

# Arachidonic acid and docosahexaenoic acid are increased in human colorectal cancer

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## Abstract

Increased arachidonic acid concentrations in experimental rodent colonic cancer have been described recently. In humans, a reduced erythrocyte stearic acid to oleic acid ratio has been reported in patients with colorectal cancer and it has been proposed that similar changes exist in the cancer tissue. The long chain fatty acids in the cancers of 15 patients with colorectal cancer were measured and compared with values in the unaffected mucosa. The values were expressed as mean (SD) mg fatty acid/g tissue and compared by analysis of variance. In the cancer tissue arachidonic acid was increased (0.703 (0.109) mg/g *v* 0.603 (0.127) mg/g,  $p < 0.05$ ) as was docosahexaenoic acid (0.211 (0.066) mg/g *v* 0.148 (0.039) mg/g,  $p < 0.001$ ). In contrast, the stearic acid to oleic acid ratio in the cancer tissue was increased rather than decreased, as previously suggested (0.36 (0.05) *v* 0.29 (0.7),  $p < 0.01$ ). Increased arachidonic acid and docosahexaenoic acid concentrations may be related to reduced lipid peroxidation, which is a feature of rapidly growing cells. Alternatively, the increased arachidonic acid values could be due to enhanced desaturase activity upon linoleic and linolenic acid, leading perhaps to increased formation of prostaglandins and other lipoxigenase products.

Colorectal cancer now represents the second commonest cause of death from cancer in Western countries and is strongly associated with a high fat intake, especially from animal sources.<sup>1,2</sup> It has been shown experimentally in rodents that a diet high in saturated fat (20%) strongly promotes the formation of colorectal tumours induced by chemical carcinogens

compared with a low saturated fat (5%) diet.<sup>3-7</sup> Analysis of cancer cell membrane fatty acids has shown a significant increase in the concentration of arachidonic acid compared with that in non-malignant mucosa in azoxymethane induced rodent colorectal cancer, irrespective of the amount or source of dietary fat.<sup>5,8,9</sup>

The latter observation may be of direct relevance to carcinogenesis, as arachidonic acid is the precursor of putative tumour promoting series 2 prostaglandins, and in particular of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>).<sup>10</sup> Indeed, reduced experimental tumour growth has been related to inhibition of tumour PGE<sub>2</sub> synthesis by indomethacin<sup>11</sup> and the methyl ester of eicosapentaenoic acid.<sup>12</sup>

There has also been some recent interest in the ratio of stearic acid to oleic acid in the red cell membranes of patients with colorectal cancer. In 1985, Wood *et al*<sup>13</sup> reported a reduced erythrocyte ratio in patients with colorectal cancer which was related to Dukes' staging, radical resection, and subsequent recurrence.<sup>13,14</sup> Moreover, a desaturating factor, supposedly showing delta-9-desaturase activity, which converts stearic acid to oleic acid, was isolated from the tissue, serum, and urine of cancer patients.<sup>15,16</sup> These authors argued that this desaturation was taking place in the cancer and could be used in cancer treatment.<sup>17</sup>

Despite the increasing interest in experimental systems, there are no published data of human colorectal cancer tissue. Therefore, we undertook the present study of colorectal tissue long chain fatty acids from patients undergoing elective surgery.

