

Changes of the mucosal n3 and n6 fatty acid status occur early in the colorectal adenoma-carcinoma sequence

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Abstract

Despite data favouring a role of dietary fat in colonic carcinogenesis, no study has focused on tissue n3 and n6 fatty acid (FA) status in human colon adenoma-carcinoma sequence. Thus, FA profile was measured in plasma phospholipids of patients with colorectal cancer (n=22), sporadic adenoma (n=27), and normal colon (n=12) (control group). Additionally, mucosal FAs were assessed in both diseased and normal mucosa of cancer (n=15) and adenoma (n=21) patients, and from normal mucosa of controls (n=8). There were no differences in FA profile of both plasma phospholipids and normal mucosa, between adenoma and control patients. There were considerable differences, however, in FAs between diseased and paired normal mucosa of adenoma patients, with increases of linoleic (p=0.02), dihomo-gammalinolenic (p=0.014), and eicosapentaenoic (p=0.012) acids, and decreases of α linolenic (p=0.001) and arachidonic (p=0.02) acids in diseased mucosa. A stepwise reduction of eicosapentaenoic acid concentrations in diseased mucosa from benign adenoma to the most advanced colon cancer was seen (p=0.009). Cancer patients showed lower α linolenate (p=0.002) and higher dihomogammalinolenate (p=0.003) in diseased than in paired normal mucosa. In conclusion changes in tissue n3 and n6 FA status might participate in the early phases of the human colorectal carcinogenesis.

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Several epidemiological studies have suggested that populations that consume high fat diets have an increased risk of colon cancer.¹ The intake of saturated fat seems to account for this association, whereas an association to polyunsaturated fat intake (linoleate) has not been seen.² The low colon cancer rate in Alaskan Eskimos, however, has been related to the high consumption of fish products rich in n3 polyunsaturated fatty acids (n3-PUFAs),³ suggesting that fish oil may be a protective factor in colon carcinogenesis.

On the other hand, experimental studies

support the idea that dietary n6-PUFAs can promote carcinogenesis. Several animal studies have shown that diets containing high proportions of n6-PUFAs increase the incidence of chemically induced colonic tumours in rats,^{4–6} whereas diets rich in n3-PUFAs tend to have an antipromotional effect.^{4–8} Fish oil seems to influence early stages of colon experimental carcinogenesis, with a decrease in proliferative indices and focal areas of dysplasia in azoxymethane treated rats.⁹ These studies support that the fatty acid composition of the diet is more important than its overall fat content in terms of colon cancer risk. Recent data also suggest a role for PUFAs on human colon carcinogenesis. Firstly, studies on the mucosal fatty acid content in patients with colorectal carcinoma have shown increased concentrations of both arachidonic (C20:4n6) and docosahexaenoic (DHA; C22:6n3) acids in tumoral mucosa.^{10–11} Nevertheless, these abnormalities could be a metabolic consequence of the established cancer rather than a causative factor for colon carcinogenesis. Secondly, n3-PUFA supplementation in healthy subjects,¹² and in patients with sporadic adenomatous polyps^{13–14} reduces the rate of colonic epithelial cell proliferation, thus decreasing the risk for colon cancer.¹⁵

In this study, a different approach to assess the possible role of PUFAs in the colorectal carcinogenesis processes was used. We investigated the changes of the mucosal fatty acid content in the human adenoma-carcinoma sequence, using biopsy specimens from patients with adenomas and carcinomas of the colon. Measurements were performed in both diseased and adjacent normal mucosa. The study of colorectal adenomas avoids the possible influence on PUFA metabolism of an established cancer. Fatty acid profile in plasma phospholipids was also evaluated.

Methods

Inclusion criteria

Patients who were found to have either sporadic polyps (with size >1 cm) or cancer of the colon during total fiberoptic colonoscopy were selected if they were not taking drugs (such as hypolipemiant, oral contraceptives, non-steroidal anti-inflammatory drugs (NSAIDs) or platelet antiaggregants) or vitamin, mineral or fish oil supplements. Likewise, only omnivore subjects taking a Western type diet were included. In addition,

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patients with associated acute or chronic diseases that could disturb plasma fatty acid pattern, or history of previous colorectal adenomatous polyps or carcinoma were excluded. The same inclusion criteria were required for patients with normal colon.

