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Association of *n*-3 and *n*-6 long-chain polyunsaturated fatty acids in plasma lipid classes with inflammatory bowel diseases

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In order to establish the biochemical basis for dietary interventions, we investigated the fatty acid composition of plasma lipid classes in patients with inactive inflammatory bowel disease. In this cross-sectional study thirty patients with ulcerative colitis (UC), twenty-one with Crohn disease (CD) and twenty-four controls were investigated (mean age: UC, 40·8 (SD 12·1); CD, 37·6 (SD 11·0); control, 31·5 (SD 8·4) years). Fatty acid composition of plasma lipids was determined by high-resolution capillary GLC. In plasma phospholipids, significantly higher values of eicosapentaenoic (20 : 5*n*-3), docosapentaenoic (22 : 5*n*-3) and γ-linolenic (18 : 3*n*-6) acids were found in control patients and patients with UC as compared to patients with CD [median % (weight by weight), control *v.* UC *v.* CD : 20 : 5*n*-3, 0·09 (interquartile range (IQR) 0·05) *v.* 0·14 (IQR 0·10) *v.* 0·16 (IQR 0·10), $P<0·05$; 22 : 5*n*-3, 0·14 (IQR 0·10) *v.* 0·27 (IQR 0·16) *v.* 0·31 (IQR 0·10), $P<0·001$; 18 : 3*n*-6, 0·02 (IQR 0·02) *v.* 0·03 (IQR 0·02) *v.* 0·05 (IQR 0·03), $P<0·05$]. When compared to the control, values of the principal *n*-3 and *n*-6 long-chain PUFA, arachidonic acid (20 : 4*n*-6) and DHA (22 : 6*n*-3) were significantly higher in patients with UC but not in patients with CD [median % (w/w), UC *v.* control: 20 : 4*n*-6, 8·43 (IQR 3·23) *v.* 6·92 (IQR 2·96), $P<0·05$; 22 : 6*n*-3, 1·22 (IQR 0·56) *v.* 0·73 (IQR 0·39), $P<0·05$]. As seen there are considerable differences between the long-chain PUFA status of patients suffering from UC or CD. The data obtained in the present study do not support the concept of eicosapentaenoic acid or DHA deficiency in patients with either UC or CD.

Crohn disease: Inflammatory bowel disease: Polyunsaturated fatty acids: Ulcerative colitis

Our knowledge concerning the primary cause and aetiology of inflammatory bowel disease (IBD) is still limited; like in many chronic diseases, the aetiology appears to be multifactorial.

Recent evidence indicates that disturbances of fatty acid status may be among the metabolic consequences of IBD. In patients with IBD, malnutrition develops as a result of reduced energy or protein intake or as a consequence of losing nutrients, or as a combination of these factors (Han *et al.* 1999). On the one hand, malnutrition has been associated with essential fatty acid deficiency (Decsi *et al.* 1998) and, on the other hand, epidemiologic studies suggest that PUFA may figure in the pathophysiology of IBD (Shoda *et al.* 1996).

The *n*-3 PUFA, eicosapentaenoic acid (20 : 5*n*-3), serves as a precursor of eicosanoids with limited inflammatory effects; moreover, abundant availability of 20 : 5*n*-3 reduces the production of the pro-inflammatory 2-series eicosanoids generated from arachidonic acid (20 : 4*n*-6; Belluzzi, 2004). In addition, *n*-3 PUFA including DHA (22 : 6*n*-3) regulate the production of some inflammatory cytokines and down-regulate the expression of a number of genes involved in inflammation (Teitelbaum & Walker, 2001; Gil, 2002); hence, changes in metabolism of *n*-3 PUFA may be of relevance in the activity of the chronic inflammatory processes in IBD.

Modification of the inflammatory process in IBD via altering the availability of the eicosanoid precursor PUFA may offer clinical benefits for the patients. However, only a few studies have addressed the question of the availability of fatty acids in patients with ulcerative colitis (UC) or Crohn disease (CD), and most studies (Esteve-Comas *et al.* 1992, 1993; Kuroki *et al.* 1997) analysed only total plasma lipid composition which is a less reliable indicator of essential fatty acid status than fatty acid composition of individual plasma lipid classes. To the best of our knowledge, the present study is the first to describe the fatty acid composition of plasma NEFA, phospholipids (PL), sterol esters and TAG lipids in patients with IBD.

