Available online at www.sciencedirect.com
ScienceDirect
Journal of Psychiatric Research 42 (2008) 58–63
JOURNAL OF PSYCHIATRIC RESEARCH
www.elsevier.com/locate/jpsychires

Omega-3 fatty acids on the forced-swimming test

Shih-Yi Huang [a], Hui-Ting Yang [a], Chih-Chiang Chiu [a,b], Carmine M. Pariante [d], Kuan-Pin Su [c,d,*]

[a] *Graduate Institute of Nutrition and Health Sciences, Taipei Medical University, Taiwan*
[b] *Laboratory of Biological Psychiatry, Taipei City Psychiatric Center, Taiwan*
[c] *Department of General Psychiatry, China Medical University and Hospital, No. 2, Yuh-Der Road, Taichung 404, Taiwan*
[d] *Stress, Psychiatry and Immunology Laboratory (SPI-Lab), Institute of Psychiatry, King's College London, UK*

Received 27 June 2006; received in revised form 11 August 2006; accepted 12 September 2006

Abstract

Objectives: Based on the findings of epidemiological data and recent clinical trials, omega-3 fatty acids seem to have a preventive and therapeutic effect on depression.
Method: We examined the effect of omega-3 fatty acids on the forced-swimming test (FST) in two groups of Sprague-Dawley rats after a six-week treatment with two different diets. Behavioral responses were observed and recorded during the 5-min test. The fatty acid composition from the whole brain tissue and the RBC membrane of the rats were analyzed.
Results: Comparing to control diet, omega-3 fatty acid diet significantly decreased the immobility time (218 ± 16 vs. 183 ± 19 s, $p = 0.001$) and increased behaviors of swimming (32 ± 7 vs. 45 ± 9 s, $p = 0.012$) and climbing (50 ± 10 vs. 73 ± 14 s, $p = 0.011$) during the FST. The group in omega-3 fatty acid diet had higher levels of docosahexaenoic acid (DHA, 50% increase) and alpha-linolenic acid (ALA, 63% increase) in the brain, and of eicosapentaenoic acid (EPA, 27% increase) in the peripheral RBC membrane. The level of brain DHA is negatively correlated to the immobility time ($r = -0.654$, $p = 0.006$) and is positively correlated to the swimming time ($r = 0.69$, $p = 0.003$).
Conclusion: The result shows that omega-3 fatty acids have a beneficial effect on preventing the development of depression-like behaviors in rats with the FST.

Keywords: Omega-3 polyunsaturated fatty acids; Docosahexaenoic acid; Eicosapentaenoic acid; Depression; Forced-swimming test

1. Introduction

Based upon the evidence from epidemiological data, biological studies in patients, and recent clinical trials, omega-3 polyunsaturated fatty acids (PUFAs) seem to be involved in the mechanisms underlying the pathogenesis and treatment of depression (Horrobin and Bennett, 1999; Su et al., 2003b). The PUFAs are classified into omega-3 (or $n-3$) and omega-6 (or $n-6$) groups. The parent essential fatty acid of omega-3 PUFAs is α-linolenic acid (ALA; C18:3$n-3$), and that of omega-6 group is linoleic acid (LA; C18:2$n-6$). The cerebral cell membrane contains high concentrations of PUFAs, some of which cannot be synthesized and therefore must be obtained from the diet. The abnormalities in PUFA composition in cell membranes can alter membrane microstructure, which could result in abnormal signal transduction and immunological dysregulation, and possibly can increase the risk of developing depression (Horrobin and Bennett, 1999; Su et al., 2003a; Chiu et al., 2003). In fact, societies in which

* Corresponding author. Address: Department of General Psychiatry, China Medical University and Hospital, No. 2, Yuh-Der Road, Taichung 404, Taiwan. Tel.: +886 4 22062121x5076; fax: +886 4 22361230.
E-mail addresses: cobolsu@gmail.com, cobol@www.cmch.org.tw (K.-P. Su).

