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A single meal has the potential to alter brain oxylipin content

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ABSTRACT

Our objective was to determine whether consumption of a single meal has the potential to alter brain oxylipin content. We examined the cerebrum of mice fed a single high-fat/high-sucrose Western meal or a low-fat/low-sucrose control meal, as well as fasted mice. We found no changes in fatty acid composition of cerebrum across the groups. The cerebral oxylipin profile of mice fed a Western meal is distinct from the profile of mice fed a low-fat/low-sucrose meal. Cerebral gene expression of cyclooxygenase 1, cyclooxygenase 2, and epoxide hydrolase 1 were elevated in Western meal-fed mice compared to low-fat/low-sucrose meal-fed mice. Mice that consumed either meal had lower gene expression of cytochrome P450, family 2, subfamily j, polypeptide 12 than fasted mice. Our data in this hypothesis-generating study indicates that the composition of a single meal has the potential to alter brain oxylipins and the gene expression of the enzymes responsible for their production.

1. Introduction

Aging is associated with cognitive decline and the development of dementia, the prevalence of which has been increasing as the population ages [1]. Further, neuroinflammation and oxidative stress are associated with aging and neurodegeneration [2]. Oxylipins are bioactive products of polyunsaturated fatty acid oxidation, which have been shown to be involved in regulating inflammation, oxidative stress, and resolution pathways in brain [3]. Less is known about the role of oxylipins in neurodegenerative diseases, however a few studies have indicated a role of oxylipins in the modulation of Alzheimer's disease progression and traumatic brain injury [4–9].

Certain dietary patterns are believed to modify the risk for developing cognitive decline [1]. Chronic consumption of a Western diet, characterized as being high in fats and sugars, has been linked to an increased risk of cognitive decline [10,11]. The Western diet has been shown to impact many aspects of brain health including oxidative stress and neuroinflammation [11]. In previous work conducted in our laboratory, we found that the consumption of a Western diet over the period of three months increased arachidonic acid derived oxylipins and altered the fatty acid profiles of the brains of mice [12]. Further, others have shown that various modifications of a chronically consumed diet have the potential to modify brain oxylipin levels [13–17].

To our knowledge, no studies have examined whether the consumption of a single meal can alter brain fatty acid or oxylipin levels.

This may have implications for acutely modifying brain signaling and inflammatory pathways in a manner that can potentially reduce the brain's resilience to external insults. For instance, frequent but acute reductions in protective anti-inflammatory oxylipins or increases in pro-inflammatory oxylipins following a Western diet could potentially accelerate age associated loss of function leading to dementia. The objective of this study was to determine whether the consumption of a single “Western diet” meal could impact brain fatty acid or oxylipin composition. We asked two basic questions: 1) does the consumption of a meal alter brain fatty acid and oxylipin levels when compared to the fasted state? and 2) does the consumption of a “Western” meal alter the fatty acid and oxylipin levels when compared to a low-fat/low-sucrose meal? We meal-trained mice for eight days, then fed one group of mice a “Western” meal, another an ingredient-matched low-fat/low-sucrose meal, and a third group was analyzed while fasting. We examined cerebrum fatty acid composition and oxylipin content. We then used the results of these analyses to identify oxylipin-generating enzymes of interest, for which we measured gene expression.

2. Materials and methods

2.1. Animals

C57Bl/6 J male mice were obtained from Jackson Laboratories at 17 weeks of age. All procedures were approved by the University of

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Abbreviations

LFLSD	low-fat/low-sucrose diet
LFLSM	low-fat/low-sucrose meal
WD	Western diet
WM	Western meal
PGD1	prostaglandin D1
DiHETrE	hydroxyeicosatrienoic acid
EpETrE	epoxyeicosatrienoic acid

EpDPE	epoxydocosapentaenoic acid
CYP2J12	cytochrome P450, family 2, subfamily j, polypeptide 12
COX	cyclooxygenase
EPHX	epoxide hydrolase
sEH	soluble epoxide hydrolase
mEH	microsomal epoxide hydrolase
DGLA	dihomo- γ -linolenic acid
AA	arachidonic acid
DHA	docosahexaenoic acid

California, Davis Institutional Animal Care and Use Committee (protocol number 20068). These procedures were carried out in accordance with the National Institutes of Health guide for the use and care of laboratory animals.

2.2. Diets

The diets used in this study were an ingredient matched low-fat/low-sucrose control diet (LFLSD, Envigo TD.08485) and a high-fat/high-sucrose Western diet (WD, Envigo TD.88137). The LFLSD contains 3.6 kcal/g with 19.1% kcal from protein, 67.9% kcal from carbohydrate, and 13.0% kcal from fat, with 120 g/kg sucrose content. The fat source for the LFLSD is 37.2 g/kg anhydrous milkfat and 12.8 g/kg soybean oil. The calculated fatty acid content of the LFLSD provided by the manufacturer can be found in **Supplemental Table 1**. The WD contains 4.5 kcal/g with 15.2% kcal from protein, 42.7% kcal from carbohydrate, and 42.0% kcal from fat, with 341.46 g/kg sucrose content and 1.5 g/kg added cholesterol. The fat source for the WD is 210 g/kg anhydrous milkfat. The measured percent fatty acid composition of the WD provided by the manufacturer can be found in **Supplemental Table 2**.

2.3. Meal training

Upon receipt at 17 weeks of age ($n = 15$), mice were given the LFLSD ad libitum for 12–14 days. Following this adjustment period, mice were trained to consume the food in a meal format for eight days. The night before the initiation of the meal training procedure, all food was removed from the cage. Each day of meal training the mice were given a meal twice daily with at least 8 h between LFLSD meals. Each meal consisted of access to an amount of food more than sufficient for their daily intake requirements (at least 5 g per mouse) for 90 min. At the end of the 90-minute period, any remaining food was removed from the cage. Water access was never restricted during this process.

2.4. Test meal and tissue collection

The day of tissue collection immediately followed the eight days of meal training. Mice were allocated to one of three groups: fasting ($n = 5$), low-fat/low-sucrose meal (LFLSM, $n = 5$), or Western meal (WM, $n = 5$). Mice in the LFLSM and WM groups were given access to a weighed amount of LFLSD or WD, respectively, for a meal period lasting 90–120 min. Any remaining food was removed, weighed, and recorded to determine the amount of food consumed. Four hours after the initiation of the meal, tissues were collected. We chose the 4 h timepoint based on previous findings that postprandial triglycerides peak at 3.5 h after consumption of a meal [18]. Due to the long meal time in our study (to ensure the mice ate a sufficient quantity), we measured the time from meal initiation we added half an hour to approximate this peak in triglycerides as best we could. For mice in the fasting group, tissue was collected at the time of meal initiation for the other groups.

Prior to tissue collection, mice were given 1–5% isoflurane until unresponsive to toe pinch. The chest cavity was then opened and the mouse was exsanguinated and the heart removed to ensure euthanasia,

prior to tissue collection. The brain was collected and snap frozen in liquid nitrogen within 5 min of death.

2.5. Free oxylipin extraction from brain

Most oxylipin and surrogate standards were obtained from Cayman Chemicals (Ann Arbor, MI, USA). 9,12,13-trihydroxyoctadecenoic (TriHOME) and 9,10,13-TriHOME were purchased from Loradan (Solna, Sweden). Solvents were obtained from Fisher Scientific and were all UPLC-MS grade.

The right hemisphere of cerebrum from each mouse (112.7–247.7 mg) was homogenized in 200 μ L of methanol containing 0.1% butylated hydroxytoluene (BHT). The samples were spiked with 10 μ L of deuterated surrogate standard solution containing 2 μ M of d11-11(12)-EpETrE, d11-14,15-DiHETrE, d4-6-keto-PGF1 α , d4-9-HODE, d4-LTB4, d4-PGE2, d4-TXB2, d6-20-HETE and d8-5-HETE in methanol. The samples were then precooled in -80 °C for 30 min and homogenized with a bead homogenizer (Nextadvance air cooling bullet blender, Troy, NY, USA) using zirconia beads. The homogenized samples were centrifuged at 4 °C at a speed of 15,871 g for 10 min. The supernatant was loaded onto 60 mg Waters Oasis HLB 3cc solid phase extraction (SPE) columns (Waters, Milford, MA, USA), pre-rinsed with one volume of ethyl acetate and two volumes of methanol, and pre-conditioned with two volumes of SPE buffer containing 5% methanol and 0.1% acetic acid in ultrapure Millipore water. The columns were rinsed twice with SPE buffer and subjected to 20 min of vacuum (≈ 20 psi). Oxylipins were eluted with 0.5 mL methanol and 1.5 mL ethyl acetate into 2 mL centrifuge tubes, dried under nitrogen and reconstituted in 100 μ L methanol. Ten μ L was analyzed by UPLC-MS/MS. The remaining brain pellets were store at -80 °C for fatty acid analysis.

2.6. UHPLC-MS/MS analysis

All measured oxylipins are listed and abbreviated in **Supplemental Table 3**. Oxylipins were analyzed by ultra-high-pressure liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) using Agilent LC system 1290 (Agilent Corporation, Santa Clara, CA, USA), coupled to an Agilent 6460 Triple Quadrupole MS system (Agilent Corporation). Oxylipin species were separated on an Agilent Eclipse Plus C18 column (2.1 \times 150 mm, 1.8 μ m, Agilent Corporation) with a binary gradient consisting of solvent A (water containing 0.1% acetic acid) and solvent B (Acetonitrile: methanol 80:15 containing 0.1% acetic acid). The column temperature was maintained at 45 °C. The gradient profile is shown in **Supplemental Table 4**. Electrospray ionization (negative mode) was used as the ion source with the experimental parameters, as follows: Gas temperature, 250 °C; Gas flow, 10 L/min; Sheath gas temperature, 300 °C; Sheath gas flow, 11 mL/min; Nebulizers 35 psi; Capillary gas, 3500 V/–3500 V. Optimization parameters and parent and product ion monitoring pairs are described **Supplemental Table 5**. Surrogate recovery data can be found in **Supplemental Table 6**, there were no differences in surrogate percent recovery between groups.

