival and neuroinflammation (Bazinet and Laye, 2014, Laye et al., 2018). Docosahexaenoic acid (DHA, 22:6 n-3) and arachidonic acid (AA, 20:4 n-6) are respectively the major n-3 and n-6 PUFAs in the brain. Despite the fact that DHA is poorly synthesized from its dietary precursor α-linolenic acid (ALA) (<1%) (Plourde and Cunnane, 2007), ALA-derived DHA has been recently reported to be sufficient for brain DHA supply (Domenichiello et al., 2014). However, preformed DHA can be provided through the diet to increase DHA levels in the brain (Orr et al., 2013, Joffre et al., 2016). N-3 LC-PUFA supplementation is the most efficient way to increase DHA levels and decrease AA levels in the brain (Murthy et al., 2002, Hiratsuka et al., 2009, Orr et al., 2010, Moranis et al., 2012, Fan et al., 2016, Lacombe et al., 2017).

N-3 LC-PUFAs exert protective actions against inflammation in the brain (Lonergan et al., 2004, Li et al., 2015, Shi et al., 2016, Dong et al., 2017, Fourrier et al., 2017). *In vivo*, fish oil supplementation providing n-3 LC-PUFAs, including DHA and eicosapentaenoic acid (EPA), is associated with a decreased cerebral expression of the pro-inflammatory cytokines tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-1β, induced by pro-inflammatory stimuli such as lipopolysaccharide (LPS, the gram-negative bacteria endotoxin), IL-1β (Dong et al., 2017) or aging (Labrousse et al., 2012, Orr et al., 2013, Dehkordi et al., 2015, Shi et al., 2016). *In vitro* data suggests that the anti-inflammatory activity of DHA in the brain is mediated through microglia, the brain's innate immune cells (De Smedt-

Peyrusse et al., 2008, Antonietta Ajmone-Cat et al., 2012, Pettit et al., 2013, Chang et al., 2015, Fourrier et al., 2017). Recent studies indicate that the mechanisms underlying the anti-inflammatory effect of DHA and EPA in the brain involve n-3 LC-PUFA derived bioactive mediators, called oxylipins, which promote the resolution of inflammation (Serhan et al., 2000, Levy et al., 2001, Hong et al., 2003, Serhan et al., 2015, Rey et al., 2016).

LC-PUFAs are converted by cyclooxygenase (COX), lipoxygenases (LOX) and cytochrome P450 (CYP) into oxylipins (Figure1). Several studies have reported their pro or anti-inflammatory activities in the brain (Orr et al., 2013, Rey et al., 2016, Laye et al., 2018). Oxylipins derived from DHA and EPA include anti-inflammatory and pro-resolving metabolites such as eicosanoids, resolvins, protectin and maresin (Bazan, 2009, Serhan et al., 2011). Oxylipins derived from AA and its precursor linoleic acid (LA) are mostly pro-inflammatory compounds and include prostaglandins(PG), leukotrienes (LT) or thromboxanes (Tx) and hydroxyoctadecadienoic acids (HODE) (Figure 1) (Calder, 2006). Oxylipin levels are tightly regulated by inflammatory stimuli or insults in peripheral tissues and immune cells (Yang et al., 2009, Balvers et al., 2012a, Willenberg et al., 2016). In the brain, n-3 and n-6 derived oxylipins are produced during injury or ischemic conditions, including from microglia (Farias et al., 2008).The expression of oxylipin's synthesizing enzymes is also tightly regulated by inflammatory stimuli (Rosenberger et al., 2004, Birnie et al., 2013, Taha et al., 2017), including in the brain (Laye et al., 2018). Administration of pro-inflammatory cytokines or LPS induces COX-2 expression in neuronal and endothelial cells (Cao et al., 1996, Nadjar et al., 2005, Rummel et al., 2006). COX-2, 5-LOX, 15-LOX and several isoforms of CYP expression are also induced in the hippocampus and cortex of rodents after traumatic brain injury (Birnie et al., 2013). A PUFA dietary intervention

modulates both cellular levels of PUFAs and oxylipins. As a result, n-3 LC-PUFA supplementation increases oxylipins derived from EPA and DHA and decreases compounds derived from AA (Balvers et al., 2012b, Hashimoto et al., 2015). Conversely, dietary n-6 PUFA supplementation increases AA-derived oxylipins and decreases oxylipins derived from EPA (Taha et al., 2016). Changes of oxylipins levels triggered by dietary n-6 and n-3 PUFA supply could be linked to the regulation of the enzymes COX-2 and 15-LOX (Rao et al., 2007, Kim et al., 2011, Taha et al., 2017). However, the influence of dietary n-3 LC-PUFAs on the conversion enzymes and oxylipin profiles in specific brain regions has been poorly addressed. N-3 LC-PUFAs are promising molecules due to their modulation of brain inflammation (Lonergan et al., 2004, Li et al., 2015, Shi et al., 2016, Dong et al., 2017, Fourrier et al., 2017). Thus, we explored whether n-3 LC-PUFA dietary supplementation provides protection in an LPS model of inflammation through differential production of oxylipins.

We hypothesized that inflammatory stimuli can differently regulate n-3 LC-PUFA derived-oxylipins production in the brain, dependent on the dietary n-3 LC-PUFA supply. To address this, we compared the oxylipin profile in the hippocampus of LPS treated mice fed with isocaloric diets with identical ALA content but supplemented or deficient in n-3 LC-PUFAs.

2. Materials and methods

2.1. Animals and treatment

All experiments were performed on male C57Bl6/J mice obtained from Janvier Labs at post-natal day 21 (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 12h light/dark cycle (07h-19h), with *ad libitum* access to food and water. A total of 88 mice were used in this study. Half of the mice were fed with a diet enriched with n-3 LC-PUFA and the other half with a diet deficient in n-3 LC-PUFA (diets are described below) for 2 months. A first cohort (supplemented and deficient n-3 LC-PUFA) was used to assess fatty acid composition in the hippocampus 24h post LPS injection (n=4/group, total 16 mice). A second cohort was used to measure inflammatory markers in the hippocampus 2h and 24h post LPS injection (n=5/group, total 40 mice). A third cohort was used to study oxylipin levels in the hippocampus 24h post LPS injection (n=8/group, total 32 mice). At post-natal day 21, animals were fed for 2 months with a diet containing 5% fat in the form of a mixture of different oils containing ALA (n-3 LC-PUFA deficient diet, with 1.6% n-3 PUFAs in the form of ALA, ratio n-6/n-3=6.7) or containing 67% fish oil that corresponds to 134 g/kg of diet (n-3 LC-PUFA supplemented diet, with 18.1% n-3 PUFAs including 1.1% ALA and 17.0% n-3 LC-PUFAs, n-6/n-3 = 0.8) (Labrousse et al., 2012, Madore et al., 2014, Delpech et al., 2015b) (Tables 1 and 2). The amounts of LA and ALA in both diets were identical.

To induce an inflammatory reaction, a dose of 125µg/kg of LPS Escherichia coli, 0127:B8, Sigma-Aldrich, Lyon, France) diluted in saline solution (Nacl 0.9%) was intraperitoneally injected after 2-months of dietary exposure (Laye et al., 1994, Mingam et al., 2008). Control mice received an injection of saline solution.

Mice were quickly anesthetized by isoflurane inhalation and euthanized by decapitation 2h or 24h after LPS injection to evaluate the time course of inflammation (Delpech et al., 2015a, Delpech et al., 2015b). Brain was rapidly removed and

carefully placed on a glass plate over dry ice to collect the hippocampus, which is impacted by LPS (Laye et al., 1994). It was immediately frozen and stored at -80°C until analysis.

2.2 Analysis of fatty acid composition

Fatty acids from the hippocampus were analyzed as previously described (Labrousse et al., 2012, Madore et al., 2014, Delpech et al., 2015b). Fatty acid composition was expressed as the percentage of total fatty acids.

