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

ORIGINAL ARTICLE

Michael MAES [1], Ivanka MIHAYLOVA [1], Marta KUBERA [2] and Eugene BOSMANS [3]

1. Clinical Research Center for Mental Health (CRC-MH), Antwerp, Belgium
2. Department of Experimental Neuroendocrinology, Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland
3. AML Laboratory, Antwerp, Belgium

Correspondence to: Michael Maes, MD., PhD.
                      Director: M-Care4U Outpatient Clinics, Olmenlaan 9, 2610 Antwerp, Belgium
                      PHONE: +32-3-4809282
                      FAX: +32-3-2889185
                      EMAIL: crc.mh@telenet.be; www.michaelmaes.com

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 immunoregulary 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 (TNFα), and interferon-γ (IFNγ); 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 IFNγ 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 IFNγ 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 ω3/6ω 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 TNFα [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, TNFα 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, IFNγ versus IL-10, and TNFα – 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 IFNγ and IL-10, and their production ratio, and on the production of TNFα.

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 IFNγ, TNFα 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% CO_2. 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% CO_2. 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 (ANOVAs) to examine the within-subject variability with the 2 positive control and the 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 PUFA 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 sigificant 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 1. Effects of the the PUFAs on the production of interferon-γ.

condition	C1	C2	C3	C4	F	p-value
control	1759 (1570)	1773 (1742)	–	–	–	–
EPA	1815 (1758)	1671 (1700)	1741 (1458)	1748 (1464)	0.25	0.9
DHA	1643 (1521)	1483 (1354)	1802 (1577)	2019 (1830)	2.2	0.06
AA	1661 (1418)	1616 (11596)	1483 (1417)	472 (575)*	13.9	$<10^{-4}$

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

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

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.

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

condition	control1	control2	C1	C2	C3	C4	F	p-value
EPA	–0.19 (1.64)	–0.18 (1.55)	1.14 (1.33)	–0.03 (1.45)	0.05 (1.28)	0.21 (1.23)	1.7	0.14
DHA	–0.22 (1.63)	–0.21 (1.54)	0.02 (1.25)	–0.10 (1.20)	0.20 (1.29)*	0.31 (1.48)*	2.8	0.0003
AA	0.06 (1.66)	0.07 (1.59)	0.24 (1.24)	0.26 (1.41)	0.23 (1.11)	–0.86 (0.95)*	7.5	$<10^{-4}$

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.

condition	C1	C2	C3	C4	F	p-value
control	2346 (916)	2360 (924)	–	–	–	–
EPA	2179 (994)	2160 (1081)	2184 (1074)	2330 (1100)	0.7	0.7
DHA	2281 (1073)	2239 (1038)	2205 (1032)	2374 (1088)	0.4	0.9
AA	2365 (937)	2029 (918)*	1822 (923)**	1298 (799)**	18.4	$<10^{-4}$

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.

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 INF(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 4 week dietary supplementation with EPA and DHA, weanling 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]. Verlengia *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 PUFA such as DHA, may have some pro-inflammatory effects may – at first sight – be in contrast to the papers reported

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 monocytic 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 PUFA 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 PUFA 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 serotonergic 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 condiderable 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.

