Abnormal plasma polyunsaturated fatty acid pattern in non-active inflammatory bowel disease

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Abstract

An abnormal plasma polyunsaturated fatty acid pattern (PUFA) (increased n3 and decreased n6 PUFA) has been reported in active inflammatory bowel disease (IBD). The possibility of a primary defect in the PUFA metabolism in IBD was hypothesised. The aim of this study was to assess plasma PUFA pattern in inactive inflammatory bowel disease and to ascertain whether patients who had had a colectomy and who were suffering from ulcerative colitis have a similar PUFA pattern than those patients with non-active ulcerative colitis and who had not had a colectomy. Plasma fatty acids were analysed by semi-capillary column gas-liquid chromatography in three groups of patients with inactive IBD (24 patients with inactive ulcerative colitis who had not had a colectomy, 15 patients with ulcerative colitis who had had a colectomy, and 27 patients with Crohn's disease). Plasma concentration and percentage of C22:6n3 and unsaturation index were significantly higher in patients with inactive ulcerative colitis without a colectomy and the Crohn's disease group ($p<0.0001$) than in controls. Plasma concentration and percentage of C22:6n3 and the unsaturation index remained significantly higher, in both the operated and non-operated ulcerative colitis patients when compared with controls ($p<0.0001$). These results suggest that in inactive IBD, an increased PUFA biosynthesis might be the cause of the high values of n3 compounds. These findings although seen in active disease, are more noticeable in remission because of the lack of artefactual factors (malnutrition, steroids, inflammation). In addition, persistence of high values in both groups of ulcerative colitis patients – that is, those who had had a colectomy and those who had not suggests the existence of a primary abnormality in the PUFA metabolism in IBD. (*Gut* 1993; 34: 1370-1373)

Polyunsaturated fatty acids (PUFA) participate in membrane function,^{1,2} including that of the immune cells^{3,4} and they are precursors of the eicosanoids.⁵⁻⁸ For these reasons, they may play an important part in the pathogenesis of inflammatory bowel disease (IBD).

We have described an abnormal PUFA pattern in active IBD.⁹ This consists of increased values of the precursor (C18:3n3) and the end product (C22:6n3) of the n3 series and decreased values of dihommo- γ -linolenic acid (C20:3n6). A stepwise decrease in PUFA values, which was more noticeable for the n6 series was also seen as the disease became more severe. Even in severe

disease, however, plasma concentrations of the n3 PUFA remained higher than those in healthy controls. These findings suggested that in IBD there might be a primary increase in PUFA biosynthesis. In active IBD this phenomenon would be partly counterbalanced by an increased fatty acid utilisation because of factors associated with disease activity, such as inflammation, hypermetabolism, malnutrition, and steroid treatment.

On the basis of the above findings, it could be hypothesised that in the absence of other factors, that is in inactive disease, the values of long chain PUFA would be even higher. Therefore, the study of patients with inactive IBD would be of interest. In addition, to ascertain the possible existence of a primary defect in PUFA metabolism, the study of patients with ulcerative colitis who had had a colectomy would be particularly important, as in such patients, the target organ has been removed. The aim of this study was to assess prospectively the plasma fatty acid pattern in patients with inactive IBD (both ulcerative colitis and Crohn's disease), and in ulcerative colitis patients after colectomy.

Patients and methods

PATIENTS

Three groups of 66 patients with inactive IBD were included in the study: 27 Crohn's disease (16 men, 11 women; median age 24 years; ranges 14-78 years), 24 ulcerative colitis patients (10 men, 14 women; median age 41 years; range: 23-75 years) and 15 patients with ulcerative colitis who had had a colectomy, four procto-

TABLE 1 Clinical features of the patients

	Patients with UC (no colectomy) (n=24)	Patients with UC (colectomy) (n=15)	CD (n=27)
<i>Location of the disease in previous attacks</i>			
Ulcerative colitis			
Rectum	6		
Left colon	12		
Universal	6	15	
Crohn's disease			
Small bowel			7
Colon			11
Ileum + colon			9
<i>Current drug treatment</i>			
Metronidazole	0	2	4
Sulphasalazine	16	0	9
5-ASA	8	0	4
<i>Nutritional state¹²</i>			
Well nourished	23	12	24
Kwashiorkor like	0	1	2
Marasmus	1	2	1
Mixed malnutrition	0	0	0

UC=ulcerative colitis, CD=Crohn's disease.

