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Selective COX-2 inhibition affects fatty acids, but not COX mRNA expression in patients with FAP

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Abstract Familial adenomatous polyposis (FAP) provides a model for sporadic colorectal cancer development. Cyclooxygenase (COX) inhibition may ameliorate polyp development, but rofecoxib was withdrawn due to cardiovascular side effects. Although this selective COX-2 inhibitor, like diet, may alter the fatty acid and eicosanoid pattern, data on the potential alteration in tissues after use, are scarce. The aims were to study if rofecoxib might influence the fatty acid distribution in serum phospholipids and duodenal lesions, mRNA for COX-1 and COX-2 in leucocytes and duodenal lesions, and finally plasma levels of PGE_2 in a randomized, double-blind, placebo controlled study ($n = 38$). Significant reductions were found for essential fatty acid index both in serum phospholipids ($P = 0.01$, 95% CI $= -0.9$; -0.1), and in duodenal lesions ($P = 0.04$, 95 CI % $= -0.9$; -0.1) after treatment. No treatment effects were found on the COX mRNA expression, or in the plasma PGE_2 levels. Dietary AA/EPA ratio was inversely associated with all the indicators of EFA status (all $P < 0.01$). These findings suggest that the effects of COX chemoprevention should be further investigated in FAP and that dietary needs should be included in the treatment of FAP.

Keywords Familial adenomatous polyposis · Cyclooxygenase · Diet · Fatty acids

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Introduction

Rofecoxib, a cyclooxygenase-2 (COX-2) inhibitor, was withdrawn because of increased risk of adverse cardiovascular events and metabolic complications [1–6]. The link between COX-2, fatty acids and risk of several diseases has been widely accepted [7, 8]. Deregulation of the COX-2/PGE_2 pathway appears to affect tumorigenesis via a number of distinct mechanisms: Promoting tumour maintenance and progression, encouraging metastatic spread, and perhaps even participating in tumour initiation [9]. COX-1 and -2 are the rate limiting enzymes in the synthesis of prostaglandins and thromboxanes [10]. Arachidonic acid (AA) is the main substrate for these enzymes, leading to the synthesis of prostaglandins which have growth promoting effects. Substituting AA with omega-3 fatty acids has been shown to lead to the production of less potent prostaglandins [11]. Since COX-2 is a fatty acid metabolising enzyme, the effects of COX-2 inhibition on fatty acid composition of different tissues is of interest.

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Although refecoxib has been used as chemoprevention against cancer, the underlying mechanisms relevant to fatty acids and colorectal cancer (CRC) have, to our knowledge, not been elucidated.

Familial adenomatous polyposis (FAP) accounts for 1% of CRCs [12], and provides a model for sporadic cancers. CRC arising in FAP patients can be largely prevented by polyp surveillance and prophylactic colectomy [13]. However, these patients remain at cancer risk at other sites, particularly duodenal cancers [14]. COX-2 inhibition is effective against colorectal polyposis in FAP [15–19]. Moreover, COX-2 expression in FAP patients was higher in the duodenum compared to the colorectal mucosa in a previous FAP study [20], offering hope for efficacy even in duodenal adenoma growth.

Biological mechanisms underlying reported associations between diet and tissues of CRC are poorly understood [21]. However, fatty acid metabolic status is an important substrate for nutrition and also for potentially COX_2 inhibitor mediated carcinogenesis. Colectomized FAP patients have been shown to have a deviant fatty acid profile with high levels of AA and docosahexaenoic acid (DHA) and low levels of linoleic acid (LA) and α-linolenic acid (ALA) in serum phospholipids [22], which is in accordance with studies in patients with other types of cancers [23, 24]. One may suspect that increased demand for essential fatty acids (EFAs) for tissue repair and membrane formation would lead to EFA deficiency, abnormal precursors of eicosanoids, and suboptimal cell function [25–27]. If alterations occur in the development of carcinogenesis, this may affect the biological functions of EFAs and their derivates. Chronic EFA deficiency may constitute a pro-tumorigenic condition when co-existing with chronically hyperproliferative states [28]. Very little, however, is known about fatty acid metabolism in FAP, although chemoprevention affecting the fatty acid derivates and the COX enzymes is often administered to this particular group of patients. Since a regular FAP treatment is COX-2 inhibition, which, like diet, may alter the fatty acid and eicosanoid pattern, the aims of this study were to investigate how rofecoxib influence the fatty acid distribution in serum and duodenal lesions, and mRNA for COX-1 and COX-2 in leucocytes and duodenal lesions, in addition to plasma levels of PGE_2.