Patients and methods

In this cross-sectional study thirty patients with inactive UC and twenty-one with CD as well as twenty-four carefully selected, clinically healthy, age-, sex-, weight- and

Abbreviations: CD, Crohn disease; IBD, inflammatory bowel disease; LCPUFA, long-chain PUFA; PL, phospholipids; UC, ulcerative colitis.

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height-matched control subjects were investigated in the First Department of Internal Medicine of the University of Pécs, Pécs, Hungary. The study was carried out in accordance with the Declaration of Helsinki II and with approval of the ethics committee of the University of Pécs. Informed written consent was obtained from each patient and control subject.

The diagnosis of UC or CD was based on histological investigations in all cases. At the time of the study, all patients were on a normal diet and no special dietary intervention was followed. Disease activity was controlled by small-dose maintenance therapy (4–8 mg methilprednisolon/d and 250–750 mg sulfasalasine/d). Members of the control group included health care providers (nurses, physicians, medical students, laboratory assistants) who received no medication and had no history of suffering from lipid disorders or absorption insufficiencies. The diet of the control subjects was assessed by a trained dietitian. The controls followed the common Hungarian diet (energy intake 9–9·5 MJ/d; protein 15–20 %; carbohydrates 45–55 %; lipids 30–35 %). Dietary histories regarding a 4-week-long period prior to the study revealed no significant differences between the energy, macro- and micronutrient intakes of the controls and IBD patients.

Anthropometric measurements were carried out by the same investigator. Body height and weight were measured in the survey unit with validated medical care instruments, and BMI was computed as weight (kg) divided by squared height (m^2).

Both in the healthy control subjects and IBD patients, venous blood samples were taken from the antecubital vein into tubes containing 2 mg/ml EDTA as anticoagulant between 08.00 and 08.30 hours, after an overnight fast. This strict postalimental time schedule was maintained in order to preclude the diet-induced dynamic changes of plasma fatty acids. The plasma was removed within 30 min and stored at −80°C until analysis. All samples were thawed only once.

TAG and cholesterol were determined with an enzymatic kit (Boehringer Mannheim, Mannheim, Germany). HDL-cholesterol was measured by the precipitation method of Steele *et al.* (1976). Platelet number was determined with an automatic cell counter (Celldyn 3700 Abbott optical LASER) and C-reactive protein concentrations with the LASER nepherlometric Beckman Immage machine in the Clinical Biochemical Institute of the University of Pécs, Pécs, Hungary.

Fatty acid analyses were carried out in the Department of Paediatrics, University of Pécs, Pécs, Hungary. For the analysis of plasma fatty acid profiles, plasma samples were melted and lipids were extracted by the addition of 3 ml chloroform and 1 ml methanol. The mixture was shaked vortically at 2000 rpm for 10 min, the underlayer was aspirated into vials and evaporated under nitrogen stream. The four fatty acid internal standards (dipentadecanoil acid, tripentadecanoin acid, cholesteryl-pentadecanoate acid and pentadecanoic acid) were added. All vials were reconstituted in 70 μl chloroform. The solvents for TLC of plasma lipids were as follows: hexane–diethyl ether–chloroform–acetic acid (21:6:3:1, by vol.) followed by chloroform–methanol–water (65:25:4, by vol.). The bands were stained with dichlorofluorescein, removed by scraping and transesterified in 3 M-HCl–methanol solution at 84°C for 45 min.