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TABLE II Plasma fatty acids in inactive inflammatory bowel disease

$\mu\text{mol/dl}$	UC (n=24)	CD (n=27)	Controls (n=107)	p
16:0*	155.6 (6.1)	126.0 (4.9)	128.9 (3.4)	0.0012
16:1†	8.5 (6.6–12.9)	10.8 (6.8–15.3)	8.4 (8.1–9.5)	0.5641
18:0*	60.1 (2.8)	51.9 (2.3)	53.2 (1.3)	0.0649
18:1*	118.3 (7.0)	104.2 (5.4)	110.6 (3.2)	0.3042
18:2n6*	169.1 (9.0)	138.4 (6.5)	137.6 (3.9)	0.0030‡
18:3n3†	2.1 (0.0–5.9)	0.0 (0.0–5.5)	0.0 (0.0–0.0)	0.0499‡
18:3n6†	0.0 (0.0–1.6)	0.0 (0.0–1.8)	0.0 (0.0–0.0)	0.2223
20:2n6†	0.5 (0.0–2.1)	0.0 (0.0–2.2)	1.0 (0.0–1.8)	0.9429
20:3n6*	15.6 (0.9)	13.7 (0.8)	13.3 (0.5)	0.1212
20:4n6*	52.0 (2.6)	43.6 (1.9)	40.6 (1.4)	0.0021‡
20:5n3†	1.8 (0.0–3.1)	1.3 (0.0–2.7)	0.7 (0.0–1.7)	0.6049
22:6n3*	16.75 (11.71–19.75)	11.6 (11.17–19.68)	7.0 (6.1–7.9)	<0.00001‡§

*Mean (SEM). One way ANOVA+Scheffé test; †median (95% CI), Kruskal-Wallis test+Mann-Whitney U test; ‡UC v controls; §CD v controls; ||CD v UC. Other abbreviations as in Table I.

TABLE III Plasma fatty acids in inactive inflammatory bowel disease

%	UC (n=24)	CD (n=27)	Controls (n=107)	p
16:0*	25.7 (0.3)	24.3 (0.4)	25.2 (0.2)	0.0515
16:1†	1.4 (0.9–2.1)	2.2 (1.3–3.1)	1.7 (1.5–1.9)	0.1456
18:0*	9.9 (0.2)	10.0 (0.3)	10.5 (0.1)	0.0811
18:1*	19.4 (0.7)	20.0 (0.6)	21.7 (0.3)	0.0061‡
18:2n6*	27.8 (0.9)	26.9 (1.0)	27.2 (0.4)	0.7817
18:3n3†	0.3 (0.0–1.0)	0.0 (0.0–0.9)	0.0 (0.0–0.0)	0.0815
18:3n6†	0.0 (0.0–0.2)	0.0 (0.0–0.3)	0.0 (0.0–0.0)	0.2480
20:2n6†	0.1 (0.0–0.3)	0.0 (0.0–0.4)	0.1 (0.0–0.3)	0.5297
20:3n6*	2.5 (0.1)	2.6 (0.1)	2.6 (0.1)	0.9068
20:4n6*	8.6 (0.3)	8.4 (0.3)	8.0 (0.2)	0.4427
20:5n3†	0.2 (0.0–0.5)	0.2 (0.0–0.6)	0.2 (0.0–0.3)	0.8298
22:6n3†	2.6 (1.9–3.5)	2.4 (2.3–3.3)	1.4 (1.3–1.6)	<0.00001‡§
UNID*	141.1 (1.7)	140.7 (1.8)	131.5 (1.1)	0.00010‡§

*Mean (SEM). One way ANOVA+Scheffé test; †median (95% CI), Kruskal-Wallis test+Mann-Whitney U test; ‡UC v controls; §CD v controls. UNID=unsaturation index. Other abbreviations as in Table I.

