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# Eicosapentaenoic acid reduces rectal polyp number and size in familial adenomatous polyposis

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## ABSTRACT

**Objective** The omega-3 polyunsaturated fatty acid eicosapentaenoic acid (EPA) has anticancer activity in vitro and in preclinical models. The present study tested whether a novel, enteric-coated formulation of EPA, as the free fatty acid (EPA-FFA), has chemopreventative efficacy in patients with familial adenomatous polyposis (FAP), in a randomised, double-blind, placebo-controlled trial.

**Methods** Patients undergoing endoscopic surveillance of their retained rectum postcolectomy were randomised to EPA-FFA (SLA Pharma) 2 g daily or placebo for 6 months. The number and size of polyps in an area of mucosa defined by a tattoo were determined before and after intervention. Global rectal polyp burden was scored (−1, 0, +1) by examination of video endoscopy records. Mucosal fatty acid content was measured by gas chromatography—mass spectrometry.

**Results** 55 patients with FAP were evaluated by an intention-to-treat analysis (EPA-FFA 28, placebo 27). Treatment with EPA-FFA for 6 months was associated with a mean 22.4% (95% CI 5.1% to 39.6%) reduction in polyp number ( $p=0.012$ ) and a 29.8% (3.6% to 56.1%) decrease in the sum of polyp diameters ( $p=0.027$ ). Global polyp burden worsened over 6 months in the placebo group (−0.34) unlike the EPA-FFA group (+0.09, difference 0.42 (0.10–0.75),  $p=0.011$ ). EPA-FFA treatment led to a mean 2.6-fold increase in mucosal EPA levels ( $p=0.018$  compared with placebo). EPA-FFA was well tolerated with an incidence of adverse events similar to placebo.

**Conclusions** EPA-FFA has chemopreventative efficacy in FAP, to a degree similar to that previously observed with selective cyclo-oxygenase-2 inhibitors. EPA holds promise as a colorectal cancer chemoprevention agent with a favourable safety profile.

**Clinical trial number** NCT00510692.

## INTRODUCTION

Familial adenomatous polyposis (FAP) is an autosomal dominant predisposition to colorectal cancer (CRC) caused by a germline mutation in the *adenomatous polyposis coli* gene.<sup>1</sup> Prophylactic removal of the colon is recommended in order to prevent CRC. There are two main surgical options, namely colectomy with ileorectal anastomosis or proctocolectomy with ileal pouch—anal anastomosis.<sup>2</sup> Colectomy with ileorectal anastomosis is usually advised in young patients with FAP in order to remove the risk of colon cancer whilst maximising the chances of a good functional outcome.<sup>2</sup> However, following ileorectal anastomosis, the rectal remnant remains vulnerable to development

## Significance of this study

### What is already known about this subject?

- ▶ Patients with familial adenomatous polyposis (FAP) with an ileorectal anastomosis require endoscopic surveillance of rectal polyps to minimise risk of rectal cancer.
- ▶ The selective cyclo-oxygenase-2 inhibitor celecoxib is licensed for use as an adjunct to endoscopic surveillance in FAP but has significant cardiovascular toxicity.
- ▶ There is a need for an alternative chemoprevention agent that is safe and well tolerated.
- ▶ The omega-3 polyunsaturated fatty acid eicosapentaenoic acid (EPA) has chemopreventative activity in preclinical colorectal cancer models and has an excellent safety profile.

### What are the new findings?

- ▶ Enteric-coated EPA in the free fatty acid form (EPA-FFA) for 6 months significantly reduced rectal polyp number and size in patients with FAP, as well as decreasing the global rectal polyp burden, compared with placebo.
- ▶ The effect of EPA-FFA was similar in magnitude to that observed with celecoxib.
- ▶ Enteric-coated EPA-FFA was safe and well tolerated.

### How might it impact on clinical practice in the foreseeable future?

- ▶ EPA-FFA should be considered for chemoprevention in patients with FAP.
- ▶ Potential chemopreventative efficacy of EPA-FFA against 'sporadic' colorectal neoplasia warrants investigation.

of multiple adenomas, leading to rectal cancer. Therefore, patients with FAP with a rectal remnant undergo regular endoscopic surveillance.<sup>2 3</sup>

Previous randomised placebo-controlled trials have demonstrated that the non-steroidal anti-inflammatory drug (NSAID) sulindac and the selective cyclo-oxygenase (COX)-2 inhibitors celecoxib and rofecoxib have chemopreventative efficacy in FAP.<sup>4–6</sup> Therefore, in principle, secondary polyp chemoprevention can be combined with endoscopic surveillance in order to reduce cancer risk in patients with FAP. To this end, celecoxib is currently licensed by the US Food and Drug Administration (FDA) and in the European Union as an adjunct to usual management in FAP. However, long-term use of COX-2 inhibitors is

associated with significant cardiovascular toxicity in older age groups<sup>7</sup> and it remains unclear whether celecoxib treatment is safe in younger individuals. Therefore, there is a need for an alternative chemoprevention agent in FAP that is safe and well tolerated.