All patients gave informed consent to the study, which was approved by the Hospital Research and Ethical Committee.

Patients

Forty nine patients with colonic adenoma or adenocarcinoma were studied. Twenty seven patients (22 men, five women; mean (SEM) age 61.9 (2) years) had sporadic adenomatous polyps (mean (SEM) size, 2.01 (0.20) cm). Most of them were tubular adenomas (n=12) and tubulovillous adenomas (n=14), and only one was a villous adenoma. Eight adenomas had severe cellular atypia considered as *in situ* carcinoma. The sites of the polyps were rectum (n=9), sigmoid (n=11), descending (n=6), and ascending colon (n=1).

Twenty two patients (16 men, six women; mean (SEM) age 61 (2.7) years) had colorectal adenocarcinomas. Cancer staging according to Dukes's was: Dukes's B, 12; and Dukes's C-D, 10. The location of the cancers were rectum (n=10), sigmoid colon (n=6), and caecum plus ascending colon (n=6).

In addition, 12 patients (six men, six women; mean (SEM) age 57 (3.6) years) with normal colon during a routine total fiberoptic colonoscopy to rule out colonic disease (haemorrhoidal bleeding, abdominal pain, irritable bowel syndrome) were also studied, and constituted the control group. There were no significant differences in sex and age between the three clinical groups.

Biopsy specimens and blood collection

Biopsy specimens were taken with endoscopic forceps. In patients with polyps or carcinoma, specimens were taken as paired samples, from the tumour and from normal looking mucosa at least 10 cm away from the lesion. If more than one polyp larger than 1 cm was found, only the largest one was studied. In patients with normal colon, biopsy specimens were taken from the rectosigmoid colon. Immediately after removal, the biopsy specimens were placed in cryovials, flash frozen in liquid nitrogen, and stored at -80°C . Polyps were removed by snare diathermy and retained for histological examination. Multiple biopsy

specimens were obtained from suspected carcinoma lesions for histological examination.

Venous blood was obtained the day after colonoscopy, before any therapeutic procedure was performed, after a 12 hour overnight fast period. Plasma was separated by centrifugation at $3000\times g$ for 10 minutes and was immediately stored at -20°C .

Fatty acid assay

The plasma lipid extraction procedure has been previously described.¹⁶ Plasma phospholipids were separated by thin layer chromatography on silica gel G-60 (Merck, Darmstadt, Germany) by using the solvent system described by Skipski and Barclay.¹⁷ Direct transesterification of fatty acids (FAs) was immediately carried out in methanol-benzene 4:1 (v/v) with acetyl chloride according to the procedure of Lepage and Roy.¹⁸ The benzene extract was evaporated under a stream of nitrogen at 40°C to complete dryness. The residue was dissolved in 100 μl of benzene and a 1 μl aliquot was injected in the chromatograph. FA methyl esters were quantified by gas-liquid chromatography in a Perkin-Elmer Autosystem chromatograph (Perkin-Elmer, Norwalk, CT) using a 30 m capillary column, 0.25 mm internal diameter, impregnated with SP-2330 as stationary phase. The identification and quantification of FA methyl esters were made by comparison with an external standard (Sigma Chemical, St Louis, MO).

FAs from C16:0 to C24:0 were measured, unidentified peaks accounting for $<0.5\%$ of the total FA. Saturated fatty acids (SFAs) were expressed as the sum of C16:0 (palmitic), C18:0 (stearic), and C24:0 (lignoceric); and monounsaturated fatty acids (MUFAs) as the sum of C16:1n7 (palmitoleic), C18:1n9 (oleic), C20:1n9 (gadoleic), C22:1n9 (erucic), and C24:1n9 (nervonic). FAs in plasma phospholipids were expressed as a percentage of the total FAs present.

Tissue samples (mean wet weight, 27 mg; range, 10 to 40 mg), were put in a 4:1 (v/v) methanol-benzene solution and shaken for about one minute in a vortex mixer. Afterwards they were homogenised by sonication in an ultrasound bath. Then, direct transesterification was performed as described above. The procedure for FA assay did not differ from that described for FA plasma phospholipids. Mucosal FAs were expressed as percentage of total FAs present.