ACKNOWLEDGEMENTS

REFERENCES

1 Maes M. Major depression and activation of the inflammatory response system. Adv Exp Med Biol. 1999; **461**: 25–46.
2 Schiepers OJ, Wichers MC, Maes M. Cytokines and major depression. Prog Neuropsychopharmacol Biol Psychiatry. 2005; **29**(2): 201–217.
3 Maes M. Evidence for an immune response in major depression: a review and hypothesis. Prog Neuropsychopharmacol Biol Psychiatry. 1995; **19**(1): 11–38.
4 Maes M, Song C, Lin A, Bonaccorso S, Scharpe S, Kenis G, Bosmans E. Anti-inflammatory effects of antidepressants (fluoxetine, trazodone, clomipramine): increased production of interleukin-10 and suppressed production of interferon-gamma. Neuropsychopharmacol 1999; **4**: 370–379.
5 Cavaillon J-M. Les Cytokines. Paris: Masson, 1996.
6 Maes M, Smith RS, Christophe A, Cosyns P, Desnyder R, Meltzer HY. Fatty acid composition in major depression: decreased ω3 fractions in cholesteryl esters and increased C20: 4ω6/C20: 5ω3 ratio in cholesteryl esters and phospholipids. J Affect Disord. 1996; **38**: 35–46.
7 Maes M, Christophe A, Delange J, Neels H, Scharpe S, Meltzer HY. Fatty acid composition in cholesteryl esters and phospholipids in depression. Psychiatry Res. 1999; **85**: 275–291.
8 Peet M, Murphy B, Shay J, Horrobin D. Depletion of omega-3 fatty acid levels in red blood cell membranes of depressive patients. Biol Psychiatry 1998; **43**(5): 315–319.
9 Mamalakis G, Tornaritis M, Kafatos A. Depression and adipose essential polyunsaturated fatty acids. Prostaglandins Leukot Essent Fatty Acids. 2002; **67**(5): 311–318.
10 Mamalakis G, Kiriakakis M, Tsibinos G, Kafatos A. Depression and adipose polyunsaturated fatty acids in an adolescent group. Prostaglandins Leukot Essent Fatty Acids. 2004; **71**(5): 289–294.

11 McNamara RK, Hahn CG, Jandacek R, Rider T, Tso P, Stanford KE, Richtand NM. Selective deficits in the omega-3 Fatty Acid docosahexaenoic Acid in the postmortem orbitofrontal cortex of patients with major depressive disorder. Biol Psychiatry. 2007; **62**(1): 17–24.

12 Severus WE. Effects of omega-3 polyunsaturated fatty acids on depression. Herz. 2006; Suppl 3: 69–74.

13 Appleton KM, Hayward RC, Gunnell D, Peters TJ, Rogers PJ, Kessler D, Ness AR. Effects of n-3 long-chain polyunsaturated fatty acids on depressed mood: systematic review of published trials. Am J Clin Nutr. 2006; **84**(6): 1308–1316.

14 Maes M, Smith RS. Editorial: Fatty acids, cytokines and major depression. Biol Psychiatry. 1998; **43**: 313–314.

15 Meydani SN, Endres S, Woods MM, Goldin BR, Soo C, Morrill-Labrode A, Dinarello CA, Gorbach SL. Oral (n-3) fatty acid supplementation suppresses cytokine production and lymphocyte proliferation: comparison between young and older women. J Nutr. 1991; **121**: 547–555.

16 Espersen GT, Grunnet N, Lervang HN, Nielsen GL, Thomsen BS, Faarvang KL, Dyerberg J, Ernst E. Decreased interleukin-1 beta levels in plasma from rheumatoid arthritis patients after dietary supplementation with n-3 polyunsaturated fatty acids. Clin Rheumatol. 1992; **11**: 393–395.

17 Endres S, Meydani SN, Ghrobani E, Schindler R, Dinarello CA. Dietary supplementation with n-3 fatty acids suppresses interleukin-2 production and mononuclear cell proliferation. J Leukoc Biol. 1993; **54**: 599–603.

18 Purasiri P, Murray A, Richardson S, Heys SD, Horrobin D, Eremin O. Modulation of cytokine production in vivo by dietary essential fatty acids in patients with colorectal cancer. Clin Sci (Colch). 1994; **87**: 711–717.

19 Soyland E, Lea T, Sandstand B, Drevon A. Dietary supplementation with very long-chain n-3 fatty acids in man decreases expression of the interleukin-2 receptor (CD25) on mitogen-stimulated lymphocytes from patients with inflammatory skin disease. Eur J Clin Invest. 1994; **24**: 236–242.