Omega ( $\omega$ )-3 polyunsaturated fatty acids (PUFAs) are found predominantly in cold-water fish such as salmon and mackerel.<sup>8</sup> There is strong preclinical evidence for anti-CRC activity of  $\omega$ -3 PUFAs,<sup>9</sup> although systematic review of epidemiological studies has not demonstrated unequivocal benefit from dietary marine  $\omega$ -3 PUFA intake on CRC risk.<sup>10</sup> One possible explanation is that regular fish consumption (2–3 times per week) provides only moderate  $\omega$ -3 PUFA exposure ( $\sim$ 500 mg per day of the two main  $\omega$ -3 PUFAs 20:5 $\omega$ 3 eicosapentaenoic acid (EPA) and 22:6 $\omega$ 3 docosahexaenoic acid (DHA), combined).<sup>11</sup> Dietary  $\omega$ -3 PUFA intake can be increased by fish oil supplements, but many of these have a range of minor side effects such as dyspepsia and halitosis. In addition,  $\omega$ -3 PUFAs are relatively poorly absorbed in the usual ethyl ester or triglyceride form.<sup>12</sup>

A 500 mg enteric-coated formulation of EPA as the free fatty acid (EPA-FFA), which is released and absorbed maximally in the small intestine minimising gastrointestinal (GI) side effects, has been developed for administration of large amounts of EPA. EPA-FFA (5% (w/w) in chow) has recently been demonstrated to reduce intestinal adenoma number by 79% in the *Apc<sup>Min/+</sup>* mouse model of FAP,<sup>13</sup> building on previous reports of the chemopreventative efficacy of dietary  $\omega$ -3 PUFAs in this well-established murine model<sup>14 15</sup> and on carcinogen-induced carcinogenesis in the rat.<sup>16</sup> Moreover, in a small Phase II study in patients with a history of sporadic colorectal adenoma, treatment with EPA-FFA 2 g per day for 3 months was safe, well tolerated and associated with reduced rectal epithelial cell proliferation and increased epithelial cell apoptosis.<sup>17</sup>

On the basis of this evidence, we performed a double-blind, randomised, placebo-controlled trial of the effect of treatment with enteric-coated EPA-FFA 2 g daily for 6 months on rectal polyps in patients with FAP with an ileorectal anastomosis, in order to test the hypothesis that EPA-FFA has chemopreventative activity in FAP.

## METHODS

The study (CTA number 18045/0208/001 0001; <http://ClinicalTrials.gov> number NCT00510692) was conducted according to ICH Good Clinical Practice and complied with the principles of the amended Declaration of Helsinki, in addition to the US FDA Code of Federal Regulations. The study sponsor was SLA Pharma (UK). Ethical approval for the study was obtained from the Wandsworth Local Research Ethics Committee. All study participants provided written informed consent.

### Study participants

Patients with FAP,  $\geq$ 18 years of age, who had previously undergone colectomy with ileorectal anastomosis were recruited through the Polyposis Registry, St Mark's Hospital, London by NJW and SKC, between December 2006 and September 2007.

Eligible subjects had an endoscopically assessable rectal remnant with three or more polyps  $\geq$ 2 mm diameter present at previous flexible sigmoidoscopy. Subjects were required to abstain from regular use of NSAIDs for the duration of the study. Use of regular low-dose aspirin was permitted.

Subjects were excluded if they had undergone colectomy in the last 12 months, were allergic to fish, suffered from a haemorrhagic disorder, were receiving oral anticoagulant

therapy, were taking a statin or had hyperlipidaemia likely to require drug therapy, were already taking any fish oil or  $\omega$ -3 PUFA supplement, or had any significant abnormality in standard haematological and biochemical parameters (see below).

### Study procedures

Prior to endoscopy, a physical examination was performed and blood was drawn for baseline measurement of standard haematological and biochemical parameters (full blood count, urea and electrolytes, liver function tests, coagulation profile and lipids). Female participants underwent a urinary  $\beta$ -human chorionic gonadotrophin pregnancy test. Phosphate enema bowel preparation was followed by routine flexible sigmoidoscopy except that an area of rectal mucosa with at least three polyps,  $\geq$ 2 mm in diameter, was identified and a reference tattoo made with 1 ml of sterile ink (Spot Endoscopic Marker; GI Supply, Camp Hill, PA, USA). An endoscope (Olympus CF200S, Olympus KeyMed, Southend-on-Sea, Essex, UK) was advanced to the ileorectal anastomosis and then withdrawn in a spiral manner to the anal verge with continuous video recording with biopsy forceps (2.4 mm (closed), Boston Scientific Natick, MA, USA) advanced to provide a reference image of known size. Three rectal biopsies were taken from macroscopically normal rectal mucosa for fatty acid analysis and snap-frozen in liquid N<sub>2</sub>, for storage at  $-70^{\circ}\text{C}$ .

