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.

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5. References

Antonietta Ajmone-Cat M, Lavinia Salvatori M, De Simone R, Mancini M, Biagioni S, Bernardo A, Cacci E, Minghetti L, 2012. Docosahexaenoic acid modulates inflammatory and antineurogenic functions of activated microglial cells. J Neurosci Res 90, 575-587.

Balvers MG, Verhoeckx KC, Bijlsma S, Rubingh CM, Meijerink J, Wortelboer HM, Witkamp RF, 2012a. Fish oil and inflammatory status alter the n-3 to n-6 balance of the endocannabinoid and oxylipin metabolomes in mouse plasma and tissues. Metabolomics 8, 1130-1147.

Balvers MG, Verhoeckx KC, Meijerink J, Bijlsma S, Rubingh CM, Wortelboer HM, Witkamp RF, 2012b. Time-dependent effect of in vivo inflammation on eicosanoid and endocannabinoid levels in plasma, liver, ileum and adipose tissue in C57BL/6 mice fed a fish-oil diet. Int Immunopharmacol 13, 204-214.

Bazan NG, 2009. Cellular and molecular events mediated by docosahexaenoic acid-derived neuroprotectin D1 signaling in photoreceptor cell survival and brain protection. Prostaglandins Leukot Essent Fatty Acids 81, 205-211.

Bazinet RP, Laye S, 2014. Polyunsaturated fatty acids and their metabolites in brain function and disease. Nat Rev Neurosci 15, 771-785.

Birnie M, Morrison R, Camara R, Strauss KI, 2013. Temporal changes of cytochrome P450 (Cyp) and eicosanoid-related gene expression in the rat brain after traumatic brain injury. BMC Genomics 14, 303.

Blais V, Rivest S, 2003. [Role of the innate immune response in the brain]. Med Sci (Paris) 19, 981-987.

Calder PC, 2006. n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. Am J Clin Nutr 83, 1505S-1519S.

Cao C, Matsumura K, Yamagata K, Watanabe Y, 1996. Endothelial cells of the rat brain vasculature express cyclooxygenase-2 mRNA in response to systemic interleukin-1 beta: a possible site of prostaglandin synthesis responsible for fever. Brain Res 733, 263-272.

Carrie I, Clement M, de Javel D, Frances H, Bourre JM, 2000. Phospholipid supplementation reverses behavioral and biochemical alterations induced by n-3 polyunsaturated fatty acid deficiency in mice. J Lipid Res 41, 473-480.

Chang PK, Khatchadourian A, McKinney RA, Maysinger D, 2015. Docosahexaenoic acid (DHA): a modulator of microglia activity and dendritic spine morphology. J Neuroinflammation 12, 34.

Chianese R, Coccurello R, Viggiano A, Scafuro M, Fiore M, Coppola G, Operto FF, Fasano S, Laye S, Pierantoni R, Meccariello R, 2018. Impact of Dietary Fats on Brain Functions. Curr Neuropharmacol 16, 1059-1085.

Christmas P, 2015. Role of Cytochrome P450s in Inflammation. Adv Pharmacol 74, 163-192.

Chung DW, Yoo KY, Hwang IK, Kim DW, Chung JY, Lee CH, Choi JH, Choi SY, Youn HY, Lee IS, Won MH, 2010. Systemic administration of lipopolysaccharide induces cyclooxygenase-2 immunoreactivity in endothelium and increases microglia in the mouse hippocampus. Cell Mol Neurobiol 30, 531-541.

Czapski GA, Gajkowska B, Strosznajder JB, 2010. Systemic administration of lipopolysaccharide induces molecular and morphological alterations in the hippocampus. Brain Res 1356, 85-94.

De Smedt-Peyrusse V, Sargueil F, Moranis A, Harizi H, Mongrand S, Laye S, 2008. Docosahexaenoic acid prevents lipopolysaccharide-induced cytokine production in microglial cells by inhibiting lipopolysaccharide receptor presentation but not its membrane subdomain localization. J Neurochem 105, 296-307.

Dehkordi NG, Noorbakhshnia M, Ghaedi K, Esmaeili A, Dabaghi M, 2015. Omega-3 fatty acids prevent LPS-induced passive avoidance learning and memory and CaMKII-alpha gene expression impairments in hippocampus of rat. Pharmacol Rep 67, 370-375.

Delpech JC, Madore C, Joffre C, Aubert A, Kang JX, Nadjar A, Laye S, 2015a. Transgenic increase in n-3/n-6 fatty acid ratio protects against cognitive deficits induced by an immune challenge through decrease of neuroinflammation. Neuropsychopharmacology 40, 525-536.

Delpech JC, Thomazeau A, Madore C, Bosch-Bouju C, Larrieu T, Lacabanne C, Remus-Borel J, Aubert A, Joffre C, Nadjar A, Laye S, 2015b. Dietary n-3 PUFAs Deficiency Increases Vulnerability to Inflammation-Induced Spatial Memory Impairment. Neuropsychopharmacology.

