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Regulation of rat plasma and cerebral cortex oxylipin concentrations with increasing levels of dietary linoleic acid

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Abstract

Linoleic acid (LA, 18:2n-6) is the most abundant polyunsaturated fatty acid in the North American diet and is a precursor to circulating bioactive fatty acid metabolites implicated in brain disorders. This exploratory study tested the effects of increasing dietary LA on plasma and cerebral cortex metabolites derived from LA, its elongation-desaturation products dihomo-gamma linolenic (DGLA, 20:3n-6) acid and arachidonic acid (AA, 20:4n-6), as well as omega-3 alpha-linolenic (α -LNA, 18:3n-3), eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). Plasma and cortex were obtained from rats fed a 0.4%, 5.2% or 10.5% energy LA diet for 15 weeks and subjected to liquid chromatography tandem mass spectrometry analysis. Total oxylipin concentrations, representing the esterified and unesterified pool, and unesterified oxylipins derived from LA and AA were significantly increased and EPA metabolites decreased in plasma at 5.2% or 10.5% energy LA compared to 0.4% energy LA. Unesterified plasma DHA metabolites also decreased at 10.5% energy LA. Total oxylipins did not significantly change in cortex, whereas unesterified LA and AA metabolites increased and unesterified EPA metabolites decreased at 5.2

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Conflicts of interest

None to declare

or 10.5% LA. DGLA and α -LNA metabolites did not significantly change in plasma or cortex. Dietary LA lowering represents a feasible approach for targeting plasma and brain LA, AA, EPA or DHA-derived metabolite concentrations.

Keywords

omega-6 linoleic acid (LA); high LA; oxylipin; metabolites; mediators; plasma; brain

1. Introduction

US intake of omega-6 linoleic acid (LA, 18:2n-6) has increased over the past century from a historic norm of 2% energy to 7% energy, owing to the growth of corn and soybean agriculture [1]. High intakes of LA could potentially be harmful to the brain because it can be converted non-enzymatically or enzymatically via 5 or 12/15lipoxygenase (LOX), cyclooxygenase (COX)-1 or 2, cytochrome P450 (CYP)-2C, 2E, 4A or 4F or soluble epoxide hydrolase (sEH) enzymes [2–4] into oxidized linoleic acid metabolites (OXLAMs) which are mostly pro-inflammatory [5–7]. OXLAMs have been linked to brain disorders including Alzheimer's disease [8], chronic migraine [9] and Parkinson's disease [10, 11]. It is not known how increased dietary LA regulates brain OXLAM composition, which would be important for understanding the role of OXLAMs in brain disorders.

COX, LOX, CYP and sEH enzymes also catalyze the formation of oxidized fatty acids known as 'oxylipins', from other fatty acid substrates including LA's elongation/desaturation product dihomo-gamma-linolenic acid (DGLA, 20:3n-6) and arachidonic acid (AA, 20:4n-6), as well as omega-3 α -linolenic acid (α -LNA) and its elongation/desaturation products eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) [12, 13]. DGLA, AA, EPA and DHA are synthesized from their dietary-essential LA or α -LNA precursors in the liver and transported through the circulation to the brain and other tissues [14–17]. In brain, COX, LOX and CYP enzymes convert LA, DGLA, AA, α -LNA, EPA or DHA into hydroxylated, epoxidized, prostacyclin or ketone metabolites that mediate the inflammatory response or resolution of inflammation (Figure 1) [18–21]. sEH catalyzes the conversion of epoxide oxylipins into dihydroxy and possibly trihydroxy fatty acid metabolites (Figure 1) [22].

Kim et al. reported that compared to an n-6 polyunsaturated fatty acid (PUFA) adequate diet containing 27.6% LA, n-6 PUFA deficiency achieved by lowering LA to 3% of total fatty acids for 15 weeks, decreased brain AA-releasing group IVA calcium-dependent phospholipase A₂ (cPLA₂) mRNA, protein and activity, and COX-2 protein, and increased group VIA DHA-releasing calcium independent phospholipase A₂ (iPLA₂) mRNA, protein and activity, and 15-LOX mRNA and protein [23]. N-6 PUFA deficiency also increased brain DHA incorporation rate and turnover within membrane phospholipids [24], and reduced the rate of loss of AA from brain membrane phospholipids [25].

The changes in COX-2 and 15-LOX following n-6 PUFA deficiency suggest possible changes in their oxidized fatty acid metabolites. Supporting this suggestion, is evidence of increased hydroxyeicosapentaenoic acid (HEPE) metabolites of EPA and reduced

hydroxyeicosatetraenoic acid (HETE) and prostaglandin (PG) F₂α metabolites of AA following 15 weeks of n-6 PUFA deficiency (low LA) in rats [25]. Brain DHA-metabolite concentrations did not change, whereas LA, DGLA and α-LNA metabolites were not measured [25].

In view of the evidence showing that dietary LA levels regulate brain AA and EPA-derived mediator concentrations and COX and LOX protein expression [23, 25], the present study tested the hypothesis that graded increments in dietary LA would alter brain oxylipin concentrations. Plasma oxylipins were also measured to determine the extent to which circulating oxylipins reflect brain oxylipin concentrations.

Rats were fed a 0.4%, 5.2% or 10.5% energy LA diet for 15 weeks, and their plasma cerebral cortex oxylipin concentrations quantified. Plasma and cortex fatty acid concentrations, which were measured in the same batch of animals and recently reported [26], are depicted in Supplementary Figure 1. The 0.4% and 5.2% energy diets are equivalent to the 3% and 27% (of total fatty acids) LA diets used in previous studies [23–25, 27]. The 10.5% energy LA diet containing 52% LA of total fatty acids was added to explore the effect of increased LA consumption on plasma and brain oxylipin metabolism at clinically relevant levels, which range from approximately 2 to 20% energy [1]. In this manuscript, we will refer to the 0.4%, 5.2% and 10.5% energy diets as the low, medium and high LA diets respectively.

Although AA, EPA and DHA-derived metabolites are often referred to as mediators because of their established roles in mediating the brain inflammatory or resolution cascades (reviewed in [28]), very little is known about what LA, DGLA or α-LNA mediate in the brain. Thus, the term metabolites or oxylipins will be used in lieu of mediators when referring to COX, LOX, CYP or sEH fatty acid products.

A total of 84 metabolites (Supplementary Table 1) derived from LA, DGLA, AA, α-LNA, EPA or DHA were quantified with liquid chromatography tandem mass spectrometry (LC-MS/MS) using an established lipidomics platform [29]. Total oxylipins, reflecting the sum of esterified and unesterified species, and unesterified (free) oxylipins were measured [30, 31], because the unesterified pool represents the bioactive form of oxylipins [32], whereas the total pool containing esterified oxylipins represents the amount potentially available for release [33].

We found that increasing dietary LA increased plasma total or unesterified LA and AA-derived metabolites, and decreased EPA and DHA metabolites. Brain (cortex) total oxylipins did not change, whereas unesterified LA and AA metabolites increased and EPA metabolites decreased following increased dietary LA intake.

2. Materials and Methods

2.1 Animals

Ethical approval for the animal protocol was obtained from the Animal Care and Use Committee of the Eunice Kennedy Schriver National Institute of Child Health and Human

Development. The protocol followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23). Male Fischer-344 (CDF) rat pups (18–21 days old) and their surrogate mothers were purchased from Charles River Laboratories (Portage, MI, USA). The pups were weaned upon arrival from their surrogate mothers and randomized to a low (n=8), moderate (n=8), or high LA diet (n=8) for 15-weeks. Animals were housed in an animal facility with regulated temperature, humidity and a 12 hour light/dark cycle. The three study diets were prepared by Dyets Inc. (Bethlehem, PA, USA) based on the AIN-93G formulation, and were isocaloric [27, 34]. The diets contained 60% carbohydrate, 20% protein, 10% fat and 10% vitamin, mineral and other additives as shown in Supplementary Table 2. The fat source was balanced to provide 0.4%, 5.2% and 10.5% energy LA. The fatty acid composition of the diets was measured by gas-chromatography (see below) and is presented in Table 1. The energy contribution of each fatty acid is shown in Supplementary Table 3. Rats had free access to food and water throughout the study; their food was replaced every 3 or 4 days.

Dietary fatty acids were extracted by the Folch method [35] and analyzed by gas chromatography [36]. Food pellets were crushed with pestle and mortar, weighed and extracted in 30 ml of chloroform/methanol (2:1 v/v) and 7.5 ml of 0.1% potassium chloride. The chloroform layer containing total lipids was separated, and the sample re-extracted with 20 ml of chloroform. The chloroform extract was dried and reconstituted in 5 ml chloroform/methanol (2:1 v/v). A portion total lipid extract (50 μ l) was methylated for 3 hours at 70 °C with 1% H₂SO₄ in methanol after adding 25 μ l of 8.11 nmol/ μ l 1,2-diheptadecanoyl-sn-glycero-3-phosphocholine as an internal standard. The fatty acid methyl esters were extracted in 3 ml heptane, further neutralized with 1.5 ml water to remove residual H₂SO₄ and reconstituted in 100 μ l isooctane. Fatty acid methyl esters were analyzed by gas-chromatography as detailed elsewhere [36].

2.2 Rat tissue collection

The rats were killed by CO₂ asphyxiation and decapitated. Trunkal blood was collected into tubes containing ethylenediaminetetraacetic acid (EDTA). Blood was centrifuged for 1 minute at 13,000 rpm and plasma collected and stored at –80°C. Brain was excised, separated into cerebrum, cerebellum and brainstem and frozen on dry-ice chilled isobutene. Samples were stored at –80°C. Only cerebral cortex tissue was available for analysis because brainstem and cerebellum were used for fatty acid composition measurements, which were recently reported [26].

2.3 Oxylipin analysis

A total of 84 oxylipins listed and abbreviated in Supplementary Table 1 were quantified using LC-MS/MS by running calibration curves for each oxylipin using authentic standards obtained from Cayman Chemicals (Ann Arbor, Michigan) or synthetic standards produced by Dr. Hammock's laboratory. Both total (unesterified and esterified) and unesterified oxylipins were measured. The oxylipin extraction and analysis was done in a blinded manner.

2.3.1 Plasma oxylipin extraction—Fatty acid metabolites were analyzed as free oxylipins and after base hydrolysis to determine the sum of both free and esterified oxylipins as previously described [31]. Oxylipins were extracted from plasma using 60 mg Waters Oasis HLB 3cc cartridges (Catalogue #: WAT094226; Milford, MA). The columns were cleaned with one wash of ethyl acetate and two washes of methanol, and conditioned with two washes of buffer containing 0.1% acetic acid and 5% methanol in Millipore water. Each column was spiked with 10 µl antioxidant solution and 10µl surrogate standard mix containing 10 nmol of d11-11(12)-EpETrE, d11-14,15-DiHETrE, d4-6-keto-PGF1a, d4-9-HODE, d4-LTB4, d4-PGE2, d4-TXB2, d6-20-HETE and d8-5-HETE. The antioxidant solution contained three antioxidants mixed at a 1:1:1 ratio (v/v/v) consisting of 0.6 mg/ml Ethylenediaminetetraacetic acid (EDTA) in water, 0.6 mg/ml butylated hydroxytoluene in methanol and 0.6 mg/ml Triphenylphosphine in water/methanol (1:1 v/v). The antioxidant solution was passed through a Millipore filter to remove solid particles. The final concentration of each antioxidant was 0.2 mg/ml.