On completion of baseline endoscopy, subjects were randomised (1:1) to either two enteric-coated, EPA-FFA 500 mg, soft-gel capsules, twice daily (ALFA; SLA Pharma AG, Liestal, Switzerland) or two identical placebo capsules (capric and caprylic acid medium-chain triglycerides; Sasol, Witten, Germany<sup>18</sup>) 500 mg twice daily for 6 months, to be taken with food. A computer-generated randomisation schedule was used to assign sequentially numbered treatment packs, which were supplied in randomised blocks of four (two EPA-FFA, two placebo) by DHP Pharma (Cricklehowell, Powys, UK). All subjects and investigators were blinded to treatment allocation.

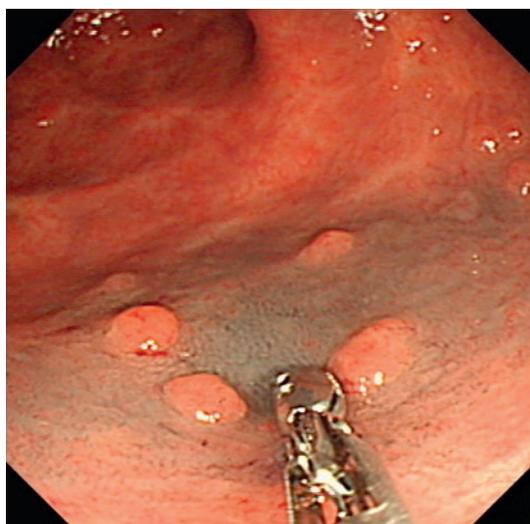
All study participants were provided with a study diary in order to record daily dosage of study treatment and adverse events (AEs). Subjects were also contacted by telephone bi-weekly during the first 4 weeks and monthly thereafter in order to check on compliance and to enquire about AEs.

At 6 months, study participants underwent a repeat physical examination and blood investigations. Subjects returned unused capsules together with the symptom diary. Video endoscopy was performed in an identical manner to the baseline examination by the same operator (NJW), who was blinded to treatment allocation. Three rectal mucosal biopsies were obtained and stored as above.

Follow-up contact was made 1–2 months after exit endoscopy in order to enquire about AEs.

### Endoscopic measurements

A photograph of the tattooed area, with the biopsy forceps visible, was selected from the video endoscopy record of the baseline and 6 month flexible sigmoidoscopy by an independent reviewer (JMH), who was blinded to treatment allocation, by comparing images on dual monitors on a frame-by-frame basis (figure 1). The number of polyps in each photograph was measured by two independent observers (RJL, AB). Individual polyp diameters were measured by consensus of the two observers using digital, Vernier callipers (Sealey AK962EV Braintree, Essex, UK) and reported as the mean of two diameters at  $90^{\circ}$  to each other, using a magnification correction factor derived from the known size of the forceps in frame.



**Figure 1** Endoscopic evaluation of the rectum. A representative image of a tattooed area of rectal mucosa containing rectal adenomas. Closed forceps abutting the mucosal surface provided a size reference.

Video endoscopy recordings of the baseline and 6 month examination for each subject were reviewed separately by five independent endoscopists, blinded to treatment allocation and the timing of the endoscopy recording. The change in global rectal polyp burden between paired video records (6 months to baseline) was scored as 'better' (+1), 'same as' (0) or 'worse' (-1). Mean scores were calculated for each subject.

### Mucosal fatty acid analysis

Homogenisation, extraction and derivatisation of rectal mucosal fatty acids (EPA, DHA, docosapentaenoic acid (DPA), arachidonic acid (AA), linolenic acid, linoleic acid, oleic acid, palmitic acid and stearic acid) were performed as described.<sup>17 18</sup> Fatty acid content was determined by gas chromatography–mass spectrometry and expressed as the percentage of the total fatty acid content.<sup>19 20</sup>

### Statistical analysis

The primary end point of the study was the number of polyps in the photograph of the tattooed area of rectum. Secondary end points were the sum of the polyp diameters in the photographed area, the global rectal polyp burden score and the mucosal fatty acid content.

A sample size of 46 patients (23 per treatment group) was estimated to be necessary to detect a difference in the change in primary end point from baseline to 6 months between the treatment groups of 3 with SD 3.5 (based on a baseline polyp number of 13 and a 23% reduction in rectal polyp number in the celecoxib 800 mg daily group in a similar study by Steinbach and colleagues<sup>5</sup>), at a 5% level of significance with 80% power. A priori, it was planned to randomise a minimum of 50 patients (25 per treatment group) in order to allow for a maximum 8% drop-out rate.<sup>17</sup>

All statistical analyses were performed on an intention-to-treat principle. The efficacy analysis set included all subjects who were randomised, took at least one dose of study medication and for whom a baseline and at least one on-treatment efficacy end point was available. The safety and tolerability analysis set included all subjects who took at least one dose of study treatment.

Treatment group differences are presented as the mean and corresponding 95% CI. The change from baseline to 6 months was analysed using analysis of covariance (ANCOVA). Treat-

ment group was the independent factor in the model and the baseline number of polyps was a covariate. Least square means (adjusted for the baseline polyp number) for each treatment group were determined and the difference in the least square means was calculated along with the corresponding 95% CI and p value. Gender and age (both continuous and categorical (<40 or ≥40 years)) were also added as covariates.