0022-3956/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jpsychires.2006.09.004

a large amount of omega-3 PUFAs is consumed appear to have a lower prevalence of major depressive disorder (Tanskanen et al., 2001b; Tanskanen et al., 2001a; Hibbeln, 1998). Consistent with this, red blood cell (RBC) membranes of patients with major depressive disorder have lower levels of omega-3 PUFAs, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Maes et al., 1996, 1999; Adams et al., 1996; Peet et al., 1998; Edwards et al., 1998). Finally, several clinical trials have been reported to show an antidepressant effect of PUFAs (Puri et al., 2001; Su et al., 2003a; Nemets et al., 2002; Peet and Horrobin, 2002). Moreover, in a preliminary trial, Stoll et al. (1999) concluded that omega-3 PUFAs improved the 4-month outcome of illness in patients with bipolar disorder. Indeed, we found that omega-3 PUFAs seem to prevent depression but not mania among patients with bipolar disorder (Su et al., 2000).

The forced-swimming test (FST), introduced by Porsolt et al. (1977) has been widely used to predict the clinical efficacy of antidepressant drugs. The rat FST is usually conducted with two sessions: a 15-min pretest session on day 1 and a 5-min test session on day 2. The ''behavioral despair'' is defined as an animal's reaction to the inability to escape from a stressful environment. The pretest forced swimming stress decreased the latency to the induction of behavioral immobility from the second test exposure. The value and limitations of this animal model have been largely discussed (Borsini and Meli, 1988; Willner, 1990; Thiebot et al., 1992). Lucki and his collaborators (Lucki et al., 1994; Detke and Lucki, 1996; Detke et al., 1995) have modified FST by scoring not only the passive behavior of the immobility time, but also the active behavior of swimming and climbing. A major feature of the FST is that administration of antidepressant agents before the first session, or between the first and second session, effectively decreases the immobility time and increase active behaviors, which is consistent with a therapeutic effect of increasing escape-directed activities (Page et al., 2003). The effects of antidepressants has made the FST a valid animal model for depression (Detke et al., 1995; Lucki, 1997).

Recently, Carlezon and his colleagues reported that dietary supplementation with omega-3 fatty acids reduced immobility in the FST when given for 30 days, but not for 3 or 10 days (Carlezon, Jr. et al., 2005). Unfortunately, the lipid profiles of brain and RBC were not reported in their study. In the present study, we examined the effect of omega-3 fatty acids on the FST and on fatty acid compositions of brain tissue and erythrocyte membrane after six-week treatment with two different diets.

2. Materials and methods

2.1. Animals and diets

Sixteen Sprague-Dawley rats (National Science Council in Taipei, Taiwan), weighing between 250 and 300 g at the start of the experiment, were used. They were housed in a temperature (22–24 °C) and a humidity-controlled (60%) room, on a 12-h light–dark (light on: 08:00–20:00 h) schedule. Food and water were available ad libitum. These conditions were maintained constant throughout the experiments.

Six weeks before the forced-swimming test, the rats were assigned to two groups on different diets. The components of these two diets were based on the AIN76 (American Institute of Nutrition-76) (American Institute of Nutrition, 1977) semi-purified diet with a modifying composition of fatty acid for this study (Table 1).

2.2. Forced-swimming test

The detailed procedures of forced-swimming test were described elsewhere (Porsolt et al., 1978; Detke and Lucki, 1996; Detke et al., 1995). Briefly, the rats were placed in vertical Plexiglass cylinders (40 cm high and 20 cm in diameter), containing water (25 °C) 30 cm-deep. They were placed into the water for a 15-min period (pretest session). At the end of this pretest phase, each rat was removed from the water, partially dried with a towel, and placed in a plastic cage illuminated with a heat lamp. Twenty-four hours later, the rats were exposed to the same experimental conditions outlined above for 5 min (test session). The immobility, swimming and climbing time were recorded and then rated by two trained raters, who were blind to the dietary treatment.

2.3. Brain and blood lipids

At the end of forced-swimming test, the rats were decapitated, the brains and RBC tissues were removed and weighted, and then frozen at −80 °C for subsequent biochemical analysis. The lipid of 2 g of brain tissues and 300 μl of RBC were homogenized and extracted with distilled water and chloroform/methanol (2:1, v/v) which contained 0.02% BHT (w/v) by using a method modified from Bligh and Dyer (1959). After a centrifuged procedure at 1500*g* for 10 min, the substratums were transferred to the

Table 1
The comparison of fatty acids in two diets: control diet vs. experiment diet*

	Control diet (%)	Experiment diet (%)
SFAs	6.04	4.87
MUFAs	76.82	38.49
Total $n-7$	0.09	0.12
Total $n-9$	76.73	38.38
PUFAs	10.75	44.16
Total $n-3$	0.39	36.80
C18:3 (ALA)	0.39	1.83
C20:5 (EPA)	0	12.36
C22:6 (DHA)	0	22.62
Total $n-6$	10.36	7.35
C18:2 (LA)	10.13	5.93
C20:4 (AA)	0	0.96

* SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

test tube for vacuum drying. Dried crude lipids were weighted before proceeding to the phospholipids separation. The crude lipids were then dissolved in 200 μl of chloroform and applied onto a solid phase extraction column (Amino disposable extraction column, Bakerbond speTM, LOT x 02550) for phospholipids separation (Edwards et al., 1998; Jumpson et al., 1997).