Destaillats F, Joffre C, Acar N, Joffre F, Bezelgues JB, Pasquis B, Cruz-Hernandez C, Rezzi S, Montoliu I, Dionisi F, Bretillon L, 2010. Differential effect of maternal diet supplementation with alpha-Linolenic adcid or n-3 long-chain polyunsaturated fatty acids on glial cell phosphatidylethanolamine and phosphatidylserine fatty acid profile in neonate rat brains. Nutr Metab (Lond) 7, 2.

Dieter P, Scheibe R, Kamionka S, Kolada A, 2002. LPS-induced synthesis and release of PGE2 in liver macrophages: regulation by CPLA2, COX-1, COX-2, and PGE2 synthase. Adv Exp Med Biol 507, 457-462.

Domenichiello AF, Chen CT, Trepanier MO, Stavro PM, Bazinet RP, 2014. Whole body synthesis rates of DHA from alpha-linolenic acid are greater than brain DHA accretion and uptake rates in adult rats. J Lipid Res 55, 62-74.

Dong Y, Xu M, Kalueff AV, Song C, 2017. Dietary eicosapentaenoic acid normalizes hippocampal omega-3 and 6 polyunsaturated fatty acid profile, attenuates glial activation and regulates BDNF function in a rodent model of neuroinflammation induced by central interleukin-1beta administration. Eur J Nutr.

Enyedi B, Kala S, Nikolich-Zugich T, Niethammer P, 2013. Tissue damage detection by osmotic surveillance. Nat Cell Biol 15, 1123-1130.

Fan C, Fu H, Dong H, Lu Y, Qi K, 2016. Maternal n-3 polyunsaturated fatty acid deprivation during pregnancy and lactation affects neurogenesis and apoptosis in adult offspring: associated

with DNA methylation of brain-derived neurotrophic factor transcripts. Nutr Res 36, 1013-1021.

Farias SE, Basselin M, Chang L, Heidenreich KA, Rapoport SI, Murphy RC, 2008. Formation of eicosanoids, E2/D2 isoprostanes, and docosanoids following decapitation-induced ischemia, measured in high-energy-microwaved rat brain. J Lipid Res 49, 1990-2000.

Fourrier C, Remus-Borel J, Greenhalgh AD, Guichardant M, Bernoud-Hubac N, Lagarde M, Joffre C, Laye S, 2017. Docosahexaenoic acid-containing choline phospholipid modulates LPS-induced neuroinflammation in vivo and in microglia in vitro. J Neuroinflammation 14, 170.

Fredman G, Ozcan L, Spolitu S, Hellmann J, Spite M, Backs J, Tabas I, 2014. Resolvin D1 limits 5-lipoxygenase nuclear localization and leukotriene B4 synthesis by inhibiting a calcium-activated kinase pathway. Proc Natl Acad Sci U S A 111, 14530-14535.

Gabbs M, Leng S, Devassy JG, Monirujjaman M, Aukema HM, 2015. Advances in Our Understanding of Oxylipins Derived from Dietary PUFAs. Adv Nutr 6, 513-540.

Hampson AJ, Grimaldi M, 2002. 12-hydroxyeicosatetrenoate (12-HETE) attenuates AMPA receptor-mediated neurotoxicity: evidence for a G-protein-coupled HETE receptor. J Neurosci 22, 257-264.

Hashimoto M, Katakura M, Tanabe Y, Al Mamun A, Inoue T, Hossain S, Arita M, Shido O, 2015. n-3 fatty acids effectively improve the reference memory-related learning ability associated with increased brain docosahexaenoic acid-derived docosanoids in aged rats. Biochim Biophys Acta 1851, 203-209.

Hiratsuka S, Koizumi K, Ooba T, Yokogoshi H, 2009. Effects of dietary docosahexaenoic acid connecting phospholipids on the learning ability and fatty acid composition of the brain. J Nutr Sci Vitaminol (Tokyo) 55, 374-380.

Hong S, Gronert K, Devchand PR, Moussignac RL, Serhan CN, 2003. Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood, and glial cells. Autacoids in anti-inflammation. J Biol Chem 278, 14677-14687.

Hung TH, Shyue SK, Wu CH, Chen CC, Lin CC, Chang CF, Chen SF, 2017. Deletion or inhibition of soluble epoxide hydrolase protects against brain damage and reduces microglia-mediated neuroinflammation in traumatic brain injury. Oncotarget 8, 103236-103260.

Ikemoto A, Ohishi M, Hata N, Misawa Y, Fujii Y, Okuyama H, 2000. Effect of n-3 fatty acid deficiency on fatty acid composition and metabolism of aminophospholipids in rat brain synaptosomes. Lipids 35, 1107-1115.

Iliff JJ, Jia J, Nelson J, Goyagi T, Klaus J, Alkayed NJ, 2010. Epoxyeicosanoid signaling in CNS function and disease. Prostaglandins Other Lipid Mediat 91, 68-84.

