Research report

Omega-3 polyunsaturated fatty acid levels in the diet and in red blood cell membranes of depressed patients

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Abstract

Background: There is a hypothesis that lack of n-3 polyunsaturated fatty acids (PUFAs) is of aetiological importance in depression. Docosahexaenoic acid, a member of the n-3 PUFA family, is a crucial component of synaptic cell membranes. The aim of this study was to measure RBC membrane fatty acids in a group of depressed patients relative to a well matched healthy control group.

Method: Red blood cell (RBC) membrane levels, and dietary PUFA intake were measured in 10 depressed patients and 14 matched healthy control subjects.

Results: There was a significant depletion of RBC membrane n-3 PUFAs in the depressed subjects which was not due to reduced calorie intake. Severity of depression correlated negatively with RBC membrane levels and with dietary intake of n-3 PUFAs. Conclusion: Lower RBC membrane n-3 PUFAs are associated with the severity of depression. Limitations: Although patient numbers were small, confounding factors were well controlled for and the results were highly significant. Results of the dietary data would tend to be weakened due to the limitations associated with dietary assessment. Clinical Relevance: The findings raise the possibility that depressive symptoms may be alleviated by n-3 PUFA supplementation.

Keywords: Depression; Polyunsaturated fatty acids; Phospholipid; Cell membranes; Diet

1. Background

It has been suggested that abnormal cell membrane fatty acid composition may be of aetiological significance in depression (Smith, 1991; Hibbeln and Salem, 1995). Cell membranes contain a phospholipid bilayer. Changes in the polyunsaturated fatty acid content of this phospholipid alters membrane micro structure. This can have profound effects upon neurotransmitter receptor function (Stubbs, 1992; Salem and Niebylski, 1995).

The polyunsaturated fatty acids (PUFAs) are classified into two main groups: omega-3 (or n-3) of which the parent essential fatty acid is alphalinolenic acid (ALA; 18:3n-3), and n-6 of which the parent essential fatty acid is linoleic acid (LA; 18:2n6). Recent studies have suggested a deficiency...
of n-3 fatty acids and a shift in the balance of fatty acids away from n-3 and towards n-6, in the depressed patients. Thus, Maes et al. (1996) reported a significant decrease of total n-3 fatty acids, and also reduced ALA (α-linolenic acid) and eicosapentaenoic acid (EPA; 20:5n-3) in serum cholesterol esters in major depressed patients relative to those with minor depression or healthy controls. They also found a significant increase of the ratio of arachidonic acid (AA; 20:4n6) to EPA in both cholesterol esters and phospholipids. Adams et al. (1996) reported that the AA to EPA ratio in plasma and red blood cell (RBC) membrane phospholipids, correlated positively with severity of depression. Recently, Peet et al. (1997) has reported a significant depletion of total n-3 PUFAs and particularly docosahexaenoic acid (DHA; 22:6n-3) in RBC membranes from drug-free depressed patients. Subsequent treatment with antidepressants had no significant effect on RBC PUFA levels. However, that study did not fully control for possible confounding factors such as stress, smoking, and diet.

There is epidemiological evidence that diet may be a contributory factor to depression. During this century there has been a substantial increase in the lifetime risk for major depression (Cross-National Collaborative Group, 1992) corresponding with a shift in diet towards more n-6 and less n-3 PUFA intake (Taylor et al., 1979; Rice, 1984; Eaton and Kanner, 1985; Leaf and Weber, 1987). These dietary changes are considered to underlie the current epidemic of coronary heart disease (Taylor et al., 1979). Depression is the strongest psychological predictor of coronary heart disease (Booth-Kewley and Friedman, 1987; Pratt et al., 1996). It can be postulated that lack of dietary n-3 fatty acids is a contributory factor not only to coronary heart disease but also to depression.

We report a further study in a separate group of depressed patients, in which RBC membrane fatty acids were measured relative to a healthy control group, taking account of stress and smoking habit, and including a full dietary analysis.

2. Methods

Ten cases with a diagnosis of a major depressive episode according to DSM IV criteria were recruited into the study. None were suffering from physical illness of severity or nature which might be associated with abnormal n-3 fatty acid levels. All were taking antidepressant medication. The cases were matched with 14 healthy control subjects with no history of psychiatric disorder. They were also matched in terms of age, gender, social class, body mass index, number of children, recent life events, smoking habits and alcohol consumption. All patients and controls were rated using the Beck Depression Inventory (BDI) (Beck et al., 1981).