2.7. Fatty acid derivatization

The remaining brain pellet from the oxylipin extraction (above) was reconstituted in 1 mL methanol. Samples were vortexed and homogenized for 1 min in the bead homogenizer. Samples were transferred to new glass tubes containing 400 μ L toluene. Two mL methanol was added to the old tubes, vortexed and the solution was transferred to new glass tubes, 600 μ L of 8% HCl (37% concentrated) in methanol solution was added. The HCl used was 37% ACS reagent (Sigma Aldrich, St. Louis, MO, USA). The samples were heated at 90 °C for 60 min on a heating block and were cooled down for 4 to 5 min thereafter at room temperature. 1 mL of water and 1 mL of hexane were then added to each sample. The samples were vortexed and centrifuged on a Beckman Coulter Allegra 6 centrifuge (Beckman Coulter, Palo Alto, CA, U.S.A.) using a GH3.8A rotor at 646 \times g for 10 min. Then 900 μ L of the hexane layer was transferred to a micro-centrifuge tube (USA Scientific, Orlando, FL, USA) containing 450 μ L of distilled water, vortexed and centrifuged for 2 min at 15,871 \times g on a 5430r Eppendorf micro-centrifuge containing a FA-45-24-11 rotor (Fisher Scientific, Pittsburgh, PA, USA). The hexane upper layer was then transferred to a new micro-centrifuge tube, dried under nitrogen and reconstituted in 100 μ L hexane. Samples were stored at -80 °C until analysis.

2.8. GC analysis

All samples were analyzed on a Perkin Elmer Clarus 500 GC system with a flame ionization detector (FID) (Perkin Elmer, Shelton, CT, U.S.A.) equipped with a FFAP capillary column (30 m \times 0.25 mm inner diameter, 0.25 μ m film thickness; Agilent Technologies, Santa Clara, Calif., U.S.A.). The injector and detector temperature were set at 240 °C and 300 °C, respectively. The split ratio was 10:1 and the injection volume was 1 μ L. The initial oven temperature was 80 °C. It was held at 80 °C for 2 min, increased by 10 °C/min to 185 °C upon injection, then to 240 °C at 5 °C/min and held at 240 °C for 13 min. The total run time was 36.5 min. Helium was the carrier gas and was maintained at a flow rate of 1.3 mL/min. A custom mix of 29 fatty acid methyl ester standards was used to identify individual fatty acids based on retention time. Data were expressed as percent of total identified fatty acid peaks.

2.9. Gene expression

The left hemisphere of cerebrum was powdered and used for analysis of gene expression. RNA was isolated from approximately 50 mg of powdered tissue using Trizol and RNeasy mini kit according to the manufacturer's instructions. RNA from each sample was quantified using a Nanodrop ND-1000 Spectrophotometer, reverse transcribed using Superscript III First Strand Synthesis Kit (Life Technologies) according to manufacturer's instructions. qRT-PCR was performed with SYBR Green PCR Mastermix (Applied Biosystems) to quantify the gene expression. Reactions were carried out in 384-well optical plates containing approximately 25 ng RNA in each well. The quantity of applied RNA was normalized by simultaneously amplifying cDNA samples with gene of interest specific primers and 18 s specific primers. The primer sequences used can be found in **Supplemental Table 7**. Primer sequences were obtained from the Primer Bank at: <https://pga.mgh.harvard.edu/primerbank/> [19], with the exception of the primers for CYP2J12 which was obtained from the publication by Graves and colleagues [20]. Transcript levels were measured by qRT-PCR using the ABI Vii7 Sequence detection system (PE Applied Biosystems). The PCR amplification parameters were: initial denaturation step at 95 °C for 10 min followed by 40 cycles, each at 95 °C for 15 s (melting) and 60 °C for 1 min (annealing and extension). A comparative threshold cycle (Ct) method was used to calculate relative changes in gene expression determined from qRT-PCR experiments [21]. The Ct, which correlates inversely with the target mRNA levels, was measured as the cycle number at which the SYBR Green emission increases above a preset

threshold level. Fold change determinations were made using the LFLSM group as a reference.

2.10. Statistical analysis

Analysis of the oxylipin and fatty acid data as well as generation of the sPLSDA plots was done using the publicly available software Metaboanalyst 4.0 [22,23]. Oxylipins for which there were less than 3 samples per group with detectable amounts were excluded from these analyses. For all other analyses, when comparing the two meal groups, data was analyzed by *t*-test or Mann-Whitney Rank Sum Test (for data that was not normally distributed). When comparing all three groups ANOVA or ANOVA on Ranks (for data that was not normally distributed). For ANOVA analyses with a significant result, a Holm-Sidak post hoc (versus Fasting group mice) test was run to determine if one or more of the meal consuming groups differed from the fasting group. Unless otherwise indicated data shown is mean \pm standard deviation.

3. Results

3.1. Food consumption during meal period

There was no difference in the body weights of the animals allocated to the fasting, low-fat/low-sucrose meal (LFLSM), and Western meal (WM) groups as determined by one-way ANOVA (Fasting 27.9 \pm 1.20 g; LFLSM 27.96 \pm 1.17 g; WM 28.42 \pm 1.20 g). Food consumption during the test meal period, determined by the difference in the weight of the food before the meal period and any remaining food at the end of the meal period, was not significantly different between the two meal consuming groups. This also holds true when the amount of food was normalized to bodyweight for each mouse. However, due to the difference in the caloric density of the two diets, the WM group did consume significantly more kilocalories during the meal period than the LFLSM group, regardless of if the caloric intake was normalized to bodyweight (**Table 1**).

3.2. Cerebral oxylipin content

Brain free oxylipin content of the right hemisphere of cerebrum was determined by UHPLC-MS/MS. The results of the analysis can be seen in **Table 2**. When addressing the question of whether consuming a meal alters brain oxylipin concentrations compared to the fasted state, prostaglandin D1 (PGD1), and 14,15-dihydroxyeicosatrienoic acid (DiHETrE) content were found to be significantly higher in the WM group compared to the fasted group, while the LFLSM group did not differ from the fasted group for any of the measured oxylipins. When comparing the two meal consuming groups, four of the measured oxylipins were significantly different between the LFLSM and WM groups. PGD1, 14,15 DiHETrE, and 8,9-Epoxyeicosatrienoic acid (EpETrE) were significantly higher in the WM group when compared to the LFLSM group. 16(17)-epoxydocosapentaenoic acid (EpDPE) was

Table 1
Bodyweight and food consumption during meal period.

	Low-fat/low-sucrose meal (LFLSM)		Western meal (WM)		<i>p</i> value
	Mean	Standard Deviation	Mean	Standard Deviation	
Bodyweight (g)	27.96	1.17	28.42	1.20	0.558
Food consumed (g)	1.78	0.62	2.6	0.62	0.071
kcal consumed	6.408	2.24	11.7	2.81	0.011
g food consumed / g bodyweight	0.064	0.024	0.092	0.022	0.095
kcal consumed / g bodyweight	0.23	0.085	0.41	0.101	0.015

Table 2
Brain oxylipin concentration expressed as pmol/g tissue.

Oxylipin	Fasting		Low-fat/low-sucrose meal (LFLSM)		Western Meal (WM)		p value	
	Mean (pmol/g)	Standard Deviation	Mean (pmol/g)	Standard Deviation	Mean (pmol/g)	Standard Deviation	ANOVA	t-test LFLSM v WM
Linoleic Acid Derived								
9-HODE	0.29	0.04	0.28	0.06	0.33	0.16	0.721	0.540
13-HODE	0.29	0.13	0.28	0.06	0.28	0.10	0.974	0.942
9,10,13-TriHOME	1.90	0.76	1.50	0.37	1.68	0.24	0.484	0.375
9,12,13-TriHOME	0.31	0.14	0.28	0.12	0.28	0.16	0.924	0.983
9-oxo-ODE	1.30	1.44	0.90	0.62	0.68	0.93	0.640	0.690
12(13)-EpOME	2.13	2.22	1.08	1.55	1.68	1.96	0.696	0.611
9,10-DiHOME	0.25	0.07	0.28	0.14	0.37	0.23	0.488	0.454
12,13-DiHOME	0.51	0.15	0.43	0.17	0.54	0.42	0.818	0.608
Dihomo- γ -linolenic acid derived								
15(S)-HETrE	1.04	0.37	1.12	0.54	1.34	0.49	0.587	0.517
PGD1	2.71	0.98	2.97	0.72	4.47	1.05	0.022 ^a	0.029
PGE1	0.82	0.41	0.85	0.33	1.07	0.52	0.621	0.460
Arachidonic acid derived								
5-HETE	5.35	2.13	4.29	1.59	6.19	2.32	0.367	0.170
8-HETE	3.36	1.53	3.27	1.04	4.72	1.56	0.224	0.121
12-HETE	11.13	7.30	9.44	3.79	10.92	3.40	0.853	0.535
15-HETE	22.39	10.51	25.17	11.29	34.61	10.20	0.207	0.203
15-oxo-ETE	2.36	0.73	2.29	1.30	2.45	0.92	0.968	0.823
PGB2	1.13	0.52	0.88	0.12	0.86	0.12	0.360	0.783
PGD2	126.52	51.77	168.76	58.59	225.06	72.29	0.075	0.213
PGE2	21.11	11.29	27.49	11.72	37.90	11.07	0.102	0.187
PGF2a	37.83	15.98	43.38	16.94	48.32	14.67	0.593	0.635
6-keto-PGF1a	8.80	5.36	8.84	3.22	11.21	3.39	0.583	0.290
PGJ2	0.81	0.46	0.95	0.36	0.93	0.28	0.827	0.916
TXB2	18.17	8.65	18.18	6.52	20.63	5.50	0.818	0.539
5(6)-EpETrE	14.64	7.19	12.25	5.02	13.11	3.28	0.782	0.755
8(9)-EpETrE	9.53	8.04	5.02	1.41	7.34	0.60	0.352	0.010
11(12)-EpETrE	19.08	16.15	12.21	5.81	11.79	3.67	0.466	0.895
14(15)-EpETrE	59.70	70.08	29.52	15.19	22.38	12.08	0.360	0.434
11,12-DiHETrE	0.29	0.06	0.33	0.09	0.40	0.09	0.127	0.246
14,15-DiHETrE	0.35	0.07	0.32	0.06	0.46	0.08	0.015 ^a	0.012
9-HETE	5.31	2.92	3.90	2.56	4.52	2.24	0.696	0.693
11-HETE	22.88	10.31	27.35	11.54	36.98	9.60	0.138	0.189
Docosahexaenoic acid derived								
10(11)-EpDPE	1.96	0.45	1.64	0.43	1.92	0.45	0.467	0.336
16(17)-EpDPE	2.00	1.57	1.17	0.67	0.33	0.47	0.075	0.048
19(20)-EpDPE	3.31	2.86	2.88	0.82	3.40	1.42	0.897	0.498
Eicosapentaenoic acid derived								
15-HEPE	5.14	2.06	2.89	0.86	3.80	1.61	0.121	0.295

significantly lower in the WM group compared to the LFLSM group. A box and whisker plot of the oxylipins that are significantly changed by fasting or meal composition can be found in Fig. 1. sPLSDA plots for the comparison of the cerebral oxylipin levels in the meal consuming groups with and without the fasting group can be seen in Supplemental Figure 1. No distinct clustering was seen when the fasting group was included in the analysis; however, when the two meal consuming groups were compared distinct clusters were formed.