2.3 Analysis of oxylipins

The different metabolites derived from LA, AA, DHA and EPA (listed in Table 3) were analyzed according to the LC-MS/MS method published by Le Faouder *et al.* (Le Faouder et al., 2013).

2.4 Quantitative Real-Time PCR

Total RNA was extracted from hippocampus using TRIzol (Invitrogen, Life Technologie™). RNA purity and concentration were determined using Nanodrop spectrophotometer (Nanodrop, Life technologies™). One microgram of RNA was reverse transcribed to synthesize cDNA using Superscript III (Invitrogen, Life Technologies™).

To measure mRNA expression, quantitative PCR was performed using the Applied Biosystems (Foster, CA) assay-on demand gene expression protocol as previously described (Mingam et al., 2008, Madore et al., 2013). We focused on the

expression levels of Interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, IL-10, COX-2, 5-LOX, 15-LOX and β2m as housekeeping gene. In brief, cDNA was amplified by real-time PCR where a target cDNA and the reference cDNA (β2m) were amplified simultaneously using an oligonucleotide probe with a 5′ fluorescent reporter dye (6-FAM) and a 3′ quencher dye (NFQ). Fluorescence was determined on an ABI PRISM 7500-sequence detection system (Applied Biosystems, California,USA). Data were analyzed using the comparative threshold cycle (Ct) method, results are expressed as relative fold change (Mingam et al., 2008, Madore et al., 2013, Madore et al., 2014, Delpech et al., 2015a) to control target mRNA expression.

2.5 Statistical analyses

Bivariate statistical analysis

All data were expressed as means ±SEM. Statistical analyses were performed using StatView. For all the experiments, a two-way ANOVA with diet (n-3 LC-PUFA deficient *versus* n-3 LC-PUFA supplemented diet) and treatment (saline *versus* LPS) as between factors was performed. In all cases, when a significant interaction was reported, ANOVAs were followed by *post hoc* Fischer test comparison. Statistical significance was set at $p<0.05$.

Principal component analysis (PCA)

PCA was used to assess the oxylipin pattern from both dietary groups under inflammatory conditions. 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

Dietary n-3 LC-PUFA supplementation modifies hippocampal fatty acid composition

We first evaluated the impact of either diet on the hippocampal fatty acid composition (Table 4). Total n-3 PUFA and n-6 PUFA levels were significantly different in the hippocampus of then-3 LC-PUFA supplemented group as compared to the n-3 LC-PUFA deficient group (DHA: $F_{(1,12)}=25.22$, $p<0.001$, EPA: $F_{(1,12)}=305.3$, $p<0.0001$, n-3 DPA: $F_{(1,12)}= 18.27$, $p<0.001$, AA: ($F_{(1,12)}=211.1$, $p<0.0001$, n-6 docosapentaenoic acid (DPA): $F_{(1,12)}=192.6$, $p<0.0001$, adrenic acid: $F_{(1,12)}=174.4$, $p<0.0001$). *Post-hoc* analysis further revealed that DHA, EPA and n-3 DPA levels were higher in the n-3 LC-PUFA supplemented group than in the n-3 LC-PUFA deficient group (DHA $p<0.001$, EPA $p<0.0001$, n-3 DPA $p<0.001$). Conversely, AA, n-6 DPA and adrenic acid levels were significantly decreased by n-3 LC-PUFA supplemented diet compared to n-3 LC-PUFA deficient diet (AA $p<0.0001$, n-6 DPA $p<0.0001$, adrenic acid $p<0.0001$). These changes resulted in significant decrease in n-6/n-3 ratio ($p<0.0001$). Monounsaturated and saturated fatty acid levels did not change significantly.

Dietary n-3 LC-PUFA supplementation and LPS injection affect hippocampal oxylipins concentration

We measured the oxylipins originating from n-6 and n-3 PUFAs in the hippocampus of mice fed with n-3 LC PUFA deficient or supplemented diets 24h after saline or LPS treatment. The LC-MS/MS detected Tx and PG (TxB2, 6kPGF1α, PGF2a, PGE2, PGD2, 15dPGJ2, 8isoPGA2), lipoxin (LxA4), epoxy fatty acids (14,15-EET, 11,12-EET, 8,9-EET, 5,6-EET), hydroxyl fatty acids (15-HETE, 8-HETE, 12-HETE, 5-HETE, 5-oxoETE) and dihydroxy fatty acids (DiHETE), all derived from AA, hydroxy fatty acids derived from LA (13-HODE and 9-HODE), hydroxy fatty acids derived from DHA (17-HDoHE and 14-HDoHE) and hydroxy fatty acids derived from EPA (18-HEPE) in the hippocampus. PGE3, LxB4, LTB4, LTB5, RvD1, RvD2, 7MaR1 and PDx were not detected in the hippocampus (Table 5).

A two-ANOVA using diet and treatment as factors revealed a significant diet effect on TxB2 ($F_{(1,28)}=13.45$, $p<0.001$), 6kPGF1α ($F_{(1,28)}=5.71$, $p<0.05$), PGE2 ($F_{(1,28)}=4.12$, $p<0.05$), 15dPGJ2 ($F_{(1,28)}=10.11$, $p<0.05$), 8isoPGA2 ($F_{(1,28)}=5.66$, $p<0.05$), 5,6-DiHETE ($F_{(1,28)}=4.60$, $p<0.05$), 14,15-EET ($F_{(1,28)}=4.77$, $p<0.05$), 15-HETE ($F_{(1,28)}=17.15$, $p<0.001$), 8-HETE ($F_{(1,28)}=6.37$, $p<0.05$) and LxA4 ($F_{(1,28)}=7.55$, $p<0.05$) derived from AA and 13-HODE ($F_{(1,28)}=7.85$, $p<0.05$) and 9-HODE ($F_{(1,28)}=4.76$, $p<0.05$) derived from LA (Table5). 17-HDoHE ($F_{(1,28)}=4.09$, $p<0.05$) derived from DHA, 18-HEPE ($F_{(1,28)}=12.17$, $p<0.001$) derived from EPA and 11,12-EET ($F_{(1,28)}=18.41$, $p<0.001$), 8,9-EET ($F_{(1,28)}=5.12$, $p<0.05$) and 5-oxoETE ($F_{(1,28)}=30.67$, $p<0.0001$) derived from AA were also altered by the diet (Table5).

However, the extent of the modifications of TxB2, 14,15-EET, 8-HETE, 5-HETE and 5-oxoETE was significantly different in LPS *versus* non-LPS treated animals (TxB2: $F_{(1,28)}=4.98$, $p<0.05$, 14,15-EET: $F_{(1,28)}=38.37$ $p<0.0001$, 8-HETE:

F(1,28)=4.57 p<0.05, 5-HETE: F(1,28)=13.66 p<0.0001 and 5-oxoETE: F(1,28)=8.05 p<0.01) (Table5). *Post hoc* analysis revealed that LPS increased TxB2 in the n-3 LC-PUFA deficient group (saline vs LPS p<0.001) whereas there were no significant differences in the n-3 LC-PUFA supplemented group. LPS increased 5-HETE, 5-oxoETE and 14,15-EET (saline vs LPS: 5-HETE p<0.001, 5-oxoETE p<0.05, 14,15-EET p<0.0001) and decreased 8-HETE (saline vs LPS p<0.05) in the n-3 LC-PUFA supplemented group whereas there were no significant differences in the n-3 LC-PUFA deficient group (Table5).

Multivariate data analysishighlightsthe separation of oxylipin pattern according to diet only in mice challenged by inflammation

We first considered the data for multivariate analysis to evaluate if diet groups or LPS groups could be discriminated by a pattern of oxylipins.