Each sample of the brain and the RBC was analyzed for the individual fatty acid by gas chromatography (Lipid Standards, FAMEs, Sigma Co., St. Louis, MO, USA). The detailed step-by-step procedures were applied according to the laboratory practice manual, as briefly described below (Maes et al., 1999; Edwards et al., 1998; Lepage et al., 1986). Extracted phospholipids were placed into 16×150 mm test tubes with Teflon-lined screw caps, and dissolved with 1 mL of 14% boron trifluoride methanol (BF_3-methanol, Sigma), for fatty acid methylation reaction. Fatty acid methyl esters were analyzed by capillary gas chromatography (Trace GC, Thermo Finnigan Co. Trace GC, Italy) equipped with a 30-m (0.32 mm ID) capillary column (cross-linked polyethylene glycol-TPA phase, Sulpelco) and frame ionized detector. Fatty acid profiles were identified according to the retention time of appropriate standard fatty acid methyl esters. Researchers who participated in the laboratory were blind to the information of coded samples.

2.4. Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences, Version 9.0 (SPSS Inc.). The difference of behavioral activities and the fatty acid profile of rats fed with two groups of diets were compared by independent *t*-test. Pearson's correlation analysis was used to examine the correlations of fatty-acid concentrations and behavioral parameters. The difference was considered statistically significant if a *p*-value was equal to or smaller than 0.05.

3. Results

3.1. Omega-3 fatty acids on behavioral activities in forced-swimming test

As shown in Table 2, there was a significant effect of omega-3 fatty acid diet on immobility time in the forced-swimming test. The immobility time of rats on omega-3 fatty acid diet was significantly shorter than that of rats on control diet. In addition, there were significant differences in the active behavior: both the climbing time and the swimming time were longer in the omega-3 group. We examined the correlations of fatty-acid concentrations and behavioral parameters. The level of brain DHA is negatively correlated to the immobility time ($r = -0.654$, $p = 0.006$) and is positively correlated to the swimming time ($r = 0.69$, $p = 0.003$). There are no significant correlations between all the behavioral parameters with the other omega-3 or omega-6 fatty acids in the brain and all the omega-3 and omega-6 fatty acids in the erythrocyte membrane.

Table 2
The effect of omega-3 fatty acid diet on behavioral responses, the level of $n-3$ and $n-6$ fatty acid in the brain tissue and the RBC membrane[a]

	Control diet group ($n = 8$)	Omega-3 diet group ($n = 8$)	p[b]
FST (s)			
Immobility	218 ± 16	183 ± 19	*0.001*[b]
Swimming	32 ± 7	45 ± 9	*0.012*[b]
Climbing	50 ± 10	73 ± 14	*0.011*[b]
Brain PUFA levels[c]			
$n-3$			
C18:3 (ALA)	1.81 ± 1.39	2.95 ± 1.83	*0.005*[b]
C20:5 (EPA)	2.94 ± 3.01	0.78 ± 1.20	0.115
C22:6 (DHA)	5.49 ± 1.06	8.26 ± 2.46	*0.001*[b]
$n-6$			
C18:2 (LA)	0.44 ± 0.29	0.45 ± 0.30	0.958
C18:3	1.27 ± 0.68	1.15 ± 0.17	0.933
C20:4 (AA)	3.89 ± 2.89	5.96 ± 1.21	0.115
C22:4	6.06 ± 3.76	7.11 ± 0.81	0.401
RBC PUFA levels[c]			
$n-3$			
C18:3 (ALA)	0.06 ± 0.05	0.08 ± 0.12	0.751
C20:5 (EPA)	0.66 ± 1.67	0.84 ± 0.66	*0.035*[b]
C22:6 (DHA)	0.67 ± 0.68	0.61 ± 0.62	0.958
$n-6$			
C18:2 (LA)	3.00 ± 1.81	3.05 ± 1.80	0.529
C18:3	0.23 ± 0.29	0.21 ± 0.25	0.562
C20:4 (AA)	3.64 ± 2.28	3.19 ± 2.85	0.462
C22:4	1.78 ± 0.93	1.43 ± 1.37	0.462

[a] The data are presented as the mean ± SD.
[b] Significantly different (for $p \leqq 0.05$).
[c] It is presented as mg/100 mg of total phospholipids.