3.3. Cerebral fatty acid composition

Brain fatty acid composition of the right hemisphere of cerebrum was determined by gas-chromatography. The results of this analysis can be seen in Table 3. None of the fatty acids were significantly different between the fasted group and either meal consuming group as determined by one-way ANOVA or the two meal-consuming groups when analyzed by *t*-test. sPLSDA plots for the comparison of the cerebral fatty acid composition of the meal consuming groups with and without the fasting group can be seen in Supplemental Figure 2. As shown, no distinct clustering was observed between the groups, consistent with the lack of statistical significance by one-way ANOVA or *t*-test.

3.4. Gene expression of oxylipin generating enzymes

RNA was isolated from the left hemisphere of cerebrum and gene expression of oxylipin producing enzymes were determined by qRT-

PCR. The results of the gene expression analysis can be seen in Fig. 2. The LFLSM and WM groups were found to have significantly lower expression of the cytochrome P450, family 2, subfamily j, polypeptide 12 (CYP2J12) than the fasted group. The expression of cyclooxygenases (COX) 1 and 2, and epoxide hydrolase (EPHX)1 were significantly higher in the WM group when compared to the LFLSM group.

4. Discussion and conclusions

The main finding of this study is that cerebral oxylipin content is affected by acute fasting and a single high-fat/high-sucrose meal. We did not observe any differences in cerebral fatty acid composition between fasted mice or mice that had consumed either meal type. Although from our data it appears that brain fatty acid composition is not affected by the consumption of a single meal, perhaps there are small changes that this study is not powered to detect. The changes in some oxylipins appear to be linked to transcriptional changes in genes involved in oxylipin synthesis and degradation. A summary of the observed changes induced by fasting and meal composition can be found in Fig. 3.

The mice in the two meal groups consumed the same amount of food as measured by weight. However, due to the caloric density of the food, the mice in the WM group consumed significantly more calories during the meal period. This does add a potential variable that could be influencing the oxylipin changes we observed. Studies have demonstrated that dietary restriction is protective in animal models of

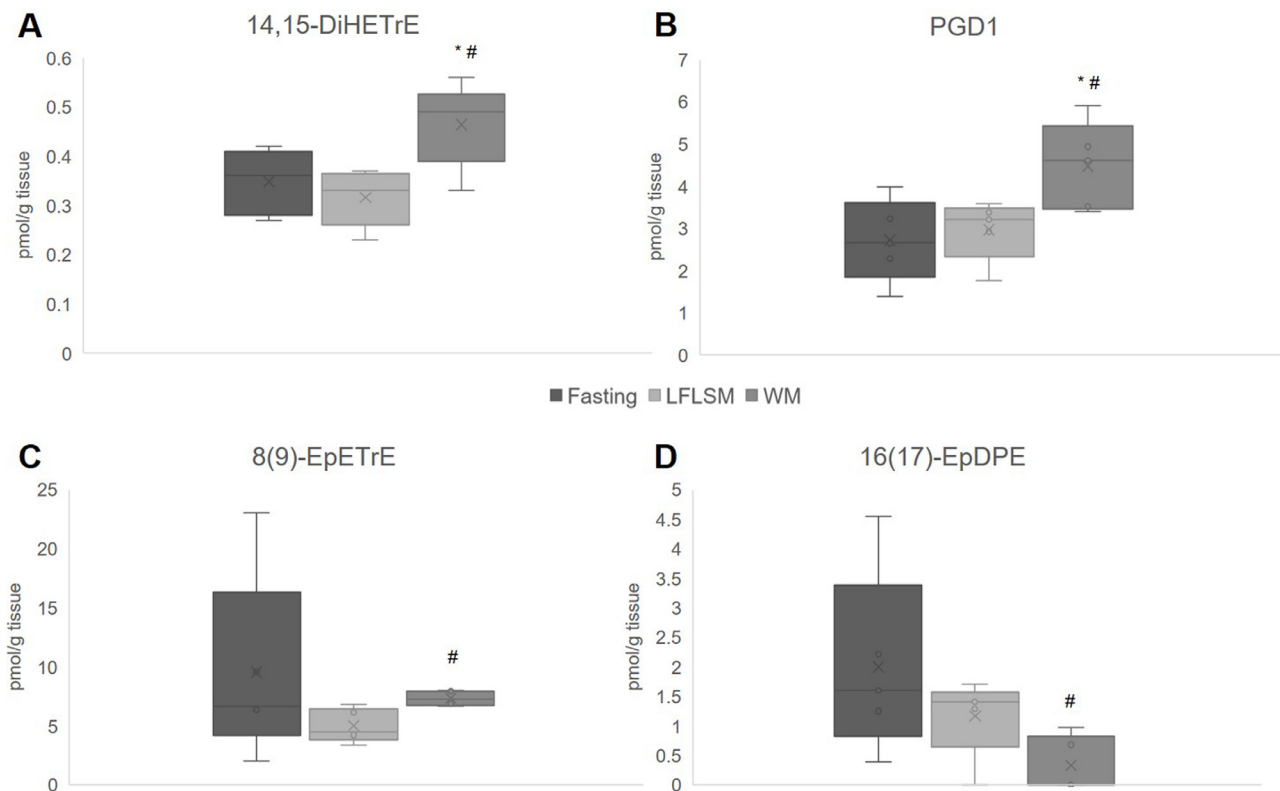


Fig. 1. Oxylipins modified by fasting or meal composition. Lipids were extracted from the right hemisphere of the cerebrum of fasting mice and mice after consuming a low-fat/low-sucrose meal (LFLSM) or Western meal (WM). Oxylipins were measured by UHPLC-MS/MS. Data shown in box and whisker plot is as follows: central line indicates median value, bottom of box indicates 25th percentile, top of box indicates 75th percentile, whiskers indicate lowest and highest values, and X indicates the mean. # $p < 0.05$ when compared to LFLSM group by *t*-test, * $p < 0.05$ when compared to Fasting group by ANOVA with Holm-Sidak post hoc (versus control) test.

neurodegeneration and can reduce neuroinflammation [2,24]. Therefore, in future studies it will be important to separate out the influence of caloric intake from meal composition.

We saw a higher level of PGD1 in the brains of mice that consumed a WM compared to those that consumed the LFLSM, as well as when compared to mice in the fasted state. PGD1, a series 1 prostaglandin, is formed by the action of COX 1 and 2 on dihomo- γ -linolenic acid (DGLA) [25]. Our data indicated that both COX1 and COX2 gene expression

were higher in mice which consumed the WM when compared to the LFLSM group, however no statistically significant differences were found when the fasting group was added to the analysis. Interestingly, none of the other products of COX metabolism were found to be elevated in the WM group. PGD1 has been shown to reduce vascular permeability induced by other prostaglandins [26] and have anti-inflammatory effects in atopic dermatitis [27]. Further, PGD1 has been shown to be generated by murine macrophages when incubated with

Table 3
Brain fatty acid percent composition (% of total detected fatty acids).

Fatty Acid	Fasting		Low-fat/low-sucrose meal (LFLSM)		Western meal (WM)		p value	
	Mean (%)	Standard Deviation	Mean (%)	Standard Deviation	Mean (%)	Standard Deviation	ANOVA	t-test LFLSM v WM
C13:0	3.9315	1.1938	3.9344	2.2779	3.5226	1.9170	0.922	0.765
C14:0	0.1720	0.0287	0.1637	0.0203	0.2632	0.1818	0.300	0.259
C14:1	0.2116	0.0673	0.2392	0.0573	0.2343	0.0366	0.708	0.876
C16:0	20.3504	6.8490	22.1384	0.3312	22.3584	1.0566	0.693	0.669
C16:1	0.7397	0.1507	0.6061	0.0983	0.8584	0.3123	0.201	0.123
C18:0	24.1695	4.6023	22.7945	1.9044	23.2786	2.3445	0.790	0.729
C18:1 cis	17.2129	3.0712	17.4695	1.3809	16.3767	0.2612	0.661	0.120
C18:1 trans	3.3693	0.3613	3.3862	0.2634	3.3037	0.2934	0.906	0.652
C18:2 n-6	0.5615	0.2565	0.6763	0.0923	0.6123	0.0366	0.537	0.187
C20:0	0.3311	0.0787	0.2766	0.1917	0.3235	0.0633	0.795	0.649
C20:1 n-9	1.9714	0.6006	2.1443	0.7324	1.8681	0.2672	0.745	0.451
C20:2 n-6	0.1186	0.0686	0.1203	0.0766	0.1512	0.0226	0.666	0.446
C20:3	0.6894	0.0963	0.6895	0.0831	0.6628	0.0403	0.821	0.536
C20:4 n-6	9.9940	1.9835	9.5567	0.8423	9.4554	1.0161	0.808	0.868
C20:5 n-3	0.0700	0.0456	0.0400	0.0365	0.0715	0.1258	0.685	0.477
C22:0	0.1289	0.1257	0.1361	0.1387	0.1126	0.1078	0.941	0.740
C22:2	0.5648	0.2260	0.6939	0.1763	0.7164	0.1779	0.439	0.846
C22:5n-6	0.1723	0.0282	0.0970	0.0915	0.1840	0.0347	0.0795	0.0786
C22:5 n-3	0.2857	0.0472	0.3127	0.0793	0.2453	0.0620	0.285	0.173
C22:6	14.9554	2.1701	14.5247	3.7033	15.4011	1.6795	0.875	0.643