In the multivariate data analysis, 23 variables corresponding to the 23 identified oxylipins in the hippocampus were analyzed to compare spectra from n-3 LC-PUFA deficient and n-3 LC-PUFA supplemented groups. The correlation matrix highlighted the most correlated variables. Only significant correlations were reported (p<0.05) (Figure 4.A). Strong correlations were observed between oxylipins: AA-metabolites (PG, Tx, lipoxin, 9-HODE, 15-HETE and 8-HETE) were positively correlated together (in blue) and negatively correlated with 18-HEPE (in red) (Figure 2.A). N-3 PUFA metabolites were positively correlated to EETs.

PCA was used to identify the oxylipin pattern that was the most responsible for differences between groups. PCA analysis showed a marked separation of variables (Figures 2.B-C.): the first component ("pattern 1") explained 33.51% of the total

variance. Pattern 1 revealed a positive score for AA-derived oxylipins: 15d-PGJ2, 8-HETE, 13-HODE, 15-HETE, TxB2, 5,6-DiHETE, 8-iso-PGA2, LxA4, PGE2, PGF2α and a negative score for 18-HEPE and to a lesser extent for 14-HDoHE, 5-oxoETE and 11,12-EET (Figures2.B-C.). The component 2 ("pattern 2") explained 20.60% of the total variance in the oxylipin pattern. Pattern 2 revealed a positive score for AA-derived oxylipins: PGF2α, PGD2, 12-HETE, 5-HETE, 14,15-EET, 5-oxoETE, 11,12-EET, 8,9-EET, 5,6-EET (Figures2.B-C.).

Then, PCA visualization of individuals showed a full separation between LPS challenged n-3 LC-PUFA deficient and n-3 LC-PUFA supplemented groups (Figure 2.D). Component 1 revealed significant diet effects ($p < 0.0001$) but no LPS effect. Component 2 exhibited diet effects ($p < 0.05$) and an interaction between diet and LPS treatment ($p < 0.05$). n-3 LC-PUFA deficient diet group challenged with LPS showed a high and positive average score for component 1 whereas n-3 LC-PUFA supplemented diet group revealed a high and positive average score for the component 2 related to n-3 PUFA metabolites and EETs.

Dietary n-3 LC-PUFA supplementation regulates LPS-induced hippocampal expression of oxylipin biosynthesis enzymes

Oxylipins are mainly generated through the action of COX-2, 5-LOX and 15-LOX. The mRNA expression of these genes was measured 2h and 24h post-LPS. 5-LOX and COX-2 mRNA expression was not affected by diets whereas 15-LOX mRNA expression was increased in the n-3 LC-PUFA supplemented group 24h post injection ($p < 0.05$) (Figure 3). LPS induced a strong increase in COX-2 mRNA expression 2h post LPS injection ($p < 0.05$) that returned to baseline 24h post-LPS

injection. LPS increased 5-LOX mRNA expression only at 24h (p<0.0001). Of note, 15-LOX mRNA expression was not modified by LPS challenge at any time.

Dietary n-3 LC-PUFA supplementation prevents LPS-induced hippocampal increase of pro-inflammatory cytokines

We thus quantified the hippocampal levels of pro-inflammatory cytokines IL-6, TNF-α, and IL-1β 2h and 24h post LPS injections in the n-3 LC-PUFA deficient and n-3 LC-PUFA supplemented groups (Figure 4). While LPS significantly increased the mRNA expression of all cytokines at both 2h and 24h post-LPS (p<0.0001 for each cytokine at each time, 2h: IL-1β $F_{(1,15)}=39.05$, TNF-α $F_{(1,15)}=48.49$; IL-6 $F_{(1,15)}=60.34$; 24h: IL-1β $F_{(1,15)}=41.62$, TNF-α $F_{(1,15)}=47.97$, IL-6 $F_{(1,15)}=102.7$), n-3 LC-PUFA supplementation significantly reduced IL-1β and TNF-α production exclusively at 24h ($F_{(1,15)}=6.31$, p<0.05 and $F_{(1,15)}=15.17$ p<0.01, respectively).

4. Discussion

In this work, we provide evidence that dietary n-3 LC-PUFA supplementation modulates hippocampal oxylipins and alters LPS-induced pro-inflammatory cytokine expression in the hippocampus, suggesting that n-3 LC-PUFAs could promote the resolution of hippocampal neuroinflammation through the release of oxylipins.

In the n-3 LC-PUFA supplemented diet, the amount of EPA and DHA corresponds to a daily intake of 40 mg/day/mouse (26.5 mg EPA and 14 mg DHA per mouse). To extrapolate to humans (Nair and Jacob, 2016), this intake represents 3.8

g/day (for a body weight of 60 kg). This is eight times more per day than the global recommendation (500 mg/day according to ISSFAL) and has been used in humans with severe inflammatory conditions such as rheumatoid arthritis (Rajaei et al., 2015) or cognitive deficit (associated to aging, cardiovascular diseases or cerebrovascular disorders) (for a recent review see Chianese et al., 2018). No studies in humans have evaluated whether this supplementation changes PUFA and oxylipin levels in the brain.

We confirmed that dietary n-3 LC-PUFA supplementation increased the levels of the n-3 derived oxylipin precursors DHA, EPA, n-3 DPA and decreased the levels of the n-6 derived oxylipin precursors AA and n-6 DPA in the hippocampus of mice compared to the n-3 LC-PUFA deficient group. A wide range of studies demonstrate that dietary n-3 PUFAs impact the lipidomic profile in the brain (Carrie et al., 2000, Labrousse et al., 2012, Skorve et al., 2015, Joffre et al., 2016). Both dietary ALA or n-3 LC-PUFA supplementation increases DHA and decreases AA in the brain with DHA supplementation most efficacious at producing these changes (Murthy et al., 2002, Hiratsuka et al., 2009, Moranis et al., 2012, Lacombe et al., 2017). We and others also reported that PUFA levels in the hippocampus were not affected by LPS despite the sensitivity of this structure to inflammatory stimuli (Orr et al., 2013, Delpech et al., 2015b).

Increasing n-3 LC-PUFA levels in the brain was associated with the modulation of oxylipin profile in the hippocampus. We analyzed both n-6 PUFA and n-3 PUFA derived oxylipins. Dietary n-3 LC-PUFA supplementation increased EPA and DHA-derived metabolites (18-HEPE and 17-HDoHE) and decreased LA-and AA-derived metabolites (TxB2, 6kPGF1a, PGE2, 15dPGJ2, 5,6-DiHETE, 14,15-EET, 15-

HETE, LxA4, 13-HODE, 9-HODE). Similarly, Hashimoto *et al.* demonstrated that an oral administration of EPA and DHA results in the generation of EPA-derived mediators and DHA derived-HDoHE, -resolvins and -protectin in the cortex of aged rats (Hashimoto et al., 2015). Moreover, high dietary levels of DHA and EPA or low dietary LA levels down-regulates the production of n-6-derived mediators, 5,6-DiHETE, 14,15-EET, 15-HETE, 13-HODE, 9-HODE, TxB2 and 6kPGF1α in the brain of rodents (Taha et al., 2016, Ostermann et al., 2017). Collectively, the evidence suggests that dietary PUFAs regulate brain PUFA-derived oxylipin concentrations. The cellular origin of oxylipins is still unclear. What is known is that all brain cells have n-3 and n-6 PUFAs in their membranes (i.e. AA, DHA, EPA, LA). In rodents, dietary PUFAs modulate PUFA content in neurons (Ikemoto et al., 2000), glial cells and microglia (Destaillats et al., 2010), suggesting that dietary PUFA content impacts PUFA composition of brain cells and potentially oxylipins derived from these cells (Laye, 2017). However, this has not been evaluated *in vivo* and further work is needed to address this.

