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ORIGINAL RESEARCH PAPER

Plant extracts and omega-3 supplementation modulate hippocampal oxylipin profile in response to LPS-induced neuroinflammation

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Abstract

Objective and design Neuroinflammation is a protective mechanism but can become harmful if chronic and/or unregulated, leading to neuronal damage and cognitive alterations. Limiting inflammation and promoting resolution could be achieved with nutrients such as grapes and blueberries polyphenols, saffron carotenoids, and omega-3, which have anti-inflammatory and proresolutive properties.

Methods This study explored the impact of 18-day supplementation with plant extracts (grape, blueberry and saffron), omega-3 or both (mix) on neuroinflammation induced by lipopolysaccharide (LPS, 250 µg/kg) in 149 mice at different time points post-LPS treatment (30 min, 2 h, 6 h). Inflammatory, oxidative and neuroprotective gene expression; oxylipin quantification; and fatty acid composition were analyzed at each time point. PCA analysis was performed with all these biomarkers.

Results Mix supplementation induced changes in the resolution of inflammation. In fact, the production of proinflammatory mediators in the hippocampus started earlier in the supplemented group than in the LPS group. Pro-resolving mediators were also found in higher quantities in supplemented mice. These changes were associated with increased hippocampal antioxidant status at 6 h post-LPS.

Conclusions These findings suggest that such dietary interventions with plant extracts, and omega-3 could be beneficial in preventing neuroinflammation and, consequently, age-related cognitive decline. Further research is needed to explore the effects of these supplements on chronic inflammation in the context of aging.

Keywords Plant extracts · Omega-3 · Neuroinflammation · Oxylipin · LPS · Mice

Abbreviations

5-oxoETE	5-oxo-eicosatetraenoic
AA	Arachidonic acid
ALA	α-linolenic acid
ApoE	Apolipoprotein E
Arg1	Arginase 1
BDNF	Brain-derived neurotrophic factor
CaMKII	Calmodulin-dependent protein kinase II
CAT	Catalase
CCL2	Chemokine (C-C motif) ligand 2
CD	Cluster of differentiation
Clec7a	C-type lectin domain family 7 member A
COX-2	Cyclooxygenase-2
CXCL	C-X-C motif chemokine ligand
DCX	Doublecortin
DHA	Docosahexaenoic acid
EET	Epoxyeicosatrienoic acid
EPA	Eicosapentaenoic acid

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GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GPx1	Glutathione peroxidase 1
GSS	Glutathione synthetase
GSTp1	Glutathione S-transferase P 1
HDHA	Hydroxy docosahexaenoic acid
HEPE	Hydroxyeicosapentaenoic acid
HETE	Hydroxyeicosatetraenoic acid
HODE	Hydroxyoctadecadienoic acid
HOME	Hydroxyoctadecenoic acid
IFNγ	Interferon-γ
IL	Interleukin
iNOS	Inducible nitric oxide synthase
LA	Linoleic acid
LOX	Lipoxygenase
LPS	Lipopolysaccharide
LT	Leukotriene
Lx	Lipoxin
MUFAs	Monounsaturated fatty acids
NF-κB	Nuclear factor-kappa B
NGF	Nerve growth factor
NRF2	Nuclear factor erythroid 2–related factor 2
Om-3	Omega-3
Om-6	Omega-6
Oxo-ODE	oxo-octadecadienoic acid
PCA	Principal component analysis
PE	Plant extracts
PG	Prostaglandin
RAGE	Receptor for advanced glycation end products
SFAs	saturated fatty acids
SOCS3	Suppressor of cytokine signaling 3
SOD	Superoxide dismutase
TGF-β	Transforming growth factorβ
TLR4	Toll-like receptor 4
TNF-α	Tumor necrosis factorα
Trem2	Triggering receptor expressed on myeloid cells 2
TrkB	Tropomyosin receptor kinase
Tspo	Translocator protein
TXB_2	Thromboxane B2

Introduction

In healthy humans, neuroinflammation serves as a vital protective mechanism which play a defensive role against pathogens. However, when it becomes chronic and unregulated, it can become detrimental due to the sustained release of cytokines and other proinflammatory molecules [1]. Chronic low-grade neuroinflammation is a characteristic feature of aging and plays a key role in the onset of age-related cognitive decline and various health issues [2]. It induces the activation of brain immune cells called microglia, which regulate inflammation. In a healthy brain, these cells express a homeostatic signature and are involved in environmental monitoring and neurogenesis. During aging, microglial cells become more reactive and produce more proinflammatory cytokines and chemokines, such as TNF-α, IL-1β, IL-6, CCL2 and CXCL10 [3–5]. They accumulate and may also polarize towards an MGnD phenotype (microglial neurodegenerative phenotype), which is characteristic of aging, thereby disrupting the inflammatory response [6]. The chronic low-grade inflammation associated with aging induces neuronal alterations leading to a decline in cognitive function, with short- and long-term memory deficits. Indeed, high levels of plasma IL-6 are correlated with impaired cognitive function in elderly people [7, 8]. Conversely, IL-6 deficiency in mice has been shown to protect against age-related cognitive decline [9]. Inflammatory processes are also intricately regulated by specific lipid mediators, called oxylipins, which, while triggering inflammation on one hand, preferably facilitate self-resolution on the other [10, 11]. Oxylipins are produced on demand and are not stocked, they can be found throughout the body in all tissues, urine and blood. Brain is equipped with enzymes allowing their synthesis [12]. There is a growing literature on the role of oxylipins in inflammation during normal or pathological aging [13, 14]. In fact, older healthy people are reported to have a different plasma oxylipin profile than healthy young people [15]. Moreover, LxA_4, a proresolving mediator, was found to be reduced in the cerebrospinal fluid and hippocampus of Alzheimer's patients and to be positively correlated with better scores on a memory test [16]. Therefore, restricting overly brain inflammatory reactions and enhancing the process of resolution are crucial for the return to homeostasis and to prevent alterations linked to an uncontrolled inflammatory response.

Strong evidence suggests that nutrition plays a significant role in influencing inflammation. Several nutrients, such as polyphenols, carotenoids and omega-3 polyunsaturated fatty acids (omega-3), have immunomodulatory properties and are good candidates to prevent impairments related to neuroinflammation. In fact, polyphenols exhibit anti-inflammatory effects through their ability to reduce LPS-induced microglial activation [17]. Notably, it has been shown that a blueberry-mulberry extract, rich in polyphenols, decreases the expression of proinflammatory markers in the brain and intestine of aged mice [18]. Furthermore, resveratrol, a stilbene present in grapes, decreases LPS-induced protein and gene expressions of IL-1β in the plasma and hippocampus of aged mice and reduces the expression of proinflammatory markers such as IL-1β and COX-2 in LPS-stimulated microglial cells [19, 20]. Studies reported that saffron carotenoids also have anti-inflammatory properties, notably by decreasing the levels of IL-6 and TNF-α

in the plasma of patients with type 2 diabetes compared to placebo [21]. In rats, saffron used as a treatment for 7 days in a model of chronic neuropathy, attenuates the production of proinflammatory factors such as IL-6, IL-1β and TNF-α in the spinal cord [22]. Moreover, it has been demonstrated in LPS-stimulated microglial cells from rats that saffron carotenoids, crocin and crocetin, decrease the expression of the proinflammatory cytokines TNF-α and IL-1β [23]. Safranal, another constituent of saffron, also decreases neuroinflammation by downregulating NF-κB signaling pathway and proinflammatory cytokines expression in mice model of epilepsy and in rat model of Alzheimer's disease [24, 25]. In addition, omega-3 have been widely described for their immunomodulatory properties. Indeed, DHA supplementation reduces the serum levels of inflammatory markers in humans [26]. In aged mice, 2-month EPA and DHA supplementation prevents the increase of proinflammatory cytokines IL-6, IL-1β and TNF-α expression in the hippocampus and plasma [27]. Oxylipin profile can be modulated by some nutrients such as polyphenols and omega-3. Indeed, despite few studies on the effects of polyphenols, Hartung et al.., reported that genistein, a flavonoid, resveratrol and its derivatives modulate the oxylipin profile and inhibit 5-LOX, an enzyme implicated in oxylipin biosynthesis, in human neutrophils [28]. Moreover, 18 days of blueberry supplementation increases plasma levels of proresolutive oxylipins and decreases those of proinflammatory derivates in humans [29]. DHA has also been shown, both in humans and mice, to increase the synthesis of proresolving oxylipins, which play a key role in resolving inflammation [12, 30–33]. To our knowledge, no studies have investigated the effects of saffron carotenoids on brain oxylipin profile.

Combining these nutrients could be pertinent for preventing neuroinflammation by having additional effects. Hence, our study explored the impact of a mix of plant extracts that were chosen according to previous results [34–39]. This mix contained polyphenols sourced from grapes and blueberries, as well as saffron carotenoids, and omega-3 fatty acids from fish, on LPS-induced neuroinflammation in mice and compare it to the effects of plant extracts or omega-3 alone.

Table 1 Composition of the supplements

	Control and LPS	LPS (PE)	LPS (om-3)	LPS (PE + om-3)
Water (µL/day)	100	-	100	-
Plant extracts (µL/day)	-	100	-	100
Memophenol™[1] (mg/day)	-	*3.69*	-	*3.69*
Saffron extract[2] (mg/day)	-	*0.1845*	-	*0.1845*
Peanut oil (µL/day)	50	50	-	-
Fish oil-omega-3 (µL/day)	-	-	50	50
DHA (mg/day)	-	-	*6.15*	*6.15*
EPA (mg/day)	-	-	*1.41*	*1.41*

[1]total polyphenol ≥ 75%: flavonoids (flavan-3-ols, flavonols, anthocyanins) ≥ 43%, flavan-3-ols monomers ≥ 20%, flavan-3-ols oligomers (DP ≤ 4) ≥ 22%, flavonols (including quercetin and glycosylated derivatives) ≥ 0.15%, anthocyanins (including malvidin 3-glucoside) ≥ 0.10%, phenolic acids ≥ 0.50%, stilbenes (including resveratrol) ≥ 300 ppm

[2]crocins (mainly trans-4-GG, trans-3-Gg; cis-4-GG, trans-2-G) > 3%, safranal > 0.2%, picrocrocin derivatives (mainly picrocrocin, HTCC) > 1%, and kaempferol derivatives (mainly kaempferol-3-sophoroside-7-glucoside, kaempferol-3-sophoroside) > 0.1%

Materials and methods

Animals and treatments

All tests were conducted with 5-month-old male C57Bl/6J mice from Janvier Labs (Le Genest-Saint-Isle, France). The mice were kept under standard housing conditions with cellulose litter in an environment controlled for temperature (21 ± 2 °C) and humidity (40%), following a 12-hour light/dark cycle (7:00–19:00), and were provided *ad libitum* access to water and food (A04, Safe, Augy, France). A total of 149 animals were used. Over an 18-day period, the mice were subjected to force-feeding through a gastric tube with 150 µL of supplements, including a plant extract containing polyphenols from grape and blueberry (3.69 mg/day, equivalent to 600 mg/day in humans) (Memophenol™ Activ'Inside, Beychac-et-Caillau, France) and carotenoids and safranal from saffron extract (0.1845 mg/day, equivalent to 30 mg/day in humans) (patent pending WO2021209455A1 Activ'Inside, Beychac-et-Caillau, France) ($n = 30$), omega-3 (om-3) in triglyceride form (containing 6.15 mg/day of DHA and 1.41 mg/day of EPA, equivalent to 1000 and 229.27 mg/day and in humans) ($n = 30$), or a combination of both ($n = 29$) (Table 1). Control mice received water and peanut oil in the same way ($n = 60$). Plant extracts and omega-3 were diluted in water or peanut oil, respectively. At the end of the experiment, mice were intraperitoneally injected with a saline solution for the control group ($n = 30$) or a solution of 250 µg/kg of LPS (lipopolysaccharide; *Escherichia coli*, 0127: B8; Sigma-Aldrich, Lyon, France, $n = 119$) to induce acute inflammation. To examine the impact of inflammation and supplementation over time, mice were euthanized at 30 min ($n = 49$), 2 h ($n = 50$), or 6 h ($n = 50$) after the injection. Hippocampus and spleen were isolated and stored at -80 °C until analysis.

