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# Arachidonic acid-containing phosphatidylcholine species are increased in selected brain regions of a depressive animal model: Implications for pathophysiology

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ABSTRACT

The Flinders Sensitive Line (FSL) rat is a genetic animal model of depression. Following recent findings that the brain fatty acid composition of FSL is characterised by increased arachidonic acid (AA), we used electrospray tandem mass spectrometry and $^1$H-NMR to examine lipid species in different brain areas. Cholesterol and sphingolipids were increased in the hypothalamus of the FSL rats. Furthermore, arachidonic acid-containing phosphatidylcholine (AA-PC) species were elevated with PC16:0/20:4, PC18:1/20:4 and PC18:0/20:4 ($p<0.003$) increased in the hypothalamus and striatum. In contrast, there was a decrease in some docosahexaenoic acid (DHA)-containing species, specifically PC18:1/22:6 ($p<0.003$) in the striatum and PE18:1/22:6 ($p<0.004$) in the prefrontal cortex. Since no significant differences were observed in the erythrocyte fatty acid concentrations, dietary or environmental causes for these observations are unlikely. The increase in AA-PC species which in this animal model may be associated with altered neuropathy target esterase activity, an enzyme involved in membrane PC homeostasis, may contribute to the depressive phenotype of the FSL rats.



## 1. Introduction

The association of depression and essential polyunsaturated fatty acids (PUFA), especially n-3 PUFA, has been variably inferred from epidemiological studies [1], examination of blood tissue in clinical studies [2] and indirectly, by the effect of n-3 PUFA supplementation in clinical trials [3–5].

Utilizing a unique animal model of depression, the Flinders Sensitive Line (FSL) rat, we showed for the first time that the brain fatty acid composition of the depressive animals differed from that of the Sprague-Dawley controls. Surprisingly, the observed difference was not in the n-3 PUFA fraction, but rather a significantly elevated concentration of arachidonic acid (AA), an n-6 PUFA, was observed in the depressive FSL strain [6]. AA is intimately involved in brain function and altered AA levels may contribute to the pathophysiology of neuropsychiatric disorders through the formation of eicosanoids and endocannabinoids, regulation of neurotransmitter levels, modulation of signal transduction and gene expression [7–9]. Since the beneficial effect of n-3PUFA treatments may be attributed, in part, to reductions of AA-derived mediators, this finding offers a model for the study of those therapies but, at the same time, raises questions regarding the pathogenesis of depression in the FSL strain and, possibly, depression in general.

The FSL rats were developed by selective breeding of Sprague-Dawley rats for increased sensitivity to the hypothermic effect of the anticholinesterase agent diisopropyl fluorophosphate (DFP) [10,11]. These rats exhibit behavioral features characteristic of depression, such as reduced locomotion, reduced activity in swim test, increased anhedonia in response to chronic mild stress, increased amount and reduced onset of rapid eye movement sleep, cognitive difficulties and reduced body weight; moreover, these behavioral abnormalities have been normalized by chronic treatment with a number of well-recognized antidepressant drugs [12]. The FSL rats are also more sensitive to directly acting muscarinic and nicotinic agonists compared to their controls, but the mechanisms underlying this hyperresponsiveness and the second messengers involved have not been elucidated [13]. DFP is an organophosphate causing delayed neuropathy, initially shown to act through the phosphorylation of an esterase [14]. Recent reports have shown that this esterase, termed neuropathy target esterase (NTE) (EC 3.1.5), is intimately involved in normal brain development and membrane phosphatidylcholine (PC) homeostasis [15–18]. Disturbed NTE activity results in altered membrane metabolism, with varying manifestations in different species [17], especially evident in the distal parts of long nerves.

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Since establishment almost three decades ago [10] the study and use of FSL rats have offered valuable insights into the neurobiology of depression [19–21]. Further to our recent findings that this model is characterised by increased AA concentration in several areas of the brain, we have now followed a lipidomic approach and conducted a detailed study of lipids species in the brains in FSL and Sprague-Dawley control rats in order to further our understanding of the involvement of lipids in this animal model of depression.

## 2. Materials and methods

### 2.1. Materials

L-α-Phosphatidylcholine, dipalmitoyl (PC), L-α-phosphatidylethanolamine, dipalmitoyl (PE), 1,2-diacyl-sn-glycero-3-phospho-D-myo-inositol (PI), 1-palmitoyl, L-α-phosphatidylserine, dipalmitoyl (PS), N-acyl-D-sphingosine-1-phosphocholine (SM), phosphate buffered saline (PBS) tablets, butylated hydroxytoluene (BHT) and anhydrous sodium sulphate were purchased from Sigma (Poole, Dorset, UK). HPLC-grade methanol and chloroform, ammonium hydroxide (10%) and absolute ethanol were purchased from Fischer Chemicals (Loughborough, Leicestershire, UK). Potassium chloride was purchased from BDH (Poole, Dorset, UK). Chloroform-$d_1$ ($CDCl_3$, 99.8%) and methanol-$d_4$ ($CD_3OD$, 99.8%) were purchased from Sigma-Aldrich (Poole, Dorset, UK).

