

Plasma Phosphatidylethanolamine and Triacylglycerol Fatty Acid Concentrations are Altered in Major Depressive Disorder Patients with Seasonal Pattern

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Abstract Disturbances in peripheral and brain lipid metabolism, including the omega-3 fatty acid docosahexaenoic acid (DHA), have been reported in major depressive disorder (MDD). However, these changes have yet to be confirmed in MDD with seasonal pattern (MDD-s), a subtype of recurrent MDD. The present exploratory study quantified plasma plasmalogen and diacyl-phospholipid species, and fatty acids within total phospholipids, cholesteryl esters, triacylglycerols and free fatty acids in non-medicated MDD-s participants ($n = 9$) during euthymia in summer or fall, and during depression in winter in order to screen for potential high sensitivity lipid biomarkers. Triacylglycerol alpha-linolenic acid concentration was significantly decreased, and myristoleic acid concentration was significantly increased, during winter depression compared to summer-fall euthymia. 1-stearoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphoethanolamine, a diacyl-phospholipid containing stearic acid and DHA, was significantly

decreased in winter depression. Concentrations of cholesteryl ester oleic acid and several polyunsaturated fatty acids between summer/fall and winter increased in proportion to the increase in depressive symptoms. The observed changes in lipid metabolic pathways in winter-type MDD-s offer new promise for lipid biomarker development.

Keywords Depression · Season · Phosphatidylethanolamine · Plasmalogen · Phospholipids · Omega-3 fatty acids · Seasonal affective disorder

Abbreviations

ALA	Alpha-linolenic acid
ARA	Arachidonic acid
di-17:0 PC	1,2-Diheptadecanoyl- <i>sn</i> -glycero-3-phosphocholine
DHA	Docosahexaenoic acid
EDTA	Ethylenediaminetetraacetic
EPA	Eicosapentaenoic acid
LNA	Linoleic acid
LC-MS/MS	Liquid chromatography tandem mass spectrometry
MDD-s	Major depressive disorder with seasonal pattern
MRM	Multiple reaction monitoring
OLA	Oleic acid
PtdCho	Phosphatidylcholine
PUFA	Polyunsaturated fatty acids
STA-ARA-Gro-3-PCho	1-Stearoyl-2-arachidonoyl- <i>sn</i> -glycero-3-phosphocholine
STA-DHA-Gro-3-PCho	1-Stearoyl-2-docosahexaenoyl- <i>sn</i> -glycero-3-phosphocholine
STA-OLA-Gro-3-PCho	1-Stearoyl-2-oleoyl- <i>sn</i> -glycero-3-phosphocholine

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STol-ARA-Gro-3-PCho	1-(1Z-octadecenyl)-2-arachidonoyl- <i>sn</i> -glycero-3-phosphocholine
STol-DHA-Gro-3-PCho	1-(1Z-octadecenyl)-docosahexaenoyl- <i>sn</i> -glycero-3-phosphocholine
STol-OLA-Gro-3-PCho	1-(1Z-octadecenyl)-2-oleoyl- <i>sn</i> -glycero-3-phosphocholine
PE	Phosphatidylethanolamine
STA-ARA-Gro-3-PEtn	1-Stearoyl-2-arachidonoyl- <i>sn</i> -glycero-3-phosphoethanolamine
STA-DHA-Gro-3-PEtn	1-Stearoyl-2-docosahexaenoyl- <i>sn</i> -glycero-3-phosphoethanolamine
STA-OLA-Gro-3-PEtn	1-Stearoyl-2-oleoyl- <i>sn</i> -glycero-3-phosphoethanolamine
STol-ARA-Gro-3-PEtn	1-(1Z-octadecenyl)-arachidonoyl- <i>sn</i> -glycero-3-phosphoethanolamine
STol-DHA-Gro-3-PEtn	1-(1Z-octadecenyl)-docosahexaenoyl- <i>sn</i> -glycero-3-phosphoethanolamine
STol-OLA-Gro-3-PEtn	1-(1Z-octadecenyl)-oleoyl- <i>sn</i> -glycero-3-phosphoethanolamine
RBC	Red blood cells
TLC	Thin layer chromatography

Introduction

Major depressive disorder with seasonal pattern (MDD-s) is a subtype of major depression characterized by recurrent episodes of depression in one season that are largely absent in other seasons. MDD-s affects 0.4–4% of the population, [1, 2] and accounts for 18% of recurrent major depressive disorder (MDD) cases in Canada [2]. It is associated with reduced quality of life and increased risk of suicide [3–5]. MDD-s is treated with light therapy and/or antidepressant medications, and often in combination with cognitive behavioral therapy [6, 7].

Disturbed fatty acid metabolism has been reported in non-seasonal MDD. As indicated in the literature review summary presented in Table 1, studies reported changes in the percentage composition (weight or mole percentage of total fatty acids) or concentration (amount per volume or total cell count) of several saturated, monounsaturated or polyunsaturated fatty acids in whole blood, red blood cells (RBC), plasma or serum of MDD or attempted suicide participants compared to healthy controls or controls with coronary heart disease or multiple sclerosis [8–25]. Circulating omega-3 polyunsaturated fatty acids (n-3 PUFA) were reported to increase in two studies [9, 10], remain unchanged in two [17, 24] and decrease in fourteen

[8, 11–16, 18–23, 25], consistent with a meta-analysis that showed an overall reduction in omega-3 fatty acids in MDD patients [26]. N-6 PUFA were decreased in three [10, 12, 19], increased in three [18, 20, 22] and unaltered in nine studies [8, 9, 11, 13, 15, 17, 21, 24, 25] whereas saturated and monounsaturated fatty acids when reported, were decreased in three [12, 13, 24], increased in four [11, 14, 17, 18] and unchanged in five studies [8, 10, 15, 21, 22]. Consistent with the reduction in circulating omega-3 fatty acids observed in most studies, McNamara *et al.* reported a 14% reduction in postmortem prefrontal cortex docosahexaenoic acid (DHA, 22:6n-3) concentration of suicide victims with MDD compared to controls who died from non-cardiovascular related causes [27].

Seasonal changes in serum fatty acid composition were also reported, but in healthy participants [28]. De Vriese *et al.* found reduced arachidonic acid (ARA, 20:4n-6), eicosa-pentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) percent composition in serum phospholipids of healthy participants during winter compared to summer, suggesting seasonal effects on serum fatty acid profile [28]. The reductions in EPA and DHA composition during winter are consistent with some MDD studies [11–13, 15], suggesting that seasonal reductions in EPA and DHA may be a risk factor for altered mood.

Most of the reported changes in fatty acid composition or concentration in MDD patients occurred in RBC, plasma or serum cholesteryl esters or phospholipids (Table 2) [11–13, 15, 19, 20, 22, 23, 25]. In humans, the incorporation of dietary fatty acids such as DHA into plasma ethanolamine glycerophospholipids and cholesteryl esters is minimal [29, 30], which means that these lipid pools are less sensitive to fluctuations in dietary DHA intake compared to the triacylglyceride pool, where ingested DHA preferentially incorporates [29], or to the total phospholipid pool, which exhibit high within-subject variability in fatty acid levels [31]. Thus, changes in specific stable lipid pools such as ethanolamine glycerophospholipids or cholesteryl esters could reflect metabolic disturbances associated with mood progression and serve as reliable disease biomarkers. It is not known whether MDD-s is associated with seasonal changes in plasma lipid pools similar to MDD.