Materials and methods

Patients were recruited from a Norwegian FAP registry. All of them had been colectomized and duodenal lesions had been verified by endoscopy and histology [29]. Biopsies were taken partly from macroscopically normal mucosa, partly from representative visible adenoma. Rikshospitalet University Hospital is a highly specialized university hospital with national responsibilities in the area of complicated treatments, such as follow-ups on the Norwegian FAP patients. Inclusion criteria were verified FAP, colectomy, 18–70 years of age and documented duodenal lesions graded as Spigelman I, II or III and the largest adenoma <10 mm. Exclusion criteria were indications for surgical treatment, suspected or documented intestinal obstruction or stenosis, patients unwilling or unable to adhere to protocol, known cardiac failure requiring medical treatment, and pregnancy. Consecutive patients were included in a randomized double-blind placebo-controlled intervention study with Rofecoxib, 25 mg × 1 daily or placebo for the duration of 1 year. Of the 47 patients initially considered for participation, 5 patients did not meet the inclusion criteria and 4 patients refused to participate. Thus, data are presented for 38 FAP patients (50% men). Eleven patients had been operated with conventional and 2 with a continent ileostomy, 20 patients with ileal-pouch-anal anastomosis (IPAA), 3 patients with ileoanal anastomosis (IAA) and 2 patients with ileorectal anastomosis (IRA). All patients were on a free diet. The main aim was to compare the effect of Rofecoxib treatment on duodenal lesions (data in preparation). Patients are previously described in detail [30, 31]. The study was interrupted because Rofecoxib was withdrawn from the market in 2004 due to severe cardiovascular side effects [32]. Eighteen patients received the intervention for 12 months (44% placebo). Of 26 patients following the scheduled treatment for at least 8 months, 50% received placebo. Of 8 patients following the treatment for less than 8 months, 50% received placebo. Mean placebo and Refecoxib duration was 9.8 ± 2.9 months and 8.8 ± 3.5 months, with mean compliance to the treatment of 85 and 90%, respectively.

Dietary intake

The habitual dietary intake has been described previously [33]. Data was gathered using a retrospective interview according to a validated food frequency questionnaire, focusing on the 1 year prior to inclusion in the study.

Fatty acids analyses in serum phospholipids and in total lipids of duodenal lesions

Total fatty acids in duodenal lesions (10–40 mg) were extracted with chloroform/methanol, transmethylated and determined by gas chromatography (vide supra). Fasting serum lipids were extracted using n-butanol and phospholipids isolated from the lipid extracts using Varian Bond Elut NH2, LCR columns (Varian, Walnut Creek, CA). Composition of fatty acids in serum phospholipids in these

FAP patients at baseline as compared to a healthy reference material ($n = 160$), has been published previously [34]. The results of the measurements are presented as weight pecentage of total fatty acid analysed. The fatty acids were 14:0 (myristic acid), 16:0 (palmitic acid), 16:1n-7 (palmitoleic acid), 18:0 (stearic acid), 18:1n-9 (oleic acid), 18:2n-6 (linoleic acid, LA), 18:3n-3 (α-linolenic acid, ALA), 20:1n-11 (eicosaenoic acid), 20:2n-6 (eicosadienoic acid), 20:4n-6 (arachidonic acid, AA), 20:5n-3 (eicosapentaenoic acid, EPA) and 22:6n-3 (docosahexaenoic acid, DHA). Dihomo-γ-linolenic acid (20:3n-6) and eruic acid (22:1n-9) were present in very small amounts, and these fatty acids are only included in sums. An estimate of delta- 9-desaturase (steaoryl-CoA desaturase, SCD) activity was calculated as ratios of 16:1n-7/16:0 and 18:1n-9/18:0. A measure of EFA status is the ratio of (sum omega-3 and sum omega 6 fatty acids) to (sum omega-7 and omega-9 fatty acids), since deficiency in ALA and LA would depress the entire omega-3 and omega-6 series[11], respectively.