## RESULTS

### Study flow and subject characteristics

The flow of patients through the study is described in a CONSORT diagram (figure 2). Of 63 patients with FAP screened for eligibility, 58 patients were randomised (placebo 29; EPA-FFA 29) and 55 subjects were included in the efficacy analyses (placebo 27; EPA-FFA 28). Two subjects in the placebo group withdrew prior to endoscopy at 6 months (see 'Compliance and adverse events') and one subject in the EPA-FFA group failed to attend for the final study visit.

The two treatment groups had similar characteristics (table 1). The broad age range within each treatment group was as expected for this Polyposis Registry. The majority of subjects were white Caucasian, with two black (one in each treatment group) and two Asian subjects (both in the placebo group). Only two subjects, both in the placebo group, were taking low-dose (75 mg daily) aspirin.

The time since colectomy varied considerably, consistent with the wide age range of the study participants (table 1), but was similar between treatment groups. The lengths of the retained rectal remnant were also well matched between the placebo and EPA-FFA group (table 1).

### Treatment with EPA-FFA reduces rectal polyp number and size compared with placebo

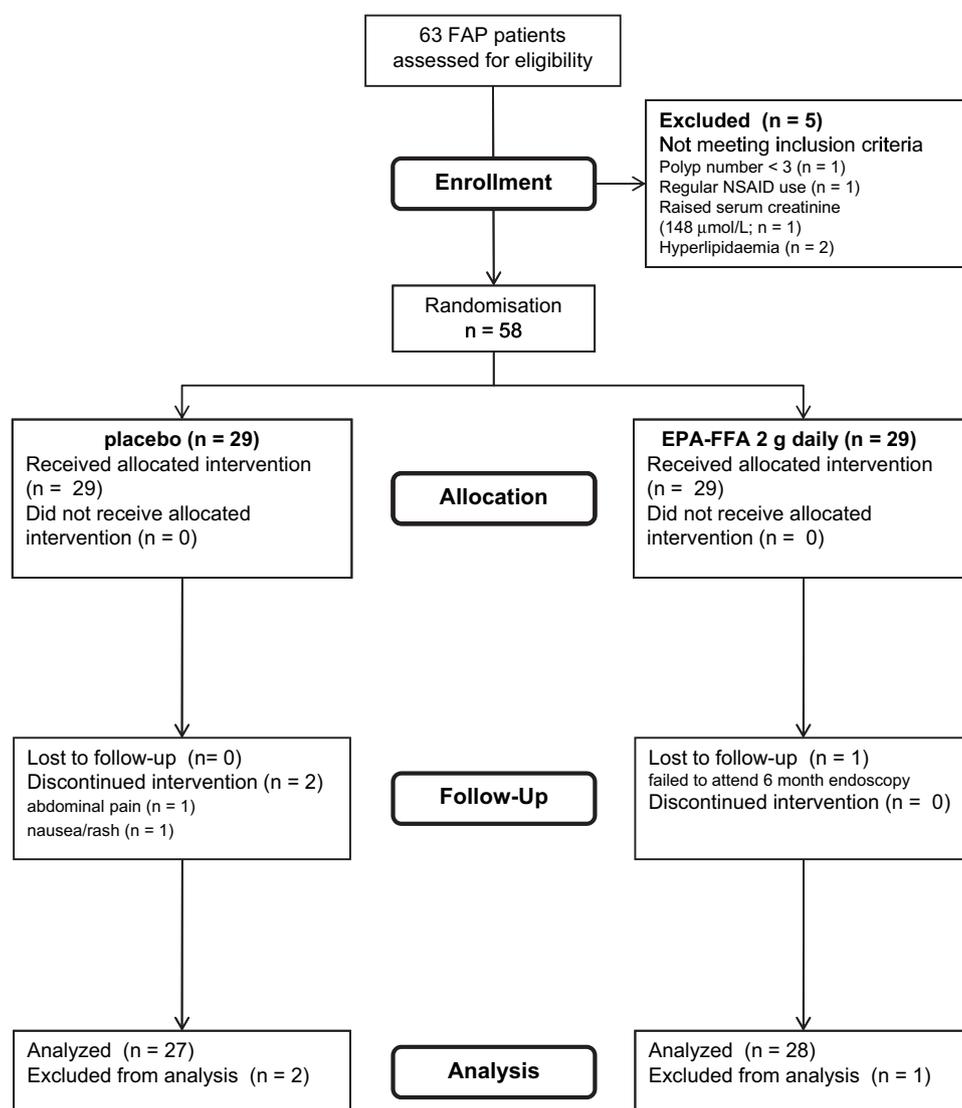
Both treatment groups had a similar mean number of polyps at baseline flexible sigmoidoscopy (table 2). However, after the 6 month intervention period, the mean number of polyps in the same mucosal field increased to 5.05 (a 9.7% increase) in the placebo group, whereas mean polyp number in the EPA-FFA group decreased to 3.61 (a 12.4% decrease). The difference between the change in polyp number between the EPA-FFA and placebo groups was -1.06 (-1.78 to -0.35; p=0.005), which represents a 22.4% (5.1% to 39.6%) decrease in polyp number in subjects taking EPA-FFA compared with placebo (p=0.012). The difference between the change in polyp number during the 6 month treatment period remained significant after inclusion of gender and age as additional covariates.