After recruitment into the study current diet was assessed using the 7-day weighed intake method. Venous blood was taken into EDTA. One patient refused venupuncture. RBCs were separated by centrifugation, washed in normal saline, frozen immediately and stored at −80°C centigrade until being air freighted frozen to Nova Scotia for fatty acid analysis. Laboratory measures were conducted blind to clinical status. Fatty acids were analysed using the method of Manku et al. (1982) by thin layer chromatography and gas chromatography.

Both n-3 and n-6 PUFAs were analysed, as well as saturated and monounsaturated fatty acids. As the focus of our hypothesis was on n-3 fatty acids, a comparison of n-3 fatty acids between patients and controls comprised the primary analysis using a probability value of 0.05. Data on other fatty acids are mentioned where appropriate. The statistical tests used to analyse the data were, 2-sample t-test, Pearson’s correlation coefficient and forward stepwise multiple regression.

3. Results

Patients and controls showed a similar distribution of age (patients 38.7 ± 10.2; controls 39.4 ± 10.9) and gender (patients 8F 2M, controls 12F 2M) and did not differ significantly in smoking habit (patients 7/10 smokers, controls 8/14 smokers) recent life events (patients 8/10 recent life events, controls 6/14) or alcohol habit (patients 1.0 ± 1.4 units per day; controls 0.8 ± 0.8 units per day). BDI score was 26.9 ± 4.7 in the depressed patients and 4.9 ± 4.2 in the controls.

As shown in Fig. 1 and Table 1 the RBC membrane n-3 PUFA levels were significantly decreased in the depressed patients relative to controls.
The n-6 PUFAs (not shown in the table) showed no significant abnormality in the patient group ($t = 1.30$, $df = 21$, $p = 0.209$, two-tailed test for total n-6). There was no significant difference between patients and controls in relation to current dietary intake of n-3 fatty acids, nor total energy intake as measured by 7-day weighed intake, although total energy intake was slightly higher in the patients (see Table 1). There were no significant relationships between the duration of depression and any of the PUFA levels.

Table 2 shows Pearson’s correlation coefficients for the BDI in relation to RBC membrane n-3 fatty acids and dietary intake of n-3 fatty acids, within the group of depressed patients. There were significant negative correlations between both RBC membrane n-3 fatty acid levels and dietary intake of n-3 fatty acid levels, and the BDI score. None of the n-6 series of PUFAs showed significant correlations with the BDI. Forward stepwise multiple regression analysis was carried out for the n-3 fatty acid series (separately for the RBC membrane and dietary data) in relation to the BDI scores. In the patient group, a single variable, ALA emerged as a predictor for BDI score. For RBC membrane ALA, the beta coefficient was -0.81, significant at the 0.008 level with an adjusted $r^2$ of 0.61. For dietary ALA the beta

Table 1

<table>
<thead>
<tr>
<th>RBC membrane[^b]</th>
<th>Current dietary[^c] n3 PUFA levels and absolute total energy intake</th>
<th>Controls</th>
<th>$p$[^d]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RBC Membranes[^b]</strong></td>
<td><strong>Patients</strong></td>
<td><strong>Controls</strong></td>
<td></td>
</tr>
<tr>
<td>18:3n3</td>
<td>0.09 (0.03)</td>
<td>0.12 (0.02)</td>
<td>0.41</td>
</tr>
<tr>
<td>20:5n3</td>
<td>0.52 (0.07)</td>
<td>0.73 (0.05)</td>
<td>0.02</td>
</tr>
<tr>
<td>22:5n3</td>
<td>1.52 (0.28)</td>
<td>2.03 (0.09)</td>
<td>0.05</td>
</tr>
<tr>
<td>22:6n3</td>
<td>3.25 (0.60)</td>
<td>4.72 (0.29)</td>
<td>0.02</td>
</tr>
<tr>
<td>Total n3</td>
<td>5.39 (0.93)</td>
<td>7.60 (0.38)</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>DIET[^c]</strong></td>
<td><strong>Patients</strong></td>
<td><strong>Controls</strong></td>
<td></td>
</tr>
<tr>
<td>18:3n3</td>
<td>1034.9 (100.4)</td>
<td>1363.4 (199.2)</td>
<td>0.21</td>
</tr>
<tr>
<td>20:5n3</td>
<td>82.3 (23.2)</td>
<td>140.3 (35.7)</td>
<td>0.23</td>
</tr>
<tr>
<td>22:5n3</td>
<td>55.7 (15.3)</td>
<td>77.1 (11.8)</td>
<td>0.27</td>
</tr>
<tr>
<td>22:6n3</td>
<td>141.4 (38.5)</td>
<td>210.7 (52.2)</td>
<td>0.33</td>
</tr>
<tr>
<td>Total n3</td>
<td>1314.3 (211.2)</td>
<td>1791.5 (211.2)</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Absolute total energy intake (kJ)</strong></td>
<td>9153.73 (769.44)</td>
<td>8478.21 (594.75)</td>
<td>0.49</td>
</tr>
</tbody>
</table>