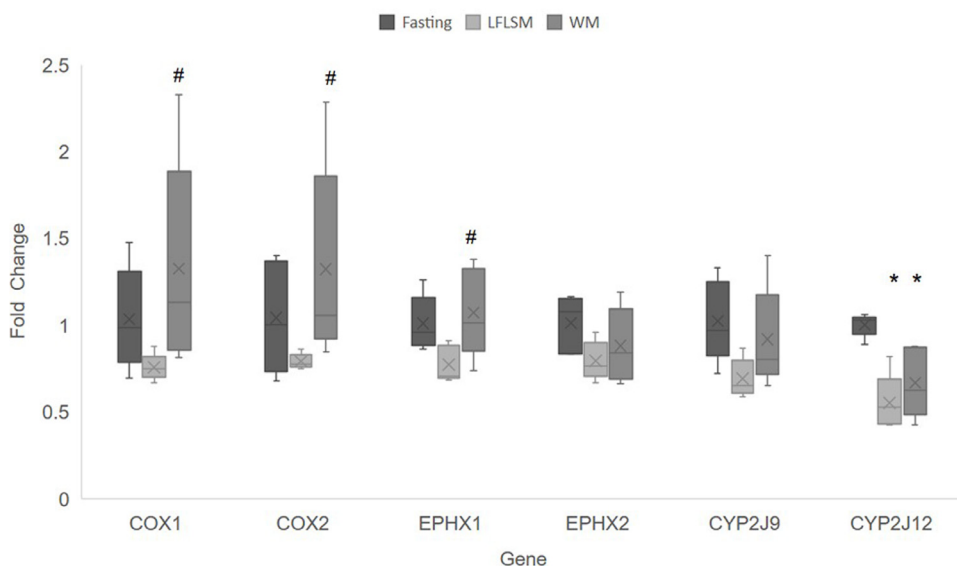


Fig. 2. Gene expression of oxylipin producing enzymes. RNA was isolated from the left hemisphere of the cerebrum of fasting mice and mice after consuming a low-fat/low-sucrose meal (LFLSM) or Western meal (WM). Gene expression was then measured by qRT-PCR. Data shown in box and whisker plot is as follows: central line indicates median value, bottom of box indicates 25th percentile, top of box indicates 75th percentile, whiskers indicate lowest and highest values, and X indicates the mean. #*p* < 0.05 when compared to LFLSM group by *t*-test, **p* < 0.05 when compared to Fasting group by ANOVA with Holm-Sidak post hoc (versus control) test.

DGLA and stimulated with lipopolysaccharide [25]. We were unable to find much information on PGD1 in the literature, particularly its role in the brain. However, at least in relation to atopic dermatitis, it appears that it may be an anti-inflammatory mediator which is induced by inflammatory stimuli as a potential feedback mechanism [25,26,27].

The cytochrome p450 epoxygenases can epoxidize any of the four double bonds of arachidonic acid (AA) producing one of the following: 5,6-EpETrE, 8,9-EpETrE, 11,12-EpETrE, or 14,15-EpETrE [28]. The action of epoxide hydrolases can then convert these products to 5,6-DiHETrE, 8,9-DiHETrE, 11,12-DiHETrE, and 14,15-DiHETrE, respectively [29]. The EpETrEs are generally considered to be beneficial, having have vasodilatory and anti-inflammatory effects which are lost upon conversion to DiHETrEs [30]. Others have demonstrated an increase in DiHETrEs and other diol products of epoxide hydrolases in the serum and brains of individuals with vascular cognitive impairment compared with controls [31,32]. Our data indicated that epoxide 8,9-EpETrE and diol 14,15-DiHETrE levels were higher in the WM group compared to the LFLSM group and fasting group. This suggests possible elevations in cytochrome P450 and soluble epoxide hydrolase metabolic pathways in mice on the WM diet, which is why we decided to look at their gene expression of the enzymes.

There are many cytochrome p450 enzymes, only some of which have described functions and expression patterns reported in the

literature. The CYP2J family of enzymes have been reported to have AA epoxygenase activity, forming EpETrEs from AA [20]. CYP2J9 and CYP2J12 have been found to be expressed in brain tissue [20,33,34]. We examined the expression of these two enzymes. We saw no significant difference in the expression of these two enzymes between the two meal consuming groups. However, CYP2J12 expression is lower in both meal consuming groups when compared to the fasted group. It seems that the expression of these cytochrome p450 enzymes do not account for the differences in 8,9-EpETrE and 14,15-DiHETrE between the LFLSM and WM groups.

Soluble epoxide hydrolase (sEH, encoded by the gene EPHX2) is generally considered the relevant epoxide hydrolase for EpETrE conversion to DiHETrEs, while microsomal epoxide hydrolase (mEH, encoded by the gene EPHX1) is considered to be the hydrolase responsible for detoxifying xenobiotics [35]. sEH has been studied in the context of neurodegenerative diseases and recovery after cerebral ischemia, with deletion or inhibition of the enzyme providing neuroprotective and anti-inflammatory effects [3,31,36,37,38,39,40,41,42,43]. Marowsky and colleagues demonstrated that both sEH and mEH are expressed in brain, although with different distributions, and are capable of catalyzing the conversion of EpETrEs to DiHETrEs [29]. Further, mEH has been shown to be upregulated in the hippocampus and cortex of Alzheimer's disease patients [44], indicating that there may also be a role

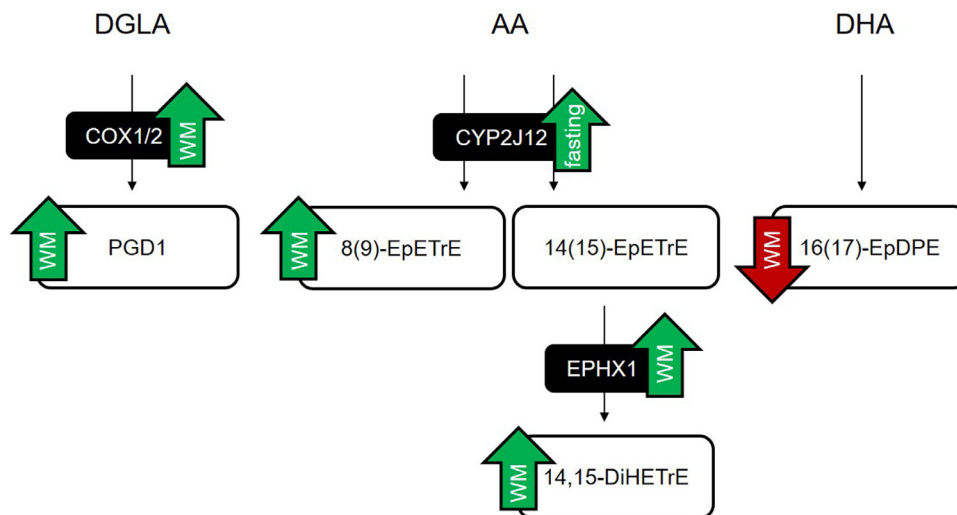


Fig. 3. Summary of observed changes induced by fasting and composition of a single meal. Black arrows connect parent fatty acids to oxylipins (solid outline), with the genes of enzymes (solid black box) capable of facilitating the production of these oxylipins shown over the arrows. Green arrows pointing up indicate a higher amount of the adjacent gene/oxylipin in the indicated group when compared to the low-fat/low-sucrose meal (LFLSM) group. Red arrows pointing down indicate a lower amount of the adjacent gene/oxylipin in the indicated group when compared to the LFLSM group. WM, Western meal. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

for mEH in neurodegeneration and cognitive decline. We did not see any difference in the expression of either epoxide hydrolase between the fasted and either meal consuming groups; however, we did see an increase in expression of EPHX1 in the WM mice when compared to the LFLSM group. While we cannot conclude that mEH is responsible for the elevated 14,15-DiHETrE levels, this study provides the basis for further investigation into the role it plays in diet induced oxylipin changes in brain.

The docosahexaenoic acid (DHA) derived oxylipin 16,17 EpDPE was lower in the WM group than in the LFLSM group. This oxylipin appears to have a vasodilatory effect through activation of BK channels [45,46]. Reduced cerebral blood flow has been found to be associated with cognitive impairment in Alzheimer's disease [47]. Therefore, a reduction of this metabolite could be detrimental to cognitive function. It is possible that the reduction in this metabolite was due to increased metabolism by one of the epoxide hydrolases into DHA-diols, which were not measured in this study.

We realize that there are a few limitations to this study. One limitation is that the brains were not subjected to high-energy microwave fixation, a process known to prevent the effects of postmortem ischemia on brain oxylipins [48]. While use of the high-energy microwave would have been ideal, we did not have access to one at the time of tissue collection. Although this means that some of the oxylipin concentrations in our samples were likely altered from the levels that existed in the brains of the mice prior to post-mortem ischemia, we took care to ensure that the tissue collection was conducted in a standardized manner for all mice. We therefore believe that the differences in the oxylipin levels between the two groups are a result of the meals consumed. Another limitation of this study is that the mice were kept on the vivarium provided bedding material, which is corn based. It is possible that the mice consumed some of this, perhaps contributing to some of the variability seen in the data. Future studies should control for this by removing any edible bedding materials. Further, during the meal training, mice were provided with the LFLSD, thus on the day of the test meal mice in the WM group received a novel diet. It is possible that the changes in oxylipin levels we observed were due to dietary change rather than the WD itself. In future studies, it would be important to test this by meal training mice on both diets and using a crossover design to assess meal composition and novelty simultaneously. This would allow analysis of which of these factors is leading to changes in oxylipin levels.