### 2.2. Animals

FSL and Sprague-Dawley male rats (230–250 g, Bar-Ilan University, Ramat-Gan, Israel) served as subjects in these experiments. The animals were maintained on a 12 h light–12 h dark cycle (lights off at 0700) with food and water available *ad libitum*. All experimental procedures were approved by the University Animal Care and Use Committees and were done in accordance with National Institutes of Health guidelines and every effort was made to minimize trauma to the animals.

### 2.3. Tissue sample preparation

Punches were taken from four brain regions (hypothalamus, nucleus accumbens, prefrontal cortex and striatum) as described previously [22]. The brain tissue was immediately frozen in liquid nitrogen, weighed, and kept at −80 °C until transfer to Bradford University, UK, in dry ice.

### 2.4. Lipid extractions

Lipids were extracted according to a modification of the Folch [23,24] method. Briefly, the brain tissue was homogenized in ice-cold chloroform-methanol (2:1, v/v) solution (5 ml) containing 1 ml water. The homogenate was centrifuged at 1000*g* for 5 min at 4 °C to separate the aqueous and organic layers. The bottom organic layer was retained whilst the aqueous layer and interface between the two layers containing the denatured proteins were re-extracted with ice-cold chloroform–methanol (5 ml). The combined organic extracts were washed with 0.5 M KCl in 50% (v/v) methanol (2 ml), dried over a small amount of sodium sulphate and filtered through tightly packed cotton wool to remove any remaining particles. The solvents were then evaporated under a stream of nitrogen.

### 2.5. $^1$H-NMR analysis

The lipid residue obtained from the previous step was re-dissolved in 0.8 ml of $CD_3OD$: $CDCl_3$ (2:1, v/v) and transferred to 5 mm NMR tubes. Samples were flushed with nitrogen in order to remove oxygen, sealed and kept at −20 °C awaiting $^1$H-NMR analysis (all samples prepared this way were analysed within 48 h). NMR spectra were recorded at room temperature on a JEOL ECA600 NMR spectrometer (JEOL UK, Welwyn, UK) operating at 600 Hz proton frequency. The spectra were acquired in the Fourier transformation mode with 32K data points, using a 45° pulse width, a relaxation delay of 1 and 2.9 s acquisition time. The residual HOD signal at approximately 4.7 ppm was suppressed by the application of a continuous and selective secondary irradiation during the relaxation delay. For each brain lipid extract 1000 scans were recorded to obtain a good quality spectrum. Chemical shifts were referenced to the residual methanol peak at 3.31 ppm. Relative quantitation of the lipids present in each sample was based on diagnostic peaks [24,25] and the results were expressed as mol% of total lipid or fatty acid chain.

### 2.6. ESI-MS/MS analysis

After NMR analysis, lipid extracts were taken to dryness under a gentle stream of nitrogen and re-dissolved in methanol–chloroform (1:2, v/v) containing BHT as an antioxidant (0.01%, w/v) (1 ml) and stored at −20 °C. For MS analysis sample aliquots were diluted 10-fold in methanol–chloroform–ammonium hydroxide–water (70:20:7:3; v/v/v/v) and infused into the electrospray (ESI) source at a flow rate of 5 μl/min using a syringe pump.

Electrospray ionisation tandem mass spectra (ESI-MS/MS) were acquired with a triple quadrupole spectrometer (Micromass Quattro Ultima, Waters, Manchester, UK) equipped with an electrospray ionisation interface. Instrument control and data acquisition were performed using the MassLynx$^{TM}$ V4.0 software. Nitrogen was used as both the nebulizing gas and desolvation gas. The desolvation gas flow was 690 L/h and the desolvation temperature was 150 °C. In the positive ion mode, the capillary and cone were maintained at 3.82 kV and 25 V, whilst in the negative mode they were maintained at 3.02 kV and 130 V. Argon was used as the collision gas for CID experiments at a pressure of $3–4 \times 10^{-4}$ mbar for precursor and neutral loss scanning, and $2–3 \times 10^{-3}$ mbar for product ion scanning.

Choline phospholipid (PC) and ethanolamine phospholipid (PE) species were detected using positive ionisation, whereas serine phospholipid (PS) and inositol phospholipid (PI) species were detected using negative ionisation [25,26]. Data acquisition was performed at the multichannel analysis (MCA) form and in the range $m/z$ 600–1000 using the following scan modes and collision energies: PC species precursors of $m/z$+184, collision energy 25 eV; PE species neutral loss of $m/z$ 141, collision energy 25 eV; PS species neutral loss of $m/z$ 87, collision energy 30 eV; PI species precursors of $m/z$ −241, collision energy 40 eV.

After conversion to centroid format according to area and correction for $^{13}$C isotope effects and correction for the decreased ion response for phospholipid species with increased acyl chain length (increasing $m/z$ values) [26], the phospholipid species were expressed as percentages of their respective totals present in each sample. The predominant molecular species present for each ion peak resolved was determined by analysis of fatty acyl ion fragments generated by collision gas-induced fragmentation under negative ionisation. The reported compositions correspond to the species that individually contributed >1 mol% to the total lipid of each respective phospholipid class.