RNA isolation and cDNA synthesis

Leucocytes were isolated from fasting blood samples, collected in EDTA tubes. The EDTA tubes were centrifuged and plasma removed. Red blood cells were lysed by red blood cell lysis buffer, and leucocytes isolated by centrifugation. Cell pellets containing leucocytes were immediately placed on dry ice and stored at −80°C. RNA was isolated by the Trizol/RNeasy hybrid protocol: Chilled Trizol reagent (Invitrogen,TM Life Technologies) was added to the frozen cells and immediately mixed by vortexing. The homogenized samples were incubated at room temperature for 5 min before the addition of 0.2 ml chloroform per ml Trizol reagent. Each tube was vortexed for 15 s, incubated at room temperature for another 5 min and centrifuged at 12,000×*g* (4°C) for 10 min. The upper aqueous phase was transferred to a new tube and an equal volume of 70% ethanol (4°C) was added during continuous mixing. Each sample was then transferred to an RNeasy mini column (Qiagen), and further RNA isolation was done by following the manufacturer's instructions. All samples were treated with Rnase-free Dnase (Qiagen) to prevent amplification of genomic DNA. RNA quality was checked by Capillary electrophoresis (RNA 600 Nano LabChip, Agilent) on an Agilent Bioanalyser 2100 system. The total RNA yield was measured on a Nanodrop ND-1000 Spectrophotometer (NanoDrop Technologies Wilmington, Delaware, USA). For cDNA synthesis, RNA from each sample was reverse-transcribed by Super Script (Invitrogen) according to the manufacturer's protocol, using oligo dT as primers.

RNA isolation from duodenal lesions

Lesions were taken and stored in RNAlater solution previous to freezing at −80°C. Each biopsy was put into a tube containing 600 µl Trizol and immediately mixed on a MixerMill (Retsch GmbH, Germany). RNA was isolated from the samples as described above.

Real time polymerase chain reaction

Gene expression analyses were carried out on a 7900HT real time PCR machine from Applied Biosystems. Based on the results from running a housekeeping gene test ("TaqMan Human Endogenous Control Plate", Applied Biosystems) with RNA from isolated human leucocytes (data not shown), Glucuronidase (GUS) and Tata binding protein (TBP) were chosen as housekeeping genes. The primers and probes were initially designed as three assays per gene, and validated for efficiency and specificity. The best of the three was then chosen. The primers and probes for the COX-1 assay were: forward primer: 5′-CTTCCAG GAGCTCGTAGGA-3′, probe: 5′-AGAAGGAGATGGCA GCAGAGTTGGAG-3′, and reverse primer: 5′-ACGCA TCAATGTCTCCATACAAT-3′. COX-2 forward primer: 5′-TGGAACATGGAATTACCCAGT-3′, probe: 5′-TGT TGAATCATTCACCAGGCAAATTGCT-3′ and reverse primer: 5′-TCCTACCACCAGCAACCCT-3′. GUS forward primer: 5′-GAAAATATGTGGTTGGAGAGCTC ATT-3′, probe: 5′-CCAGCACTCTCGTCGGTGACTGTT CA-3′ and reverse primer: 5′-CCGAGTGAAGATCCCC TTTTTA-3′. TBP forward primer: 5′-CTGGAAAAGTTG TATTAACAGGTGC-3′, probe: 5′-AGCAGAAATTTATG AAGCATTTGAAAACATCTACCCTATT-3′ and reverse primer: 5′-CATTACGTCGTCTTCCTGAATC-3′.

TaqMan Universal PCR Master Mix (Applied Biosystems) was added as reaction mix. The reaction conditions were initiated by a step of 2 min. at 50°C and 10 min. at 95°C, followed by 40 cycles of denaturation at 95°C for 15 s. and annealing at 60°C for 1 min. Standards and samples were analysed in triplicates for all assays. A combination of cDNA from several samples was made and diluted in order to make a dilution curve that was included on each plate. The average of the three values for each gene was divided by the average of the corresponding GUS and TBP values, generating a normalized value of the gene expression which is a unit less value used to compare the relative amount of mRNA for each gene in the different samples. For comparison, we also analysed leucocytes from 59 Norwegian blood donors (mean age: 41 ± 12 years, 49% men). Normal duodenal tissue ($n = 11$) was provided from clinicians in the hospital (anonymous, but healthy samples).

PGE_2 in plasma

Plasma samples were analysed for PGE_2 by ELISA (R&D, London, UK), according to the manufacturer's instructions.

Ethics approval

The study was performed in accordance with the Helsinki Declaration. Patients were informed by a physician, and thorough written information was provided to all patients. The protocol was explained to the subjects, who gave their consent before inclusion. No honorarium was offered. The patients were informed specifically of the option to withdraw at any stage of the study with no requirement to provide an explanation for withdrawal. The study protocol (RH01/01) was approved by the Norwegian health authorities and the Regional Committee of Medical Ethics 20/06/2002 (reference: S-02127). At cessation of the study, all patients were informed personally about the reasons for drug withdrawal and any consequences relevant to the individual patient. Retrospective registration was done 02/13/09 at ClinicalTrials.gov.