Quantitative real time PCR

Total RNA from hippocampus was extracted using the TRIzol extraction protocol (Invitrogen, Life Technologies,

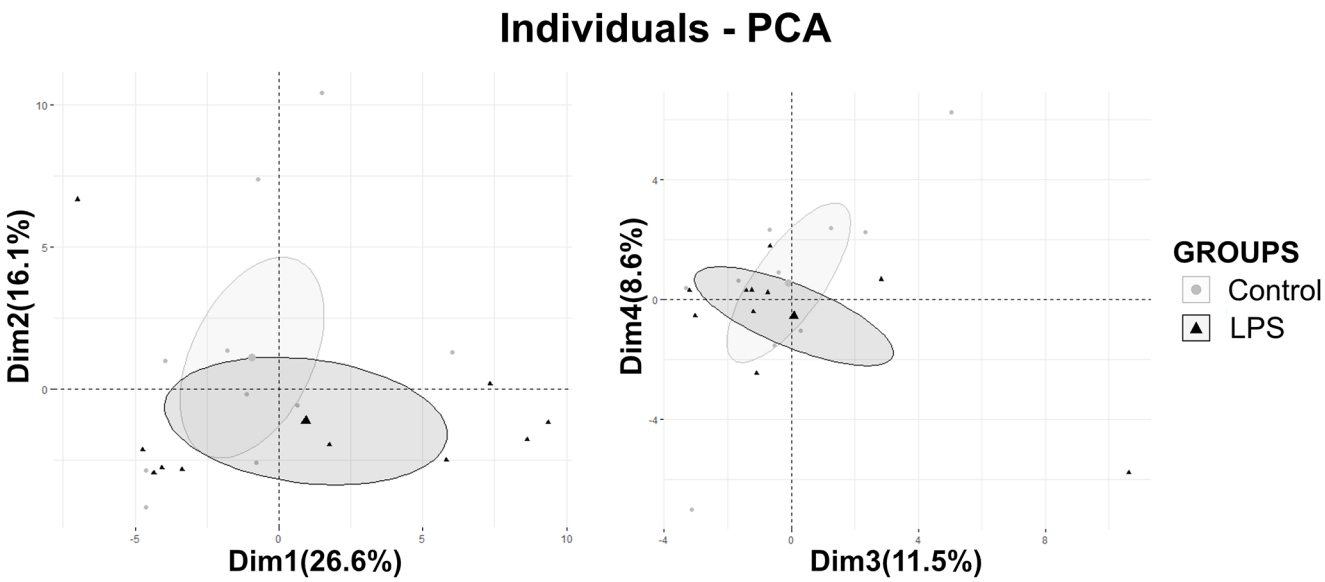

Fig. 1 Multivariate analysis of control and LPS groups at 30 min: individual map of PCA

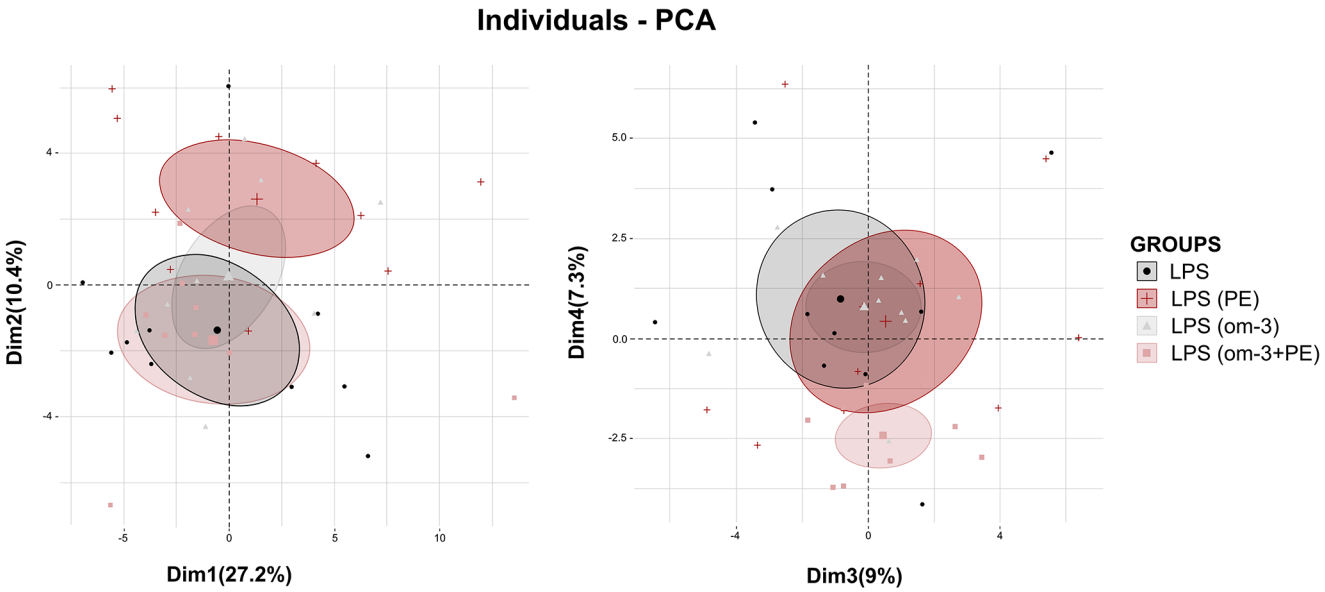

Fig. 2 Multivariate analysis of supplemented and non-supplemented LPS groups at 30 min: individual map of PCA

France). The quantity and purity of the RNA were assessed using a Nanodrop photospectrometer (Nanodrop One, Life Technologies, France). Two micrograms of RNA were subjected to reverse transcription to generate complementary DNA (cDNA) using Superscript IV (Invitrogen, Life Technologies, France). Subsequently, the cDNAs were amplified through PCR with TaqMan® primers specific to the target genes studied, as described in previous studies [12, 40]. We focused on IL-6 (Mm00446190_m1), IL-1β (Mm00434228_m1), TNFα (Mm00443258_m1), TGF-β1 (Mm01178820_m1), CCL2 (Mm00441242_m1), CXCL9 (Mm00434946_m1), CXCL10 (Mm00445235_m1), IFNγ (Mm01168134_m1), NF-κB (Mm00476361_m1), TLR4 (Mm00445273_m1), CD11b (Mm00434455_m1), iNOS (Mm00440502_m1), CD68 (Mm03047343_m1), CD86 (Mm00444540_m1), Tspo (Mm00437828_m1), SOCS3 (Mm00545913_s1), CD36 (Mm00432403_m1), Arg1 (Mm00475988_m1), Glut5 (Mm00600311_m1), P2ry12 (Mm00446026_m1), COX-2 (Mm00478374_m1), CD206 (Mm00485148_m1), CD11c (Mm00498698_m1), Trem2 (Mm04209424_g1), ApoE (Mm01307193_g1), Clec7a (Mm01183349_m1), Bax (Mm00432051_m1), Bcl2 (Mm00477631_m1), Caspase-3 (Mm01195085_m1), RAGE (Mm00545815_m1), Catalase (Mm00437992_m1), GPx1 (Mm00656767_g1), SOD1 (Mm01344233_g1), SOD2 (Mm01313000_m1), NRF2 (Mm00477784_m1),

GSS (Mm00515065_m1), GSTp1 (Mm04213618_gH), BDNF (Mm04230607_s1), TrkB (Mm00435422_m1), NGF (Mm00443039_m1), TrkA (Mm01219406_m1), DCX (Mm00438400_m1), b3-tubulin (Mm00727586_s1), CaMKII (Mm00437967_m1), 5-LOX (Mm01182747_m1), 12-LOX (Mm00545833_m1) and 15-LOX (Mm00507789_m1) in hippocampus. GAPDH (Mm99999915_g1) was used as reference gene. Fluorescence levels were assessed using a LightCycler® 480 instrument II (Roche, La Rochelle, France), and the final quantification was analyzed through the comparative threshold cycle (Ct) method. The outcomes are presented as relative fold changes, calculated in reference to the control target mRNA expression, as outlined in previous studies [12, 40].

Measurement of IL-6 in the spleen

The measurement of IL-6 level in spleen tissue was conducted using the Mouse IL-6 ELISA kit (Sigma-Aldrich, Lyon, France) according to the manufacturer protocol. The concentration of IL-6 in each sample, expressed in pg/mL/mg of protein, was determined by spectrophotometry (Victor3V, PerkinElmer, France) based on the standard range provided by the kit supplier.

Lipids analysis

Lipids were extracted from the hippocampus at CSGA lab (CSGA, INRAE UMR 1324, Dijon, France), and the fatty acids were methylated following established procedures outlined in a previous study [41]. The resulting fatty acid methyl esters (FAMEs) were analyzed through gas chromatography using a Hewlett Packard Model 5890 series II gas chromatograph (Palo Alto, CA, USA). This system was equipped with a split/spitless injector, a flame ionization detector (Palo Alto, CA, USA), and a CPSIL-88 column (100 m×0.25 mm internal diameter; film thickness, 0.20 µm; Varian, Les Ulis, France). Hydrogen served as the carrier gas at an inlet pressure of 210 kPa. The procedure involved maintaining the oven temperature at 60 °C for 5 min, followed by an increase to 165 °C at a rate of 15 °C/min, holding for 1 min, and then raising it to 225 °C at 2 °C/min. The final temperature was maintained at 225 °C for 17 min. The injector and detector temperatures were kept at 250 °C and 280 °C, respectively. Identification of FAMEs was accomplished by comparing them with commercially available standards. The fatty acid composition was expressed as a percentage of the total detected fatty acids.

Oxylipin quantification

Metabolites derived from linoleic acid (LA), arachidonic acid (AA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) were isolated from the hippocampus and subjected to analysis through mass spectrometry (LC-MS/MS) at the METATOUL platform (MetaboHUB, INSERM UMR 1048, I2MC, Toulouse, France). The analytical procedures closely followed the methods outlined by Le Faouder et al. [42]. The obtained results were expressed as pg/mg of protein.

Statistical analyses

Principal component analysis (PCA) was performed to differentiate the main profiles of biomarkers using R Software (R version 4.1.1, RStudio environment). Indeed, this statistical approach is suitable to large-scale data: PCA aims to summarize the variability of the data while maximizing their variance in order to identify profiles, called components. The components were calculated as a weighted linear combination of the initial variables, with higher weights for the most contributory variables. We conducted independent PCAs, between Control and LPS groups and between all LPS groups with or without supplementation, at 3 times (30 min, 2 h and 6 h after LPS injection), each time on the 90 available biomarkers (gene expression and oxylipin concentration in hippocampus, protein expression in spleen). The number of selected components was based on the bar plot (Cattell criteria) and on the percentage of the information contained in the selected components. For each component, biomarkers were considered contributors when their absolute correlation with the component was ≥ 0.50. Two-dimensional plots of individuals were drawn to illustrate two components at the same time (on the x-axis and y-axis).

The group variable was not considered in the construction of PCAs but was added as an additional variable to describe the components. In this way, a one-factor analysis of variance was performed between each component and group variable (coordinates of the individuals) to identify whether the group was characteristic of the component, retained for a critical probability of the overall Fisher test < 0.05. In the significant case, Student t-tests were achieved to compare the average of the category with the general mean, in order to identify potential characteristic category of each component (i.e. potential distinction according to the LPS, LPS PE, LPS om-3 or LPS PE + om-3). The distinction of a group in a component was considered when the associated critical probabilities p-value < 0.05 (Student t-test). To visualize group distinction, barycenter of individuals in each category and their confidence ellipses were added on the plots.

Statistical analyses of oxylipins and fatty acid composition were performed using GraphPad Prism software (version 10.1.0, GraphPad Software, Boston, MA, USA). For fatty acid analysis, the 5 groups were compared using a 2-way ANOVA (with time and treatment as factors), followed by Dunnett's post hoc test when appropriate. For oxylipin analysis, following PCA, the 4 groups treated with LPS were compared at each time point using a 1-way ANOVA followed by Tukey's post hoc test when appropriate or a Kruskal-Wallis test followed by Dunn's post hoc test when appropriate, when data were non-normal. To complete the oxylipin analysis, a heatmap based on the z-score calculated for each data point compared with the mean of the data group was generated using R software (R version 4.4.1, corrplot package version 0.92) for the three time points studied in the 5 groups.

Results

Principal component analysis (PCA) was performed at each time point of euthanasia on the 90 available biomarkers. At each time point, the effect of LPS treatment was evaluated comparing Control vs. LPS groups and the effect of supplementations was evaluated comparing non-supplemented vs. supplemented LPS groups. For each analysis, we will only describe the number of components that explain at least 50% of the total variance.

30 Min post-LPS: multivariate analysis

LPS-treated mice did not significantly differ from control mice after 30 min of exposure

The first 3 components from the PCA analysis explained 54.2% of the total variance (first component, 'dim 1': 26.6%; second component, 'dim 2': 16.1%; third component, 'dim 3': 11.5%) (Fig. 1). No significant differences between LPS and Control group were revealed on these dimensions meaning that the profiles between Control and LPS groups cannot be distinguished.

Supplementations increased inflammatory and antioxidant responses in 30 min LPS-treated animals

The first 4 components derived from the PCA analysis explained 53.9% of the total variance (first component, 'dim 1': 27.2%; second component, 'dim 2': 10.4%; third component, 'dim 3': 9%; fourth component, 'dim 4': 7.3%) (Fig. 2). Table 2 shows the correlations between biomarkers and components. PE supplemented group was significantly different from the other groups on dim 2, for which it showed positive scores ($p = 0.001$). Specifically, dim 2 revealed a positive correlation with proinflammatory markers CCL2, TNFα, IL-1β, NFκB, CXCL10, CXCL9, microglial markers Clec7a, SOCS3, CD11c, CD206, ApoE and antioxidant markers GPx1 and Catalase. PE + om-3 supplemented group was significantly different from the other groups on dim 4, for which it showed negative scores ($p = 0.001$). Dim 4 showed a positive correlation with antioxidant marker GSS and C16-2OH, a hydroxy fatty acid and a negative correlation with PGD_2, LTB_4, PGE_2, derived from AA and 10-HODE, derived from LA. No distinction between the supplemented groups were found on dimensions 1 and 3.

