Incorporation of fatty acids from fish oil and olive oil into colonic mucosal lipids and effects upon eicosanoid synthesis in inflammatory bowel disease

K Hillier, R Jewell, L Dorrell, C L Smith

Abstract
The incorporation of the fatty acids in fish and olive oil into the colonic mucosa of patients with inflammatory bowel disease was examined during 12 weeks' dietary supplementation with the oils, and the influence on colonic mucosal prostaglandin and thromboxane generation was measured. With a dietary supplement of 18 g fish oil daily, concentrations of the major polyunsaturated fatty acids in fish oil, eicosapentaenoic acid and docosahexaenoic acid, were significantly raised in mucosal lipids. The first time these were measured, after three weeks' supplementation, the mean increases in eicosapentaenoic and docosahexaenoic acid were seven fold and 1·5 fold respectively, and these increases were maintained during the 12 week study. Arachidonic acid values fell throughout the study and this reduction was significant at 12 weeks. Mucosal prostaglandin E2 (PGE2), thromboxane B2, and 6-keto prostaglandin F1α synthesis were suppressed, and this reached significance (p<0·05) at three and 12 weeks for PGE2 and at 12 weeks for thromboxane B2. The predominant fatty acid in olive oil is oleic acid. Supplementation with 18 g/day resulted in a significant increase in oleic acid in colonic mucosa at 12 weeks (p<0·05) and a fall in stearic acid and docosahexaenoic acid; there was no significant change in eicosanoid synthesis. It is concluded that colonic lipids and prostaglandin and thromboxane synthesis can be readily altered by dietary supplementation with fish oil. The extent of incorporation of the fatty acids present in oils is dependent upon the individual fatty acid.

It is claimed that diets enriched in fish lipids offer therapeutic benefit in several diseases. Epidemiological studies have shown that a high intake of fish oils may be related to a reduced incidence of cardiovascular and asthmatic disease. In clinical studies, supplementation with fish oils has led to improvements in patients with psoriasis and rheumatoid arthritis, reductions in plasma triglyceride and cholesterol concentrations, and in thrombocyte aggregability. It has been suggested that fish oil lipids lead to improvements in ulcerative colitis, but this has not been confirmed. Feeding studies in laboratory animals have also shown improvements in induced atherosclerosis, lupus, and colitis.

In normal western diets the preponderant intake of oils from vegetable sources such as sunflowers provides relatively high amounts of linoleic acid (C18:2) (Fig 1). This can be metabolised to arachidonic acid (C20:4) and hence to dienoic eicosanoids such as prostaglandin E2 (PGE2), thromboxane A2 (TxA2), and leukotriene C4 (LTC4). The predominant polyunsaturated fatty acids present in fish oils are eicosapentaenoic acid (C20:5) and docosahexaenoic acid (C22:6). C20:5, which is absent in vegetable oils, has been shown to be metabolised to a family of trienoic eicosanoids, for example, PGE3, TxA3, and also LTC5 which have subtly different biological properties to the dienoic eicosanoids and LTC4 derived from arachidonic acid. It is hypothesised that fish oils may reduce inflammation via the production of relatively larger amounts of 3 series prostaglandins and 5 series leukotrienes which possess reduced inflammatory potential. In addition, fish oils may inhibit the cyclo-oxygenase enzymes that form prostaglandins and thromboxanes.

The synthesis of several eicosanoids is increased in inflammatory bowel disease and may be responsible for some of the pathophysiological features of the disease. Mucosal samples taken from patients with active disease have increased capacity to synthesis PGE2, TxA2, and leukotrienes. The fatty acids in fish oil, eicosapentaenoic acid and docosahexaenoic acid are present in fish lipids.
The increased eicosanoids present may result, in part, from the large amounts of arachidonic acid (C20:4) found in the mucosa of patients with inflammatory bowel disease compared with normal mucosa.\textsuperscript{16,17} It has been hypothesised that modulation of excessive formation of some eicosanoids may be beneficial in inflammatory bowel disease; benefit may accrue both from reducing the directly pro-inflammatory effects inherent in some eicosanoids but also indirectly as some eicosanoids can act to increase the synthesis or action of other inflammatory mediators. In addition, increased supplementation of diets with fish oils has been shown to reduce interleukin formation,\textsuperscript{18} free radical generation,\textsuperscript{19} and platelet activating factor formation,\textsuperscript{20} all of which are increased in inflammatory bowel disease. Despite these wide ranging effects and therapeutic potential, no studies on the incorporation of dietary fish oil fatty acids in large bowel mucosa in man have been reported.