In addition, the unsaturation index (UI) was calculated as previously described¹⁹ according to this equation:

$$\text{UI} = \sum (\text{FA per cent value} \times \text{number of double bonds})$$

Nutritional status

Protein-energy nutritional status was evaluated measuring the following nutritional parameters: triceps skinfold thickness, mid-arm muscle circumference, body mass index (BMI), serum albumin, prealbumin, and retinol binding protein. Triceps skinfold thickness and mid-arm muscle circumference values were

TABLE 1 Nutritional status of the patients studied. Values are mean (SEM)

	Controls (n=12)	Polyps (n=27)	Cancer (n=22)	p Value
TSF (%)	92.3 (7.3)	90.3 (6.1)	76.3 (10.2)	NS
MAMC (%)	112 (3.0)	111.3 (2.6)	107.1 (4.8)	NS
BMI (kg/m^2)	27.5 (1.18)	26.9 (0.69)	25.7 (1.08)	NS
Serum albumin (g/l)	46 (0.60)	45.7 (0.83)	39.2 (1.04)*	0.001
Prealbumin (mg/dl)	28.1 (1.1)	27.5 (1.3)	22.1 (1.76)*	0.02
RBP (ng/dl)	4.2 (0.15)	4.12 (0.22)	2.98 (0.31)*	0.004
Vitamin A (ratio)	1.07 (0.08)	1.04 (0.04)	1.15 (0.15)	NS
Vitamin E (ratio)	1068 (48.2)	1074.2 (41.5)	1075.4 (57.3)	NS
Vitamin C (mg/l)	7.5 (1.03)	8.5 (0.73)	5.9 (0.98)	NS

*Cancer v control and polyp groups. TSF=triceps skinfold thickness, MAMC=mid-arm muscle circumference, RBP=retinol binding protein.

TABLE II Per cent distribution of FAs in plasma phospholipids. Values are mean (SEM)

	Controls (n=12)	Polyps (n=27)	Cancer (n=22)	p Value
SFAs	44.9 (0.45)	45.7 (0.52)	47.3 (0.35)*	0.003
MUFAs	15.9 (0.53)	16.5 (0.47)	16.9 (0.53)	NS
n3-PUFA				
C18:3n3	0.11 (0.01)	0.12 (0.01)	0.10 (0.01)	NS
C20:5n3	0.70 (0.07)	0.73 (0.06)	0.46 (0.03)*	0.0001
C22:5n3	0.47 (0.05)	0.66 (0.05)	0.58 (0.06)	NS
C22:6n3	3.45 (0.15)	3.71 (0.21)	3.42 (0.19)	NS
n6-PUFA				
C18:2n6	18.95 (0.91)	17.35 (0.52)	16.6 (0.42)†	0.05
C18:3n6	0.19 (0.02)	0.15 (0.01)	0.17 (0.02)	NS
C20:2n6	0.42 (0.02)	0.49 (0.06)	0.43 (0.02)	NS
C20:3n6	2.84 (0.18)	2.89 (0.17)	2.90 (0.14)	NS
C20:4n6	10.2 (0.32)	9.95 (0.49)	9.38 (0.37)	NS
C22:4n6	1.69 (0.09)	1.58 (0.07)	1.45 (0.06)	NS
C22:5n6	0.14 (0.01)	0.17 (0.01)	0.20 (0.01)	NS
UI	138.9 (1.3)	137.8 (2.5)	130.7 (2.2)†	0.01

*Cancer v control and adenoma groups, †cancer v control group.

expressed as per cent of the median value of the reference population living in our area.²⁰

In addition, plasma vitamins A, E, and C concentrations were measured. The aliquot for vitamin C assay was mixed with 5% metaphosphoric acid at a 1:9 v/v ratio before freezing. Vitamins A and E were measured by high performance liquid chromatography and vitamin C by fluorescence spectrophotometry as described.^{21 22} Vitamin A was expressed as vitamin A:retinol binding protein molar ratio, and vitamin E as vitamin E:(cholesterol+triglycerides) molar ratio.

Statistical methods

Statistical analysis was performed using the Biomedical Data Processing statistical package, BMDP-PC90 (BMDP, Statistical Software, Los Angeles, CA, 1990).²³

Results were expressed as mean (SEM). For unpaired data, one way analysis of variance was used to assess differences between means. Levene's test was used to assess the equality of group variability. If variances were not assumed to be equal, the Brown-Forsythe test was used. Post-hoc pairwise comparisons were performed by the Tukey test to identify which group differences account for the significant overall *F* value. In this multiple comparison test the significance level is adjusted for the number of comparisons made.