Statistical analysis

This group of patients is very rare, and the present sample size is close to the maximum possible number of eligible Norwegian FAP patients. Results are expressed as mean values ± standard deviation (SD), or as stated. The effect of each treatment was analysed separately in each group by Student's paired t tests. Differences in mean values between groups are tested by Independent-Samples *T* tests. Relations between variables were investigated using Pearson's correlation coefficients (*r*). We included the results from all the included patients in the tables. However, since the trial was interrupted, we have also provided footnotes that include analysis excluding the cases that followed the treatment for less than 8 months. Statistical significance was set to $P \leq 0.05$. All statistical analyses were performed with the SPSS 12.0 for Windows.

Results

Relative composition of fatty acids in serum phospholipids

The composition of fatty acids in serum phospholipids at baseline and after the intervention, expressed as weight percentage of total fatty acids analysed, are presented in Table 1.

In both groups, the relative amounts of several fatty acids increased in serum phospholipids, whereas the relative amount of DHA ($P < 0.03$) decreased in the intervention period. However, a significant treatment effect was observed only for myristic acid ($P = 0.02$, 95% CI = −0.4; −0.04) and eicosaenoic acid ($P = 0.01$, 95% CI = 0.02; 0.1). After exclusion of the patients who received treatment for less than 8 months, significant treatment effects were found also for the different estimates of essential fatty acid status: EFA index ($P = 0.01$, 95% CI = −0.9; −0.1), PUFA/(SAT + MUFA) ($P = 0.02$, 95% CI = −0.3; −0.1), and sum omega-7 and omega-9 fatty acids ($P = 0.01$, 95% CI = 1.3; 8.1).

Relative composition of fatty acids in duodenal lesions

Significant treatment effects were observed in duodenal lesions for LA, PUFA, omega-6 fatty acids, EFA index, ratio PUFA/(SAT + MUFA), oleic acid, MUFA, omega-9 fatty acids, and sum omega-7 + omega-9 fatty acids (all $P < 0.05$) (Table 2). Exclusion of the cases that had received treatment for less than 8 months did not change these results.

Gene expression in blood and duodenal lesions

No significant treatment effects were found on mRNA expression levels of COX-1 and COX-2, nor plasma levels of PGE_2. In the Rofecoxib group separately, however, the duodenal expression levels of COX-1 was increased ($P = 0.03$, 95% CI = −0.20; −0.01), but this reached statistical significance for only one of the two housekeeping genes (Table 3).

The mRNA COX-1 and COX-2 expression in leucocytes from FAP patients was lower than in healthy individuals, but only significantly so when using GUS as the housekeeping gene ($P < 0.001$, 95% CI = −0.5; −0.2, and $P < 0.01$, 95% CI = −1.6; −0.2, respectively). The COX-1 expression in duodenal lesions from FAP patients was lower than in normal lesions, regardless of choice of housekeeping gene (both $P < 0.01$). The levels of COX-2 expression in duodenal lesions and plasma PGE_2 did not vary between FAP patients and healthy subjects (data not shown).

Relationship to dietary intake of fatty acids

The dietary AA/EPA ratio was inversely associated with duodenal EFA index ($r = -0.4$, $P = 0.01$) and ratio PUFA/(SAT + MUFA) in both serum phospholipids and duodenal lesions (both $r = -0.4$, $P < 0.01$). In serum phospholipids, habitual use of omega-3 fatty acid supplements was associated with higher levels of EPA ($P = 0.01$), DHA ($P = 0.02$), PUFA ($P = 0.006$), omega-3 fatty acids ($P = 0.007$), ratio omega-3/omega-6 ($P = 0.01$) and ratio

Table 1 Fatty acid composition of serum phospholipids (weight %) from patients with familial adenomatous polyposis before and after treatment with Rofecoxib