In the present study, we sought to address whether the symptomatic phase of MDD-s is associated with changes in plasma phospholipid molecular species and fatty acid concentrations (nmol/mL) in various lipid pools. In view of the reported changes in plasma or serum phospholipid and cholesteryl ester fatty acids in depressed patients and the potential impact of season on serum fatty acids [9, 10, 12, 13, 17, 20, 22, 23, 25, 28], we hypothesized that plasma phospholipids species and cholesteryl ester fatty acids would be altered in non-medicated MDD-s patients during winter compared to summer-fall. We focused on MDD-s because it is the most predictable subtype of MDD in which

Table 1 Summary of changes in fatty acid percent composition or concentrations in patients with depression relative to healthy controls

Reference	Matrix	Lipid fraction	Reported changes in percent composition	Reported changes in concentration (mg per dL, L or cells)
MDD patients				
Ellis and Sanders [9]	Plasma	Choline glycerophospholipids	↑ EPA (123%) ↑ DHA (75%)	
Fehily <i>et al.</i> [10]	Plasma	Choline glycerophospholipids	↓ LNA (17%) ↑ EPA (43%) ↑ DHA (36%)	
	RBC	Ethanolamine glycerophospholipids	↑ DHA (14%)	
Maes <i>et al.</i> [13].	Serum	Choline glycerophospholipids	↓ LNA (14%)	
		Phospholipids	↓ 20:3n-9 (22%)	
		Cholesteryl ester	↑ 16:1 trans (40%) ↓ 16:1n-7 (30%) ↓ ALA (40%) ↓ EPA (30%)	
Peet <i>et al.</i> [14]	RBC	Total lipids	↑ 16:0 (17%) ↑ 18:0 (18%) ↓ LNA (20%) ↓ 20:3n-3 (32%) ↓ n-3 DPA (36%) ↓ DHA (43%)	
			↓ EPA (28%) ↓ DHA (31%)	
			↓ EPA (28%) ↓ DHA (31%)	
			↓ EPA (28%) ↓ DHA (31%)	
			↓ EPA (28%) ↓ DHA (31%)	
Edwards <i>et al.</i> [8] ^a	RBC	Phospholipids	↓ EPA (28%) ↓ DHA (31%)	
Maes <i>et al.</i> [12] ^b	Serum	Phospholipids	↑ Monounsaturates (8%) ↓ 22:4n-6 (65%) ↑ 22:5n-6 (%) ↓ EPA (31%) ↓ 22:5n-3 (11%)	↓ Saturates (18%) ↓ Monounsaturates (12%) ↓ LNA (21%) ↓ ARA (26%) ↓ 22:4n-6 (74%) ↓ ALA (31%) ↓ EPA (34%) ↓ DHA (20%)
		Cholesteryl esters	↓ ALA (25%) ↓ EPA (34%)	↓ Saturates (19%) ↓ Monounsaturates (13%) ↓ LNA (17%) ↓ 20:2n6 (48%) ↓ ARA (20%) ↓ ALA (36%) ↓ EPA (44%)
Tiemeier <i>et al.</i> [22] in elderly adults ≥60 years ^b	Plasma	Phospholipids	↑ ARA (3%) ↓ DHA (5%)	
Frasure-Smith <i>et al.</i> [20] in acute coronary heart disease patients ^a	Plasma	Phospholipids	↑ 18:3n-6 (36%) ↑ 22:4n-6 (16%) ↓ DHA (14%)	
Amin <i>et al.</i> [19] in acute coronary heart disease patients ^a	RBC	Phospholipids (membrane)	↓ 20:3n-6 (13%) ↓ 22:5n-3 (12%) ↓ DHA (13%)	
Aupperle <i>et al.</i> [24]. in multiple sclerosis patients	RBC	Phospholipids (membrane)	↓ 18:1n-9 (8%)	
Feart <i>et al.</i> [21] in elderly adults; mean age 74.6.	Plasma	Total lipids	↓ EPA (16%)	
Rees <i>et al.</i> [25]. Women in 3rd trimester of pregnancy	Plasma	Phospholipids	↓ DHA (26%)	
Riemer <i>et al.</i> [23] ^c	Serum	Cholesteryl esters	↓ DHA (27%)	

Table 1 continued

Reference	Matrix	Lipid fraction	Reported changes in percent composition	Reported changes in concentration (mg per dL, L or cells)
Assies <i>et al.</i> [18]	Plasma	Total lipids		↑ 14:0 (46%) ↑ 16:0 (19%) ↑ 20:0 (12%) ↑ 14:1n-5 (142%) ↑ 16:1n-7 (60%) ↑ 18:1n-7 (21%) ↑ 16:1n-9 (30%) ↑ 18:1n-9 (23%) ↓ 24:1n-9 (16%) ↑ LNA (19%) ↑ 18:3n-6 (21%) ↑ 22:2n-6 (detected) ↑ ALA (25%)
	RBC	Total lipids		↑16:0 (5%) ↓ 20:0 (11%) ↓22:0 (20%) ↓24:0 (31%) ↓ 16:1n-9 (52%) ↓ 24:1n-9 (32%) ↑18:3n-6 (50%) ↓ 20:3n-6 (9%) ↓ ARA (12%) ↑22:2n-6 (detected) ↓ 22:4n-6 (18%) ↓ 22:5n-6 (19%) ↑ 18:4n-3 (500%) ↓ 22:5n-3 (25%) ↓ DHA (26%)
Sublette <i>et al.</i> [16] ^c	Plasma	Total lipids		↓ ALA (15%) ↓ DHA (23%)
Rizzo <i>et al.</i> [15] ^d	RBC	Phospholipids	↓ EPA (48-50%)	
	Whole blood	Total lipids	↓ EPA (57-64%) ↓ DHA (42-46%)	
Vařeka <i>et al.</i> [17]	Plasma	Cholesteryl ester	↑ 16:1n-7 (22%) ^d	
		Choline glycerophospholipid (Phosphatidylcholine)	↔ ^e	
Suicide patients				
Huan <i>et al.</i> [11]	RBC	Phospholipids	↑ 18:0 (5%) ↓ EPA (30%) ↓ 22:5n-3(12%) ↓ DHA (17%)	
Healthy volunteers during winter relative to summer				
De Vriese <i>et al.</i> [28]	Serum	Phospholipids	↓ ARA (5%) ↓ EPA (29%) ↓ DHA (19%)	