## Patients and methods

### PATIENTS

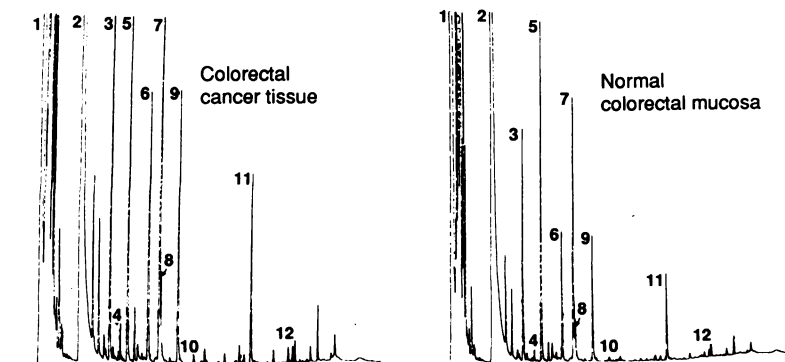
The study group consisted of 15 consecutive patients with colorectal cancer who presented to one surgeon (JPN) for elective surgery at Dudley Road Hospital, Birmingham. There were 10 women and five men with a mean age of 73.3 (range 58-88) years. Those patients presenting with anaemia, bowel obstruction, or weight loss of more than 10% were excluded. None of the patients were receiving chemotherapy or had received radiotherapy. All were omnivores on a free range diet. The sites of the cancers were rectum (n=11), caecum (n=2), and sigmoid colon (n=2). The cancer staging according to Dukes was: Dukes' A, one; Dukes' B, nine; and Dukes' C, five (two also with liver metastasis). Three of the tumours were well differentiated, nine were moderately well differentiated, and three were poorly differentiated.

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Chromatographs of human colorectal cancer tissue compared with unaffected mucosa. The peaks are as follows: 1=solvent front, 2=butylated hydroxytoluene, 3=palmitic acid, 4=palmitoleic acid, 5=margaric acid (internal standard), 6=stearic acid, 7=oleic acid, 8=cis-vaccenic acid, 9=linoleic acid, 10=alpha-linolenic acid, 11=arachidonic acid, and 12=docosahexaenoic acid.

## TISSUE

Immediately after resection the bowel was divided longitudinally to reveal the bowel lumen. Small slices of the cancer were obtained by sharp dissection and frozen in liquid nitrogen. The mucosa furthest away from the cancer was dissected off the muscle and frozen similarly. The tissue was stored in liquid nitrogen until analysis.

## SOURCES OF MATERIALS

Unless otherwise stated, all chemicals and reagents were of analar grade and were obtained from the Sigma Chemical Company Ltd, Poole, Dorset, UK.

## FATTY ACID ANALYSIS

Fatty acids were analysed by a modification of a previously described method.<sup>18</sup> After thawing, aliquots of tissue were weighed (200–1000 mg) and homogenised on ice in 10 ml of 0.1 mmol/l Tris HCl buffer (pH 8.9) in a Polytron homogeniser (setting=6) for 30 seconds. A 2 ml aliquot of tissue homogenate was placed in a screw topped glass tube to which was added 5 ml of methanol/6 mol/l HCl (5:1 by volume), and 0.2 ml of methanol containing 20 g/l of the antioxidant butylated hydroxytoluene, and the internal standard, margaric acid (17:0, 1 g/l). The fatty acids were transesterified by incubating at 90°C under nitrogen for four hours. After cooling, the fatty acid methyl esters were extracted into 8 ml of hexane and washed with saturated NaCl solution. The samples were dried over anhydrous sodium sulphate and evaporated to dryness under nitrogen at 37°C. The samples were then dissolved in 0.2 ml of hexane and sealed in brown glass ampoules. They were stored at –20°C and analysed within one week. Fatty acid standards were prepared as described previously.<sup>18</sup>

Fatty acid methyl esters were analysed by gas liquid chromatography using a Pye Unicam Series 304 chromatograph (Philips Scientific, Cambridge, UK) and a fused silica tube capillary column (50 m WCOT CPSil88, 0.33 mm internal diameter, Chrompak UK Ltd) with flame

ionisation detection. The gases used were helium as the carrier gas (2.7 bar; flow rate 0.483 ml/minute) with air and hydrogen for the detector. The injection temperature was set at 270°C for each run and the oven temperature was initially ramped at 2°C/minute from 150°C to 200°C, then ramped at 5°C/minute between 200°C and 240°C, and finally held for six minutes. Detection was at 300°C.