colectomy and 11 colectomy plus ileal pelvic pouch (eight men, seven women; median age 31 years, range: 15–46 years). Patients were considered inactive as assessed by the Truelove and Witts' index¹⁰ for ulcerative colitis and the Van Hess' index¹¹ for Crohn's disease. No patient with ileal pelvic pouch had clinical, endoscopic, or histological signs of pouchitis. Table I shows the clinical features of the patients studied.¹² The results of the fatty acid state of all of these patients had been reported previously in active phase.⁹

CONTROLS

A previously reported group of 107 healthy, well nourished subjects (46 men, 61 women; median age: 32.5 years; ranges 18–76 years) acted as a control group.⁹

Informed consent was obtained from each patient and healthy controls. The study was performed in conformance with the 1975 Declaration of Helsinki ethical guidelines and was approved by the Research and Ethical Committees of the Hospital.

PLASMA FATTY ACID ASSAY

In all patients and healthy controls, a 5 ml venous blood sample for plasma fatty acid measurement was taken after a 14 hour overnight fast. Blood samples in all ulcerative colitis and Crohn's disease patients were obtained three months after they had been considered inactive and in ulcerative colitis patients who had had a colectomy between three to six months after completion of all operations. The methods used

for plasma fatty acid assay have been described in detail elsewhere.^{9,13,14} Heptadecanoic acid (C17:0) was added as an internal standard to allow fatty acid concentrations in plasma total lipids to be determined as absolute values, and not only as percentages.

Fatty acid from 14:0 to 22:6n3 were measured. Unidentified peaks accounted for less than 0.5% of the total fatty acid. Results are expressed as a total fatty acid concentration ($\mu\text{mol/dl}$) and as a percentage distribution of each fatty acid. The unsaturation index (UNID) was calculated according to the formula¹⁵:

$$\text{UNID} = \sum (\text{fatty acid percentage} \times \text{number of double bonds}).$$

STATISTICAL ANALYSIS

For statistical analysis the Statistical Package for Social Sciences SPSS/PC+ (SPSS Inc, Chicago, Illinois, 1985) was used.¹⁶ Variables with normal distribution and homogeneous variance were compared by means of parametric tests, otherwise their non-parametric counterparts were used. One way analysis of variance with 'a posterior' Scheffé test or Kruskal-Wallis one way analysis of variance by ranks were used. When the Kruskal-Wallis test disclosed a significant p value, the Mann-Whitney U test was carried out to detect where the differences occurred. Results are expressed as mean (SEM) or median and the 95% confidence intervals (CI)¹⁷ for parametric and non-parametric variables respectively. Because age distribution was different among the groups studied and this is a factor influencing plasma lipids,¹⁸ the results were adjusted for the effect of age by means of analysis of variance using age as a covariate.

Results

PLASMA FATTY ACIDS IN INACTIVE INFLAMMATORY BOWEL DISEASE

Tables II and III detail respectively the plasma fatty acid concentration and the percentages of the different fatty acids in patients with inactive ulcerative colitis and Crohn's disease.

The most striking finding in the plasma fatty acid profile in inactive IBD was the considerable increase of docosahexaenoic acid (C22:6n3), the final product of the n3 series, both in ulcerative colitis and Crohn's disease when compared with controls. This fact was seen when the results were expressed either as absolute concentration or as a percentage.

Arachidonic acid (C20:4n6), the main product of the n6 series, showed the same tendency. Statistical significance was only obtained, however, between the ulcerative colitis patients and controls, when the values were expressed as absolute plasma concentration. Absolute plasma values of linoleic acid (C18:2n6), the essential precursor of the n6 series, and α -linolenic acid (C18:3n3), the essential precursor of the n3 series, were also increased in ulcerative colitis (Table II).

As a consequence of the increase in long chain and highly unsaturated PUFA, the values of the

TABLE IV Plasma fatty acids in inactive ulcerative colitis. Influence of the colectomy on the plasma fatty acid pattern