ARA arachidonic acid (20:4n-6), ALA alpha-linolenic acid (18:3n-3), DHA docosahexaenoic acid (22:6n-3), EPA eicosapentaenoic acid (20:5n-3), LNA linoleic acid (18:2n-6), MDD major depressive disorder, MDD-s major depressive disorder with seasonal pattern, RBC red blood cells. 14:0 myristic acid, 20:0 arachidic acid, 22:0 behenic acid, 24:0 lignoceric acid, 14:1n-5 myristoleic acid, 16:1n-7 palmitoleic acid, 18:1n-7 vaccenic acid, 16:1n-9 hypogeic acid, 18:1n-9 oleic acid (OLA), 24:1n-9 nervonic acid, 18:4n-3 octadecatetraenoic acid, 22:5n-3 n-3 docosapentaenoic acid, 18:3n-6 gamma-linoleic acid, 20:3n-6 dihomogamma-linolenic acid, 20:2n-6 eicosadienoic acid, 22:2n-6 docosadienoic acid, 22:4n-6 docosatetraenoic acid, 22:5n-6 n-6 docosapentaenoic acid

^a Saturated and monounsaturated fatty acids not reported

^b Only total saturated and monounsaturated fatty acids reported

^c Only EPA and DHA reported

^d Study done with elderly women

^e Although no change in individual fatty acids were found, total monounsaturated fatty acids and n-6 polyunsaturated fatty acids increased and decreased in cholesteryl esters, respectively, and total saturated fatty acids increased in phosphatidylcholine

to observe prospective changes in lipid biomarkers between mood states. Moreover, unlike MDD, lipid metabolism in MDD-s remains understudied.

Fatty acids were measured in plasma free fatty acids, triacylglycerols, cholesteryl esters, and total phospholipids. Diacyl-phospholipid and plasmalogen molecular species, which make up the majority of the total phospholipid pool, were also quantified. Diacyl-phospholipids contain two fatty acids bound to the stereospecifically-numbered (*sn*)-1 and 2 positions via an ester bond, whereas plasmalogens contain a vinyl-ether bond instead of an ester bond at the *sn*-1 position (reviewed in [32]). The *sn*-1 position typically contains a saturated fatty acid, whereas the *sn*-2 position contains a PUFA such as ARA or DHA [33].

We found seasonal changes in triacylglyceride myristoleic acid and n-3 fatty acid concentrations within diacyl-phospholipids and triacylglycerides, as well as positive associations between the change in depressive score and the change in cholesteryl ester oleate and several PUFA concentrations.

Experimental Procedures

Materials

Standard choline plasmalogens, (1-(1Z-octadecenyl)-2-oleoyl-*sn*-glycero-3-phosphocholine (STol-OLA-Gro-3-PCho), 1-(1Z-octadecenyl)-2-arachidonoyl-*sn*-glycero-3-phosphocholine (STol-ARA-Gro-3-PCho), 1-(1Z-octadecenyl)-docosahexaenoyl-*sn*-glycero-3-phosphocholine (STol-DHA-Gro-3-PCho)), ethanolamine plasmalogens (1-(1Z-octadecenyl)-oleoyl-*sn*-glycero-3-phosphoethanolamine (STol-OLA-Gro-3-PEtn), 1-(1Z-octadecenyl)-arachidonoyl-*sn*-glycero-3-phosphoethanolamine (STol-ARA-Gro-3-PEtn), 1-(1Z-octadecenyl)-docosahexaenoyl-*sn*-glycero-3-phosphoethanolamine (STol-DHA-Gro-3-PEtn)), diacyl-phosphatidylcholine (1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (STA-OLA-Gro-3-PCho), 1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (STA-ARA-Gro-3-PCho), 1-stearoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine (STA-DHA-Gro-3-PCho)) and diacyl-phosphatidylethanolamine (1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (STA-OLA-Gro-3-PEtn), 1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphoethanolamine (STA-ARA-Gro-3-PEtn), 1-stearoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphoethanolamine (STA-DHA-Gro-3-PEtn)) were obtained from Avanti Polar Lipids (Alabaster, AL, USA).

Standard free heptadecanoic acid (17:0), free palmitic acid (16:0), tripalmitin (tri-16:0), and cholesteryl-palmitate used for the thin layer chromatography (TLC) or fatty acid analysis were obtained from NuChek Prep Inc. (Elysian,

MN, USA). 1,2-diheptadecanoyl-*sn*-glycero-3-phosphocholine (di-17:0 PC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine were obtained from Avanti Polar Lipids (Alabaster, AL, USA).

Participants

Ethical approval for the present study was obtained from the Sunnybrook Research Ethics Board. All participants provided informed written consent prior to beginning the study. A total of 15 medication-free participants were recruited. Participants were aged between 18 and 65 years and had a history of MDD-s based on at least two episodes of depression that presented a seasonal pattern over the past 3 years were recruited. Participants 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 from the study. None of the participants received light or cognitive behavioral therapy during winter.

Each participant came for a baseline visit in summer or early fall (August–October) and a follow-up visit during winter (December–February). A structured clinical interview was used to diagnose depressive episodes based on DSM-5 criteria, and the Beck Depression Inventory-II (BDI-II) was used to assess depressive symptom severity [34]. The BDI-II is a 21-item self-report scale that measures the severity of depressive symptoms. Each item is endorsed on a 4-point scale that ranges from 0 to 3, corresponding to statements of increasing severity. The individual items are summed to create a total score between 0 and 63. Although it is recommended that total scores be interpreted according to the characteristics of each individual population, a total score of 0–13 is often considered minimal, 14–19 mild, 20–28 moderate, and 29–63 severe.

During each visit, a blood sample was collected in K₂-ethylenediaminetetraacetic (EDTA) tubes and centrifuged for 10 min at 1000×g at 4 °C. Samples were collected in the morning at 9:00 AM (±1:00 h), after a ≥10 h overnight fast. Plasma was isolated and stored at −80 °C until analysis. Only participants who were euthymic in summer and depressed in winter were included in the final analysis. Lipid assays described below were performed in a blinded and randomized manner.

Diacyl-Phospholipid and Plasmalogen Extraction

Phospholipids containing plasmalogens and diacyl-phospholipids were extracted from plasma by methanol precipitation and solid phase extraction as established by Otoki *et al.* [35]. Eighty microliters of plasma were mixed with 20 µL of 25 µM 2Na-EDTA in water and 500 µL of

methanol containing 0.002% butylated hydroxytoluene. Surrogate standards were not added because deuterated standards for most of the phospholipid species analyzed (plasmalogens in particular) do not exist. The samples were centrifuged at $10,000\times g$ for 10 min at $-10\text{ }^{\circ}\text{C}$, and 600 μL of the supernatant was collected. The remaining precipitate was re-extracted with 500 μL of methanol and subjected to centrifugation at $10,000\times g$ for 10 min at $-10\text{ }^{\circ}\text{C}$. The resulting 500 μL of the supernatant was combined to the first supernatant, to yield a total of 1100 μL of methanol solution. Four hundred μL of the pooled methanol extract was loaded onto a silica Sep-Pak solid phase extraction cartridge (100 mg; Waters, Milford, MA, USA) equilibrated with methanol. The eluted methanol was collected. The cartridge was then loaded with an additional 1.6 mL of methanol to elute residual phospholipids, yielding 2 mL of methanol in total. Ten microliters of the final aliquot was injected into an Agilent liquid chromatography tandem mass spectrometry (LC–MS/MS) system operated in multiple reaction monitoring (MRM) mode to analyze plasmalogen and diacyl-phospholipid molecular species. The following phospholipid species were analyzed: STol-OLA-Gro-3-PCho, STol-ARA-Gro-3-PCho, STol-DHA-Gro-3-PCho, STol-OLA-Gro-3-PEtn, STol-ARA-Gro-3-PEtn, STol-DHA-Gro-3-PEtn, STA-OLA-Gro-3-PCho, STA-ARA-Gro-3-PCho, STA-DHA-Gro-3-PCho, STA-OLA-Gro-3-PEtn, STA-ARA-Gro-3-PEtn and STA-DHA-Gro-3-PEtn.

