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

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\textbf{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 epoxyketoctadecadienoic acid) and lipoxigenase-derived 13-hydroxyoctadecadienoic 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-dihydroxyeicosatrienoic 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 hypersonnia, 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 secretation, 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 (Fornier 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 (Gabbis et al., 2015; Serhan et al., 2008). Oxylipins can be formed non-enzymatically due to auto-oxidation or enzymatically by cyclooxygenase (COX), lipooxygenase (LOX), cytochrome p450 (CYP) or soluble epoxide hydrolase (sEH) enzymes (Arnold et al., 2010b; Fer et al., 2008; Gabbis 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 antidepressant-like effects, and the likely involvement of fatty acid epoxides or diols in depression-like behavior (Ren et al., 2016). These 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 polyunsaturated 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 unesterified and esterified fractions, and free (unesterified) oxylipins. Unesterified oxylipins are considered to constitute the bioactive pool, whereas total oxylipins include the esterified pool, the primary transport 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, hypothyroidism, neurological or neurodegenerative conditions, cancer, inflammatory 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 1000g 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-DiHETE, d4-6-keto-PGF1a, d4-9-HODE, d4-LTB4, d4-PGE2, d4-TXB2, d6-20-HETE and d8-5-HETE in methanol (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 antioxidant 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-
same color code. Adapted from Zivkovic et al. (2012).


acids (DiHODEs), dihydroxyeicosatrienoic acids (DiHETEs) and dihydroxydocosapentaenoic acids (DiHDPES). On this
via the soluble epoxide hydrolase (sEH) to their respective diols, dihydroxyoctadecamonoenoic acids (DiHOMEs), dihydroxyeicosatrienoic acids (DiHETrEs), dihydroxyoctadecadienoic
esters (EpETrEs), epoxyoctadecadienoic acids (EpODEs), epoxyeicosatetraenoic acids (EpETEs) and epoxydocosapentaenoic acids (EpDPEs). These epoxy-metabolites can be converted
are also synthetized through the LOX pathway. Finally, the cytochrome p450 pathway generates epoxy-metabolites, such as epoxyoctamonoemoic acids (EpOMEs), epoxyeicosatrienoic
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),
leukotrienes, lipoxins and ketones, such as 12(S)-HETE, 15(S)-HETE, 12(S)-Resolvin D1, 15(S)-Resolvin D1, 12(S)-Resolvin E1, 9,12,13-TriHOME, 9,10,13-TriHOME and 11,12-,15-

Fig. 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

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 prostanooids 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, PGE2a, PGE2, PGD2, PGJ2, PGK2, PGI3, PGD3, 15-deoxy-PGJ2, resolvin E1, 9,12,13-TriHOME, 9,10,13-TriHOME and 11,12,15-TriHETE. 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-glycero-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

As a result of BDI-II scores in the winter. After excluding these subjects, we present paired samples t-tests and their p values comparing concentrations between summer-fall to those in 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.

3.2. 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 calculated 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-DIEHETE (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-DiHETErE 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-DiHETErE, decreased during winter compared to summer-fall, while sEH-derived total 7,8-DiHDPE, total 19,20-DiHDPE, and total 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 pathway 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 conversion 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 et al., 2010).
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 antidepressant treatments, and the descriptive finding that the changes did not occur in 2 asymptomatic participants, increases confidence. Future studies should confirm the 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 account for possible seasonal effects on oxylipins independent of depression status. Regardless of whether the changes may be seasonal or state-specific, the knowledge that oxylipins change is 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 (Cumpane 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.

Ethics approval and consent to participate

This study was approved by the Research Ethics Board of Sunnybrook Health Sciences Centre (ID 300-2014).
Table 2
Free plasma oxylipin concentrations in summer-fall and winter.

