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Publication Date

2017-06-01

DOI

10.1016/j.psychres.2017.02.056

Peer reviewed

Altered soluble epoxide hydrolase-derived oxylipins in patients with seasonal major depression: An exploratory study

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Abstract

Many cytochrome p450-derived lipids promote resolution of inflammation, in contrast to their soluble epoxide hydrolase(sEH)-derived oxylipin breakdown products. Here we compare plasma oxylipins and precursor fatty acids between seasons in participants with major depressive disorder with seasonal pattern (MDD-s). Euthymic participants with a history of MDD-s recruited in summer-fall were followed-up in winter. At both visits, a structured clinical interview (DSM-5 criteria) and the Beck Depression Inventory II (BDI-II) were administered. Unesterified and total oxylipin pools were assayed by liquid chromatography tandem mass-spectrometry (LC- MS/MS). Precursor fatty acids were measured by gas chromatography. In nine unmedicated participants euthymic at baseline who met depression criteria in winter, BDI-II scores increased from 4.9 ± 4.4 to 19.9 ± 7.7. Four sEH-derived oxylipins increased in winter compared to summer-fall with

moderate to large effect sizes. An auto-oxidation product (unesterified epoxyketooctadecadienoic acid) and lipoxygenase-derived 13-hydroxyoc- tadecadienoic acid also increased in winter. The cytochrome p450-derived 20-COOH-leukotriene B4 (unesterified) and total 14(15)-epoxyeicosatetraenoic acid, and the sEH-derived 14,15-dihydroxyeicostrienoic acid (unesterified), decreased in winter. We conclude that winter depression was associated with changes in cytochrome p450- and sEH-derived oxylipins, suggesting that seasonal shifts in omega-6 and omega-3 fatty acid metabolism mediated by sEH may underlie inflammatory states in symptomatic MDD-s.

1. Introduction

Unlike most other fields of medicine, the clinical management of major depressive disorder (MDD) remains without clinically useful biomarkers with which to predict and monitor the course of the disease. The field also lacks a unified pathophysiological model with which to integrate the observed biological phenomena. For instance, highly reproducible evidence supports elevated concentrations of inflammatory cytokines (Dowlati et al., 2010) and lower levels of omega-3 fatty acids (Lin et al., 2010) in peripheral blood of depressed patients compared to controls. Further evidence suggests that inflammatory markers such as cytokines may decline with treatment and/or symptomatic improvement (Hannestad et al., 2011); however, those markers lack clinical utility, in part due to poor sensitivity and specificity for the disease state, or a lack of quantitative assays that can be appropriately standardized between laboratories (Noble et al., 2008). The forgoing findings, however, do suggest the potential importance of inflammatory and lipid pathways in the pathophysiology of MDD, and in principle, the potential utility of these biomarkers to monitor MDD.

Of all the MDD subtypes, MDD with seasonal pattern (MDD-s; formerly referred to as Seasonal Affective Disorder) is perhaps the best validated and most predictable, with an onset of depressive episodes that typically occurs in winter (Rosenthal et al., 1984). Based on clinical diagnostic criteria, the prevalence of MDD-s has been estimated at 0.4% in the USA (Blazer et al., 1998) and 1.7–4.0% in Canada where it accounts for 18% of recurrent MDD cases (Levitt et al., 2000). Moreover, MDD-s is an excellent model of atypical depression because it is characterized mostly by hypersomnia, carbohydrate craving, increased appetite and weight gain (Garvey et al., 1988; Rosenthal et al., 1984). MDD-s is thought to be caused by a shift in circadian rhythm associated with a longer nocturnal melatonin secretion, and a dysregulation of monoamine neurotransmitter (serotonin, dopamine, and norepinephrine) systems (Lam and Levitan, 2000; Sohn and Lam, 2005). MDD-s can be treated with antidepressants such as selective serotonin reuptake inhibitors, cognitive behavioral therapy or light therapy (Westrin and Lam, 2007), but many who suffer from MDD-s do not seek treatment, facilitating observation of both unmedicated depressed and euthymic states within the same individuals within a short period of time. The effectiveness of treatment is limited, leading to a high rate of recurrence (Forneris et al., 2015; Gartlehner et al., 2015; Kaminski-Hartenthaler et al., 2015; Nussbaumer et al., 2015) and a necessity for a more comprehensive understanding of the pathophysiology.

The oxidation of polyunsaturated fatty acids (PUFA) produces bioactive lipid mediators known as oxylipins, which are involved in regulating pro-inflammatory and resolution pathways in blood and various tissues (Gabbs et al., 2015; Serhan et al., 2008). Oxylipins can be formed non-enzymatically due to auto-oxidation or enzymatically by cyclooxygenase (COX), lipoxygenase (LOX), cytochrome p450 (CYP) or soluble epoxide hydrolase (sEH) enzymes (Arnold et al., 2010b; Fer et al., 2008; Gabbs et al., 2015; Moghaddam et al., 1996; Morisseau et al., 2010; Nieves and Moreno, 2006; Reinaud et al., 1989; Yamamoto et al., 1988). CYP enzymes catalyze the epoxidation of PUFA into their epoxide metabolites that are converted to their corresponding diols by sEH (Imig and Hammock, 2009; Morisseau et al., 2010; Zeldin et al., 1993) (Fig. 1).

Changes in oxylipin metabolism may be related to depression. In mice, direct inhibition of sEH was reported to reduce immobility in the forced swim test and the tail suspension test, demonstrating anti- depressant-like effects, and the likely involvement of fatty acid epoxides or diols in depression-like behavior (Ren et al., 2016). Those findings are consistent with evidence of increased levels of the sEH protein in postmortem brain samples from patients with MDD or bipolar disorder compared to healthy controls (Ren et al., 2016); however, it remains to be determined whether peripheral blood concentrations of these lipid mediators are altered in depressed states in living people. A recent meta-analysis reported increased lipid peroxidation in major depression that was normalized following antidepressant treatment (Mazereeuw et al., 2015), but the basis for these changes remains unclear.