A standard curve for each of the compounds was established by plotting standard concentration on the y-axis and standard area on the x-axis. Concentrations were calculated based on an equation derived from each of the external standard curves as follows:

$$\text{Concentration} = \frac{\text{Area of compound in sample} \times \text{slope of external standard curve}}{(80 \times (400/1100) \times (10/2000)),}$$

where the slope is derived from the standard curve plot, 80 μL is the volume of plasma used for the extraction, 1100 μL is the total volume of the methanol extract, 400 μL is the volume of methanol extract used for the solid phase extraction, 2000 μL is the final elution volume following solid phase extraction (i.e. 400 μL methanol phospholipid extract + 1600 μL used to elute residual phospholipids), and 10 μL is the volume injected into the LC–MS/MS.

The standard percentage recovery was determined by spiking 80 μL of a plasma sample with 200 pmol of diacyl phospholipid and plasmalogen standard mix and calculating the amount recovered with the following equation:

$$\text{Percent recovery} = \text{Observed value/expected value} \times 100.$$

The observed value is the measured amount of each phospholipid (pmol) in spiked plasma minus the amount (pmol) in non-spiked plasma. The expected value is $200\text{ pmol} \times (400/1100) \times (10/2000)$ where 1100 μL is the total volume of methanol extract, 400 μL is the volume of methanol extract used for the solid phase extraction, 2000 μL is the volume of elution following solid phase extraction and 10 μL is the volume injected in the instrument. The percentage recovery was 105% for STol-OLA-Gro-3-PCho, 134% for STol-ARA-Gro-3-PCho, 118% for STol-DHA-Gro-3-PCho, 89% for STol-OLA-Gro-3-PEtn, 124% for STol-ARA-Gro-3-PEtn, 123% for STol-DHA-Gro-3-PEtn, 123% for STol-OLA-Gro-3-PEtn, 36% for STol-ARA-Gro-3-PEtn, 69% for STol-DHA-Gro-3-PEtn, 85% for STA-OLA-Gro-3-PEtn, 109% for STA-ARA-Gro-3-PEtn and 78% STA-DHA-Gro-3-PEtn. The low percentage recovery for STol-ARA-Gro-3-PEtn is likely due to the low amount of STol-ARA-Gro-3-PEtn spiked into the sample (200 pmol) relative to the total amount of STol-ARA-Gro-3-PEtn present in 80 μL of human blood (~3600 pmol).

Matrix effects reflecting the extent of ion suppression were calculated using the following equation:

$$\text{Matrix effects} = \frac{(\text{Area in plasma spiked with 200 pmol standard})}{(\text{Area in non-spiked plasma} + \text{Area in standard alone})} \times 100.$$

A value close to 100% reflects minimal matrix effects. In this study, calculated matrix effect values were between 84 and 107%, suggesting minimal matrix interference.

LC–MS/MS analysis

Plasmalogen and diacyl-phospholipids were analyzed by LC–MS/MS consisting of an Agilent LC system 1290 (Agilent Corporation, Palo Alto, CA, USA) equipped with a vacuum degasser, a quaternary pump and an autosampler, and an Agilent 6460 Triple Quadrupole MS system (Agilent Corporation).

Samples and standards were separated on a C8 column ($2.0 \times 100\text{ mm}$; GL Science, Tokyo, Japan) with a binary gradient consisting of solvent A (methanol: water 68:32 (v/v) containing 0.1 mM sodium acetate) and solvent B (methanol containing 0.1 mM sodium acetate) [33]. The column temperature was maintained at $40\text{ }^{\circ}\text{C}$. The gradient profile is shown in Supplementary Table 1. Samples were analyzed in MRM mode. Electrospray ionization was used as the ion source with the following experimental parameters: Gas temperature, $300\text{ }^{\circ}\text{C}$; Sheath Gas temperature, $220\text{ }^{\circ}\text{C}$; Sheath Gas flow, 11 ml/min; Nebulizers, 45 psi; Capillary gas, 3500/–3500 V. Other optimization parameters and MRM pairs are described in Supplementary Table 2.

Lipid Extraction and Analysis

Total lipids were extracted from plasma by the Folch method and fractionated into phospholipids, triacylglycerols, cholesteryl esters and unesterified (free) fatty acids by thin layer chromatography (TLC) [36].

Two hundred microliters of plasma were added to 3 mL of chloroform: methanol (2:1 v/v) containing unesterified heptadecanoic acid (17:0) as an internal standard for unesterified fatty acids, followed by 0.75 mL 0.1 M NaCl. The samples were vortexed and centrifuged. The lower chloroform phase containing total lipids was pipetted into a new test-tube. Lipids were re-extracted with additional 2 mL of chloroform. The chloroform extracts were pooled, dried under nitrogen, reconstituted in 100 μ L chloroform: methanol (2:1 v/v) and plated on pre-washed and heat-activated silica gel-60 TLC plates (EM Separation Technologies, Gibbstown, NJ, USA). Plates were washed in a TLC tank containing 100 mL of chloroform: methanol (2:1 v/v). The solvent front was allowed to migrate to the top of the plates, which were then kept in a vacuum oven chamber at 80 °C for at least 2 h.

Total lipids extracted from plasma were re-dissolved in 100 μ L chloroform/methanol and re-plated to ensure that all lipids were transferred to the TLC plates. Lipids were resolved using heptane: diethylether: glacial acetic acid (60:40:3, v/v/v) solvent system to separate phospholipids, triacylglycerides, cholesteryl esters and free fatty acids [37]. Authentic standards (free palmitic acid, tripalmitin, cholesteryl-palmitate and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine) were run on separate lanes on the plates to identify lipid bands under ultraviolet light, after spraying with 0.02% dichlorofluorescein solution in methanol. The bands were scraped into test tubes containing di-17:0 PC as an internal standard for phospholipids, triacylglycerides and cholesteryl esters.

Each lipid fraction was transesterified in methanolic acid according to the method of Ichihara and Fukubayashi [38]. Four hundred microliters of toluene, 3 mL methanol and 600 μ L HCl-methanol (8:92, v/v) were added to each lipid fraction. 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. The upper hexane layer containing fatty acid methyl esters was dried under nitrogen and reconstituted in 5 μ L of hexane for the unesterified fatty acid fraction and 50 μ L of hexane for the esterified fractions.