20 Gallai V, Sarchielli P, Trequattrini A, Franceschini M, Floridi A, Firenze C, Alberti A, Di Benedetto D, Stragliotto E. Cytokine secretion and eicosanoid production in the peripheral blood mononuclear cells of MS patients undergoing dietary supplementation with n-3 polyunsaturated fatty acids. J Neuroimmunol. 1995; **56**: 143–153.

21 Caughey GE, Mantzioris E, Gibson RA, Cleland LG, James ML. The effect on human tumor necrosis factor " and interleukin 1β production of diets enriched in n-3 fatty acids from vegetable oil or fish oil. J Am Clin Nutr. 1996; **63**: 116–122.

22 Nanji AA, Zakim D, Rahemtulla A, Daly T, Miao L, Zhao S, Khwaja S, Tahan SR, Dannenberg AJ. Dietary saturated fatty acids downregulate cyclooxygenase-2 and tumor necrosis factor alfa and reverse fibrosis in alcohol-induced liver disease in the rat. Hepatology. 1997; **26**: 1538–1545.

23 Calder PC. Immunoregulatory and anti-inflammatory effects of n-3 polyunsaturated fatty acids. Braz J Med Biol Res. 1998; **31**: 467–90.

24 Tashiro T, Yamamori H, Takagi K, Hayashi N, Furukawa K, Nakajima N. n-3 versus n-6 polyunsaturated fatty acids in critical illness. Nutrition. 1998; **14**: 551–553.

25 Hayashi N, Tashiro T, Yamamori H, Takagi K, Morishima Y, Otsubo Y, Sugiura T, Furukawa K, Nitta H, Nakajima N, Suzuki N, Ito I. Effects of intravenous omega-3 and omega-6 fat emulsion on cytokine production and delayed type hypersensitivity in burned rats receiving total parenteral nutrition. JPEN J Parenter Enteral Nutr. 1998; **22**: 363–367.

26 Thies F, Nebe-von-Caron G, Powell JR, Yaqoob P, Newsholme EA, Calder PC. Dietary supplementation with gamma-linolenic acid or fish oil decreases T lymphocyte proliferation in healthy older humans. J Nutr. 2001; **131**(7): 1918–1927.

27 Verlengia R, Gorjao R, Kanunfre CC, Bordin S, Martins De Lima T, Martins EF, Curi R. Comparative effects of eicosapentaenoic acid and docosahexaenoic acid on proliferation, cytokine production, and pleiotropic gene expression in Jurkat cells. J Nutr Biochem. 2004; **15**(11): 657–665.

28 Switzer KC, Fan YY, Wang N, McMurray DN, Chapkin RS. Dietary n-3 polyunsaturated fatty acids promote activation-induced cell death in Th1-polarized murine CD4+ T-cells. J Lipid Res. 2004; **45**(8): 1482–1492.

29 Zhang P, Smith R, Chapkin RS, McMurray DN. Dietary (n-3) polyunsaturated fatty acids modulate murine Th1/Th2 balance toward the Th2 pole by suppression of Th1 development. J Nutr. 2005; **135**(7): 1745–1751.

30 Fritsche KL, Anderson M, Feng C. Consumption of eicosapentaenoic acid and docosahexaenoic acid impair murine interleukin-12 and interferon-gamma production in vivo. J Infect Dis. 2000; **182** Suppl 1: S54–61.

31 Irons R, Fritsche KL. Omega-3 polyunsaturated fatty acids impair in vivo interferon-gamma responsiveness via diminished receptor signaling. Infect Dis. 2004; **191**(3): 481–486.

32 Damsgaard CT, Lauritzen L, Kjaer TM, Holm PM, Fruekilde MB, Michaelsen KF, Frokiaer H. Fish oil supplementation modulates immune function in healthy infants. J Nutr. 2007; **137**: 1031–1036.