Table 2 Multivariate analysis of supplemented and non-supplemented LPS groups at 30 min

dim 2				dim 4			
		correlation	p-value			correlation	p-value
Proinflammation	CCL2	0.753	$p < 0.001$	Antioxidant	GSS	0.552	$p < 0.001$
	TNFα	0.679	$p < 0.001$	Hydroxy fatty acids	C16-2OH	0.542	$p < 0.001$
	IL-1β	0.644	$p < 0.001$	AA-derived oxylipins	PGD_2	-0.504	$p = 0.001$
	NFκB	0.607	$p < 0.001$		LTB_4	-0.516	$p < 0.001$
	CXCL10	0.597	$p < 0.001$		PGE_2	-0.553	$p < 0.001$
	CXCL9	0.585	$p < 0.001$	LA-derived oxylipins	10-HODE	-0.631	$p < 0.001$
MGnD microglia	Clec7a	0.653	$p < 0.001$				
	CD11c	0.568	$p < 0.001$				
	ApoE	0.512	$p < 0.001$				
Proresolutive microglia	SOCS3	0.608	$p < 0.001$				
	CD206	0.545	$p < 0.001$				
Antioxidant	GPx1	0.582	$p < 0.001$				
	Catalase	0.567	$p < 0.001$				

Correlation between each variable and the principal component score from PCA (dim 2 and 4)

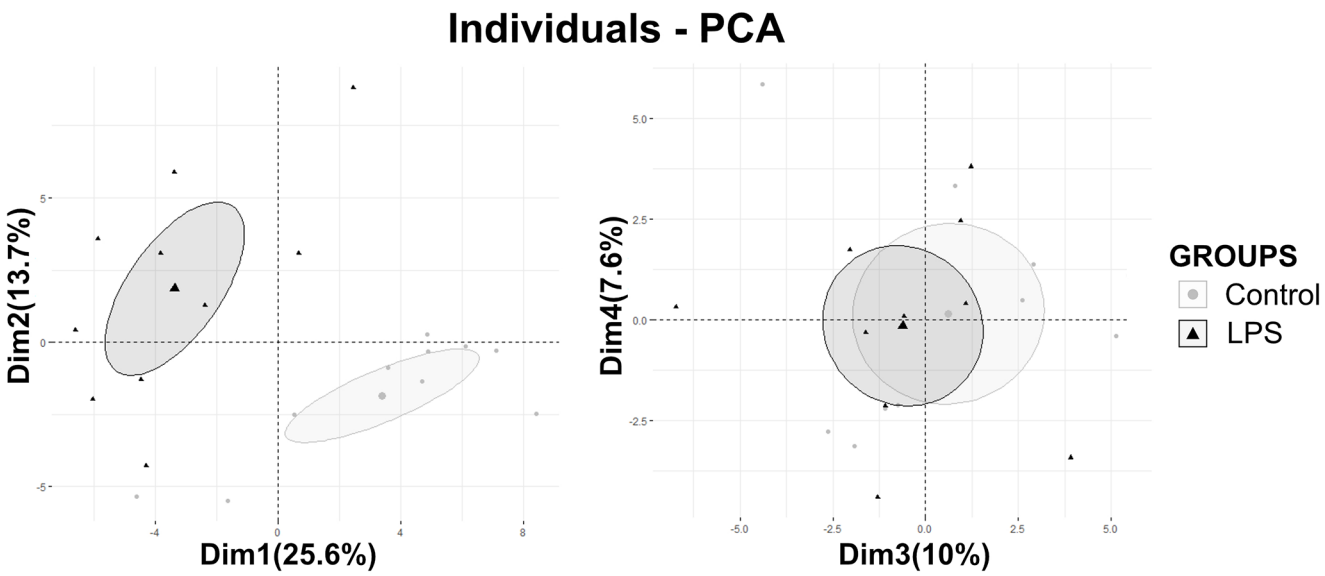

Fig. 3 Multivariate analysis of control and LPS groups at 2 h: individual map of PCA

2 H post-LPS: multivariate analysis

LPS modulated the oxylipin profile and increased proinflammatory and antioxidant markers after 2 h of exposure

The first 4 components from the PCA analysis explained 56.9% of the total variance (first component, 'dim 1': 25.6%; second component, 'dim 2': 13.7%; third component, 'dim 3': 10%; fourth component, 'dim 4': 7.6%) (Fig. 3). Control and LPS groups significantly differed from each other on dim 1 and dim 2. In fact, LPS group exhibited negative scores on dim 1 and positive scores on dim 2 whereas it was the opposite for Control group (dim 1: $p < 0.001$ and dim 2: $p = 0.016$). Table 3 shows the correlation between biomarkers and components. Dim 1 was positively correlated with various LA- and AA-derived oxylipins and hydroxy fatty acids, as well as 12-LOX, an oxylipin biosynthesis enzyme and Clec7a, a microglial marker. Dim 1 was also negatively correlated with PGE_2 and LTB_4, derived from AA, COX-2, involved in oxylipin biosynthesis, microglial marker CD86 and proinflammatory cytokines and chemokines TNFα, IL-1β, CCL2, IL-6 (both in spleen and hippocampus), CXCL10 and CXCL9. Dim 2 revealed only positive correlation with oxylipins derived from AA, DHA and LA, pro-inflammatory factors NFκB, IL-6 (both in spleen and hippocampus), TNFα and IL-1β, microglial marker SOCS3, antioxidant markers Sod2, Catalase, GPx1, Sod1 and NRF2, and 15-LOX, an oxylipin biosynthesis enzyme. No significant differences between LPS group and Control group were found on dimensions 3 and 4.

PE + om-3 supplementation modulated oxylipin composition in 2 h LPS-treated animals

The first 5 components derived from the PCA analysis accounted for 55.1% of the total variance, with the breakdown as follows: the first component (dim 1) contributing 23.7%, the second component (dim 2) 10.9%, the third component (dim 3) 8%, the fourth component (dim 4) 6.7% and the fifth component (dim 5) 5.8% (Fig. 4). PE + om-3 group was significantly different from LPS group on dim 4, for which it showed positive scores ($p < 0.001$) whereas LPS group showed negative scores ($p < 0.001$). Table 4 shows the correlation between biomarkers and components. Dim 4 was positively correlated with LxA_4, derived from AA and C10-3OH, a hydroxy fatty acid, and negatively correlated with LTB_4, also derived from AA. No distinction between the LPS groups were found on dimensions 1, 2, 3 and 5.

6 H post-LPS: multivariate analysis

LPS increased the proinflammatory response, decreased neuroprotection and modulated oxylipin composition after 6 h of exposure

The first 2 components from the PCA analysis explained 54.2% of the total variance (first component, 'dim 1': 36.9%; second component, 'dim 2': 17.3%) (Fig. 5). Control group was significantly different from LPS group on dim 1 and dim 2. In fact, LPS group exhibited negative scores on dim 1 and positive scores on dim 2 whereas it was the opposite for Control group (dim 1: $p = 0.039$ and dim 2: $p < 0.001$). Table 5 shows the correlation between biomarkers and components. Dim 1 was positively correlated with various LA-,

Table 3 Multivariate analysis of control and LPS groups at 2 h

dim 1				dim 2			
		correlation	p-value			correlation	p-value
LA-derived oxylipins	13-oxo-ODE	0.820	$p<0.001$	AA-derived oxylipins	15-HETE	0.679	$p<0.001$
	9-oxo-ODE	0.716	$p<0.001$		12-HETE	0.572	$p=0.008$
	9,10-DiHOME	0.698	$p<0.001$	DHA-derived oxylipins	17-HDHA	0.580	$p=0.007$
	12,13-DiHOME	0.639	$p=0.002$		14-HDHA	0.560	$p=0.010$
	9-HODE	0.602	$p=0.005$	LA-derived oxylipins	9,12,13-TriHOME	0.524	$p=0.018$
	9,12,13-TriHOME	0.565	$p=0.009$		13-HODE	0.509	$p=0.022$
AA-derived oxylipins	14,15-EET	0.850	$p<0.001$	Proinflammation	NFκB	0.819	$p<0.001$
	TXB_2	0.799	$p<0.001$		IL-6 (hippocampus)	0.622	$p=0.003$
	5,6-EET	0.784	$p<0.001$		TNFα	0.615	$p=0.004$
	8,9-EET	0.756	$p<0.001$		IL-6 (spleen)	0.587	$p=0.006$
	8-HETE	0.750	$p<0.001$		IL-1β	0.575	$p=0.008$
	11,12-EET	0.740	$p<0.001$	Proresolutive microglia	SOCS3	0.772	$p<0.001$
	LxA_4	0.717	$p<0.001$	Antioxidant	Sod2	0.729	$p<0.001$
	5-HETE	0.717	$p<0.001$		Catalase	0.649	$p=0.002$
	$PGF_{2\alpha}$	0.661	$p=0.001$		GPx1	0.623	$p=0.003$
	5-oxo-ETE	0.583	$p=0.007$		Sod1	0.585	$p=0.007$
	$5dPGJ_2$	0.543	$p=0.013$		NRF2	0.547	$p=0.012$
	PGE_2	-0.505	$p=0.023$	Oxylipins biosynthesis	15-LOX	0.574	$p=0.008$
	LTB_4	-0.574	$p=0.008$				
Hydroxy fatty acids	C16:1-3OH	0.831	$p<0.001$				
	C10-3OH	0.821	$p<0.001$				
	C16-3OH	0.818	$p<0.001$				
	C8-3OH	0.816	$p<0.001$				
	C18:1-3OH	0.808	$p<0.001$				
	C12-3OH	0.790	$p<0.001$				
	C18-3OH	0.729	$p<0.001$				
	C18-2OH	0.697	$p<0.001$				
	C16-2OH	0.686	$p<0.001$				
Oxylipins biosynthesis	12-LOX	0.777	$p<0.001$				
	COX-2	-0.543	$p=0.013$				
MGnD microglia	Clec7a	0.537	$p=0.015$				
Proinflammatory microglia	CD86	-0.508	$p=0.022$				
Proinflammation	TNFα	-0.564	$p=0.010$				
	IL-1β	-0.590	$p=0.006$				
	CCL2	-0.598	$p=0.005$				
	IL-6 (hippocampus)	-0.626	$p=0.003$				
	IL-6 (spleen)	-0.628	$p=0.003$				
	CXCL10	-0.750	$p<0.001$				
	CXCL9	-0.778	$p<0.001$				

Correlation between each variable and the principal component score from PCA (dim 1 and 2)

AA- and DHA-derived oxylipins and hydroxy fatty acids as well as neurotrophic factor BDNF and negatively correlated with LTB_4, derived from AA, and IDO1. Dim 2 was positively correlated with PGE_2 and PGD_2, derived from AA, 3 hydroxy fatty acids, proinflammatory markers IL-1β, NFκB, IL-6 (both in spleen and hippocampus), CCL2, TNFα, IDO1, TLR4, CXCL9, CXCL10 and iNOS, microglial markers SOCS3, Arg1, CD86, CD11b and ApoE, oxidative markers NRF2, RAGE and GSS, oxylipin biosynthesis enzymes 15-LOX, 5-LOX, COX-2, and apoptosis marker Bax. Dim 2 was negatively correlated with microglial markers Trem2 and Glut5, oxylipin biosynthesis enzyme 12-LOX and neurogenesis marker DCX.

PE + om-3 supplementation induced a change in oxylipin profile in 6 h LPS-treated animals

The first 5 components derived from the PCA analysis explained 53.8% of the total variance. Specifically, the first component (dim 1) contributed 29.4%, the second

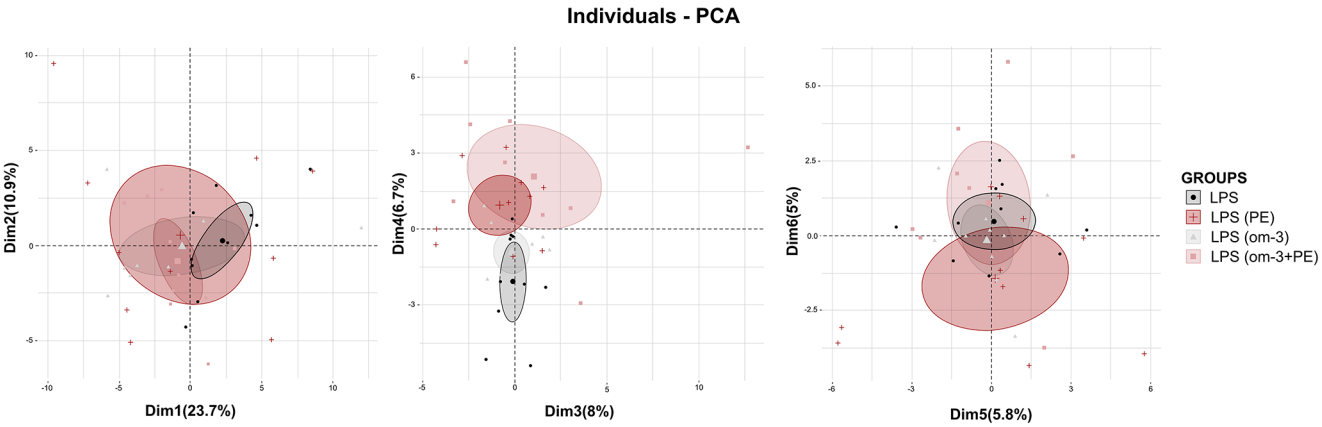

Fig. 4 Multivariate analysis of supplemented and non-supplemented LPS groups at 2 h: individual map of PCA

Table 4 Multivariate analysis of supplemented and non-supplemented LPS groups at 2 h

dim 4			
		correlation	p-value
AA-derived oxylipins	LxA_4	0.555	$p<0.001$
	LTB_4	-0.589	$p<0.001$
Hydroxy fatty acids	C10-3OH	0.536	$p<0.001$

Correlation between each variable and the principal component score from PCA (dim 4)

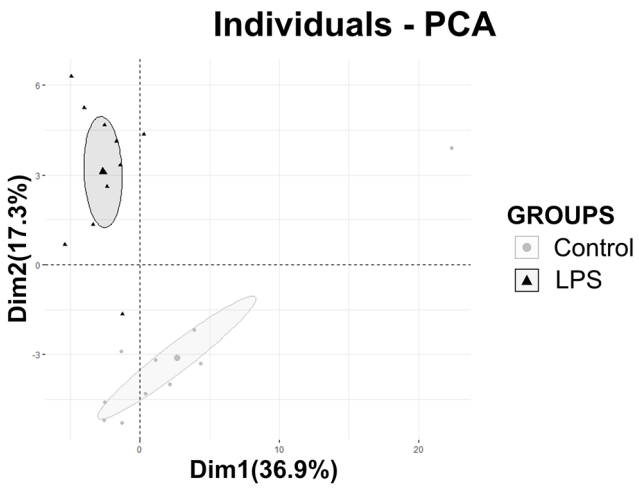

Fig. 5 Multivariate analysis of control and LPS groups at 6 h: individual map of PCA

component (dim 2) 10.2%, the third component (dim 3) 8.2% and the fourth component (dim 4) 6.2% (Fig. 6). Table 6 shows the correlation between biomarkers and components. LPS group showed negative scores on dim 1 ($p=0.001$) whereas PE + om-3 supplemented group showed positive scores ($p=0.040$). Dim 1 was positively correlated with oxylipins derived from LA, AA, EPA and DHA and hydroxy fatty acids as well as microglial markers Tspo and ApoE, neuroprotective receptor TrkA and antioxidant markers GPx1, Sod2 and Sod1. PE + om-3 group was significantly different from the other groups on dim 3, for which it revealed negative scores ($p<0.001$). Dim 3 was positively correlated with PGD_2, derived from AA, microglial markers CD68, Glut5, CD11b and ApoE and C18-3OH. No distinctions of supplemented groups were found on dim 2 and 4.