Fatty acid methyl esters were analyzed by gas-chromatography. One microliter of sample 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 μ m thickness; Agilent, Santa Clara, CA, USA). The detector and injector temperatures were set at 300 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; Table 2).

Statistical Analysis

Statistical analysis was performed on GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA, USA). Due to the small sample size ($n = 9$), a non-parametric Wilcoxon's rank test was used to assess the differences between summer-fall and winter. The relation between change in fatty acid concentrations in the different lipid pools and change in BDI-II score was evaluated using a Spearman's correlation. Corrections for multiple independent statistical tests were not performed due to the exploratory nature of the study. Statistical significance was set at $p < 0.05$ (two-tailed). All data are expressed as medians (interquartile range; 25th and 75th percentiles).

Results

Of 15 participants recruited, 4 dropped out before the end of the study and 2 did not meet the DSM-5 depression criteria in winter. The remaining participants (4 men, 5 women) were 46.7 ± 14 years of age and had an average of 3.3 previous episodes of depression. Median BDI-II scores significantly increased in the remaining 9 participants between summer-fall and winter (summer-fall, 5.0 (interquartile range 0.5–8.5); winter, 20.0 (interquartile range 13.5–27.0); $p = 0.0039$) [39], indicating minimal symptoms in summer-fall and generally mild to moderate severity in winter. BDI-II scores ranged between 0–12 in summer-fall and 9–28 in winter.

Table 2 shows fatty acid concentrations within free fatty acids, phospholipids, triacylglycerides and cholesteryl esters. There were no significant differences in free or esterified fatty acid concentrations between summer-fall and winter, except for alpha-linolenic acid (ALA) and myristoleic acid (14:1n-5) within triacylglycerides. ALA was significantly reduced by 34% and myristoleic (14:1n-5) was increased by 28% in triacylglycerides in winter as compared to summer-fall.

Table 2 Plasma fatty acid concentrations (μM) in free fatty acids, phospholipids, triacylglycerides and cholesteryl esters in MDD-s patients during summer-fall and winter

	Summer-fall	Winter
Free fatty acids		
12:0	86.5 (73.1–158.7)	97.3 (58.2–151.3)
14:0	18.3 (13.4–21.8)	22.0 (16.3–25.3)
16:0	193.6 (106–311.9)	189.2 (108.2–294.3)
18:0	43.6 (36.1–55.8)	44.6 (36.5–56.9)
Total SFA	422.5 (293.1–471.8)	344.9 (284.3–467.8)
14:1 n-5	19.1 (12.7–36.6)	23.6 (15.1–33.2)
16:1 n-7	137.8 (112.4–201.2)	132.8 (99.0–219.3)
18:1 n-9	131.7 (70.8–186.7)	161.4 (78.4–223.6)
Total MUFA	332.3 (241.9–369)	360 (211.9–369)
18:2 n-6	33.0 (26.2–49.4)	39.2 (23.2–62.2)
18:3 n-6	–	–
20:2 n-6	–	–
20:3 n-6	–	–
20:4 n-6	–	–
Total n-6 PUFA	33.0 (26.2–49.4)	39.2 (23.2–62.2)
18:3 n-3	–	–
20:5 n-3	16.2 (12.4–21.8)	15.1 (14.3–19.7)
22:5 n-3	–	–
22:6 n-3	–	–
Total n-3 PUFA	16.2 (12.4–21.8)	15.1 (14.3–19.7)
Total FA	764.4 (598.7–863.0)	686.4 (618.1–962.4)
Phospholipids		
12:0	148.7 (90.0–160.9)	126.6 (87.55–186.7)
14:0	0.0 (0.0–11.7)	0.0 (0.0–15.8)
16:0	1267.1 (1154–1860)	1229.5 (1034–2068)
18:0	671.5 (605–796.8)	620.2 (564–897.2)
Total SFA	2199 (1838–2822)	2089 (1804–2986)
14:1 n-5	21.4 (0.0–25.5)	25.9 (0.0–34.8)
16:1 n-7	89.7 (12.6–130.0)	80.9 (42.4–117.7)
18:1 n-9	421.3 (304.2–512.8)	417.7 (275.3–577)
Total MUFA	524.5 (404.4–632.9)	559.6 (445.2–642.6)
18:2 n-6	884.5 (621.3–1140)	1027.5 (711–1247)
18:3 n-6	–	–
20:2 n-6	–	–
20:3 n-6	83.2 (60.8–164)	114.5 (58.1–188.2)
20:4 n-6	399.5 (323.3–399.5)	413.6 (321.3–557.3)
Total n-6 PUFA	1373 (1074–1678)	1492 (1125–1964)
18:3 n-3	–	–
20:5 n-3	31.2 (22.6–57.2)	46.7 (32.7–60.1)
22:5 n-3	29.5 (0.0–40.8)	29.8 (10.6–49.8)
22:6 n-3	127.7 (100.1–168.2)	138.1 (121.8–170.3)
Total n-3 PUFA	187.7 (157.9–227)	229 (182.3–277.3)
Total FA	4468.5 (3597–5173)	4591.9 (3668–5946)
Triglycerides		
12:0	103.4 (75.0–138.8)	99.8 (52.8–147.6)
14:0	20.5 (11.3–51.5)	25.3 (12.6–38.3)

Table 2 continued

	Summer-fall	Winter
16:0	458.7 (236.6–956.2)	552.0 (266.9–1100)
18:0	69.0 (47.1–137.0)	243.7 (146.8–283.2)
Total SFA	680.9 (399.3–1214)	762.1 (433.4–1315)
14:1 n-5	32.2 (25.8–46.5)	41.1 (32.0–46.9)*
16:1 n-7	255.5 (192.6–264.8)	243.7 (146.8–283.2)
18:1 n-9	584.9 (272.2–624.7)	435.4 (336.9–893.9)
Total MUFA	787.4 (518.4–939.6)	695.3 (612.5–1112)
18:2 n-6	272.8 (182.0–306.3)	216.8 (119.4–346.1)
18:3 n-6	–	–
20:2 n-6	–	–
20:3 n-6	–	–
20:4 n-6	17.2 (12.0–24.4)	21.3 (19.4–29.3)
Total n-6 PUFA	272.8 (196.2–331)	238.7 (148.6–370.4)
18:3 n-3	16.0 (12.7–27.0)	10.5 (3.1–19.9)*
20:5 n-3	21.8 (16.4–29.4)	21.3 (19.4–29.3)
22:5 n-3	–	–
22:6 n-3	9.4 (3.2–19.8)	9.8 (2.0–15.2)
Total n-3 PUFA	51.9 (43.9–72.9)	39.4 (34.8–63.2)*
Total FA	1967.3 (1207–2344)	2110.2 (1252–2678)
Cholesteryl esters		
12:0	268.7 (154.9–371.6)	202.9 (149.3–257.9)
14:0	23.6 (17.1–34.6)	23.1 (15.5–31.8)
16:0	474.3 (417.8–621.9)	476.1 (378.2–544.9)
18:0	35.1 (8.0–47.0)	35.9 (23.1–43.2)
Total SFA	775.7 (634.7–1114)	717.8 (647.3–831.0)
14:1 n-5	23.2 (0.0–48.3)	31.4 (19.1–31.5)
16:1 n-7	265.2 (215.7–389.5)	283.4 (219.9–318.5)
18:1 n-9	702.0 (580.6–927.6)	704.5 (624.4–810.3)
Total MUFA	973.2 (902.2–1383)	1010 (886.2–1140)
18:2 n-6	2035.6 (1635–2708)	2230.7 (1653–2526)
18:3 n-6	23.4 (13.9–41.6)	29.8 (12.7–46.8)
20:2 n-6	13.9 (0.0–28.8)	29.8 (0.0–37.7)
20:3 n-6	–	–
20:4 n-6	260.6 (217.6–369.4)	199.4 (171.2–342.7)
Total n-6 PUFA	2341 (1890–3115)	2635 (1862–2875)
18:3 n-3	24.4 (13.8–47.6)	16.0 (14.0–18.0)
20:5 n-3	40.1 (23.1–85.7)	34.5 (25.6–56.2)
22:5 n-3	–	–
22:6 n-3	22.8 (20.0–35.1)	25.8 (16.9–29.5)
Total n-3 PUFA	93.1 (61.2–187.9)	72.2 (49.1–95.9)
Total FA	4333.1 (3592–5806)	4438.1 (3456–4918)