[^b]: Calculated using 2-sample $t$-test.
[^c]: RBC values are presented as mg/100mg of total phospholipid.
[^d]: Measured via the 7-day weighed intake method. Fatty acid values are presented as mg/100g of total energy intake per day.
The RBC membrane and dietary data for the patients and controls were pooled and applied to a forward stepwise multiple regression analysis. DHA with a beta coefficient of $-0.92$, significant at the 0.0003 level, and LA with a beta coefficient of 0.48, significant at the 0.03 level were the variables that emerged as predictors of BDI score in the RBC membranes. Their adjusted $r^2$ value was 0.45. For the pooled dietary data, none of the dietary n-3 or n-6 series were predictors of the BDI score. However, 18:1n7 was predictive of the BDI score with a beta coefficient of $-0.46$, significant at the 0.0235 level, with an adjusted $r^2$ value of 0.18.

Table 3 shows the Pearson’s correlation coefficients for dietary intake of n-3 PUFAs in relation to RBC membrane n-3 PUFA levels. Significant positive correlations between the dietary and RBC membrane n-3 PUFAs were found. In the depressed subjects current absolute intake of ALA was found to correlate significantly and positively with the following RBC membrane PUFAs:-ALA, EPA, docosapentaenoic acid (DPA:n-3; 22:5n3), DHA and total n-3.

In the non-depressed subjects dietary DPA:n-3 correlated with RBC ALA ($r = 0.68$, $P = 0.007$), dietary DHA correlated with RBC DPA:n-3 ($r = 0.56$, $P = 0.036$) and dietary dihomogamma-linolenic acid (DHGLA; 20:3n6) correlated with RBC DPA:n-3 ($r = 0.55$, $P = 0.042$) and RBC arachidonic acid (ArA; 20:4n6) ($r = 0.55$, $P = 0.042$).

The n-3 fatty acids were compared between smokers and non-smokers, and between subjects with recent stressful life events and those with none. It was found that neither smoking nor the recent occurrence of life events had any significant effect upon RBC membrane levels of individual n-3 fatty acids or total n-3 fatty acids.

Absolute total energy intake (7-day weighed intake) was $9153.9 \pm 2433.2$ KJouls (2178.7 ± 578.1 Kcals) in patients and $8478.2 \pm 2225.4$ KJouls (2021.8 ± 532.6 Kcals) in controls. Using a Pearson’s
correlation coefficient test, no significant correlation between total energy intake and severity of depression was found ($r = 0.04, P = 0.9$).

4. Discussion

We have confirmed our previous finding (Peet et al., 1997) that PUFAs of the n-3 series are significantly depleted in RBC membranes from depressed patients. The absolute values and the magnitude of the differences in the n-3 PUFA series were not significantly different from the previous findings.

Although patient numbers were small, the results are highly significant and in the predicted direction. The findings are consistent with other recent studies of fatty acid levels in phospholipid and cholesterol esters in plasma from depressed patients (Adams et al., 1996; Maes et al., 1996). There is an earlier study (Fehily et al., 1981) reporting increased levels of EPA and DHA in erythrocyte membranes of depressed patients. No research diagnostic criteria were used in this study and so comparisons are difficult. Results from recent studies have been very consistent in finding decreases of these fatty acids in depressed patients.