Together, these data highlight that oxylipin profile depends on the dietary intake, the more n-3 LC-PUFAs are in the diet, the more n-3 PUFA derived oxylipins and the less n-6 PUFA derived oxylipins are in the hippocampus. However, oxylipin production may be more finely tuned by the regulation of their biosynthetic enzymes. These enzymes are involved in numerous pathways of oxylipin synthesis (Gabbs et al., 2015). 15-LOX mRNA expression was higher in the n-3 LC-PUFA supplemented group than in the n-3 LC-PUFA deficient group 24h after LPS. This is consistent with studies showing that a dietary n-3 PUFAs deprivation decreased 15-LOX mRNA expression in the brain (Rao et al., 2007, Kim et al., 2011). 15-LOX is involved in the conversion of AA in 15-HETE, DHA in 17-HDoHE, EPA in 18-HEPE and LA in 13-

HODE. The n-3 LC-PUFA supplemented group was characterized by 15-HETE and 13-HODE decrease and 17-HDoHE increase. These data suggest that 15-LOX promotes production of DHA-derived metabolites instead of AA-derived metabolites in the n-3 LC-PUFA supplemented group. This diet effect on 15-LOX was not observed after 2h LPS. We could speculate that 2h after LPS, in the inflammatory phase, 15-LOX is not yet targeted and mobilized. We previously showed *in vitro* in microglial cells that 15-LOX mRNA expression was not increased before 24h LPS stimulation (Rey et al., 2016). The actions of 15-LOX have been described as both beneficial and detrimental in the brain. 15-LOX is initially implicated in neurodegeneration and neurotoxicity through increase of oxidative stress (Pratico et al., 2004, Wang et al., 2015, Yigitkanli et al., 2017). However, some studies suggest that 15-LOX-metabolites of AA have neuroprotective properties *via* the nuclear receptor peroxisome proliferator-activated receptor PPARγ activation (Sun et al., 2015) and that 15-LOX contributes to the preservation of cognitive performance through RvD1 formation (Shalini et al., 2017). Moreover, 15-LOX could differently influence inflammation depending on their polymorphism (Tarannum and Faizuddin, 2017).

Enzymes that catalyze the formation of oxylipins are also regulated during inflammation. LPS-induced inflammation increases the mRNA expression of COX-2 at the early phase of inflammation and of 5-LOX in the late stage of inflammation (Quan et al., 1998, Chung et al., 2010, Czapski et al., 2010, Rey et al., 2016). Moreover, LPS induces AA-derived mediator production, converted through COX-2 and 5-LOX, in microglia cell culture (Wang et al., 2004). 5-LOX function is complex and depends on its cellular localization. In the nucleus, 5-LOX promotes pro-inflammatory LT synthesis, while in the cytoplasm it generates anti-inflammatory

LxA4 (Fredman et al., 2014). COX-2 is implicated, at least in part, in the conversion of AA in PG and Tx (Salinas et al., 2007). Together with our data, it appears that LPS promotes eicosanoid production through COX-2 and 5-LOX pathways in the brain, leading to the increased pro-inflammatory cytokines.

Interestingly, pro-inflammation cytokines IL-1β and TNF-α mRNA expression induced by LPS in the hippocampus was decreased in n-3 LC-PUFA supplemented group compared to n-3 LC-PUFA deficient group. This result is in agreement with previous work *in vivo* and *in vitro*. Fish oil supplementation providing n-3 LC-PUFAs, including DHA and EPA, is associated with a decreased cerebral expression of the pro-inflammatory cytokines TNF-α, IL-6 and IL-1β induced by peripheral or intracerebral administration of LPS or aging (Labrousse et al., 2012, Orr et al., 2013, Dehkordi et al., 2015, Shi et al., 2016). Dietary EPA supplementation also decreases TNF-α expression in the hippocampus following IL-1β central injection (Dong et al., 2017). Moreover, increasing endogenously n-3/n-6 PUFA ratio in the hippocampus of transgenic Fat-1 mice encoding n-3 PUFA desaturase protects from pro-inflammatory cytokine increase in response to LPS (Delpech et al., 2015a). *In vitro*, DHA reduces LPS-induced pro-inflammatory cytokine expression through the regulation of the pro-inflammatory signaling pathway nuclear factor kappa B (NFκB) in microglia (De Smedt-Peyrusse et al., 2008, Antonietta Ajmone-Cat et al., 2012, Pettit et al., 2013, Chang et al., 2015, Fourrier et al., 2017). Neuroinflammation is a host defense mechanism that could be deleterious in uncontrolled conditions. Cytokines and lipid mediators released during inflammation act together to allow the resolution of inflammation (Woodroofe and Cuzner, 1993, Woodroofe, 1995, Blais and Rivest, 2003, Laye, 2010, Serhan, 2011, Solito and Sastre, 2012).

LPS induced an increase in n-6 PUFA derived mediators, TxB2 and 8-HETE, in the n-3 LC-PUFA deficient group that was blunted in the n-3 LC-PUFA supplemented group. TxB2 is the product of TxA2 degradation which is involved in neuroinflammation (Yu et al., 2014). These results confirmed previous *in vitro* studies indicating LPS treatment increases oxylipins derived from AA in macrophages (Dieter et al., 2002, Le Faouder et al., 2013) and in microglia (Slepko et al., 1997, Wang et al., 2014, Jung et al., 2016). *In vivo*, brain ischemia strongly increases both n-3 and n-6 oxylipins in rat brain (Farias et al., 2008). Moreover, in mice, LPS treatment induces expression of almost all n-3 and n-6 oxylipins in plasma, serum, adipose tissue and ileum (Yang et al., 2009, Balvers et al., 2012a, Willenberg et al., 2016). Recent studies reported that a chronic administration of LPS in the cerebral ventricles induces the production of n-6 PUFA derived-prostaglandins in the brain (Rosenberger et al., 2004, Taha et al., 2017). In our study, multivariate analysis clearly revealed a pro-inflammatory oxylipin pattern in the n-3 LC-PUFA deficient group challenged by LPS, whereas the n-3 LC-PUFA supplemented group challenged by LPS exhibited an anti-inflammatory oxylipin pattern. Interestingly, 14,15-EET, 5-HETE and 5-oxoETE derived from AA were included in the pattern of anti-inflammatory oxylipins. Numerous studies have established that EETs derived from AA by CYP action exhibit a range of biological activities in peripheral tissues such as vasodilatation, anti-inflammation and cellular signaling regulation (Iliff et al., 2010). Indeed, EETs act mainly on NFκB signaling pathway (Christmas, 2015) and suppress the expression of IL-1β and TNF-α in a model of acute lung injury (Zhou et al., 2017). Inhibition of enzymes degrading EET protects against brain damage and reduces microglia-mediated neuroinflammation in traumatic brain injury (Hung et al., 2017). 5-oxoETE is involved in many pathophysiological processes through mainly

pro-inflammatory activities (Powell and Rokach, 2015). In contrast, a recent study suggests that this mediator could play an important protective role in the detection of tissue damage and the stimulation of leukocyte migration at the site of injury (Enyedi et al., 2013, Powell and Rokach, 2015). HETEs derived from LOX pathways are described mostly as pro-inflammatory compounds, although some are reported to have anti-inflammatory properties (Hampson and Grimaldi, 2002). The degradation of 5-HETE produces the anti-inflammatory 5-oxoETE (Powell and Rokach, 2015), suggesting an inflammatory protection in the n-3 LC-PUFA supplemented group. The anti-inflammatory status of the n-3 LC-PUFA supplemented group was also supported by the strong increase of the resolvins and protectins precursors: 18-HEPE, 17-HDoHE and 14-HDoHE in the hippocampus. Resolvins and protectins derived from n-3 PUFAs have been reported able to down-regulate the inflammatory response in the central nervous system and microglia *in vitro* (Zhao et al., 2011, Rey et al., 2016).