Values are median and interquartile ranges (25th and 75th percentile). Wilcoxon's rank test was used to assess the differences between summer-fall and winter

SFA saturated fatty acids, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids, FA fatty acid

* Significant difference between summer-fall and winter by Wilcoxon's test; $p < 0.05$; –, not detected

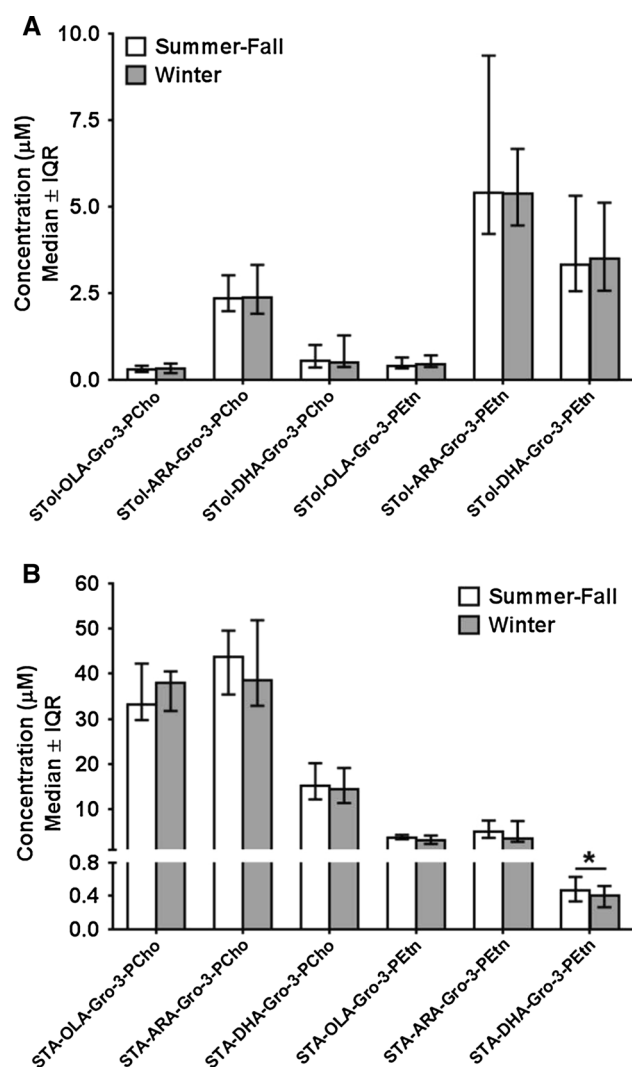


Fig. 1 Plasma concentration of different plasmalogen species (a) and diacyl-phospholipids (b) in summer-fall and winter. Values are medians $\pm$ interquartile ranges (IQR; 25th and 75th percentiles) of 9 subjects. Wilcoxon's rank test was used to assess the differences between summer-fall and winter. Asterisk significant difference between summer-fall and winter assessed by a Wilcoxon's rank test ($p < 0.05$). STol-OLA-Gro-3-PCho, 1-(1Z-octadecenyl)-2-oleyl-*sn*-glycero-3-phosphocholine; STol-ARA-Gro-3-PCho, 1-(1Z-octadecenyl)-2-arachidonyl-*sn*-glycero-3-phosphocholine; STol-DHA-Gro-3-PCho, 1-(1Z-octadecenyl)-docosahexaenoyl-*sn*-glycero-3-phosphocholine; STol-OLA-Gro-3-PEtn, 1-(1Z-octadecenyl)-oleyl-*sn*-glycero-3-phosphoethanolamine; STol-ARA-Gro-3-PEtn, 1-(1Z-octadecenyl)-arachidonyl-*sn*-glycero-3-phosphoethanolamine; STol-DHA-Gro-3-PEtn, 1-(1Z-octadecenyl)-docosahexaenoyl-*sn*-glycero-3-phosphoethanolamine; STA-OLA-Gro-3-PCho, 1-stearyl-2-oleyl-*sn*-glycero-3-phosphocholine; STA-ARA-Gro-3-PCho, 1-stearyl-2-arachidonyl-*sn*-glycero-3-phosphocholine; STA-DHA-Gro-3-PCho, 1-stearyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine; STA-OLA-Gro-3-PEtn, 1-stearyl-2-oleyl-*sn*-glycero-3-phosphoethanolamine; STA-ARA-Gro-3-PEtn, 1-stearyl-2-arachidonyl-*sn*-glycero-3-phosphoethanolamine; STA-DHA-Gro-3-PEtn, 1-stearyl-2-docosahexaenoyl-*sn*-glycero-3-phosphoethanolamine

Figure 1a, b show the concentration of plasmalogen and diacyl-phospholipid species, respectively. STA-DHA-Gro-3-PEtn, a diacyl-phospholipid bearing DHA, was significantly reduced by 13.8% in winter compared to summer-fall ($p = 0.0273$). No significant differences were observed in plasmalogen or other diacyl-phospholipid species.

In an exploratory manner, we assessed correlations between seasonal changes in fatty acids within the various lipid pools and the change in BDI-II score, to test whether changes in specific lipid concentrations fluctuate with depressive severity. Significant correlations were seen in one fatty acid (16:1n-7) in phospholipids and in 8 fatty acids in cholesteryl esters (Supplementary Table 3). Figure 2a–f plots this relationship for main cholesteryl ester fatty acids, including oleic acid (OLA, 18:1n-9), linoleic acid (LNA, 18:2n-6), ARA, ALA and EPA, as well as total fatty acids. As shown, the change in OLA, LNA, ARA, ALA, EPA and total fatty acids ($p < 0.05$) was positively correlated to the change in BDI-II between summer-fall and winter.