<table>
<thead>
<tr>
<th>Metabolite*</th>
<th>Summer-fall (n=9)</th>
<th>Winter (n=9)</th>
<th>d</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>12(13)-EpOME</td>
<td>8.7 ± 7.7</td>
<td>8.4 ± 3.1</td>
<td>-0.05</td>
<td>0.16</td>
<td>0.87</td>
</tr>
<tr>
<td>9(10)-EpOME</td>
<td>4.5 ± 4.6</td>
<td>4.7 ± 3.0</td>
<td>0.06</td>
<td>0.16</td>
<td>0.87</td>
</tr>
<tr>
<td>5,6-DiHETrE</td>
<td>3.9 ± 3.0</td>
<td>5.3 ± 6.0</td>
<td>0.23</td>
<td>0.7</td>
<td>0.5</td>
</tr>
<tr>
<td>9,12,13-TriHOME</td>
<td>3.6 ± 3.2</td>
<td>3.2 ± 0.8</td>
<td>-0.49</td>
<td>1.48</td>
<td>0.18</td>
</tr>
<tr>
<td>EKODE</td>
<td>0.9 ± 1.3</td>
<td>1.6 ± 1.8</td>
<td>0.71</td>
<td>0.65</td>
<td>0.06</td>
</tr>
<tr>
<td>13-HODE</td>
<td>17.6 ± 9.6</td>
<td>24.5 ± 12.3</td>
<td>0.52</td>
<td>1.57</td>
<td>0.15</td>
</tr>
<tr>
<td>9-HODE</td>
<td>8.9 ± 5.5</td>
<td>10.6 ± 4.7</td>
<td>0.35</td>
<td>1.04</td>
<td>0.33</td>
</tr>
<tr>
<td>15-oxo-ODE</td>
<td>1.7 ± 0.9</td>
<td>2.4 ± 2.1</td>
<td>0.38</td>
<td>1.15</td>
<td>0.28</td>
</tr>
<tr>
<td>9-oxo-ODE</td>
<td>11.2 ± 11.8</td>
<td>14.6 ± 12.6</td>
<td>0.26</td>
<td>0.78</td>
<td>0.46</td>
</tr>
<tr>
<td>Total OxLAMs</td>
<td>66.4 ± 35.8</td>
<td>82.6 ± 28.0</td>
<td>0.61</td>
<td>1.83</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Values are mean ± SD. t-scores and p values for paired samples t-tests are also reported. Concentrations are expressed in nM. *-LNA, α-linolenic acid; AA, arachidonic acid; DGLA, di-homo-gamma-linoleic acid; DHA, docosahexaenoic acid; DHdPPE, dihydroyxocosapentaenoic acid; DHETE, dihydroxyeicosatetraenoic acid; DHODE, dihydroxyoctadecadienoic acid; DHOME, dihydroxoyctadecamonoenoic acid; EKODE, epoxyoetycatedecadienoic acid; EPA, eicosapentaenoic acid; EpDPE, epixyocaproponenoic acid; EpETE, epoxyeicosatetraenoic acid; EpHETE, epixyotetraenoic acid; EpODE, epoxyoctadecadienoic acid; HETE, hydroxyeicosatetraenoic acid; HETE, hydroxyeicosatetrienoic acid; HODE, hydroxyoctadecadienoic acid; HTETE, hydroxyoctadecatrienoic acid; LTBA, leukotriene B4; OxLAMs, oxidized linoleic acid metabolites; oxo-ETE, oxo-eicosatetraenoic acid; oxo-ODE, oxo-octadecadienoic acid; PGF2, prostaglandin F2.

Competing interests
No relevant competing interests.

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Consent for publication
Not applicable.

Availability of data and material
The datasets generated during and/or analyzed during the current study are not publicly available due to privacy considerations but some data could be available from the corresponding author on reasonable request.
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Authors’ contributions

WS and AJL designed the clinical study and carried out recruitment and participant assessments. HY, JO, JY, BDH and AYT designed and carried out the lipidomic assays. MH, WS and AYT planned and conducted the statistical analyses. MH, AYT and WS contributed to drafting the manuscript. JOY, JY, BDH and AJL edited/critically reviewed the manuscript. All authors approved the final version.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psychres.2017.02.056.

References