	Placebo group			Rofecoxib group			95% CI[b]		
	At baseline $n = 17$	After intervention $n = 15$	P value[a]	At baseline $n = 18$	After intervention $n = 15$	P value[a]	P value[b,c]	Lower	Upper
Fatty acids (weight %) in serum phospholipids									
Myristic acid	0.1 ± 0.01	0.8 ± 0.2	<0.01	0.01 ± 0.01	0.6 ± 0.2	<0.01	0.02	−0.4	−0.04
Palmitoleic acid	0.3 ± 0.4	0.6 ± 0.3	<0.001	0.4 ± 0.3	0.6 ± 0.3	0.007	0.9	−0.2	0.2
ALA	0.1 ± 0.1	0.2 ± 0.2	<0.01	0.01 ± 0.04	0.2 ± 0.2	<0.01	0.4	−0.1	0.1
Eicosaenoic acid	0.1 ± 0.1	0.01 ± 0.01	0.9	0.01 ± 0.01	0.1 ± 0.1	<0.01	0.01	0.02	0.1
Eicosadienoic acid	0.2 ± 0.5	0.3 ± 0.3	0.3	0.1 ± 0.2	0.3 ± 0.2	<0.001	0.7	−0.3	0.4
EPA	1.7 ± 1.0	1.9 ± 0.9	0.6	1.5 ± 0.8	1.9 ± 0.8	0.03	0.6	−0.6	0.9
DHA	11.2 ± 2.7	9.9 ± 2.2	0.02	11.0 ± 3.2	9.9 ± 1.9	0.03	0.8	−1.4	1.9
Palmitoleic/ palmitic acid	0.01 ± 0.1	0.02 ± 0.1	<0.001	0.01 ± 0.1	0.1 ± 0.1	0.004	0.9	−0.1	0.1
EPA/DHA	0.2 ± 0.1	0.2 ± 0.1	0.2	0.1 ± 0.1	0.2 ± 0.1	0.006	0.8	−0.1	0.1

Results are presented as mean ± standard deviation

[a] P value: between baseline and intervention

[b] P value: treatment effect: difference between end value and start value

[c] The results for palmitic acid, stearic acid, oleic acid, LA, AA, SAT, MUFA, PUFA, sum omega-3 fatty acids, sum omega-6 fatty, omega-3/ omega-6 fatty acids acids, sum omega-9 fatty acids, LA/AA, EFA index[d], PUFA/(SAT + MUFA) and sum omega-7 and omega-9 fatty acids were not significant (all subjects) (data not shown in Table). After exclusion of the patients that were compliant for less than 8 months ($n = 4$, 50% placebo), similar treatment effects were found for the same fatty acids: myristic acid ($P = 0.02$, 95% CI = −0.4; −0.1) and eicosaenoic acid ($P = 0.02$, 95% CI = 0.01; 0.1), and additionally for EFA index ($P = 0.01$, 95% CI = −0.9;−0.1), PUFA/(SAT + MUFA) ($P = 0.02$, 95% CI = −0.3; −0.1), and sum omega-7 and omega-9 fatty acids ($P = 0.01$, 95% CI = 1.3; 8.1)

[d] EFA index: (sum omega-3 fatty acids and sum omega 6 fatty acids) / (sum omega-7 and omega-9 fatty acids)

PUFA/(SAT + MUFA) ($P = 0.008$). Use of omega-3 fatty acid supplements was associated with lower duodenal levels of oleic acid ($P = 0.04$) and MUFA ($P = 0.03$), and higher duodenal levels of EPA ($P = 0.002$), DHA ($P = 0.01$), ratio EPA/DHA ($P = 0.04$) and EFA index ($P = 0.046$) (data not shown).

We wanted to see if the habitual intake of omega-3 and omega-6 fatty acids were related to the treatment effects. Results were significant for the habitual intake of omega-6 fatty acids in particular as compared to the changes of fatty acid composition in duodenal lesions (Table 4). No significant coefficients of correlation were found as compared to the changes in fatty acid composition in serum phospholipids, expression of COX-1 and COX-2, and plasma levels of PGE_2 (data not shown).

Discussion

Rofecoxib has no likely therapeutic future because of concerns relative to cardiovascular toxicity. However, investigation of the effects of this selective COX-2 inhibitor on nutrition-related parameters is still relevant in order to improve treatment and care of FAP.

In the present study, comparable treatment effects were observed on the fatty acid composition in serum phospholipids and duodenal lesions, presumably and most importantly the non-beneficial effects involving EFAs. Treatment effects were observed neither on mRNA expression levels of COX-1 and COX-2 in duodenal lesions and leucocytes, nor on the levels of plasma PGE_2. Dietary intake of fatty acids was reflected in the composition of fatty acids in both serum phospholipids and duodenal lesions. Furthermore, intake of omega-3 fatty acids was beneficially related to increased levels of estimates of EFA status.