Discussion

To our knowledge, this is the first study to probe seasonal changes in lipidomic markers in unmedicated MDD-s patients. We found statistically significant reductions in triacylglyceride ALA and STA-DHA-Gro-3-PEtn, and increased triacylglyceride myristoleic acid concentrations in plasma of non-medicated MDD-s patients during winter compared to summer-fall. Changes in cholesteryl ester OLA, LNA, ARA, ALA, EPA and total fatty acids between summer-fall and winter were positively correlated to the change in BDI-II scores.

The reduction in triacylglyceride ALA and increase in myristoleic acid during winter may be due to changes in their dietary intake or metabolism. We are not aware of clinical data on fatty acid intake in MDD-s patients. Two studies reported no difference in fatty acid intake between control and MDD patients, although seasonal shifts in consumption in relation to mood were not addressed [8, 16]. Energy intake, habitual food intake patterns and physical activity are known to change between seasons [40, 41]. A detailed analysis of seasonal patterns in fatty acid consumption in relation to physical activity in MDD-s patients could clarify whether the observed triacylglyceride fatty acid changes were related to diet or metabolic disturbances.

Although phospholipid DHA did not change between seasons in this study, STA-DHA-Gro-3-PEtn, an ethanolamine phospholipid containing stearic acid and DHA, decreased by 14% in winter depression compared to

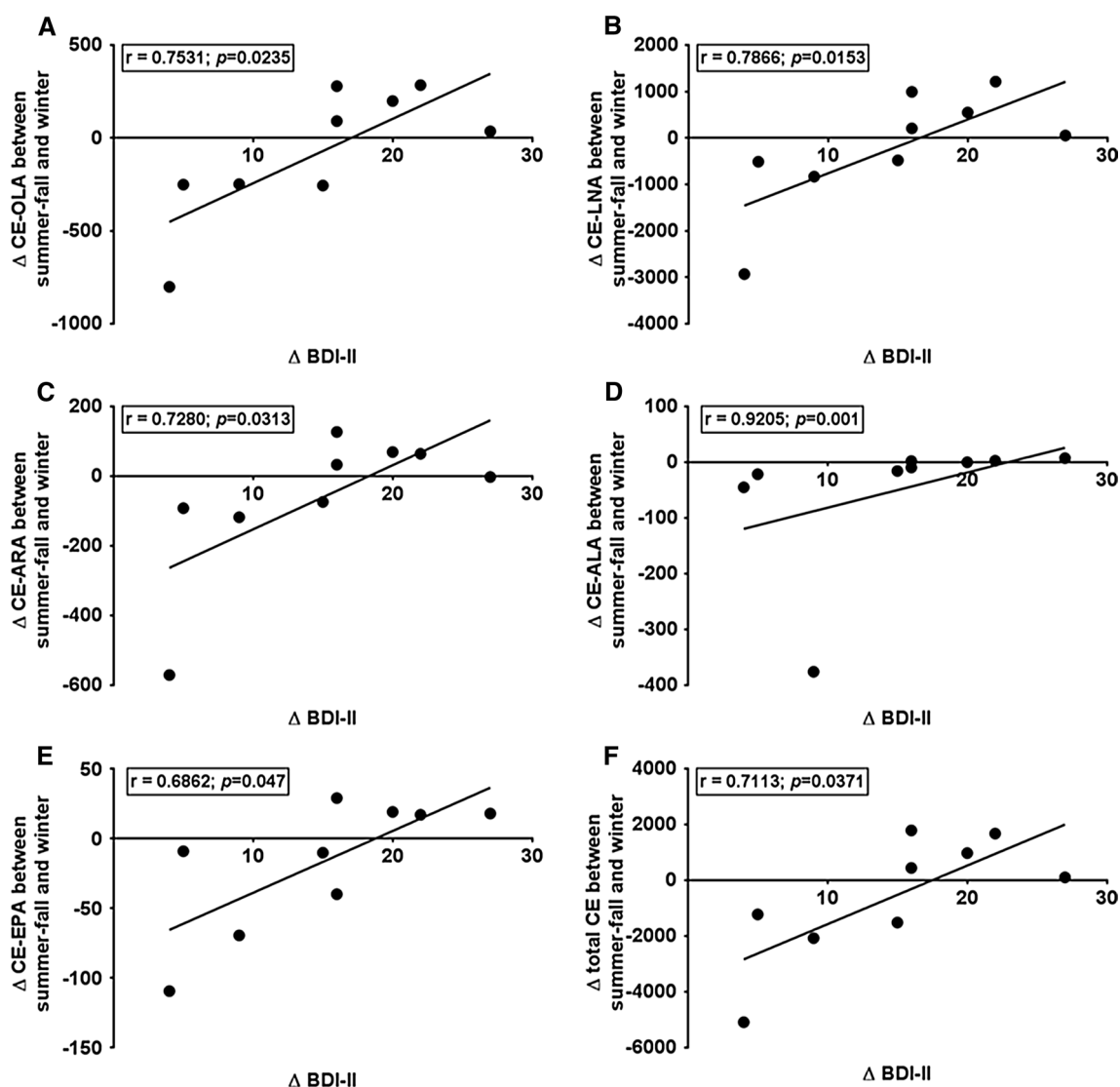


Fig. 2 Spearman's correlation between the change in BDI-II score and change in cholesteryl-esters (CE) fatty acids between winter and summer-fall. **a** Oleic acid (OLA; 18:1 n-9), **b** linoleic acid (LNA;

18:2 n-6), **c** arachidonic acid (ARA; 20:4 n-6), **d** α -linolenic acid (ALA; 18:3 n-3), **e** eicosapentaenoic acid (EPA; 20:5 n-3) and **f** total fatty acids

euthymia in summer-fall. This suggests that measuring fatty acids in total phospholipids may not yield the same level of sensitivity as measuring individual phospholipid species with LC-MS/MS. Total phospholipid DHA concentration was either unchanged [18] or reduced [13] in plasma of MDD patients compared to healthy controls. The inconsistency between studies may reflect inadequate sensitivity of measuring total phospholipid DHA concentration. Specific phospholipid species such as STA-DHA-Gro-3-PETn may change more reliably with mood states.

The 14% reduction in STA-DHA-Gro-3-PETn concentration in winter relative to summer-fall likely reflects metabolic disturbances in DHA or stearic acid turnover within phosphatidylethanolamine rather than seasonal shifts in

DHA or stearic acid intake. This is because dietary DHA is preferentially incorporated into plasma phosphatidylcholine, but not phosphatidylethanolamine, as evidenced by one study that showed limited responsiveness of phosphatidylethanolamine to dietary DHA intake compared to phosphatidylcholine following 28 days of fish oil supplementation (3.48 g EPA and 2.28 g DHA) [30]. Instead, reduced synthesis or increased utilization may explain the STA-DHA-Gro-3-PETn reduction. Tracer studies involving labeled STA-DHA-Gro-3-PETn, stearic acid or DHA could be used in future studies to probe STA-DHA-Gro-3-PETn turnover in MDD-s patients [42, 43]. The reduction in STA-DHA-Gro-3-PETn, if confirmed in larger cohorts, might justify its development as a diagnostic biomarker of mood

symptoms in MDD-s patients. It would also be worthwhile to assess whether this marker is specific to MDD-s or whether it also generalizes to other MDD subtypes.