In view of preclinical and post-mortem evidence implicating sEH in depressive disorders, we hypothesized that fatty acid diols produced through sEH activity would be higher in the plasma of MDD-s patients during the winter compared to summer. Therefore, in patients with a history of MDD-s recruited in the summer and followed into winter depression, we quantified plasma oxylipins and their precursor fatty acids in both states. We screened for 84 oxidized fatty acid metabolites in plasma (Supplementary Table 1) derived from different polyunsa-turated fatty acids (Fig. 1). To our knowledge, no data are currently available on plasma concentrations of these metabolites in any subtype of major depression, and we explored both total oxylipins, representing unesterifed and esterified fractions, and free (unesterified) oxylipins. Unesterifed oxylipins are considered to constitute the bioactive pool, whereas total oxylipins include the esterified pool, the primary trans port and storage form that could be released via lipase enzymes (Shearer and Newman, 2008).

2. Methods

2.1. Participants

Ethical approval for this study was obtained from the local Research Ethics Board. All participants provided informed written consent prior to beginning the study. Participants aged between 18–65 years were recruited. All participants had a history of MDD-s based on at least two episodes of depression that presented a seasonal pattern over the past 3 years. Subjects who used an antidepressant, hypnotic or antipsychotic, or had abnormal liver, kidney or lung function, anemia, hypothyroid- ism, neurological or neurodegenerative conditions, cancer, inflamma- tory disease, other acute medical conditions or infection were excluded. Each participant underwent 2 study visits, a baseline visit during the summer or

early fall and a follow-up during the winter. At each visit, depressive status was assessed using a Structured Clinical Interview for DSM-5 criteria (American Psychiatric Association, 2013). Participants also completed a Beck Depression Inventory-II (BDI-II) (Beck et al., 1961). A blood sample was collected in K2-EDTA tubes and centrifuged for 10 min at 1000*g* at 4 °C. Plasma was isolated and stored at −80 °C until analysis. Only participants who were euthymic in the summer and depressed in the winter were included in the final analysis.

2.2. Oxylipin extraction by solid phase extraction

Free (unesterified) and total (unesterified and esterified) oxylipins were extracted by solid phase extraction (SPE), as previously described (Schuchardt et al., 2013; Yang et al., 2009).

To analyze free oxylipins, 10 μL surrogate standard solution, 10 μL of antioxidant solution and 1 mL of SPE buffer containing 5% methanol and 0.1% acetic acid in ultrapure water were added to 200 μL of plasma. The surrogate standard solution contained 500 nM 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 metha-nol (i.e. 5 pmol of each deuterated oxylipin per sample). The antioxidant solution contained 0.2 mg/mL of butylated hydroxytoluene (BHT) and triphenylphosphine (TPP) and 1 mg/mL ethylenediamine-tetraacetic acid (EDTA) in methanol/water (50/50, v/v). The antiox- idant solution was filtered through a Millipore filter prior to use, to remove solid particles.

For total oxylipins, 50–100 μL of plasma were mixed with 10 μL surrogate standard solution (500 nM), 10 μL of antioxidant solution and 100 μL of extraction buffer (0.1% acetic acid and 0.1% BHT in methanol) and kept in −80 °C overnight (Arnold et al., 2010b). Samples were then hydrolyzed in 200 μL of 0.25 M sodium carbonate solution at 60 °C for 30 min under constant shaking. The samples were allowed to cool, and 25 μL acetic acid and 1575 μL ultrapure water were added. The pH was confirmed to be between 4 and 6 by spiking a litmus paper with a few μL from one of the samples.

Oxylipins were extracted by SPE with 60 mg Waters Oasis HLB 3 cc cartridges (Waters, Milford, MA, USA). The columns were first rinsed with one volume of ethyl acetate and two volumes of methanol and conditioned with two volumes of SPE buffer. Plasma samples prepared as described above were poured onto the column, which was topped to volume with SPE buffer. The column was washed twice with SPE buffer and dried under vacuum suction (~20 psi) for 20 min. Oxylipins adsorbed to the SPE column were eluted with 0.5 mL methanol and 1.5 mL ethyl acetate into a 2 mL centrifuge tube containing 6 μL of 30% glycerol in methanol. The collected fraction was dried in a speed- vac. The samples were reconstituted in 50 μL of methanol containing 200 nM 1-cyclohexyl ureido, 3-dodecanoic acid as a recovery standard and filtered by centrifugation using Ultrafree-MC-VV polyvinylidene fluoride filters (0.1 μm; Millipore, Bedford, MA, USA).

2.3. Oxylipin analysis by LC-MS/MS

A total of 84 oxylipins listed and abbreviated in Supplementary Table 1 were analyzed by UPLC-MS/MS as previously described (Yang et al., 2009; Zivkovic et al., 2012) on an Agilent 1200SL (Agilent Corporation, Palo Alto, CA, USA) UPLC system connected to a

4000 QTRAP tandem mass spectrometer (Applied Biosystems Instrument Corporation, Foster City, CA, USA) equipped with an electrospray ionization source (Turbo V). An Agilent 2.1×150 mm Eclipse Plus C18 column with a 1.8 μm particle size was used to separate the oxylipins on the UPLC. Calibrations curves for each oxylipin were obtained using standards obtained from Cayman Chemicals (Ann Arbor, MI, USA) or synthetic standards produced by Dr. Hammock's laboratory.