We examined the incorporation of fatty acids from fish oil (Maxepa) into the mucosa of the large bowel in patients with inflammatory bowel disease during 12 weeks of dietary supplementation. This was compared with the incorporation from fatty acids in olive oil and effects of the supplements on prostaglandin, thromboxane, and prostacyclin synthesis were also assessed. A preliminary communication of results in 13 patients has previously been presented in abstract form.\textsuperscript{21}

Patients and methods

Twenty patients with active ulcerative colitis or Crohn’s colitis were admitted to the study. On admission, mucosal tissue specimens were taken from inflamed colonic sites during colonoscopy. One biopsy specimen was immediately frozen in liquid nitrogen and stored at ~70°C until analysed for eicosanoids and fatty acids. Histological examination of a second specimen verified the diagnosis.

Patients were allocated to receive 6×1 g capsules of fish oil (Maxepa) or olive oil three times a day on a random open basis for a 12 week study period. Patients were asked not to change their conventional diet. The total consumption per day of 18 g Maxepa provided 3·2 g daily C20:5, 2·2 g daily C22:6, 1·7 g daily palmitoleic acid (C16:1), and 2.4 g daily oleic acid (C18:1). The olive oil consumption provided 13·9 g per day C18:1. No restrictions were put on the intake of drugs. No patient was taking non-steroidal anti-inflammatory drugs.

Eleven patients received Maxepa; nine had active ulcerative colitis and two active Crohn’s colitis. There were eight men and three women with an age range of 32–79 years. All patients were receiving between 15–30 mg prednisolone daily on admission to the trial, reducing to 0–20 mg after 12 weeks. Six were taking additional sulphasalazine or mesalazine on admission to the study.

In both groups, the protocol followed reduced steroids every three weeks if improvement in the patient’s condition was maintained. At a dosage of greater than 20 mg per day, reduction was by 10 mg, and at a dosage of less than 20 mg, by 5 mg.

Patients attended the clinic three, six, and 12 weeks after admission to the study for reassessment and colonoscopy. The study was approved by the Southampton District Ethical Committee and informed consent was obtained from the patients.

Dietary oils

The batches of Maxepa used contained 13·5% C18:1, 18·0% C20:5, and 12% C22:6. The olive oil contained 77·3% C18:1, 7·2% C18:2, and trace amounts of other fatty acids. Both fish oil and olive oil contained 1 IU vitamin E per ml and 0·2% w/w natural peppermint oil.

Fatty acid analysis

Transmethylation of total lipids was by a modification of the method of Folch et al.\textsuperscript{22} Portions of biopsy specimens were rinsed with 1 ml 1·15% KCl and homogenised in 1 ml chloroform/methanol (2:1 v/v). The homogenise was washed with an additional 1 ml chloroform/methanol and the total homogenate stood at room temperature in 2 ml chloroform/methanol for 20 minutes. It was then centrifuged at 1000 g for five minutes. The supernatant was removed and evaporated to dryness under nitrogen at 30°C. The residue was reconstituted in 20 μl dry methanol, and after the addition of 20 μl sodium methoxide (2·2% in dry methanol) was retained under nitrogen for 30 minutes. To the reaction, 20 μl acetycloride/dry methanol (1·19 v/v) was added and the tubes were retained under nitrogen for a further five minutes. The samples were extracted twice with 100 μl hexane, the pooled upper layers were evaporated to dryness, and the residue was reconstituted in 50 μl hexane. Samples were stored under nitrogen at ~20°C until analysed.