Fifty to one hundred µl of ice-thawed plasma was added directly to the column, which was then topped to volume with buffer containing 0.1% acetic acid and 5% MeOH in Millipore water. The solid phase extraction chamber plug, to which the column was connected, was opened to allow the solution to pass through. In doing so, the oxylipins and surrogate oxylipin standards adsorb to the column filter and other polar compounds (sugars, proteins, etc.) elute into the extraction chamber. The column was washed with the buffer twice and then dried under vacuum suction (~20psi) for 20 minutes. Oxylipins were eluted into a 2 ml centrifuge tube containing 6µl of 30% glycerol (in methanol) by washing the column with 0.5 ml methanol and 1.5 ml ethyl acetate. The collected methanol/ethyl acetate extract containing oxylipins was dried in a vacuum centrifuge and reconstituted in 50 µl of 200 nM 1-cyclohexyl ureido, 3-dodecanoic acid in methanol solution as a recovery standard.

To measure total oxylipins (esterified and free), 10 µl of antioxidant mix and 10 µl surrogate standard were mixed with 45–100 µl of the remaining plasma, which was then hydrolyzed in 100 µl of 0.5 M sodium carbonate solution (26.5 mg per ml of 1:1 v/v methanol/water) at 60 °C for 30 min under constant shaking. The samples were neutralized with 25 µl acetic acid and 1575 µl water was added. The pH was confirmed to be neutral after adding the acetic acid by spiking a litmus paper with a few microliters from one of the samples. The hydrolyzed oxylipins were poured into pre-cleaned and pre-conditioned solid phase extraction columns and washed with buffer and extraction solvents as described above.

2.3.2 Cerebral cortex oxylipin extraction—Ice-cold methanol (400 µl) containing 0.1% acetic acid and 0.1% butylated hydroxytoluene (BHT) was added to approximately 0.35 g of frozen cortex, following the addition of 10 µl antioxidant mix and 20 µl surrogate standard solution containing 200 nmol of d11-11(12)-EpETrE, d11-14,15-DiHETrE, d4-6-keto-PGF1a, d4-9-HODE, d4-LTB4, d4-PGE2, d4-TXB2, d6-20-HETE and d8-5-HETE in methanol. The samples were cooled in a –80°C freezer for 30 minutes and then homogenized for 5 minutes at 30 vibrations per second using a bead homogenizer. The homogenized samples were stored overnight in a –80°C freezer and then centrifuged at high speed in a 5145R Eppendorf microcentrifuge (Hauppauge, NY) for 10 minutes. Approximately half of the supernatant was subjected to direct solid phase extraction to

extract free oxylipins. The remaining supernatant was hydrolyzed in equal volumes of 0.5 M sodium carbonate solution (26.5 mg per ml of 1:1 v/v methanol/water) at 60 °C for 30 min under constant shaking. Following neutralization with 25 µl acetic acid and addition of 1575 µl water, the hydrolyzed oxylipins were subjected to solid phase extraction.

All samples stored in –80°C until LC-MS/MS analysis. Samples were analyzed within a week following extraction.

2.3.3 LC-MS/MS analysis—Oxylipins were analyzed as previously described [29, 31, 37] on an Agilent 1200 SL (Agilent Corporation, Palo Alto, CA) UPLC system connected to a 4000 QTrap tandem mass spectrometer (Applied Biosystems Instrument Corporation, Foster City, CA) equipped with an electrospray source (Turbo V). The UPLC was equipped with an Agilent 2.1 × 150 mm Eclipse Plus C18 column with a 1.8 µm particle size.

The autosampler was kept at 4 °C, and the column at 50 °C. The mobile phase A contained water with 0.1% glacial acetic acid and mobile phase B consisted of acetonitrile/methanol (84:15) with 0.1% glacial acetic acid. Gradient elution was performed at a flow rate of 0.25 ml/min. The total run time was 21.5 minutes. Solvent B was held at 35% for 0.25 minutes, then increased to 45% between 0.25–1 min, 55% from 1 – 3 min, 66% from 3 – 8.5 min, 72% from 8.5 to 12.5 min, 82% from 12.5–15 min and 95% from 15–16.5 min, maintained at 95% to 18 min, and finally lowered to 35% from 18 to 18.1 min and held at 35% between 18.1 and 21 min. This gradient enables the separation of oxylipins according to polarity, with the most polar oxylipins eluting first (prostaglandins and leukotrienes) followed by less polar hydroxy and epoxy fatty acids. The instrument was operated in negative electrospray ionization mode and using optimized multiple reaction monitor (MRM) conditions of the parent and fragmentation product ion [37]. Analyst software 1.4.2 was used to quantify the peaks according to the standard curves used to establish the response factor for each oxylipin after correcting for the surrogate standard recovery.

Oxylipins that were base-hydrolyzed and used d4-PGE2 as a surrogate standard were not quantified due to degradation of the d4-PGE2 during the hydrolysis step [31]. Oxylipins that were three or more times lower than the minimum standard concentration used for the standard curve were not analyzed, because they likely represent baseline noise.

2.3.4 Statistical analysis—The oxylipin measurements were completed for all 24 plasma samples (8 per group), but only 19 out of 24 cortex samples were analyzed because of sample loss during homogenization (n=3) or samples not injected into the LC-MS/MS due to syringe failure (n=2), an issue that was realized during the semi-automated data analysis. These samples were not re-injected due to possible storage effects on oxylipin levels. Thus, the sample size for the cortex was 6, 6, and 7 for the 0.4%, 5.2% and 10.5% energy dietary LA groups, respectively.

Dietary data are presented as mean ± standard deviation (SD). Oxylipin data are presented as medians and interquartile ranges. Oxylipin values that were below the detection or quantitation limit were imputed as one-half the minimum value of the other samples. Oxylipin data were analyzed using Kruskal Wallis one-way analysis of variance (ANOVA).

In an exploratory manner, we conducted Dunn's post-hoc test to determine differences amongst the groups. Oxylin values that were below the detection or quantitation limit were imputed as one-half the limit of quantitation. Oxylin data are presented as medians and interquartile ranges. The reference ('control') group is the low (0.4%) LA diet. Statistical significance was accepted at $p \leq 0.05$.

3. Results

3.1 Dietary fatty acid composition

Dietary LA was altered as a controlled variable by replacing coconut or olive oil with high-LA safflower oil as shown in Supplementary Table 2. The fatty acid composition of the diets is in Table 1. As shown, the low, medium and high LA diets contained 3.1%, 29.7% and 51.9% LA of total fatty acids, respectively. This provided 0.4%, 5.2% and 10.5% energy LA as shown in Supplementary Table 3. The fatty acid composition of the low and medium LA diets is consistent with previous reports that used the same diets [23, 24, 27].

3.2 Plasma oxylin concentrations

Plasma concentrations of several total or unesterified oxylin derived from LA, AA, EPA and DHA were significantly affected by dietary LA.

Total di-hydroxylated (9–10 DiHOME, 12,13 DiHOME) metabolites of LA were 3 to 8 times higher in the 5.2% and 10.5% LA groups compared to 0.4% controls. Similar changes in di-hydroxylated LA metabolites were seen within the unesterified pool, in which the epoxidized metabolites, 9(10)-EpOME and 12(13)-EpOME, were increased by 6–12 fold in the 5.2 and 10.5% LA diets relative to 0.4% LA. There were no significant differences between the 5.2% and 10.5% LA diets.

Total AA-derived 15-oxo-ETE (a ketone metabolite) the dihydroxylated metabolite, 8,9-diHETrE were significantly higher by 3–4 fold in the 5.2% LA group compared to 0.4% LA controls. 5,6-DiHETrE, 14,15-DiHETrE and TBX₂ were significantly increased by 4 to 6 fold in the 5.2% and 10.5% LA groups compared to 0.4% controls. Similar 4 to 6 fold increments in 15-oxo-ETE, 14,15-DiHETrE, 11,12-DiHETrE and TBX₂ were detected within the unesterified oxylin pool. Unesterified mono-hydroxylated 12-HETE and epoxidized 11(12)-EpETrE and 14(15)-EpETrE AA metabolites also significantly increased by 4 to 10 fold in the 5.2% and 10.5% LA groups compared to 0.4% LA controls. Conversely, total LXA₄ was reduced by 88–95% with the 5.2% and 10.5% LA diets compared to 0.4% LA controls.

Total and unesterified mono-hydroxy (HEPEs), epoxy (EpETEs) and di-hydroxy (diHETEs) EPA metabolites were significantly decreased by the 5.2% and 10.5% LA diets compared to 0.4% LA, with the exception of total 8,15-diHETE, which was significantly higher in the 5.2% LA compared to 0.4% LA controls. Changes ranged from 2 to 89 fold within the total oxylin pool and from 2 to 9 within the unesterified pool.

No change in total DHA-metabolites was detected. Unesterified dihydroxylated DHA-metabolites (10,11-DiHDPE, 13,14-DiHDPE, 16,17-DiHDPE and 19,20-DiHDPE), however, decreased significantly by 2–4 folds in the 10.5% group compared to 0.4% LA.

Total and unesterified metabolites of α -LNA and DGLA did not differ significantly between the groups.

3.2 Cerebral cortex oxylipin concentrations

Total and unesterified hydroxy (9- and 13-HODE), epoxy (9(10)- and 12(13)-EpOME), and dihydroxy (9,10- and 12,13-DiHOME) LA-metabolites were significantly increased in the 10.5% LA groups compared to 0.4% LA. The increase in LA metabolites ranged from approximately 2 to 3 fold, except for total 9(10)-EpOME, which increased by 92 fold. Unesterified 9-oxo-ODE and tri-hydroxy (9,10,13- and 9,12,13-TriHOME) LA-metabolites were significantly increased in the 10.5% group relative to 0.4% LA controls by 2 to 3 fold.

Except for an 1.8-fold higher concentration in 8,9-DiHETrE in the 10.5% LA group compared to 0.4% LA, no change in total AA-derived metabolites was observed. AA-derived unesterified 11,12-DiHETrE, 14,15-DiHETrE and PGD₂ were significantly higher by 1.5–1.6 fold in the 10.5% LA groups compared to 0.4% LA. No significant changes in other unesterified AA metabolites were detected.

EPA-derived total and unesterified hydroxy (5 and 15-HEPE), epoxy (8(9)-, 14(15)- and 17(18)-EpETE) and di-hydroxy (17,18-DiHETE) metabolites were significantly reduced in the 5.2% and 10.5% LA groups relative to 0.4% LA by 3 to 19 fold. Unesterified PGE₃ was also significantly reduced by 86% in the 5.2% and 10.5% LA compared to the 0.4% LA group.

Total and unesterified α -LNA, DGLA and DHA-derived metabolites did not significantly change in cerebral cortex.

3.3 Summary of oxylipin changes

Table 4 summarizes significant changes in plasma and cortex total and unesterified oxylipin concentrations. As indicated, changes in total oxylipins reflected changes in the unesterified pool in both plasma and cortex.