The autosampler temperature was kept at 4 °C and the column at 50 °C. The mobile phase A contained 0.1% acetic acid in ultrapure water and the mobile phase B contained acetonitrile/methanol /acetic acid (84/16/0.1). Gradient elution was performed at a flow rate of 0.25 mL/min for a total run time of 21.5 min as follows: solvent B was held at 35% for 0.25 min, increased to 45% from 0.25 to 1 min, to 55% B from 1 to 3 min, to 65% B from 3 to 8.5 min, to 72% from 8.5 to 12.5 min, to 82% B from 12.5 to 15 min, to 95% B from 15 to 16.5 min, held at 95% for 1.5 min, decreased to 35% from 18 to 18.1 min and held at 35% for 2.9 min. The gradient enables the separation of oxylipins according to their polarity, with the most polar compounds such as prostanoids eluting first. The instrument was operated in negative electrospray ionization mode and used optimized multiple reaction monitoring conditions of the parent and fragmentation product ion listed in Supplementary Table 1 to measure each oxylipin (Yang et al., 2009). Peaks were quantified according to external standard curves and corrected for the surrogate standard recovery using Analyst software 1.4.2.

Because d4-PGE2 is degraded during the hydrolysis process, total oxylipins that were quantified with d4-PGE2 as an internal standard were not analyzed. These included THF-diol, EKODE, PGE1, PGD1, PGF2a, PGE2, PGD2, PGJ2, PGB2, PGE3, PGD3, 15-deoxy-PGJ2, resolvin E1, 9,12,13-TriHOME, 9,10,13-TriHOME and 11,12-,15-TriHETrE. The limit of quantification (LOQ) was set to three times the lowest standard concentration on the standard curve. Oxylipins with > 30% of values below the LOQ were excluded from the statistical analysis.

2.4. Total fatty acid analysis

Plasma fatty acids were directly derivitized in methanolic acid as previously described (Ichihara and Fukubayashi, 2010). Briefly, 400 μL toluene, 3 mL methanol and 600 μL HCl-methanol (8/92, v/v) were added to 100 μL of plasma spiked with 1,2-diheptadecanoyl-*sn*-gly- cero-3-phosphocholine (17:0 PC, Avanti Polar Lipids Inc., Alabaster, AL, USA) as an internal standard. Samples were vortexed and heated at 90 °C for 1 h. After cooling for a few minutes, 1 mL of hexane and 1 mL of distilled water were added. Samples were vortexed and the phases were allowed to separate for a few minutes. One microliter of the upper hexane layer containing fatty acid methyl esters was injected at a split ratio of 10:1 into a 3800 Varian gas chromatograph equipped with a flame ionization detector (Varian Inc., Walnut Creek, CA, USA). Fatty acids were separated on a DB-23 capillary column (30 m length; 0.25 mm ID; 0.25 μm thickness; Agilent, Santa Clara, CA, USA). The detector and injector temperatures were set at 300 °C and 250 °C, respectively. The oven temperature program was set at 50 °C for 2 min, increased by 10 °C/min to 180 °C, held at 180 °C for 5 min, increased by 5 °C/min to 240 °C and held at 240 °C for 5 min. Helium was used as a

carrier gas at a flow rate of 1.3 mL/min. A custom mix of 31 fatty acid methyl ester standards (NuChek Prep, Elysian, MN, USA) was used to identify the individual fatty acids. Results were expressed as concentration (nmol/mL) and percent of identified fatty acids.

2.5. Statistical analysis

A paired samples *t*-test was used to compare BDI-II scores between summer-fall and winter (GraphPad Prism 6.0, GraphPad Software Inc., San Diego, CA, USA).

In an exploratory manner, differences in plasma fatty acid and oxylipin concentrations were evaluated by their observed effect sizes (Cohen, 1992). For each variable, an effect size corresponding to Cohen's *d* was calculated as the difference between summer-fall and winter mean concentrations, divided by the standard deviation of the change (Lakens, 2013). We considered that medium effect sizes ($|d| > 0.5$) were likely to be clinically meaningful. For reference, we also present paired samples *t*-tests and their *p* values comparing concentrations in summer-fall to those in winter (GraphPad Prism 6.0, GraphPad Software Inc., San Diego, CA, USA); however, due to the exploratory nature of the study, we focus on effect sizes, which may be more useful in planning confirmatory studies. All results are expressed as mean ± SD.

3. Results

3.1. Participants and characteristics

A total of 15 participants were recruited into the study. Four participants dropped out before the end of the study and two did not meet depression criteria in the winter. After excluding these subjects, nine met study criteria for current MDD-s and were included in this analysis. These nine participants were 46.7 ± 14 years old and included 4 men and 5 women. The average number of previous depressive episodes was 3.3 ± 1.2. A significant increase in BDI-II score (t=5.785, df=8, *p*=0.0004) was observed during winter (19.9 ± 7.7) compared to summer-fall (4.9 ± 4.4). Based on their BDI-II scores (Beck et al., 1996), the participants were not depressed during summer-fall and mildly to moderately depressed during winter.

3.2. Plasma fatty acid concentrations

Summer-fall and winter plasma fatty acid concentrations, and percent composition, are summarized in Table 1. A medium effect size was found for plasma α-linolenic acid; plasma α-linolenic acid (α-LNA, 18:3 n-3) was decreased in winter compared to summer-fall, both when the data were expressed as concentrations (−28%; *d*=−0.52; t=1.56; df=8; *p*=0.16; Table 1 and Fig. 2) and percent composition (−37%; *d*=−0.69; t=2.08; df=8; *p*=0.07; Table 1). No differences in other fatty acids were observed between summer-fall and winter.

