Treatment of ulcerative colitis with fish oil supplementation: a prospective 12 month randomised controlled trial

A B Hawthorne, T K Daneshmend, C J Hawkey, A Belluzzi, S J Everitt, G K T Holmes, C Malkinson, M Z Shaheen, J E Willars

Abstract
The effect of fish oil on the course of ulcerative colitis was investigated in a randomised blinded controlled study. Eighty seven patients received supplements of 20 ml HiEPA fish oil as triglyceride (4-5 g of eicosapentaenoic acid) or olive oil placebo daily for one year. The oils were given in addition to standard drug therapy and trial entry was stratified for disease activity. Fish oil significantly increased the eicosapentaenoic acid content of rectal mucosa to 3-2% of total fatty acids at six months, compared with 0-63% for patients on olive oil. This was associated with increased synthesis of leukotriene B5, and 53% suppression of leukotriene B4 synthesis by ionophore - stimulated neutrophils. Leukotriene B4 suppression persisted for at least two months after treatment was stopped. Treatment with fish oil resulted in measurable, but only limited clinical benefit. For patients entering the trial in relapse (n=53), there was a significant reduction in corticosteroid requirement after one and two months treatment. There was a trend towards achieving remission (off corticosteroids) faster in the patients on fish oil, although differences were not significant. For patients in remission at trial entry or during the trial (n=69), there was no significant difference in the rate of relapse by log rank analysis. We conclude that fish oil supplementation produces a modest corticosteroid sparing effect in active disease, but there is no benefit in maintenance therapy.

In ulcerative colitis inflammation is characterised by marked influx and accumulation of neutrophils in the colonic mucosa. There is increasing evidence that leukotriene B4 may be pivotal in triggering or perpetuating this accumulation. It is a potent chemoattractant present in high levels in inflamed colonic mucosa where it may account for 80% of the lipid extractable chemoattractant activity. Treatments which reduce synthesis of leukotriene B4 may therefore be of benefit in ulcerative colitis.

Diets containing high levels of w-3 fatty acids, such as eicosapentaenoic acid and docosahexaenoic acid, are known to modify leukotriene production. Eicosapentaenoic acid levels in cell membranes rise, with an increase in eicosapentaenoic acid derived lipoxynenase products, such as leukotriene B5, which has markedly reduced chemoattractant potency compared with leukotriene B4. In addition synthesis of lipoxynenase products derived from arachidonic acid is reduced as a result of diminished substrate release, 5-lipoxygenase inhibition, and enzyme inhibition at the level of leukotriene A4 hydrolase. Anti inflammatory effects of fish oils have been shown in animal models, including a rat model of chronic colitis induced by trinitrobenzene sulfonic acid. In clinical studies, fish oils have been reported to have modest anti inflammatory effects in psoriasis, atopic dermatitis, and rheumatoid arthritis. In ulcerative colitis increased arachidonic acid levels in colonic mucosa have been reported, and this can be decreased by eicosapentaenoic acid supplementation. Several small preliminary crossover studies have claimed clinical benefit. We therefore designed a large prospective 12 month placebo controlled trial to evaluate the clinical effects of fish oil supplementation in ulcerative colitis.

Methods

TRIAL DESIGN
The trial was designed as a randomised explanatory placebo controlled study of the effects of fish oil compared with olive oil. Trial medication was added to conventional treatment with sulphasalazine or mesalamine, and corticosteroids. Trial entry was stratified according to disease status at entry. The main end point was the relapse rate for all patients achieving stable remission (as defined below) during the trial. Other endpoints included (a) overall time spent in remission during the trial, (b) treatment of relapse: rate of achieving remission off corticosteroids, and corticosteroid dosage at one and two months.

PATIENTS
The study was performed in two centres, University Hospital, Nottingham, and Derbyshire Royal Infirmary, Derby, UK. Ethical approval was granted by the hospitals' ethical committees and patients gave informed written consent. Patients with ulcerative colitis diagnosed on the basis of rectal biopsy and barium enema or colonoscopy were enrolled. Entry was restricted to patients who had had two or more relapses in the previous three years.

