Why fish oils may not always be adequate treatments for depression or other inflammatory illnesses: Docosahexaenoic acid, an omega-3 polyunsaturated fatty acid, induces a Th-1-like immune response

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Abstract

BACKGROUND: We have shown that a depletion of ω3 polysaturated fatty acids (PUFAs) plays a role in the pathophysiology of depression, in part because ω3 PUFAs have anti-inflammatory effects. ω3 PUFAs are frequently employed to treat depression. Most if not all antidepressants have negative immunoregulatory effects by decreasing the production of proinflammatory cytokines, such as interferon-γ (IFNγ) and/or increasing that of anti-inflammatory cytokines, such as interleukin-10 (IL-10).

AIM: The aim of the present study was to examine the immunoregulatory effects of the ω3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and the ω6 PUFA, arachidonic acid (AA), on the production of interferon-γ (IFNγ), interleukin-10 (IL-10) and tumor necrosis factor-α (TNFα).

METHODS: This study examines the ex vivo effects of EPA (4.5 μM, 9 μM, 18 μM and 45 μM), DHA (1.3 μM, 3 μM, 6 μM and 13 μM) and AA (8 μM, 16 μM, 32 μM and 80 μM) on the LPS + PHA-stimulated production of IFNγ, IL-10 and TNFα, and on the IFNγ/IL-10 production ratio. Results: We found that EPA did not have any significant effects on the above cytokines. DHA significantly increased the IFNγ/IL-10 production ratio, caused by a greater reduction in IL-10 than in IFNγ. AA significantly decreased TNFα production.

DISCUSSION: The results show that DHA induces a Th-1-like immune response and that AA has anti-inflammatory effects by decreasing the production of TNFα. Thus, the immune effects of ω3 PUFAs are not compatible with what is expected from antidepressive substances. The results of the present study show that treatment with fish oils, containing DHA, should be avoided in the treatment of depression. Toward this end, highly concentrated and pure EPA seems to be indicated.
INTRODUCTION

There is now evidence that activation of the inflammatory response system (IRS) and a decreased omega-3 fatty acid status play a role in the pathophysiology of depression [1,2]. As reviewed elsewhere, major depression is accompanied by IRS activation. The findings include: a) an increased production of pro-inflammatory cytokines, e.g. monocytic and Th-1-like cytokines, including interleukin-1 (IL-1), IL-6, tumor necrosis factor-alpha (TNFa), and interferon-γ (IFNg); b) an acute phase response with increased production of positive acute phase proteins; and increased prostaglandin levels [1,3]. It is also known that the above cytokines have behavioural effects, inducing depressogenic and anxiogenic effects [1,3]. Different antidepressants have a common immunoregulatory effect, i.e. decreasing the production of IFNg and/or increasing that of IL-10 [4]. IL-10 is an anti-inflammatory cytokine, which is produced by monocytes and Th-2-like T cells, and which antagonizes Th-1-like cell functions, including IFNg production [5].

We were the first to show that major depression is accompanied by decreased plasma levels of ω3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and by reductions in the ω3/ω6 PUFA and EPA/arachidonic acid (AA) ratios [6,7]. Since these first papers, decreased ω3 PUFA were also established in the red blood cell membrane, adipose tissues and in the brain of depressed patients [8–11]. Another hot topic in psychiatric research revolves around the clinical efficacy of ω3 PUFAs, such as EPA and DHA, in treating depression [12,13].

Both ω3 and ω6 PUFAs are potent modulators of the IRS [14]. We have posited that the above changes in PUFA contents in depression may in part explain the IRS activation in depression [6,7]. Indeed, ω3 PUFAs have anti-inflammatory effects and, thus, a depletion in ω3 PUFAs may cause an increased inflammatory potency and greater IRS responses [7]. EPA, for example, inhibits the synthesis of eicosanoids, such as PGE2 [15]. Administration of ω3 PUFAs significantly reduces the serum concentrations or the stimulated production of proinflammatory cytokines, such as IL-1, IL-6, and TNFa [15–24]. ω6 PUFAs, on the other hand, have proinflammatory capacities: AA is the precursor of proinflammatory eicosanoids of the prostaglandin-2 series, such as PGE2; AA increases the production of IL-1, TNFa and IL-6 [15,19,24,25]. Therefore, an imbalance of ω6 to ω3 PUFAs may cause an overproduction of proinflammatory cytokines [17]. Consumption of ω3 rich diets (fish oils), on the other hand, leads to a replacement of AA in the cell membrane by EPA, which decreases the production of the inflammatory eicosanoids [23].