3.3. Plasma free and total oxylipin concentrations

Of the 84 oxylipins analyzed by LC-MS/MS, 28 free and 36 total oxylipins were detected in plasma and their changes between visits were explored (Table 2). Concentrations of nine oxylipin species changed between study visits with medium to large effect sizes (Fig. 2). Exploring free (unesterified) oxylipins (Table 2), concentrations of unesterified AA-derived 20-COOH-LTB4 (a CYP product of 5-LOX- derived LTB4) and 14,15-DiHETrE (sEH-

derived metabolite) were decreased by 54% (d=−0.83; t=2.5; df=8; p=0.04) and 14% (d= −0.91; t=2.72; df=8; p=0.03;), respectively, in winter compared to summer- fall. A medium effect size was also observed for some LA-derived metabolites; unesterified EKODE (auto-oxidation product), 13-HODE (12/15-LOX-derived metabolite) and 12,13-DiHOME (sEH-derived metabolite) were increased by 77% (d=0.71; t=0.65; df=8; p=0.06), 39% (d=0.52; t=1.57; df=8; p=0.15) and 33% (d=0.53; t=1.6; df=8; p=0.15), respectively, in winter compared to summer-fall (Fig. 2). No change in unesterified ALA-, EPA- and DHA-derived metabolite concentrations were observed between summer-fall and winter.

Exploring total oxylipin concentrations (Table 2), medium effect sizes were reported. Total EPA-derived 14(15)-EpETE (CYP-derived metabolite) was decreased by 40% (d=−0.5; t=1.51; df=8; p=0.17) in winter compared to summer-fall (Fig. 2). Concentrations of total LA- derived 12,13-DiHOME and DHA-derived 19,20-DiHDPE and 7,8- DiHDPE, three sEH-derived metabolites, were increased by 56% (d=0.62; t=1.86; df=8; p=0.10), 65% (d=0.58; t=1.74; df=8; p=0.12) and 93% (d=0.51; t=1.52; df=8; p=0.17), respectively, in winter compared to summer-fall (Fig. 2). No other changes in total oxylipin concentrations were observed between summer-fall and winter.

In a post-hoc descriptive analysis of two participants who did not become symptomatic over the course of the study, no clear differences between the seasons were observed for free and total 12,13-DiHOME, total 19,20-DiHDPE, free 14,15-DiHETrE and free 20-COOH-LTB4 (Supplementary Fig. 1). Moreover, free 13-HODE decreased during winter, while total 14(15)-EpETE increased, two patterns that were not observed in the participants who became depressed.

4. Discussion

This exploratory targeted lipidomic study showed for the first time a possible association between plasma oxylipin concentrations and a state of winter depression. More specifically, concentrations of 4 products of the sEH pathway increased, while one sEH substrate decreased, emerging as the most consistent candidate biomarkers. Of these, the omega-6 derived sEH product 12,13-DiHOME increased in winter depression consistently in both free and total oxylipin pools. Because the assays used here are quantitative, the findings offer new potential to develop standardized laboratory tests for MDD-s, if the results can be confirmed in further studies and differentiated from seasonal effects.

The present findings suggest the involvement of the sEH pathway in the winter depression state of MDD-s. Total 14(15)-EpETE, a sEH substrate, as well as sEH-derived free 14,15-DiHETrE, decreased during winter compared to summer-fall, while sEH-derived total 7,8-DiHDPE, total 19,20-DiHDPE, and total and free 12,13-DiHOME were increased during winter (Fig. 2). The results from 2 asymptomatic participants suggest that some of these changes may be specific to a depressed state, independent of seasonal changes, although this result would require further study in order to draw any conclusions. The involvement of sEH pathways in MDD-s is consistent with evidence of increased sEH protein in postmortem brains of depressed patients compared to healthy controls, and with decreased depressive-like behavior due to inhibition of sEH in mice (Ren et al., 2016). The present results suggest

that MDD-s may be associated with increased LA- and DHA-derived sEH diols, and with decreased AA-derived sEH diols and EPA-derived CYP epoxidized products. While preliminary in nature, these differences in omega-3 and omega-6 fatty acid metabolism might be explained by preferential selectivity of CYP and sEH enzymes towards EPA and then DHA compared to AA as previously reported (Arnold et al., 2010a; Fischer et al., 2014; Morisseau et al., 2010).

AA-, EPA- and DHA-derived epoxides derived from the CYP path- way have been described as anti-inflammatory metabolites (Jiang et al., 2014; Lopez-Vicario et al., 2015; Morisseau et al., 2010; Node et al., 1999). Inhibiting sEH activity, and therefore inhibiting their conver- sion to their respective diols, reduces LPS-induced inflammatory pain (Inceoglu et al., 2006), acute systemic inflammation (Schmelzer et al., 2005), colitis (Norwood et al., 2010), lung inflammation (Smith et al., 2005) and depressive-like behaviors in an inflammation-induced depression model (Ren et al., 2016). The sEH-derived diols seem to be less anti-inflammatory than their respective epoxides (Morisseau and Hammock, 2013). The decreased EPA-derived epoxides along with the increased DHA- and LA-derived diols observed in winter depression suggest an increase in sEH activity that could underlie a state of increased inflammation and the development of mood symptoms.

The main limitations of this study were the small sample size and a lack of a control group without MDD-s; however, the prospective within-subjects study design, without confounding influences of anti- depressant treatments, and the descriptive finding that the changes did not occur in 2 asymptomatic participants, increases confidence. Future studies should confirm the present observations of altered LA, AA, EPA and DHA metabolite concentrations in larger cohorts with a parallel control arm of never-depressed healthy participants that would ac- count for possible seasonal effects on oxylipins independent of depression status. Regardless of whether the changes may be seasonal or state-specific, the knowledge that oxylipin changes are likely to occur in winter depression is important in and of itself, since winter depression is common and these lipid mediators regulate inflammation and oxidative stress pathways relevant to depression (Dowlati et al., 2010; Maes et al., 2010).

Another limitation is that dietary fatty acid intake, which can influence plasma oxylipin concentrations in humans (Nording et al., 2013; Ramsden et al., 2012; Schebb et al., 2014), was not assessed. However, plasma concentrations of the dietary fatty acid precursors (LA, AA, EPA and DHA) of the implicated oxylipin species did not change between summer and winter in this study, suggesting that shifts in their metabolism were likely more important than changes in dietary fatty acid intake per se. On the other hand, α-LNA concentrations decreased in winter compared to summer-fall. This change likely reflects a decrease in dietary α-LNA intake, since α-LNA is essential and cannot be synthesized endogenously. It is unlikely that α-LNA was metabolically utilized for oxylipin synthesis because none of the α- LNA-derived oxylipins changed between seasons. Most likely, α-LNA was metabolically shunted towards increased β-oxidation or recycling into sterols or saturated and monounsaturated fatty acids during winter (Cunnane et al., 2003, 1994; Menard et al., 1998). This possibility along with altered α-LNA intake levels between seasons should be explored in future adequately powered studies.