Joffre C, Gregoire S, De Smedt V, Acar N, Bretillon L, Nadjar A, Laye S, 2016. Modulation of brain PUFA content in different experimental models of mice. Prostaglandins Leukot Essent Fatty Acids 114, 1-10.

Jung YS, Park JH, Kim H, Kim SY, Hwang JY, Hong KW, Bae SS, Choi BT, Lee SW, Shin HK, 2016. Probucol inhibits LPS-induced microglia activation and ameliorates brain ischemic injury in normal and hyperlipidemic mice. Acta Pharmacol Sin 37, 1031-1044.

Kim HW, Rao JS, Rapoport SI, Igarashi M, 2011. Dietary n-6 PUFA deprivation downregulates arachidonate but upregulates docosahexaenoate metabolizing enzymes in rat brain. Biochim Biophys Acta 1811, 111-117.

Labrousse VF, Nadjar A, Joffre C, Costes L, Aubert A, Gregoire S, Bretillon L, Laye S, 2012. Short-term long chain omega3 diet protects from neuroinflammatory processes and memory impairment in aged mice. PLoS One 7, e36861.

Lacombe RJS, Giuliano V, Colombo SM, Arts MT, Bazinet RP, 2017. Compound-specific isotope analysis resolves the dietary origin of docosahexaenoic acid in the mouse brain. J Lipid Res 58, 2071-2081.

Laye S, 2010. Polyunsaturated fatty acids, neuroinflammation and well being. Prostaglandins Leukot Essent Fatty Acids 82, 295-303.

Laye S, Nadjar A, Joffre C, Bazinet RP, 2018. Anti-Inflammatory Effects of Omega-3 Fatty Acids in the Brain: Physiological Mechanisms and Relevance to Pharmacology. Pharmacol Rev 70, 12-38.

Laye S, Nadjar, A., Joffre, C., and Bazinet, R.P., 2017. Anti-inflammatory effects of omega-3 fatty acids in the brain: Physiological mechanisms and relevance to pharmacology

Pharma Rev.

Laye S, Parnet P, Goujon E, Dantzer R, 1994. Peripheral administration of lipopolysaccharide induces the expression of cytokine transcripts in the brain and pituitary of mice. Brain Res Mol Brain Res 27, 157-162.

Le Faouder P, Baillif V, Spreadbury I, Motta JP, Rousset P, Chene G, Guigne C, Terce F, Vanner S, Vergnolle N, Bertrand-Michel J, Dubourdeau M, Cenac N, 2013. LC-MS/MS method for rapid and concomitant quantification of pro-inflammatory and pro-resolving polyunsaturated fatty acid metabolites. J Chromatogr B Analyt Technol Biomed Life Sci 932, 123-133.

Levy BD, Clish CB, Schmidt B, Gronert K, Serhan CN, 2001. Lipid mediator class switching during acute inflammation: signals in resolution. Nat Immunol 2, 612-619.

Li Q, Leung YO, Zhou I, Ho LC, Kong W, Basil P, Wei R, Lam S, Zhang X, Law AC, Chua SE, Sham PC, Wu EX, McAlonan GM, 2015. Dietary supplementation with n-3 fatty acids from weaning limits brain biochemistry and behavioural changes elicited by prenatal exposure to maternal inflammation in the mouse model. Transl Psychiatry 5, e641.

Lonergan PE, Martin DS, Horrobin DF, Lynch MA, 2004. Neuroprotective actions of eicosapentaenoic acid on lipopolysaccharide-induced dysfunction in rat hippocampus. J Neurochem 91, 20-29.

Madore C, Joffre C, Delpech JC, De Smedt-Peyrusse V, Aubert A, Coste L, Laye S, Nadjar A, 2013. Early morphofunctional plasticity of microglia in response to acute lipopolysaccharide. Brain Behav Immun 34, 151-158.

Madore C, Nadjar A, Delpech JC, Sere A, Aubert A, Portal C, Joffre C, Laye S, 2014. Nutritional n-3 PUFAs deficiency during perinatal periods alters brain innate immune system and neuronal plasticity-associated genes. Brain Behav Immun 41, 22-31.

Mingam R, Moranis A, Bluthe RM, De Smedt-Peyrusse V, Kelley KW, Guesnet P, Lavialle M, Dantzer R, Laye S, 2008. Uncoupling of interleukin-6 from its signalling pathway by dietary n-3-polyunsaturated fatty acid deprivation alters sickness behaviour in mice. Eur J Neurosci 28, 1877-1886.

Moranis A, Delpech JC, De Smedt-Peyrusse V, Aubert A, Guesnet P, Lavialle M, Joffre C, Laye S, 2012. Long term adequate n-3 polyunsaturated fatty acid diet protects from depressive-like behavior but not from working memory disruption and brain cytokine expression in aged mice. Brain Behav Immun 26, 721-731.

Murthy M, Hamilton J, Greiner RS, Moriguchi T, Salem N, Jr., Kim HY, 2002. Differential effects of n-3 fatty acid deficiency on phospholipid molecular species composition in the rat hippocampus. J Lipid Res 43, 611-617.