3.2. Omega-3 fatty acids on erythrocyte and brain polyunsaturated fatty acid levels

As shown in the Table 2, there was a significant effect of the diet on the composition of polyunsaturated fatty acids in the rat's brain. The levels of DHA and ALA in the omega-3 fatty acid group were significantly higher than those in the control group. Surprisingly, EPA was lower in the omega-3 fatty acid group, but the difference did not reach statistical significance. There was no significant difference in other fatty acids between groups. In contrast to results in the brain, the erythrocyte level of EPA in the omega-3 fatty acid group was significantly higher than that in the control group, while there was no significant difference in DHA and ALA between two groups. There was no difference in omega-6 PUFAs in the brain or erythrocyte membrane.

4. Discussion

To the best of our knowledge, this is the first study designed to evaluate the effect of 6 weeks of omega-3 fatty acids on the FST model of depression and on brain and RBC lipid composition. The major finding of this study

is that the dietary supplement of omega-3 fatty acids attenuated the immobility time and increased behaviors of swimming and climbing in the FST. This is consistent with a recent article by Carlezon and his colleagues, which revealed that dietary supplementation with omega-3 fatty acids reduced immobility when given for 30 days, but not for 3 or 10 days (Carlezon, Jr. et al., 2005). However, the lipid profiles of brain and RBC were not reported in their study.

The result of our study supports that omega-3 fatty acids have an effect on the development of depression-like behaviors in rats tested with the FST. A cross-national study has revealed a significant inverse correlation between annual prevalence of major depression and fish consumption (Hibbeln, 1998). Fish and seafood are the major source of omega-3 PUFAs in the human diet, therefore the infrequent consumption of fish could mean a low intake of omega-3 fatty acids, which in turn could contribute to an elevated risk of depression. Further supporting the role of low omega-3 fatty acids in the risk of depression, several studies of patients with depression have reported reduced omega-3 fatty acids in plasma or RBC membranes (Maes et al., 1996, 1999; Adams et al., 1996; Peet et al., 1998; Edwards et al., 1998). Moreover, several clinical trials have been reported to show an antidepressant effect of PUFAs. The EPA monotherapy revealed antidepressant effect in a case of treatment-resistant major depressive disorder (Puri et al., 2001). Significant benefits of omega-3 PUFAs augmentation on antidepressant medications were also demonstrated in three double-blind, placebo-controlled trials (Su et al., 2003a; Nemets et al., 2002; Peet and Horrobin, 2002).

One of the biological mechanisms to explain this result is the regulation of neurotransmitters and signal transduction by PUFAs. The change of fatty acid concentration in the brain, induced by chronic deficiency in dietary omega-3 fatty acid, could alter serotonergic and dopaminergic neurotransmission and then lead to an increase in 5-HT_2 receptors and decrease in D_2 receptors in the frontal cortex (Delion et al., 1997, 1996, 1994). The upregulation of 5-$HT_{2A/C}$ is thought to play a role in the pathophysiology of depression (Maes and Meltzer, 1995). Furthermore, high cerebrospinal fluid concentration of 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of serotonin and an indicator of brain serotonin turnover, has been shown to be positively associated with high plasma concentration of omega-3 PUFAs among healthy subjects (Hibbeln et al., 1998), and lower serotonergic activity has been well established in the pathophysiology of depression (Risch and Nemeroff, 1992b). Biochemical studies have also shown that omega-3 PUFAs could increase CSF 5-HIAA concentration (Nizzo et al., 1978), a finding which is commonly associated with the improvement of depressive symptoms (Risch and Nemeroff, 1992a).