5. Conclusions

This preliminary study suggests that oxylipin concentrations fluctuate between depression states in a small MDD-s cohort. The most consistent findings were changes in sEH oxylipin metabolites of long chain dietary omega-6 (LA, AA) and omega-3 (EPA and DHA) fatty acids. Owing to their spectra of biological activities, these changes in oxylipin mediators during the onset of mood symptoms would be consistent with previously established inflammatory and oxidative phenomenology. Of itself, this study does not establish whether these changes may be causal, and therefore whether sEH may be a drug target in depression; however, the medium to large effect sizes observed in these quantitative assays suggest new potential for the development of standardized clinical biomarkers. Larger confirmatory studies including controls are needed, particularly to account for potential confounders such as seasonal effects that may be unrelated to depressive states.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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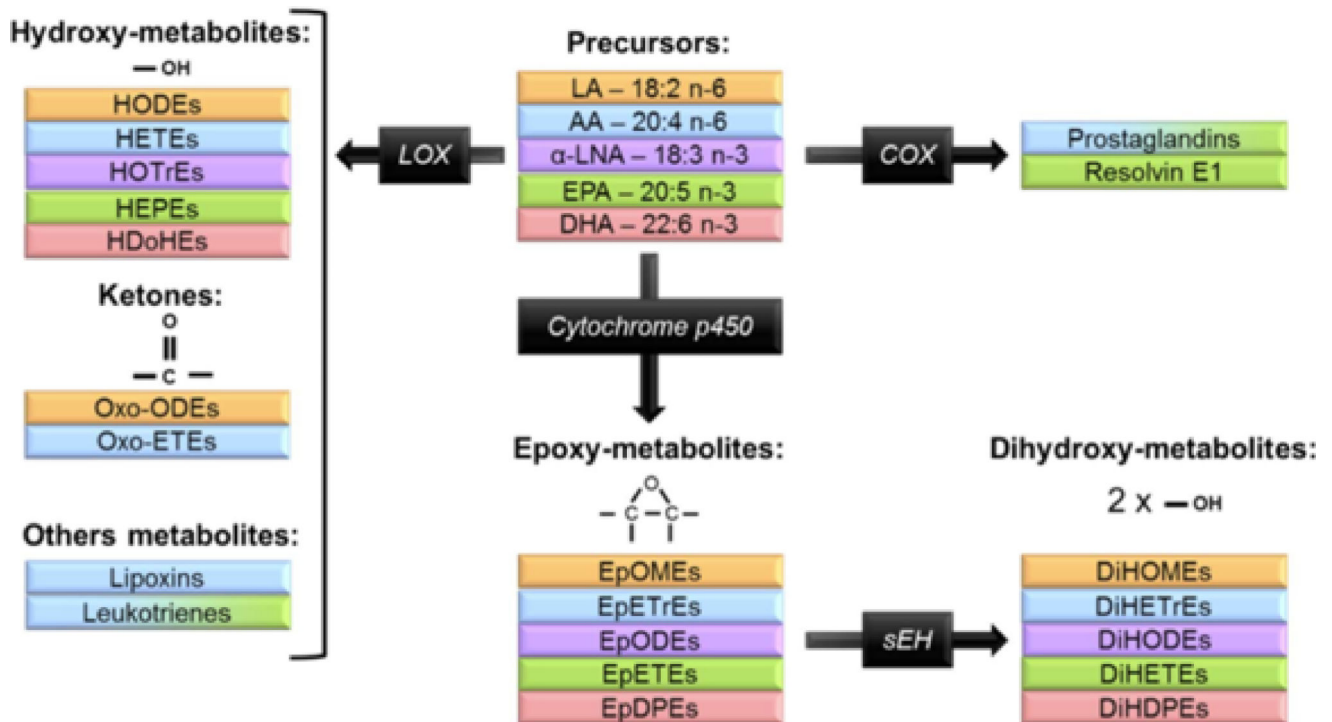
Figure 1.
Enzymatic oxylipin synthesis pathways. Oxylipins are synthetized from fatty acids, such as linoleic acid (LA), arachidonic acid (AA), α-linolenic acid (α-LNA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) through three main enzymatic pathways. The cyclooxygenase pathway (COX) produces prostaglandins and resolvins from AA and EPA. The lipoxygenase (LOX) pathway produces hydroxy-metabolites, such as hydroxyoctadecadienoic acids (HODEs), hydroxyeicosatetraenoic acids (HETEs), hydroxyoctadecatrienoic