Nadjar A, Tridon V, May MJ, Ghosh S, Dantzer R, Amedee T, Parnet P, 2005. NFkappaB activates in vivo the synthesis of inducible Cox-2 in the brain. J Cereb Blood Flow Metab 25, 1047-1059.

Nair AB, Jacob S, 2016. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm 7, 27-31.

Orr SK, Palumbo S, Bosetti F, Mount HT, Kang JX, Greenwood CE, Ma DW, Serhan CN, Bazinet RP, 2013. Unesterified docosahexaenoic acid is protective in neuroinflammation. J Neurochem.

Orr SK, Tong JY, Kang JX, Ma DW, Bazinet RP, 2010. The fat-1 mouse has brain docosahexaenoic acid levels achievable through fish oil feeding. Neurochem Res 35, 811-819.

Ostermann AI, Reutzel M, Hartung N, Franke N, Kutzner L, Schoenfeld K, Weylandt KH, Eckert GP, Schebb NH, 2017. A diet rich in omega-3 fatty acids enhances expression of soluble epoxide hydrolase in murine brain. Prostaglandins Other Lipid Mediat.

Pettit LK, Varsanyi C, Tadros J, Vassiliou E, 2013. Modulating the inflammatory properties of activated microglia with Docosahexaenoic acid and Aspirin. Lipids Health Dis 12, 16.

Plourde M, Cunnane SC, 2007. Extremely limited synthesis of long chain polyunsaturates in adults: implications for their dietary essentiality and use as supplements. Appl Physiol Nutr Metab 32, 619-634.

Powell WS, Rokach J, 2015. Biosynthesis, biological effects, and receptors of hydroxyeicosatetraenoic acids (HETEs) and oxoeicosatetraenoic acids (oxo-ETEs) derived from arachidonic acid. Biochim Biophys Acta 1851, 340-355.

Pratico D, Zhukareva V, Yao Y, Uryu K, Funk CD, Lawson JA, Trojanowski JQ, Lee VM, 2004. 12/15-lipoxygenase is increased in Alzheimer's disease: possible involvement in brain oxidative stress. Am J Pathol 164, 1655-1662.

Quan N, Whiteside M, Herkenham M, 1998. Cyclooxygenase 2 mRNA expression in rat brain after peripheral injection of lipopolysaccharide. Brain Res 802, 189-197.

Rajaei E, Mowla K, Ghorbani A, Bahadoram S, Bahadoram M, Dargahi-Malamir M, 2015. The Effect of Omega-3 Fatty Acids in Patients With Active Rheumatoid Arthritis Receiving DMARDs Therapy: Double-Blind Randomized Controlled Trial. Glob J Health Sci 8, 18-25.

Rao JS, Ertley RN, DeMar JC, Jr., Rapoport SI, Bazinet RP, Lee HJ, 2007. Dietary n-3 PUFA deprivation alters expression of enzymes of the arachidonic and docosahexaenoic acid cascades in rat frontal cortex. Mol Psychiatry 12, 151-157.

Rey C, Nadjar A, Buaud B, Vaysse C, Aubert A, Pallet V, Laye S, Joffre C, 2016. Resolvin D1 and E1 promote resolution of inflammation in microglial cells in vitro. Brain Behav Immun 55, 249-259.

Rosenberger TA, Villacreses NE, Hovda JT, Bosetti F, Weerasinghe G, Wine RN, Harry GJ, Rapoport SI, 2004. Rat brain arachidonic acid metabolism is increased by a 6-day intracerebral ventricular infusion of bacterial lipopolysaccharide. J Neurochem 88, 1168-1178.

Rummel C, Sachot C, Poole S, Luheshi GN, 2006. Circulating interleukin-6 induces fever through a STAT3-linked activation of COX-2 in the brain. Am J Physiol Regul Integr Comp Physiol 291, R1316-1326.

Salinas G, Rangasetty UC, Uretsky BF, Birnbaum Y, 2007. The cycloxygenase 2 (COX-2) story: it's time to explain, not inflame. J Cardiovasc Pharmacol Ther 12, 98-111.

Serhan CN, 2011. The resolution of inflammation: the devil in the flask and in the details. FASEB J 25, 1441-1448.

Serhan CN, Chiang N, Dalli J, 2015. The resolution code of acute inflammation: Novel pro-resolving lipid mediators in resolution. Semin Immunol 27, 200-215.

Serhan CN, Clish CB, Brannon J, Colgan SP, Chiang N, Gronert K, 2000. Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. J Exp Med 192, 1197-1204.

Serhan CN, Krishnamoorthy S, Recchiuti A, Chiang N, 2011. Novel anti-inflammatory--pro-resolving mediators and their receptors. Curr Top Med Chem 11, 629-647.

Shalini SM, Ho CF, Ng YK, Tong JX, Ong ES, Herr DR, Dawe GS, Ong WY, 2017. Distribution of Alox15 in the Rat Brain and Its Role in Prefrontal Cortical Resolvin D1 Formation and Spatial Working Memory. Mol Neurobiol.