In conclusion, while it has previously been shown that the oxylipin content of the brain can be modified by changes in the composition of a chronically consumed diet [12–17], in this study we demonstrate that a single meal exposure is sufficient to produce differences in oxylipin content. We also demonstrate that fasting alters brain oxylipin content. We did not see any evidence of a significant shift in fatty acid composition of the brain, which led us to look further at the gene expression of the enzymes which generate oxylipins from their precursor fatty acids. It appears that there is a change in the expression of some of these enzymes. It is possible that there are differences in the activity and substrate preference of these enzymes, which was not addressed by this study. Caveats to be noted and taken into consideration are that although there was no significant difference in the amount of food consumed, the higher caloric density of the WD led to a higher caloric intake during the meal period in the WM group mice and these mice received a novel diet for the test meal. While we can conclude that a single meal has the potential to impact brain oxylipin levels, it remains to be elucidated if this is a function of meal composition, quantity of caloric intake, exposure to a novel diet, or some interaction of these factors.

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CRediT authorship contribution statement

J.E. Norman: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Project administration. **H.H. Aung:** Investigation, Writing - review & editing. **Y. Otoki:** Investigation, Formal analysis. **Z. Zhang:** Investigation, Formal analysis. **A.Y. Taha:** Methodology, Formal analysis, Resources, Supervision, Writing - review & editing. **J.C. Rutledge:** Conceptualization, Supervision, Writing - review & editing, Funding acquisition.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.plefa.2020.102062](https://doi.org/10.1016/j.plefa.2020.102062).

References

- [1] A.M. Abbatecola, M. Russo, M. Barbieri, Dietary patterns and cognition in older persons. *Curr. Opin. Clin. Nutr. Metab. Care* 21 (2018) 10–13, <https://doi.org/10.1097/MCO.0000000000000434>.
- [2] T.A. Prolla, M.P. Mattson, Molecular mechanisms of brain aging and neurodegenerative disorders: lessons from dietary restriction, (2001) 11.
- [3] W. Swardfager, M. Hennebelle, D. Yu, B.D. Hammock, A.J. Levitt, K. Hashimoto, A.Y. Taha, Metabolic/inflammatory/vascular comorbidity in psychiatric disorders; soluble epoxide hydrolase (sEH) as a possible new target, *Neurosci. Behav. Rev.* 87 (2018) 56–66, <https://doi.org/10.1016/j.neubiorev.2018.01.010>.
- [4] G.Y. Sun, A. Simonyi, K.L. Fritsche, D.Y. Chuang, M. Hannink, Z. Gu, C.M. Greenleaf, J.K. Yao, J.C. Lee, D.Q. Beversdorf, Docosahexaenoic acid (DHA): An essential nutrient and a nutraceutical for brain health and diseases, *Prostaglandins Leukot. Essent. Fatty Acids* 136 (2017) 3–13, <https://doi.org/10.1016/j.plefa.2017.03.006>.
- [5] J.G. Devassy, S. Leng, M. Gabbs, M. Monirujjaman, H.M. Aukema, Omega-3 polyunsaturated fatty acids and oxylipins in neuroinflammation and management of alzheimer disease, *Adv. Nutr* 7 (2016) 905–916, <https://doi.org/10.3945/an.116.012187>.
- [6] N. Iwamoto, K. Kobayashi, K. Kosaka, The formation of prostaglandins in the postmortem cerebral cortex of Alzheimer-type dementia patients, *J. Neurol* 236 (1989) 80–84, <https://doi.org/10.1007/bf00314401>.
- [7] X. Wang, M. Zhu, E. Hjorth, V. Cortés-Toro, H. Eyjolfssdottir, C. Graff, I. Nennesmo, J. Palmblad, M. Eriksdotter, K. Sambamurti, J.M. Fitzgerald, C.N. Serhan, A.-C. Granholm, M. Schultzberg, Resolution of inflammation is altered in Alzheimer's disease, *Alzheimers Dement, J. Alzheimers Assoc* 11 (2015) 40–50, <https://doi.org/10.1016/j.jalz.2013.12.024>.
- [8] W.J. Lukiw, J.-G. Cui, V.L. Marcheselli, M. Bodker, A. Botkjaer, K. Gotlinger, C.N. Serhan, N.G. Bazan, A role for docosahexaenoic acid-derived neuroprotectin D1 in neural cell survival and Alzheimer disease, *J. Clin. Invest.* 115 (2005) 2774–2783, <https://doi.org/10.1172/JCI25420>.
- [9] M. Zhu, X. Wang, E. Hjorth, R.A. Colas, L. Schroeder, A.-C. Granholm, C.N. Serhan, M. Schultzberg, Pro-resolving lipid mediators improve neuronal survival and increase Aβ42 phagocytosis, *Mol. Neurobiol.* 53 (2016) 2733–2749, <https://doi.org/10.1007/s12035-015-9544-0>.
- [10] M. Wang, J.E. Norman, V.J. Srinivasan, J.C. Rutledge, Metabolic, inflammatory, and microvascular determinants of white matter disease and cognitive decline, *Am. J. Neurodegener. Dis.* 5 (2016) 171–177.
- [11] H. Francis, R. Stevenson, The longer-term impacts of Western diet on human cognition and the brain, *Appetite* 63 (2013) 119–128, <https://doi.org/10.1016/j.appet.2012.12.018>.