acids (HOTrEs) and hydroxydocosahexaenoic acid (HDOHEs). Leukotrienes, lipoxins and ketones, such as oxo-octadecadienoic acids (oxo-ODEs) and oxo-eicosatetraenoic acids (oxo-ETEs), are also synthetized through the LOX pathway. Finally, the cytochrome p450 pathway generates epoxy-metabolites, such as epoxyoctamonoemoic acids (EpOMEs), epoxyeicosatrienoic acids (EpETrEs), epoxyoctadecadienoic acids (EpODEs), epoxyeicosatetraenoic acids (EpETEs) and epoxydocosapentaenoic acids (EpDPEs). These epoxy-metabolites can be converted via the soluble epoxide hydrolase (sEH) to their respective diols, dihydroxyoctadecamonoenoic acids (DiHOMEs), dihydroxyeicosatrienoic acids (DiHETrEs), dihydroxyoctadecadienoic acids (DiHODEs), dihydroxyeicostetraenoic acids (DiHETEs) and dihydroxydocosapentaenoic acids (DiHDPES). On this figure, precursors and their respective metabolites share the same color code. Adapted from Zivkovic et at. (2012).
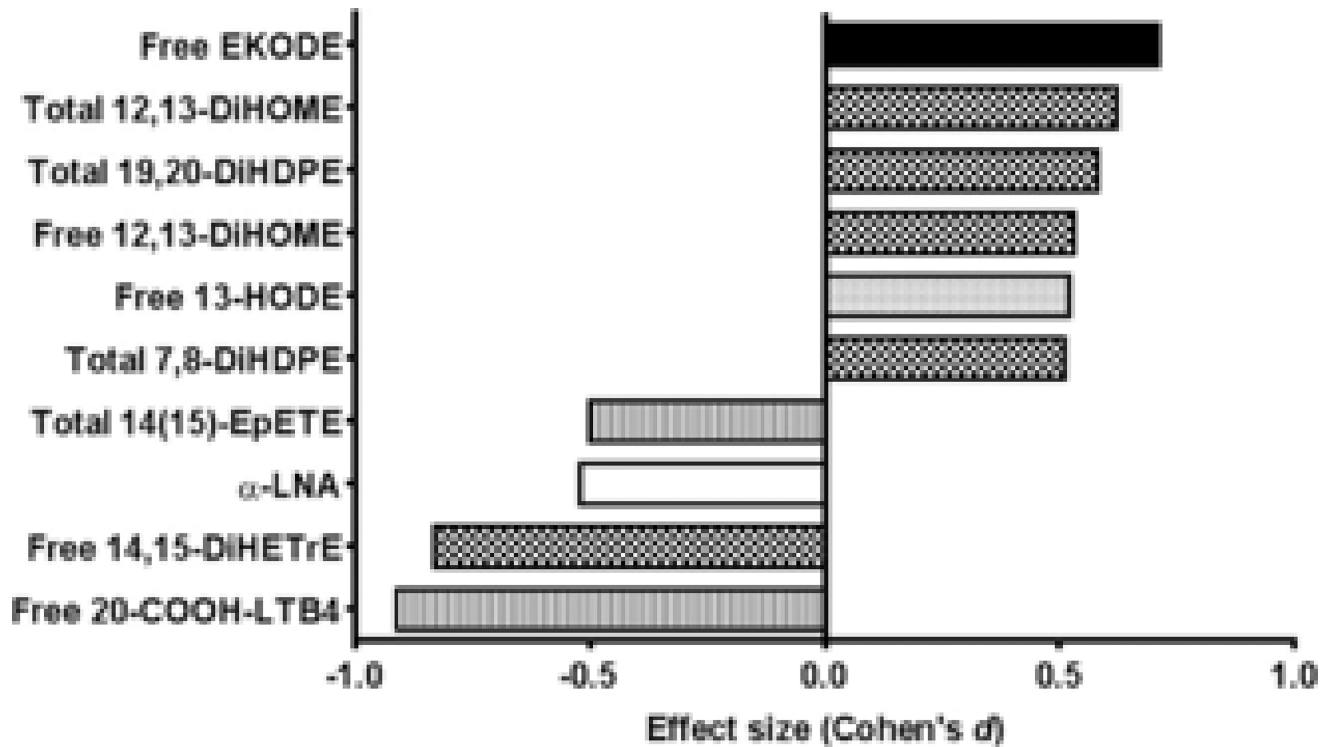
Figure 2.
Largest effect sizes in oxidized fatty acid metabolites from summer-fall euthymia to winter depression (n=9). Data are expressed as effect sizes (Cohen's *d*). White bar refers to a fatty acid precursor, black bar to autooxidative product; squared bars to sEH- derived oxylipins; dotted bar to lipoxygenase-derived oxylipins and striped bars to cytochrome p450-derived oxylipins. EKODE, epoxyketooctadecadienoic acid; DiHDPE, dihydroxydocosapentaenoic acid; DiHOME, dihydroxyoctadecamonoenoic acid; DiHETrE, dihydroxyeicosatrienoic acid; α-LNA, α-linolenic acid; EpETE, epoxyeicosatetraenoic acid; LTB4, leukotriene B4.