33 Gorjao R, Verlengia R, Lima TM, Soriano FG, Boaventura MF, Kanunfre CC, Peres CM, Sampaio SC, Otton R, Folador A, Martins EF, Curi TC, Portiolli EP, Newsholme P, Curi R. Effect of docosahexaenoic acid-rich fish oil supplementation on human leukocyte function. Clin Nutr. 2006; **25**(6): 923–938.

34 Vaisman N, Zaruk Y, Shirazi I, Kaysar N, Barak V. The effect of fish oil supplementation on cytokine production in children. Eur Cytokine Netw. 2005; **16**(3): 194–198.

35 Trebble TM, Arden NK, Wootton SA, Mullee MA, Calder PC, Burdge GC, Fine DR, Stroud MA. Peripheral blood mononuclear cell fatty acid composition and inflammatory mediator production in adult Crohn's disease. Clin Nutr. 2004; **23**(4): 647–655.

36 Weldon SM, Mullen AC, Loscher CE, Hurley LA, Roche HM. Docosahexaenoic acid induces an anti-inflammatory profile in lipopolysaccharide-stimulated human THP-1 macrophages more effectively than eicosapentaenoic acid. J Nutr Biochem. 2007; **18**(4): 250–258.

37 Bhattacharya A, Sun D, Rahman M, Fernandes G. Different ratios of eicosapentaenoic and docosahexaenoic omega-3 fatty acids in commercial fish oils differentially alter pro-inflammatory cytokines in peritoneal macrophages from C57BL/6 female mice. J Nutr Biochem. 2007; **18**(1): 23–30.

38 Tappia PS, Man WJ, Grimble RF. Influence of unsaturated fatty acids on the production of tumour necrosis factor and interleukin-6 by rat peritoneal macrophages. Mol Cell Biochem. 1995; **143**(2): 89–98.

39 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. Int J Clin Pract. 2001; **55**(8): 560–563.

40 Freeman MP, Hibbeln JR, Wisner KL, Brumbach BH, Watchman M, Gelenberg AJ. Randomized dose-ranging pilot trial of omega-3 fatty acids for postpartum depression. Acta Psychiatr Scand 2006; **113**(1): 31–5.

41 Kenis G, Maes M. Effects of antidepressants on the production of cytokines. Int J Neuropsychopharmacol. 2002; **5**(4): 401–412.

42 Hardardottir I, Kinsella JE. Increasing the dietary (n-3) to (n-6) polyunsaturated fatty acid ratio increases tumor necrosis factor production by murine resident peritoneal macrophages without an effect on elicited peritoneal macrophages. J Nutr. 1992; **122**(10): 1942–1951.

43 Ferreri NR, Sarr T, Askenase PW, Ruddle NH. Molecular regulation of tumor necrosis factor-alpha and lymphotoxin production in T cells. Inhibition by prostaglandin E2. J Biol Chem. 1992; **267**(13): 9443–9.

44 Dong YL, Herndon DN, Yan TZ, Waymack JP. Blockade of prostaglandin products augments macrophage and neutrophil tumor necrosis factor synthesis in burn injury. J Surg Res. 1993; **54**(5): 480–485.

45 Mincke E, Cosyns P, Christophe AB, De Vriese S, Maes M. Lower omega-3 polyunsaturated fatty acids and lower docosahexaenoic acid in men with pedophilia. Neuroendocrinol Lett 2006; **27**(6): 719–723.

46 Sliwinski S, Croonenberghs J, Christophe A, Deboutte D, Maes M. Polyunsaturated fatty acids: do they have a role in the pathophysiology of autism? Neuroendocrinol Lett 2006; **27**(4): 465–471.

47 Maes M, Mihaylova I, Leunis JC. In chronic fatigue syndrome, the decreased levels of omega-3 poly-unsaturated fatty acids are related to lowered serum zinc and defects in T cell activation. Neuroendocrinol Lett 2005; **26**(6): 745–751.