Although the effects of ω3 and ω6 PUFAs on monocytic cytokines, such as IL-1 and IL-6, are relatively well known, the exact role of short-term administration of EPA, DHA and AA on the production of the Th1 versus Th2 cytokines, IFNg versus IL-10, and TNFa – which are all three highly relevant to depression – are less well delineated. The aims of the present study were to examine the in vivo effects of EPA, DHA and AA on the stimulated production of IFNg and IL-10, and their production ratio, and on the production of TNFa.

SUBJECTS AND METHODS

Subjects

After an overnight fast, blood was drawn from 18 healthy volunteers (9 males and 9 females, mean age = 31.3±4.2 years) between 8:30 and 9:30 a.m. All subjects gave written informed consent after full explanation of the study design. We excluded the following subjects: i) age under 18 or over 40; ii) a past or present history of psychiatric disorders (axis-I); iii) subjects who ever had been taken major psychotropic medication, such as antidepressants, antipsychotics and anxiolytics and subjects with alcohol or any other drug of dependence abuse; iv) subjects with any organic disorder, such as diabetes, autoimmune diseases, cardio-vascular disorders; v) smokers; vi) subjects who suffered from an infectious, allergic or inflammatory response for at least 4 weeks prior to blood sampling. The subjects abstained from caffeine and alcohol for at least 12 hours prior to blood sampling.

Methods

The effects of EPA, DHA and AA on the production of IFNg, TNFa and IL-10, were examined by stimulating diluted whole blood with PHA and LPS. Blood was diluted four times in RPMI 1640 culture medium (BioWhittaker, Verviers, Belgium) supplemented with L-glutamine and antibiotics (100 U/ml penicilline and 100 μg/ml streptomycine). The suspension was homogenized and plated in a 24-well tissue culture plates (Costar, The Netherlands) at 1 ml/well. After the addition of the 0.25 ml blood suspension, the plates were incubated for 30 min at 37°C and 5% CO2. The PUFAs, EPA sodium salt, DHA sodium salt and AA sodium salt (Sigma-Aldrich, Bornem, Belgium) were dissolved in sterile medium, whereas medium alone served as the corresponding control. 0.25 ml of whole blood from each of the volunteers was cultured with 2 mediums alone (in order to have 2 control conditions for optimal use in the statistical analyses) with 4 concentrations of each of the PUFAs. 20 μl of each of the concentrated solutions of the PUFAs were added to the wells and gently mixed with the medium. The final concentrations yielded EPA 4.5 μM, 9 μM, 18 μM and 45 μM; DHA 1.3 μM, 3 μM, 6 μM and 13 μM, and AA 8 μM, 16 μM, 32 μM and 80 μM. We decided to employ these PUFA concentrations because these are the concentrations reached in blood-brain during physiological conditions and during treatment with PUFAs. The plates were placed on a gyratory shaker for 10 min to mix to contents of the wells, and afterwards incubated for 20 min at 37°C and 5% CO2. The cultures were stimulated with LPS (5 μg/ml; E. coli 026:B6; lyophylized and sterilized by gamma-ir-
radiation; Sigma, Belgium) and PHA (1 μg/ml; Murex Diagnostics Ltd, Dartford, England). Plates were mixed for 10 min and transferred to the incubator. Supernatant for the assay of TNFα was collected after 24 h; and a supernatant for the measurement of IFNγ and IL-10 was collected after 72 h. After incubation, the plates were mixed for 10 min. before centrifugation at 800 × g and 41 °C for 15 min. Supernatants were carefully aspirated and transferred to labeled eppendorf tubes. Samples were stored at −20 °C until assayed for cytokines.

Cytokines were determined using commercially available enzyme linked immunosorbent assays (ELISA) from NIBSC (IFNγ assays) and BD, OptEIA™ ELISA Sets (TNFα and IL-10 assays). All assays were performed according to the manufacturer instructions. Determinations were performed in a single run by the same operator (IM). The intra-assay variations were lower than 8% for all assays.

Statistics
We employed repeated measure (RM) design analyses of variance (ANOVA) to examine the within-subject variability with the 2 positive control and each of the PUFAs at 4 different concentrations. When the overall within-subject analysis yielded significant results, we used the least significant difference (LSD) in order to examine the differences in the production of cytokines between the positive controls and each of the PUFAs concentrations. The IFNγ/IL-10 ratio was computed as: z-transformed IFNγ value minus the z-transformed IL-10 value. ANOVAs were employed to ascertain differences between group means. Relationships between variables were assessed by means of Pearson’s product moment correlation coefficients.