Depleted RBC membrane n-3 fatty acid levels showed a significant association with the severity of depression, such that the lowest levels were associated with more severe depression. This is consistent with the report of Adams et al. (1996) in that the AA to EPA ratio in plasma and erythrocyte membrane phospholipids correlated positively with severity of depression. Furthermore, in this study a similar relationship was found for dietary n-3 fatty acids in the depressed patients, such that a diet higher in n-3 fatty acids was associated with less severe depression. This did not simply reflect loss of appetite because of more severe depression. The depressed patients did not significantly differ from controls in absolute total energy intake. Indeed absolute total energy intake was slightly higher in the cases, but there was no relationship between absolute total energy intake and severity of depression. Also, dietary n-6 PUFA intake did not significantly differ between patients and controls, but intake was found to be higher in the patients. Furthermore, dietary n-6 PUFA did not correlate with severity of depression, except in the multiple regression analysis for the whole study population, in which dietary intake of LA was positively, rather than negatively, associated with severity of depression. Therefore, this appears to be a more specific relationship between severity of depression and n-3 fatty acids.

DHA is a member of the n-3 PUFA family and is a fatty acid which is of particular interest with regard to depression. DHA is especially concentrated in synaptic membranes of the retina and cerebral cortex (Salem et al., 1986; Salem and Niebylski, 1995). Changes in membrane phospholipid composition, particularly related to DHA content, alters the configuration and function of membrane-related proteins including enzymes, transporter proteins and receptors (Salem et al., 1986; Stubbs, 1992; Salem and Niebylski, 1995; Litman and Mitchell, 1996; Slater et al., 1996; Witt et al., 1996). This provides the rationale linking membrane phospholipid abnormalities with current receptor-based theories. It is of great interest that reduced membrane DHA emerged as a significant predictor of depression in the multiple regression analysis across the whole study population.

There are several possible mechanisms by which RBC membrane n-3 fatty acids could be depleted in depressed patients. We took care to address possible confounding factors by carefully matching the subjects. Neither smoking nor stressful life events were found to have a significant impact upon n-3 fatty acid levels. Leng et al. (1994) found no effect of smoking on plasma PUFA levels in physically healthy people. Also, there is previous evidence that psychological stress in normals leads to reduced plasma levels of AA, but leaves DHA unaffected (Williams et al., 1992). The depressed patients were all taking antidepressant medication, but Peet et al. (1997) found that antidepressant treatment did not affect PUFA levels. Nevertheless, further studies in drug naive patients would be informative.

One mechanism whereby n-3 PUFA could be depleted in the RBC membranes of the depressed patients could be reduced dietary intake. Although no significant difference in n-3 fatty acid intake between patients and controls was found using the 7-day weighed dietary assessment, n-3 fatty acid intake was reduced in the patients. Furthermore, significant negative correlations were found between
the BDI and ALA and total n-3 fatty acids in the diet of the patient group. Given the well-documented limitations associated with dietary assessment (Willett, 1990), the lack of a detected significant difference in n-3 PUFA intake between the depressed and non-depressed subjects could be due to limitations in the dietary assessment method which would tend to weaken the dietary data.

The significant positive correlations between the dietary and RBC membrane n-3 PUFAs of the depressed subjects support the findings of previous studies where a link between dietary n-3 fatty acids and tissue levels was found (Neuringer and Connor, 1986; Carlson et al., 1989; and Bjerve et al., 1993). As shown in Table 3, in the depressed subjects, current absolute intake of ALA was found to correlate significantly and positively with RBC ALA, RBC EPA, RBC DPA, n-3, RBC DHA and RBC total n-3 PUFAs. Thus, where the diet was lower in n-3 PUFAs, as was found in the depressed patients, the RBC membrane composition of n-3 PUFAs was also reduced. No such relationship was apparent in the controls. The correlations between dietary ALA and the RBC membrane n-3 PUFAs also indicates that the metabolic pathway of ALA desaturation and elongation is not abnormal. However, this does not exclude other metabolic abnormalities of lipid metabolism affecting incorporation or breakdown of PUFAs.

The relationship between dietary n-3 fatty acids, RBC membrane n-3 fatty acids and severity of depression supports the possible therapeutic use of n-3 fatty acids in the treatment of depression.

References