Fatty acid methyl esters were analysed by gas liquid chromatography on a Packard 438A chromatograph (wall coated, open tubular fused silica capillary column, length 51 m; internal diameter 0·22 mm; liquid phase CP SILE88, film thickness 0·21 μm; carrier gas H2; split-flow ratio 100:1; column flow 1 ml/minute; injection temperature 270°C; FID 300°C; temperature programme 175°C for three minutes, increased to 210°C at 5°C/minute and maintained at 210°C for 25 minutes). Peak identification was by comparison with a known standard mixture, each profile being recorded on a Spectra Physics SP4270 integrator. Fatty acid values were expressed as a percentage of the total major fatty acids present.

Eicosanoid synthesis and analysis

Biopsies were preincubated with 1 ml Krebs
Incorporation of fatty acids from fish oil and olive oil into colonic mucosal lipids and effects upon eicosanoid synthesis in inflammatory bowel disease

TABLE 1 The mean (SEM) percentage fatty acid composition of human colonic mucosa in patients with active inflammatory bowel disease during 12 weeks' dietary supplementation with enriched fish oil capsules (Maxepa) or olive oil. The second figure in parentheses is number of patients

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Week 0</th>
<th>Week 3</th>
<th>Week 6</th>
<th>Week 12</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C20:4 – olive oil</strong></td>
<td>8.5 (1.0)</td>
<td>8.6 (1.3)</td>
<td>7.5 (2.0)</td>
<td>5.1 (3.3)</td>
</tr>
<tr>
<td><strong>C20:4 – fish oil</strong></td>
<td>10.0 (2.9)</td>
<td>7.9 (1.0)</td>
<td>8.3 (0.7)</td>
<td>5.6 (0.8)</td>
</tr>
<tr>
<td><strong>C20:5 – olive oil</strong></td>
<td>0.29 (0.05)</td>
<td>0.36 (0.04)</td>
<td>0.38 (0.7)</td>
<td>0.2 (0.5)</td>
</tr>
<tr>
<td><strong>C20:5 – fish oil</strong></td>
<td>0.44 (0.06)</td>
<td>3.1 (0.5)</td>
<td>3.2 (0.7)</td>
<td>3.1 (0.5)</td>
</tr>
<tr>
<td><strong>C22:6 – olive oil</strong></td>
<td>1.7 (0.2)</td>
<td>1.7 (0.2)</td>
<td>1.7 (0.2)</td>
<td>1.0 (0.8)</td>
</tr>
<tr>
<td><strong>C22:6 – fish oil</strong></td>
<td>2.1 (0.21)</td>
<td>3.3 (0.5)</td>
<td>3.1 (0.4)</td>
<td>2.9 (0.3)</td>
</tr>
<tr>
<td><strong>Eicosapentaenoic acid</strong></td>
<td>12.9 (1.1)</td>
<td>12.9 (1.3)</td>
<td>14.0 (1.4)</td>
<td>9.4 (1.3)</td>
</tr>
<tr>
<td><strong>Arachidonic acid</strong></td>
<td>13.2 (0.7)</td>
<td>13.6 (1.0)</td>
<td>14.0 (1.9)</td>
<td>13.4 (0.9)</td>
</tr>
<tr>
<td><strong>Lynsacosaenoic acid</strong></td>
<td>21.7 (1.9)</td>
<td>22.1 (2.6)</td>
<td>20.9 (2.5)</td>
<td>29.9 (2.7)</td>
</tr>
<tr>
<td><strong>Linolenic acid</strong></td>
<td>19.5 (1.4)</td>
<td>19.6 (1.0)</td>
<td>17.3 (1.6)</td>
<td>19.0 (1.9)</td>
</tr>
<tr>
<td><strong>Maxepa</strong></td>
<td>1.9 (0.3)</td>
<td>1.8 (0.3)</td>
<td>2.0 (0.4)</td>
<td>1.3 (0.5)</td>
</tr>
<tr>
<td><strong>Olive oil</strong></td>
<td>1.8 (0.2)</td>
<td>1.1 (0.1)</td>
<td>1.25 (0.1)</td>
<td>1.2 (0.2)</td>
</tr>
</tbody>
</table>

Patients took 6 g Maxepa or olive oil three times daily.