A second hypothesis is that omega-3 PUFAs play an important role in the mechanism of mood stabilization by targeting parts of the "arachidonic acid cascade" (Rapoport and Bosetti, 2002). The "arachidonic acid cascade" hypothesis in mood disorders has been supported by a number of evidences, including the higher levels of AA and increased activity of phospholipase A_2 (PLA_2), a major metabolic enzyme of AA, in patients with mood disorders (Maes et al., 1996, 1999; Chiu et al., 2003; Noponen et al., 1993) and the inhibitory effect on PLA_2 activity of mood stabilizers (Chang et al., 2001; Rintala et al., 1999; Chang and Jones, 1998; Ghelardoni et al., 2004). Furthermore, AA is the major substrate for prostaglandin E_2 (PGE_2), which is important in the development of animal's sickness behavior, a series of behavioral changes that resemble depressive symptoms (Maddock and Pariante, 2001; Song et al., 2003). PGE_2have also been found to be elevated in the plasma (Lieb et al., 1983), spinal fluid (Linnoila et al., 1983), and saliva (Ohishi et al., 1988) of patients with depression. It has been reported that omega-3 PUFA treatment could reduce PGE_2 synthesis (James et al., 2000), attenuating cytokine-induced sickness, stress and anxiety-like behaviors (Song et al., 2004, 2003). Interestingly, by reducing prostaglandin production, PUFAs also have the ability of inhibiting the function of membrane steroid transporters in the brain like the multidrug resistance p-glycoprotein (MDR PGP) (Murck et al., 2004). Membrane steroid transporters have been identified as fundamental mechanism regulating tissue sensitivity to glucocorticoid hormones. Among these, the MDR PGP localized on the endothelial cells of the blood–brain-barrier (BBB) has a physiological role in the access of glucocorticoids to the brain and in the regulation of HPA axis activity (Pariante et al., 2004). An overactive MRD PGP in depressed patients could reduce the access of glucocorticoids to the brain and hence inducing glucocorticoid resistance, a condition that participates to the pathogenesis of depressive symptoms (Pariante, 2006). Indeed, one of the mechanisms by which antidepressants increase glucocorticoid sensitivity in vitro is by inhibiting membrane steroid transporters that expels glucocorticoids out of the cells, and therefore by increasing the intracellular levels of glucocorticoids: an effect that is independent of any neurotransmitter actions (Pariante et al., 2004). Therefore, it is intriguing that PUFAs, similar to other antidepressants, have also been shown to inhibit the function of MDR PGP, by reducing PGE2 levels (Murck et al., 2004).

DHA is a major structural component of phospholipid in neuronal cell membranes, while EPA is not present in neuronal cell membranes. It has been reported that DHA is more important in brain functioning than EPA (Peet and Stokes, 2005). This is supported in our finding that the level of brain DHA, but not EPA, is negatively correlated to the immobility time and is positively correlated to the swimming time. However, EPA (Nemets et al., 2002; Su et al., 2003a; Peet and Horrobin, 2002), rather than DHA (Marangell et al., 2003), appears to be the effective agent in clinical studies for depression. The contradiction of clinical and basic studies raise questions about different modes of action of DHA and EPA. EPA, but not DHA, has other important physiological functions,

including a role as precursor for eicosanoids and modulator of cytokines (Fenton et al., 2000). It has been proposed that depression is accompanied by the increased secretion of eicosanoids, such as prostaglandins, and by excessive secretion of proinflammatory cytokines (Maes and Smith, 1998). EPA can act as the inhibitor of PLA_2 reduce the secretion of eicosanoids and proinflammatory cytokines (Song et al., 2004, 2003), which might associated with the improvement of loss of interest, fatigue, loss of energy, poor appetite and inability to concentrate, in patients with depression (Capuron and Miller, 2004).

The other important finding of this study is a significant effect of omega-3 fatty acid diet on the levels of brain and RBC polyunsaturated fatty acids. The dietary supplement of omega-3 fatty acids only increased the DHA and ALA, but not EPA in the brain; and increased EPA, but not ALA and DHA, in the peripheral RBC. EPA was lower in the brain in the omeg-3 fatty acid group, although the difference did not reach statistical significance. Unfortunately, a limitation of the study was that PUFA levels were assessed after FST; therefore, we cannot clarify whether this discrepancy is due to the experimental diet having different effects on the brain and RBC, or the forced swimming stress having different effects on the brain and RBC. Most studies revealing omega-3 fatty acid deficits in patients with major depressive disorder were designed to assess peripheral tissues only (Maes et al., 1996, 1999; Adams et al., 1996; Peet et al., 1998; Edwards et al., 1998), and therefore it is important to clarify the relationship between brain and RBC omega-3 fatty acid.