- [12] J.M. Rutkowski, L.L. Lee, M. Puchowicz, M.S. Golub, D.E. Befroy, D.W. Wilson, S. Anderson, G. Cline, J. Bini, K. Borkowski, T.A. Knotts, J.C. Rutledge, on behalf of the M.M.P.C.I.W. Group, Reduced cognitive function, increased blood-brain-barrier transport and inflammatory responses, and altered brain metabolites in LDLR^{-/-} and C57BL/6 mice fed a western diet, *PLoS One* 13 (2018) e0191909, <https://doi.org/10.1371/journal.pone.0191909>.
- [13] A.I. Ostermann, M. Reutzel, N. Hartung, N. Franke, L. Kutzner, K. Schoenfeld, K.-H. Weylandt, G.P. Eckert, N.H. Schebb, A diet rich in omega-3 fatty acids enhances expression of soluble epoxide hydrolase in murine brain, *Prostaglandins Other Lipid Mediat* 133 (2017) 79–87, <https://doi.org/10.1016/j.prostaglandins.2017.06.001>.
- [14] C. Rey, J.C. Delpech, C. Madore, A. Nadjar, A.D. Greenhalgh, C. Amadiou, A. Aubert, V. Pallet, C. Vaysse, S. Layé, C. Joffre, Dietary n-3 long chain PUFA supplementation promotes a pro-resolving oxylipin profile in the brain, *Brain Behav. Immun.* 76 (2019) 17–27, <https://doi.org/10.1016/j.bbi.2018.07.025>.
- [15] A.I. Ostermann, P. Waindok, M.J. Schmidt, C.-Y. Chiu, C. Smyl, N. Rohwer, K.-H. Weylandt, N.H. Schebb, Modulation of the endogenous omega-3 fatty acid and oxylipin profile in vivo—a comparison of the fat-1 transgenic mouse with C57BL/6 wildtype mice on an omega-3 fatty acid enriched diet, *PLoS One* 12 (2017) e0184470, <https://doi.org/10.1371/journal.pone.0184470>.
- [16] A.Y. Taha, M. Hennebelle, J. Yang, D. Zamora, S.I. Rapoport, B.D. Hammock, C.E. Ramsden, Regulation of rat plasma and cerebral cortex oxylipin concentrations with increasing levels of dietary linoleic acid, *Prostaglandins Leukot. Essent. Fatty Acids* 138 (2018) 71–80, <https://doi.org/10.1016/j.plefa.2016.05.004>.
- [17] C.E. Ramsden, M. Hennebelle, S. Schuster, G.S. Keyes, C.D. Johnson, I.A. Kirpich, J.E. Dahlen, M.S. Horowitz, D. Zamora, A.E. Feldstein, C.J. McClain, B.S. Muhlhauser, M. Makrides, R.A. Gibson, A.Y. Taha, Effects of diets enriched in linoleic acid and its peroxidation products on brain fatty acids, oxylipins, and aldehydes in mice, *BBA - Mol. Cell Biol. Lipids*. 1863 (2018) 1206–1213, <https://doi.org/10.1016/j.bbalip.2018.07.007>.
- [18] D.A. Hyson, T.G. Paglieroni, T. Wun, J.C. Rutledge, Postprandial lipemia is associated with platelet and monocyte activation and increased monocyte cytokine expression in normolipemic men, *Clin. Appl. Thromb.* 8 (2002) 147–155, <https://doi.org/10.1177/107602960200800211>.
- [19] X. Wang, A. Spandios, H. Wang, B. Seed, PrimerBank: a PCR primer database for quantitative gene expression analysis, 2012 update, *Nucleic Acids Res* 40 (2012) D1144–D1149, <https://doi.org/10.1093/nar/gkr1013>.
- [20] J.P. Graves, A. Gruzdev, J.A. Bradbury, L.M. DeGraff, H. Li, J.S. House, S.L. Hoopes, M.L. Edin, D.C. Zeldin, Quantitative polymerase chain reaction analysis of the mouse Cyp2j subfamily: tissue distribution and regulation, *Drug Metab. Dispos* 43 (2015) 1169–1180.
- [21] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻ΔΔCT Method, *Methods* 25 (2001) 402–408, <https://doi.org/10.1006/meth.2001.1262>.
- [22] J. Chong, J. Xia, MetaboAnalystR: an R package for flexible and reproducible analysis of metabolomics data, *Bioinformatics* 34 (2018) 4313–4314, <https://doi.org/10.1093/bioinformatics/bty528>.
- [23] J. Chong, O. Soufan, C. Li, I. Caraus, S. Li, G. Bourque, D.S. Wishart, J. Xia, MetaboAnalyst 4, towards more transparent and integrative metabolomics analysis, *Nucleic Acids Res* 46 (2018) W486–W494, <https://doi.org/10.1093/nar/gky310>.
- [24] E. Bok, M. Jo, S. Lee, B.-R. Lee, J. Kim, H.-J. Kim, Dietary restriction and neuroinflammation: a potential mechanistic link, *Int. J. Mol. Sci.* 20 (2019) 464, <https://doi.org/10.3390/ijms20030464>.
- [25] S. Kakutani, H. Kawashima, T. Tanaka, A. Shiraishi-Tateishi, Y. Kiso, Uptake of dihomogamma-linolenic acid by murine macrophages increases series-1 prostaglandin release following lipopolysaccharide treatment, *Prostaglandins Leukot. Essent. Fatty Acids*. 83 (2010) 23–29, <https://doi.org/10.1016/j.plefa.2010.02.032>.
- [26] PROCEEDINGS OF THE British Pharmacological Society, *Br. J. Pharmacol* 55 (1975), <https://doi.org/10.1111/j.1476-5381.1975.tb07634.x> 239P-317P.
- [27] Y. Amagai, K. Oida, A. Matsuda, K. Jung, S. Kakutani, T. Tanaka, K. Matsuda, H. Jang, G. Ahn, Y. Xia, H. Kawashima, H. Shibata, H. Matsuda, A. Tanaka, Dihomogamma-linolenic acid prevents the development of atopic dermatitis through prostaglandin D1 production in NC/Tnd mice, *J. Dermatol. Sci.* 79 (2015) 30–37, <https://doi.org/10.1016/j.jdermsci.2015.03.010>.
- [28] A.A. Spector, H.-Y. Kim, Cytochrome P450 epoxygenase pathway of polyunsaturated fatty acid metabolism, *Biochim Biophys Acta*. 1851 (2015) 356–365, <https://doi.org/10.1016/j.bbalip.2014.07.020>.
- [29] A. Marowsky, J. Burgener, J.R. Falck, J.-M. Fritschy, M. Arand, Distribution of soluble and microsomal epoxide hydrolase in the mouse brain and its contribution to cerebral epoxyeicosatrienoic acid metabolism, *Neuroscience* 163 (2009) 646–661, <https://doi.org/10.1016/j.neuroscience.2009.06.033>.
- [30] A.A. Spector, Arachidonic acid cytochrome P450 epoxygenase pathway, *J. Lipid Res* 50 (2009) S52–S56, <https://doi.org/10.1194/jlr.R800038-JLR200>.
- [31] J.W. Nelson, J.N. Young, R. Borkar, R.L. Woltjer, J.F. Quinn, L.C. Silbert, M.R. Grafe, N.J. Alkayed, Role of soluble epoxide hydrolase in age-related vascular cognitive decline, *Prostaglandins Other Lipid Mediat* 0 (2014) 30–37, <https://doi.org/10.1016/j.prostaglandins.2014.09.003>.
- [32] D. Yu, M. Hennebelle, D.J. Sahlas, J. Ramirez, F. Gao, M. Masellis, H. Cogo-Moreira, R.H. Swartz, N. Herrmann, P.C. Chan, J.A. Pettersen, D.T. Stuss, S.E. Black, A.Y. Taha, W. Swardfager, Soluble epoxide hydrolase-derived linoleic acid oxylipins in serum are associated with periventricular white matter hyperintensities and vascular cognitive impairment, *Transl. Stroke Res* (2018), <https://doi.org/10.1007/s12975-018-0672-5>.
- [33] H.J. Renaud, J.Y. Cui, M. Khan, C.D. Klaassen, Tissue Distribution and gender-divergent expression of 78 Cytochrome P450 mRNAs in Mice, *Toxicol. Sci.* 124 (2011) 261–277, <https://doi.org/10.1093/toxsci/krf240>.
- [34] C.M. Davis, X. Liu, N.J. Alkayed, Cytochrome P450 eicosanoids in cerebrovascular function and disease, *Pharmacol. Ther.* 179 (2017) 31–46, <https://doi.org/10.1016/j.pharmthera.2017.05.004>.
- [35] R. Vaclavikova, D.J. Hughes, P. Soucek, Microsomal epoxide hydrolase 1 (EPHX1): gene, structure, function, and role in human disease, *Gene* 571 (2015) 1–8, <https://doi.org/10.1016/j.gene.2015.07.071>.
- [36] M. Hennebelle, Y. Otoki, J. Yang, B.D. Hammock, A. J. Levitt, A.Y. Taha, W. Swardfager, Altered soluble epoxide hydrolase-derived oxylipins in patients with seasonal major depression: an exploratory study, *Psychiatry Res.* 252 (n.d.) 94–101.
- [37] C.-H. Wu, S.-K. Shyue, T.-H. Hung, S. Wen, C.-C. Lin, C.-F. Chang, S.-F. Chen, Genetic deletion or pharmacological inhibition of soluble epoxide hydrolase reduces brain damage and attenuates neuroinflammation after intracerebral hemorrhage, *J. Neuroinflammation* 14 (2017) 230, <https://doi.org/10.1186/s12974-017-1005-4>.
- [38] K. Hashimoto, Role of soluble epoxide hydrolase in metabolism of PUFAs in psychiatric and neurological disorders, *Front. Pharmacol.* (2019) 10, <https://doi.org/10.3389/fphar.2019.00036>.
- [39] X. Qin, Q. Wu, L. Lin, A. Sun, S. Liu, X. Li, X. Cao, T. Gao, P. Luo, X. Zhu, X. Wang, Soluble epoxide hydrolase deficiency or inhibition attenuates MPTP-induced Parkinsonism, *Mol. Neurobiol.* 52 (2015) 187–195, <https://doi.org/10.1007/s12035-014-8833-3>.
- [40] H.-J. Huang, Y.-T. Wang, H.-C. Lin, Y.-H. Lee, A.M.-Y. Lin, Soluble epoxide hydrolase inhibition attenuates MPTP-induced neurotoxicity in the nigrostriatal dopaminergic system: involvement of α-synuclein aggregation and ER stress, *Mol. Neurobiol.* 55 (2018) 138–144, <https://doi.org/10.1007/s12035-017-0726-9>.
- [41] Y. Chen, H. Tian, E. Yao, Y. Tian, H. Zhang, L. Xu, Z. Yu, Y. Fang, W. Wang, P. Du, M. Xie, Soluble epoxide hydrolase inhibition Promotes White Matter Integrity and Long-Term Functional Recovery after chronic hypoperfusion in mice, *Sci. Rep.* 7 (2017) 7758, <https://doi.org/10.1038/s41598-017-08227-z>.
- [42] J.J. Iliff, N.J. Alkayed, Soluble epoxide hydrolase inhibition: targeting multiple mechanisms of ischemic brain injury with a single agent, *Future Neurol* 4 (2009) 179–199.
- [43] Q. Ren, M. Ma, J. Yang, R. Nonaka, A. Yamaguchi, K. Ishikawa, K. Kobayashi, S. Murayama, S.H. Hwang, S. Saiki, W. Akamatsu, N. Hattori, B.D. Hammock, K. Hashimoto, Soluble epoxide hydrolase plays a key role in the pathogenesis of Parkinson's disease, *Proc. Natl. Acad. Sci.* 115 (2018) E5815–E5823, <https://doi.org/10.1073/pnas.1802179115>.
- [44] M. Liu, A. Sun, E.-J. Shin, X. Liu, S.-G. Kim, C.R. Runyons, W. Markesbery, H.-C. Kim, G. Bing, Expression of microsomal epoxide hydrolase is elevated in Alzheimer's hippocampus and induced by exogenous β-amyloid and trimethyl-tin, *Eur. J. Neurosci.* 23 (2006) 2027–2034, <https://doi.org/10.1111/j.1460-9568.2006.04724.x>.
- [45] K. Sato, D. Chino, N. Nishioka, K. Kanai, M. Aoki, K. Obara, S. Miyauchi, Y. Tanaka, Pharmacological evidence showing significant roles for potassium channels and CYP epoxygenase metabolites in the relaxant effects of docosahexaenoic acid on the rat aorta contracted with U46619, *Biol. Pharm. Bull.* 37 (2014) 394–403, <https://doi.org/10.1248/bpb.b13-00746>.
- [46] R. Wang, Q. Chai, T. Lu, H.-C. Lee, Activation of vascular BK channels by docosahexaenoic acid is dependent on cytochrome P450 epoxygenase activity, *Cardiovasc. Res.* 90 (2011) 344–352, <https://doi.org/10.1093/cvr/cvq411>.
- [47] A.E. Leeuwis, M.R. Benedictus, J.P.A. Kuijter, M.A.A. Binnewijzend, A.M. Hooghiemstra, S.C.J. Verfaillie, T. Koene, P. Scheltens, F. Barkhof, N.D. Prins, W.M. van der Flier, Lower cerebral blood flow is associated with impairment in multiple cognitive domains in Alzheimer's disease, *Alzheimers Dement* 13 (2017) 531–540, <https://doi.org/10.1016/j.jalz.2016.08.013>.
- [48] M. Hennebelle, A.H. Metherel, A.P. Kitson, Y. Otoki, J. Yang, K.S.S. Lee, B.D. Hammock, R.P. Bazinet, A.Y. Taha, Brain oxylipin concentrations following hypercapnia/ischemia: effects of brain dissection and dissection time, *J. Lipid Res.* (n.d.) 10.1194/jlr.D084228.

Supplemental Data

Supplemental Table 1. Calculated fatty acid content of LFLSD (Envigo TD.08485)

Fatty acid	g/kg diet
Saturated fat	25.4
Monounsaturated fat	13.7
Polyunsaturated fat	9.3
4:0	0.7
6:0	0.6
8:0	0.4
10:0	0.9
12:0	1.1
14:0	4.1
16:0	12.2
16:1	0.6
18:0	5.3
18:1 (oleic)	13.0
18:2 (linoleic)	8.3
18:3 (linolenic)	1.2

Supplemental Table 2. Typical fatty acid profile of WD (Envigo TD.88137)

Fatty acid	% of total fatty acids
Saturated fat	61.8
Monounsaturated fat	27.3
Polyunsaturated fat	4.7
4:0	2.1
6:0	1.5
8:0	1.1
10:0	2.6
12:0	3.3
14:0	10.6
16:0	28.9
16:1	1.5
18:0	12.5
18:1 (oleic)	20.9
18:1 isomers	4.0
18:2 (linoleic)	2.3
18:2 isomers	1.3
18:3 (linolenic)	0.7

Supplemental Table 3. Measured oxylipins and abbreviations.