Shi Z, Ren H, Huang Z, Peng Y, He B, Yao X, Yuan TF, Su H, 2016. Fish Oil Prevents Lipopolysaccharide-Induced Depressive-Like Behavior by Inhibiting Neuroinflammation. Mol Neurobiol.

Skorve J, Hilvo M, Vihervaara T, Burri L, Bohov P, Tillander V, Bjorndal B, Suoniemi M, Laaksonen R, Ekroos K, Berge RK, Alexson SE, 2015. Fish oil and krill oil differentially modify the liver and brain lipidome when fed to mice. Lipids Health Dis 14, 88.

Slepko N, Minghetti L, Polazzi E, Nicolini A, Levi G, 1997. Reorientation of prostanoid production accompanies "activation" of adult microglial cells in culture. J Neurosci Res 49, 292-300.

Solito E, Sastre M, 2012. Microglia function in Alzheimer's disease. Front Pharmacol 3, 14.

Sun L, Xu YW, Han J, Liang H, Wang N, Cheng Y, 2015. 12/15-Lipoxygenase metabolites of arachidonic acid activate PPARgamma: a possible neuroprotective effect in ischemic brain. J Lipid Res 56, 502-514.

Taha AY, Blanchard HC, Cheon Y, Ramadan E, Chen M, Chang L, Rapoport SI, 2017. Dietary Linoleic Acid Lowering Reduces Lipopolysaccharide-Induced Increase in Brain Arachidonic Acid Metabolism. Mol Neurobiol 54, 4303-4315.

Taha AY, Hennebelle M, Yang J, Zamora D, Rapoport SI, Hammock BD, Ramsden CE, 2016. Regulation of rat plasma and cerebral cortex oxylipin concentrations with increasing levels of dietary linoleic acid. Prostaglandins Leukot Essent Fatty Acids.

Tarannum F, Faizuddin M, 2017. Effect of Alox-15 Polymorphism on GCF Levels of Lipoxin-A4 in Chronic Periodontitis: A Preliminary Study. Braz Dent J 28, 140-147.

Wang L, Yuan R, Yao C, Wu Q, Christelle M, Xie W, Zhang X, Sun W, Wang H, Yao S, 2014. Effects of resolvin D1 on inflammatory responses and oxidative stress of lipopolysaccharide-induced acute lung injury in mice. Chin Med J (Engl) 127, 803-809.

Wang T, Qin L, Liu B, Liu Y, Wilson B, Eling TE, Langenbach R, Taniura S, Hong JS, 2004. Role of reactive oxygen species in LPS-induced production of prostaglandin E2 in microglia. J Neurochem 88, 939-947.

Wang X, Zhu M, Hjorth E, Cortes-Toro V, Eyjolfsdottir H, Graff C, Nennesmo I, Palmblad J, Eriksdotter M, Sambamurti K, Fitzgerald JM, Serhan CN, Granholm AC, Schultzberg M, 2015. Resolution of inflammation is altered in Alzheimer's disease. Alzheimers Dement 11, 40-50 e41-42.

Willenberg I, Rund K, Rong S, Shushakova N, Gueler F, Schebb NH, 2016. Characterization of changes in plasma and tissue oxylipin levels in LPS and CLP induced murine sepsis. Inflamm Res 65, 133-142.

Woodroofe MN, 1995. Cytokine production in the central nervous system. Neurology 45, S6-10.

Woodroofe MN, Cuzner ML, 1993. Cytokine mRNA expression in inflammatory multiple sclerosis lesions: detection by non-radioactive in situ hybridization. Cytokine 5, 583-588.

Yang J, Schmelzer K, Georgi K, Hammock BD, 2009. Quantitative profiling method for oxylipin metabolome by liquid chromatography electrospray ionization tandem mass spectrometry. Anal Chem 81, 8085-8093.

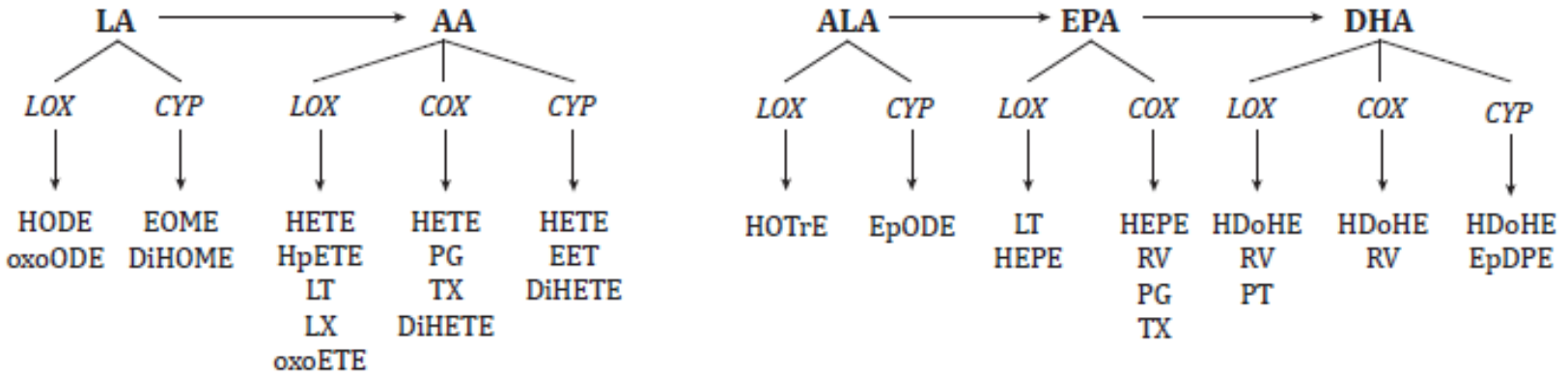
Yigitkanli K, Zheng Y, Pekcec A, Lo EH, van Leyen K, 2017. Increased 12/15-Lipoxygenase Leads to Widespread Brain Injury Following Global Cerebral Ischemia. Transl Stroke Res 8, 194-202.