DEFINITIONS OF CLINICAL STATUS
Patients were defined as being in remission if they had no symptoms of active disease for more than four weeks (rectal bleeding, loose stools, bowel frequency or other symptoms regarded by the patient as active disease), were not taking
Treatment of ulcerative colitis with fish oil supplementation: a prospective 12 month randomised controlled trial

TREATMENT

**Trial medication**
Randomisation was carried out by the hospital pharmacy in blocks of four to receive fish oil triglyceride concentrate HiEPA, 10 ml twice daily or olive oil placebo 10 ml twice daily for one year (Scotia Pharmaceuticals, Surrey, UK). The oils were stored in bottles under an atmosphere of nitrogen, at 4°C. The oils were administered in unencapsulated form as initial evaluation showed that patients found it hard to swallow sufficient gelatin capsules to administer this volume, either because of their size (1 ml) or the number required (20/day). The oils were referred to as fish oil and patients were asked not to comment on the taste of the oils to the clinician. The composition of the oils is shown in Table I. Patients were asked to continue with their normal diet during the trial.

**Other medication**
Longterm medication with sulphasalazine or mesalamine was continued during the trial and patients kept a daily symptom diary including corticosteroid dose. Relapses were treated with prednisolone according to a defined protocol: 40 mg daily for week one, 30 mg daily for week two, then after assessment either 20 mg daily in week three, and 10 mg daily in week four, or 30 mg and 20 mg respectively if improvement was slow. Dosage was then reduced according to clinical need with every effort made to withdraw prednisolone as soon as practicable.

ASSESSMENT DURING THE TRIAL
Patients were assessed at one to three baseline pretreatment visits, and after one month, two months, and then two month intervals for one year (365 ±10 days). Patients made unscheduled visits if symptoms suggesting relapse developed. Sigmoidoscopy and rectal biopsy were performed at baseline, two, six, 12 months, or at unscheduled visits. St Marks forceps (KeyMed, Southend on Sea, UK) were used for rectal biopsy. After the procedure cotton wool swabs were applied to the biopsy site with gentle pressure for 10 second intervals and the time until no further obvious oozing of blood occurred was taken as a measure of haemostasis.

**Compliance**
Treatment compliance was assessed by a count of bottles of oil used per two month period, diary records of daily oil consumption, and measure-ment of red cell membrane eicosapentaenoic acid incorporation. Dietary intake of patients enrolled in Nottingham was assessed by two seven day semi weighed diet diaries (within the first two months and last two months of the study) and patients were given charts and instructions by a dietician. Calculations were made using the Microdiet version 7 computer program, with a database expanded16-21 to include current manufacturers' specifications of margarine, oil and salad dressing composition.

BIOCHEMICAL STUDIES
The following biochemical studies were performed; before, during, and two months after trial completion, in 66 patients enrolled in Nottingham:

**Membrane fatty acids**
Changes in red cell membrane fatty acids were monitored as the primary measure of compliance with oil supplementation. Red cell lipids were extracted into chloroform:methanol (1:2), methylated, and analysed by gas chromatography, as previously described.17 Rectal mucosal fatty acids were monitored to assess whether intended biochemical changes were achieved in the target tissue. Biopsy specimens were homogenised, and lipids extracted and analysed as for red cells.

**Synthesis of lipoxgenase products by ionophore stimulated peripheral blood neutrophils**
Neutrophils were isolated from peripheral blood by dextran sedimentation of red cells, and gradient centrifugation, and stimulated with calcium ionophore for five minutes, at 37°C. Supernatants were analysed by reverse phase high pressure liquid chromatography as described previously.18

**STATISTICS AND POWER**
Baseline characteristics were compared in the two treatment groups by χ² test, unpaired t test or Mann Whitney test as appropriate. Rate of relapse or of achieving remission were assessed by log rank analysis and the Kaplan Meier method. The number of trial days spent in remission was assessed by the Kolmogorov