*p = <0.05, †p <0.01 using Wilcoxon's ranking test and refer to comparison with values at entry to the trial (0 weeks). C20:4 = arachidonic acid, C20:5 = eicosapentaenoic acid, C22:6 = docosahexaenoic acid, C18:1 = oleic acid, C18:2 = linoleic acid, C18:3 = γ-linolenic acid, C18:4 = stearic acid, C18:6 = α-linolenic acid, C20:3 = dihomoy-γ-linolenic acid, C18:3 = linolenic acid, C16:0 = palmitic acid, C16:1 = palmitoleic acid, C18:2 = linoleic acid.

For clarity some fatty acids which show no change through the 12 week period have been omitted. Values on entry to the study were as follows with the figures in the fish oil group shown in brackets:

C14:0, 14 (0.2); C16:0, 19.1 (1); C18:0, 19 (0.3); C18:1, 17 (0.2); C18:2, 17 (1.0); C18:3, 11 (1.2), C20:3, 11 (1.2); C22:5, 11 (2.0); C22:6, 10 (0.8). C16:0, 13 (0.3), n = 9 for the olive oil group and n = 11 for the fish oil group. The data are expressed for the individual fatty acids as a percentage of the total fatty acids analysed including those that did not alter throughout the 12 week study period.

Results

FATTY ACID STUDIES

The results of ulcerative colitis and Crohn's disease patients were not different, and data are therefore presented together. Both fish oil and olive oil were well accepted, with only one patient complaining about the taste of fish oil. Table I shows the percentage fatty acid distribution in mucosa from patients taking fish oil or olive oil for up to 12 weeks. In the Maxepa group between eight and 10 patients of the 11 recruited attended regularly during the study period. In the olive oil group five patients of nine recruited continued for the full 12 weeks study period.

On admission to the study (week 0) there was no significant difference in the percentages of individual fatty acids in the colonic mucosa of patients allocated to the fish oil or the olive oil group. The distribution of fatty acids in colonic mucosal lipids at week 0 before beginning the dietary supplements is noticeable in that C20:5 and C22:6, which are prevalent in fish oils, make up only a small percentage of total fatty acids present, whereas C18:1, present in large amounts in olive oil makes up about 20% of total

Figure 2: Ratio of arachidonic acid (AA):eicosapentaenoic acid (EPA) in colonic mucosa of individual patients taking 18 g fish oil daily or 18 g olive oil daily. In the fish oil group the ratio fell significantly by three weeks and remained suppressed throughout the 12 week study (p<0.08 at three, six, and 12 weeks compared with 0 weeks using Wilcoxon's rank test). Changes in the olive oil group were not significant.

TOTAL PROTEIN ANALYSIS

Each biopsy specimen was hydrolysed in 0.1 N NaOH (1 ml) at 50°C for approximately five hours and analysed for total protein content by a modification of the method by Lowry et al.
mucosal fatty acids. The main unsaturated fatty acids present were C18:1, C18:2, and C20:4 while the main saturated fatty acids present were palmitic acid (C16:0) and C18:0. Table I shows that fish oil consumption had a considerable effect on C20:5 and C22:6 values. After three weeks, C20:5 had increased sevenfold (p<0.01) and C22:6 almost doubled (p<0.01). The increases were maintained during the study period. In the olive oil group, there were no significant changes in C20:5 and C22:6. The range of C20:5 on admission was 0.14–0.7% and after three weeks it was 0.86–4.9%. These data suggest that compliance in taking the capsules was acceptable. The individual patient values for the ratio of C20:4/C20:5 are shown in Figure 2. At entry to the study, the ratio of C20:4/C20:5 in 10 of the 11 patients in the fish oil group ranged from 14.1 to 42.0, but in one patient the ratio was 74.2. In the olive oil group the ratio in eight out of nine patients was 14.2–39.0 but in one patient it was 92.1. There was no dietary or other understood reason for high C20:4/C20:5 ratios in these two patients. The ratio of C20:4/C20:5 fell precipitously in all but one patient and remained low during the 12 week study period. Dihomo-γ-linolenic acid (C20:3) was also significantly reduced in the fish oil group at three weeks and six weeks. In the olive oil group there was no increase in the C18:1 values in the colonic mucosa until 12 weeks supplementation (p<0.05), despite the fact that the diet provided 13.9 g per day C18:1 in olive oil. There was also a fall in C18:0 and C22:6 after 12 weeks in patients taking olive oil.