Several of the present estimated changes suggest insufficient EFA status [35, 36]. Generally, in EFA deficiency, both palmitoleic acid and oleic acid are elongated and desaturated when delta-6 desaturase is stimulated, and this process is considered as an ineffective attempt to replace EFAs with the long chain metabolites, primarily 5, 8, 11 eicosatrienoic acid (mead acid). EFA deficiently also induces an increased activity of delta-9 desaturase, which might account for the abnormal increase in palmitoleic acid in serum phospholipids in both the placebo and Rofecoxib group separately. In many tumour cells, the metabolism of EFA is clearly abnormal, since there is a partial or complete loss of delta-6 desaturase. A dietary deficiency of

Table 2 Fatty acid composition of duodenal lesions (weight %) from patients with familial adenomatous polyposis before and after treatment with Rofecoxib

	Placebo group			Rofecoxib group			95% CI[2]		
	At baseline $n = 16$	After intervention $n = 12$	P value[a]	At baseline $n = 17$	After intervention $n = 13$	P value[a]	P value[b, c]	Lower	Upper
Fatty acids (weight %) in duodenal lesions									
Oleic acid	20.2 ± 4.3	20.2 ± 2.8	0.3	18.7 ± 0.9	21.4 ± 3.6	0.04	0.04	0.2	6.7
LA	24.9 ± 3.3	25.3 ± 2.8	0.3	27.3 ± 2.3	25.3 ± 3.7	0.07	0.03	−6.4	−0.3
MUFA	21.2 ± 1.2	21.1 ± 2.8	0.3	19.6 ± 1.4	22.4 ± 3.9	0.04	0.03	0.3	6.9
PUFA	39.4 ± 6.0	40.4 ± 2.6	0.2	42.4 ± 2.0	39.2 ± 5.4	0.04	0.03	−9.7	−0.7
Sum omega-6 fatty acids	35.2 ± 5.1	36.2 ± 2.3	0.2	38.3 ± 2.2	35.3 ± 5.5	0.07	0.03	−9.1	−0.5
Sum omega-9 fatty acids	20.3 ± 4.3	20.2 ± 2.7	0.3	18.8 ± 1.4	21.4 ± 3.6	0.04	0.04	0.2	6.7
EPA/DHA	0.3 ± 0.1	0.4 ± 0.1	0.3	0.3 ± 0.01	0.4 ± 0.1	0.03	0.9	−0.1	0.1
EFA index[d]	2.0 ± 0.6	2.0 ± 0.4	0.3	2.2 ± 0.3	1.9 ± 0.5	0.04	0.04	−0.9	−0.1
PUFA/(SAT + MUFA)	0.7 ± 0.2	0.7 ± 0.1	0.3	0.7 ± 0.1	0.7 ± 0.1	0.04	0.03	−0.2	−0.1
Sum omega-7 + omega-9 fatty acids	4.4 ± 3.3	3.6 ± 1.4	0.3	3.2 ± 0.8	4.1 ± 2.2	0.3	0.03	0.3	6.9

Results are presented as mean ± standard deviation

[a] P value: between baseline and intervention

[b] P value: treatment effect: difference between end value and start value

[c] The results for myristic acid, palmitic acid, palmitolic acid, stearic acid, ALA, eicosaenoic acid, eicosadienoic acid, AA, EPA, DHA, SAT, sum omega-3 fatty acids, omega-3/omega-6 fatty acids, palmitolic acid/palmitic acid, oleic acid/stearic acid and AA/LA, were not significant (all subjects) (data not shown in Table). After exclusion of the patients that were compliant for less than 8 months ($n = 3$), similar treatment effects were found for the same fatty acids: oleic acid ($P = 0.01$, 95% CI = 1.2; 7.8), LA ($P = 0.046$, 95% CI = −0.7; −0.1), MUFA ($P = 0.009$, 95% CI = 1.3; 8.1), PUFA ($P = 0.02$, 95% CI = −10.9; −1.3), omega-6 fatty acids ($P = 0.03$, 95% CI = −10.2; −0.7), omega-9 fatty acids ($P = 0.01$, 95% CI = −1.2; −7.8), EFA index ($P = 0.01$, 95% CI = 1.2; 7.8), omega-9 fatty acids ($P = 0.01$, 95% CI = −1.2; −7.8), EFA index ($P = 0.01$, 95% CI = −0.9; −0.1), PUFA/(SAT + MUFA) ($P = 0.02$, 95% CI = −0.3; −0.1), and sum omega-7 and omega-9 fatty acids ($P = 0.01$, 95% CI = 1.3; 8.1)

[d] EFA index: sum omega-3 fatty acids + sum omega-6 fatty acids/sum omega-7 fatty acids + sum omega-9 fatty acids

EFAs does not predictably produce tumours, but it may predispose to the development of cancer [37]. We did not introduce any conscious dietary intervention that would have accounted for the significant increases in several FAs post treatment in the placebo group. One may suspect that increased demand for EFAs for tissue repair and membrane formation would lead to EFA deficiency, abnormal precursors of eicosanoids, and suboptimal cell function (1–3). Chronic EFA deficiency may constitute a pro-tumorigenic condition when co-existing with chronically hyperproliferative states, and disease development might also explain some of the findings. It may be that serum phospholipids are poor short term indicators of EFAD status except in severe EFAD. This may offer an explanation for why significant findings were predominately found in duodenal lesions which may reflect dietary intake over a longer period of time.