$\mu\text{mol/dl}$	UC patients		Controls (n=107)	p
	Operated (n=15)	Non-operated (n=24)		
16:0*	121.3 (7.3)	155.6 (6.1)	128.9 (3.4)	0.0013§
16:1†	6.6 (1.0–10.3)	8.5 (6.6–12.9)	8.4 (8.1–9.5)	0.1631
18:0*	52.2 (2.2)	60.1 (2.8)	53.2 (1.3)	0.0615
18:1*	90.1 (6.6)	118.3 (7.0)	110.6 (3.2)	0.0319
18:2n6*	147.5 (8.6)	169.1 (9.0)	137.6 (3.9)	0.0037§
18:3n3†	0.0 (0.0–4.9)	2.1 (0.0–5.9)	0.0 (0.0–0.0)	0.0808
18:3n6†	0.0 (0.0–1.9)	0.0 (0.0–1.6)	0.0 (0.0–0.0)	0.3176
20:2n6†	0.0 (0.0–2.3)	0.5 (0.0–2.1)	1.0 (0.0–1.8)	0.7593
20:3n6*	12.7 (1.4)	15.6 (0.9)	13.3 (0.5)	0.1082
20:4n6*	45.4 (2.4)	52.0 (2.6)	40.6 (1.4)	0.0022§
20:5n3†	0.0 (0.0–2.3)	1.8 (0.0–3.1)	0.7 (0.0–1.7)	0.6853
22:6n3*	13.9 (9.6–16.1)	16.7 (11.7–19.7)	7.0 (6.1–7.9)	<0.00001‡§

*Mean (SEM). One way ANOVA+Scheffé test; †median (95% CI), Kruskal-Wallis test+Mann-Whitney U test; ‡operated v controls; §non-operated v controls; ||operated v non-operated.

TABLE V Plasma fatty acids in inactive ulcerative colitis. Influence of the colectomy on the plasma fatty acid pattern

%	UC patients		Controls (n=107)	p
	Operated (n=15)	Non-operated (n=24)		
16:0*	24.4 (0.5)	25.7 (0.3)	25.2 (0.2)	0.2147
16:1†	1.2 (0.2–2.1)	1.4 (0.9–2.1)	1.7 (1.5–1.9)	0.0236‡
18:0*	10.7 (0.3)	9.9 (0.2)	10.5 (0.1)	0.2266
18:1*	18.1 (0.7)	19.4 (0.7)	21.7 (0.3)	0.0002‡§
18:2n6*	29.9 (1.0)	27.8 (0.9)	27.2 (0.4)	0.0918
18:3n3†	0.3 (0.0–1.2)	0.3 (0.0–1.0)	0.0 (0.0–0.0)	0.1199
18:3n6†	0.0 (0.0–0.4)	0.0 (0.0–0.2)	0.0 (0.0–0.0)	0.3530
20:2n6†	0.0 (0.0–0.4)	0.1 (0.0–0.3)	0.1 (0.0–0.3)	0.3782
20:3n6†	2.6 (2.0–3.3)	2.4 (2.2–2.9)	2.5 (2.3–2.8)	0.9194
20:4n6†	9.1 (8.3–10.6)	8.6 (7.9–9.9)	7.8 (7.1–8.7)	0.0625
20:5n3†	0.0 (0.0–0.9)	0.2 (0.0–0.5)	0.2 (0.0–0.3)	0.9017
22:6n3†	2.3 (1.8–2.8)	2.6 (1.9–3.5)	1.4 (1.3–1.6)	<0.00001‡§
UNID*	142.0 (1.7)	141.7 (1.7)	131.5 (1.1)	<0.00001‡§

*Mean (SEM). One way ANOVA+Scheffé test; †median (95% CI), Kruskal-Wallis test+Mann-Whitney U test; ‡operated v controls; §non-operated v controls. UNID=unsaturation index.

UNID were also significantly high, both in inactive ulcerative colitis and Crohn's disease, compared with controls (Table III).

Changes in saturated and monoenic fatty acids depended on a diminished percentage of oleic acid (C18:1n9) and an increased concentration of palmitic acid (C16:0) in ulcerative colitis with respect to the control group and an increased percentage of palmitoleic acid (C16:1) in Crohn's disease (Table III).

After adjusting for age as described in the statistical methods section, the significance of the differences seen did not change.

INFLUENCE OF COLECTOMY FOR ULCERATIVE COLITIS ON PLASMA FATTY ACID PATTERN (Tables IV and V)

Plasma docosahexaenoic acid (C22:6n3), either as a percentage or an absolute concentration, was significantly higher both in operated and non-operated ulcerative colitis patients when compared with controls. In addition, when expressed as plasma concentration, C22:6n3 was significantly higher in non-operated than in operated patients (Table IV). UNID was also significantly increased in both groups of patients compared with controls (Table V).