In conclusion, our result shows that omega-3 fatty acids have a beneficial effect on preventing the development of depression-like behaviors in rats with the FST. The other limitations are that: (1) there was no active control group, e.g. rats with antidepressant treatment; and (2) this study did not examine the length of time required to produce behavioral changes from dietary exposure. Furthermore, future studies should examined specific regions of the brain rather than the whole brain.

Declaration of interest

None.

Acknowledgements

The work was supported by the grants from the China Medical University (CMU93-M-24 and CMU94-105), the Department of Health (DOH 94F044), and the National Science Council (NSC 94-2314-B-039-027 and NSC93-2320-B-039-001) in Taiwan.

References

Adams PB, Lawson S, Sanigorski A, Sinclair AJ. Arachidonic acid to eicosapentaenoic acid ratio in blood correlates positively with clinical symptoms of depression. Lipids 1996;31(Suppl.):S157–61.

American Institute of Nutrition. Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies. Journal of Nutrition 1977;107:1340–8.

Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Canadian Journal of Medical Sciences 1959;37:911–7.

Borsini F, Meli A. Is the forced swimming test a suitable model for revealing antidepressant activity? Psychopharmacology (Berlin) 1988;94:147–60.

Capuron L, Miller AH. Cytokines and psychopathology: lessons from interferon-alpha. Biological Psychiatry 2004;56:819–24.

Carlezon Jr WA, Mague SD, Parow AM, Stoll AL, Cohen BM, Renshaw PF. Antidepressant-like effects of uridine and omega-3 fatty acids are potentiated by combined treatment in rats. Biological Psychiatry 2005;57:343–50.

Chang MC, Jones CR. Chronic lithium treatment decreases brain phospholipase A2 activity. Neurochemistry Research 1998;23:887–92.

Chang MC, Contreras MA, Rosenberger TA, Rintala JJ, Bell JM, Rapoport SI. Chronic valproate treatment decreases the in vivo turnover of arachidonic acid in brain phospholipids: a possible common effect of mood stabilizers. Journal of Neurochemistry 2001;77:796–803.

Chiu CC, Huang SY, Su KP, Lu ML, Huang MC, Chen CC, et al. Polyunsaturated fatty acid deficit in patients with bipolar mania. European Neuropsychopharmacology 2003;13:99–103.

Delion S, Chalon S, Herault J, Guilloteau D, Besnard JC, Durand G. Chronic dietary alpha-linolenic acid deficiency alters dopaminergic and serotonergic neurotransmission in rats. Journal of Nutrition 1994;124:2466–75.

Delion S, Chalon S, Guilloteau D, Besnard JC, Durand G. alpha-Linolenic acid dietary deficiency alters age-related changes of dopaminergic and serotoninergic neurotransmission in the rat frontal cortex. Journal of Neurochemistry 1996;66:1582–91.

Delion S, Chalon S, Guilloteau D, Lejeune B, Besnard JC, Durand G. Age-related changes in phospholipid fatty acid composition and monoaminergic neurotransmission in the hippocampus of rats fed a balanced or an $n-3$ polyunsaturated fatty acid-deficient diet. Journal of Lipid Research 1997;38:680–9.

Detke MJ, Lucki I. Detection of serotonergic and noradrenergic antidepressants in the rat forced swimming test: the effects of water depth. Behavioural Brain Research 1996;73:43–6.

Detke MJ, Rickels M, Lucki I. Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. Psychopharmacology (Berlin) 1995;121:66–72.

Edwards R, Peet M, Shay J, Horrobin D. Omega-3 polyunsaturated fatty acid levels in the diet and in red blood cell membranes of depressed patients. Journal of Affective Disorder 1998;48:149–55.

Fenton WS, Hibbeln J, Knable M. Essential fatty acids, lipid membrane abnormalities, and the diagnosis and treatment of schizophrenia. Biological Psychiatry 2000;47:8–21.

Ghelardoni S, Tomita YA, Bell JM, Rapoport SI, Bosetti F. Chronic carbamazepine selectively downregulates cytosolic phospholipase A2 expression and cyclooxygenase activity in rat brain. Biological Psychiatry 2004;56:248–54.

Hibbeln JR. Fish consumption and major depression. Lancet 1998;351:1213.

Hibbeln JR, Linnoila M, Umhau JC, Rawlings R, George DT, Salem Jr N. Essential fatty acids predict metabolites of serotonin and dopamine in cerebrospinal fluid among healthy control subjects, and early- and late-onset alcoholics. Biological Psychiatry 1998;44:235–42.