Fatty acids were analysed by high-resolution capillary GLC using a Finnigan 9001 gas chromatograph (Finnigan/Trometrics Inc., Austin, TX, USA) with split injection (ratio of 1:25), automatic sampler (A200SE; CTC Analytic, Zwingen, Switzerland) and flame-ionisation detector with a DB-23 cyanopropyl column of 40 m length (J & W Scientific, Folsom, CA, USA). The temperature programme was the following: temperature of injector at 80°C for 0·1 min, temperature increase by 180°C/min up to 280°C, temperature of detector at 280°C, temperature of column area at 60°C for 0·2 min, temperature increase by 40°C/min up to 180°C, 5 min isotherm period, temperature increase by 1·5°C/min up to 200°C, 8·5 min isotherm period, temperature increase by 40°C/min up to 240°C and 13 min isotherm period. The constant linear velocity was 0·3 m/s (referred to 100°C). Peak identification was verified by comparison with authentic standards. Fatty acid results were expressed as percentages (weight by weight) of fatty acids detected with a chain length between twelve and twenty-four carbon atoms.

Statistical analysis

Results were evaluated with SPSS for Windows, release 11.5 (SPSS Inc., Chicago, IL, USA). All data except fatty acids are presented as means and standard deviations and were evaluated by ANOVA followed by the least significant difference comparison test to compare mean values of subgroups. The fatty acid data are presented as median and range from the first to the third quartile values, because skewed distributions were found in several parameters, especially in fatty acids present at low concentrations. Fatty acids were analysed by the Kruskal–Wallis non-parametric ANOVA followed by Mann–Whitney's two-sided rank test to compare median values of subgroups. Results were regarded as statistically significant at $P<0·05$.

Results

The clinical features of the study subjects are shown in Table 1. The ages of the patients with UC were significantly higher than those of the controls. In patients with CD, the values of total cholesterol were significantly lower, whereas those of C-reactive protein were significantly higher compared with healthy controls.

The fatty acid composition of plasma NEFA, PL and TAG lipids are shown in Tables 2, 3 and 4, respectively. Because nearly no significant differences were seen in plasma sterol esters, these values are not presented in tabulated form but mentioned in the text only.

SFA

Patients with either UC or CD had significantly lower values of palmitic acid (16 : 0) in plasma PL, stearic acid (18 : 0) and eicosanoic acid (20 : 0) values in plasma TAG, and total SFA in plasma PL and TAG than the corresponding values of healthy controls.

Values of 16 : 0 in NEFA and TAG as well as values of 18 : 0 in NEFA were significantly lower in patients with CD than in healthy controls.

Table 1. Clinical characteristic of patients with ulcerative colitis and Crohn disease and healthy controls (Mean values and standard deviations)

	Healthy control (*n* 24)		Ulcerative colitis (*n* 30)		Crohn disease (*n* 21)	
	Mean	SD	Mean	SD	Mean	SD
Gender (male/female)	9/15		15/15		11/10	
Age (years)	31·5	8·8	40·8*	12·1	37·6	11·0
Weight (kg)	72·9	15·4	72·1	15·3	69·2	14·7
Height (cm)	171·2	11·7	169·8	9·5	170·9	11·9
BMI (kg/m^2)	24·6	2·5	24·7	3·9	23·6	3·4
TAG (mmol/l)	1·55	0·77	1·45	0·94	1·63	0·83
Total cholesterol (mmol/l)	5·61	0·94	5·48	1·20	4·75*	0·95
HDL-cholesterol (mmol/l)	1·58	0·39	1·57	0·35	1·60	0·45
C-reactive protein (mg/l)	3·41	1·61	8·48	14·01	12·81*	12·51
Platelet count (× 1000/mm^3)	288	39	279	76	287	66

Mean values were significantly different from those of the control group: *$P<0·05$.

Table 2. Fatty acid composition (% weight per weight) of plasma NEFA in patients with ulcerative colitis, Crohn disease and healthy controls

(Median values and interquartile range)