Oxylipin analysis

As the effect of supplementations seemed to be driven by a change in oxylipin profile, we investigated the impact of supplementations on the oxylipin concentrations derived from LA, AA, EPA and DHA at each time point. The generated heatmap demonstrated that gene regulation and oxylipin production were dependent on the time point and the group considered (Fig. 7). Interestingly, heatmap clearly highlighted the underexpression of oxylipins at 30 min post-LPS in the PE + om-3 group compared with the other groups and the overexpression of oxylipins at 6 h in the supplemented groups compared with the LPS and control groups.

After 30 min of exposure (Table 7), supplementations affected the amount of prostaglandins derived from AA: 8-iso-PGA_2 ($F_{(3,35)}=3.457$, $p=0.027$), PGD_2 ($F_{(3,35)}=3.463$, $p=0.027$), PGE_2 ($H_{(3)}=13.030$, $p=0.005$), $PGF_{2\alpha}$ ($H_{(3)}=13.870$, $p=0.003$), $15dPGJ_2$ ($F_{(3,35)}=6.589$, $p=0.001$) and that of 18-HEPE ($F_{(3,35)}=29.160$, $p<0.001$), derived from EPA. Indeed, $PGF_{2\alpha}$ concentration was significantly higher in PE group than in LPS group ($p=0.006$). PE + om-3 supplementation significantly increased PGD_2, PGE_2, $PGF_{2\alpha}$ and decreased $15dPGJ_2$ compared to LPS group (PGD_2: $p=0.049$, PGE_2: $p=0.040$, $PGF_{2\alpha}$: $p=0.010$, $15dPGJ_2$: $p=0.040$). Moreover, PE + om-3 supplementation significantly increased PGD_2 ($p=0.031$) and PGE_2 ($p=0.011$) compared to om-3 group and decreased 8-iso-PGA_2 compared to PE supplementation ($p=0.015$) and $15dPGJ_2$ compared to all other groups ($p=0.001$ vs. PE, $p=0.006$ vs. om-3). Finally, om-3 and PE + om-3

Table 5 Multivariate analysis of control and LPS groups at 6 h

dim 1				dim 2			
		correlation	p-value			correlation	p-value
LA-derived oxylipins	13-oxo-ODE	0.968	$p<0.001$	AA-derived oxylipins	PGE_2	0.563	$p<0.001$
	9-oxo-ODE	0.962	$p<0.001$		PGD_2	0.472	$p=0.036$
	13-HODE	0.951	$p<0.001$	Hydroxy fatty acids	C12-3OH	0.589	$p<0.001$
	9-HODE	0.946	$p<0.001$		C16:1-3OH	0.556	$p=0.011$
	9,10-DiHOME	0.943	$p<0.001$		C14-3OH	0.501	$p=0.025$
	9,12,13-TriHOME	0.906	$p<0.001$	Proinflammation	IL-1β	0.808	$p<0.001$
	9,10,13-TriHOME	0.782	$p<0.001$		NFκB	0.760	$p<0.001$
DHA-derived oxylipins	14-HDHA	0.959	$p<0.001$		IL-6 (HC)	0.759	$p<0.001$
	17-HDHA	0.928	$p<0.001$		CCL2	0.748	$p<0.001$
AA-derived oxylipins	5-HETE	0.975	$p<0.001$		TNFα	0.739	$p<0.001$
	5,6-EET	0.973	$p<0.001$		IL-6 (spleen)	0.654	$p<0.001$
	14,15-EET	0.969	$p<0.001$		IDO1	0.653	$p<0.001$
	5-oxo-ETE	0.959	$p<0.001$		TLR4	0.614	$p<0.001$
	11,12-EET	0.959	$p<0.001$		CXCL9	0.605	$p<0.001$
	12-HETE	0.954	$p<0.001$		CXCL10	0.595	$p<0.001$
	8,9-EET	0.950	$p<0.001$		iNOS	0.488	$p=0.029$
	TXB_2	0.949	$p<0.001$	Proresolutive microglia	SOCS3	0.823	$p<0.001$
	8-HETE	0.949	$p<0.001$		Arg1	0.559	$p=0.010$
	15-HETE	0.946	$p<0.001$	Proinflammatory microglia	CD86	0.654	$p<0.001$
	LxA_4	0.940	$p<0.001$		CD11b	0.646	$p<0.001$
	$PGF_{2\alpha}$	0.922	$p<0.001$	MGnD microglia	ApoE	0.510	$p=0.021$
	8-iso-PGA_2	0.912	$p<0.001$		Trem2	-0.539	$p=0.014$
	6k$PGF_{1\alpha}$	0.853	$p<0.001$	Homeostatic microglia	Glut5	-0.721	$p<0.001$
	PGE_2	0.737	$p<0.001$	Oxidation	NRF2	0.672	$p<0.001$
	15dPGJ_2	0.624	$p=0.003$		RAGE	0.497	$p=0.026$
	LTB_4	-0.507	$p=0.023$		GSS	0.497	$p=0.026$
Hydroxy fatty acids	C18:1-3OH	0.958	$p<0.001$	Oxylipins biosynthesis	15-LOX	0.629	$p<0.001$
	C16-3OH	0.936	$p<0.001$		5-LOX	0.548	$p=0.012$
	C16-2OH	0.925	$p<0.001$		COX-2	0.559	$p=0.010$
	C18-2OH	0.923	$p<0.001$		12-LOX	-0.670	$p=0.001$
	C18-3OH	0.879	$p<0.001$	Apoptosis	Bax	0.557	$p=0.011$
	C10-3OH	0.879	$p<0.001$	Neurogenesis	DCX	-0.536	$p=0.015$
	C8-3OH	0.852	$p<0.001$				
	C12-3OH	0.600	$p=0.005$				
Neuroprotection	BDNF	0.571	$p=0.009$				
Proinflammation	IDO1	-0.504	$p=0.024$				

Correlation between each variable and the principal component score from PCA (dim 1 and 2)

supplementations significantly increased 18-HEPE compared to LPS and PE groups ($p<0.001$ for all).

After 2 h of exposure (Table 8), supplementations modulated the concentrations of LA-derived 9-oxo-ODE ($F_{(3,35)}=6.195$, $p=0.002$) and 13-oxo-ODE ($H_{(3)}=12.110$, $p=0.007$), as well as AA-derived 8,9-EET ($F_{(3,35)}=4.129$, $p=0.013$), 5-HETE ($F_{(3,34)}=6.513$, $p=0.001$), 8-HETE ($F_{(3,35)}=5.740$, $p=0.003$), 12-HETE ($H_{(3)}=11.560$, $p=0.009$), 15-HETE ($F_{(3,35)}=4.026$, $p=0.015$), 5-oxo-ETE ($F_{(3,35)}=8.448$, $p<0.001$), LTB_4 ($F_{(3,28)}=3.715$, $p=0.023$), PGD_2 ($F_{(3,35)}=3.380$, $p=0.029$) and PGE_2 ($H_{(3)}=8.090.$, $p=0.044$). 18-HEPE ($F_{(3,35)}=23.99$, $p<0.001$) and 14-HDHA ($F_{(3,35)}=3.021$, $p=0.043$), derived respectively from EPA and DHA, were also affected. In fact, PE supplementation significantly decreased 14-HDHA ($p=0.038$), 9-oxo-ODE ($p=0.016$), 5-oxo-ETE ($p=0.002$), 5-HETE ($p=0.003$), 8-HETE ($p=0.009$), 15-HETE ($p=0.040$) and LTB_4 ($p=0.033$) compared to LPS group. Om-3 supplementation significantly decreased 5-oxo-ETE compared to LPS group ($p=0.052$). PE + om-3 supplementation significantly decreased 9-oxo-ODE ($p=0.001$), 13-oxo-ODE ($p=0.005$), 8,9-EET ($p=0.008$), 5-oxo-ETE ($p<0.001$) 5-HETE ($p=0.003$), 8-HETE ($p=0.004$), 12-HETE (0.006), 15-HETE ($p=0.016$) and LTB_4 ($p=0.053$) and tented to increase PGD_2 ($p=0.061$) compared to LPS group. Moreover, PE supplementation significantly increased PGE_2 and

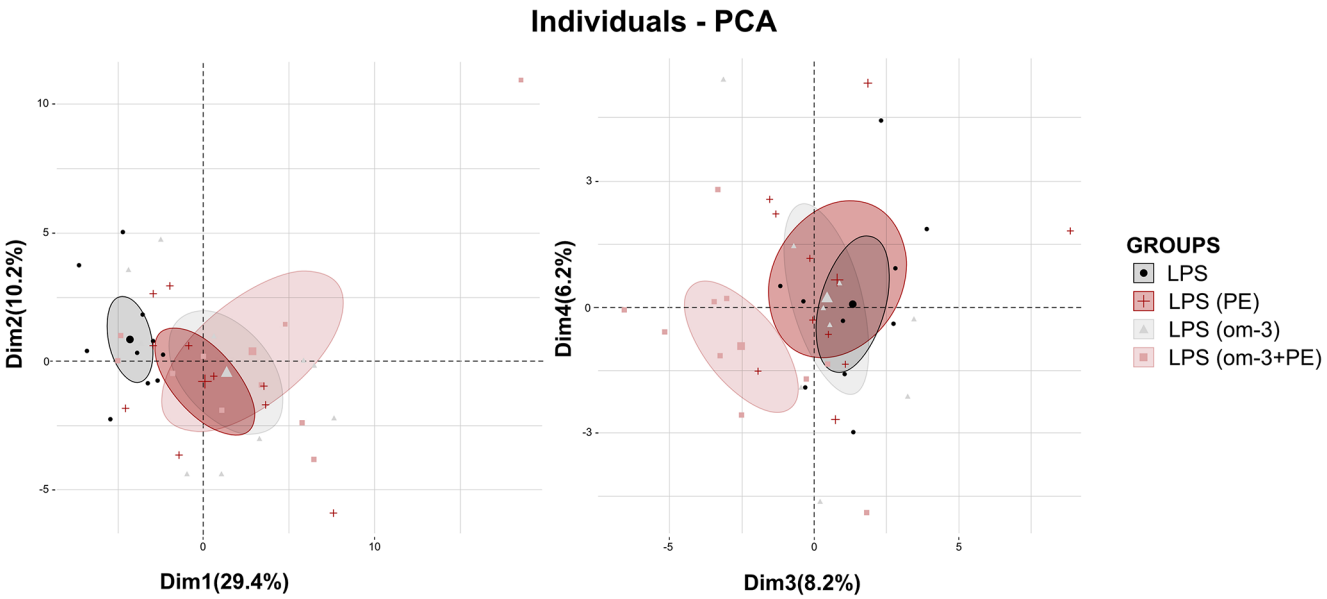

Fig. 6 Multivariate analysis of supplemented and non-supplemented LPS groups at 6 h: individual map of PCA

PE + om-3 supplementation significantly increased PGD_2 compared to om-3 group ($p = 0.049$ and $p = 0.034$, respectively). Finally, om-3 and PE + om-3 supplementations significantly increased 18-HEPE compared to LPS and PE groups ($p < 0.001$ for all).