Changes in plasma did not entirely reflect changes in cortex. In total oxylipin pool, incremental amounts of dietary LA intakes affected 16 and 12 metabolites in plasma and cortex, respectively, but overlapping changes were observed in only 7 metabolites – 9,10- and 12,13-DiHOME, 5-HEPE, 8(9)-, 14(15)- and 17(18)-EpETE and 17,18-DiHETE. Unesterified oxylipin concentrations changed in 19 metabolites in both plasma and cortex, but overlapping changes were detected in only 9 metabolites – 9(10)- and 12(13)-EpOME, 9,10- and 12,13-DiHOME, 11,12- and 14,15-DiHETrE, 5- and 15-HEPE and 17,18-DiHETE.

4. Discussion

This exploratory study showed significant changes in plasma and cortex oxylipin concentrations following increased dietary LA intake. In plasma, LA and AA-derived metabolites increased and EPA and DHA-derived metabolites decreased within total or unesterified oxylipins following increased dietary LA (Table 2). Cortex total oxylipins did not change, whereas unesterified LA and AA metabolites increased and EPA (but not DHA) metabolites decreased following graded increments in dietary LA (Table 3). Changes in plasma did not reflect changes in cortex with the exception of 7 or 9 oxylipins (Table 4).

The discrepancy between changes in plasma and cortex total and unesterified oxylipins suggests that plasma oxylipins are not representative biomarkers of brain oxylipin levels. Causes of this discrepancy could be due to their differing compartmental half-lives or degradation of plasma oxylipins upon entering the brain. The extent of oxylipin entry into the brain is not known. Akanuma et al. reported that the elimination of labeled PGE₂ through the blood brain barrier is reduced following intracerebrovascular injection of bacterial lipopolysaccharide, suggesting mechanisms that regulate PGE₂ (and possibly other oxylipin) partitioning in and out of brain [38].

The increase in plasma and cortex LA and AA-derived oxylipin concentrations was statistically similar between the 5.2% and 10.5% LA diets relative to 0.4% LA, with a few notable exceptions (Table 4). Mono-hydroxylated, mono-oxygenated (ketones) and epoxidized LA or AA metabolites did not increase beyond 5.2% LA compared to 0.4% LA, whereas many of the epoxide di or tri-hydroxylated sEH degradation products (Figure 1) were significantly higher at 10.5% LA compared to 5.2% or 0.4% LA (Table 4). This suggests that the degradation of CYP-derived epoxidized metabolites by plasma and cortex sEH was enhanced when LA intake increased beyond 5.2% energy. Future studies should confirm this hypothesis by measuring sEH enzyme activity following graded increments in dietary LA. It would be valuable to also identify the enzyme that converts hydroxy, epoxy or ketone metabolites into trihydroxylated products (Figure 1).

The concomitant increase in cortex AA-metabolites and decrease in EPA-metabolites is consistent with a study by Lin et al. which reported higher brain AA-derived HETEs and PGF₂ α , and lower EPA-derived HEPEs in rats on a 24% LA diet (similar to our 5.2% LA group) compared to rats on a 2% LA diet (similar to our 0.4% LA group) [25]. The reciprocal changes in AA and EPA metabolites following graded LA increments could potentially be explained by two processes – 1) precursor fatty acid availability and 2) COX, LOX, sEH or CYP selectivity towards fatty acids. Brain AA concentration exceeds EPA concentration by > 600-fold [25], yet CYP 4F2, CYP 2E1 and CYP 2J2 preferentially metabolize EPA over AA [13]. Thus, under moderate or high dietary LA conditions, the reduction in cortex EPA concentration (Supplementary Figure 1-B) is substrate limiting to COX, LOX, sEH or CYP enzymes. When LA is lowered, however, cortex EPA concentration increases by approximately 10-fold (Supplementary Figure 1-B) and becomes available for CYP-mediated epoxylation. A similar process likely governs plasma AA and EPA-metabolite levels, but at different substrate thresholds, since plasma AA and EPA

concentrations are within the same order of magnitude (Supplementary Figure 1-A). Substrate preference for COX, LOX and sEH enzymes remains to be confirmed.

The increase in plasma LA-derived oxylipins and decrease in DHA-derived oxylipin concentrations are likely related to changes in their precursor fatty acid pool, which increased and decreased respectively with increased dietary LA intake (Supplementary Figure 1-A). LA lowering was reported to decrease plasma LA-derived metabolites (OXLAMs) in humans [39], consistent with the findings of this study. In mice and humans, fish oil supplementation raised circulating DHA concentrations in association with increased plasma DHA metabolites [13, 31].

Consistent with the findings of Lin et al., [25], brain DHA-metabolites were not altered by dietary LA (Table 2), despite the reported increase in brain DHA incorporation rate and turnover (acylation-reacylation rate) within membrane phospholipids [23]. The increase in DHA turnover reflects an increase in the rate of phospholipid remodelling and does not necessarily imply an increase in the rate of formation of DHA-metabolites. It is possible that the rate of formation of DHA-metabolites increased without causing significant accumulation if they are rapidly metabolized. This interpretation would be consistent with the Lin et al study which showed increased rate of loss of DHA from brain following LA lowering [23]. Alternative pathways of loss may also include β -oxidation products or recycling into sterols in response to increased LA incorporation by the brain [40, 41].

In plasma, the concentration of total oxylipins exceeded unesterified oxylipins by 5–10 fold, whereas in cortex, total and unesterified oxylipins were similarly concentrated. This suggests that the majority of oxylipins in plasma are esterified to triacylglycerol, phospholipid, cholesteryl ester or other lipids, whereas in cortex, most oxylipins are unesterified. In plasma, oxylipins are released from the esterified fraction via lipoprotein lipase [33]. The abundance of unesterified oxylipins in cortex is consistent with the notion that they are actively produced when needed via COX, LOX, CYP or sEH enzymes, rather than stored in membrane phospholipids [22]. This is in agreement with the changes seen in this study, which occurred in the cortex unesterified fraction but not the total oxylipin pool.

Changes in oxylipins in response to dietary LA are likely underestimated in this study because the rats were not subjected to head-focused microwave fixation, which stops brain lipid metabolism [42–45]. Brain unesterified fatty acids and their eicosanoid and docosanoid metabolites were reported to increase following decapitation or ischemia compared to microwave fixation [43, 44]. Thus, baseline oxylipin values in this study reflect an ischemic state, which may have masked dietary effects or underestimated the amount of oxylipins esterified to phospholipids. Lin et al. measured brain unesterified AA, EPA and DHA metabolite concentrations in microwave-fixated rat brain following 15 weeks of a low (2% of fatty acid, 0.4% energy) or moderate (24% of fatty acids, 5.2% energy) LA diet [25]. Of the measured AA and EPA metabolites that overlapped with this study, they observed higher AA-derived HETE concentrations in the moderate LA group compared to the low LA group, whereas this study did not find significant changes in HETEs [25]. In both studies, however, AA-derived PGE₂ was unaltered and EPA-derived HEPE metabolites increased in the moderate LA group compared to low LA controls [25].

The findings of this study are strengthened by the blinded measurements, which eliminate analytical bias associated with sample handling or LC-MS/MS peak integrations [46, 47], and by measuring oxylipins in both total and unesterified fractions. Limitations include the possibility of statistical false positives and false negatives associated with the small sample size (6–8 per group). Many metabolites were measured on each rat and several statistical tests were performed to identify differences in the diet groups, thus inflating type I errors. Power calculations were not performed prior to initiating the study because it was exploratory, and we did not know the magnitude of variance and change to expect.

In summary, graded increments in dietary LA increased plasma and cortex LA and AA metabolites, and decreased EPA or DHA metabolites. Changes in plasma did not entirely reflect in cortex, thus pointing out the limitation of using plasma measurements as relevant biomarkers of brain oxylipin concentrations. This could be overcome by using positron emitting tomography to image fatty acid incorporation into the brain, as a way to quantify the amount lost due to metabolism (into oxylipins and other signaling molecules) [48, 49]. The changes in brain oxylipins suggest that dietary LA lowering could be used to target disturbed LA, AA or EPA metabolism in brain disorders [9, 49–51].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AA	arachidonic acid
ANOVA	one-way analysis of variance
BHT	butylated hydroxytoluene
cPLA₂	calcium-dependent phospholipase A ₂
COX	cyclooxygenase
CYP	cytochrome P450
DGLA	dihomo-gamma-linoleic acid
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
EDTA	ethylenediaminetetraacetic acid

EPA	eicosapentaenoic acid
GC	gas-chromatography
HEPE	hydroxyeicosapentaenoic acid
HETE	hydroxyeicosatetraenoic acid
IQR	interquartile ranges
iPLA₂	calcium-independent phospholipase A ₂
LA	linoleic acid
α-LNA	α -linolenic acid
LOX	lipoxygenase
MRM	multiple reaction monitoring
OXLAM	oxidized linoleic acid metabolite
PUFA	polyunsaturated fatty acid
PG	prostaglandin
PUFA	polyunsaturated fatty acid
sEH	soluble epoxide hydrolase
SD	standard deviation

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Highlights

- Increased LA intake raised omega-6 and reduced omega-3 derived oxylipins in rat plasma and cortex.

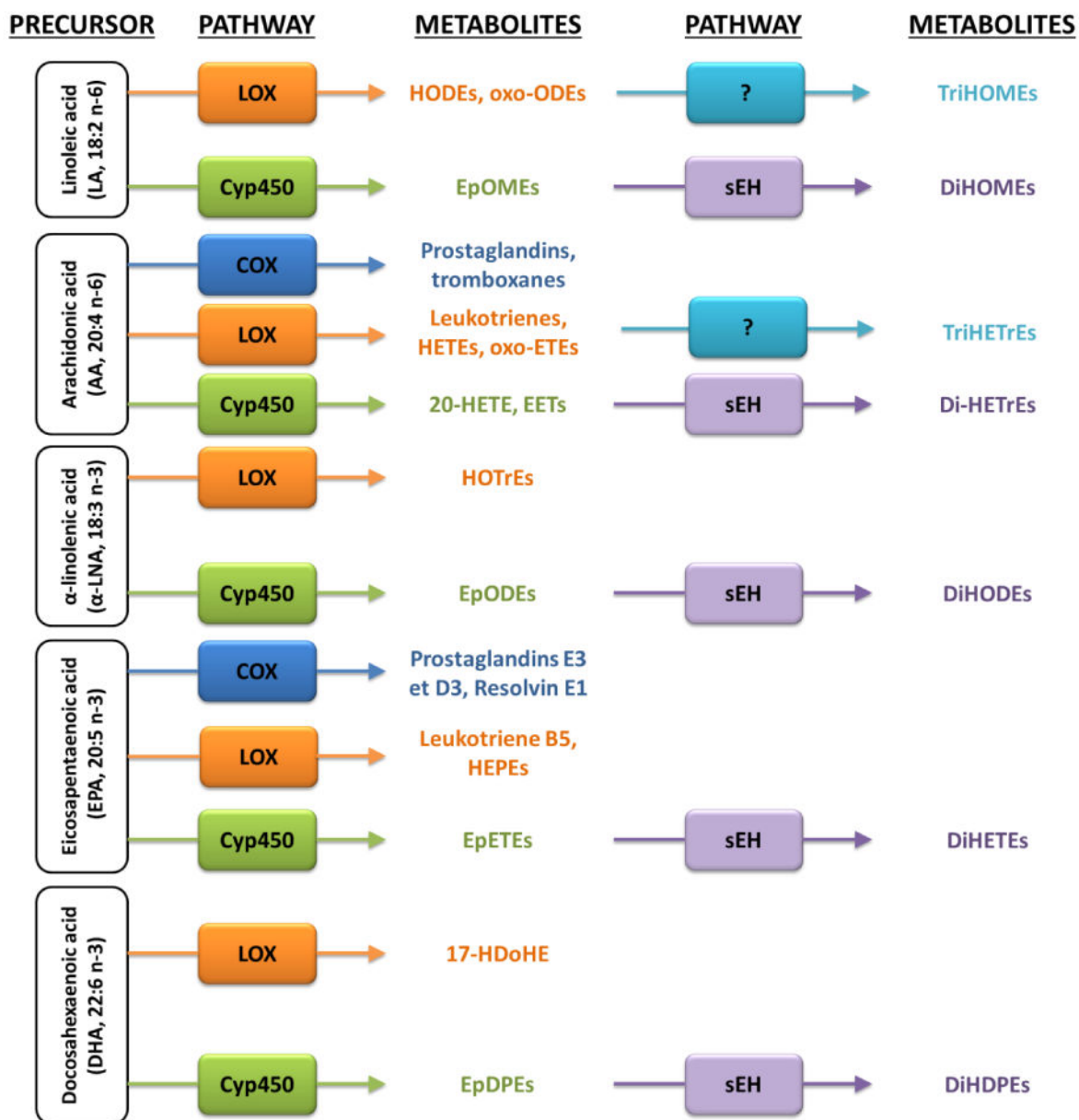


Figure 1.