RESULTS

Table 1 shows the effects of the PUFAs on the production of IFNγ. EPA and DHA did not significantly alter the production of IFNγ. AA significantly reduced the production of IFNγ and this effect was significant at 80 μM but not at the other concentrations.

Table 2 shows the effects of the PUFAs on the production of IL-10. EPA did not have any significant effect on the production of IL-10. DHA significantly reduced the production of IL-10; this effect was significant at p<0.05 for DHA 1.3 μM and 3.0 μM, and at p<0.01 for DHA at 6 μM and 13 μM. AA suppressed the production of IL-10 at 16 μM and 32 μM.

Table 3 shows the effects of the PUFAS on the IFNγ/IL10 production ratio. EPA did not significantly affect the production ratio. DHA significantly increased the IFNγ/IL-10 production ratio and this effect was significant at 6 μM and 13 μM. AA significantly decreased the IFNγ/IL-10 production ratio and this effect was significant at 80 μM.

Table 4 shows the effects of the PUFAs on the production of TNFα. EPA and DHA did not have any significant effects of the production of TNFα. AA significantly reduced the production of TNFα and this effect was significant at p<0.05 for AA 16 μM and at p<0.01 at AA 32 μM and 80 μM.

<table>
<thead>
<tr>
<th>Condition</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>1759 (1570)</td>
<td>1773 (1742)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>EPA</td>
<td>1815 (1758)</td>
<td>1671 (1700)</td>
<td>1741 (1458)</td>
<td>1748 (1464)</td>
<td>0.25</td>
<td>0.9</td>
</tr>
<tr>
<td>DHA</td>
<td>1643 (1521)</td>
<td>1483 (1354)</td>
<td>1802 (1577)</td>
<td>2019 (1830)</td>
<td>2.2</td>
<td>0.06</td>
</tr>
<tr>
<td>AA</td>
<td>1661 (1418)</td>
<td>1616 (11596)</td>
<td>1483 (1417)</td>
<td>472 (575)*</td>
<td>13.9</td>
<td>&lt;10^-4</td>
</tr>
</tbody>
</table>

All results are shown as mean (± SD) and in pg/ml; All results of RM design ANOVAs with the 2 control conditions and the 4 concentrations (C1-C4) of each of the PUFAs as repeated measurements (all df=5/85). *p<0.01 by LSD.

<table>
<thead>
<tr>
<th>Condition</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>F</th>
<th>p-value</th>
</tr>
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<tr>
<td>control</td>
<td>1855 (1210)</td>
<td>1857 (1070)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>EPA</td>
<td>1570 (843)</td>
<td>1652 (981)</td>
<td>1618 (861)</td>
<td>1468 (796)</td>
<td>2.2</td>
<td>0.06</td>
</tr>
<tr>
<td>DHA</td>
<td>1553 (855)*</td>
<td>1573 (881)*</td>
<td>1478 (927)**</td>
<td>1501 (898)**</td>
<td>7.8</td>
<td>0.0003</td>
</tr>
<tr>
<td>AA</td>
<td>1618 (912)</td>
<td>1569 (946)*</td>
<td>1507 (788)*</td>
<td>1908 (977)</td>
<td>2.9</td>
<td>0.01</td>
</tr>
</tbody>
</table>

All results are shown as mean (± SD) and in pg/ml; All results of RM design ANOVAs with the 2 control conditions and the 4 concentrations (C1-C4) of each of the PUFAs as repeated measurements (all df=5/85). *p<0.05, **p<0.01 by LSD.

Table 1. Effects of the PUFAs on the production of interferon-γ.

Table 2. Effects of the PUFAs on the production of interleukin-10.
DISCUSSION

The major finding of this study is that the acute in vivo administration of PUFAs to human immune cells results in significant effects on the Th1/Th2 production ratio and on the production of TNFα. Thus, DHA induces a Th-1-like immune response and AA has anti-inflammatory effects by decreasing the IFNγ/IL-10 production ratio and TNFα as well.