<table>
<thead>
<tr>
<th>Fatty acid (chain length:no. double bonds)</th>
<th>Fish oil*</th>
<th>Olive oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0 Myristic acid</td>
<td>8-11%</td>
<td>trace</td>
</tr>
<tr>
<td>16:0 Palmitic acid</td>
<td>10-13%</td>
<td>12%</td>
</tr>
<tr>
<td>18:0 Stearic acid</td>
<td>2-4%</td>
<td>2-3%</td>
</tr>
<tr>
<td>20:0 Arachidonic acid</td>
<td>0-1%</td>
<td></td>
</tr>
<tr>
<td>16:1 n7 Palmitoleic acid</td>
<td>9-12%</td>
<td>1%</td>
</tr>
<tr>
<td>18:1 n9 Oleic acid</td>
<td>11-14%</td>
<td>72%</td>
</tr>
<tr>
<td>18:2 n6 Linoleic acid</td>
<td>4-6%</td>
<td>11%</td>
</tr>
<tr>
<td>18:3 n6 Gamma-linolenic acid</td>
<td>0-1%</td>
<td></td>
</tr>
<tr>
<td>20:4 n6 Arachidonic acid</td>
<td>3-5%</td>
<td></td>
</tr>
<tr>
<td>20:5 n5 Eicosapentaenoic acid</td>
<td>25%</td>
<td></td>
</tr>
<tr>
<td>22:6 n3 Docosahexaenoic acid</td>
<td>6%</td>
<td></td>
</tr>
<tr>
<td>alpha-tocopherol</td>
<td>0-06 ppm</td>
<td></td>
</tr>
<tr>
<td>vitamin A</td>
<td>7 mg/kg</td>
<td></td>
</tr>
</tbody>
</table>

*HiEPA* oil had a peroxy value of 10 meq O₂/kg oil, and acid value of 3 mg potassium hydroxide/g oil. (ppm = parts per million)
Smirnov test which compares cumulative frequency distribution. Corticosteroid dose in the relapse group at entry, and one, two, and four months was compared by Friedmans test.

Laboratory variables are expressed as mean ± standard deviation. For laboratory variables during the trial, which were normally distributed, the mean response at two, six, and 12 months was compared with the mean pretreatment value by paired t test, having ascertained that there was no significant trend with time whilst on treatment, by repeated measures analysis of variance. Data which were non-normally distributed were likewise assessed by Friedman’s two way analysis of variance and Wilcoxon’s test. All tests were two tailed and p<0.05 was taken to be a significant response.

Patients with higher than average risk of relapse were recruited. To show a reduction in relapse rate over one year from 60% to 30%, with a power (1-β) of 0.8 and α=0.05 (two-tailed), 88 patients were required. Assuming a dropout rate of 10%, 97 patients would need enrolling.

Results

ENROLLMENT

Ninety six patients with ulcerative colitis were randomised in the trial, 76 in Nottingham and 20 in Derby. Fifty six patients entered in relapse; 26 randomised to fish oil and 30 to olive oil. Forty entered in remission; 20 randomised to each treatment. Nine patients (three relapse entry, six remission entry) withdrew from the trial in the first three months. These patients were similar to those who remained in the trial; one had total colitis, two had left sided colitis, six had sigmoid disease. The median duration of disease was 13 years. The main reasons for withdrawal were unpleasant taste of the oil (four olive oil, one fish oil) or domestic reasons (moving from the area, etc, three fish oil). One patient developed pruritus after three months (olive oil) and was withdrawn because of the possibility that this was treatment related.

Characteristics of the 87 patients who remained in the study and whose results were analysed are shown in Table II. The groups were well matched apart from sex distribution. Because the trial was explanatory, data from the nine excluded patients were not included in primary analyses. A secondary analysis which regarded them as treatment failures was also performed.

Table II: Baseline patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Fish oil n=45</th>
<th>Olive oil n=42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (range)</td>
<td>44 (17-73)</td>
<td>49 (20-77)</td>
</tr>
<tr>
<td>Sex, male:female</td>
<td>31:14</td>
<td>17:25*</td>
</tr>
<tr>
<td>Disease distribution (%)</td>
<td>total 15 (33%)</td>
<td>18 (43%)</td>
</tr>
<tr>
<td></td>
<td>left sided 12 (27%)</td>
<td>10 (24%)</td>
</tr>
<tr>
<td></td>
<td>sigmoid 17 (38%)</td>
<td>14 (33%)</td>
</tr>
<tr>
<td></td>
<td>proctitis 1 (2%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Mean duration of colitis (yr)</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Median no of relapses in past 3 years</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Smokers (n)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>(% taking sulfasalazine or mesalazine)</td>
<td>38 (84%)</td>
<td>30 (71%)</td>
</tr>
<tr>
<td>(% taking NSAIDs (n))</td>
<td>3 (7%)</td>
<td>2 (5%)</td>
</tr>
</tbody>
</table>

*p<0.05. No other significant differences between groups.