Horrobin DF, Bennett CN. Depression and bipolar disorder: relationships to impaired fatty acid and phospholipid metabolism and to diabetes, cardiovascular disease, immunological abnormalities, cancer, ageing and osteoporosis. Possible candidate genes. Prostaglandins Leukotrienes and Essential Fatty Acids 1999;60:217–34.

James MJ, Gibson RA, Cleland LG. Dietary polyunsaturated fatty acids and inflammatory mediator production. American Journal of Clinical Nutrition 2000;71:343S–8S.

Jumpson J, Lien EL, Goh YK, Glandinin T. Small changes of dietary ($n-6$) and ($n-3$) fatty acid content ratio alter phosphatidylethanolamine and phosphatidylcholine fatty acid composition during development of neuronal and glial cells in rats. Journal of Nutrition 1997;127:724–31.

Lepage P, Helynck G, Chu JY, Luu B, Sorokine O, Trifilieff E, et al. Purification and characterization of minor brain proteolipids: use of fast atom bombardment-mass spectrometry for peptide sequencing. Biochimie 1986;68:669–86.

Lieb J, Karmali R, Horrobin D. Elevated levels of prostaglandin E2 and thromboxane B2 in depression. Prostaglandins Leukotrienes and Medicine 1983;10:361–7.

Linnoila M, Whorton AR, Rubinow DR, Cowdry RW, Ninan PT, Waters RN. CSF prostaglandin levels in depressed and schizophrenic patients. Archives of General Psychiatry 1983;40:405–6.

Lucki I. The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. Behavioural Pharmacology 1997;8:523–32.

Lucki I, Singh A, Kreiss DS. Antidepressant-like behavioral effects of serotonin receptor agonists. Neuroscience and Biobehavioral Reviews 1994;18:85–95.

Maddock C, Pariante CM. How does stress affect you? An overview of stress, immunity, depression and disease. Epidemiological Psychiatry Society 2001;10:153–62.

Maes M, Meltzer HYM. The serotonin hypothesis of major depression. In: Psychopharmacology, the fourth generation of progress. New York: Raven Press; 1995. p. 933–41.

Maes M, Smith RS. Fatty acids, cytokines, and major depression. Biological Psychiatry 1998;43:313–4.

Maes M, Smith R, Christophe A, Cosyns P, Desnyder R, Meltzer H. Fatty acid composition in major depression: decreased omega 3 fractions in cholesteryl esters and increased C20: 4 omega 6/C20:5 omega 3 ratio in cholesteryl esters and phospholipids. Journal of Affective Disorder 1996;38:35–46.

Maes M, Christophe A, Delanghe J, Altamura C, Neels H, Meltzer HY. Lowered omega3 polyunsaturated fatty acids in serum phospholipids and cholesteryl esters of depressed patients. Psychiatry Research 1999;85:275–91.

Marangell LB, Martinez JM, Zboyan HA, Kertz B, Kim HF, Puryear LJ. A double-blind, placebo-controlled study of the omega-3 fatty acid docosahexaenoic acid in the treatment of major depression. American Journal of Psychiatry 2003;160:996–8.

Murck H, Song C, Horrobin DF, Uhr M. Ethyl-eicosapentaenoate and dexamethasone resistance in therapy-refractory depression. International Journal of Neuropsychopharmacology 2004;7:341–9.

Nemets B, Stahl Z, Belmaker RH. Addition of omega-3 fatty acid to maintenance medication treatment for recurrent unipolar depressive disorder. American Journal of Psychiatry 2002;159:477–9.

Nizzo MC, Tegos S, Gallamini A, Toffano G, Polleri A, Massarotti M. Brain cortex phospholipids liposomes effects on CSF HVA, 5-HIAA and on prolactin and somatotropin secretion in man. Journal of Neural Transmission 1978;43:93–102.

Noponen M, Sanfilipo M, Samanich K, Ryer H, Ko G, Angrist B, et al. Elevated PLA2 activity in schizophrenics and other psychiatric patients. Biological Psychiatry 1993;34:641–9.

Ohishi K, Ueno R, Nishino S, Sakai T, Hayaishi O. Increased level of salivary prostaglandins in patients with major depression. Biological Psychiatry 1988;23:326–34.

Page ME, Brown K, Lucki I. Simultaneous analyses of the neurochemical and behavioral effects of the norepinephrine reuptake inhibitor reboxetine in a rat model of antidepressant action. Psychopharmacology (Berlin) 2003;165:194–201.