Oxylipins listed in grey were excluded from analyses due to detection in less than 3 samples per group.

Oxylipin	Abbreviation
10(11)-epoxydocosapentaenoic acid	10(11)-EpDPE
11(12)-epoxyeicosateteaenoic acid	11(12)-EpETE
11(12)-epoxyeicosatrienoic acid	11(12)-EpETrE
12(13)-epoxyoctadecamonoenoic acid	12(13)-EpOME
13(14)-epoxydocosapentaenoic acid	13(14)-EpDPE
14(15)-epoxyeicosateteaenoic acid	14(15)-EpETE
14(15)-epoxyeicosatrienoic acid	14(15)-EpETrE
16(17)-epoxydocosapentaenoic acid	16(17)-EpDPE
17(18)-epoxyeicosateteaenoic acid	17(18)-EpETE
19(20)-epoxydocosapentaenoic acid	19(20)-EpDPE
5(6)-epoxyeicosatrienoic acid	5(6)-EpETrE
7(8)-epoxydocosapentaenoic acid	7(8)-EpDPE
8(9)-epoxyeicosateteaenoic acid	8(9)-EpETE
8(9)-epoxyeicosatrienoic acid	8(9)-EpETrE
9(10)-epoxyoctadecamonoenoic acid	9(10)-EpOME
11,12-dihydroxyeicosatrienoic acid	11,12-DiHETrE
12,13-dihydroxyoctadecamonoenoic acid	12,13-DiHOME
14,15-dihydroxyeicosatetraenoic acid	14,15-DiHETE
14,15-dihydroxyeicosatrienoic acid	14,15-DiHETrE
17,18-dihydroxyeicosatetraenoic acid	17,18-DiHETE
5,15-dihydroxyeicosatetraenoic acid	5,15-DiHETE
5,6-dihydroxyeicosatetraenoic acid	5,6-DiHETE
5,6-dihydroxyeicosatrienoic acid	5,6-DiHETrE
8,15-dihydroxyeicosatetraenoic acid	8,15-DiHETE
8,9-dihydroxyeicosatrienoic acid	8,9-DiHETrE
9,10-dihydroxyoctadecamonoenoic acid	9,10-DiHOME
6-keto-prostaglandin F1- α	6-keto-PGF1a
13- hydroxyoctadecatrienoic acid acid	13-HOTrE
13-hydroxyoctadecadienoic acid	13-HODE
17- hydroxydocosahexaenoic acid	17-HDoHE
9- hydroxyoctadecatrienoic acid acid	9-HOTrE
9-hydroxyoctadecadienoic acid	9-HODE
20-COOH- Leukotriene B4	20-COOH-LTB4
20-OH-Leukotriene B4	20-OH-LTB4
6-trans-leukotriene B4	6-trans-LTB4
Leukotriene B3	LTB3
Leukotriene B4	LTB4
Leukotriene C4	LTC4
Leukotriene D4	LTD4
Leukotriene E4	LTE4
Lipoxin A4	LXA4
15-deoxy-Prostaglandin J2	15-deoxy-PGJ2
9,10,13-trihydroxyoctadecamonoenoic acid	9,10,13-TriHOME
9,12,13-trihydroxyoctadecamonoenoic acid	9,12,13-TriHOME
Prostaglandin B2	PGB2

Prostaglandin D1	PGD1
Prostaglandin D2	PGD2
Prostaglandin D3	PGD3
Prostaglandin E1	PGE1
Prostaglandin E2	PGE2
Prostaglandin E3	PGE3
Prostaglandin F2- α	PGF2a
Prostaglandin J2	PGJ2
Resolvin E1	Resolvin E1
Tromboxane B2	TXB2
13-oxo-octadecadienoic acid	13-oxo-ODE
20-hydroxyeicosatetraenoic acid	20-HETE
9-oxo-octadecadienoic acid	9-oxo-ODE
11-hydroxyeicosatetraenoic acid	11-HETE
12-hydroxyeicosapentaenoic acid	12-HEPE
12-hydroxyeicosatetraenoic acid	12-HETE
12-oxo-eicosatetraenoic acid	12-oxo-ETE
15-hydroxyeicosapentaenoic acid	15-HEPE
15-hydroxyeicosatetraenoic acid	15-HETE
15-oxo-eicosatetraenoic acid	15-oxo-ETE
15(S)-hydroxyeicosatrienoic acid	15(S)-HETrE
5-hydroxyeicosapentaenoic acid	5-HEPE
5-hydroxyeicosatetraenoic acid	5-HETE
5-oxo-eicosatetraenoic acid	5-oxo-ETE
8-hydroxyeicosapentaenoic acid	8-HEPE
8-hydroxyeicosatetraenoic acid	8-HETE
9-hydroxyeicosatetraenoic acid	9-HETE

Supplemental table 4. Gradient condition of LC-MS/MS analysis for oxylipin separation

min	B conc (%)	flow (min/mL)
0	35	0.3
3	40	0.25
4	48	
10	60	
20	70	
24	85	
24.5	85	0.25
24.6	100	0.35
26	100	
26.1	35	0.35
27.3	35	0.3
28	stop	

Supplemental Table 5. MRM pairs, optimized parameters and internal standards.

Compound name	Precursor Ion (m/z)	Product Ion (m/z)	Frag (V)	CE (V)	Cell Acc (V)	Rt (min)	Internal standard
20-COOH-LTB4	365.2	347.2	120	7	4	4.0	d4-LTB4
Resolvin E1	349.3	195	115	10	4	4.6	d4-PGE2
d4-6-keto-PGF1a	373.3	167.1	90	19	4	4.7	-
6-keto-PGF1a	369.3	163.2	90	22	4	4.7	d4-6-keto-PGF1a
20-OH-LTB4	351.2	195.2	95	13	4	5.0	d4-LTB4
d4-TXB2	373.3	173.2	105	10	4	6.1	-
TXB2	369.2	169.1	80	10	4	6.1	d4-TXB2
PGE3	349.3	269.2	120	7	4	6.2	d4-PGE2
PGD3	349.3	269.2	120	7	4	6.5	d4-PGE2
9,12,13-TriHOME	329.2	211.1	125	16	4	6.6	d4-PGE2
9,10,13-TriHOME	329.2	171.1	110	16	4	6.7	d4-PGE2
PGF2a	353.2	309.2	120	10	4	6.7	d4-PGE2
d4-PGE2	355.2	275.3	90	7	4	7.0	-
PGE2	351.2	271.3	80	10	4	7.0	d4-PGE2
PGD1	353.3	317.2	75	7	4	7.2	d4-PGE2
PGE1	353.3	317.2	75	7	4	7.3	d4-PGE2
PGD2	351.2	271.3	80	10	4	7.3	d4-PGE2
LTD4	495.3	177.1	55	13	4	7.8	d4-LTB4
LXA4	351.2	115.2	95	10	4	8.1	d4-LTB4
LTC4	624.3	272.1	70	22	4	9.6	d4-LTB4
LTE4	438.2	333.3	90	13	4	9.7	d4-LTB4
PGJ2	333.3	189.2	90	10	4	9.9	d4-PGE2
PGB2	333.3	175.1	125	13	4	10.0	d4-PGE2
6-trans-LTB4	335.2	195.1	125	7	4	10.8	d4-LTB4
5,15-DiHETE	335.2	173.2	95	7	4	10.8	d11-14,15-DiHETrE
5,6-DiHETE	335.2	115.2	90	4	4	10.9	d11-14,15-DiHETrE
8,15-DiHETE	335.2	235.2	90	7	4	11.1	d11-14,15-DiHETrE
17,18-DiHETE	335.3	247.2	105	7	4	11.7	d11-14,15-DiHETrE
d4-LTB4	339.2	197.2	80	10	4	11.9	-

LTB4	335.2	195.1	125	7	4	11.9	d4-LTB4
14,15-DiHETE	335.3	207.2	95	7	4	12.3	d11-14,15-DiHETrE
9,10-DiHOME	313.2	201.2	130	16	4	12.42	d11-14,15-DiHETrE
12,13-DiHOME	313.2	183.2	130	16	4	12.7	d11-14,15-DiHETrE
14,15-DiHETrE	337.2	207.1	130	10	4	13.8	d11-14,15-DiHETrE
d11-14,15-DiHETrE	348.2	207.1	125	10	6	13.8	-
LTB3	337.2	195.2	120	7	4	14.2	d4-LTB4
11,12-DiHETrE	337.2	167.1	120	13	4	14.8	d11-14,15-DiHETrE
9-HOTrE	293.2	171.2	110	4	4	15.3	d4-9HODE
8,9-DiHETrE	337.2	127.1	85	13	4	15.6	d11-14,15-DiHETrE
13-HOTrE	293.2	195.1	125	10	4	15.6	d4-9HODE
15-deoxy-PGJ2	315.2	271.2	130	4	4	16.1	d4-PGE2
d6-20-HETE	325.2	281.2	110	7	4	16.5	-
20-HETE	319.2	275.1	125	10	4	16.5	d6-20-HETE
15-HEPE	317.2	219.2	90	4	4	16.6	d8-5-HETE
5,6-DiHETrE	337.2	145.1	85	7	4	16.7	d11-14,15-DiHETrE
8-HEPE	317.2	155.2	115	7	4	17.0	d8-5-HETE
12-HEPE	317.2	179.2	110	4	4	17.2	d8-5-HETE
5-HEPE	317.2	115.1	115	4	4	17.8	d8-5-HETE
d4-9HODE	299.2	172.3	90	13	4	18.0	-
13-HODE	295.2	195.2	95	13	4	18.0	d4-9HODE
9-HODE	295.2	171.1	120	10	4	18.0	d4-9HODE
15-HETE	319.2	219.2	120	4	4	19.1	d8-5-HETE
17(18)-EpETE	317.2	215.2	130	4	4	19.2	d-11-11(12)EpEtrE
13-oxo-ODE	293.2	195.1	95	13	4	19.3	d6-20-HETE
17-HDoHE	343.2	281.2	95	4	4	19.5	d4-9HODE
11-HETE	319.2	167.2	100	7	4	19.5	d8-5-HETE
9-HETE	319.2	167.2	80	7	4	19.8	d8-5-HETE
9-oxo-ODE	293.2	185.1	90	13	4	20.0	d6-20-HETE
15-oxo-ETE	317.2	113.1	125	10	6	20.1	d8-5-HETE
14(15)-EpETE	317.2	207.2	100	4	4	20.2	d-11-11(12)EpEtrE