Oxylipin formation pathways from fatty acids. Linoleic acid (LA, 18:2n-6), dihomo-gamma linolenic acid (DGLA, 20:3n-6), arachidonic acid (AA, 20:4n-6), α -linolenic acid (α -LNA, 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) are precursors for cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P45 (CYP) enzymes, which convert them into mono-hydroxylated, epoxidized or ketone products. Soluble epoxide hydrolase (sEH) catalyzes the formation of dihydroxylated metabolites from epoxidized metabolites. sEH may catalyze tri-hydroxylated metabolite formation, but this remains to be tested.

Table 1

Dietary fatty composition, expressed as percent of total fatty acids.

	0.4% Group	5.2% Group	10.5% Group
10:0	33.4 ± 1.3	20.6 ± 1.7	7.1 ± 0.1
12:0	0.7 ± 0.1	0.5 ± 0.1	0.4 ± 0.03
14:0	20.5 ± 0.2	11.0 ± 0.1	4.1 ± 0.01
16:0	13.3 ± 0.3	9.9 ± 0.2	8.2 ± 0.02
16:1n-7	0.1 ± 0.002	0.1 ± 0.002	0.1 ± 0.001
18:0	15.1 ± 0.4	14.5 ± 0.7	9.9 ± 0.2
18:1n-9	7.8 ± 0.1	8.7 ± 0.2	13.7 ± 0.03
18:2n-6	3.1 ± 0.05	29.7 ± 0.6	51.9 ± 0.1
18:3n-3	6.1 ± 0.1	5.1 ± 0.1	4.6 ± 0.01

Data are mean ± SD of n = 3 per diet.

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Table 2

Plasma oxylin concentrations (pmol/ml plasma)

Compound	TOTAL				UNESTERIFIED				
	0.4% Group Median (IQR)	5.2% Group Median (IQR)	10.5% Group Median (IQR)	0.4% Group Median (IQR)	5.2% Group Median (IQR)	10.5% Group Median (IQR)	0.4% Group Median (IQR)	5.2% Group Median (IQR)	10.5% Group Median (IQR)
n-6 Derivatives LA									
9-HODE	54.1 (35.9, 121.5)	46.5 (25.5, 115.9)	81.0 (54.7, 102.9)	43.2 (27.9, 74.8)	28.0 (20.0, 45.3)	37.6 (32.2, 58.8)	43.2 (27.9, 74.8)	28.0 (20.0, 45.3)	37.6 (32.2, 58.8)
13-HODE	86.0 (55.3, 168.6)	68.1 (40.1, 180.6)	106.8 (80.5, 131.9)	76.1 (51.4, 119.8)	49.5 (39.1, 77.5)	74.6 (60.0, 106.5)	76.1 (51.4, 119.8)	49.5 (39.1, 77.5)	74.6 (60.0, 106.5)
Total HODE	147.5 (91.2, 282.7)	114.6 (65.5, 296.6)	188.4 (133.8, 234.1)	119.3 (79.3, 194.5)	76.3 (60.2, 122.8)	114.7 (92.5, 162.5)	119.3 (79.3, 194.5)	76.3 (60.2, 122.8)	114.7 (92.5, 162.5)
9-oxo-ODE	251.9 (108.7, 353.1)	265.4 (204.1, 329.4)	498.5 (123.1, 770.0)	128.3 (80.7, 222.5)	200.5 (109.0, 357.0)	265.6 (151.3, 362.3)	128.3 (80.7, 222.5)	200.5 (109.0, 357.0)	265.6 (151.3, 362.3)
13-oxo-ODE	19.8 (11.8, 34.7)	27.9 (18.6, 46.5)	58.9 (6.2, 102.5)	14.2 (6.8, 23.2)	19.1 (9.8, 60.8)	13.4 (9.3, 19.2)	14.2 (6.8, 23.2)	19.1 (9.8, 60.8)	13.4 (9.3, 19.2)
9(10)-EpOME	120.1 (59.5, 391.7)	208.4 (114.1, 310.7)	196.2 (125.4, 499.5)	14.2 (9.2, 25.2) ^a	93.5 (49.5, 185.0) ^b	175.0 (68.0, 520.0) ^b	14.2 (9.2, 25.2) ^a	93.5 (49.5, 185.0) ^b	175.0 (68.0, 520.0) ^b
12(13)-EpOME	600.5 (153.2, 1,255.0)	1,037.5 (364.7, 2,060.0)	583.8 (382.8, 2,765.3)	14.2 (12.9, 29.9) ^a	82.0 (39.6, 182.8) ^b	169.0 (73.0, 529.0) ^b	14.2 (12.9, 29.9) ^a	82.0 (39.6, 182.8) ^b	169.0 (73.0, 529.0) ^b
Total EpOME	848.6 (212.7, 1,518.6)	1,321.1 (478.8, 2,295.4)	740.3 (547.8, 3,264.8)	27.9 (22.5, 55.1) ^a	175.4 (88.2, 367.8) ^b	346.3 (141.0, 1,059.3) ^b	27.9 (22.5, 55.1) ^a	175.4 (88.2, 367.8) ^b	346.3 (141.0, 1,059.3) ^b
9,10-DiHOME	4.1 (3.4, 4.8) ^a	16.6 (11.5, 21.0) ^b	32.3 (21.5, 45.6) ^b	2.8 (2.2, 4.5) ^a	8.1 (7.1, 8.7) ^{ab}	16.1 (14.6, 24.1) ^b	2.8 (2.2, 4.5) ^a	8.1 (7.1, 8.7) ^{ab}	16.1 (14.6, 24.1) ^b
12,13-DiHOME	5.7 (4.8, 6.2) ^a	13.3 (6.9, 15.4) ^b	18.3 (12.0, 26.5) ^b	3.5 (2.1, 3.9) ^a	5.9 (5.5, 8.2) ^b	8.7 (7.9, 13.1) ^b	3.5 (2.1, 3.9) ^a	5.9 (5.5, 8.2) ^b	8.7 (7.9, 13.1) ^b
Total DiHOME	9.2 (8.8, 10.2) ^a	31.3 (18.3, 35.3) ^b	52.7 (33.4, 69.7) ^b	5.8 (4.8, 8.3) ^a	13.9 (13.1, 16.4) ^{ab}	24.0 (22.3, 37.2) ^b	5.8 (4.8, 8.3) ^a	13.9 (13.1, 16.4) ^{ab}	24.0 (22.3, 37.2) ^b
9,10,13-TriHOME				2.0 (1.2, 2.5)	2.1 (1.0, 3.3)	4.0 (1.8, 6.3)	2.0 (1.2, 2.5)	2.1 (1.0, 3.3)	4.0 (1.8, 6.3)
9,12,13-TriHOME				7.8 (6.4, 13.8)	10.3 (3.8, 14.9)	14.1 (7.6, 25.4)	7.8 (6.4, 13.8)	10.3 (3.8, 14.9)	14.1 (7.6, 25.4)
Total TriHOME				9.5 (7.8, 16.7)	12.2 (5.1, 18.2)	17.9 (9.3, 31.7)	9.5 (7.8, 16.7)	12.2 (5.1, 18.2)	17.9 (9.3, 31.7)
EKODE				13.0 (9.1, 16.4)	16.5 (10.6, 24.2)	21.6 (14.0, 27.1)	13.0 (9.1, 16.4)	16.5 (10.6, 24.2)	21.6 (14.0, 27.1)
DGLA									
15(S)-HETE	3.4 (1.4, 8.0)	2.3 (0.6, 4.0)	3.1 (0.5, 7.6)	3.9 (2.8, 4.6)	4.6 (0.8, 7.7)	2.1 (1.5, 4.1)	3.9 (2.8, 4.6)	4.6 (0.8, 7.7)	2.1 (1.5, 4.1)
AA									
5-HETE	23.5 (18.1, 35.9)	80.8 (68.5, 108.4)	138.0 (43.8, 212.6)	2.9 (2.3, 3.5)	5.8 (4.6, 8.3)	5.2 (4.4, 10.3)	2.9 (2.3, 3.5)	5.8 (4.6, 8.3)	5.2 (4.4, 10.3)
8-HETE	26.5 (16.1, 33.5)	28.0 (16.7, 34.3)	33.6 (13.8, 57.9)	2.2 (0.4, 8.0)	6.1 (1.7, 13.6)	3.2 (1.1, 6.7)	2.2 (0.4, 8.0)	6.1 (1.7, 13.6)	3.2 (1.1, 6.7)
12-HETE	13.5 (9.8, 24.5)	42.2 (30.8, 56.6)	64.1 (15.5, 123.5)	8.0 (3.7, 14.9) ^a	59.3 (24.1, 77.3) ^b	29.2 (20.1, 57.3) ^b	8.0 (3.7, 14.9) ^a	59.3 (24.1, 77.3) ^b	29.2 (20.1, 57.3) ^b
15-HETE	22.0 (13.4, 33.8)	54.9 (33.2, 71.9)	79.0 (18.4, 138.3)	5.8 (2.1, 8.0)	11.7 (5.8, 20.5)	8.5 (6.1, 11.9)	5.8 (2.1, 8.0)	11.7 (5.8, 20.5)	8.5 (6.1, 11.9)
20-HETE	20.3 (6.5, 32.9)	14.1 (12.3, 21.1)	16.5 (4.0, 43.7)	9.4 (6.6, 15.6)	5.1 (2.0, 17.4)	5.6 (3.4, 10.3)	9.4 (6.6, 15.6)	5.1 (2.0, 17.4)	5.6 (3.4, 10.3)
5-oxo-EETE	15.9 (3.4, 25.2)	27.5 (22.7, 37.8)	41.9 (2.3, 65.9)	9.3 (6.1, 15.9)	10.4 (6.2, 16.5)	4.5 (2.3, 17.6)	9.3 (6.1, 15.9)	10.4 (6.2, 16.5)	4.5 (2.3, 17.6)
15-oxo-EETE	9.9 (5.5, 11.7) ^a	36.2 (24.6, 62.9) ^b	35.6 (8.4, 91.1) ^{ab}	2.1 (1.1, 3.1) ^a	11.6 (4.8, 14.8) ^b	6.4 (4.5, 12.0) ^b	2.1 (1.1, 3.1) ^a	11.6 (4.8, 14.8) ^b	6.4 (4.5, 12.0) ^b