Pariante CM. The glucocorticoid receptor: part of the solution or part of the problem? Journal of Psychopharmacology 2006;20:79–84.

Pariante CM, Thomas SA, Lovestone S, Makoff A, Kerwin RW. Do antidepressants regulate how cortisol affects the brain? Psychoneuroendocrinology 2004;29:423–47.

Peet M, Horrobin DF. A dose-ranging study of the effects of ethyl-eicosapentaenoate in patients with ongoing depression despite apparently adequate treatment with standard drugs. Archives of General Psychiatry 2002;59:913–9.

Peet M, Stokes C. Omega-3 fatty acids in the treatment of psychiatric disorders. Drugs 2005;65:1051–9.

Peet M, Murphy B, Shay J, Horrobin D. Depletion of omega-3 fatty acid levels in red blood cell membranes of depressive patients. Biological Psychiatry 1998;43:315–9.

Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. Nature 1977;266:730–2.

Porsolt RD, Anton G, Blavet N, Jalfre M. Behavioural despair in rats: a new model sensitive to antidepressant treatments. European Journal of Pharmacol 1978;47:379–91.

Puri BK, Counsell SJ, Hamilton G, Richardson AJ, Horrobin DF. Eicosapentaenoic acid in treatment-resistant depression associated with symptom remission, structural brain changes and reduced neuronal phospholipid turnover. International Journal of Clinical Practice 2001;55:560–3.

Rapoport SI, Bosetti F. Do lithium and anticonvulsants target the brain arachidonic acid cascade in bipolar disorder? Archives of General Psychiatry 2002;59:592–6.

Rintala J, Seemann R, Chandrasekaran K, Rosenberger TA, Chang L, Contreras MA, et al. 85 kDa cytosolic phospholipase A2 is a target for chronic lithium in rat brain. Neuroreport 1999;10:3887–90.

Risch SC, Nemeroff CB. Neurochemical alterations of serotonergic neuronal systems in depression. Journal of Clinical Psychiatry 1992a;53(Suppl.):3–7.

Risch SC, Nemeroff CB. Neurochemical alterations of serotonergic neuronal systems in depression. Journal of Clinical Psychiatry 1992b;53(Suppl.):3–7.

Song C, Li X, Leonard BE, Horrobin DF. Effects of dietary $n-3$ or $n-6$ fatty acids on interleukin-1beta-induced anxiety, stress, and inflammatory responses in rats. Journal of Lipid Research 2003;44:1984–91.

Song C, Leonard BE, Horrobin DF. Dietary ethyl-eicosapentaenoic acid but not soybean oil reverses central interleukin-1-induced changes in behavior, corticosterone and immune response in rats. Stress 2004;7:43–54.

Stoll AL, Severus WE, Freeman MP, Rueter S, Zboyan HA, Diamond E, et al. Omega 3 fatty acids in bipolar disorder: a preliminary double-blind, placebo-controlled trial. Archives of General Psychiatry 1999;56:407–12.

Su KP, Shen WW, Huang SY. Are omega3 fatty acids beneficial in depression but not mania? Archives of General Psychiatry 2000;57:716–7.

Su KP, Huang SY, Chiu CC, Shen WW. Omega-3 fatty acids in major depressive disorder. A preliminary double-blind, placebo-controlled trial. European Neuropsychopharmacology 2003a;13:267–71.

Su KP, Shen WW, Huang SY. The use of omega-3 fatty acids for the management of depression and psychosis during pregnancy and breast-feeding. In: Phospholipid spectrum disorder in psychiatry and neurology. Carnforth: Marius Press; 2003b. p. 391–9.

Tanskanen A, Hibbeln JR, Hintikka J, Haatainen K, Honkalampi K, Viinamaki H. Fish consumption, depression, and suicidality in a general population. Archives of General Psychiatry 2001a;58:512–3.

Tanskanen A, Hibbeln JR, Tuomilehto J, Uutela A, Haukkala A, Viinamaki H, et al. Fish consumption and depressive symptoms in the general population in Finland. Psychiatric Services 2001b;52:529–31.

Thiebot MH, Martin P, Puech AJ. Animal behavioural studies in the evaluation of antidepressant drugs. British Journal of Psychiatry 1992:44–50.

Willner P. Animal models of depression: an overview. Pharmacological Therapy 1990;45:425–55.