The first finding of this study is that acute administration of EPA and DHA did not have any significant effects on IFNγ production. Thies et al. [26] showed that dietary supplementation with EPA, but not with other ω6 or ω3 PUFAs, can decrease lymphocyte proliferation but not the production of IFNγ by mitogen-stimulated human peripheral blood mononuclear cells (PBMC). Other research, however, often showed contradictory results. Thus, Gallai et al. [20] reported that a 6-month dietary supplementation with n-3 PUFAs led to a significant decrease in the levels of IFNγ produced by stimulated PBMCs in normal persons and in patients with multiple sclerosis. Verlangia et al. [27] reported that both EPA and DHA decreased INFγ production in Jurkat T cells. Switzer et al. [28] found that ω3 PUFAs enhance the deletion of proinflammatory Th1 cells possibly as a result of alterations in membrane microdomain fatty acid composition. In mice, it was found that dietary fish oil increased the Th2/Th1 ratio in the presence of homologous mouse serum [29]. After a week dietary supplementation with EPA and DHA, weaning female C3H mice had significantly lower circulating IFNγ after challenge with live Listeria monocytogenes [30]. Finally, in mice it was shown that ω3 PUFAs diminish IFNγ signaling in macrophages [31]. The discrepancies between our study and some of the above papers may be explained by differences in the duration of treatment. Thus, most previous studies have examined the effects of a long-standing diet with mixtures of ω3 PUFAs on cytokine levels [17], whereas we examined the effects of an acute administration of pure EPA or DHA. Thus, the findings of studies, which examine the (sub)chronic effects of treatments with ω3 PUFAs are – in part – a result of alterations in membrane microdomain fatty acid composition, whereas in our study the direct effects of EPA and DHA are examined.

The second finding of this study is that DHA has positive immunoregulatory effects by decreasing IL-10 production and, as a result, increasing the IFNγ/IL-10 production ratio, whereas EPA did not have any significant effects on IL-10. One previous study on the effects of PUFAs in late infancy reported that fish oil supplementation tended to reduce the stimulated production of IL-10 [32]. Verlangia et al. [27] reported that EPA was able to decrease IL-10 production. In another study [33], DHA-rich fish oil raised the secretion of IL-10, results which were in sharp contrast to those described using EPA-rich fish oil supplementation. In children, IL-10 production was significantly higher in lymphocyte cultures after 12 weeks supplementation with 300 mg/day of ω3 PUFAs [34]. In any case, the findings reported here that ω3 PUFAs such as DHA, may have some pro-inflammatory effects may – at first sight – be in contrast to the papers reported

Table 3. Effects of the PUFAs on the interferon-γ / interleukin-10 production ratio.

<table>
<thead>
<tr>
<th>condition</th>
<th>control1</th>
<th>control2</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPA</td>
<td>−0.19 (1.64)</td>
<td>−0.18 (1.55)</td>
<td>1.14 (1.33)</td>
<td>−0.03 (1.45)</td>
<td>0.05 (1.28)</td>
<td>0.21 (1.23)</td>
<td>1.7</td>
<td>0.14</td>
</tr>
<tr>
<td>DHA</td>
<td>−0.22 (1.63)</td>
<td>−0.21 (1.54)</td>
<td>0.02 (1.25)</td>
<td>−0.10 (1.20)</td>
<td>0.20 (1.29)*</td>
<td>0.31 (1.48)*</td>
<td>2.8</td>
<td>0.0003</td>
</tr>
<tr>
<td>AA</td>
<td>0.06 (1.66)</td>
<td>0.07 (1.59)</td>
<td>0.24 (1.24)</td>
<td>0.26 (1.41)</td>
<td>0.23 (1.11)</td>
<td>−0.86 (0.95)*</td>
<td>7.5</td>
<td>&lt;10^−4</td>
</tr>
</tbody>
</table>

All results are shown as mean (± SD) and in pg/ml; All results of RM design ANOVAs with the 2 control conditions and the 4 concentrations (C1-C4) of each of the PUFAs as repeated measurements (all df=5/85). *p<0.05 by LSD.

C1-C4: EPA: C1=4.5 μM, C2=9 μM, C3=18 μM and C4=45 μM; DHA: C1=1.3 μM, C2=3 μM, C3=6 μM and C4=13 μM; AA: C1=8 μM, C2=16 μM, C3=32 μM and C4=80 μM.

Table 4. Effects of the PUFAs on the production of tumour necrosis factor-α.

<table>
<thead>
<tr>
<th>condition</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>F</th>
<th>p-value</th>
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<td>control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPA</td>
<td>2346 (916)</td>
<td>2360 (924)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DHA</td>
<td>2179 (994)</td>
<td>2160 (1081)</td>
<td>2184 (1074)</td>
<td>2330 (1100)</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>AA</td>
<td>2281 (1073)</td>
<td>2239 (1038)</td>
<td>2205 (1032)</td>
<td>2374 (1088)</td>
<td>0.4</td>
<td>0.9</td>
</tr>
</tbody>
</table>

All results are shown as mean (± SD) and in pg/ml; All results of RM design ANOVAs with the 2 control conditions and the 4 concentrations (C1-C4) of each of the PUFAs as repeated measurements (all df=5/85). *p<0.05, **p<0.01 by LSD.