Compound	TOTAL					UNESTERIFIED				
	0.4% Group Median (IQR)	5.2% Group Median (IQR)	10.5% Group Median (IQR)	0.4% Group Median (IQR)	5.2% Group Median (IQR)	10.5% Group Median (IQR)	0.4% Group Median (IQR)	5.2% Group Median (IQR)	10.5% Group Median (IQR)	
5(6)-EpETe	19.7 (11.2, 92.1)	83.4 (32.0, 197.9)	85.3 (66.8, 307.4)							
8(9)-EpETe	0.5 (0.3, 1.2)	0.8 (0.4, 1.3)	0.7 (0.5, 1.0)							
11(12)-EpETe	277.1 (109.6, 514.7)	951.9 (409.7, 2,399.3)	596.3 (332.0, 2,079.0)	3.3 (1.9, 9.9) ^a	20.2 (12.6, 73.8) ^b	31.6 (14.2, 84.7) ^b				
14(15)-EpETe	400.9 (165.8, 1,157.2)	1,821.9 (812.5, 5,082.1)	1,193.8 (620.0, 4,524.4)	2.9 (0.9, 4.1) ^a	14.5 (7.3, 34.7) ^b	17.3 (10.8, 52.6) ^b				
5,6-DHETe	2.4 (2.2, 2.9) ^a	10.0 (6.5, 15.6) ^b	13.1 (9.4, 16.2) ^b	0.1 (0.0, 0.3)	0.2 (0.2, 0.5)	0.4 (0.1, 0.5)				
8,9-DHETe	0.7 (0.3, 1.1) ^a	2.3 (1.4, 2.8) ^b	1.1 (0.8, 1.9) ^{ab}							
11,12-DHETe	0.6 (0.5, 1.0)	2.1 (0.9, 3.5)	1.6 (1.1, 2.3)	0.4 (0.1, 0.6) ^a	1.1 (0.8, 1.3) ^b	1.1 (0.7, 2.1) ^b				
14,15-DHETe	0.5 (0.2, 0.8) ^a	2.8 (2.0, 4.2) ^b	2.8 (2.2, 4.7) ^b	0.3 (0.2, 1.0) ^a	1.8 (1.3, 2.4) ^b	1.9 (1.4, 2.1) ^b				
TXB2	0.5 (0.4, 0.8) ^a	1.8 (1.3, 3.2) ^b	1.9 (1.2, 2.1) ^b	0.4 (0.3, 0.8) ^a	1.6 (1.3, 2.0) ^b	1.6 (1.1, 3.2) ^b				
LTB3	3.1 (1.2, 4.1)	1.7 (0.6, 3.0)	2.4 (0.4, 4.9)							
LTB4	1.3 (0.1, 4.1)	0.1 (0.0, 1.1)	0.2 (0.1, 1.2)							
6-trans-LTB4	1.6 (0.0, 5.4)	0.7 (0.0, 3.4)	1.6 (0.3, 2.6)							
20-COOH-LTB4	14.2 (7.3, 25.8)	14.7 (10.3, 23.1)	12.5 (9.0, 35.6)	3.2 (1.3, 5.0)	2.0 (1.2, 3.4)	3.9 (1.6, 6.0)				
20-OH-LTB4	1.6 (1.4, 1.8)	0.7 (0.0, 1.7)	1.5 (0.2, 3.2)							
LXA4	5.8 (2.1, 6.7) ^a	0.7 (0.5, 1.8) ^b	0.3 (0.1, 1.8) ^b	0.3 (0.1, 0.5)	0.1 (0.0, 0.3)	0.3 (0.2, 0.5)				
11,12,15-TrHETe				0.3 (0.2, 0.4)	0.4 (0.3, 0.5)	0.5 (0.4, 0.8)				
n-3 Derivatives α-LNA										
9-HOTe	2.3 (0.2, 6.3)	1.8 (0.3, 2.8)	2.2 (1.4, 2.7)	7.3 (1.6, 11.0)	2.7 (1.9, 5.4)	3.0 (2.4, 6.4)				
9(10)-EpODE	26.2 (18.7, 103.1)	12.4 (6.9, 36.9)	12.5 (8.8, 31.3)	15.1 (9.3, 29.1)	13.4 (9.1, 42.3)	30.1 (8.6, 74.2)				
12(13)-EpODE	55.4 (19.5, 116.6)	30.1 (11.4, 45.3)	11.7 (6.0, 53.8)	5.8 (3.6, 16.0)	4.3 (2.7, 19.1)	10.0 (3.5, 30.6)				
15(16)-EpODE	466.4 (145.3, 790.0)	217.2 (78.2, 328.6)	99.7 (63.3, 375.7)	34.8 (14.1, 57.1)	24.9 (19.8, 67.2)	45.2 (18.1, 110.4)				
15,16-DiHODE	3.8 (3.1, 4.8)	4.2 (3.5, 5.9)	4.6 (3.9, 6.3)	3.8 (3.2, 5.8)	3.2 (2.6, 3.7)	3.6 (2.9, 4.8)				
EPA										
5-HEPE	62.4 (31.7, 72.8) ^a	10.4 (4.4, 14.9) ^b	6.3 (2.8, 16.2) ^b	9.5 (4.7, 14.4) ^a	1.4 (0.9, 4.2) ^{ab}	1.6 (0.3, 1.7) ^b				
12-HEPE	2,284.6 (683.6, 3,233.9) ^a	145.4 (80.6, 338.1) ^b	25.8 (0.1, 66.3) ^b							
15-HEPE	6.3 (4.9, 13.4)	3.9 (1.6, 6.6)	2.7 (0.1, 5.7)	9.9 (6.6, 15.2) ^a	4.7 (1.3, 11.9) ^{ab}	3.5 (2.2, 4.2) ^b				
8(9)-EpETE	41.9 (19.3, 140.5) ^a	2.9 (1.0, 11.1) ^{ab}	0.0 (0.0, 1.4) ^b							
11(12)-EpETE	171.4 (57.1, 267.0) ^a	8.9 (5.2, 21.9) ^b	4.0 (1.4, 5.1) ^b	6.4 (4.8, 10.5) ^a	1.0 (0.3, 2.7) ^b	1.5 (0.8, 3.2) ^b				
14(15)-EpETE	293.9 (131.8, 559.8) ^a	23.0 (6.4, 53.9) ^b	3.9 (3.1, 21.7) ^b	3.9 (3.7, 6.0)	3.0 (1.3, 5.2)	1.1 (0.4, 3.4)				

Compound	UNESTERIFIED							
	0.4% Group Median (IQR)	5.2% Group Median (IQR)	10.5% Group Median (IQR)	0.4% Group Median (IQR)	5.2% Group Median (IQR)	10.5% Group Median (IQR)	0.4% Group Median (IQR)	5.2% Group Median (IQR)
17(18)-EpETE	382.5 (220.8, 803.9) ^a	29.5 (10.9, 93.1) ^b	10.9 (7.4, 19.1) ^b	9.9 (8.6, 15.4) ^a	4.6 (2.4, 7.3) ^b	2.2 (1.6, 9.3) ^b		
Total EpETE	951.2 (431.2, 1,712.8) ^a	62.9 (26.0, 190.0) ^b	18.2 (13.1, 46.5) ^b					
5,15-DiHETE	0.8 (0.5, 1.6)	0.9 (0.3, 1.7)	1.7 (0.9, 2.0)					
8,15-DiHETE	0.7 (0.3, 1.1) ^a	2.3 (1.4, 2.8) ^b	1.1 (0.8, 1.9) ^{ab}					
17,18-DiHETE	1.7 (1.0, 3.6) ^a	0.7 (0.3, 1.0) ^{ab}	0.5 (0.3, 0.8) ^b	3.7 (3.1, 4.2) ^a	0.8 (0.3, 1.2) ^b	0.4 (0.2, 0.8) ^b		
PGD3				0.7 (0.4, 1.0)	0.4 (0.3, 0.6)	0.4 (0.4, 0.6)		
PGE3				0.1 (0.1, 0.2)	0.1 (0.1, 0.2)	0.1 (0.0, 0.1)		
DHA								
7(8)-EpDPE	1346.7 (627.4, 4,955.1)	489.8 (386.9, 941.5)	515.8 (199.5, 1,132.4)	96.2 (46.4, 187.1)	139.8 (47.5, 301.8)	108.8 (30.2, 260.8)		
10(11)-EpDPE	155.1 (37.6, 345.1)	75.7 (29.3, 112.0)	31.3 (19.8, 114.2)	8.3 (4.4, 11.1)	6.0 (3.8, 17.3)	3.2 (2.8, 9.7)		
13(14)-EpDPE	218.8 (78.7, 417.7)	103.6 (29.8, 245.8)	42.6 (19.8, 189.3)	2.8 (1.4, 4.1)	4.5 (1.0, 9.7)	1.0 (0.3, 4.4)		
16(17)-EpDPE	236.8 (75.9, 524.5)	133.8 (46.9, 332.6)	48.7 (31.8, 234.1)					
19(20)-EpDPE	504.3 (164.2, 1,047.8)	231.9 (95.6, 722.6)	133.4 (67.0, 448.5)	3.5 (2.8, 4.6)	6.6 (1.5, 21.0)	3.0 (0.9, 10.1)		
Total EpDPE	2,851.7 (1,074.4, 6,809.5)	1,241.8 (588.6, 2,201.9)	696.8 (401.6, 2,118.5)	105.6 (55.4, 204.6)	157.4 (61.3, 339.4)	119.0 (34.6, 281.4)		
17-HDoHE	14.5 (8.7, 35.6)	3.1 (1.9, 13.2)	6.0 (1.3, 17.1)	5.1 (4.2, 8.4)	4.7 (0.6, 7.1)	2.8 (1.3, 5.6)		
10,11-DiHDPE				0.7 (0.4, 0.9) ^a	0.2 (0.1, 0.4) ^{ab}	0.2 (0.1, 0.3) ^b		
13,14-DiHDPE				0.4 (0.3, 0.4) ^a	0.2 (0.2, 0.4) ^{ab}	0.1 (0.1, 0.3) ^b		
16,17-DiHDPE	1.0 (0.6, 1.6)	0.9 (0.6, 1.7)	1.0 (0.5, 1.5)	1.3 (0.7, 2.2) ^a	0.5 (0.3, 1.0) ^{ab}	0.4 (0.3, 0.7) ^b		
19,20-DiHDPE	6.8 (4.4, 9.6)	2.5 (1.3, 4.2)	2.6 (1.3, 4.5)	3.3 (2.3, 6.5) ^a	2.1 (1.5, 3.7) ^{ab}	1.6 (1.2, 2.0) ^b		

Data are presented as median and interquartile ranges (IQR) of n=8 per group. The lower and upper boundaries of the IQR represent 25th and 75th percentile values, respectively. Data were analyzed by Kruskal Wallis one-way analysis of variance (ANOVA) followed by Dunn's post-hoc test. Different alphabetical superscripts mean that the groups differed significantly (P<0.05) from each other.