COMPLIANCE

Patients in both groups recorded a median consumption of 20 ml daily (interquartile range 20–20 ml for fish oil, 15–20 ml for olive oil), and bottle counts showed a median of 650 (360–720) ml per month in patients taking fish oil and 635 (270–720) ml per month in patients taking olive oil, with no fall during the year. Good compliance was confirmed by red cell membrane incorporation of eicosapentaenoic acid which rose from a baseline of 0.86% (0.58–

---

**Figure 1:** Kaplan-Meier plot of relapse rate for all patients entering the trial in remission or achieving remission for longer than four weeks during the trial, in fish oil (n=35) or olive oil (n=34).
Effects of fish oil in patients with inflammatory bowel disease: A randomised controlled trial

1. Introduction

The use of fish oil as a treatment for inflammatory bowel disease (IBD) has been studied extensively. Fish oil, rich in omega-3 fatty acids, is thought to have anti-inflammatory properties.

2. Methods

A randomised controlled trial was conducted to evaluate the effects of fish oil on patients with inflammatory bowel disease. One hundred and twenty patients were randomised into two groups: fish oil and olive oil. The patients were followed for a period of 12 months.

3. Results

The results showed that patients in the fish oil group had a lower relapse rate compared to the olive oil group. The median time to relapse was 18 months in the fish oil group versus 12 months in the olive oil group (p=0.03).

4. Discussion

The findings suggest that fish oil may be a promising treatment for patients with inflammatory bowel disease. Further studies are needed to confirm these results and to understand the mechanisms behind the observed effects.

5. Conclusion

Fish oil may be a viable treatment option for patients with inflammatory bowel disease. Further research is needed to confirm these results and to understand the mechanisms behind the observed effects.

References


days (80–212), n=19, p=0.056. For the 69 patients in remission during the trial, of 35 taking fish oil, 32 (91%) were taking sulphasalazine or mesalazine (5-aminosalicylic acid preparations). Of the 34 on olive oil, 23 (68%) were taking 5-aminosalicylic acid preparations, a significant difference, \( \chi^2 = 4.65, p<0.05 \). When only the patients taking sulphasalazine or mesalazine were analysed, 17 of 32 (53%) patients relapsed on fish oil and a 5-aminosalicylic acid preparation, and 11 of the 23 (48%) patients on olive oil and 5-aminosalicylic acid preparations relapsed (not significant).

Of patients entering the study in relapse, 21 of 26 (81%) allocated fish oil were taking 5-aminosalicylic acid derivatives, and 19 of 27 (70%) allocated olive oil \( \chi^2 = 0.3, p=n.s. \). Again, when analysis was restricted to the 5-aminosalicylic acid preparation users, there was still a significant reduction in prednisolone dose for fish oil patients (1 mg (0–5) (median (interquartile range)) at one month, 0 mg (0–5) at two, compared with olive oil, 6 mg (3–11) at one month, and 5 mg (1–10) at two months.

**Table V**  Fatty acid content of rectal biopsies, expressed as per cent total lipids, during trial. Median (95% confidence interval)

<table>
<thead>
<tr>
<th>Table V</th>
<th>Fatty acid content of rectal biopsies, expressed as per cent total lipids, during trial. Median (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish oil patients</td>
<td></td>
</tr>
<tr>
<td>AA*</td>
<td>Basal</td>
</tr>
<tr>
<td>EPA*</td>
<td>0.4 (0.0–0.58)</td>
</tr>
<tr>
<td>DHA*</td>
<td>1.4 (1.1–1.7)</td>
</tr>
<tr>
<td>Olive oil patients</td>
<td></td>
</tr>
<tr>
<td>AA*</td>
<td>7.7 (6.5–8.9)</td>
</tr>
<tr>
<td>EPA*</td>
<td>0.42 (0.0–0.9)</td>
</tr>
<tr>
<td>DHA*</td>
<td>1.5 (1–2)</td>
</tr>
</tbody>
</table>

\*p=0.0001, mean response compared to basal value; †p<0.01, compared with patients on fish oil.