Yu L, Yang B, Wang J, Zhao L, Luo W, Jiang Q, Yang J, 2014. Time course change of COX2-PGI2/TXA2 following global cerebral ischemia reperfusion injury in rat hippocampus. Behav Brain Funct 10, 42.

Zhao Y, Calon F, Julien C, Winkler JW, Petasis NA, Lukiw WJ, Bazan NG, 2011. Docosahexaenoic acid-derived neuroprotectin D1 induces neuronal survival via secretase- and PPARgamma-mediated mechanisms in Alzheimer's disease models. PLoS One 6, e15816.

Zhou Y, Liu T, Duan JX, Li P, Sun GY, Liu YP, Zhang J, Dong L, Lee KSS, Hammock BD, Jiang JX, Guan CX, 2017. Soluble Epoxide Hydrolase Inhibitor Attenuates Lipopolysaccharide-Induced Acute Lung Injury and Improves Survival in Mice. Shock 47, 638-645.

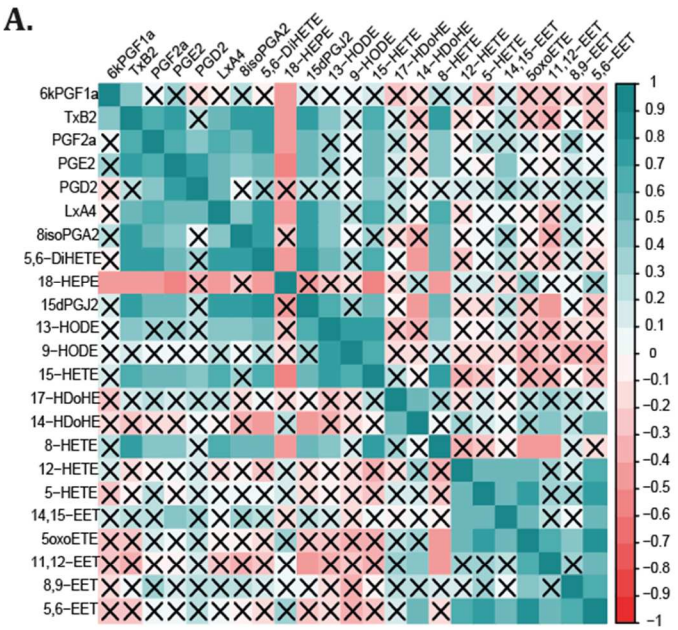
Figure 1. Oxylipin synthesis pathways



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.

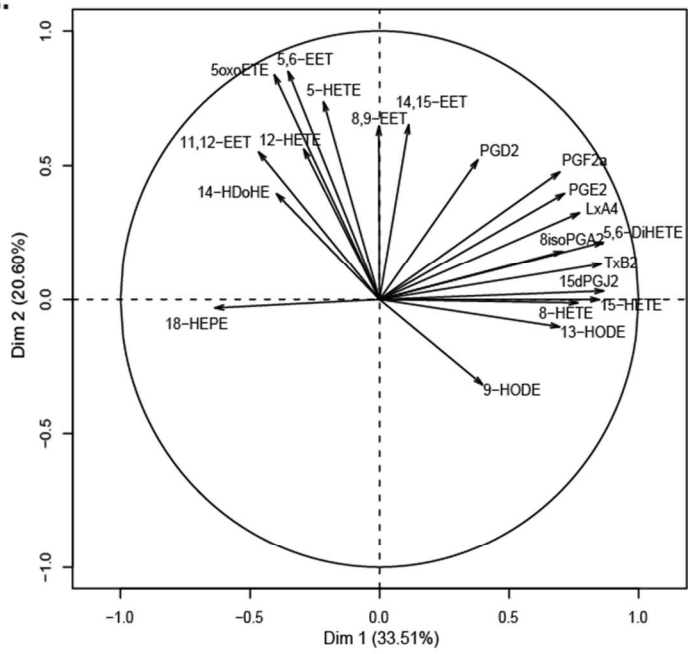
A.