The fatty acids were identified from their retention times and based on the use of authentic fatty acid methyl ester standards. The coefficients of variation of the nine major fatty acid methyl esters of interest ranged from 0.9% to 3.6% for within batch variation and from 2.9% to 9.1% for inter batch variation.<sup>18</sup>

The results were expressed in absolute units – that is as mg fatty acid per g weight wet tissue, and were based on the mean of at least two traces. Relative values (%) are also given for comparison with other studies.

## STATISTICAL ANALYSIS

The data were entered into the University of Birmingham mainframe computer (IBM3090) and analysed by analysis of variance. The significance of the F ratios was determined from tables (Documenta-Geigy). Significance was taken as  $p < 0.05$ .

## Results

A typical chromatogram is shown in the Figure. The total fatty acid content in mucosa was mean (SD) 6.271 (1.515) mg/g compared with 6.581 (1.411) mg/g in the cancer (NS). The comparison of individual fatty acids, along with 95% confidence intervals of the values, is shown in the Table. The stearic acid:oleic acid ratios were mean (SD) 0.29 (0.07) in the mucosa compared with 0.36 (0.05) in the cancer tissue ( $p < 0.01$ ). The total polyunsaturated fatty acids in mucosa were 1.734 (0.334) mg/g compared with 1.905 (0.390) mg/g in the cancer (NS); similarly there was no significant difference for total unsaturated fatty acids – 4.22 (1.09) mg/g *v* 4.361 (0.0972) mg/g respectively.

Comparison of fatty acids in normal mucosa compared with those in cancer (n=15, paired samples). Values are mean (SD) (95% confidence intervals)

Fatty acid		Mucosa		Cancer	
		Fatty acid (mg/g tissue)	%	Fatty acid (mg/g tissue)	%
16:0	Palmitic acid	1.472 (0.374) (1.265, 1.680)	23.3 (1.7)	1.568 (0.331) (1.384, 1.753)	22.0 (5.7)
16:1 (n-7)	Palmitoleic acid	0.142 (0.075) (0.100, 0.184)	2.1 (0.8)	0.171 (0.086) (0.123, 0.219)	2.8 (1.3)
18:0	Stearic acid	0.577 (0.120) (0.511, 0.644)	9.4 (1.8)	0.695 (0.134)* (0.621, 0.769)	10.6 (1.1)
18:1 (n-9)	Oleic acid	2.080 (0.608) (1.743, 2.417)	32.8 (3.6)	1.954 (0.504) (1.674, 2.233)	29.6 (2.6)
18:1 (n-7)	Cis-vaccenic acid	0.264 (0.164) (0.174, 0.355)	4.0 (1.6)	0.314 (0.147) (0.232, 0.395)	4.4 (1.4)
18:2 (n-6)	Linoleic acid	0.959 (0.262) (0.814, 1.104)	15.4 (2.8)	0.991 (0.283) (0.834, 1.148)	14.8 (2.4)
18:3 (n-3)	Alpha linolenic acid	0.026 (0.018) (0.016, 0.035)	0.4 (0.3)	0.017 (0.012) (0.010, 0.0236)	0.3 (0.2)
20:4 (n-6)	Arachidonic acid	0.603 (0.127) (0.533, 0.673)	9.9 (2.1)	0.703 (0.109) (0.642, 0.763)	10.9 (1.7)
22:6 (n-3)	Docosahexaenoic acid	0.148 (0.039) (0.126, 0.169)	2.4 (0.7)	0.211 (0.066)** (0.174, 0.248)	3.1 (0.9)

\* $p < 0.05$ ; \*\* $p < 0.01$ .