C1-C4: EPA: C1=4.5 μM, C2=9 μM, C3=18 μM and C4=45 μM; DHA: C1=1.3 μM, C2=3 μM, C3=6 μM and C4=13 μM; AA: C1=8 μM, C2=16 μM, C3=32 μM and C4=80 μM.
in the Introduction showing that ω3 PUFAs have anti-inflammatory effects. However, most studies mentioned in the Introduction have examined the long-term effects of fish oils or EPA on the production or plasma levels of monocyctic cytokines. The present study, however, focused on the effects of an acute administration of the pure EPA or DHA on the Th-1 and Th-2-like cytokines, IFNγ versus IL-10.

Another finding of this study is that acute administration of EPA and DHA did not affect the production of TNFα. Also here, the effects of long-term treatment are quite different. Thus, long-term ω3 PUFAs administration to healthy women reduced TNFα production [15]. The production of TNFα by mononuclear cells of healthy male subjects receiving supplemental intakes of 0.3, 1.0 and 2.0 g EPA+DHA/day as consecutive 4-week courses was significantly decreased with increasing ω3 PUFAs intake [35]. In another study [36], it was shown that DHA may be more effective than EPA in alleviating LPS-induced TNFα reduction in human THP-1 monocyte-derived macrophages. In peritoneal macrophages from C57BL/6 female mice, TNFα production was decreased after 8 h and 24 hr in oil containing EPA and DHA mixtures [37]. In B-lymphocyte cell lines (Raji) EPA and DHA (25 μM) decreased the production of TNFα [27]. Incubation of rat peritoneal macrophages with 100 μM DHA and EPA increased TNFα (21% and 15% respectively) [38].

It should be stressed, that there is much debate whether ω3 PUFAs have a clinical efficacy in the treatment of depression [13,39,40]. The immune effects of ω3 PUFAs reported here are not compatible with what is expected from antidepressive substances. Indeed, it has been shown that antidepressive treatments induce a Th-2 shift, i.e. they decrease the IFNγ/IL-10 production ratio by increasing IL-10 and/or decreasing IFNγ production. Thus SSRIs, tricyclic antidepressants, lithium and moclobemide all have similar effects on the Th1/Th2 ratio [7,41]. Considering the cytokine hypothesis of depression [1], we would predict that EPA does not have a clinical efficacy in depression since this PUFA does not induce a Th-2 shift. On the contrary, since DHA seems to induce a Th-1 shift, DHA treatment could aggravate the IRS response in depression. Of course, the putative antidepressive effects of ω3 PUFAs could be attributed to other mechanisms, such as effects on the serotoninergic system or on gene expression. So, if researchers want to examine the antidepressive effects of ω3 PUFAs they should employ pure EPA without addition of DHA. Finally, we suggest that the results of those outcome studies, which used fish oils with considerable amounts of DHA, should be reconsidered using pure EPA substances. Indeed, DHA contents in the fish oil mixtures employed in those studies could have interfered with the clinical efficacy of ω3 PUFAs by aggravating an existing Th1 response in depression.

Finally, we found that AA significantly reduced the production of TNFα and at higher concentrations also IFNγ production. In mice fed a PUFA diet, Hardardottir and Kinsella [42] detected that with an increasing ω3/ω6 ratio, there was a decrease in prostaglandin secretion by macrophages which partly explained the increased production of TNFα. Indeed, PGE2 is known to inhibit TNFα accumulation [43]. Also, the blockade of prostaglandin products augments macrophage and neutrophil TNFα synthesis in burn injury [44].

In summary, the results show that DHA has some positive immunoregulatory effects by induction of a Th-1-like immune response and that AA has some anti-inflammatory effects by decreasing the production of TNFα. The immune effects of the ω3 PUFAs reported here are not compatible with what is expected from established antidepressive substances, which have negative immunoregulatory effects by decreasing the IFNγ/IL10 production ratio. The results that DHA has pro-inflammatory effects suggest that treatments of depressed patients with fish oils containing DHA should be avoided since DHA could aggravate the IRS response in depression. Highly concentrated EPA is probably better if one chooses to treat depression and other conditions, such as autism, pedophilia and chronic fatigue syndrome [45–47], with fish oils.

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