To conclude, the present results show that dietary n-3 LC-PUFA supplementation promotes an oxylipin anti-inflammatory profile after peripheral inflammatory challenge. Changes in oxylipin levels could be linked to the decreased inflammatory response in the hippocampus of n-3 LC-PUFA supplemented group. These results highlight that oxylipins may be involved in the crucial and beneficial role assigned to n-3 PUFAs during neuroinflammatory episode.

5. References

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Figure 1. Oxylipin synthesis pathways
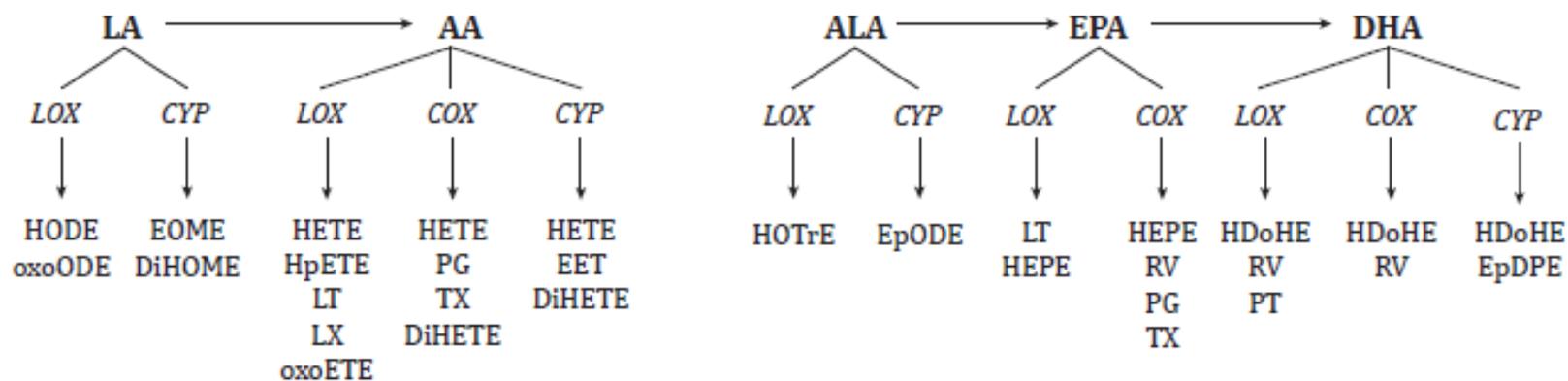
AA: arachidonic acid, ALA: alpha linolenic acid, COX: cyclooxygenase, CYP: cytochrome 450, DHA: docosahexaenoic acid, DiHETE : dihydroxy-eicosatetraenoic acid, DiHOME : dihydroxy-octadecaenoic acid, EET: epoxy-eicosatrienoic acid, EPA: eicosapentaenoic acid, EpDPE : epoxy-docosapentaenoic acid, EpODE : epoxy-octadecadienoic acid, EOME : epoxy-octadecenoic acid, HDoHE : hydroxy-docosahexaenoic acid, HETE: hydroxyeicosatetraenoic acid, HEPE: hydroxy-eicosapentaenoic acid; HODE: hydroxy-octadecadienoic acid, HOTrE: hydroxy-octadecatrienoic acid, HpETE : hydroperoxy-eicosatetraenoic acid, LA: linoleic acid, LOX: lipoxygenase, LT: leukotriene, LX: lipoxin, oxoODE : keto-octadecadienoic acid, oxoETE : keto-eicosatetraenoic, PG: prostaglandin; PT : protectin, Rv : resolvin, TX : thromboxane.

Figure 2. Multivariate analysis of oxylipins. (A) Correlation matrix of the 23 variables (blue: positive correlation, red:negative correlation, X no significant correlation). (B) Correlations between each variable and the principal component score from PCA ([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 (>|0.20|) within each pattern). (C) Correlation circle from PCA. (D) Individuals map of PCA analysis.

B.

	Loadings[a]	
	Pattern 1	Pattern 2
6kPGF1a	0.100	-0.077
TxB2	0.308[b]	0.061
PGF2a	0.251[b]	0.219[b]
PGE2	0.257[b]	0.182
PGD2	0.137	0.240[b]
LxA4	0.278[b]	0.149
8isoPGA2	0.255[b]	0.083
5,6-DiHETE	0.311[b]	0.099
15dPGJ2	0.312[b]	0.015
13-HODE	0.250[b]	-0.047
9-HODE	0.144	-0.146
15-HETE	0.306[b]	0.000
8-HETE	0.276[b]	-0.006
12-HETE	-0.105	0.258[b]
5-HETE	-0.078	0.339[b]
5oxoETE	-0.146	0.385[b]
14,15-EET	0.041	0.300[b]
11,12-EET	-0.168	0.253[b]
8,9-EET	-0.001	0.297[b]
5,6-EET	-0.127	0.391[b]
18-HEPE	-0.229[b]	-0.015
17-HDoHE	0.020	0.177
14-HDoHE	-0.143	0.182