Presently, the estimated activity of SCD in serum phospholipids was increased in both the placebo and Rofecoxib group, but no treatment effect was found. The dietary content of palmitoleic acid is low, and most palmitoleic acid in serum is derived from the desaturation of palmitic acid catalysed by SCD. The level of the other main product of SCD desaturation, oleic acid, was increased after treatment in duodenal lesions. The strong correlation of high levels of MUFA and neoplastic phenotype suggest that the regulation of SCD might play a role in cancer development. SCD is the central lipogenic enzyme catalyzing in vivo reactions in the synthesis of MUFAs, particularly oleic acid and palmitoleic acid, which are the major MUFAs of membrane phospholipids, triglycerides, wax esters, and cholesteryl esters. An increased desaturation estimated as a decreased ratio of stearic acid/oleic acid [38], was not found in this investigation.

Treatment lowered the levels of LA and omega-6 fatty acids in duodenal lesions as compared to placebo. A positive correlation was found between dietary intake of omega-6 fatty acids and duodenal amounts of LA at baseline.

Table 3 mRNA expression of COX-1 and COX-2 in leucocytes and duodenal lesions and levels of plasma PGE_2 in patients with familial adenomatous polyposis before and after treatment with Rofecoxib

	Placebo group			Rofecoxib group			P value[b,c]	95% CI[2]	
	At baseline $n = 15$	After intervention $n = 13$	P value[a]	At baseline $n = 18$	After intervention $n = 15$	P value[a]			
Leucocytes									
COX-1 G	0.6 ± 0.3	0.4 ± 0.2	0.45	0.6 ± 0.2	0.7 ± 0.7	0.4	0.3	−0.20	0.61
COX-1 T	2.4 ± 1.2	2.0 ± 0.8	0.63	2.9 ± 1.5	3.4 ± 2.4	0.3	0.3	−0.68	2.20
COX-2 G	1.5 ± 1.9	1.2 ± 0.6	0.69	1.8 ± 1.2	1.6 ± 1.4	0.5	0.9	−1.54	1.40
COX-2 T	4.6 ± 3.3	5.8 ± 3.1	0.19	8.3 ± 5.0	6.7 ± 5.1	0.2	0.08	−7.72	0.65
Duodenal lesions									
COX-1 G	1.1 ± 1.2	1.2 ± 0.2	0.19	1.1 ± 0.2	1.2 ± 0.2	0.1	0.8	−0.15	0.20
COX-1 T	0.9 ± 0.2	0.9 ± 0.2	0.62	0.9 ± 0.2	1.01 ± 0.2	0.03	0.2	−0.05	0.20
COX-2 G	1.2 ± 0.2	1.2 ± 0.3	0.39	1.3 ± 0.3	1.2 ± 0.4	0.9	0.6	−0.21	0.36
COX-2 T	1.04 ± 0.2	1.0 ± 0.3	0.19	1.1 ± 0.3	1.04 ± 0.3	0.9	0.3	−0.13	0.39
Plasma									
Plasma PGE_2	521 ± 306	690 ± 556	0.28	650 ± 701	615 ± 825	0.6	0.2	−129	503

Results are presented as mean ± standard deviation

[a] P value between baseline and intervention

[b] Treatment effect: difference between end value and start value

[c] After exclusion of the patients that received the treatment for less than 8 months ($n = 4$, 50% placebo), no treatment effect was found

G glucuronidase (GUS) chosen as housekeeping gene

T tata binding protein (TBP) chosen as housekeeping gene

COX cyclooxygenase

Table 4 Pearson coefficients (r) of correlation between dietary intake of omega-3 and omega-6 fatty acids and the treatment effect (intervention–baseline): changes in fatty acid composition in duodenal lesions (weight %) from patients with familial adenomatous polyposis ($n = 38$)

Differences (stop–baseline) in duodenal lesions	Dietary intake (% of total fat)		
	Sum omega-3 fatty acids	EPA + DHA	Sum omega-6 fatty acids
Myristic acid	−0.48	−0.01	−0.58*
Stearic acid	0.72**	0.22	0.56*
ALA	−0.48	0.20	−0.82**
EPA	0.02	−0.52	0.76**
DHA	−0.03	−0.52	0.70**
Omega-3 fatty acids	−0.35	−0.59*	0.44
Ratio ALA/LA	−0.42	0.17	−0.75**
Ratio AA/LA	0.44	−0.11	0.58*

Only significant results are shown

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

Dietary intake of omega-6 fatty acids will increase the formation of AA; hence, more PGE_2 is formed [39]. In this study the dietary ratio of omega-6 fatty acids/omega-3 fatty acid correlated positively to PGE_2 values. Dietary omega-3 fatty acids are regarded as beneficial, as their desaturated metabolites competitively inhibit the production of AA-derived eicosanoids such as prostaglandins and leucotrienes, which are potent mediators, and some of these mediators are known to be involved in inflammation. On the other hand, lipoxins, which are anti-inflammatory, can also be synthesized from AA[40].