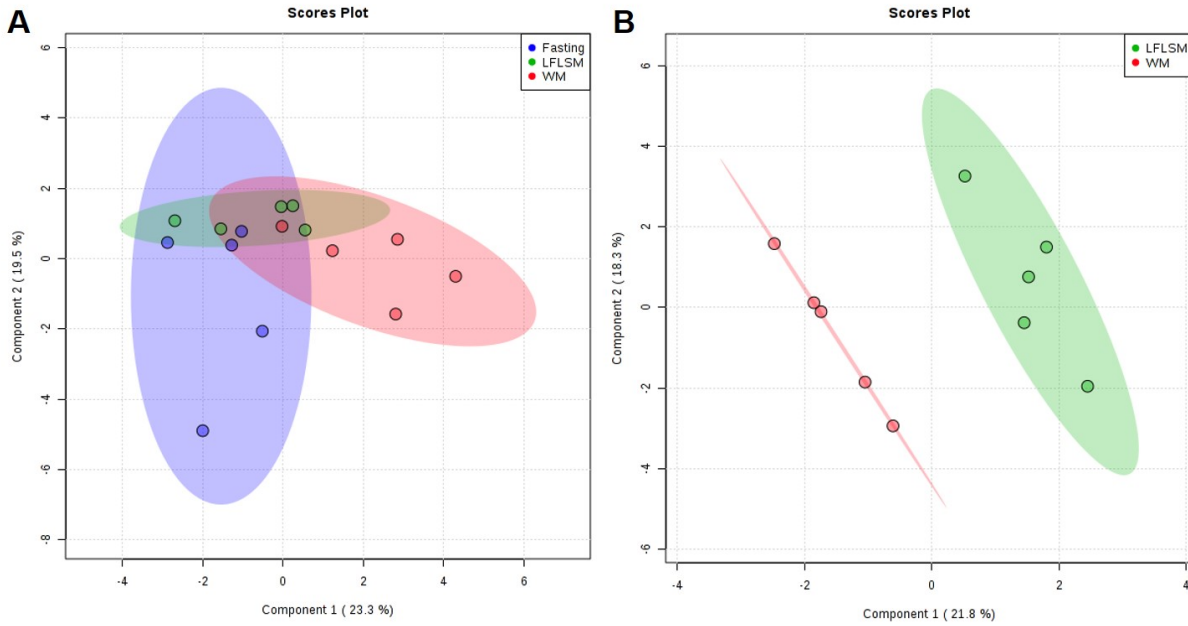
8-HETE	319.2	155.2	120	7	4	20.4	d8-5-HETE
11(12)-EpETE	317.2	167.2	90	4	4	20.5	d-11-11(12)EpEtrE
12-HETE	319.2	179.2	120	7	4	20.5	d8-5-HETE
8(9)-EpETE	317.2	127.2	115	4	4	20.8	d-11-11(12)EpEtrE
12-oxo-ETE	317.2	153.1	115	7	4	21.1	d8-5-HETE
15(S)-HETrE	321.2	221.2	85	7	4	21.1	d8-5-HETE
5-HETE	319.2	115.1	90	10	4	21.2	d8-5-HETE
d8-5-HETE	327.2	116.1	75	7	4	21.2	-
12(13)EpOME	295.3	195.2	95	7	4	22.5	d-11-11(12)EpEtrE
19(20)-EpDPE	343.2	241.2	130	7	4	22.5	d-11-11(12)EpEtrE
14(15)-EpETrE	319.2	219.3	130	4	4	22.8	d-11-11(12)EpEtrE
9(10)-EpOME	295.3	171.1	100	7	4	22.8	d-11-11(12)EpEtrE
16(17)-EpDPE	343.2	233.2	130	4	4	23.3	d-11-11(12)EpEtrE
13(14)-EpDPE	343.2	193.2	80	4	4	23.4	d-11-11(12)EpEtrE
5-oxo-ETE	317.2	273.2	120	7	4	23.4	d8-5-HETE
11(12)-EpETrE	319.2	167.2	105	4	4	23.5	d-11-11(12)EpEtrE
d-11-11(12)EpEtrE	330.2	167.2	80	7	4	23.5	-
10(11)-EpDPE	343.2	153.2	90	4	4	23.6	d-11-11(12)EpEtrE
7(8)-EpDPE	343.2	113.1	85	4	4	23.8	d-11-11(12)EpEtrE
8(9)-EpETrE	319.2	167.2	90	4	4	23.9	d-11-11(12)EpEtrE
5(6)-EpETrE	319.2	191.1	115	4	4	24.2	d-11-11(12)EpEtrE

Supplemental Table 6. Recovery of surrogates by group.

Surrogate	Fasting		Low-fat/low-sucrose meal (LFLSM)		Western Meal (WM)		p value	
	Mean (%)	Standard Deviation	Mean (%)	Standard Deviation	Mean (%)	Standard Deviation	ANOVA	t-test LFLSM v WM
d11-11(12)-EpEtrE	34.643	19.264	29.940	9.119	33.956	19.408	0.891	0.686
d11-14,15-DiHETrE	59.941	15.646	58.773	13.197	59.124	14.311	0.991	0.969
d4-6keto-PGF1 α	56.338	11.087	59.775	6.913	60.113	12.572	0.822	0.959
d4-9-HODE	51.735	18.148	49.774	16.198	52.259	20.367	0.975	0.836
d4-LTB4	40.559	11.143	40.326	14.728	42.168	11.507	0.969	0.831
d4-PGE2	64.338	12.363	65.267	7.004	65.322	10.095	0.985	0.992
d4-TXB2	57.683	8.988	57.720	6.528	61.773	8.261	0.659	0.414
d6-20-HETE	64.828	14.835	61.253	15.328	61.216	14.326	0.908	0.997
d8-5-HETE	32.091	18.161	26.703	11.899	27.586	18.461	0.859	0.931

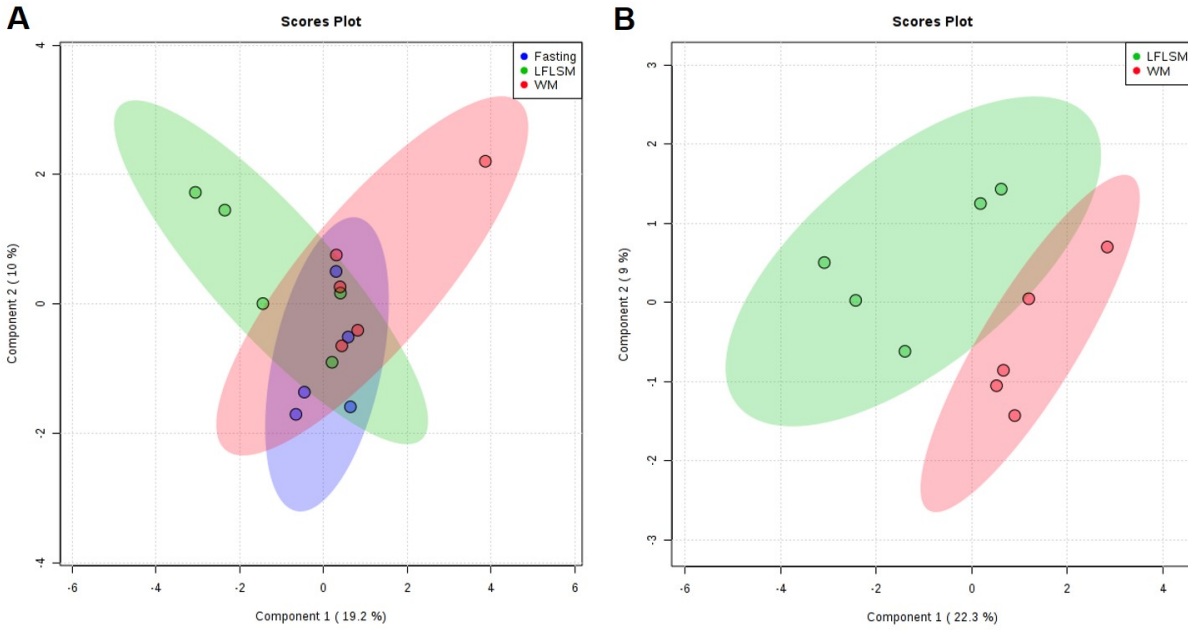
Supplemental Table 7. Primer sequences used for qRT-PCR

Gene	Forward Primer	Reverse Primer
18s	GCAATTATTCCCCATGAACG	GGCCTCACTAAACCATCCAA
COX1	AGCCTGAGCGGGAATAGTG	ATGGGCAGTTACGATAACATTGT
COX2	AATTGCTCTCCCCTCTCTACG	GGTTTTAGGTCGTTTGTGGGAT
EPHX1	GGAGACCTTACCACTTGAAGATG	GCCCGGAACCTATCTATCCTCT
EPHX2	CTTGGTGCGTACCAGACGG	TTCTAGGTAGATTGGCTCCA
CYP2J9	GGGAATGTTCTAAGCCTGGATTT	GAGTGACTGGGCGATTCAAAA
CYP2J12	AAGGAGGCTGACTGTCTTGTGG	GACTGTCCTCATACTCAAAGCGC



Supplemental Figure 1. sPLSDA plots of free oxylipin content of cerebrum.

The lipids from the right hemisphere of cerebrum were extracted from fasting mice and mice after consuming a low-fat/low-sucrose meal (LFLSM) or Western meal (WM). The free oxylipin content was determined by UHPLC/MS/MS. sPLSDA plots are shown of all three groups **A** or the two meal consuming groups **B**.



Supplemental Figure 2. sPLSDA plots of fatty acid composition of cerebrum.

The lipids from the right hemisphere of cerebrum were extracted from fasting mice and mice after consuming a low-fat/low-sucrose meal (LFLSM) or Western meal (WM). Fatty acid composition was determined by gas chromatography. sPLSDA plots are shown of all three groups **A** or the two meal consuming groups **B**.