Paired *t* test or the non-parametric Wilcoxon signed rank test were used to compare mucosal FAs values of adenomas and carcinomas with the values in adjacent normal mucosa.

As FA status cannot be measured by a single

TABLE III FAs (%) in normal mucosa from control, adenoma, and cancer patients. Values are mean (SEM)

	Controls (n=8)	Polyps (n=21)	Cancer (n=15)	p Value
SFAs	33 (1.11)	35.7 (0.69)	34.5 (0.54)	NS
MUFAs	34.6 (2.22)	31.4 (1.12)	33.8 (1.24)	NS
n3-PUFA				
C18:3n3	0.23 (0.02)	0.24 (0.04)	0.19 (0.02)	NS
C20:5n3	0.76 (0.18)	0.63 (0.08)	0.43 (0.07)	0.09
C22:6n3	2.71 (0.33)	2.90 (0.18)	2.41 (0.16)	NS
n6-PUFA				
C18:2n6	14.6 (0.80)	13.8 (0.47)	15.1 (0.84)	NS
C18:3n6	0.19 (0.04)	0.21 (0.03)	0.20 (0.05)	NS
C20:3n6	1.61 (0.14)	1.78 (0.08)	1.73 (0.14)	NS
C20:4n6	11.4 (0.88)	12.3 (0.55)	10.9 (0.57)	NS
C22:4n6	0.83 (0.08)	0.94 (0.05)	0.77 (0.05)	NS
UI	138.8 (0.08)	139.4 (2.7)	133.5 (2.2)	NS

variable, many variables must be considered together. This necessitates a large number of statistical comparisons, but it is the only satisfactory way of comparing FA data.

Significance was defined at $p < 0.05$. Marginal *p* values (< 0.10) are also described.

Results

Nutritional status

There were no significant differences in anthropometric parameters, BMI, serum proteins, and plasma vitamins between control and adenoma patients. Cancer patients, however, had significantly low values of serum albumin, prealbumin, and retinol binding protein compared with both control and adenoma groups (Table 1).

Fatty acid concentrations in plasma phospholipids

Table II describes FA concentrations in plasma phospholipids. There were no differences between control and adenoma groups. Cancer patients had significantly increased values of SFAs compared with both control and adenoma groups. In addition, both eicosapentaenoic (EPA, C20:5n3) and linoleic (C18:2n6) acid concentrations and the UI were significantly lower in the cancer group.

Mucosal fatty acids

Analysis of FAs could not be performed in 20% of the specimens because of technical reasons. As a consequence, about 30% of the paired comparisons could not be made. Thus, only 21 adenoma, 15 cancer, and eight control patients were included in this analysis. There were no significant differences in the clinical characteristics and FA values in plasma phospholipids between this subgroup and the whole group of patients.

Analysis of the normal looking mucosas obtained from patients with colorectal polyps and cancer showed no significant differences in the FA pattern compared with patients with normal colons. A trend was seen, however, to

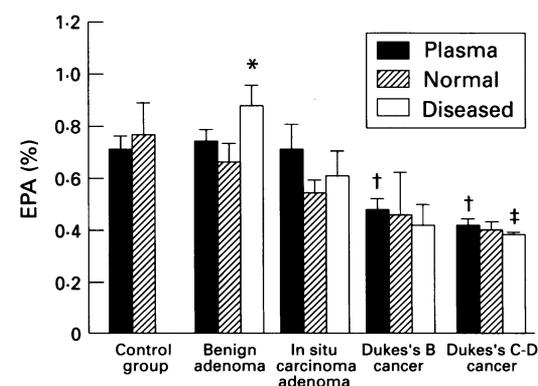


Figure 1: Changes of eicosapentaenoic acid (EPA; C20:5n3) content in plasma phospholipids, diseased mucosa, and adjacent normal mucosa of adenoma and carcinoma patients. Patients with normal colon constituted the control group. * $p = 0.04$ v associated normal mucosa, † $p < 0.05$ v plasma of both control group and benign adenoma, ‡ $p = 0.009$ v diseased mucosa of benign adenoma.