Table 3

Cerebral cortex oxylipin concentrations (pmol/g wet weight)

Compound	TOTAL					UNESTERIFIED				
	0.4% Group Median (IQR)	5.2% Group Median (IQR)	10.5% Group Median (IQR)	0.4% Group Median (IQR)	5.2% Group Median (IQR)	10.5% Group Median (IQR)				
n-6 Derivatives s La										
9-HODE	15.5 (13.8, 20.5) ^a	32.4 (27.4, 39.7) ^{ab}	43.3 (40.2, 48.1) ^b	12.7 (12.5, 18.9) ^a	29.0 (26.8, 32.0) ^{ab}	30.7 (27.5, 40.3) ^b				
13-HODE	21.8 (19.6, 25.4) ^a	43.6 (34.9, 50.5) ^{ab}	59.0 (57.4, 65.9) ^b	17.4 (15.2, 23.7) ^a	40.8 (34.5, 44.1) ^{ab}	48.6 (40.5, 54.0) ^b				
Total HODE	37.3 (33.4, 45.9) ^a	76.0 (62.3, 90.2) ^{ab}	101.5 (99.2, 114.0) ^b	30.1 (27.7, 42.6) ^a	69.8 (61.3, 76.3) ^{ab}	79.2 (67.6, 94.4) ^b				
9-oxo-ODE	10.4 (7.7, 15.0)	19.9 (13.6, 20.9)	18.4 (13.1, 22.5)	12.3 (10.1, 16.2) ^a	15.9 (11.7, 19.7) ^{ab}	21.0 (18.2, 29.7) ^b				
13-oxo-ODE	7.9 (6.7, 8.9)	8.3 (5.5, 9.4)	6.9 (5.0, 12.2)	9.8 (7.4, 12.5)	8.0 (6.1, 10.3)	9.7 (6.2, 13.2)				
9(10)-EpOME	13.3 (9.5, 307.0) ^a	24.3 (11.7, 576.6) ^{ab}	1,226.1 (586.7, 1,837.4) ^b	11.2 (6.3, 12.8) ^a	19.4 (13.3, 27.2) ^{ab}	25.3 (18.7, 29.8) ^b				
12(13)-EpOME	12.2 (9.1, 16.1)	16.7 (10.6, 17.0)	27.2 (18.8, 40.9)	12.5 (7.0, 15.2) ^a	24.0 (16.6, 32.2) ^{ab}	27.3 (22.5, 43.2) ^b				
Total EpOME	32.2 (22.9, 316.2) ^a	57.9 (28.4, 593.6) ^{ab}	1,252.5 (605.5, 1,860.3) ^b	23.7 (13.3, 28.0) ^a	43.5 (29.8, 59.3) ^{ab}	52.6 (41.2, 73.0) ^b				
9,10-DiHOME	2.0 (1.2, 2.5) ^a	3.0 (2.9, 3.6) ^{ab}	4.7 (4.2, 5.0) ^b	1.5 (1.1, 2.3) ^a	2.9 (2.4, 3.4) ^{ab}	4.3 (3.7, 4.7) ^b				
12,13-DiHOME	2.3 (1.9, 3.2) ^a	3.5 (3.4, 3.6) ^{ab}	4.4 (3.4, 5.2) ^b	1.7 (1.7, 3.2) ^a	3.1 (2.7, 3.7) ^{ab}	4.0 (3.3, 4.7) ^b				
Total DiHOME	4.4 (3.1, 5.7) ^a	6.5 (6.4, 7.0) ^{ab}	9.3 (7.5, 9.9) ^b	3.2 (2.8, 5.5) ^a	6.0 (5.0, 7.1) ^{ab}	8.3 (7.2, 9.3) ^b				
9,10,13-TriHOME				0.3 (0.2, 0.4) ^a	0.8 (0.5, 1.0) ^{ab}	1.0 (0.7, 1.1) ^b				
9,12,13-TriHOME				3.0 (2.0, 3.9) ^a	6.6 (6.0, 7.3) ^{ab}	7.1 (4.8, 8.7) ^b				
Total TriHOME				3.3 (2.2, 4.4) ^a	7.4 (6.7, 8.2) ^{ab}	8.1 (5.5, 9.6) ^b				
EKODE				0.4 (0.2, 0.6)	0.5 (0.5, 0.5)	0.5 (0.4, 0.6)				
DGLA										
PGDI				2.2 (2.0, 3.2)	2.5 (2.4, 2.7)	2.7 (2.6, 2.8)				
15(S)-HETE	16.5 (7.9, 19.7)	12.1 (10.2, 13.7)	9.5 (8.5, 14.4)	12.7 (7.4, 23.5)	11.6 (8.4, 12.8)	11.3 (8.8, 14.5)				
AA										
5-HETE	50.4 (34.9, 61.1)	54.9 (53.8, 78.1)	58.1 (44.1, 72.6)	41.0 (27.1, 46.3)	55.6 (37.6, 61.9)	53.0 (43.9, 54.8)				
8-HETE	36.8 (21.6, 48.7)	35.2 (32.0, 46.1)	37.7 (33.1, 58.7)	40.0 (25.8, 46.7)	44.4 (31.8, 50.9)	47.4 (41.6, 54.2)				
9-HETE	166.3 (119.8, 219.9)	206.2 (198.9, 240.4)	212.3 (180.2, 325.1)	167.0 (118.0, 236.6)	229.6 (173.5, 297.2)	244.4 (211.5, 297.2)				
12-HETE	387.0 (157.3, 592.8)	360.5 (315.0, 686.9)	461.0 (359.9, 620.9)	392.3 (220.7, 737.7)	534.9 (375.6, 693.6)	693.4 (420.9, 780.0)				
15-HETE	187.3 (130.8, 259.3)	251.0 (238.5, 296.6)	217.4 (208.0, 374.0)	217.9 (135.3, 313.8)	323.8 (239.8, 329.1)	308.8 (254.6, 381.7)				
20-HETE	12.3 (9.6, 15.8)	10.1 (8.0, 18.8)	10.3 (8.9, 14.5)	14.9 (11.2, 16.5)	14.1 (10.5, 15.0)	12.3 (11.3, 19.4)				