Cholesteryl ester fatty acid concentrations did not differ significantly between summer-fall and winter, yet significant positive associations between summer-fall to winter changes in BDI-II scores and cholesteryl ester OLA, LNA, ARA, ALA, EPA and total fatty acids concentrations were observed. Maes *et al.* reported an inverse association between depressive scores and cholesteryl ester ALA and EPA percent composition or concentrations [12], which is opposite to our findings. It is not clear whether some of these associations reflect metabolic perturbations related directly to mood symptoms, or differences in dietary fatty acid intake, either of which may have differed between the populations studied. MDD-s is known to be a good model for atypical depression, which often involves hypersomnia and overeating [44], two factors that impact fatty acid intake and potentially cholesteryl ester composition between seasons.

The observed reduction in ALA triacylglyceride concentration is in general agreement with two MDD studies which reported reduced ALA concentration in serum or plasma cholesteryl ester, phospholipid and total lipid [12, 16], and opposite to one MDD study which showed increased plasma total lipid ALA concentration [18]. Except for myristoleic acid, we did not observe changes in the concentration of other fatty acids that were previously found altered in various MDD studies. Notwithstanding dietary, activity, age or gender confounders, differences in lipid profile could be due to divergent lipid metabolic pathways that are impacted in MDD and MDD-s. Thus, the decrease in ALA could be related to reduced intake or increased utilization for β -oxidation or synthesis into EPA, possibilities that merit follow-up with detailed dietary assessments and tracers that inform on net oxidation and liver synthesis-secretion rates [45].

Limitations of this study include the small sample size, absence of dietary or physical activity information and lack of a control group to disentangle effects of season from those of depression. However, the study is strengthened by a within-subjects design, which increases power by accounting for between-subjects variability. Multiple independent comparisons used in this study increase the risk of false positives and should be confirmed in larger studies. The between-group variation reported herein, however, is valuable for designing adequately powered follow-up studies. Overall, the present findings provide a starting point for larger confirmatory studies to characterize disturbed lipid metabolic pathways in MDD-s.

In summary, this exploratory study identified seasonal disturbances in lipid metabolism in unmedicated MDD-s participants. The changes in ethanolamine phospholipid

DHA concentration in particular, and associations between cholesteryl ester n-3 fatty acids and symptom severity, add to the evidence suggesting the involvement of n-3 fatty acids in MDD, extending the finding to MDD-s. The STA-DHA-Gro-3-PEtn changes identify a novel species that can be measured reliably for potential use as a biomarker of progression or response to therapy. Replication of these findings in adequately powered and controlled studies is merited.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no competing interest.

Ethical approval The study was approved by the Sunnybrook Research Ethics Board and complied with ethical standards.

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Supplementary Material:

Plasma phosphatidylethanolamine and triacylglycerol fatty acid concentrations are altered in major depressive disorder patients with seasonal pattern

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Supplementary Table 1: Gradient condition of LC-MS/MS

time (min)	Solvent B (%)	flow (ml/min)
0	0	0.3
17		0.35
17.1		0.2
20	98	0.2
25	100	0.2
28	100	0.2
28.1	100	0.35
30	100	0.35
30.1	0	0.35
31.5	0	0.3
33	Stop	

Supplementary Table 2: Optimal MS/MS parameters for PL detection

PL	Position <i>sn1/sn2</i>	Precursor ion <i>m/z</i>	Product ion <i>m/z</i>	Fragmentor V	Collision energy
choline-plasmalogen	18:0/18:1	794.6	307.3	70	40
	18:0/20:4	816.6	307.3	70	45
	18:0/22:6	840.6	307.3	70	48
ethanolamine-plasmalogen	18:0/18:1	752.5	307.3	120	42
	18:0/20:4	774.5	307.3	120	45
	18:0/22:6	798.5	307.3	100	42
choline-diacyl-phospholipids	18:0/18:1	810.6	147.0	100	40
	18:0/20:4	832.6	147.0	100	45
	18:0/22:6	856.6	147.0	90	48
ethanolamine-diacyl-phospholipids	18:0/18:1	768.6	164.0	90	30
	18:0/20:4	790.6	164.0	90	35
	18:0/22:6	814.6	164.0	100	35

Abbreviations: *sn*, stereospecifically-numbered.

Supplementary Table 3. Spearman's correlation between change in BDI-II score and fatty acid concentrations in the different lipid pool. Spearman's correlation r and p values are indicated when significant ($p < 0.05$). -, non detectable; ns, non significant; LC-MS/MS, liquid chromatography tandem mass spectrometry; GC, gas chromatography; PCp, phosphocholine plasmalogens; PC, phosphatidylcholine; PEp, phosphoethanolamine plasmalogen; PE, phosphatidylethanolamine; FFA, free fatty acid; CE, cholesteryl ester; PL, phospholipid; TG, triglyceride; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

		LC-MS/MS analysis				GC analysis			
		PCp	PC	PEp	PE	FFA	CE	PL	TG
SFA	Δ 12:0	-	-	-	-	ns	$r = 0.6862$; $p = 0.047$	ns	ns
	Δ 14:0	-	-	-	-	ns	ns	ns	ns
	Δ 16:0	-	-	-	-	ns	ns	ns	ns
	Δ 18:0	-	-	-	-	ns	ns	ns	ns
MUFA	Δ 14:1 n-5	-	-	-	-	ns	ns	ns	ns
	Δ 16:1 n-7	-	-	-	-	ns	ns	$r = 0.7866$; $p = 0.0153$	ns
	Δ 18:1 n-9	ns	ns	ns	Ns	ns	$r = 0.7531$; $p = 0.0235$	ns	ns
n-6 PUFA	Δ 18:2 n-6	-	-	-	-	ns	$r = 0.7866$; $p = 0.0153$	ns	ns
	Δ 18:3 n-6	-	-	-	-	-	$r = 0.7531$; $p = 0.0235$	-	-
	Δ 20:2 n-6	-	-	-	-	-	$r = 0.7320$; $p = 0.0303$	-	-
	Δ 20:3 n-6	-	-	-	-	-	-	ns	-
	Δ 20:4 n-6	ns	ns	ns	ns	-	$r = 0.7280$; $p = 0.0313$	ns	ns
n-3 PUFA	Δ 18:3 n-3	-	-	-	-	-	$r = 0.9205$; $p = 0.004$	-	ns

	Δ 20:5 n-3	-	-	-	-	ns	$r = 0.6862;$ $p = 0.0470$	ns	ns
	Δ 22:5 n-3	-	-	-	-	-	-	ns	-
	Δ 22:6 n-3	ns	Ns	ns	ns	-	ns	ns	ns
Δ Total FA		-	-	-	-	ns	$r = 0.7113;$ $p = 0.0371$	ns	ns