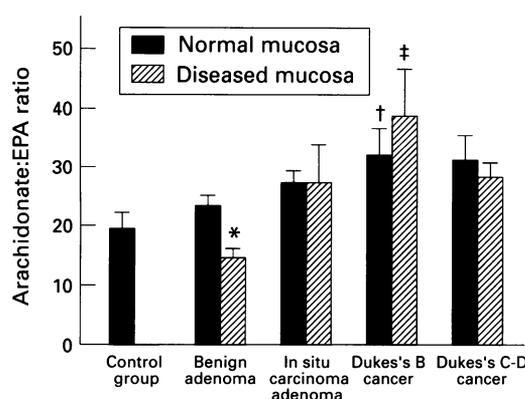


Figure 2: Ratio of arachidonic acid:eicosapentaenoic acid (EPA) in both diseased mucosa and adjacent normal mucosa of adenoma and carcinoma patients. Patients with normal colon constituted the control group. * $p=0.01$ v associated normal mucosa; † $p=0.05$ v control group; and ‡ $p=0.03$ v diseased mucosa of benign adenoma.

low EPA values in the cancer group (Table III, Fig 1), and the arachidonate:EPA ratio was significantly increased in cancer patients (Fig 2).

As described in Table IV, there were noticeable changes in mucosal FA status of both adenomas and colorectal cancer compared with their associated normal mucosa. In both groups, there was an increase in SFAs and a decrease in MUFAs (mainly caused by low values of oleic acid) in the diseased mucosa. n3-PUFA pattern in diseased mucosa was significantly different from that in paired normal mucosa in adenoma patients, with an increase of both EPA and DHA, and a decrease of α linolenic acid (C18:3n3). In cancer patients, only low values of α linolenic acid were seen. On the other hand, the diseased mucosa from colorectal cancer had significantly lower values of both EPA and DHA than diseased mucosa from adenomas.

Mucosal n6-PUFA pattern was also different in both adenomas and colorectal cancer compared with their associated normal mucosa (Table IV). In polyps, there was a significant increase in linoleic and dihomo-gammalinolenic (DGLA, C20:3n6) acids, and a decrease in arachidonic acid values in the diseased mucosa. On the other hand, cancer patients had higher concentrations of DGLA and adrenic acid (C22:4n6) in carcinoma mucosa than in the associated normal mucosa.

The comparison of the FA pattern in the

diseased mucosae of benign adenoma, adenoma with in situ carcinoma, Dukes's B and Dukes's C-D colorectal cancer disclosed significant differences in the concentrations of EPA, with a stepwise reduction of this FA from benign adenoma to the more advanced colon cancer (Table V, Fig 1). In addition, the arachidonate:EPA ratio was significantly higher in diseased mucosa from cancer patients (mainly in Dukes's B cancer) than in diseased mucosa from benign adenoma patients (Fig 2). A trend to low concentrations of DHA in Dukes's B and C-D cancer patients were also seen. On the other hand, the UI was significantly lower in Dukes's C-D cancer patients.

Discussion

This study shows for the first time that mucosal n3 and n6 FA status is considerably changed early in the colorectal adenoma-dysplasia-carcinoma sequence. Despite the fact that the assessment of the mucosal FA pattern could only be made in 70% of the patients, the number of cases was large enough to perform reliable statistical paired comparisons. Moreover, the subgroup of patients in whom this analysis was performed were representative of the whole series of patients, in terms of clinical features and plasma FA profile. On the other hand, interpretation of the results must be cautious as multiple statistical comparisons have been performed, a fact inherent to the studies on fatty acid status, and some of the observed significances might have arisen by chance. In this sense, only highly significant results and those with biological plausibility are discussed.