	Control (*n* 21)		Ulcerative colitis (*n* 30)		Crohn disease (*n* 24)	
Fatty acid	Median	IQR	Median	IQR	Median	IQR
SFA						
16 : 0	29·99	5·12	26·38*	3·81	27·34	5·39
18 : 0	12·64	3·94	19·57*	14·07	16·81	8·44
20 : 0	0·52	0·27	0·47	0·35	0·47	0·33
Sum of SFA	46·25	4·06	50·26	12·63	45·36	12·69
cis-MUFA						
18 : 1*n*-7	25·22	5·06	26·07	8·49	28·37	6·82
18 : 1*n*-9	1·74	0·28	1·78	0·65	1·75	0·97
Sum of MUFA‡	29·50	5·30	29·55	9·29	32·23	6·63
trans-Fatty acids						
16 : 1,t	0·64	0·36	0·59	0·38	0·51	0·38
18 : 1*n*-9,t	3·99	1·11	4·42	3·22	3·97	2·05
18 : 2*n*-6,tt	1·01	0·45	0·95	0·49	0·79*	0·43
Sum of *trans*-fatty acids	5·92	1·47	6·27	3·71	5·61	2·25
n-6 PUFA						
18 : 2*n*-6	14·87	4·99	9·56**	5·85	12·27	7·05
18 : 3*n*-6	0·16	0·15	0·21	0·31	0·15	0·26
20 : 2*n*-6	0·25	0·09	0·23	0·14	0·27	0·16
20 : 3*n*-6	0·20	0·09	0·11*	0·13	0·13*	0·15
20 : 4*n*-6	1·09	0·55	0·43*	0·37	0·56*	0·55
22 : 4*n*-6	0·07	0·08	0·07	0·07	0·06	0·07
Sum of *n*-6 LCPUFA§	1·67	0·59	1·01*	0·63	1·09*	0·07
Sum of *n*-6 PUFA¶	16·78	5·89	11·15*	5·68	13·23	7·67
n-3 PUFA						
18 : 3*n*-3	0·26	0·15	0·24	0·23	0·27	0·30
20 : 3*n*-3	0·17	0·08	0·14	0·09	0·11	0·09
20 : 5*n*-3	0·05	0·08	0·02	0·08	0·05	0·05
22 : 5*n*-3	0·05	0·08	0·05	0·11	0·06	0·06
22 : 6*n*-3	0·11	0·10	0·09	0·11	0·08	0·13
Sum of *n*-3 LCPUFA¶	0·44	0·29	0·37	0·30	0·32	0·43
Sum of *n*-3 PUFA‡‡	0·71	0·37	0·62	0·43	0·72	0·50

IQR, interquartile range; LCPUFA, long-chain PUFA.
Mean values were significantly different from those of the control group: *$P<0·05$; **$P<0·001$.
‡ Sum of MUFA = 16 : 1*n*-7 + 18 : 1*n*-7 + 18 : 1*n*-9 + 20 : 1*n*-9 + 22 : 1*n*-9 + 24 : 1*n*- 9.
§ Sum of *n*-6 LCPUFA = 20 : 2*n*-6 + 20 : 3*n*-6 + 20 : 4*n*-6 + 22 : 4*n*-6 + 22 : 5*n*-6.
‖ Sum of *n*-6 PUFA = sum of *n*-6 LCPUFA + 18 : 2*n*-6 + 18 : 3*n*-6.
¶ Sum of *n*-3 LCPUFA = 20 : 3*n*-3 + 20 : 5*n*-3 + 22 : 5*n*-3 + 22 : 6*n*-3.
‡‡ Sum of *n*-3 PUFA = sum of *n*-3 LCPUFA + 18 : 3*n*-3.

Table 3. Fatty acid composition of plasma TAG in patients with ulcerative colitis, Crohn disease (CD) and healthy controls (Median values and interquartile range)