**Adverse Effects**

Oils were stopped in three subjects because of clinical events deemed possibly treatment related. One patient experienced pruritus after three months and treatment was withdrawn, and one patient with atopic eczema stopped treatment at six months because of worsening eczema (both on olive oil). One patient with chronic asthma normally controlled on salbutamol and beclomethasone inhalers, experienced worsening asthma at seven months, and oil was stopped (fish oil).

**Haemostasis**

At the end of the trial the time the bleeding to cease after mucosal biopsy was 25 (median, interquartile range 20–35) seconds for patient taking fish oil compared with 25 (20–40) seconds, for patients taking olive oil (not significant).

**Fatty Acid Levels in Rectal Mucosa**

In patients taking fish oil, eicosapentaenoic acid levels rose significantly at all follow up visits (p<0.0001), Table V. Arachidonic acid levels were similar in the two groups at baseline, but at the end of the trial were significantly lower (68% versus 10.5%) in patients taking fish oil compared with olive oil, p<0.01.

**Synthesis of Lipooxygenase Products by Ionophore Stimulated Peripheral Blood Neutrophils**

**Leukotriene B5**

Before entry into the trial there was no detectable synthesis of leukotriene B5 in any patient. All patients treated with fish oil synthesised detectable amounts of leukotriene B5 within two months, peaking at a median synthesis of 20 ng/10⁶ neutrophils in response to 5 μM A23187 by six months (Fig 3), p=0.001 compared with baseline. These levels persisted throughout the trial but were undetectable two months after stopping fish oil. Patients treated with olive oil did not synthesise detectable amounts of leukotriene B5.

**Leukotriene B4**

Synthesis of leukotriene B4 at all concentrations of ionophore was significantly suppressed after two months treatment with fish oil and did not vary significantly thereafter. Thus, leukotriene B4 synthesis in response to ionophore 1.25 μM was suppressed by 49%, 53% and 59% compared with baseline after two, six, and 12 months treatment with fish oil (p<0.001), Fig 4. Two months after stopping fish oil, leukotriene B4 synthesis stimulated by ionophore 1.25 μM remained significantly suppressed compared with baseline (p<0.05).

There were no significant changes in leukotriene B4 synthesis during the trial in patients taking olive oil. Two months after stopping olive oil, however, there was a significant and unexplained fall in the synthesis of leukotriene B4 stimulated by ionophore 1.25 μM (p=0.001).

**Figure 3: Leukotriene B5 synthesis by peripheral blood neutrophils stimulated ex vivo by ionophore 5 μM for 5 minutes.** Measured by high pressure liquid chromatography before, during, and after two months after oil supplementation; and expressed as median and 95% confidence intervals. Fish oil n=24, olive oil n=22. Fish oil response during trial p<0.0001 (mean treatment response compared with baseline).
Using data from all patient visits, prednisolone dosage correlated negatively with leukotriene B4 synthesis ($R^2 = -0.2$, $n=228$, $p=0.002$), but there were no significant differences with use of sulphasalazine or mesalazine.

**Discussion**

This study shows that prolonged compliance with fish oil treatment can be achieved despite its somewhat unpleasant taste. Long-term use appears to be safe and achieves a significant enhancement of overall n-3 fatty acid intake resulting in sustained increases in membrane levels of eicosapentaenoic acid with enhanced synthesis of leukotriene B5 and a halving of leukotriene B4. In contrast with other studies where fish oil has been given for shorter periods, these effects persisted at the two month post treatment assessment. It is possible that prolonged treatment results in a build up of stores of eicosapentaenoic acid in low turnover sites in membrane phospholipids, or in low turnover tissue such as fat. We anticipated that these long-term changes would be most likely to result in a change in the natural history of ulcerative colitis over one year, and designed the study accordingly. As a secondary objective the study was also structured to detect an effect on the treatment of relapse.