Selective COX-2 inhibitors were developed with the goal of inhibiting PGE_2 synthesis in areas of inflammation. PGE_2 is known to be a tumour promoter, and is often found in high

concentrations in cancer cells. The levels of neither COX-2 in lesions, nor plasma PGE_2, were reduced after treatment. The levels of COX-2 in duodenal lesions and plasma PGE_2 also did not vary between FAP patients and healthy subjects. A significant change in the Rofecoxib treated group, separately, in duodenal biopsy expression levels of COX-1 was found, but only when TBP was used as the housekeeping gene. Notably, the outcome for the comparisons between FAP patients and healthy subjects regarding both COX-1 and COX-2 expression in leucocytes was also dependent on the choice of housekeeping gene. We will not speculate on the importance of these findings.

The intake of omega-3 fatty acids was lower than the minimum recommendation of 0.5% of energy in the patients not taking omega-3 supplements [41, 42]. Notably, cancer patients are generally advised to take supplements with very long chain omega-3 fatty acids, and to follow the general dietary guidelines [43]. FAP patients are at high cancer risk, and the present study suggests that dietary modification should follow chemoprevention in FAP patients. Fatty acids are nearly 100% absorbed, and previous studies have shown that the correlation between FA in diet and serum phospholipids lipids is high. Correlations between individual FAs in diet and duodenal biopsies have, to our knowledge, not been reported previously. Thus, the results are biologically meaningful since simple dietary advice might change the EFA status even in a FAP patient. We would suggest that the nutrient intake among FAP patients should at least meet the recommendations for healthy subjects, and in particular with regard to essential fatty acids. Clinicians should thus involve clinical nutritionists in the treatment of such patients.

The design, a prospective double-blind randomized placebo-controlled trial is a key strength to this study. A good sample of FAP patients was recruited for the study, and the number enrolled is close to the maximum possible in terms of eligible Norwegian patients. Certainly sample size imposes obvious restrictions on conclusions. We could have applied the Bonferroni correction to address the problem of multiple comparisons by adjusting the *P*-values. However, the comparable effects were observed both in blood samples and duodenal biopsies. Exclusion of the patients who received treatment for less than 8 months from the statistical analysis, however, did not change the outcome of the study. We do not think that poor compliance may explain any lack of effect, since in both groups, compliance was close to 90%. Findings were similar in duodenal lesions and serum phospholipids, which supports the relevance of investigating fatty acid metabolism in FAP. To the best of our knowledge no previous study has investigated the fatty acid composition of duodenal lesions from FAP patients. This drug might have a direct influence on the fatty acid metabolism pathways in the gut epithelium. One possible explanation for the lack of modulation of COX_2 levels is possibly that the drug was simply not getting to the epithelium. In this regard, direct measures of rofecoxib in both the blood and in the duodenum, by the chromatographic methods otherwise employed, should be implemented in future comparable studies. The delay in processing and publishing the clinical data is unfortunate, but with the drug withdrawn, the urgency to publish was somewhat precluded. Initially after the withdrawal of the drug, our focus was on assuring the cardiovascular status of our participants and various aspects of follow-up. Moreover, we still believe that the conclusions drawn are valid, and in contrast to other papers, this interdisciplinary paper suggests the relevance of clinical nutrition for better treatment of such patients. Future FAP studies should study fatty acid status and metabolism, molecular mechanisms relevant to essential fatty acid metabolism, inflammation and angiogenesis, as well as nutrition requirements.

In conclusion, the study indicated that several significant Rofecoxib-related, non-beneficial changes were found in the fatty acid distribution of both serum phospholipids and duodenal lesions. In contrast, no treatment effects on the mRNA expression of COX-1 and COX-2 in the same tissues, nor in the levels of plasma PGE_2, were found. Dietary intake of fatty acids was reflected in the composition of fatty acids in both serum phospholipids and duodenal lesions. Furthermore, both dietary and supplemental intake of omega-3 fatty acids was found to be beneficially related to EFA status.

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