Compound	TOTAL						UNESTERIFIED					
	0.4% Group Median (IQR)	5.2% Group Median (IQR)	10.5% Group Median (IQR)	0.4% Group Median (IQR)	5.2% Group Median (IQR)	10.5% Group Median (IQR)	0.4% Group Median (IQR)	5.2% Group Median (IQR)	10.5% Group Median (IQR)	0.4% Group Median (IQR)	5.2% Group Median (IQR)	10.5% Group Median (IQR)
5-oxo-ETE	8.6 (5.8, 16.3)	9.0 (8.6, 12.8)	9.6 (8.0, 18.1)	26.1 (14.5, 30.6)	26.8 (20.8, 27.2)	30.2 (21.4, 31.3)	26.1 (14.5, 30.6)	26.8 (20.8, 27.2)	30.2 (21.4, 31.3)	26.1 (14.5, 30.6)	26.8 (20.8, 27.2)	30.2 (21.4, 31.3)
12-oxo-ETE	88.7 (71.6, 147.3)	145.7 (54.0, 147.7)	79.6 (70.3, 144.7)	258.0 (180.7, 281.3)	325.9 (304.3, 359.3)	276.9 (249.3, 401.1)	258.0 (180.7, 281.3)	325.9 (304.3, 359.3)	276.9 (249.3, 401.1)	258.0 (180.7, 281.3)	325.9 (304.3, 359.3)	276.9 (249.3, 401.1)
15-oxo-ETE	13.3 (10.8, 19.3)	18.6 (9.5, 20.5)	15.5 (14.3, 28.7)	28.8 (18.2, 44.7)	40.1 (24.0, 41.9)	41.5 (34.5, 56.0)	28.8 (18.2, 44.7)	40.1 (24.0, 41.9)	41.5 (34.5, 56.0)	28.8 (18.2, 44.7)	40.1 (24.0, 41.9)	41.5 (34.5, 56.0)
5(6)-EpETE	3.2 (2.1, 43.8)	4.1 (4.1, 27.3)	1.9 (0.3, 142.5)	114.7 (54.2, 172.7)	141.1 (94.3, 191.9)	188.6 (126.6, 223.5)	114.7 (54.2, 172.7)	141.1 (94.3, 191.9)	188.6 (126.6, 223.5)	114.7 (54.2, 172.7)	141.1 (94.3, 191.9)	188.6 (126.6, 223.5)
11(12)-EpETE	0.2 (0.2, 125.6)	4.7 (0.2, 106.2)	0.2 (0.1, 7.6)	386.9 (171.7, 510.4)	472.5 (237.4, 649.8)	596.0 (391.9, 874.2)	386.9 (171.7, 510.4)	472.5 (237.4, 649.8)	596.0 (391.9, 874.2)	386.9 (171.7, 510.4)	472.5 (237.4, 649.8)	596.0 (391.9, 874.2)
14(15)-EpETE	1.1 (0.4, 66.7)	3.3 (0.9, 79.8)	1.5 (0.6, 2.8)	360.6 (140.8, 530.6)	348.3 (173.1, 656.6)	562.0 (389.0, 1,043.7)	360.6 (140.8, 530.6)	348.3 (173.1, 656.6)	562.0 (389.0, 1,043.7)	360.6 (140.8, 530.6)	348.3 (173.1, 656.6)	562.0 (389.0, 1,043.7)
5,6-DHETE	2.0 (1.5, 2.7)	2.2 (1.8, 2.3)	3.6 (3.0, 4.1)	0.4 (0.3, 0.6)	0.6 (0.5, 0.8)	0.8 (0.6, 1.0)	0.4 (0.3, 0.6)	0.6 (0.5, 0.8)	0.8 (0.6, 1.0)	0.4 (0.3, 0.6)	0.6 (0.5, 0.8)	0.8 (0.6, 1.0)
8,9-DHETE	1.0 (0.8, 1.2) ^a	1.3 (1.2, 1.6) ^{ab}	1.8 (1.7, 2.0) ^b	0.9 (0.6, 1.1)	1.5 (1.1, 1.6)	1.8 (1.2, 1.9)	0.9 (0.6, 1.1)	1.5 (1.1, 1.6)	1.8 (1.2, 1.9)	0.9 (0.6, 1.1)	1.5 (1.1, 1.6)	1.8 (1.2, 1.9)
11,12-DHETE	1.3 (0.9, 2.0)	1.6 (1.4, 2.0)	2.0 (2.0, 2.1)	1.3 (1.0, 1.5) ^a	1.8 (1.6, 2.0) ^{ab}	2.1 (1.8, 2.4) ^b	1.3 (1.0, 1.5) ^a	1.8 (1.6, 2.0) ^{ab}	2.1 (1.8, 2.4) ^b	1.3 (1.0, 1.5) ^a	1.8 (1.6, 2.0) ^{ab}	2.1 (1.8, 2.4) ^b
14,15-DHETE	2.4 (2.1, 3.5)	2.8 (2.6, 3.7)	3.6 (3.5, 3.8)	2.1 (1.7, 2.8) ^a	3.3 (2.6, 3.5) ^{ab}	3.5 (3.3, 3.9) ^b	2.1 (1.7, 2.8) ^a	3.3 (2.6, 3.5) ^{ab}	3.5 (3.3, 3.9) ^b	2.1 (1.7, 2.8) ^a	3.3 (2.6, 3.5) ^{ab}	3.5 (3.3, 3.9) ^b
PGD2				94.2 (88.3, 118.8) ^a	132.1 (128.6, 135.5) ^{ab}	139.3 (137.0, 150.7) ^b	94.2 (88.3, 118.8) ^a	132.1 (128.6, 135.5) ^{ab}	139.3 (137.0, 150.7) ^b	94.2 (88.3, 118.8) ^a	132.1 (128.6, 135.5) ^{ab}	139.3 (137.0, 150.7) ^b
PGE2				8.4 (7.7, 14.2)	13.0 (12.1, 15.7)	13.3 (12.9, 14.3)	8.4 (7.7, 14.2)	13.0 (12.1, 15.7)	13.3 (12.9, 14.3)	8.4 (7.7, 14.2)	13.0 (12.1, 15.7)	13.3 (12.9, 14.3)
PGF2a				46.5 (33.9, 65.2)	66.9 (63.6, 92.5)	74.9 (72.2, 84.5)	46.5 (33.9, 65.2)	66.9 (63.6, 92.5)	74.9 (72.2, 84.5)	46.5 (33.9, 65.2)	66.9 (63.6, 92.5)	74.9 (72.2, 84.5)
PGJ2				3.1 (2.5, 8.3)	5.4 (3.9, 6.6)	7.1 (5.9, 7.8)	3.1 (2.5, 8.3)	5.4 (3.9, 6.6)	7.1 (5.9, 7.8)	3.1 (2.5, 8.3)	5.4 (3.9, 6.6)	7.1 (5.9, 7.8)
TXB2	46.6 (40.0, 55.8)	63.6 (56.1, 67.4)	72.3 (59.8, 83.5)	56.8 (54.7, 65.2)	69.2 (66.1, 85.4)	78.4 (71.4, 85.8)	56.8 (54.7, 65.2)	69.2 (66.1, 85.4)	78.4 (71.4, 85.8)	56.8 (54.7, 65.2)	69.2 (66.1, 85.4)	78.4 (71.4, 85.8)
6-trans-LTB4	0.5 (0.1, 0.9)	0.0 (0.0, 1.3)	0.6 (0.1, 1.1)				0.5 (0.1, 0.9)	0.0 (0.0, 1.3)	0.6 (0.1, 1.1)			
6-keto-PGF1a	840.2 (87.3, 2,478.4)	424.8 (83.8, 3,798.8)	1,200.8 (432.5, 1,518.1)	36.7 (33.5, 55.5)	61.0 (52.7, 69.1)	54.7 (49.6, 73.2)	36.7 (33.5, 55.5)	61.0 (52.7, 69.1)	54.7 (49.6, 73.2)	36.7 (33.5, 55.5)	61.0 (52.7, 69.1)	54.7 (49.6, 73.2)
n-3 Derivative s α-LNA												
9-HOTHE	0.6 (0.3, 0.8)	1.0 (0.8, 1.0)	0.8 (0.8, 1.2)	0.6 (0.5, 1.0)	0.9 (0.7, 1.0)	1.1 (0.7, 1.4)	0.6 (0.5, 1.0)	0.9 (0.7, 1.0)	1.1 (0.7, 1.4)	0.6 (0.5, 1.0)	0.9 (0.7, 1.0)	1.1 (0.7, 1.4)
13-HOTHE	1.3 (0.9, 1.6)	1.6 (1.1, 1.6)	1.7 (1.1, 1.9)	1.5 (0.9, 1.9)	1.3 (1.2, 1.7)	1.8 (1.4, 2.2)	1.5 (0.9, 1.9)	1.3 (1.2, 1.7)	1.8 (1.4, 2.2)	1.5 (0.9, 1.9)	1.3 (1.2, 1.7)	1.8 (1.4, 2.2)
9(10)-EpODE	0.9 (0.7, 1.1)	0.8 (0.6, 1.5)	1.6 (1.1, 2.2)	1.4 (0.7, 1.6)	1.5 (0.9, 2.3)	1.8 (1.4, 2.9)	1.4 (0.7, 1.6)	1.5 (0.9, 2.3)	1.8 (1.4, 2.9)	1.4 (0.7, 1.6)	1.5 (0.9, 2.3)	1.8 (1.4, 2.9)
12(13)-EpODE	0.7 (0.2, 0.9)	0.2 (0.1, 0.4)	0.8 (0.5, 1.0)	0.6 (0.5, 1.4)	0.9 (0.2, 1.7)	0.9 (0.7, 1.5)	0.6 (0.5, 1.4)	0.9 (0.2, 1.7)	0.9 (0.7, 1.5)	0.6 (0.5, 1.4)	0.9 (0.2, 1.7)	0.9 (0.7, 1.5)
15(16)-EpODE	3.6 (2.1, 4.2)	2.4 (2.4, 3.3)	3.9 (3.1, 5.1)	3.7 (2.5, 5.1)	4.2 (2.2, 8.0)	4.0 (2.9, 9.5)	3.7 (2.5, 5.1)	4.2 (2.2, 8.0)	4.0 (2.9, 9.5)	3.7 (2.5, 5.1)	4.2 (2.2, 8.0)	4.0 (2.9, 9.5)
15,16-DIHODE	0.6 (0.3, 1.0)	0.7 (0.5, 0.7)	1.0 (0.8, 1.1)	0.4 (0.4, 0.9)	0.7 (0.5, 0.9)	0.9 (0.8, 1.2)	0.4 (0.4, 0.9)	0.7 (0.5, 0.9)	0.9 (0.8, 1.2)	0.4 (0.4, 0.9)	0.7 (0.5, 0.9)	0.9 (0.8, 1.2)
EPA												
5-HEPE	2.8 (2.6, 3.7) ^a	1.0 (0.8, 1.2) ^b	0.7 (0.4, 1.0) ^b	4.2 (2.5, 5.6) ^a	1.0 (0.8, 1.1) ^b	1.0 (0.4, 1.0) ^b	4.2 (2.5, 5.6) ^a	1.0 (0.8, 1.1) ^b	1.0 (0.4, 1.0) ^b	4.2 (2.5, 5.6) ^a	1.0 (0.8, 1.1) ^b	1.0 (0.4, 1.0) ^b
12-HEPE	98.6 (46.4, 127.9)	75.0 (52.0, 86.1)	106.9 (56.3, 232.6)	134.6 (43.1, 235.4)	72.2 (51.0, 286.1)	241.8 (127.1, 398.8)	134.6 (43.1, 235.4)	72.2 (51.0, 286.1)	241.8 (127.1, 398.8)	134.6 (43.1, 235.4)	72.2 (51.0, 286.1)	241.8 (127.1, 398.8)
15-HEPE	12.5 (8.1, 16.8) ^a	3.0 (1.5, 3.0) ^b	1.2 (1.1, 3.0) ^b	15.1 (8.5, 20.3) ^a	2.1 (1.6, 4.3) ^{ab}	1.6 (1.1, 6.3) ^b	15.1 (8.5, 20.3) ^a	2.1 (1.6, 4.3) ^{ab}	1.6 (1.1, 6.3) ^b	15.1 (8.5, 20.3) ^a	2.1 (1.6, 4.3) ^{ab}	1.6 (1.1, 6.3) ^b