	Control (*n* 21)		Ulcerative colitis (*n* 30)		Crohn disease (*n* 24)	
Fatty acid	Median	IQR	Median	IQR	Median	IQR
SFA						
16 : 0	31·31	6·97	25·45*	4·44	27·42	7·99
18 : 0	7·93	3·78	4·82**	1·93	4·67**	0·95
20 : 0	0·32	0·28	0·21**	0·06	0·19**	0·07
Sum of SFA	42·04	10·06	33·10**	5·08	33·50*	7·68
cis-MUFA						
18 : 1*n*-7	32·69	4·99	36·31**	5·25	36·70*	5·77
18 : 1*n*-9	1·74	0·50	1·93	0·40	1·79	0·78
Sum of MUF0A‡	37·01	4·31	41·14**	5·13	41·19*	5·04
trans-Fatty acids						
16 : 1,t	0·81	0·21	0·73	0·41	0·69	0·35
18 : 1*n*-9,t	5·00	2·40	2·71*	2·12	1·97**	1·79
18 : 2*n*-6,tt	1·03	0·46	0·59*	0·56	0·67*	0·44
Sum of *trans*-fatty acids	6·71	2·67	4·33**	2·65	2·95**	2·39
n-6 PUFA						
18 : 2*n*-6	11·14	8·36	19·19*	5·52	17·81*	7·40
18 : 3*n*-6	0·13	0·10	0·20	0·22	0·23*	0·15
20 : 2*n*-6	0·16	0·07	0·25**	0·08	0·27*	0·13
20 : 3*n*-6	0·09	0·05	0·15**	0·08	0·16*	0·10
20 : 4*n*-6	0·42	0·24	0·62**	0·31	0·77**	0·32
22 : 4*n*-6	0·04	0·02	0·06**	0·02	0·09**	0·03
Sum of *n*-6 LCPUFA§	0·76	0·30	1·12**	0·37	1·24**	0·39
Sum of *n*-6 PUFA‖	11·88	8·71	20·80**	5·75	20·00*	7·02
n-3 PUFA						
18 : 3*n*-3	0·20	0·16	0·23	0·11	0·27*	0·17
20 : 3*n*-3	0·04	0·03	0·07	0·05	0·06	0·03
20 : 5*n*-3	0·01	0·01	0·02*	0·02	0·03**	0·02
22 : 5*n*-3	0·01	0·02	0·05**	0·04	0·06**	0·03
22 : 6*n*-3	0·04	0·02	0·07**	0·04	0·05*	0·05
Sum of *n*-3 LCPUFA¶	0·11	0·10	0·20**	0·14	0·25**	0·11
Sum of *n*-3 PUFA‡‡	0·31	0·20	0·45*	0·24	0·53*	0·26

IQR, interquartile range; LCPUFA, long-chain PUFA.
Mean values were significantly different from those of the control group: *$P<0·05$; **$P<0·001$.
‡ Sum of MUFA = 16 : 1*n*-7 + 18 : 1*n*-7 + 18 : 1*n*-9 + 20 : 1*n*-9 + 22 : 1*n*-9 + 24 : 1*n*- 9.
§ Sum of *n*-6 LCPUFA = 20 : 2*n*-6 + 20 : 3*n*-6 + 20 : 4*n*-6 + 22 : 4*n*-6 + 22 : 5*n*-6.
‖ Sum of *n*-6 PUFA = sum of *n*-6 LCPUFA + 18 : 2*n*-6 + 18 : 3*n*-6.
¶ Sum of *n*-3 LCPUFA = 20 : 3*n*-3 + 20 : 5*n*-3 + 22 : 5*n*-3 + 22 : 6*n*-3.
‡‡ Sum of *n*-3 PUFA = sum of *n*-3 LCPUFA + 18 : 3*n*-3.

cis-*MUFA*

Values of vaccenic acid (18 : 1*n*-7) and those of total MUFA in plasma TAG were significantly higher in patients with UC and CD than in controls.

Trans-isomeric fatty acids

Compared with healthy controls, values of *trans*-octadecadienoic acid (18 : 2*n*-6,tt) were significantly lower in patients with UC and CD in plasma PL and TAG and in patients with CD in plasma NEFA. Values of *trans*-octadecenoic acid (18 : 1*n*-9,t) and values of total *trans*-isomeric fatty acids were significantly lower in patients with UC and CD in plasma TAG compared with healthy controls.