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

C.



D.

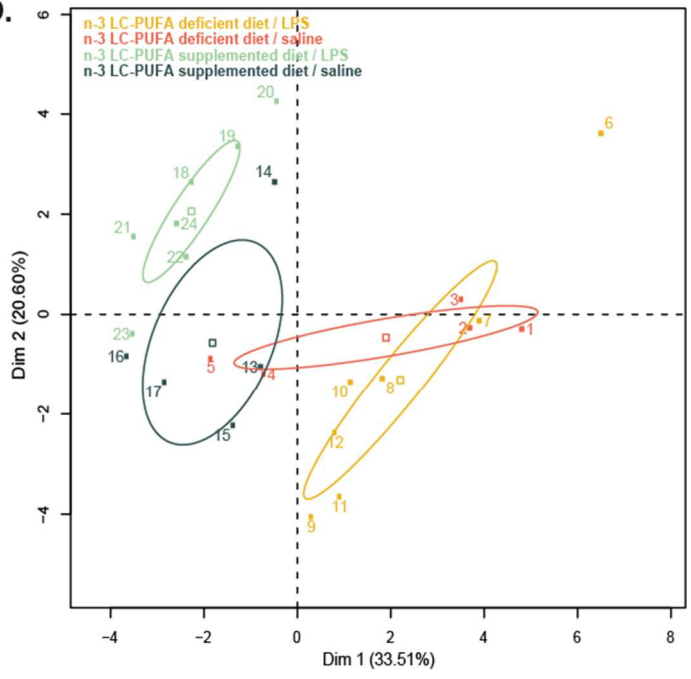


Figure 3.Oxylipin synthesis enzyme expression in the hippocampus of mice fed with an n-3 LC-PUFA deficientor n-3 LC-PUFA supplemented diet for 2 months, 2h and 24h following LPS injection. Data are presented as means ±SEM (n=5-6). Data were analyzed by a two-way ANOVA.