Compound	TOTAL						UNESTERIFIED					
	0.4% Group Median (IQR)	5.2% Group Median (IQR)	10.5% Group Median (IQR)	0.4% Group Median (IQR)	5.2% Group Median (IQR)	10.5% Group Median (IQR)	0.4% Group Median (IQR)	5.2% Group Median (IQR)	10.5% Group Median (IQR)	0.4% Group Median (IQR)	5.2% Group Median (IQR)	10.5% Group Median (IQR)
8(9)-EpETE	3.8 (2.0, 7.0) ^a	0.3 (0.1, 0.9) ^b	0.6 (0.1, 1.1) ^b	5.6 (2.3, 6.8) ^a	0.3 (0.1, 0.9) ^b	0.6 (0.3, 0.9) ^b	8.7 (3.4, 12.8)	2.6 (1.5, 4.7)	6.5 (2.8, 7.3)	11.1 (4.0, 16.0) ^b	0.9 (0.5, 1.7) ^b	1.1 (0.9, 1.7) ^b
11(12)-EpETE	6.3 (3.9, 6.6)	1.9 (1.4, 2.4)	3.4 (1.8, 6.5)	7.5 (2.8, 12.3) ^a	0.8 (0.2, 1.6) ^b	0.9 (0.5, 1.3) ^b	11.1 (4.0, 16.0) ^b	0.9 (0.5, 1.7) ^b	1.1 (0.9, 1.7) ^b	33.5 (11.2, 46.7) ^a	6.4 (2.4, 8.7) ^b	9.5 (5.2, 11.0) ^b
14(15)-EpETE	5.2 (4.5, 6.4) ^a	0.3 (0.1, 0.4) ^b	0.3 (0.2, 0.3) ^b	11.1 (4.0, 16.0) ^b	0.6 (0.1, 1.0) ^b	0.9 (0.5, 1.7) ^b	33.5 (11.2, 46.7) ^a	1.1 (0.9, 1.5)	1.4 (1.1, 1.8)	1.4 (1.1, 1.6)	4.6 (3.6, 6.8)	4.6 (3.6, 6.8)
17(18)-EpETE	7.1 (5.5, 12.8) ^a	0.9 (0.8, 0.9) ^b	0.6 (0.1, 1.0) ^b	1.3 (1.1, 1.9) ^a	0.5 (0.4, 0.6) ^b	0.5 (0.4, 0.6) ^b	0.7 (0.6, 0.9) ^a	0.1 (0.1, 0.2) ^b	0.1 (0.1, 0.2) ^b	706.5 (386.9, 986.8)	737.3 (297.9, 1,003.4)	778.0 (467.3, 931.8)
Total EpETE	22.6 (15.8, 32.3) ^a	4.1 (2.8, 4.9) ^b	6.1 (2.3, 8.6) ^b	706.5 (386.9, 986.8)	737.3 (297.9, 1,003.4)	778.0 (467.3, 931.8)	706.5 (386.9, 986.8)	737.3 (297.9, 1,003.4)	778.0 (467.3, 931.8)	706.5 (386.9, 986.8)	737.3 (297.9, 1,003.4)	778.0 (467.3, 931.8)
5,15-DiHETE	2.1 (1.6, 2.4)	1.7 (1.6, 2.9)	2.6 (1.8, 3.3)	49.8 (26.7, 74.3)	64.8 (35.0, 75.6)	61.3 (37.7, 73.5)	49.8 (26.7, 74.3)	52.5 (20.8, 76.1)	61.3 (37.7, 73.5)	49.8 (26.7, 74.3)	52.5 (20.8, 76.1)	61.3 (37.7, 73.5)
8,15-DiHETE	5.7 (4.8, 6.2)	6.7 (6.7, 7.0)	7.4 (5.2, 8.3)	35.8 (18.3, 52.7)	45.0 (32.5, 59.9)	41.8 (24.9, 63.7)	35.8 (18.3, 52.7)	34.6 (14.8, 53.9)	41.8 (24.9, 63.7)	35.8 (18.3, 52.7)	34.6 (14.8, 53.9)	41.8 (24.9, 63.7)
17,18-DiHETE	2.0 (1.6, 2.0) ^a	0.4 (0.3, 0.5) ^b	0.6 (0.5, 0.6) ^b	37.4 (19.5, 59.1)	50.4 (33.5, 74.1)	47.0 (28.9, 71.3)	37.4 (19.5, 59.1)	31.2 (15.2, 52.2)	47.0 (28.9, 71.3)	63.5 (33.0, 94.8)	61.2 (22.4, 87.5)	72.2 (50.5, 115.4)
PGE3	801.8 (712.0, 841.5)	766.0 (663.3, 996.1)	1,019.1 (711.6, 1,331.6)	893.1 (484.4, 1,267.8)	964.5 (371.2, 1,273.1)	1,058.9 (609.3, 1,257.2)	893.1 (484.4, 1,267.8)	964.5 (371.2, 1,273.1)	1,058.9 (609.3, 1,257.2)	893.1 (484.4, 1,267.8)	964.5 (371.2, 1,273.1)	1,058.9 (609.3, 1,257.2)
DHA	23.4 (14.9, 41.3)	19.7 (19.4, 21.0)	23.2 (21.1, 24.4)	20.3 (11.8, 39.6)	15.2 (12.6, 17.8)	14.4 (12.3, 22.3)	20.3 (11.8, 39.6)	15.2 (12.6, 17.8)	14.4 (12.3, 22.3)	20.3 (11.8, 39.6)	15.2 (12.6, 17.8)	14.4 (12.3, 22.3)
7(8)-EpDPE	608.6 (530.4, 673.3)	576.0 (514.5, 744.7)	753.8 (545.1, 973.3)	706.5 (386.9, 986.8)	737.3 (297.9, 1,003.4)	778.0 (467.3, 931.8)	706.5 (386.9, 986.8)	737.3 (297.9, 1,003.4)	778.0 (467.3, 931.8)	706.5 (386.9, 986.8)	737.3 (297.9, 1,003.4)	778.0 (467.3, 931.8)
10(11)-EpDPE	41.5 (39.7, 47.6)	53.1 (33.9, 55.9)	64.8 (35.0, 75.6)	49.8 (26.7, 74.3)	64.8 (35.0, 75.6)	61.3 (37.7, 73.5)	49.8 (26.7, 74.3)	52.5 (20.8, 76.1)	61.3 (37.7, 73.5)	49.8 (26.7, 74.3)	52.5 (20.8, 76.1)	61.3 (37.7, 73.5)
13(14)-EpDPE	31.4 (30.2, 40.9)	31.8 (27.5, 43.1)	45.0 (32.5, 59.9)	35.8 (18.3, 52.7)	45.0 (32.5, 59.9)	41.8 (24.9, 63.7)	35.8 (18.3, 52.7)	34.6 (14.8, 53.9)	41.8 (24.9, 63.7)	35.8 (18.3, 52.7)	34.6 (14.8, 53.9)	41.8 (24.9, 63.7)
16(17)-EpDPE	40.1 (28.7, 41.3)	33.8 (29.9, 48.6)	50.4 (33.5, 74.1)	37.4 (19.5, 59.1)	50.4 (33.5, 74.1)	47.0 (28.9, 71.3)	37.4 (19.5, 59.1)	31.2 (15.2, 52.2)	47.0 (28.9, 71.3)	63.5 (33.0, 94.8)	61.2 (22.4, 87.5)	72.2 (50.5, 115.4)
19(20)-EpDPE	69.5 (62.4, 80.6)	71.3 (57.4, 103.6)	103.4 (65.5, 133.8)	63.5 (33.0, 94.8)	103.4 (65.5, 133.8)	115.4 (72.2, 158.6)	63.5 (33.0, 94.8)	61.2 (22.4, 87.5)	72.2 (50.5, 115.4)	893.1 (484.4, 1,267.8)	964.5 (371.2, 1,273.1)	1,058.9 (609.3, 1,257.2)
Total EpDPE	801.8 (712.0, 841.5)	766.0 (663.3, 996.1)	1,019.1 (711.6, 1,331.6)	893.1 (484.4, 1,267.8)	964.5 (371.2, 1,273.1)	1,058.9 (609.3, 1,257.2)	893.1 (484.4, 1,267.8)	964.5 (371.2, 1,273.1)	1,058.9 (609.3, 1,257.2)	893.1 (484.4, 1,267.8)	964.5 (371.2, 1,273.1)	1,058.9 (609.3, 1,257.2)
17-HDoHE	23.4 (14.9, 41.3)	19.7 (19.4, 21.0)	23.2 (21.1, 24.4)	20.3 (11.8, 39.6)	15.2 (12.6, 17.8)	14.4 (12.3, 22.3)	20.3 (11.8, 39.6)	15.2 (12.6, 17.8)	14.4 (12.3, 22.3)	20.3 (11.8, 39.6)	15.2 (12.6, 17.8)	14.4 (12.3, 22.3)
4,5-DiHDPE	3.7 (2.8, 6.3) ^{ab}	2.7 (2.6, 2.8) ^a	4.9 (3.7, 5.9) ^b	0.7 (0.3, 0.8)	0.4 (0.3, 0.4)	0.6 (0.2, 0.6)	0.7 (0.3, 0.8)	0.4 (0.3, 0.4)	0.6 (0.2, 0.6)	0.7 (0.3, 0.8)	0.4 (0.3, 0.4)	0.6 (0.2, 0.6)
7,8-DiHDPE	0.3 (0.1, 0.4)	0.3 (0.2, 0.3)	0.3 (0.3, 0.4)	0.4 (0.3, 0.5)	0.3 (0.3, 0.5)	0.4 (0.3, 0.5)	0.4 (0.3, 0.5)	0.3 (0.2, 0.4)	0.4 (0.3, 0.5)	0.4 (0.3, 0.5)	0.3 (0.2, 0.4)	0.4 (0.3, 0.5)
10,11-DiHDPE	0.4 (0.3, 0.5)	0.3 (0.3, 0.5)	0.5 (0.4, 0.6)	0.4 (0.3, 0.6)	0.5 (0.4, 0.6)	0.6 (0.5, 0.7)	0.4 (0.3, 0.6)	0.4 (0.4, 0.5)	0.5 (0.4, 0.6)	0.5 (0.3, 0.7)	0.5 (0.5, 0.5)	0.6 (0.5, 0.7)
13,14-DiHDPE	0.5 (0.4, 0.7)	0.5 (0.4, 0.6)	0.7 (0.6, 0.8)	0.5 (0.3, 0.7)	0.7 (0.6, 0.8)	0.8 (0.7, 0.9)	0.5 (0.3, 0.7)	0.5 (0.5, 0.5)	0.6 (0.5, 0.7)	1.1 (0.8, 1.6)	1.3 (0.9, 1.3)	1.3 (1.0, 1.6)
16,17-DiHDPE	1.2 (1.1, 1.5)	1.2 (1.2, 1.2)	1.3 (1.3, 1.7)	1.1 (0.8, 1.6)	1.3 (1.3, 1.7)	1.6 (1.5, 1.7)	1.1 (0.8, 1.6)	1.3 (0.9, 1.3)	1.3 (1.0, 1.6)	6.1 (4.3, 7.4)	5.5 (4.8, 5.8)	5.9 (5.3, 6.3)
19,20-DiHDPE	5.9 (5.0, 8.2)	6.0 (5.5, 6.1)	6.7 (6.2, 8.0)	6.1 (4.3, 7.4)	6.0 (5.5, 6.1)	6.7 (6.2, 8.0)	6.1 (4.3, 7.4)	5.5 (4.8, 5.8)	5.9 (5.3, 6.3)	6.1 (4.3, 7.4)	5.5 (4.8, 5.8)	5.9 (5.3, 6.3)

Data are presented as median and interquartile ranges (IQR) of n=6-7 per group. The lower and upper boundaries of the IQR represent 25th and 75th percentile values, respectively. The 0.4% and 5.2% LA groups contained six samples each and the 10.5% LA group contained seven samples. Data were analyzed by Kruskal Wallis one-way analysis of variance (ANOVA) followed by Dunn's post-hoc test. Different alphabetical superscripts mean that the groups differed significantly (P<0.05) from each other.

Table 4

Plasma and cortex oxylipins that changed significantly in the total or unesterified pools

	TOTAL		UNESTERIFIED	
	Plasma	Cortex	Plasma	Cortex
n-6 Derivatives LA				
9-HODE	↔	↑(10.5%)	↔	↑(10.5%)
13-HODE	↔	↑(10.5%)	↔	↑(10.5%)
Total HODE	↔	↑(10.5%)	↔	↑(10.5%)
9-oxo-ODE	↔	↔	↔	↑(10.5%)
9(10)-EpOME	↔	↑(10.5%)	↑↑	↑(10.5%)
12(13)-EpOME	↔	↔	↑↑	↑(10.5%)
Total EpOME	↔	↑(10.5%)	↑↑	↑(10.5%)
9,10-DiHOME	↑↑	↑(10.5%)	↑(10.5%)	↑(10.5%)
12,13-DiHOME	↑↑	↑(10.5%)	↑↑	↑(10.5%)
Total DiHOME	↑↑	↑(10.5%)	↑(10.5%)	↑(10.5%)
9,10,13-TriHOME	ND	ND	↔	↑(10.5%)
9,12,13-TriHOME	ND	ND	↔	↑(10.5%)
Total TriHOME	ND	ND	↔	↑(10.5%)
AA				
5-HETE	↔	↔	↔	↔
12-HETE	↔	↔	↑↑	↔
15-oxo-EETE	↑(5.2%)	↔	↑↑	↔
11(12)-EpETrE	↔	↔	↑↑	↔
14(15)-EpETrE	↔	↔	↑↑	↔
5,6-DiHETrE	↑↑	↔	↔	↔
8,9-DiHETrE	↑(5.2%)	↑(10.5%)	ND	↔
11,12-DiHETrE	↔	↔	↑↑	↑(10.5%)
14,15-DiHETrE	↑↑	↔	↑↑	↑(10.5%)
PGD2	ND	ND	ND	↑(10.5%)
TXB2	↑↑	↔	↑↑	↔
LXA4	↓↓	ND	↔	ND
n-3 Derivatives EPA				
5-HEPE	↓↓	↓↓	↓(10.5%)	↓↓
12-HEPE	↓↓	↔	ND	↔
15-HEPE	↔	↓↓	↓(10.5%)	↓(10.5%)
8(9)-EpETE	↓(10.5%)	↓↓	ND	↓↓
11(12)-EpETE	↓↓	↔	↓↓	↔
14(15)-EpETE	↓↓	↓↓	↔	↓↓
17(18)-EpETE	↓↓	↓↓	ND	↓↓
Total EpETE	↓↓	↓↓	↓↓	↓↓
8,15-DiHETE	↑(5.2%)	↔	ND	↔
17,18-DiHETE	↓(10.5%)	↓↓	↓↓	↓↓

	TOTAL		UNESTERIFIED	
	Plasma	Cortex	Plasma	Cortex
PGE3	ND	ND	↔	↓↓
DHA				
10,11-DiHDPE	ND	↔	↓(10.5%)	↔
13,14-DiHDPE	ND	↔	↓(10.5%)	↔
16,17-DiHDPE	↔	↔	↓(10.5%)	↔
19,20-DiHDPE	↔	↔	↓(10.5%)	↔

One arrow means that the 5.2% and/or 10.5% LA group differed significantly from 0.4% LA controls. Parenthesis indicate the specific group (5.2% or 10.5%) that differed from the 0.4% LA group. Two arrows mean that both the 5.2% and 10.5% LA groups differed significantly from 0.4% LA. A left-right arrow (↔) means that there were no significant differences between any groups. ND means that the metabolites were not detected.