EPA-FFA treatment was also associated with a decrease in polyp size such that the sum of polyp diameters increased by 17.2% during 6 months treatment with placebo, whereas the sum of polyp diameters decreased by 12.6% in the EPA-FFA treatment group. This represents a 29.8% (3.6% to 56.1%) overall decrease in polyp size in the EPA-FFA treatment group compared with the placebo group (p=0.027).

### Treatment with EPA-FFA reduces the global rectal polyp burden score compared with placebo

In addition to individual polyp measurements in a carefully defined area of rectal mucosa, we also assessed whether there was evidence of widespread changes in polyp burden throughout the rectal mucosa by examination of complete video endoscopy records. Consistent with the polyp number and size data, the video review panel scores demonstrated that there was a 'worsening' (ie, increased rectal polyp burden after 6 months in the placebo group; table 2). In contrast, there was a modest

**Figure 2** CONSORT study flow diagram. EPA-FFA, eicosapentaenoic acid as the free fatty acid; FAP, familial adenomatous polyposis; NSAID, non-steroidal anti-inflammatory drug.



improvement evident in the EPA-FFA group (table 2). The difference in change in rectal polyp burden between the EPA-FFA group and placebo group was statistically significant ( $p=0.011$ ).

**Table 1** Subject characteristics

		Treatment group	
		Placebo (n=27)	EPA-FFA (n=28)
Age at study entry (years)	Mean (SD)	42.5 (13.8)	39.5 (11.4)
	Range	18.4–73.6	18.3–64.7
Gender	Male n (%)	15 (55.6)	13 (46.4)
	Female n (%)	12 (44.4)	15 (53.6)
History of tobacco use	Never smoked n (%)	16 (59.3)	15 (53.6)
	Current smoker n (%)	4 (14.8)	5 (17.9)
	Former smoker n (%)	7 (25.9)	8 (28.6)
Time since colectomy (years)	Mean (SD)	15.4 (9.3)*	15.9 (9.6)
	Range	1.0–29.7	1.0–42.5
Length of rectal remnant† (cm)	Mean (SD)	20.6 (4.0)	20.4 (5.7)
History of other surgery‡	n (%)	18 (66.6)	11 (39.3)
Dyspepsia at baseline	n (%)	1 (3.7)	4 (14.3)

\*Not recorded in two cases.

†Measured at baseline endoscopy from the ileorectal anastomosis to the anal verge.

‡Surgery other than colectomy including appendectomy, cholecystectomy, hernia repair, pancreaticoduodenectomy, division of adhesions, small bowel resection, oophorectomy and sterilisation.

EPA-FFA, eicosapentaenoic acid as the free fatty acid.

### Mucosal fatty acid content

There was a significant increase in rectal mucosal EPA and DPA content after EPA-FFA treatment compared with placebo (table 3). Although the mean percentage EPA content increased from 0.73 to 1.30% in the placebo group, there was a rise from 0.97 to 2.50% in the EPA-FFA group, which represents a 2.6-fold increase in mucosal EPA content that was significantly different from placebo ( $p=0.018$ ). Similarly, there was a 1.8-fold increase in mucosal DPA content in the EPA-FFA group, which was significantly higher ( $p=0.010$ ) than the change observed in placebo-treated individuals (table 3). However, the mean mucosal AA content did not change significantly during EPA-FFA treatment and there was no significant difference in mucosal AA levels compared with the placebo group (table 3). There was no significant difference in the mucosal content of the other fatty acids that were analysed (data not shown).

### Compliance and adverse events

Compliance based on counting of unused capsules was similar in both treatment groups. The mean number of days exposure to placebo was 169 (SD 42) compared with 167 (45) days in the EPA-FFA group. This level of EPA-FFA exposure was equivalent to a mean daily dose of 1.78 (0.38) g of EPA-FFA.

AEs were analysed for all randomised study subjects ( $n=58$ ). There were two withdrawals in the placebo group due to

**Table 2** Endoscopic polyp measurements

	Placebo			EPA-FFA		
	BL	6 m	Δ (6 months–BL) (95% CI)	BL	6 m	Δ (6 months–BL) (95% CI)
No. polyps* (mean±SD)	4.50±2.63	5.05±3.30	0.54 (0.03 to 1.05)	4.13±2.47	3.61±2.31	–0.52 (–1.02 to –0.02)
Difference between groups (95% CI; p value)	–1.06 (–1.78 to –0.35) p=0.005					
% Change in polyp no.				9.7 (–2.6 to 22.0)		
Difference between groups† (95% CI; p value)	–22.4 (–39.6 to –5.1) p=0.012					
% Change in polyp diameter*				17.2 (–1.7 to 36.2)		
Difference between groups† (95% CI; p value)	–29.8 (–56.1 to –3.6) p=0.027					
Change in global rectal polyp burden‡				–0.34 (–0.56 to –0.11)		
Difference between groups (95% CI; p value)	0.42 (0.10 to 0.75) p=0.011			0.09 (–0.14 to 0.32)		

\*Evaluable subjects were 22 (placebo) and 23 (EPA-FFA) for polyp number; and 19 (placebo) and 21 (EPA-FFA) for polyp diameter.