After 6 h of exposure (Table 9), supplementations significantly impacted the concentration of most oxylipins derived from LA: 9-HODE ($F_{(3,36)} = 5.133$, $p = 0.005$), 13-HODE ($F_{(3,36)} = 4.213$, $p = 0.012$), 9,10-DiHOME ($F_{(3,36)} = 4.511$, $p = 0.009$), 12,13-DiHOME ($F_{(3,36)} = 9.159$, $p < 0.001$), 9-oxo-ODE ($F_{(3,36)} = 6.698$, $p = 0.001$), 13-oxo-ODE ($F_{(3,36)} = 5.257$, $p = 0.004$), 9,10,13-TriHOME ($F_{(3,36)} = 4.767$, $p = 0.007$), 9,12,13-TriHOME ($H_{(3)} = 15.570$, $p = 0.001$). Most AA-derived oxylipins were also modulated by the supplementations: 5,6-EET ($F_{(3,36)} = 3.368$, $p = 0.029$), 8,9-EET ($F_{(3,36)} = 10.74$, $p < 0.001$), 11,12-EET ($F_{(3,35)} = 6.812$, $p = 0.001$), 14,15-EET ($H_{(3)} = 12.73$, $p = 0.005$), 5-HETE ($F_{(3,36)} = 6.736$, $p = 0.001$), 8-HETE ($F_{(3,36)} = 6.363$, $p = 0.001$), 15-HETE ($F_{(3,36)} = 4.274$, $p = 0.011$), 5-oxo-ETE ($F_{(3,36)} = 9.646$, $p < 0.001$), LTB_4 ($F_{(3,26)} = 4.729$, $p = 0.009$), LxA_4 ($H_{(3)} = 11.20$, $p = 0.011$), 8-iso-PGA_2 ($H_{(3)} = 21.67$, $p < 0.001$), PGD2 ($F_{(3,36)} = 6.050$, $p = 0.002$), $PGF_{2\alpha}$ ($F_{(3,36)} = 2.877$, $p = 0.049$), $15dPGJ_2$ ($F_{(3,36)} = 10.21$, $p < 0.001$), TXB_2 ($F_{(3,36)} = 7.746$, $p < 0.001$). The same results were found for EPA-derived 18-HEPE ($H_{(3)} = 27.41$, $p < 0.001$) and DHA-derived 14-HDHA ($F_{(3,36)} = 5.365$, $p = 0.004$) and 17-HDHA ($F_{(3,36)} = 6.470$, $p = 0.001$). Indeed, compared to LPS group, all supplementations significantly increased 9,10-DiHOME (trend at $p = 0.090$ vs. PE, $p = 0.007$ vs. om-3, $p = 0.048$ vs. PE + om-3), 12,13-DiHOME ($p = 0.003$ vs. PE, $p < 0.001$ vs. om-3 and vs. PE + om-3), 9-oxo-ODE ($p = 0.040$ vs. PE, $p = 0.012$ vs. om-3, $p < 0.001$ vs. PE + om-3), 9,10,13-TriHOME ($p = 0.028$ vs. PE, $p = 0.007$ vs. om-3, trend at $p = 0.065$ vs. PE + om-3), $15dPGJ_2$ ($p = 0.002$ vs. PE, $p = 0.012$ vs. om-3, $p < 0.001$ vs. PE + om-3), TXB_2 ($p = 0.004$ vs. PE, $p < 0.001$ vs. om-3, $p = 0.005$ vs. PE + om-3) and 8,9-EET ($p = 0.050$ vs. PE, $p = 0.010$ vs. om-3, $p < 0.001$ vs. PE + om-3). Compared to LPS group, PE supplementation induced a higher level of 9,12,13-TriHOME ($p = 0.011$), 5-HETE ($p = 0.010$), LTB_4 ($p = 0.006$), LxA_4 ($p = 0.014$) and 8-iso-PGA_2 ($p < 0.001$). Om-3 supplementation increased the level of 9-HODE ($p = 0.055$), 13-HODE ($p = 0.046$), 13-oxo-ODE ($p = 0.028$), 9,12,13-TriHOME ($p = 0.002$), LxA_4 ($p = 0.040$), 8-iso-PGA_2 ($p = 0.017$) and tended to increase 11,12-EET ($p = 0.060$), 14,15-EET ($p = 0.091$) and $PGF_{2\alpha}$ ($p = 0.055$) levels compared to LPS group. PE + om-3 supplementation increased the level of 9-HODE ($p = 0.003$), 13-HODE ($p = 0.010$), 13-oxo-ODE ($p = 0.003$), 11,12-EET ($p < 0.001$), 14,15-EET ($p = 0.003$), 5-HETE ($p < 0.001$), 8-HETE ($p < 0.001$), 15-HETE ($p = 0.006$), 8-iso-PGA_2 ($p < 0.001$), 14-HDHA ($p = 0.004$) and 17-HDHA ($p < 0.001$) and decreased that of PGD_2 ($p = 0.005$), compared to LPS group. Moreover, PE + om-3 supplementation increased the concentrations of 8,9-EET, 14-HDHA and 17-HDHA compared to PE group ($p = 0.028$, $p = 0.030$, $p = 0.050$, respectively) and decreased that of PGD_2 compared to om-3 group ($p = 0.004$). In addition, PE + om-3 supplementation increased the concentrations of 5-oxo-ETE compared to the other groups ($p < 0.001$ vs. LPS, $p = 0.012$ vs. PE, $p = 0.017$ vs. om-3). Finally, 18-HEPE was also significantly higher in om-3 and PE + om-3 groups than in LPS and PE groups ($p < 0.001$ vs. LPS, om-3 vs. PE $p = 0.006$, PE + om-3 vs. PE $p = 0.003$).

Table 6 Multivariate analysis of supplemented and non-supplemented LPS groups at 6 h

dim 1				dim 3			
		correlation	p-value			correlation	p-value
LA-derived oxylipins	9-HODE	0.963	$p<0.001$	AA-derived oxylipin	PGD_2	0.592	$p<0.001$
	13-HODE	0.945	$p<0.001$	Proinflammatory microglia	CD68	0.583	$p<0.001$
	13-oxo-ODE	0.925	$p<0.001$		CD11b	0.516	$p<0.001$
	9-oxo-ODE	0.919	$p<0.001$	Homeostatic microglia	Glut5	0.579	$p<0.001$
	9,12,13-TriHOME	0.861	$p<0.001$	MGnD microglia	ApoE	0.516	$p<0.001$
	12,13-DiHOME	0.856	$p<0.001$	Hydroxy fatty acids	C18-3OH	0.511	$p<0.001$
	9,10-DiHOME	0.856	$p<0.001$				
	9,10,13-TriHOME	0.726	$p<0.001$				
	10-HODE	0.668	$p<0.001$				
AA-derived oxylipins	TXB2	0.833	$p<0.001$				
	15-HETE	0.796	$p<0.001$				
	8-iso-PGA_2	0.778	$p<0.001$				
	5-HETE	0.776	$p<0.001$				
	11,12-EET	0.774	$p<0.001$				
	14,15-EET	0.768	$p<0.001$				
	15dPGJ$_2$	0.758	$p<0.001$				
	LTB_4	0.753	$p<0.001$				
	8,9-EET	0.727	$p<0.001$				
	8-HETE	0.704	$p<0.001$				
	5,6-EET	0.684	$p<0.001$				
	5-oxo-ETE	0.648	$p<0.001$				
	$PGF_{2\alpha}$	0.619	$p<0.001$				
	6kPGF$_{1\alpha}$	0.611	$p<0.001$				
	12-HETE	0.540	$p<0.001$				
	LxA_4	0.505	$p<0.001$				
DHA-derived oxylipins	17-HDHA	0.703	$p<0.001$				
	14-HDHA	0.642	$p<0.001$				
EPA-derived oxylipin	18-HEPE	0.555	$p<0.001$				
Hydroxy fatty acids	C18:1-3OH	0.825	$p<0.001$				
	C16-3OH	0.775	$p<0.001$				
	C8-3OH	0.774	$p<0.001$				
	C16:1-3OH	0.664	$p<0.001$				
	C12-3OH	0.649	$p<0.001$				
	C16-2OH	0.631	$p<0.001$				
	C18-2OH	0.619	$p<0.001$				
	C10-3OH	0.612	$p<0.001$				
	C14-3OH	0.595	$p<0.001$				
Neuroprotection	TrkA	0.618	$p<0.001$				
Proinflammatory microglia	Tspo	0.617	$p<0.001$				
MGnD microglia	ApoE	0.517	$p<0.001$				
Antioxidant	GPx1	0.537	$p<0.001$				
	Sod2	0.527	$p<0.001$				
	Sod1	0.515	$p<0.001$				

Correlation between each variable and the principal component score from PCA (dim 1 and 3)

Fatty acid analysis

We then analyzed the hippocampal composition of fatty acids that are precursors of oxylipin compounds (Fig. 8A-C). Time of exposure to LPS and treatment significantly impacted the proportions of total SFAs (time: $F_{(2,128)}=3.522$, $p=0.032$; treatment: $F_{(4,128)}=8.757$, $p<0.001$) and total omega-6 + omega-3 (om6 + om-3) ($F_{(2,128)}=13.51$, $p<0.001$; treatment: $F_{(4,128)}=19.97$, $p<0.001$). At each time point, the omega-6 and omega-3 proportions followed the same evolution as total om-6 + om-3 (data not shown). Moreover, an interaction between time and treatment was

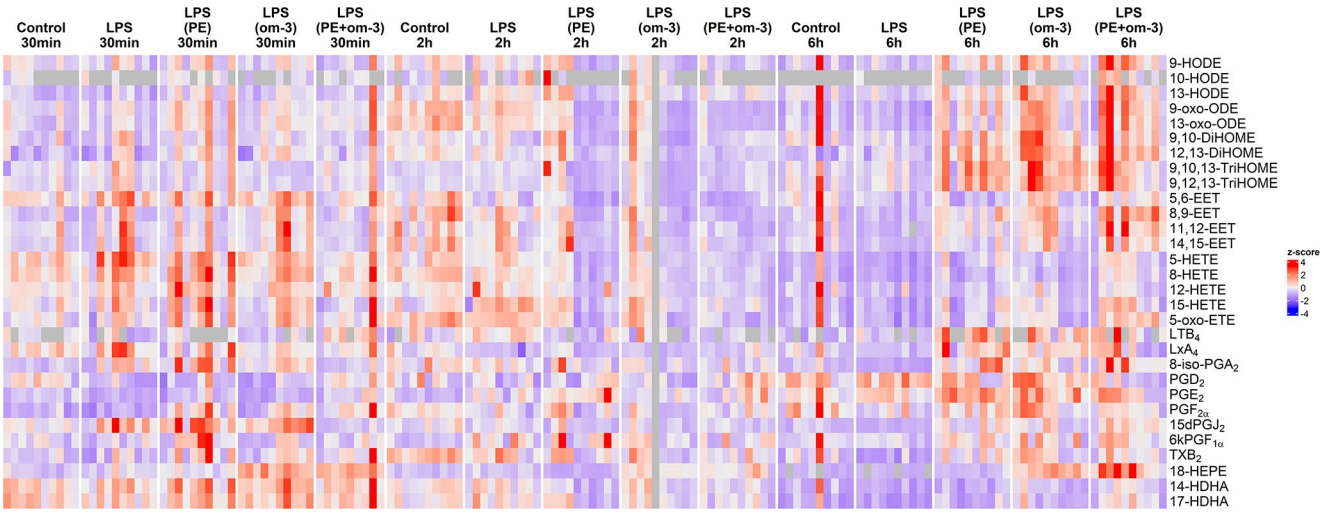

Fig. 7 Heatmap representation of oxylipin concentrations in LPS groups. Red indicates higher concentration and blue indicates lower concentration ($n = 9$–10/group)

observed for the proportions of total SFAs ($F_{(8,128)} = 3.931$, $p < 0.001$), total MUFAs ($F_{(8,128)} = 2.984$, $p = 0.004$) and total om-6 + om-3 ($F_{(8,128)} = 9.801$, $p < 0.001$). Indeed, compared to control group, LPS significantly decreased the proportion of total SFAs ($p < 0.001$) and increased that of total om-6 + om-3 ($p < 0.001$), 30 min after exposure but not later. Concerning the effect of the supplementations, after 30 min of exposure, all of them significantly increased total SFAs proportion ($p < 0.001$ for all) and decreased total om-6 + om-3 proportions compared to LPS ($p < 0.001$ for all). After 2 h of exposure, no effects of the supplementations were observed. After 6 h of exposure, all the supplementation significantly increased total MUFAs (PE: $p = 0.011$, om-3: $p = 0.021$, PE + om-3: $p = 0.007$) and decreased total om-6 + om-3 proportion ($p < 0.001$ for all) compared to LPS group.

Discussion

Neuroinflammation, when it becomes excessive, unregulated and prolonged, can switch from a protective to a detrimental mechanism [1]. Several mechanisms contribute to the resolution of inflammation, including the inhibition of proinflammatory markers, the production of anti-inflammatory cytokines, and the generation of oxylipins [10, 43]. This study provides evidence that a combination of polyphenols and carotenoids from plant extracts (Memophenol™ and a patented saffron extract) and omega-3 from fish induced a different resolution of inflammation through a switch in hippocampal oxylipin profile after LPS injection.