Table 1

Plasma fatty acid in concentrations (nmol/mL) and composition (% of identified fatty acids) in summer-fall and winter.

	Fatty acid concentrations (nmol/mL)					Fatty acid composition (% of identified fatty acids)				
	Summer-Fall (n=9)	Winter (n=9)	d	t	p	Summer-Fall (n=9)	Winter (n=9)	d	t	p
Lauric Acid (12:0)	229.9 ± 65.9	239.5 ± 76.6	0.11	0.32	0.75	2.1 ± 0.3	2.1 ± 0.4	−0.03	0.09	0.93
Myristic Acid (14:0)	153.7 ± 89.2	143 ± 65.3	−0.11	0.35	0.74	1.3 ± 0.2	1.2 ± 0.1	−0.4	1.2	0.26
Palmitic Acid (16:0)	2528 ± 1568	2531 ± 1491	0.02	0.05	0.96	21.4 ± 3.0	20.8 ± 1.7	−0.28	0.83	0.43
Stearic Acid (18:0)	803.1 ± 347.2	847.5 ± 451.3	0.08	0.26	0.8	7.1 ± 0.8	7.0 ± 0.5	−0.29	0.88	0.41
Saturated fatty acids	3715 ± 2050	3791 ± 2071	0.03	0.09	0.93	31.9 ± 2.7	31.1 ± 1.2	−0.39	1.16	0.28
Palmitoleic acid (16:1 n-7)	172.9 ± 181.9	157.2 ± 118.3	−0.08	0.25	0.81	1.3 ± 0.7	1.2 ± 0.4	−0.21	0.63	0.54
Oleic acid (18:1 n-9)	2190 ± 1284	2452 ± 1668	0.16	0.49	0.64	18.6 ± 3.3	19.3 ± 3.2	0.46	1.39	0.2
Monounsaturated fatty acids	2362 ± 1457	2609 ± 1781	0.14	0.41	0.69	19.9 ± 3.7	20.5 ± 3.4	0.38	1.13	0.29
LA (18:2 n-6)	3741 ± 1127	4098 ± 1780	0.16	0.49	0.63	34.2 ± 5.4	34.7 ± 3.7	0.17	0.5	0.63
DGLA (20:3 n-6)	200 ± 175.7	204.2 ± 151.2	0.02	0.06	0.95	1.6 ± 0.6	1.6 ± 0.6	−0.07	0.22	0.83
AA (20:4 n-6)	849 ± 329.6	955.5 ± 647.6	0.15	0.46	0.66	7.6 ± 1.4	7.6 ± 1.1	−0.02	0.06	0.95
n-6 polyunsaturated fatty acids	4797 ± 1572	5287 ± 2597	0.16	0.48	0.64	43.5 ± 4.8	44.1 ± 3.2	0.19	0.57	0.59
α-LNA (18:3 n-3)	87.8 ± 37	63.2 ± 27.7 *	−0.52	1.56	0.16	0.8 ± 0.4	0.5 ± 0.1 *	−0.69	2.08	0.07
EPA (20:5 n-3)	118.6 ± 89.1	125.6 ± 77.3	0.1	0.29	0.77	1.2 ± 1.1	1.2 ± 0.9	0.04	0.12	0.9
DHA (22:6 n-3)	248.6 ± 103.9	251.2 ± 78.7	0.02	0.05	0.96	2.3 ± 0.9	2.3 ± 0.8	−0.02	0.06	0.96
n-3 polyunsaturated fatty acids	489.5 ± 175.3	482.6 ± 206.5	−0.02	0.07	0.94	4.6 ± 2.0	4.3 ± 1.7	−0.31	0.94	0.37
Total fatty acids	11352 ± 5099	12171 ± 6556	0.11	0.32	0.75					

Values are mean ± SD. t-scores and p values of a paired t-test analysis are also reported. LA, linoleic acid; DGLA, dihomo-gamma-linolenic acid; AA, arachidonic acid; α-LNA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid

*
medium effect size (Cohen's |d| > 0.5).

Table 2

Free and total plasma oxylipin concentrations in summer-fall and winter.

	Free					Total				
	Summer-fall (n=9)	Winter (n=9)	d	t	p	Summer-fall (n=9)	Winter (n=9)	d	t	p
12(13)-EpOME	8.7 ± 7.7	8.4 ± 3.1	-0.05	0.16	0.87	313.2 ± 177.6	326.9 ± 363.5	0.03	0.09	0.93
9(10)-EpOME	4.5 ± 4.6	4.7 ± 3.0	0.06	0.16	0.87	159 ± 70	190.4 ± 203.4	0.13	0.39	0.7
12,13-DiHOME	5.4 ± 1.7	7.2 ± 4.6 *	0.53	1.6	0.15	9.1 ± 4.0	14.2 ± 10.0 *	0.62	1.86	0.1
9,10-DiHOME	3.9 ± 3.0	5.3 ± 6.0	0.23	0.7	0.5	9.8 ± 7.3	15.6 ± 14.6	0.4	1.21	0.26
9,12,13-TriHOME	3.6 ± 3.2	3.2 ± 0.8	-0.49	1.48	0.18					
EKODE	0.9 ± 1.3	1.6 ± 1.8 *	0.71	0.65	0.06					
13-HODE	17.6 ± 9.6	24.5 ± 12.3 *	0.52	1.57	0.15	74.6 ± 35.8	73.0 ± 34.8	-0.04	0.11	0.92
9-HODE	8.9 ± 5.5	10.6 ± 4.7	0.35	1.04	0.33	65.8 ± 27.8	66.2 ± 37.4	0.01	0.03	0.97
13-oxo-ODE	1.7 ± 0.9	2.4 ± 2.1	0.38	1.15	0.28	10.7 ± 7.9	6.6 ± 10.7	0.32	0.95	0.37
9-oxo-ODE	11.2 ± 11.8	14.6 ± 12.6	0.26	0.78	0.46	60.5 ± 62.5	69.4 ± 68.4	0.08	0.25	0.81
Total OxLAMs	66.4 ± 35.8	82.6 ± 28.0 *	0.61	1.83	0.1	702.8 ± 265.5	762.3 ± 569.2	0.08	0.25	0.8
Total DGLA metabolites (15(S)-HETrE)	0.6 ± 0.4	0.9 ± 0.7	0.43	1.29	0.23	4.4 ± 7.2	3.1 ± 4.1	-0.14	0.43	0.68
14(15)-EpETrE						419.4 ± 415.9	308 ± 386.9	-0.18	0.55	0.6
11(12)-EpETrE						167.7 ± 158.3	144.6 ± 168.7	-0.09	0.27	0.79
5(6)-EpETrE	5.1 ± 6.1	4.3 ± 3.0	-0.18	0.55	0.6	369.2 ± 197.3	463.7 ± 595.5	0.14	0.41	0.69
14,15-DiHETrE	0.7 ± 0.3	0.6 ± 0.2 **	-0.91	2.72	0.03					
11,12-DiHETrE	0.5 ± 0.2	0.5 ± 0.2	0.3	0.89	0.4					
8,9-DiHETrE						2.3 ± 1.8	2.8 ± 2.1	0.17	0.52	0.61
5,6-DiHETrE						10.7 ± 6.9	11.0 ± 4.9	0.07	0.2	0.85
PGF2α	7.9 ± 2.4	8.7 ± 2.2	0.35	1.04	0.33					
20-COOH-LTB4	1.1 ± 0.6	0.5 ± 0.3 **	-0.83	2.5	0.04	5.2 ± 2.7	5.5 ± 2.7	0.07	0.2	0.84
20-HETE	2.6 ± 0.8	2.4 ± 1.5	-0.1	0.31	0.76	17.0 ± 22.7	10.9 ± 5.8	-0.26	0.78	0.46
5-HETE						18.0 ± 22.0	21.0 ± 15.9	0.13	0.38	0.71
15-HETE						17.3 ± 16.9	21.2 ± 23.5	0.14	0.42	0.69
12-HETE	8.6 ± 5.9	6.0 ± 4.8	-0.44	1.33	0.22	8.8 ± 8.2	13.4 ± 13.8	0.25	0.75	0.47
15-oxo-ETE	0.4 ± 0.1	0.4 ± 0.6	0.12	0.36	0.73	3.5 ± 4.4	3.7 ± 4.7	0.04	0.12	0.9