†A negative difference between treatment groups indicates a decrease in the EPA-FFA group compared with the placebo group.

‡A negative value implies an increased global polyp burden. A positive value implies a decreased global polyp burden. Evaluable subjects were 25 in each of the placebo and EPA-FFA groups. BL, baseline; EPA-FFA, eicosapentaenoic acid as the free fatty acid.

abdominal pain (1) and nausea/rash (1). There was one withdrawal due to nausea and epigastric discomfort in the EPA-FFA group. The most commonly reported AEs were GI disorders (table 4).

Diarrhoea was reported by a similar number of individuals in both groups. The frequency of other AEs was similar in the two treatment groups, except for nausea which was reported by nine subjects taking EPA-FFA compared with three subjects in the placebo group (table 4).

Serious AEs were reported in three subjects in the EPA-FFA treatment group (acute bowel obstruction in two subjects, breast cancer in one subject). None of these was considered to be related to study treatment. No bleeding episodes were reported during the intervention period in either treatment group.

There were no remarkable changes in physical examination or haematological (including coagulation profile) and biochemical parameters during the study.

## DISCUSSION

This randomised, placebo-controlled trial has demonstrated that administration of EPA, as the free fatty acid, for 6 months has antineoplastic activity in patients with FAP compatible with activity as a secondary chemoprevention agent. Polyp number, polyp size and overall polyp burden all decreased significantly after treatment with EPA-FFA 2 g daily compared with the placebo group.

The relatively short intervention period and the presence of polyps at baseline, combined with similar observations in previous randomised controlled trials of COX inhibitors in patients with FAP<sup>4–6</sup> means that the observed antineoplastic activity is almost certainly a combination of regression of existing adenomas and prevention of de novo tumour growth. Previous randomised controlled trials of celecoxib and rofecoxib in FAP<sup>5, 6</sup> predicted eventual activity as secondary chemo-

prevention agents in patients with a history of ‘sporadic’ colorectal neoplasia.<sup>21–23</sup> Therefore, our data are also likely to have important implications for possible chemoprevention of ‘sporadic’ colorectal neoplasia, as well as for postcolectomy management of patients with FAP.

The close methodological similarities between our study and that of Steinbach *et al*<sup>5</sup> allow a tentative comparison of the efficacy of EPA-FFA and the selective COX-2 inhibitor celecoxib. Both studies measured polyp number in a defined area of mucosa (although the celecoxib study also included patients with FAP with colonic polyps) and used video assessment of whole endoscopy records after 6 months treatment. The sample size calculation for the present study was based on the previous celecoxib study, although, in the event, the baseline number of rectal polyps at baseline was considerably lower than the number of colonic and rectal polyps reported by Steinbach *et al*.<sup>5</sup> Figure 3 demonstrates the effect on rectal polyps of treatment with EPA-FFA 2 g daily or celecoxib 800 mg daily compared with its respective placebo comparator. Although there are differences in the changes that were observed in the placebo arm of these two studies, the net differences in polyp number and global polyp burden compared with placebo seen with these two agents are remarkably similar. A prospective, within-study comparison would be required in order to determine the relative efficacies of these two agents in FAP.

Management of duodenal polyposis in patients with FAP is often challenging and celecoxib has previously been suggested for treatment of severe duodenal polyposis.<sup>2, 24</sup> Efficacy of EPA-FFA against rectal polyposis in FAP should now prompt evaluation of EPA-FFA for treatment of patients with duodenal adenomas.

An important aspect of our study was confirmation that oral administration of EPA-FFA is associated with increased rectal

**Table 3** Fatty acid content of rectal mucosa

Fatty acid	Placebo (n=26)			EPA-FFA (n=26)			Difference EPA-FFA–placebo (95% CI)	p Value
	Baseline (SD)	6 months (SD)	Change (95% CI)	Baseline (SD)	6 months (SD)	Change (95% CI)		
EPA	0.73* (0.72)	1.30 (1.09)	0.54 (–0.06 to 1.13)	0.97 (0.89)	2.50 (1.98)	1.56 (0.97 to 2.15)	1.03 (0.18 to 1.87)	0.018
DPA	0.75 (0.65)	0.76 (0.66)	0.04 (–0.20 to 0.28)	0.64 (1.15)	1.17 (0.69)	0.49 (0.25 to 0.73)	0.45 (0.11 to 0.79)	0.010
DHA	1.39 (0.61)	1.42 (1.05)	–0.11 (–0.46 to 0.24)	1.92 (1.30)	1.71 (0.94)	–0.07 (–0.42 to 0.28)	0.04 (–0.47 to 0.55)	0.875
AA	9.63 (2.18)	9.88 (1.86)	0.61 (–0.10 to 1.31)	8.47 (1.68)	8.82 (1.91)	–0.01 (–0.72 to 0.69)	–0.62 (–1.63 to 0.40)	0.228

\*Data are presented as the mean (%) of the total mucosal fatty acid pool.