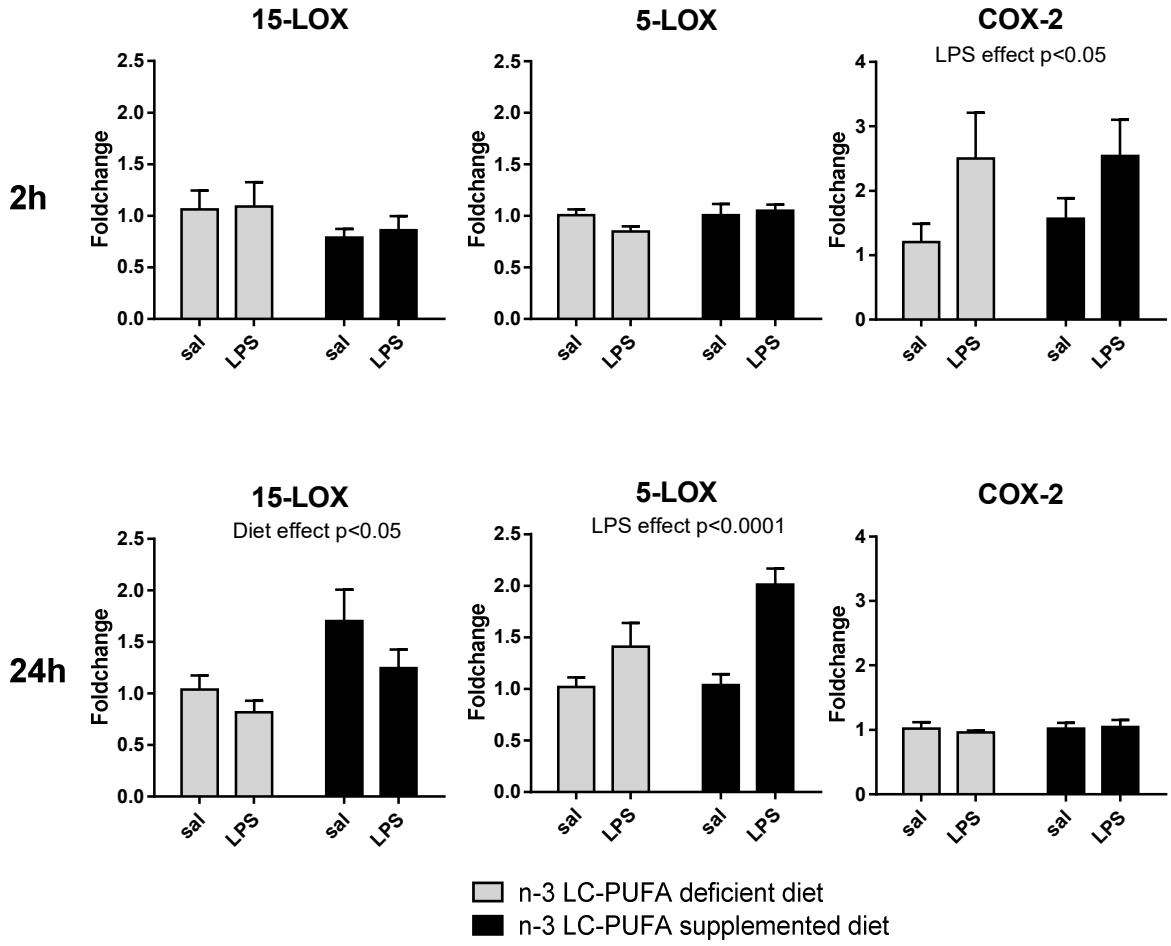


Figure 4. Cytokine expression in the hippocampus of mice fed withan n-3 LC-PUFA deficientor n-3 LC-PUFA supplemented diet for 2 months, 2h and 24h following LPS injection. Data are presented as means ±SEM (n=6). Data were analyzed by a two-way ANOVA followed by a *post hoc* Fischer test comparison.

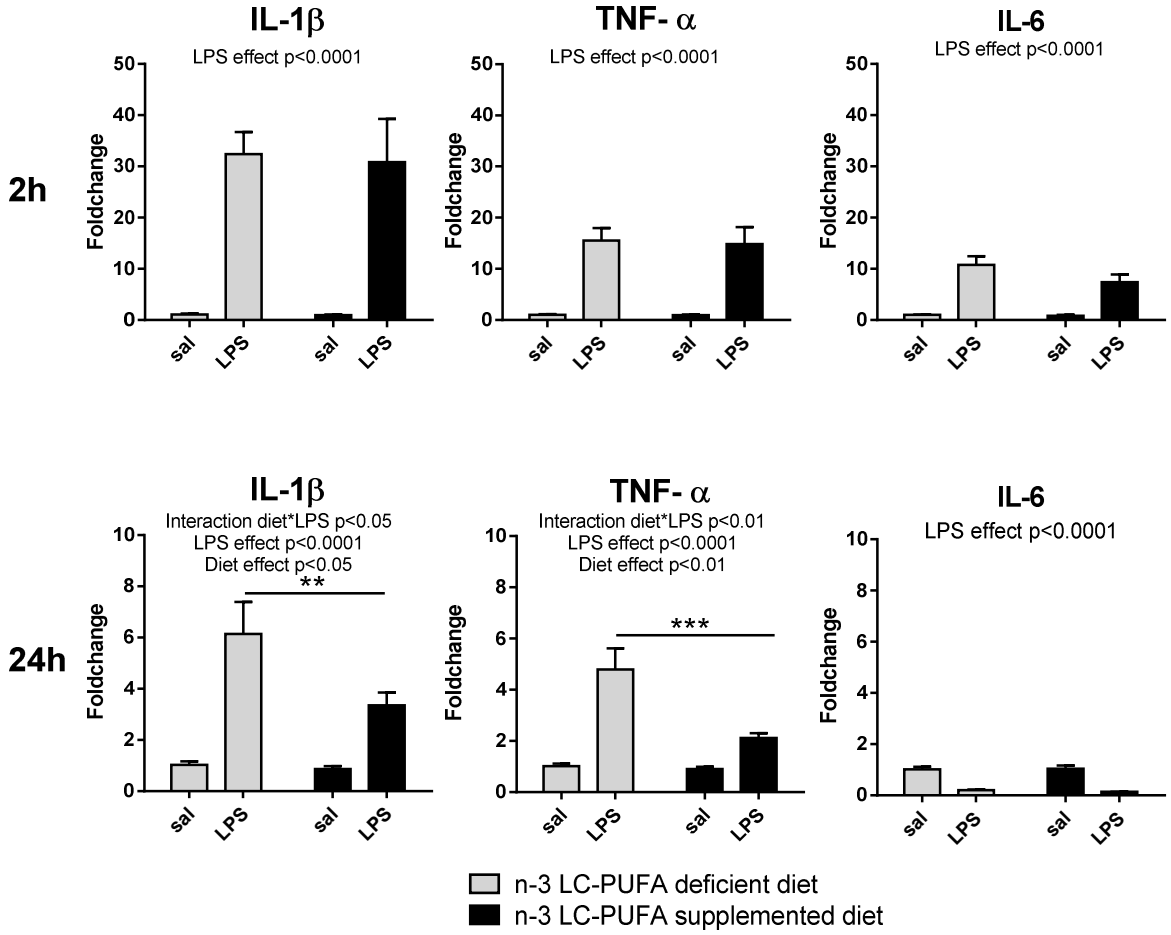


Table 1.Composition of the diets (g/kg diet)

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 acid	DHA	7(S),8(R),17(S)-Trihydroxy-4Z,9E,11E,13Z,15E,19Zdocosahexaenoic
RvD2 acid	DHA	7S,16R,17S-trihydroxy-4Z,8E,10Z,12E,14E,19Z-docosahexaenoic
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		Statisticaleffect		
	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 ($p<0.05$).

		n-3 LC-PUFA deficient diet		n-3 LC-PUFA supplemented diet		Statistical effect		
Oxylipin	Pathway	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