As the FA pattern in both plasma phospholipids and normal mucosa was not different between patients with either adenomatous polyps or normal colon, the observed changes in the FA status of the diseased mucosa from adenomas do not seem a consequence of differences in either fat intake or hepatic FA biosynthesis. Thus, changes of tissue lipid biochemistry might participate in the colon carcinogenesis processes. The observed changes, however, are not always readily interpretable on the basis of the present knowledge on mucosal FA metabolism. Tissue FA pattern may be the result of a combination of different phenomena including liver biosynthetic

TABLE IV FAs (%) in paired normal and diseased mucosa from colonic adenomas and colorectal carcinomas. Values are mean (SEM)

	Colon adenoma (n=21)			Colon cancer (n=15)		
	Normal mucosa	Diseased mucosa	p Value	Normal mucosa	Diseased mucosa	p Value
SFAs	35.7 (0.7)	37.3 (0.8)	0.001	34.5 (0.5)	38.0 (0.97)	0.0002
MUFAs	31.4 (1.12)	29.0 (0.8)	0.07	33.8 (1.2)	30.8 (1.06)	0.053
n3-PUFAs						
C18:3n3	0.24 (0.03)	0.14 (0.01)	0.001	0.19 (0.02)	0.13 (0.02)	0.002
C20:5n3	0.63 (0.07)	0.80 (0.09)	0.012	0.43 (0.07)	0.40 (0.07)†	NS
C22:6n3	2.90 (0.18)	3.22 (0.19)	0.06	2.40 (0.16)	2.61 (0.18)‡	NS
n6-PUFAs						
C18:2n6	13.8 (0.5)	14.9 (0.4)	0.02	15.1 (0.84)	13.8 (0.66)	NS
C18:3n6	0.21 (0.03)	0.23 (0.05)	NS	0.20 (0.05)	0.19 (0.04)	NS
C20:3n6	1.78 (0.08)	2.26 (0.18)	0.014	1.73 (0.14)	2.20 (0.24)	0.003
C20:4n6	12.3 (0.55)	11.2 (0.37)	0.02	10.9 (0.56)	10.7 (0.49)	NS
C22:4n6	0.94 (0.05)	0.94 (0.05)	NS	0.76 (0.05)	1.06 (0.14)	0.03
UI	139.4 (2.7)	138.6 (2.6)	NS	133.5 (2.2)	130.8 (2.6)*	NS

* $p=0.04$ v diseased mucosa of adenoma, † $p=0.002$ v diseased mucosa of adenoma, ‡ $p=0.03$ v diseased mucosa of adenoma.

TABLE V FAs (%) in diseased mucosa from patients with colon adenoma and colon cancer. Comparison of benign colon adenoma, adenoma with in situ carcinoma, Dukes's B, and Dukes's C–D colorectal cancer. Values are mean (SEM)

	Benign adenoma (n=15)	In situ carcinoma adenoma (n=6)	Dukes's B cancer (n=8)	Dukes's C–D cancer (n=7)	P Value
SFAs	37.8 (1.0)	35.8 (0.9)	37.1 (1.3)	39.0 (1.39)	NS
MUFAs	29.4 (0.97)	28.1 (1.55)	30.8 (1.72)	30.9 (1.31)	NS
n3-PUFAs					
C18:3n3	0.15 (0.02)	0.09 (0.02)	0.15 (0.04)	0.12 (0.02)	NS
C20:5n3	0.88 (0.11)	0.61 (0.14)	0.42 (0.12)	0.38 (0.03)*	0.009
C22:6n3	3.09 (0.22)	3.55 (0.36)	2.48 (0.19)	2.75 (0.32)	0.10
n6-PUFAs					
C18:2n6	14.4 (0.4)	16.1 (0.9)	14.2 (1.05)	13.4 (2.82)	NS
C18:3n6	0.26 (0.07)	0.18 (0.05)	0.22 (0.07)	0.15 (0.05)	NS
C20:3n6	2.30 (0.25)	2.17 (0.15)	2.55 (0.42)	1.81 (0.15)	NS
C20:4n6	10.6 (0.45)	12.4 (0.40)	10.9 (0.69)	10.4 (0.73)	NS
C22:4n6	0.94 (0.05)	0.95 (0.13)	1.10 (0.25)	1.01 (0.10)	NS
UI	135.8 (3.4)	145.3 (1.7)	133.1 (3.4)	128.2 (3.9)	0.05

*Dukes's C–D v benign adenoma, †Dukes's C–D v adenoma with in situ carcinoma.

activities, selective plasma FA uptake, esterification reactions, own synthetic activity, molecular selection of FAs in association with specific membrane proteins, and increased utilisation of particular FAs.²⁴ Whatever the mechanism, the findings of this study may be of relevance in the understanding of the role of lipids in colorectal carcinogenesis.