AA, arachidonic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FFA, free fatty acid.

**Table 4** Adverse events (AEs)

	Placebo (n=29)		EPA-FFA (n=29)	
	n*	%†	n	%
Diarrhoea	10 (9)	34.5	9 (9)	31.0
Abdominal pain	6 (6)	20.7	9 (8)	27.6
Abdominal distension	5 (5)	17.2	3 (3)	10.3
Upper abdominal pain	5 (5)	17.2	6 (6)	20.7
Epigastric discomfort	5 (5)	17.2	3 (3)	10.3
Nausea	3 (3)	10.3	9 (9)	31.0
Headache	5 (5)	17.2	8 (8)	27.6
Dizziness	—	—	5 (5)	17.2
Malaise	5 (5)	17.2	3 (3)	10.3

\*n=number of AEs reported during the 6 month treatment period. The figure in parentheses is the number of subjects reporting a particular AE.

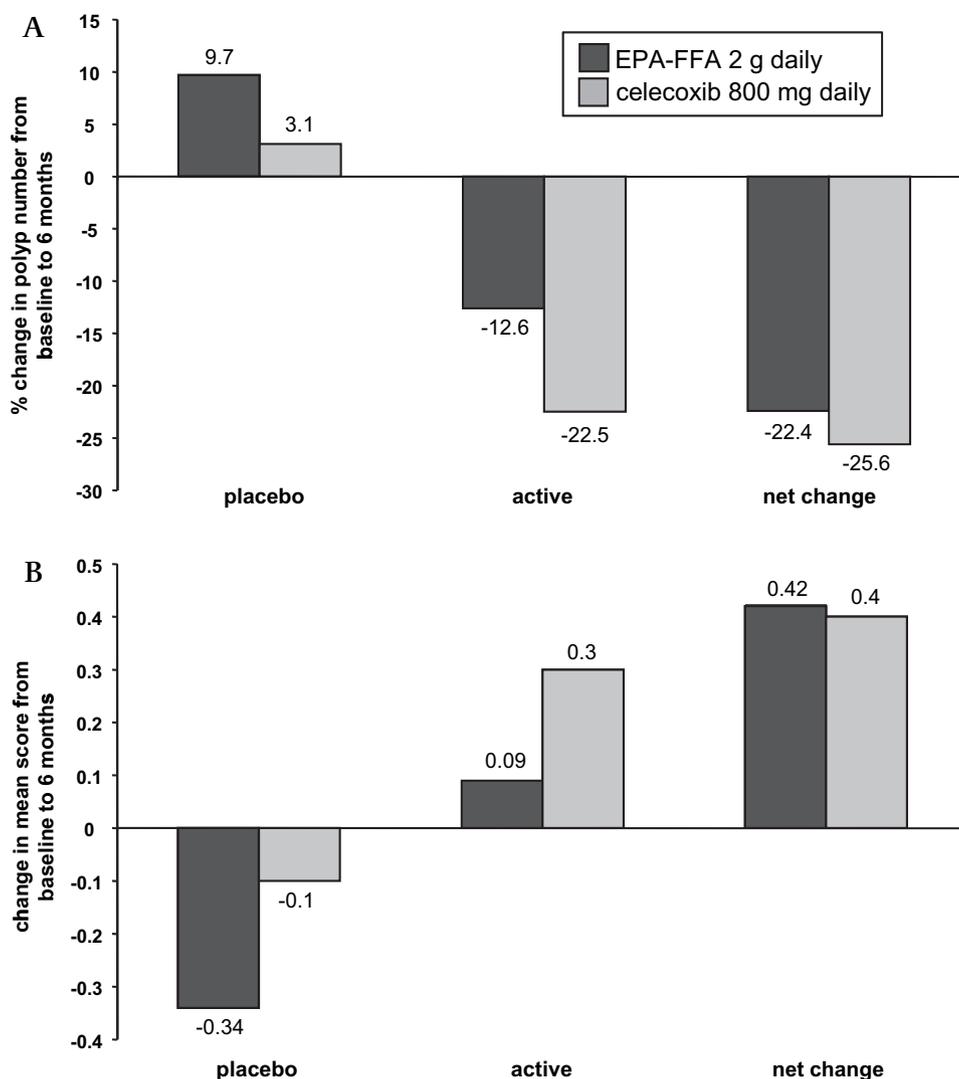
†The percentage of subjects in each group reporting a given AE.  
EPA-FFA, eicosapentaenoic acid as the free fatty acid.