In the n6 series, the plasma concentration of linoleic (C18:2n6) and arachidonic acid (C20:4n6) was only significantly increased in non-operated ulcerative colitis patients with

respect to the control group, although the same trend was seen for the operated group.

The increase of plasma long chain PUFA content in both groups of patients was associated with a significant decrease in the percentage of oleic acid (C18:1n9) in both groups of patients and by the reduction in the percentage of palmitoleic acid (C16:1n7) in operated patients (Table V).

After adjusting for the effect of age, the results did not change except for the oleic acid concentration, which showed no differences among the three groups ($p=0.151$).

Discussion

Before discussing the results of this study, an aspect of the methods used has to be noted. Fatty acid values in this paper are expressed both as a percentage of the total fatty acids and as an absolute plasma concentration. Expressing the results as plasma concentration permitted us to show that any change in a given fatty acid is real, whereas its percentage may only reflect compensatory changes because of increases or decreases in other fatty acids. The results, however, should also be given as a percentage distribution. This provides complementary information, as it helps to ascertain whether changes in the plasma concentrations result in variations in the relative amounts of fatty acids – that is, in qualitative changes of plasma lipids.

In a previous paper,⁹ we reported increased n3 and decreased n6 PUFA values in active IBD. It was then hypothesised that in active IBD a primary enhancement in PUFA synthesis coexisted with an increased utilisation related to the inflammatory phenomena. In this series of inactive IBD patients, plasma n3 PUFA are also increased. This, together with high values of n6 PUFA and UNID, in the absence of factors associated with active disease, further supports this hypothesis. The fact that high n3 PUFA values occur in both active and non-active disease can be explained because n3 series has the highest affinity for PUFA biosynthetic enzyme systems.^{19,20}

The increase in n6 long chain PUFA is particularly noticeable in non-active, non-operated ulcerative colitis patients, their values being significantly higher than those in healthy controls. Also, significantly increased values of the precursors of both n3 (C18:3n3) and n6 (C18:2n6) series were seen in this group of patients. As a high intake of essential fatty acids, particularly of α -linolenic acid (C18:3n3), was ruled out in all cases, this finding could be attributed to a negative feedback effect^{21–23} upon δ -6 desaturase activity, caused by the excessive amount of products of both n3 (C22:6n3) and n6 series (C20:4n6). From these data, it could be speculated that the hypothesis of an increased PUFA biosynthesis is more active in ulcerative colitis than in Crohn's disease patients.

Differences in age distribution may account, at least in part, for the differences seen in plasma fatty acid concentrations between operated and non-operated patients and between ulcerative colitis patients and controls. After adjusting for

age as a covariate, however, the significance of the results did not change, except for oleic acid concentration, which showed no differences between operated and non-operated ulcerative colitis patients.

Malnutrition may be another important factor that explains fatty acid differences among groups because PUFA deficiency has been described in malnourished patients.^{24,25} Malnutrition only occurred, however, in four (10%) ulcerative colitis and three (11%) Crohn's disease patients in this series. Moreover, vitamin and trace element deficiencies, which have been reported in active IBD,^{26,27} could be additional factors influencing PUFA biosynthesis. Unfortunately, data on the micronutrient state in this series of non-active patients are not available.

A further argument in favour of the existence of an increased PUFA biosynthesis in IBD is the persistence of increased values of docosahexaenoic acid (C22:6n3) three to six months after colectomy in patients with ulcerative colitis. In addition, this finding suggests that it may be because of a primary metabolic defect. Although three to six months seems to be enough time to reach a stable state after colectomy, the possibility that plasma PUFA pattern took longer to return to normal has to be ruled out in future studies. Thus, a study comparing larger groups of patients with both proctocolectomy and colectomy plus ileal pouch patients, at least two years after the completion of surgery, has to be performed to confirm these results.

On the other hand, the hypothesis of a genetically determined primary defect in PUFA metabolism in IBD should be assessed by measuring PUFA profile in healthy relatives of IBD patients.

These results further support our reluctance to use long chain n3 PUFA in treatment for either acute attacks of IBD or for maintenance treatment of the disease.

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