Table II shows the prostaglandin and thromboxane synthesis by mucosal specimen in both groups. On admission to the study (week 0) there was no significant difference in eicosanoid synthesis between the fish oil and olive oil groups. The fish oil diet resulted in a significant reduction in PGE2 synthesis at three weeks and 12 weeks, although the decline at six weeks did not reach significance. At six weeks, however, all patients tested had PGE2 values lower than the individual patient’s admission values at all three sites. PG12 synthesis (measured as the stable metabolite 6-keto-PGF1α) was lower than the individual patient’s admission values in seven of nine, six of eight, and seven of nine patients at three, six, and 12 weeks after taking fish oil but the figures did not reach statistical significance. TxA2 synthesis was lower than individual patient’s admission values in seven of nine, seven of eight, and eight of nine patients at three, six, and 12 weeks after taking fish oil but this only reached a level of significance of p<0.05 after 12 weeks. The olive oil diet had no significant effect on the synthesis of prostaglandins or thromboxanes; the sample size at 12 weeks was, however, small.

The current study was not performed primarily as a clinical trial to assess therapeutic benefit. Nonetheless, after three weeks, nine of 10 taking fish oil were improved endoscopically and symptomatically and six of eight taking olive oil were improved. There was no difference in the steroid intake in the two groups.

### Discussion

Supplementation of diets with fish oil significantly and relatively rapidly increased the incorporation of C20:5 and C22:6 fatty acids in colon mucosal lipids and there was, at the same time, a small but significant fall (p<0.05) in dihomo-γ-linolenic acid (C20:3). At this time we do not have information on the cell types into which these fatty acids are incorporated or if the incorporation is uniform in different cell types.

There is considerable variability in the incorporation of dietary supplements of fatty acids into mucosal lipids. For example, we have found no increase in the amounts of ruminal fatty acids in colon mucosal lipids in patients taking fish oil despite this fatty acid making up 13.5% of the total fatty acids present and providing 2.4 g per day C18:1. Even with the provision of 13.9 g C18:1 per day in olive oil, a significant increase in colonic mucosa was not seen until 12 weeks of dietary supplementation. In laboratory animal studies, supplementation of the diets of rats with corn oil (46% C18:2) resulted in an increase in C18:2 in gastric mucosal lipids and liver and yet duodenal mucosa showed no increase in C18:2. The reasons for this are, as yet, unclear but this illustrates that every tissue, and indeed cell type, is different in its ability to synthesize fatty acid from fatty acid incorporation from dietary oils.

Three weeks after starting the fish oil supplement, the increases in C20:5 and C22:6 in the mucosa had reached their optimum; as no earlier samples were taken we cannot say precisely when the peak was achieved. It is also possible that C20:4, which was significantly reduced after 12 weeks’ fish oil supplementation, may continue to fall after 12 weeks as fatty acid compartments with a slow turnover become progressively depleted of this fatty acid.