Acute LPS injection is a model widely used to study neuroinflammation, as it induced the release of pro- and anti-inflammatory factors [44]. LPS is associated with memory deficits and neurodegenerative disorders such as Alzheimer's or Parkinson's diseases in humans [45–47] As expected, we observed, at 2 h and 6 h, an increase in the expression of proinflammatory molecules such as NFκB, IL-6, IL-1β, TNFα, CCL2, CXCL9 and CXCL10, as well as activated microglial markers, of proinflammatory and proresolutive phenotypes, in LPS-treated animals, as previously reported by Chataigner et al.., and Hasegawa-Ishii et al. [30, 48]. 2 h after injection, an increase in antioxidant enzymes expression in the hippocampus was observed in LPS-treated animals, as already shown by Essadek et al.., suggesting an increase in the body's defense system against cellular free radicals [49]. This was accompanied by an increase in the levels of proinflammatory oxylipins PGE_2, LTB_4, 12-HETE and 15-HETE, as well as proresolutive DHA derivates 14-HDHA and 17-HDHA. Same changes in oxylipin profile 2 h after LPS treatment were previously reported in mice hippocampus [30]. 6 h after treatment, LPS group showed a lower expression of Glut5, a marker of microglial homeostasis, and Trem2, which was positively linked to the inhibition of neuroinflammation in BV2 microglia [50, 51]. These changes were associated with higher concentrations of proinflammatory mediators PGE_2, PGD_2 and LTB_4 as well as higher gene expression of their biosynthesis enzymes COX-2, 5-LOX and 15-LOX, but not for 12-LOX, which was less expressed in LPS group than in control group. Prostaglandins and leukotrienes production begins within a few minutes, at the start of the inflammatory response, and peaks after a few hours [52]. This production is followed by a switch in oxylipins class to lipoxins and other proresolving mediators production, lasting from a few hours to a few days [53]. In our study, the higher levels of proinflammatory mediators in LPS group were observed at 6 h. A study quantifying the plasma level of some oxylipins between the basal

Table 7 Effect of supplementations on oxylipins concentrations in hippocampus 30 min post-LPS treatment

Oxylipin (pg/mg of protein)	30 min			
	LPS	LPS (PE)	LPS (om-3)	LPS (PE + om-3)
LA-derived oxylipins				
9-HODE	499.77 ± 55.62	579.39 ± 65.62	523.36 ± 45.54	463.07 ± 77.19
10-HODE	3.82 ± 0.86	3.00 ± 0.34	1.61 ± 0.51	2.80 ± 0.37
13-HODE	458.58- ± 55.77	689.97 ± 80.42	559.79 ± 50.39	599.69 ± 112.15
9,10-DiHOME	116.53 ± 14.03	127.51 ± 14.21	114.00 ± 6.87	112.88 ± 142.72
12,13-DiHOME	49.89 ± 10.17	81.95 ± 8.68	66.37 ± 10.46	63.37 ± 8.46
9-oxo-ODE	579.33 ± 47.40	761.88 ± 72.06	611.65 ± 40.39	685.30 ± 84.46
13-oxo-ODE	559.06 ± 46.64	810.95 ± 75.02	668.78 ± 47.34	733.30 ± 92.79
9,10,13-TriHOME	432.96 ± 36.37	399.57 ± 41.72	324.48 ± 18.06	413.27 ± 36.23
9,12,13-TriHOME	511.71 ± 46.63	495.09 ± 55.53	416.85 ± 18.69	489.08 ± 51.52
AA-derived oxylipins				
5,6-EET	739.01 ± 73.46	684.23 ± 73.15	738.96 ± 51.80	600.24 ± 70.37
8,9-EET	418.29 ± 95.49	414.68 ± 61.65	603.04 ± 80.13	400.05 ± 68.90
11,12-EET	184.95 ± 51.16	163.17 ± 35.99	234.18 ± 53.46	126.88 ± 31.71
14,15-EET	173.32 ± 48.60	154.07 ± 29.76	207.04 ± 40.04	128.47 ± 27.05
5-HETE	12236.25 ± 1436.67	11278.97 ± 1359.16	10732.84 ± 572.05	8765.17 ± 910.06
8-HETE	684.47 ± 62.76	771.88 ± 93.74	688.48 ± 51.38	628.00 ± 78.36
12-HETE	2007.81 ± 184.41	2760.11 ± 426.60	1993.16 ± 167.73	2020.33 ± 216.11
15-HETE	4325.54 ± 389.91	4969.80 ± 596.42	4322.89 ± 326.77	3712.30 ± 615.67
5-oxo-ETE	9250.67 ± 729.10	11015.46 ± 1125.02	10062.46 ± 799.93	10192.79 ± 1264.45
LTB_4	11.34 ± 3.27	14.65 ± 2.02	10.74 ± 1.66	21.46 ± 3.66
LxA_4	723.92 ± 100.53	628.42 ± 99.50	555.67 ± 31.07	574.37 ± 72.11
8-iso-PGA_2	336.56 ± 55.58 $^{a, b}$	481.70 ± 88.29 ^{a}	357.35 ± 34.46 $^{a, b}$	213.84 ± 31.16 ^{b}
PGD_2	1988.49 ± 231.07 ^{a}	2228.61 ± 367.54 $^{a, b}$	1907.27 ± 212.69 ^{a}	3066.38 ± 272.57 ^{b}
PGE_2	643.76 ± 44.70 ^{a}	918.51 ± 136.41 $^{a, b}$	611.02 ± 64.94 ^{a}	1138.12 ± 145.36 ^{b}
$PGF_{2\alpha}$	2981.16 ± 218.37 ^{a}	5679.13 ± 670.63 ^{b}	4709.28 ± 538.61 $^{a, b}$	5751.16 ± 749.57 ^{b}
15dPGJ_2	68.01 ± 12.82 ^{a}	91.44 ± 16.72 ^{a}	80.93 ± 9.93 ^{a}	19.53 ± 3.84 ^{b}
6k$PGF_{1\alpha}$	266.17 ± 42.42	530.38 ± 117.69	274.79 ± 60.94	309.62 ± 50.02
TXB_2	2009.90 ± 209.76	2758.92 ± 351.62	2398.08 ± 169.60	2554.16 ± 324.55
EPA-derived oxylipin				
18-HEPE	18.47 ± 3.33 ^{a}	22.16 ± 2.52 ^{a}	53.13 ± 4.12 ^{b}	52.18 ± 3.76 ^{b}
DHA-derived oxylipins				
14-HDHA	656.44 ± 52.73	767.80 ± 84.58	769.63 ± 53.16	733.09 ± 98.42
17-HDHA	585.22 ± 44.31	667.55 ± 56.86	692.26 ± 53.91	705.46 ± 110.68

Values with superscripts (a, b, c) differ significantly ($n = 9$–10/group). Data are presented as mean ± SEM and are expressed in pg/mg of protein

condition and 96 h post-LPS already described a peak at 3–6 h, depending on the derivate [54]. The expression of the neuroprotective and neurogenesis markers BDNF and DCX also decreased, as already described in the literature but after 2 days of LPS injections [55]. LPS treatment was also associated with changes in fatty acid composition. Specifically, total SFAs levels decreased after 30 min of treatment but not later, and this decrease was offset by the increase in total om-6 + om-3 levels at the same time. Similarly, a previous study showed that 24 h of LPS treatment on BV2 microglial murine cells induced an increase in total om-6 and total om-3, associated with an increase in total SFAs and a decrease in total MUFAs [56]. We hypothesized that in our study, the decrease in SFAs was due to their oxidation to yield energy to fight against inflammation, with the increase in om-6 + om-3 as a consequence since the results are expressed in % of total fatty acids.

In this study, the mix of plant extracts (Memophenol™ and a patented saffron extract) and omega-3 had anti-inflammatory and proresolutive effects, mostly driven by changes in oxylipins. Indeed, at 30 min post-LPS, the mix increased the amount of proresolutive EPA-derived 18-HEPE. 18-HEPE has been demonstrated to suppress LPS-induced TNFα expression in macrophage cell culture [57]. Surprisingly, while most of the studies described lower AA-oxylipins levels after omega-3 supplementation, PE + om-3 also increased some prostaglandins, such as PGD_2, PGE_2 and $PGF_{2\alpha}$, and decreased others, such as 15dPGJ_2 and 8-iso-PGA_2, which is produced by the oxidation of AA

Table 8 Effect of supplementations on oxylipins concentrations in hippocampus 2 h post-LPS treatment

Oxylipin (pg/mg of protein)	2 h			
	LPS	LPS (PE)	LPS (om-3)	LPS (PE + om-3)
LA-derived oxylipins				
9-HODE	527.72 ± 45.02	492.59 ± 75.40	454.53 ± 53.28	428.85 ± 36.47
10-HODE	3.80 ± 1.28	12.19 ± 10.74	3.99 ± 1.19	2.16 ± 1.81
13-HODE	734.39 ± 59.99	694.53 ± 102.16	664.22 ± 67.80	661.56 ± 45.03
9,10-DiHOME	108.83 ± 8.81	114.32 ± 17.35	102.00 ± 17.55	107.89 ± 4.89
12,13-DiHOME	69.64 ± 5.41	66.23 ± 9.95	62.40 ± 10.66	52.72 ± 2.34
9-oxo-ODE	755.01 ± 42.89 [a]	498.03 ± 58.96 [b]	567.31 ± 92.10 [a, b]	423.29 ± 24.09 [b]
13-oxo-ODE	783.47 ± 31.72 [a]	545.28 ± 69.25 [a, b]	582.24 ± 93.35 [a, b]	441.03 ± 32.68 [b]
9,10,13-TriHOME	366.80 ± 10.63	362.43 ± 73.51	307.88 ± 37.72	315.67 ± 16.35
9,12,13-TriHOME	431.12 ± 15.33	399.63 ± 44.59	380.61 ± 48.65	400.16 ± 22.47
AA-derived oxylipins				
5,6-EET	456.34 ± 41.41	357.91 ± 41.54	400.08 ± 45.65	342.67 ± 25.93
8,9-EET	566.46 ± 62.40 [a]	365.31 ± 71.56 [a, b]	433.55 ± 100.48 [a, b]	238.93 ± 21.23 [b]
11,12-EET	169.62 ± 34.55	136.60 ± 35.28	124.89 ± 38.56	77.13 ± 18.62
14,15-EET	177.85 ± 27.97	146.03 ± 44.56	139.08 ± 33.48	89.47 ± 13.87
5-HETE	8687.93 ± 483.86 [a]	5974.22 ± 635.47 [b]	7267.27 ± 632.75 [a, b]	6011.76 ± 256.07 [b]
8-HETE	574.93 ± 26.64 [a]	412.55 ± 42.49 [b]	477.98 ± 41.98 [a, b]	396.99 ± 22.46 [b]
12-HETE	2055.56 ± 266.21 [a]	1458.20 ± 217.13 [a, b]	1464.90 ± 147.06 [a, b]	1178.80 ± 76.63 [b]
15-HETE	4933.11 ± 388.15 [a]	3666.96 ± 332.01 [b]	4110.44 ± 355.17 [a, b]	3489.06 ± 197.34 [b]
5-oxo-ETE	11383.55 ± 210.49 [a]	7964.80 ± 726.40 [b]	8980.97 ± 947.29 [b]	7296.41 ± 431.54 [b]
LTB_4	21.06 ± 1.63 [a]	11.50 ± 2.25 [b]	15.08 ± 4.29 [a, b]	11.07 ± 1.31 [b]
LxA_4	320.49 ± 35.63	384.73 ± 34.39	351.22 ± 16.76	428.66 ± 33.80
8-iso-PGA_2	322.14 ± 30.79	298.17 ± 69.33	264.70 ± 34.54	260.16 ± 34.60
PGD_2	2320.52 ± 273.99 [a, b]	2651.40 ± 305.58 [a, b]	2184.07 ± 241.13 [a]	3368.82 ± 315.77 [b]
PGE_2	1205.12 ± 144.23 [a, b]	1769.87 ± 203.45 [a]	1145.44 ± 80.72 [b]	1392.47 ± 196.93 [a, b]
$PGF_{2\alpha}$	4460.68 ± 346.77	5187.24 ± 603.67	4231.33 ± 417.79	4881.66 ± 319.24
15dPGJ_2	28.60 ± 3.69	22.57 ± 7.16	23.97 ± 5.37	23.53 ± 3.09
6k$PGF_{1\alpha}$	423.18 ± 75.57	573.55 ± 145.90	383.03 ± 53.36	365.89 ± 66.31
TXB_2	2737.90 ± 210.02	2516.78 ± 233.77	2350.98 ± 237.65	2359.31 ± 144.08
EPA-derived oxylipin				
18-HEPE	12.71 ± 2.00 [a]	3.47 ± 1.07 [a]	29.94 ± 1.95 [b]	29.46 ± 4.32 [b]
DHA-derived oxylipins				
14-HDHA	569.16 ± 43.68 [a]	403.78 ± 38.59 [b]	526.77 ± 53.89 [a, b]	462.98 ± 31.51 [a, b]
17-HDHA	531.63 ± 47.24	421.07 ± 59.65	476.90 ± 42.74	428.65 ± 39.26

Values with superscripts (a, b, c) differ significantly ($n = 10$/group). Data are presented as mean ± SEM and are expressed in pg/mg of protein

[12, 58, 59]. As the proinflammatory mediator production begins earlier with the mix supplementation, this may suggest that the time course of inflammation resolution is different than that in the LPS group. Indeed, at 2 h post-LPS, PE + om-3 supplementation induced lower concentrations of 9-oxo-ODE, 13-oxo-ODE, 5-HETE, 8-HETE, 12-HETE, 15-HETE, 5-oxo-ETE and LTB_4, mainly described as pro-inflammatory [60]. Higher concentrations of the proresolving mediators LxA_4, reduced in AD patients, and 18-HEPE, were also observed, indicating a switch to a proresolutive profile [16, 53]. Finally, at 6 h post-LPS, mice supplemented with the mix showed a completely different oxylipin profile, characterized by an increase in almost all oxylipins detected and a decrease in PGD_2. The proresolutive properties of both 14-HDHA and 17-HDHA have been extensively documented; notably they suppress the inflammatory response within the central nervous system (CNS) and in microglial cells [12, 61, 62]. Moreover, oxidized linoleic acid metabolites, such as 13-HODE, 9-HODE, 9,10-DiHOME, 9,10,13-TriHOME and 12,13-DiHOME, which were highly overexpressed by the mix, regulate neuronal morphogenesis in vitro [63]. 13-HODE and 18-HEPE are largely described as potent anti-inflammatory molecules. Indeed, 13-HODE is a ligand for PPARα and PPARγ, whose activation reduces the expression of TNFα, IL-1β and IL-6 [64]. 18-HEPE is a metabolic precursor of resolvin E involved in the resolution of inflammation and is significantly increased in the plasma of subjects supplemented with blueberries [29]. These changes were accompanied by an increase in the gene expression of antioxidant enzymes and a decrease in the

Table 9 Effect of supplementations on oxylipins concentrations in hippocampus 6 h post-LPS treatment