	Free					Total				
	Summer-fall (n=9)	Winter (n=9)	d	t	p	Summer-fall (n=9)	Winter (n=9)	d	t	p
5-oxo-ETE	1.5 ± 1.4	2.2 ± 1.7	0.44	1.33	0.22	14.3 ± 17.3	16.6 ± 18.8	0.08	0.24	0.81
Total AA metabolites	28.3 ± 13.5	25.7 ± 8.1	−0.26	0.8	0.45	1053 ± 795.2	1022 ± 1129	−0.02	0.06	0.95
15(16)-EpODE	6.0 ± 4.0	5.4 ± 3.3	−0.11	0.32	0.75	55.6 ± 41.2	51.2 ± 52.8	−0.06	0.17	0.86
12(13)-EpODE						6.9 ± 4.0	8.1 ± 11.6	0.09	0.27	0.79
9(10)-EpODE	0.5 ± 0.7	0.7 ± 0.4	0.17	0.51	0.62	6.4 ± 6.1	8.6 ± 11.3	0.15	0.44	0.67
15,16-DiHODE	11.5 ± 8.2	11.8 ± 8.3	0.03	0.08	0.94	20.1 ± 16.0	28.1 ± 19.8	0.37	1.11	0.3
9-HOTrE	0.6 ± 0.3	0.6 ± 0.2	−0.01	0.03	0.97	2.2 ± 1.7	1.9 ± 1.5	−0.09	0.27	0.79
Total α-LNA metabolites	18.7 ± 11.6	18.4 ± 10.6	−0.01	0.05	0.96	91.2 ± 58.7	98.0 ± 77.2	0.06	0.19	0.86
17(18)-EpETE						35.2 ± 29.9	29.1 ± 31.2	−0.16	0.48	0.64
14(15)-EpETE						26.1 ± 18.8	15.7 ± 16.5 *	−0.5	1.51	0.17
11(12)-EpETE						12.4 ± 11.6	14.2 ± 16.7	0.08	0.24	0.82
8(9)-EpETE						4.3 ± 3.5	5.4 ± 4.2	0.23	0.68	0.51
17,18-DiHETE	0.4 ± 0.3	0.5 ± 0.3	0.28	0.83	0.43					
Total EPA metabolites	0.4 ± 0.3	0.5 ± 0.3	0.28	0.83	0.43	78.1 ± 57.4	64.5 ± 64.7	−0.17	0.5	0.63
19(20)-EpDPE						78.0 ± 52.2	69.7 ± 77.8	−0.08	0.22	0.83
16(17)-EpDPE						42.6 ± 26.2	25.7 ± 18.6	−0.47	1.4	0.2
13(14)-EpDPE						34.8 ± 28.1	24.2 ± 28.1	−0.21	0.64	0.54
10(11)-EpDPE	0.5 ± 0.6	0.6 ± 0.5	0.09	0.27	0.79	28.8 ± 21.0	28.4 ± 26.4	−0.01	0.03	0.97
19,20-DiHDPE	2.1 ± 1.4	2.2 ± 1.1	0.15	0.44	0.67	2.6 ± 1.8	4.3 ± 2.5 *	0.58	1.74	0.12
16,17-DiHDPE 7,8-DiHDPE	0.4 ± 0.2	0.4 ± 0.3	0.02	0.07	0.94	1.5 ± 1.5	2.9 ± 2.7 *	0.51	1.52	0.17
Total DHA metabolites	3.0 ± 1.6	3.3 ± 1.5	0.17	0.5	0.63	188.3 ± 122.8	155.2 ± 144.2	−0.14	0.43	0.68

Values are mean ± SD. t-scores and p values for paired samples t-tests are also reported Concentrations are expressed in nM. α-LNA, α-linolenic acid; AA, arachidonic acid; DGLA, di- homo-gamma-linoleic acid; DHA, docosahexaenoic acid; DiHDPE, dihydroxydocosapentaenoic acid; DiHETE, dihydroxyeicosatetraenoic acid; DiHETrE, dihydroxyeicosatrienoic acid; DiHODE, dihydroxyoctadecadienoic acid; DiHOME, dihydroxyoctadecamonoenoic acid; EKODE, epoxyketooctadecadienoic acid; EPA, eicosapentaenoic acid; EpDPE, epoxydoco- sapentaenoic acid; EpETE, epoxyeicosatetraenoic acid; EpETrE, epoxyeicosatrienoic acid; EpODE, epoxyoctadecadienoic acid; EpOME, epoxyoctadecamonoenoic acid; HETE, hydroxyeicosatetraenoic acid; HETrE, hydroxyeicosatrienoic acid; HODE, hydroxyoctadecadienoic acid; HOTrE, hydrooctadecatrienoic acid; LTB4, leukotriene B4; OxLAMs, oxidized linoleic acid metabolites; oxo-ETE, oxoeicosatetraenoic acid; oxoODE, oxooctadecadienoic acid; PGF2α, prostaglandin F2α.

*
 medium effect size (Cohen's |d| > 0.5).

**
 large effect size (|d| > 0.8).