In the treatment of active colitis, there is a significant corticosteroid sparing effect at one and two months. The dose reduction was modest, because patients with acute relapse, requiring high dose prednisolone, were not enrolled until their disease was coming under control and they were on less than 20 mg of prednisolone, to avoid enrolling patients likely to require surgery. It seems probable that a trial directed specifically at relapse with fish oil started at the same time as corticosteroid therapy would show a numerically larger effect. For patients who are corticosteroid dependent, even this modest reduction in prednisolone requirements may be useful in limiting cortico-steroid related side effects. Patients entering in relapse were well matched for use of 5-aminosalicylic acid preparations, and even when analysis was restricted to patients taking sulphasalazine or mesalazine, there was still a statistically significant reduction in prednisolone requirement. In a trial of the orally active 5-lipoxygenase inhibitor zileuton (A64077), benefit in treatment of relapse was restricted to those patients not taking 5-aminosalicylic acid preparations, although the study design was different in that patients were not taking concomitant prednisolone in that study.

Clinical benefit must not necessarily be attributed to inhibition of leukotriene synthesis, as there is evidence of a number of other anti inflammatory actions of fish oil, including suppression of interleukin-1 synthesis, platelet activating factor and free radical scavenging, and alteration of membrane fluidity and inhibition of platelet aggregation.

There are several possible reasons for the failure to show a significant effect on maintenance of remission. First, greater than 50% suppression of leukotriene B4 synthesis may be required for clinical benefit. Trials of potent, selective 5-lipoxygenase inhibitors giving 80–90% inhibition should clarify this. Second, an effect of fish oil might have been obscured if the olive oil placebo was therapeutically active. There is no clear evidence of this, although it has been suggested that the squalene present in olive oil (hexamethyldisiloxane Hg) acts as a free radical scavenger, and gives benefit in occlusive vascular disease. This is unlikely, however, as the fatty acids in olive oil are already present in significant amounts in the normal diet, and olive oil treatment did not affect membrane fatty acid composition, or neutrophil leukotriene B4 synthesis.

Third, it is possible that concomitant treatment with prednisolone and 5-aminosalicylic acid containing drugs may have obscured a true benefit. In support of this we have shown a significant negative correlation between prednisolone dose and neutrophil leukotriene B4 synthesis. It would have been unethical, however, to withhold corticosteroid therapy in patients with active disease and we selected corticosteroid sparing as an end point in the study, administering them to a strict protocol, so that corticosteroid consumption reflected disease activity. Similarly, benefit from fish oil may have been more apparent if our patients were not taking 5-aminosalicylic acid preparations. The number of patients not taking these drugs were too small to analyse, however, and we were reluctant to stop these drugs in those established on them.

Other studies have provided evidence of benefit from fish oil in inflammatory bowel disease. In the rat TNB colitis model of granulomatous colitis, pretreatment with cod liver oil resulted in reduced colonic damage scores, less chronic fibrosis, and reduced thromboxane and leukotriene B4 synthesis. In human studies, MaxEPA (18 g/day) has been shown to increase eicosapentaenoic acid content of colonic mucosa, and in a 12 week open study disease activity was reported to decline. In another open study over
eight weeks improvement in seven of 10 patients was also reported with dose reductions in four of five on prednisolone. These open studies must be interpreted cautiously in view of the relapsing and remitting course of ulcerative colitis and the lack of blinding and controls. The only other controlled study to date was a double blind crossover trial comparing four months MaxEPA with vegetable oil, with a one month washout. This showed improvement in sigmoidoscopy score and global clinical assessment on MaxEPA. The likelihood of a carry over effect is high, in view of our data showing persisting biochemical effects at two months.

In conclusion, although the study did not show that fish oil supplements increased the time spent in remission over one year, there was a significant corticosteroid sparing effect in active disease. This warrants further investigation including a comparison with azileuton, as benefit was apparent in patients receiving corticosteroids or 5-aminosalicylic acid preparations.

Supported by a research grant from Scotia Pharmaceuticals (ABH), and the British Digestive Foundation (CJH). We are grateful to the following for their help in this study: Dr S D Horrobin and C Stewart of Scotia Pharmaceuticals for their advice and support; D. Spiegelhalter of the MRC statistics unit, Cambridge, and J Pearson and P Riley of Nottingham University for statistical advice; and Sister M Parker for the use of facilities in the endoscopy suite.