Figure 3.Oxylipin synthesis enzyme expression in the hippocampus of mice fed with an n-3 LC-PUFA deficientor n-3 LC-PUFA supplemented diet for 2 months, 2h and 24h following LPS injection. Data are presented as means ±SEM (n=5-6). Data were analyzed by a two-way ANOVA.
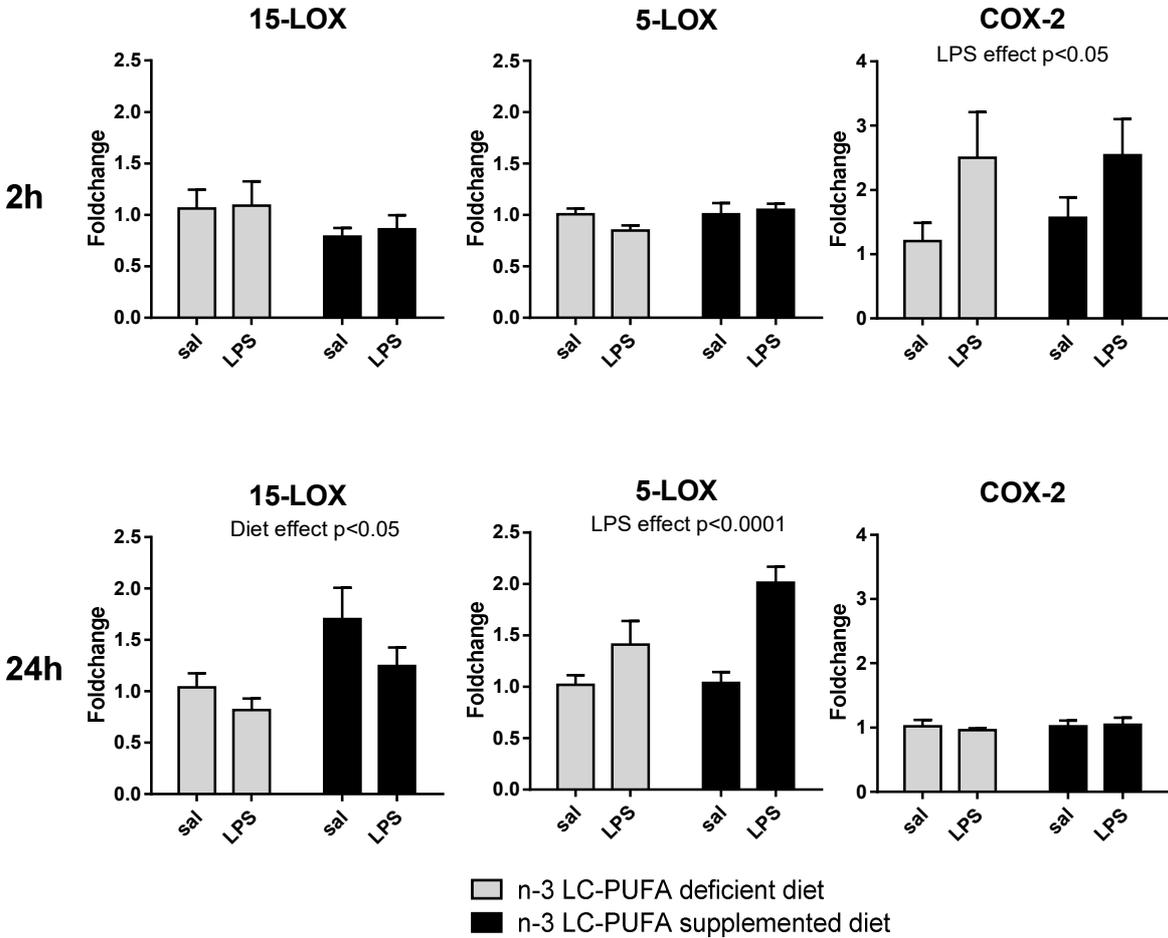
Figure 4. Cytokine expression in the hippocampus of mice fed withan n-3 LC-PUFA deficientor n-3 LC-PUFA supplemented diet for 2 months, 2h and 24h following LPS injection. Data are presented as means ±SEM (n=6). Data were analyzed by a two-way ANOVA followed by a *post hoc* Fischer test comparison.
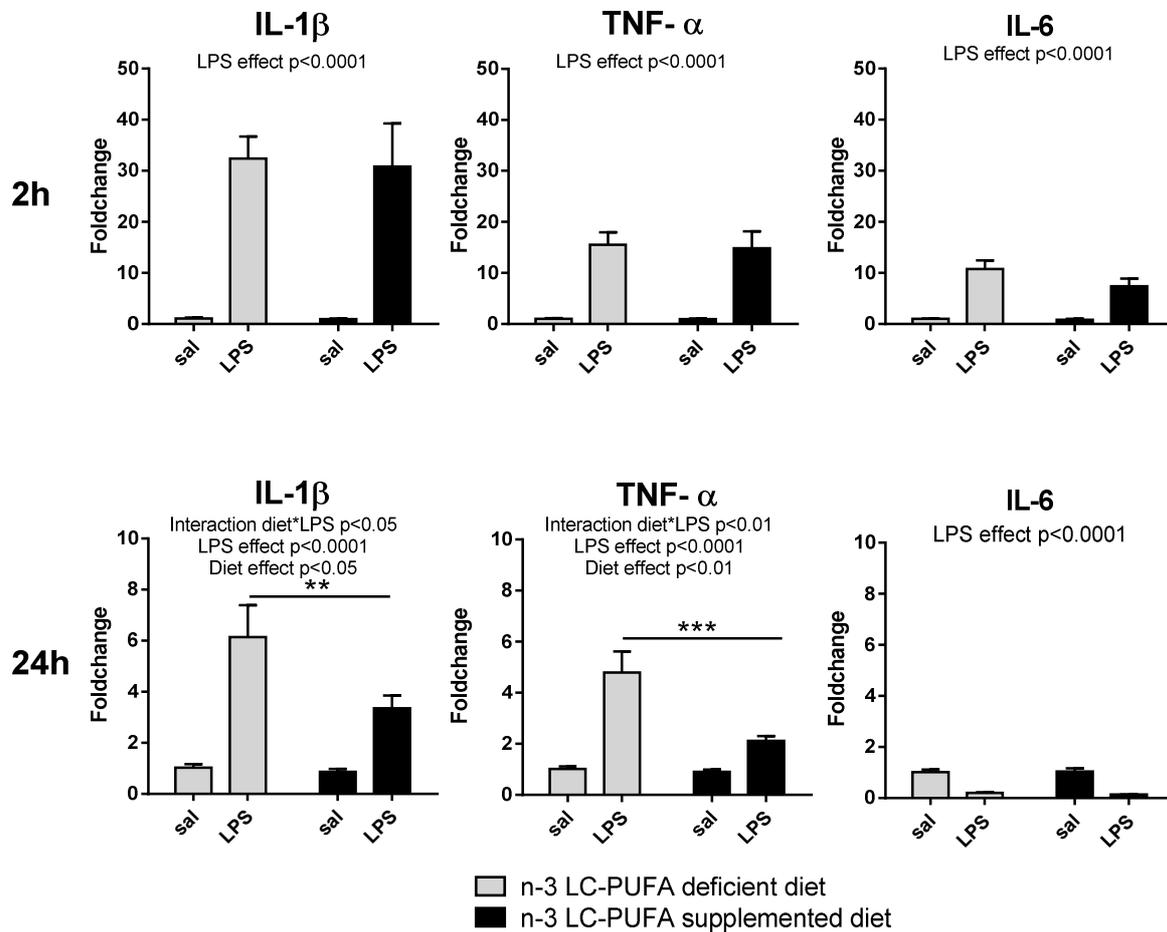
Table 1.Composition of the diets (g/kg diet)

Ingredient	Amount
Casein	180
Cornstarch	460
Sucrose	230
Cellulose	20
Fat [1]	50
Mineral mix [2]	50
Vitamin mix [3]	10

[1]: for detailed composition, see Table 2

[2]: composition (g/kg): sucrose, 110.7; $CaCO_3$, 240; K_2HPO_4, 215; $CaHPO_4$, 215; $MgSO_4,7H_2O$, 100; NaCl, 60; MgO, 40; $FeSO_4,7H_2O$, 8; $ZnSO_4,7H_2O$, 7; $MnSO_4,H_2O$, 2; $CuSO_4,5H_2O$, 1; $Na_2SiO_7,3H_2O$, 0.5; $AlK(SO_4)_2,12H_2O$, 0.2; K_2CrO_4, 0.15; NaF, 0.1; $NiSO_4,6H_2O$, 0.1; H_2BO_3, O.1; $CoSO_4,7H_2O$, 0.05; KIO_3, 0.04; $(NH_4)_6Mo_7O_{24},4H_2O$, 0.02; LiCl, 0.015; Na_2SeO_3, 0.015; NH_4VO_3, 0.01

[3]: composition (g/kg): sucrose, 549.45; retinyl acetate, 1; cholecalciferol, 0.25; DL- -tocopheryl acetate, 20; phylloquinone, 0.1; thiamin HCl, 1; riboflavin, 1; nicotinic acid, 5; calcium pantothenate, 2.5; pyridoxine HCl, 1; biotin, 1; folic acid, 0.2; cyanobalamin, 2.5; choline HCl, 200; DL-methionin, 200; p-aminobenzoic acid, 5; inositol, 10

Table 2. Fatty acid composition of the dietary lipids (% wt of total fatty acids).

Fatty acid	n-3 LC-PUFA deficient diet	n-3 LC-PUFA supplemented diet
16:0	22.6	19.6
18:0	3.3	4.1
other SFA	1.8	7.6
total SFA	**27.7**	**31.3**
18:1n-9	57.9	24.6
18:1n-7	1.5	2.9
other MUFA	0.6	9.1
total MUFA	**60.0**	**36.6**
18:2n-6 (LA)	10.6	12.9
18:3 n-6	-	0.3
20:2 n-6	-	0.2
20:4 n-6 (AA)	0.1	0.6
total n-6 PUFAs	**10.7**	**14.0**
18:3 n-3 (ALA)	1.6	1.1
20:5 n-3 (EPA)	ND	10.6
22:5 n-3 (DPA n-3)	ND	0.9
22:6 n-3 (DHA)	ND	5.6
total n-3 PUFAs	**1.6**	**18.1**
total PUFAs	**12.3**	**32.1**
ratio n-6/n-3	**6.7**	**0.8**

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

Table 3. Classification and formal name of each PUFA metabolites quantified by LC-MS/MS (Le Faouder 2013).