One of the most striking findings in this study is the appreciable change in mucosal EPA concentrations. There was a stepwise reduction of mucosal EPA values from benign adenoma to the most advanced colon cancer, mainly in the diseased mucosa, whereas plasma concentrations were only decreased in cancer patients. In addition, EPA content in adenoma tissue was increased compared with adjacent normal mucosa. These results reinforce the suggestion that long chain n3-PUFA (fish oil) may influence early stages of colon carcinogenesis.^{9 13 14} Recent work in humans shows that low dose fish oil supplementation (2.5 g/day for one month) normalises the rectal mucosal cell proliferation pattern in patients with sporadic colonic adenomas.¹⁴ In that study, doubling the rectal mucosal EPA content after supplementation was enough to significantly decrease the epithelial proliferative indices. However, whether the stepwise reduction of tissue EPA values in the adenoma-dysplasia-carcinoma sequence, as seen in this study, is related to increased rectal cytokinetics has to be evaluated.

Mucosal arachidonate values, expressed as µg/g of tissue, were reported to be significantly higher in colorectal cancer than in normal adjacent mucosa.¹⁰ In two other studies, however, there were no significant differences when expressed as a proportion of total FAs.^{11 25} In this study, values of this FA, also expressed as a proportion of total FAs, were not increased, but the mucosal arachidonate:EPA ratio was significantly higher in colon cancer compared with adenoma and control patients. Several studies have suggested that effects of PUFAs in tumour development and progression may be mediated by modulation of prostaglandin synthesis and metabolism.^{7 10–12 26} Arachidonic acid gives rise to the 2-series prostaglandin, EPA generates the 3-series prostaglandin, and DGLA produces the 1-series prostaglandin. Competitive effects occur between these precursor FAs in the

cyclooxygenase reaction. Therefore, a high arachidonate:EPA ratio implies more substrate for prostaglandin E₂ synthesis. In fact, increased local production of prostaglandin E₂ has been reported in human colon cancer and colonic adenomas compared with paired normal mucosa.^{10 25 27} In addition, it has been postulated that the inhibitory effect of n3-PUFA upon experimental colon cancer and human rectal epithelial proliferation may result from the suppression of excessive production of prostaglandin E₂.^{7 12–14} On the other hand, concentrations of DGLA were increased in both adenoma and cancer diseased mucosae compared with paired normal tissue. Thus, 1-series prostaglandin might also participate in colorectal carcinogenesis.

It has been recently shown that the cyclooxygenase 2 gene expression is considerably increased in most human colorectal cancers and in a subset of adenomas compared with the adjacent normal mucosa.²⁸ Therefore, changes in the tissue FA pattern of our patients would also be caused by differences in FA utilisation to generate eicosanoids.

Tissue PUFAs may also exert growth regulating functions by the inositol lipid cycle,²⁴ through the activation of protein kinase C.²⁹ Enrichment of cell membranes with n3 or n6-PUFAs have opposite effects on this signalling pathway in a human colon cancer cell line,³⁰ and it has been shown that arachidonate may stimulate cell proliferation by activating protein kinase C and other kinases.^{31 32} Recent data suggest that changes in the inositol lipid cycle may occur early in the adenoma-carcinoma sequence.³³ On the other hand, it has been shown that n3 and n6-PUFAs may have opposite regulatory effects on the activity and expression of the gene product of cellular ras proto-oncogenes.^{34 35}

An important physical property of cell membranes is their fluidity, one of the major factors regulating it being the unsaturation in the FAs chains.²⁴ Therefore, the finding of a decrease in the UI only in the patients with advanced colon cancer, suggests that cell membrane fluidity is reduced late in colon carcinogenesis. An increase of SFAs and a decrease of MUFAs tissue values seem to account for the low UI seen. Changes in fluidity may affect the efficiency of diverse metabolic processes that occur in the membrane matrix. Therefore, the role of membrane fluidity in tumour progression and dissemination should be studied.

In summary, although many questions remain, results of this study suggest that changes in tissue PUFA biochemistry may participate in the human colorectal carcinogenesis. Further investigation is necessary to determine the putative role of these changes and whether or not longterm dietary manipulation may modify human tumour growth and progression.

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Changes of the mucosal n3 and n6 fatty acid status occur early in the colorectal adenoma-carcinoma sequence.

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