mucosal levels of EPA in humans in conjunction with its anti-neoplastic activity. This finding is consistent with data from Anti and colleagues, who have previously described that rectal mucosal EPA levels increased significantly after 6 months treatment with a fish oil preparation containing both EPA and DHA.<sup>25</sup> EPA is understood to incorporate into the phospholipid bilayer of cell membranes,<sup>26</sup> where it may compete with, and possibly displace, the  $\omega$ -6 PUFA AA.<sup>27</sup> However, we did not observe a statistically significant decrease in the mucosal AA

content of rectal mucosa in our study, unlike previous human studies measuring plasma  $\omega$ -3 PUFA levels.<sup>27</sup> We also observed an increase in mucosal DPA content relative to other fatty acids in individuals taking EPA-FFA, but no increase in the other main  $\omega$ -3 PUFA DHA, which is naturally present at relatively low levels in the human rectum compared with other tissues such as cerebral cortex and the retina.<sup>27</sup> EPA can undergo metabolic interconversion to DHA via DPA.<sup>26</sup> However, conversion of DPA to DHA, via elongase and  $\Delta^6$ -desaturase activities, is known to be the rate-limiting step in DHA biosynthesis, and this step is, in fact, downregulated by dietary EPA administration.<sup>28</sup> Therefore, a plausible hypothesis is that exogenous EPA administration leads to increased incorporation of both EPA and its metabolite DPA, but not DHA, in rectal mucosa of patients with FAP.

The mechanistic basis of the antineoplastic activity of EPA remains unclear. Both COX-dependent and COX-independent mechanisms of action of EPA have been described.<sup>9 29–31</sup> On the one hand, EPA can act as an alternative (although poorly efficient) substrate for COX-2, rather than its usual  $\omega$ -6 PUFA substrate AA,<sup>32</sup> leading to reduced synthesis of protumourigenic prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) in favour of production of the equivalent '3-series' PG (PGE<sub>3</sub>), which has recently been demonstrated to have antitumourigenic activity in human lung cancer cells.<sup>33</sup> A 'PGE<sub>2</sub> to PGE<sub>3</sub> switch' has recently been demonstrated in vivo in colorectal mucosa of rats treated with a fish oil preparation and pectin.<sup>34</sup> If a COX-2-dependent mechanism of action makes

**Figure 3** Comparison of the effects of eicosapentaenoic acid as the free fatty acid (EPA-FFA) and celecoxib in patients with familial adenomatous polyposis from this study and the study of Steinbach *et al.*<sup>5</sup> (A) The percentage change in polyp number in a defined area of colorectal mucosa. (B) The change in global polyp burden measured by video panel assessment. Note that a positive difference between the baseline and 6 month examination implies a decreased global polyp burden ('better').



a major contribution to the anti-CRC activity of EPA, a negative interaction between combined selective COX-2 inhibition and EPA treatment might be expected. On the other hand, anti-neoplastic activity of EPA independent of expression of either COX-1 or COX-2 has been described in HCT116 human CRC cells.<sup>31</sup> Several alternative mechanisms of action of EPA and other  $\omega$ -3 PUFAs have been proposed, including alteration of T cell and colonocyte membrane 'lipid raft' function and antioxidant properties.<sup>29</sup>

Treatment with EPA-FFA 2 g daily for 6 months was very well tolerated. The frequency of AEs in the EPA-FFA group was similar to that in the placebo group. The most common AE in both treatment groups was diarrhoea, which may reflect the absence of physiological control of faecal water content by the colon postcolectomy in patients with FAP. Delivery of EPA-FFA in enteric-coated capsules may explain the absence of minor upper GI AEs that have previously been associated with prolonged use of other  $\omega$ -3 PUFA preparations.<sup>25 35 36</sup>

A fundamental requirement for a cancer chemoprevention agent is an excellent safety profile. Selective COX-2 inhibitors have demonstrable efficacy for chemoprevention of FAP and 'sporadic' colorectal neoplasia.<sup>5 6 21–23</sup> However, concerns regarding cardiovascular safety are now likely to limit their long-term use as chemoprevention agents in all but the highest risk individuals.<sup>37 38</sup> In contrast,  $\omega$ -3 PUFA preparations have beneficial cardiovascular properties and are indicated for treatment of hypertriglyceridaemia, as well as for secondary prevention post-myocardial infarction.<sup>39</sup> Moreover, EPA alone has antiplatelet activity similar to aspirin.<sup>40</sup> Therefore, it is possible that EPA-FFA treatment may combine CRC chemopreventative efficacy with cardiovascular benefits, which is a particularly attractive therapeutic strategy for middle-to-old age populations relevant to secondary prevention of 'sporadic' colorectal neoplasia.

In summary, we report that a novel formulation of EPA, as the free fatty acid, has antineoplastic activity in patients with FAP to a degree similar to that observed with the selective COX-2 inhibitor celecoxib. EPA-FFA holds promise as a primary and secondary chemoprevention agent for FAP and for 'sporadic' colorectal neoplasia that has a favourable safety and tolerability profile.

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