Compound	Precursor	Formal name
9-HODE	LA	9-hydroxy-10E,12Z-octadecadienoic acid
13-HODE	LA	13-hydroxy-9Z,11E-octadecadienoic acid
6kPGF1α	AA	6-oxo-9α,11α,15(S)-Trihydroxy-prost-13E-en-1-oic acid
PGE2	AA	9-Oxo-11α,15(S)-dihydroxy-prosta-5Z,13E-dien-1-oic acid
PGF2α	AA	9α,11α,15S-trihydroxy-prosta-5Z,13E-dien-1-oic acid
PGD2	AA	9α,15S-dihydroxy-11-oxo-prosta-5Z,13E-dien-1-oic acid
15dPGJ2	AA	11-oxo-prosta-5Z,9,12E,14E-tetraen-1-oic acid
8isoPGA2	AA	9-oxo-15S-hydroxy-(8β)-prosta-5Z,10,13E-trien-1-oic acid
TXB2	AA	9α,11,15(S)-Trihydroxythromba-5Z,13E-dien-1-oic acid
LxB4	AA	5(S),14(R),15(S)-Trihydroxy-6E,8Z,10E,12E eicosatetraenoic acid
LTB4	AA	5(S),12(R)-Dihydroxy-6Z,8E,10E,14Z-eicosatetraenoic acid
LxA4	AA	5(S),6(R),15(S)-Trihydroxy-7E,9E,11Z,13E eicosatetraenoic acid
5-HETE	AA	5(S)-Hydroxy-6E,8Z,11Z,14Z-eicosatetraenoic acid
8-HETE	AA	8-Hydroxy-5Z,9E,11Z,14Z-eicosatetraenoic acid
12-HETE	AA	12(S)-Hydroxy-5Z,8Z,10E,14Z-eicosatetraenoic acid
15-HETE	AA	15(S)-Hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic acid
19-HETE	AA	19(S)-Hydroxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid
20-HETE	AA	20-Hydroxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid
5-Oxo-ETE	AA	5-Oxo-6E,8Z,11Z,14Z-eicosatetraenoic acid
5,6-EET	AA	5(6)-Epoxy-8Z,11Z,14Z-eicosatrienoic acid
8,9-EET	AA	8(9)-Epoxy-5Z,11Z,14Z-eicosatrienoic acid
11,12-EET	AA	11(12)-Epoxy-5Z,8Z,14Z-eicosatrienoic acid
14,15-EET	AA	14(15)-Epoxy-5Z,8Z,11Z-eicosatrienoic acid
5,6-DiHETE	AA	11-Trans-5(S),6(R)-dihydroxy-7E,9E,11E,14Zeicosatetraenoic acid
17-HDoHE	DHA	17-Hydroxy-4Z,7Z,10Z,13Z,15E,19Zdocosahexaenoic acid
14-HDoHE	DHA	14-Hydroxy-4Z,7Z,10Z,12E,16Z,19Zdocosahexaenoic acid
PDx	DHA	10(S),17(S)-Dihydroxy-4Z,7Z,11E,13Z,15E,19Zdocosahexaenoic acid
RvD1	DHA	7(S),8(R),17(S)-Trihydroxy-4Z,9E,11E,13Z,15E,19Zdocosahexaenoic acid
RvD2	DHA	7S,16R,17S-trihydroxy-4Z,8E,10Z,12E,14E,19Z-docosahexaenoic acid
7-MaR1	DHA	7(S),14(R)-Dihydroxy-4Z,8E,10Z,12Z,16Z,19Zdocosahexaenoic acid
18-HEPE	EPA	18-Hydroxy-5Z,8Z,11Z,14Z,16E-eicosapentaenoic acid
PGE3	EPA	9-Oxo-11α,15(S)-dihydroxy-prosta-5Z,13E,17Z-trien-1-oic acid
LTB5	EPA	5(S),12(R)-Dihydroxy-6Z,8E,10E,14Z,17Zeicosapentaenoic acid

Table 4. Fatty acid composition of the hippocampus of mice fed with an n-3 LC-PUFA deficient or an n-3 LC-PUFA supplemented diet for 2 months and treated with LPS 24 hours prior to sacrifice. Data are presented as means ±SEM (n=4) and are expressed as % of total fatty acids. Data were analyzed by a two-way ANOVA (diet x LPS) followed, when a significant interaction was revealed (p<0.05), by a *post hoc* Fischer test comparison. Values with different superscripts (a,b,c) differ significantly (p<0.05).

| | n-3 LC-PUFA deficient diet | | n-3 LC-PUFA supplemented diet | | Statistical effect | | |
	Saline	LPS	Saline	LPS	Diet	LPS	Diet x LPS
14:0	0.13 ± 0.01	0.15 ± 0.00	0.14 ± 0.01	0.12 ± 0.04	-	-	-
16:0	19.18 ± 0.60	19.59 ± 0.39	19.50 ± 0.55	19.24 ± 1.62	-	-	-
17:0	0.11 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	p<0.05	-	-
18:0	21.80 ± 0.20	21.66 ± 0.05	21.47 ± 0.13	22.43 ± 1.63	-	-	-
20:0	0.32 ± 0.02	0.31 ± 0.03	0.31 ± 0.04	0.31 ± 0.04	-	-	-
22:0	0.40 ± 0.05	0.38 ± 0.05	0.36 ± 0.05	0.34 ± 0.05	-	-	-
24:0	0.60 ± 0.13	0.53 ± 0.05	0.56 ± 0.07	0.53 ± 0.05	-	-	-
SFAs	**42.57 ± 0.57**	**42.78 ± 0.30**	**42.50 ± 0.35**	**43.12 ± 0.50**	-	-	-
16:1n-9	0.14 ± 0.01	0.15 ± 0.01	0.17 ± 0.04	0.13 ± 0.02	-	-	-
16:1n-7	0.43 ± 0.02	0.45 ± 0.02	0.56 ± 0.02	0.51 ± 0.12	p<0.05	-	-
18:1n-9	13.84 ± 0.56	14.08 ± 0.34	14.66 ± 0.34	14.10 ± 0.82	-	-	-
18:1n-7	3.06[a,b] ± 0.09	3.14[a] ± 0.02	3.03[a,b] ± 0.04	2.93[b] ± 0.14	p<0.05	-	p<0.05
20:1n-9	0.96 ± 0.17	0.97 ± 0.10	0.88 ± 0.09	0.83 ± 0.06	-	-	-
20:1n-7	0.24 ± 0.03	0.26 ± 0.03	0.26 ± 0.02	0.24 ± 0.02	-	-	-
22:1n-9	0.09 ± 0.03	0.11 ± 0.00	0.11 ± 0.02	0.11 ± 0.01	-	-	-
24:1n-9	1.39 ± 0.17	1.35 ± 0.18	1.19 ± 0.18	1.07 ± 0.13	p<0.05	-	-
MUFAs	**20.20 ± 0.99**	**20.58 ± 0.61**	**20.93 ± 0.62**	**20.04 ± 0.97**	-	-	-
18:2n-6	0.27 ± 0.03	0.24 ± 0.03	0.22 ± 0.01	0.25 ± 0.05	-	-	-
20:3n-6	0.37 ± 0.01	0.37 ± 0.02	0.34 ± 0.05	0.36 ± 0.03	-	-	-
20:4n-6	10.46 ± 0.33	10.17 ± 0.25	8.15 ± 0.22	8.26 ± 0.34	p<0.0001	-	-
22:4n-6	2.27 ± 0.15	2.20 ± 0.04	1.42 ± 0.05	1.45 ± 0.18	p<0.0001	-	-
22:5n-6	0.49 ± 0.03	0.46 ± 0.05	0.19 ± 0.05	0.21 ± 0.02	p<0.0001	-	-
n-6	**13.90 ± 0.50**	**13.51 ± 0.27**	**10.38 ± 0.23**	**10.60 ± 0.58**	p<0.0001	-	-
20:5n-3	0.08 ± 0.04	0.08 ± 0.01	0.33 ± 0.04	0.34 ± 0.02	p<0.0001	-	-
22:5n-3	0.22 ± 0.03	0.19 ± 0.03	0.58 ± 0.06	0.37 ± 0.25	p<0.001	-	-
22:6n-3	15.20 ± 1.00	14.67 ± 0.20	17.36 ± 0.21	18.01 ± 1.93	p<0.001	-	-
n-3	**15.49 ± 0.96**	**14.94 ± 0.19**	**18.27 ± 0.27**	**18.72 ± 1.68**	p<0.0001	-	-
20:3 n-9	0.22 ± 0.03	0.25 ± 0.03	0.25 ± 0.03	0.24 ± 0.02	-	-	-
PUFAs	**29.61 ± 1.20**	**28.70 ± 0.45**	**28.89 ± 0.18**	**29.57 ± 2.24**	-	-	-
dma16:0	1.83 ± 0.14	1.94 ± 0.17	1.82 ± 0.18	1.71 ± 0.48	-	-	-
dma18:0	3.55 ± 0.26	3.6 ± 0.16	3.7 ± 0.07	3.6 ± 0.41	-	-	-
dma18:1	2.24 ± 0.30	2.4 ± 0.07	2.2 ± 0.04	2.0 ± 0.50	-	-	-
DMA	**7.62 ± 0.65**	**7.9 ± 0.35**	**7.7 ± 0.23**	**7.3 ± 1.38**	-	-	-
n-6/n-3	**0.90 ± 0.05**	**0.9 ± 0.01**	**0.6 ± 0.02**	**0.6 ± 0.02**	p<0.0001	-	-
DHA/AA	**1.45 ± 0.08**	**1.4 ± 0.02**	**2.1 ± 0.08**	**2.2 ± 0.15**	p<0.0001	-	-