## Discussion

To our knowledge, this is the first study to show compositional changes of fatty acids in human colorectal cancer. The fatty acid profile of the unaffected mucosa was similar to that of control subjects in a study of inflammatory bowel disease.<sup>19</sup> Also, the rectal mucosa fatty acid profile of patients undergoing surgery for haemorrhoidectomy was found not to differ from the normal rectal mucosa from patients with colorectal cancer ascertained by packed column chromatography, thereby excluding a mucosal lipid field change in these patients.<sup>9</sup> Thus, the use of non-malignant mucosal fatty acids as a comparison for cancer tissue fatty acids is valid. Moreover, as diet may have subtle influences on colonic mucosal fatty acid profiles of both rodents<sup>5 8 12 20 21</sup> and humans (rather than age or sex),<sup>9</sup> such a comparison will provide more precise information relating to cancer tissue per se.

Consistent with experimental data,<sup>5 8 9</sup> an increased arachidonic acid concentration was found in the cancer tissue. Given that this is the precursor of series 2 prostaglandins, it is relevant that PGE<sub>2</sub> has been shown to be increased in human colorectal cancer tissue.<sup>22</sup> Evidence suggests that PGE<sub>2</sub> in particular is a potent tumour promoter.<sup>10</sup> Not only has chemically induced colon carcinogenesis in rodents been significantly reduced by inhibitors of series 2 prostaglandins,<sup>11 12 23</sup> but the growth of the human colonic cell lines COLO-320 and HT-29 implanted into nude mice is also reduced by dietary n-3 lipids.<sup>24 25</sup> Furthermore, the reduced tumour growth of colonic tumours is related to reduced tumour content of arachidonic acid by dietary n-3 lipids.<sup>12 4 25</sup> Nevertheless, arachidonic acid and prostaglandins in colorectal cancer tissue obtained from patients remains to be established. In particular, it will be important to determine the activity of the cyclo-oxygenase systems involved as this is likely to be a more important determinant of prostaglandin synthesis than substrate availability.

In contrast to the hypothesis of Wood, Habib *et al.*,<sup>13-17</sup> the stearic acid:oleic acid ratio was not reduced but increased. This is perhaps not surprising given that this ratio in red cells was not found to be significantly different between patients with colorectal cancer and age and sex matched control subjects in two separate studies.<sup>26 27</sup> Although evidence was produced to support the reduced stearic acid:oleic acid red cell ratio in experimental colon cancer,<sup>17 28</sup> Nicholson<sup>9</sup> has shown that dietary influences predominate. Moreover, adipose tissue linoleic acid was inversely correlated with mucosal oleic acid<sup>9</sup> confirming the potent inhibitory effect of linoleic acid (only available from the diet) on delta-9-desaturase activity.<sup>29</sup>

An unexpected finding was the significant increase in docosahexaenoic acid in the cancer tissue. An important feature of rapidly growing cells is that they generally exhibit decreased lipid peroxidation while slowly dividing cells show increased values.<sup>30-32</sup> In particular, after partial hepatic resection, cycles of cell division are directly linked with considerably reduced concentrations of lipid peroxides.<sup>30-32</sup> Conversely,

the growth of cancer cells *in vitro* can be inhibited by stimulating lipid peroxidation.<sup>33-35</sup> Moreover, a number of cancer cell lines have been shown to lack important antioxidant systems.<sup>36</sup> The source of lipid peroxides is long chain polyunsaturated fatty acids, particularly arachidonic acid and docosahexaenoic acid. Increased tissue concentrations of these two fatty acids can be used as an important measure of reduced lipid peroxidation.<sup>37</sup> Therefore, the findings in this study are consistent with such a process taking place.

The basis for the increase in arachidonic acid values in the cancer tissue remains to be established. This increase could be due to increased conversion because of enhanced desaturase activity upon the precursors linoleic acid and bis homo-gamma-linolenic acid.<sup>38</sup> Alternatively, there may be decreased utilisation of arachidonic acid to form lipid peroxides or prostaglandins and leukotrienes. Clearly a combination of these processes may be occurring: thus it is possible that there is both increased delta-6-desaturase activity and prostanoid production along with decreased lipid peroxidation.

In conclusion, differences in the fatty acid content of human colorectal cancer tissue have been described. This study therefore justifies further investigations in order to determine the mechanism involved.

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