Oxylipin (pg/mg of protein)	6 h			
	LPS	LPS (PE)	LPS (om-3)	LPS (PE + om-3)
LA-derived oxylipins				
9-HODE	402.50 ± 30.98 [a]	651.37 ± 59.37 [a, b]	689.29 ± 70.24 [a, b]	816.95 ± 118.46 [b]
10-HODE	3.23 ± 0.00	2.26 ± 1.00	1.49 ± 0.54	4.81 ± 1.14
13-HODE	532.10 ± 39.48 [a]	811.47 ± 61.77 [a, b]	906.75 ± 93.72 [b]	989.50 ± 153.11 [b]
9,10-DiHOME	102.99 ± 6.57 [a]	156.38 ± 13.73 [a, b]	179.30 ± 18.79 [b]	162.65 ± 19.53 [b]
12,13-DiHOME	53.87 ± 5.79 [a]	106.74 ± 9.00 [b]	118.43 ± 10.44 [b]	115.00 ± 13.18 [b]
9-oxo-ODE	391.04 ± 31.12 [a]	733.70 ± 73.44 [b]	793.91 ± 87.29 [b]	915.29 ± 127.18 [b]
13-oxo-ODE	411.35 ± 34.76 [a]	692.01 ± 71.82 [a, b]	838.20 ± 99.90 [b]	958.68 ± 161.18 [b]
9,10,13-TriHOME	350.42 ± 29.08 [a]	613.91 ± 63.03 [b]	661.91 ± 71.01 [b]	581.50 ± 78.73 [a, b]
9,12,13-TriHOME	442.63 ± 35.13 [a]	741.14 ± 68.60 [b]	804.39 ± 70.22 [b]	740.96 ± 128.99 [a, b]
AA-derived oxylipins				
5,6-EET	401.61 ± 25.50	398.29 ± 40.14	534.52 ± 52.79	534.78 ± 46.23
8,9-EET	255.17 ± 27.84 [a]	531.70 ± 60.90 [b]	596.79 ± 70.89 [b, c]	832.92 ± 107.34 [c]
11,12-EET	53.24 ± 10.64 [a]	163.37 ± 20.80 [a, b]	189.97 ± 36.49 [a, b]	287.91 ± 56.36 [b]
14,15-EET	57.27 ± 7.98 [a]	125.41 ± 20.09 [a, b]	149.17 ± 32.60 [a, b]	205.05 ± 39.85 [b]
5-HETE	4033.42 ± 241.85 [a]	6774.06 ± 828.47 [b]	5557.49 ± 323.20 [a, b]	7438.19 ± 693.53 [b]
8-HETE	274.52 ± 18.57 [a]	374.19 ± 37.20 [a, b]	349.96 ± 27.52 [a]	472.16 ± 41.17 [b]
12-HETE	1031.58 ± 163.44	1189.57 ± 99.29	1365.35 ± 180.14	1326.85 ± 144.13
15-HETE	2613.63 ± 167.58 [a]	3575.20 ± 360.43 [a, b]	3495.94 ± 218.48 [a, b]	4166.99 ± 422.17 [b]
5-oxo-ETE	6364.41 ± 390.91 [a]	7957.95 ± 580.42 [a]	8081.49 ± 576.60 [a]	10544.22 ± 642.28 [b]
LTB_4	11.48 ± 1.73 [a]	33.42 ± 3.57 [b]	25.11 ± 4.09 [a, b]	26.30 ± 6.68 [a, b]
LxA_4	362.06 ± 18.56 [a]	736.60 ± 121.51 [b]	617.95 ± 34.39 [b]	548.03 ± 100.83 [a, b]
8-iso-PGA_2	98.95 ± 8.58 [a]	422.96 ± 71.04 [b]	291.56 ± 43.08 [b]	439.74 ± 84.28 [b]
PGD_2	4323.30 ± 259.26 [a]	3573.79 ± 381.20 [a, b]	4372.37 ± 367.52 [a]	2588.25 ± 336.72 [b]
PGE_2	1618.59 ± 110.65	2268.82 ± 140.42	2073.62 ± 207.11	1714.90 ± 239.59
$PGF_{2\alpha}$	4533.23 ± 404.62	5840.77 ± 411.30	6221.79 ± 617.76	5039.73 ± 311.10
15dPGJ_2	6.65 ± 1.32 [a]	39.03 ± 6.74 [b]	33.34 ± 7.17 [b]	49.78 ± 5.78 [b]
6k$PGF_{1\alpha}$	276.77 ± 30.91	443.78 ± 48.27	449.39 ± 86.84	462.62 ± 61.63
TXB_2	1560.67 ± 119.60 [a]	2451.52 ± 165.96 [b]	2603.70 ± 205.20 [b]	2418.39 ± 176.62 [b]
EPA-derived oxylipin				
18-HEPE	5.74 ± 1.10 [a]	9.72 ± 1.75 [a]	47.87 ± 3.74 [b]	60.13 ± 10.85 [b]
DHA-derived oxylipins				
14-HDHA	299.68 ± 21.44 [a]	333.21 ± 28.29 [a]	402.88 ± 36.51 [a, b]	461.74 ± 36.24 [b]
17-HDHA	216.91 ± 21.18 [a]	312.43 ± 38.00 [a]	353.13 ± 47.21 [a, b]	467.89 ± 50.59 [b]

Values with superscripts (a, b, c) differ significantly ($n = 9$–10/group). Data are presented as mean ± SEM and are expressed in pg/mg of protein

gene expression of microglial markers CD68 and CD11b, both described as proinflammatory [65, 66]. All these modifications of oxylipin concentrations could be interpreted as a modification of inflammation resolution kinetic. Furthermore, all supplementations counteracted the LPS effect on fatty acid composition. Indeed, they increased total SFAs and decreased total om-6 + om-3 levels at 30 min. At 6 h post-LPS, all supplementations also increased total MUFAs levels and decreased total om-6 + om-3 levels. The lower proportion of om-6 + om-3 may be explained by their oxidation to produce oxylipins or by the increase in SFA level.

Moreover, the combination of plant extracts and omega-3 exhibited a greater effect than each nutrient alone. In fact, PE supplementation only showed antioxidant effect 30 min after LPS treatment, associated with a proinflammatory effect. After 2 h and 6 h of treatment, PE supplementation also induced changes in the oxylipin profile, but to a lesser extent. Indeed, it decreased the levels of some LA- and AA-derived oxylipins at 2 h and increased the levels of others at 6 h. Similarly, om-3 supplementation increased only the 18-HEPE concentration after 30 min of LPS. This effect was also observed after 2 h and 6 h, accompanied by other changes in the oxylipin profile, mostly by an increase in LA-derived oxylipins at 6 h. Om-3 supplementation did increase DHA-derived oxylipin levels compared to LPS group, in accordance with previous studies [12, 30]. As PE supplementation had no effect on either 14- or 17-HDHA, metabolite precursors of maresin and resolvin D that are pro-resolving molecules, but PE + om-3 supplementation

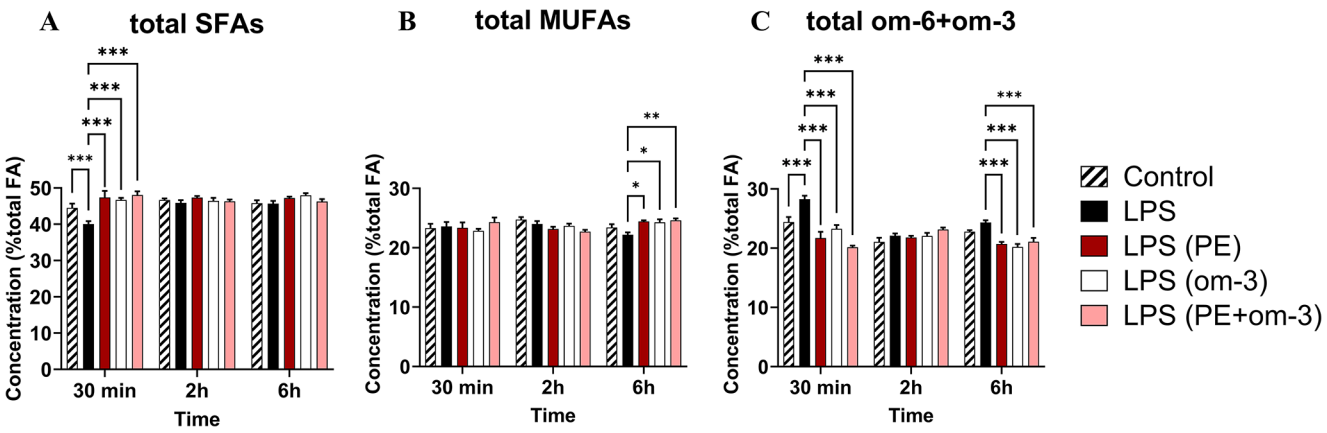

Fig. 8 Effect of LPS and supplementations on fatty acid concentrations in hippocampus. (**A**) Total SFAs concentration. (**B**) Total MUFAs concentration. (**C**C) Total omega-6 + omega-3 concentration. (*$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$ vs. LPS by 2-WAY ANOVA and Dunnett's post-hoc test, $n = 9$–10/group). Data are presented as mean ± SEM

did, these results suggest that plant extracts and omega-3 exerted a potentiating effect.

To our knowledge, this study is the first to provide interesting initial evidence on the potential beneficial effects of a mix containing grapes and blueberries polyphenols, saffron carotenoids and fish omega-3 on neuroinflammation and oxylipin profile. However, it has some limitations. Indeed, this study focused on a relatively short-term period with limited time points (6 h maximum post-LPS injection), further studies on chronic inflammatory models, are necessary to conclude on the long-term effects of the nutrients. Behavioral assessments could also be added to understand how these changes impacted functional changes in mice. Moreover, even if many biomarkers have been measured, the exact mechanisms by which the supplementations exert their effects remain unclear. Further analysis of the pathways involved could provide a more complete and detailed overview of key players in this biological process. In addition, the use of only male mice limits the generalizability of our findings, especially given sex-related differences in inflammatory responses [67]. Further studies have to be conducted in female mice.

Conclusion

This study presented evidence of the additional effect of a mix containing PE (Memophenol™ and a patented saffron extract) and omega-3 on LPS-induced neuroinflammation, as it showed modulations of some genes and oxylipins not found with the two other supplementations. The mix supplementation, for only 18 consecutive days, induced a shift in the oxylipin profile, demonstrating a different resolution of inflammation than without supplementation or with nutrients alone. Thus, our results confirmed the interest of a multinutrient approach to limit alterations induced by age, such as chronic low-grade inflammation.

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Author contributions LP, DG, SL, VP, ALD and CJ devised the project, the main conceptual ideas and proof outline. MM, ALD and CJ conceived and designed experiments. MM, and ED conducted research. MM and ED analyzed data. MM, JB, and AP performed statistical analysis. MM wrote the manuscript with support of LP, ALD and CJ. All authors have read and agreed to the published version of the manuscript.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate Animal husbandry and experimental procedures were performed in accordance with the EU Directive 2010/63/EU for animal experiments and were approved by the National Ethics Committee for the Care and Use of Animals (approval ID A27756).

Consent for publication Not applicable.