AA: arachidonic acid; DHA: docosahexaenoic acid; DMA: dimethylacetals; MUFAs: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFAs: saturated fatty acids.

Table 5. Oxylipin concentrations in the hippocampus of mice fed with an n-3 LC-PUFA deficient diet or n-3 LC-PUFA supplemented diet for 2 months and treated with LPS 24 hours prior to sacrifice. Data are presented as means ± SEM (n=8) and are expressed in pg/mg protein. Data were analyzed by a two-way ANOVA (diet x treatment) followed, when a significant interaction was revealed (p<0.05), by a *post hoc* Fischer test comparison. Values with different superscripts (a,b,c) differ significantly (p<0.05).

Oxylipin	Pathway	n-3 LC-PUFA deficient diet		n-3 LC-PUFA supplemented diet		Statistical effect		
		Saline	LPS	Saline	LPS	Diet	LPS	Diet x LPS
AA-oxylipins								
Thromboxanes, prostaglandins								
TxB2	COX	2205.6[a] ± 219.91	3206.6[b] ± 313.24	1902.8[a] ± 139.32	1961.5[a] ± 110.73	p<0.001	p<0.05	p<0.05
6kPGF1α	COX	305.7 ± 33.39	405.2 ± 52.84	256.6 ± 31.45	273.2 ± 29.15	p<0.05	-	-
PGF2 α	COX	7393.7 ± 705.07	7586.9 ± 796.88	6810.5 ± 491.51	6606.6 ± 408.05	-	-	-
PGE2	COX	1280.0 ± 89.67	1549.1 ± 117.02	1164.7 ± 126.61	1190.0 ± 129.58	p<0.05	-	-
PGD2	COX	973.8 ± 122.31	994.0 ± 166.32	1073.6 ± 213.63	954.3 ± 262.48	-	-	-
15dPGJ2	COX	61.0 ± 9.33	67.4 ± 7.74	43.0 ± 2.42	44.9 ± 3.13	p<0.05	-	-
8isoPGA2	no enz	475.2 ± 57.81	511.7 ± 48.96	385.8 ± 40.55	389.8 ± 22.24	p<0.05	-	-
Lipoxin								
LxA4	LOX	168.5 ± 15.86	133.8 ± 24.30	119.2 ± 16.01	88.0 ± 10.04	p<0.05	-	-
Epoxy fatty acids								
14,15-EET	CYP450	178.8[a] ± 20.56	134.2[a] ± 28.88	0[b] ± 0.00	219.7[c] ± 20.13	p<0.05	p<0.001	p<0.0001
11,12-EET	CYP450	0.0 ± 0.00	0.0 ± 0.00	177.0 ± 66.07	250.0 ± 64.24	p<0.001	-	-
8,9-EET	CYP450	874.2 ± 50.14	724.7 ± 133.45	1174.0 ± 130.88	889.2 ± 64.10	p<0.05	p<0.05	-
5,6-EET	CYP450	566.4 ± 38.52	494.4 ± 102.99	578.6 ± 51.98	685.2 ± 26.34	-	-	-
Hydroxy fatty acids								
15-HETE	LOX	4666.0 ± 377.63	4768.2 ± 363.28	3480.6 ± 273.37	3111.6 ± 328.29	p<0.001	-	-
8-HETE	LOX	534.3[a] ± 18.74	598.1[a] ± 65.01	515.5[a] ± 61.74	371.1[b] ± 22.88	p<0.05	-	p<0.05
12-HETE	LOX	1041.4 ± 32.99	1068.3 ± 198.11	1010.8 ± 132.78	1421.1 ± 100.27	-	-	-
5-HETE	LOX	6001.5[a] ± 186.28	4627.6[a,b] ± 702.12	4625.1[b] ± 508.55	7655.3[c] ± 738.04	-	-	p<0.001
5-oxoETE	LOX	6908.7[a,b] ± 353.58	5645.5[a] ± 657.69	8454.80[b] ± 700.11	10443.6[c] ± 478.49	p<0.0001	-	p<0.05
Dihydroxy fatty acids								
5,6-DiHETE	CYP450	24.7 ± 5.42	25.3 ± 4.61	18.3 ± 3.46	14.4 ± 1.54	p<0.05	-	-
LA-oxylipins								
Hydroxy fatty acids								
13-HODE	LOX	1135.2 ± 155.32	962.4 ± 89.06	813.1 ± 88.01	689.4 ± 72.71	p<0.05	-	-
9-HODE	LOX	797.4 ± 135.59	637.1 ± 59.60	579.1 ± 36.53	502.1 ± 54.34	p<0.05	-	-
DHA-oxylipins								
Hydroxy fatty acids								

17-HDoHE	LOX	1108.5[a] ± 43.79	1328.8[a,b] ± 105.46	1542.4[b] ± 131.13	1328.8[a,b] ± 108.09	$p<0.05$	-	-
14-HDoHE	LOX	970.4 ± 112.69	988.1 ± 62.66	1151.9 ± 103.93	1135.9 ± 36.80	-	-	-
EPA-oxylipins								
Hydroxy fatty acids								
18-HEPE	COX	0.0 ± 0.00	0.0 ± 0.00	42.4 ± 17.17	30.0 ± 11.64	$p<0.001$	-	-

5-oxoETE: 5-oxo-eicosatetraenoic; 18-HEPE: hydroxy-eicosapentaenoic acid ; AA : arachidonic acid ; COX : cyclooxygenase ; CYP450 : cytochrome P450 ; DHA : docosahexaenoic acid ; EET: epoxy-eicosatrienoic acid; EPA : eicosapentaenoic acid ; HDoHE: hydroxy-docosahexaenoic acid; HETE: hydroxy-eicosatetraenoic acid; HODE: hydroxy-octadecadienoic acid; LxA4: lipoxin A4 ; .LOX : lipoxygenase ; PG: prostaglandin; TxB2: thromboxane B2