The relatively rapid exchange between plasma C20:5 and colon mucosal phospholipids noted in this study seems to be much slower in other cell types such as platelets and red cells. In the latter study, volunteers took consecutive incremental doses of cod liver oil of 10, 20, 40, and then 20 ml per day changing from one dose level to another after four weeks. Platelet and red cell concentrations of C20:5 rose only slowly when doses were increased and in red cells even continued to increase despite a lowering of the dose to 20 ml per day after 12 weeks. The return to normal fatty acid concentrations after stopping the cod liver oil took several weeks. In contrast to the relatively rapid changes seen in colonic mucosal fatty acids in patients taking fish oil, significant changes in C18:1 and C18:0 values were not seen until between six and 12 weeks after beginning

### Table 11

<table>
<thead>
<tr>
<th>Week 0</th>
<th>Week 3</th>
<th>Week 6</th>
<th>Week 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE2 - olive oil</td>
<td>22.7 (4.6) (9)</td>
<td>40.5 (13.1) (8)</td>
<td>17.9 (7.2) (5)</td>
</tr>
<tr>
<td>PGE2 - fish oil</td>
<td>25.1 (4.7) (11)</td>
<td>11.2 (2.1) (9)*</td>
<td>14.0 (2.7) (8)</td>
</tr>
<tr>
<td>TxB2 - olive oil</td>
<td>10.5 (2.1) (9)</td>
<td>8.7 (1.6) (8)</td>
<td>10.1 (3.9) (5)</td>
</tr>
<tr>
<td>TxB2 - fish oil</td>
<td>10.3 (1.4) (11)</td>
<td>7.8 (1.4) (9)</td>
<td>8.0 (3.3) (8)</td>
</tr>
<tr>
<td>6-keto-F2α - olive oil</td>
<td>8.9 (2.0) (9)</td>
<td>14.4 (1.6) (8)</td>
<td>10.0 (4.5) (5)</td>
</tr>
<tr>
<td>6-keto-F2α - fish oil</td>
<td>13.1 (3.2) (11)</td>
<td>6.6 (1.5) (9)</td>
<td>7.8 (1.6) (8)</td>
</tr>
</tbody>
</table>

* = p<0.05 Wilcoxon’s rank test and refers to comparison with values at entry to the trial (9 weeks)
supplementation in those taking olive oil. These data indicate that there are lipid pools where turnover of fatty acids proceed at different rates; they also suggest that loading doses of fatty acids may effectively increase the fatty acid concentration in lipid pools, which can then be maintained on lower continuing doses. The long term effects of fish oil supplementation have also been described by Endres et al. During a six week feeding study with fish oil, interleukin-1 production by peripheral blood mononuclear cells was suppressed. On stopping the fish oil diet interleukin-1 production fell further until the nadir was reached 10 weeks after ceasing supplementation and had returned to normal 10 weeks after that.

The therapeutic potential, if any, of fish oil in the treatment of inflammatory bowel disease is unproven. In a small open study, 12 weeks’ administration of fish oil improved patients’ symptoms and the histological appearance of the rectal mucosa. During the 12 weeks, sulphasa-lazine and steroids were withdrawn. In a controlled double blind clinical trial carried out over one year, fish oil or olive oil were randomly administered. There was no difference in relapse rate or percentage of time spent in remission, and steroid treatment was not different in the two groups. It is questionable whether olive oil is a suitable ‘placebo’ in such a study. Although there are, as yet, no data to substantiate this, it is possible that the consumption of large amounts of C18:1 may have biological effects.

In an animal model, where a large bowel inflammatory colitis was induced in rats by the administration of trinitrobenzene sulphonic acid, fish oil, but not olive oil, reduced the chronic severity of the lesions, although the severity of the initial injury produced was similar in the olive oil and fish oil groups.

The pharmacological effects of fish oil are such that it might be expected to benefit inflammatory bowel disease. It causes a suppression of the production of many potentially damaging mediators that have been shown to be produced in excess within the colonic mucosal cells in these disorders. Interleukin-1, platelet-activating factor, and prostaglandin are all produced in greater amounts compared with normal mucosa and Hawkey et al have also shown that there is an increased ratio of thromboxane to PG2 in the colonic mucosa in Crohn’s disease. It is of importance that some mediators that are produced act synergistically but others are, in fact, antagonistic; for example, PGE2 has been shown to inhibit interleukin synthesis. Further studies should determine whether different fats and varied dosage schedules have the ability to modulate selectively the production of particular mediators and whether this can favourably influence inflammatory bowel disease.