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References

1. Lyman M, Lloyd DG, Ji X, Vizcaychipi MP, Ma D. Neuroinflammation: the role and consequences. Neurosci Res. 2014;79:1–12.
2. Franceschi C, Garagnani P, Parini P, Giuliani C, Santoro A. Inflammaging: a new immune–metabolic viewpoint for age-related diseases. Nat Rev Endocrinol. 2018;14:576–90.
3. Marschallinger J, Iram T, Zardeneta M, Lee SE, Lehallier B, Haney MS, et al. Lipid-droplet-accumulating microglia represent a dysfunctional and proinflammatory state in the aging brain. Nat Neurosci. 2020;23:194–208.
4. Sierra A, Gottfried-Blackmore AC, McEwen BS, Bulloch K. Microglia derived from aging mice exhibit an altered inflammatory profile. Glia. 2007;55:412–24.
5. Semple BD, Kossmann T, Morganti-Kossmann MC. Role of chemokines in CNS health and pathology: a focus on the CCL2/CCR2 and CXCL8/CXCR2 networks. J Cereb Blood Flow Metab off J Int Soc Cereb Blood Flow Metab. 2010;30:459–73.
6. Yang S, Qin C, Hu Z-W, Zhou L-Q, Yu H-H, Chen M, et al. Microglia reprogram metabolic profiles for phenotype and function changes in central nervous system. Neurobiol Dis. 2021;152:105290.
7. Cohen HJ, Pieper CF, Harris T, Rao KMK, Currie MS. The Association of Plasma IL-6 levels with functional disability in Community-Dwelling Elderly. J Gerontol Biol Sci Med Sci. 1997;52A:M201–8.
8. Mooijaart SP, Sattar N, Trompet S, Lucke J, Stott DJ, Ford I, et al. Circulating interleukin-6 concentration and cognitive decline in old age: the PROSPER study. J Intern Med. 2013;274:77–85.
9. Braida D, Sacerdote P, Panerai AE, Bianchi M, Aloisi AM, Iosuè S, et al. Cognitive function in young and adult IL (interleukin)-6 deficient mice. Behav Brain Res. 2004;153:423–9.
10. Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. Nature. 2014;510:92–101.
11. Haeggström JZ, Funk CD. Lipoxygenase and Leukotriene pathways: Biochemistry, Biology, and roles in Disease. Chem Rev. 2011;111:5866–98.
12. Rey C, Delpech JC, Madore C, Nadjar A, Greenhalgh AD, Amadieu C, et al. Dietary n-3 long chain PUFA supplementation promotes a pro-resolving oxylipin profile in the brain. Brain Behav Immun. 2019;76:17–27.
13. Caligiuri SPB, Parikh M, Stamenkovic A, Pierce GN, Aukema HM. Dietary modulation of oxylipins in cardiovascular disease and aging. Am J Physiol-Heart Circ Physiol. 2017;313:H903–18.
14. Devassy JG, Leng S, Gabbs M, Monirujjaman M, Aukema HM. Omega-3 polyunsaturated fatty acids and oxylipins in Neuroinflammation and management of Alzheimer Disease. Adv Nutr. 2016;7:905–16.
15. Caligiuri SPB, Aukema HM, Ravandi A, Pierce GN. Elevated levels of pro-inflammatory oxylipins in older subjects are normalized by flaxseed consumption. Exp Gerontol. 2014;59:51–7.
16. Wang X, Zhu M, Hjorth E, Cortés-Toro V, Eyjolfsdottir H, Graff C, et al. Resolution of inflammation is altered in Alzheimer's disease. Alzheimers Dement. 2015;11:40.
17. Gallardo-Fernández M, Hornedo-Ortega R, Alonso-Bellido IM, Rodríguez-Gómez JA, Troncoso AM, García-Parrilla MC, et al. Hydroxytyrosol decreases LPS- and α-Synuclein-Induced Microglial Activation in Vitro. Antioxidants. 2019;9:36.
18. Li H, Xiao C, Wang F, Guo X, Zhou Z, Jiang Y. Blueberry–Mulberry Extract alleviates cognitive impairment, regulates gut metabolites, and inhibits inflammation in aged mice. Foods. 2023;12:860.
19. Abraham J, Johnson RW. Consuming a Diet supplemented with Resveratrol reduced infection-related neuroinflammation and deficits in working memory in aged mice. Rejuvenation Res. 2009;12:445–53.
20. Meng T, Xiao D, Muhammed A, Deng J, Chen L, He J. Anti-inflammatory action and mechanisms of Resveratrol. Molecules. 2021;26:229.
21. Mobasseri M, Ostadrahimi A, Tajaddini A, Asghari S, Barati M, Akbarzadeh M, et al. Effects of saffron supplementation on glycemia and inflammation in patients with type 2 diabetes mellitus: a randomized double-blind, placebo-controlled clinical trial study. Diabetes Metab Syndr Clin Res Rev. 2020;14:527–34.
22. Amin B, Abnous K, Motamedshariaty V, Hosseinzadeh H. Attenuation of oxidative stress, inflammation and apoptosis by ethanolic and aqueous extracts of Crocus sativus L. stigma after chronic constriction injury of rats. Acad Bras Ciênc. 2014;86:1821–32.
23. Nam KN, Park Y-M, Jung H-J, Lee JY, Min BD, Park S-U, et al. Anti-inflammatory effects of crocin and crocetin in rat brain microglial cells. Eur J Pharmacol. 2010;648:110–6.
24. Yan J, Li T, Ji K, Zhou X, Yao W, Zhou L, et al. Safranal alleviates pentetrazole-induced epileptic seizures in mice by inhibiting the NF-κB signaling pathway and mitochondrial-dependent apoptosis through GSK-3β inactivation. J Ethnopharmacol. 2024;333:118408.
25. Baluchnejadmojarad T, Mohamadi-Zarch S-M, Roghani M. Safranal, an active ingredient of saffron, attenuates cognitive deficits in amyloid β-induced rat model of Alzheimer's disease: underlying mechanisms. Metab Brain Dis. 2019;34:1747–59.
26. Alfano CM, Imayama I, Neuhouser ML, Kiecolt-Glaser JK, Smith AW, Meeske K, et al. Fatigue, inflammation, and ω-3 and ω-6 fatty acid intake among breast Cancer survivors. J Clin Oncol. 2012;30:1280–7.
27. Labrousse VF, Nadjar A, Joffre C, Costes L, Aubert A, Grégoire S et al. Short-Term Long Chain Omega3 Diet Protects from Neuroinflammatory Processes and Memory Impairment in Aged Mice. Schunck W-H, editor. PLoS ONE. 2012;7:e36861.
28. Hartung NM, Fischer J, Ostermann AI, Willenberg I, Rund KM, Schebb NH, et al. Impact of food polyphenols on oxylipin biosynthesis in human neutrophils. Biochim Biophys Acta BBA - Mol Cell Biol Lipids. 2019;1864:1536–44.
29. Nieman DC, Sakaguchi CA, Omar AM, Davis KL, Shaffner CE, Strauch RC, et al. Blueberry intake elevates post-exercise anti-inflammatory oxylipins: a randomized trial. Sci Rep. 2023;13:11976.
30. Chataigner M, Martin M, Lucas C, Pallet V, Layé S, Mehaignerie A, et al. Fish Hydrolysate Supplementation containing n-3 long chain polyunsaturated fatty acids and peptides prevents LPS-Induced Neuroinflammation. Nutrients. 2021;13:824.
31. Joffre C, Rey C, Layé S. N-3 polyunsaturated fatty acids and the resolution of Neuroinflammation. Front Pharmacol. 2019;10:1022.
32. Schuchardt JP, Ostermann AI, Stork L, Fritzsch S, Kohrs H, Greupner T, et al. Effect of DHA supplementation on oxylipin levels in plasma and immune cell stimulated blood. Prostaglandins Leukot Essent Fat Acids. 2017;121:76–87.
33. Gabbs M, Zahradka P, Taylor CG, Aukema HM. Time Course and Sex effects of α-Linolenic acid-Rich and DHA-Rich supplements

on human plasma oxylipins: a Randomized double-blind crossover trial. J Nutr. 2021;151:513–22.
34. Bensalem J, Servant L, Alfos S, Gaudout D, Layé S, Pallet V, et al. Dietary polyphenol supplementation prevents alterations of spatial Navigation in Middle-aged mice. Front Behav Neurosci. 2016;10:9.
35. Bensalem J, Dudonné S, Gaudout D, Servant L, Calon F, Desjardins Y, et al. Polyphenol-rich extract from grape and blueberry attenuates cognitive decline and improves neuronal function in aged mice. J Nutr Sci. 2018;7:e19.
36. Bensalem J, Dudonné S, Etchamendy N, Pellay H, Amadieu C, Gaudout D, et al. Polyphenols from grape and blueberry improve episodic memory in Healthy Elderly with Lower Level of Memory performance: a bicentric Double-Blind, randomized, placebo-controlled clinical study. J Gerontol Ser A. 2019;74:996–1007.
37. Jackson PA, Forster J, Khan J, Pouchieu C, Dubreuil S, Gaudout D, et al. Effects of Saffron Extract supplementation on Mood, Well-Being, and response to a Psychosocial Stressor in healthy adults: a Randomized, Double-Blind, Parallel Group, Clinical Trial. Front Nutr. 2021;7:606124.
38. De Monchaux C, De Smedt-Peyrusse V, Morael J, Vancassel S, Capuron L, Gaudout D, et al. Prevention of stress-induced depressive-like behavior by Saffron extract is associated with modulation of Kynurenine pathway and monoamine neurotransmission. Pharmaceutics. 2021;13:2155.
39. Monchaux de Oliveira C, Morael J, Guille A, Amadieu C, Vancassel S, Gaudout D, et al. Saffron extract interferes with lipopolysaccharide-induced brain activation of the kynurenine pathway and impairment of monoamine neurotransmission in mice. Front Nutr. 2023;10:1267839.
40. Mingam R, Moranis A, Bluthé R-M, De Smedt-Peyrusse V, Kelley KW, Guesnet P, et al. Uncoupling of interleukin-6 from its signalling pathway by dietary n-3-polyunsaturated fatty acid deprivation alters sickness behaviour in mice. Eur J Neurosci. 2008;28:1877–86.
41. Larrieu T, Madore C, Joffre C, Layé S. Nutritional n-3 polyunsaturated fatty acids deficiency alters cannabinoid receptor signaling pathway in the brain and associated anxiety-like behavior in mice. J Physiol Biochem. 2012;68:671–81.
42. Le Faouder P, Baillif V, Spreadbury I, Motta J-P, Rousset P, Chêne G, et al. LC–MS/MS method for rapid and concomitant quantification of pro-inflammatory and pro-resolving polyunsaturated fatty acid metabolites. J Chromatogr B. 2013;932:123–33.
43. Barnig C, Bezema T, Calder PC, Charloux A, Frossard N, Garssen J, et al. Activation of Resolution pathways to prevent and fight chronic inflammation: lessons from Asthma and Inflammatory Bowel Disease. Front Immunol. 2019;10:1699.
44. Layé S, Parnet P, Goujon E, Dantzer R. Peripheral administration of lipopolysaccharide induces the expression of cytokine transcripts in the brain and pituitary of mice. Mol Brain Res. 1994;27:157–62.
45. Reichenberg A, Yirmiya R, Schuld A, Kraus T, Haack M, Morag A, et al. Cytokine-associated emotional and cognitive disturbances in humans. Arch Gen Psychiatry. 2001;58:445–52.
46. Zhao Y, Cong L, Jaber V, Lukiw WJ. Microbiome-Derived Lipopolysaccharide Enriched in the Perinuclear Region of Alzheimer's Disease Brain. Front Immunol. 2017;8:1064.
47. Zhao Y, Walker DI, Lill CM, Bloem BR, Darweesh SKL, Pinto-Pacheco B, et al. Lipopolysaccharide-binding protein and future Parkinson's disease risk: a European prospective cohort. J Neuroinflammation. 2023;20:170.
48. Hasegawa-Ishii S, Inaba M, Shimada A. Widespread time-dependent changes in tissue cytokine concentrations in brain regions during the acute phase of endotoxemia in mice. Neurotoxicology. 2020;76:67–74.
49. Essadek S, Bouchab H, El Kebbaj R, Gondcaille C, El Kamouni S, Savary S, et al. Effects of a short-term Lipopolysaccharides Challenge on Mouse Brain and Liver Peroxisomal antioxidant and β-oxidative functions: protective action of Argan Oil. Pharmaceuticals. 2022;15:465.
50. Butovsky O, Jedrychowski MP, Moore CS, Cialic R, Lanser AJ, Gabriely G, et al. Identification of a unique TGF-β–dependent molecular and functional signature in microglia. Nat Neurosci. 2014;17:131–43.
51. Li C, Zhao B, Lin C, Gong Z, An X. TREM2 inhibits inflammatory responses in mouse microglia by suppressing the PI3K/NF-κB signaling. Cell Biol Int. 2019;43:360–72.
52. Chiang N, Serhan CN. Structural elucidation and physiologic functions of Specialized Pro-resolving mediators and their receptors. Mol Aspects Med. 2017;58:114–29.
53. Levy BD, Clish CB, Schmidt B, Gronert K, Serhan CN. Lipid mediator class switching during acute inflammation: signals in resolution. Nat Immunol. 2001;2:612–9.
54. Graves JP, Bradbury JA, Gruzdev A, Li H, Duval C, Lih FB, et al. Expression of Cyp2c/Cyp2j subfamily members and oxylipin levels during LPS-induced inflammation and resolution in mice. FASEB J. 2019;33:14784–97.
55. Jing W, Song S, Sun H, Chen Y, Zhao Q, Zhang Y, et al. *Mahuang-Fuzi-Xixin* Decoreversesverses Depression-Like Behavior in LPS-Inmiced Miregulatinglating inflammasomemasomneurogenesisenesis. Neural Plast. 2019;2019:1–13.
56. Button EB, Mitchell AS, Domingos MM, Chung JH-J, Bradley RM, Hashemi A, et al. Microglial cell activation increases saturated and decreases monounsaturated fatty acid content, but both lipid species are Proinflammatory. Lipids. 2014;49:305–16.
57. Weylandt KH, Krause LF, Gomolka B, Chiu C-Y, Bilal S, Nadolny A, et al. Suppressed liver tumorigenesis in fat-1 mice with elevated omega-3 fatty acids is associated with increased omega-3 derived lipid mediators and reduced TNF-α. Carcinogenesis. 2011;32:897–903.
58. Balvers MGJ, Verhoeckx KCM, Bijlsma S, Rubingh CM, Meijerink J, Wortelboer HM, et al. 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. 2012;8:1130–47.
59. Leng S, Winter T, Aukema HM, Dietary ALA. EPA and DHA have distinct effects on oxylipin profiles in female and male rat kidney, liver and serum. J Nutr Biochem. 2018;57:228–37.
60. Turnbull J, Jha RR, Ortori CA, Lunt E, Tighe PJ, Irving WL, et al. Serum levels of proinflammatory lipid mediators and Specialized Proresolving molecules are increased in patients with severe Acute Respiratory Syndrome Coronavirus 2 and correlate with markers of the adaptive Immune response. J Infect Dis. 2022;225:2142–54.
61. Rey C, Nadjar A, Buaud B, Vaysse C, Aubert A, Pallet V, et al. Resolvin D1 and E1 promote resolution of inflammation in microglial cells in vitro. Brain Behav Immun. 2016;55:249–59.
62. Gabbs M, Leng S, Devassy JG, Monirujjaman M, Aukema HM. Advances in our understanding of Oxylipins Derived from Dietary PUFAs. Adv Nutr. 2015;6:513–40.
63. da Costa Souza F, Grodzki ACG, Morgan RK, Zhang Z, Taha AY, Lein PJ. Oxidized linoleic acid metabolites regulate neuronal morphogenesis in vitro. Neurochem Int. 2023;164:105506.
64. Ricote M, Huang J, Fajas L, Li A, Welch J, Najib J, et al. Expression of the peroxisome proliferator-activated receptor γ (PPARγ) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density lipoprotein. Proc Natl Acad Sci U S A. 1998;95:7614–9.
65. Schmid MC, Khan SQ, Kaneda MM, Pathria P, Shepard R, Louis TL, et al. Integrin CD11b activation drives anti-tumor innate immunity. Nat Commun. 2018;9:5379.

66. Zhang J, Li S, Liu F, Yang K. Role of CD68 in tumor immunity and prognosis prediction in pan-cancer. Sci Rep. 2022;12:7844.
67. Villa A, Gelosa P, Castiglioni L, Cimino M, Rizzi N, Pepe G, et al. Sex-specific features of Microglia from Adult mice. Cell Rep. 2018;23:3501–11.