n-*6 PUFA*

In plasma PL, the values of γ-linolenic acid (18 : 3*n*-6) and docosatetraenoic acid (22 : 4*n*-6) were significantly higher in patients with UC and CD than in the controls, whereas the values of 20 : 4*n*-6 and the sum of *n*-6 PUFA were significantly higher in patients with UC only. In plasma TAG, values of linoleic acid (18 : 2*n*-6), eicosadienoic acid (20 : 2*n*-6), dihomo-γ-linolenic acid (20 : 3*n*-6), 20 : 4*n*-6, 22 : 4*n*-6, the sum of *n*-6 PUFA and the sum of *n*-6 long-chain PUFA (LCPUFA) were significantly higher in patients with UC and CD compared with healthy controls. In contrast, a different picture was seen in plasma NEFA, where the values of 20 : 3*n*-6, 20 : 4*n*-6 and the sum of LCPUFA were significantly lower in patients with UC and CD than in healthy controls. Values of 18 : 2*n*-6 and the sum of *n*-6 PUFA were also significantly lower in patients with UC compared with healthy controls in plasma NEFA. Values of 20 : 2*n*-6 and 20 : 3*n*-3 were significantly lower in patients with UC compared with healthy controls in plasma sterol esters (data not shown).

n-*3 PUFA*

In plasma PL, docosatrienoic acid (20 : 3*n*-3) values were significantly higher in patients with UC and CD and 22 : 6*n*-3 values were higher in patients with UC compared with control subjects. In plasma TAG, values of α-linolenic acid (18 : 3*n*-3)

Table 4. Fatty acid composition of plasma phospholipids in patients with ulcerative colitis, Crohn disease and healthy controls

(Median values and interquartile range)

	Control (*n* 21)		Ulcerative colitis (*n* 30)		Crohn disease (*n* 24)	
Fatty acid	Median	IQR	Median	IQR	Median	IQR
SFA						
16 : 0	35·92	4·42	31·47**	3·46	32·65*	3·41
18 : 0	17·59	2·48	17·28	2·12	16·69	1·74
20 : 0	0·66	0·13	0·62	0·25	0·67	0·39
Sum of SFA	55·46	4·87	52·17**	3·87	52·03*	4·93
cis-MUFA						
18 : 1*n*-7	7 8·33	1·72	8·25	1·24	8·78	1·03
18 : 1*n*-9	1·06	0·19	1·18	0·31	1·08	0·37
Sum of MUFA‡	11·44	1·44	11·84	2·06	12·18	1·90
trans-Fatty acids						
16 : 1,t	0·13	0·03	0·12	0·05	0·13	0·04
18 : 1*n*-9,t	0·49	0·19	0·44	0·52	0·43	0·37
18 : 2*n*-6,tt	0·33	0·16	0·22*	0·18	0·22*	0·15
Sum of *trans*-fatty acids	1·00	0·32	0·88	0·69	0·71	0·53
n-6 PUFA						
18 : 2*n*-6	19·91	5·67	21·57	7·49	19·71	4·44
18 : 3*n*-6	0·02	0·02	0·03*†	0·02	0·05**	0·03
20 : 2*n*-6	0·42	0·15	0·38	0·10	0·37	0·15
20 : 3*n*-6	2·06	0·79	2·11	0·93	2·01	1·00
20 : 4*n*-6	6·92	2·96	8·43*	3·23	8·02	3·85
22 : 4*n*-6	0·15	0·10	0·22**	0·14	0·26**	0·13
Sum of *n*-6 LCPUFA§	9·65	3·33	11·42	4·30	10·79	3·44
Sum of *n*-6 PUFA‖	29·40	4·35	32·91*	7·33	32·27	5·88
n-3 PUFA						
18 : 3*n*-3	0·05	0·04	0·06	0·03	0·07	0·04
20 : 3*n*-3	0·27	0·08	0·21*	0·08	0·21*	0·06
20 : 5*n*-3	0·09	0·05	0·14*	0·10	0·16*	0·10
22 : 5*n*-3	0·14	0·10	0·27**	0·16	0·31**	0·10
22 : 6*n*-3	0·73	0·39	1·22*	0·56	0·92	0·82
Sum of *n*-3 LCPUFA¶	1·26	0·48	1·96*	0·79	1·73*	1·11
Sum of *n*-3 PUFA‡‡	1·33	0·47	2·01**	0·79	1·64*	1·10

IQR, interquartile range; LCPUFA, long-chain PUFA.
Mean values were significantly different from those of the control group: *$P<0·05$; **$P<0·001$.
Mean value was significantly different from that of the Crohn disease group: †$P<0·05$.
‡ Sum of MUFA = 16: 1*n*-7 + 18 : 1*n*-7 + 18 : 1*n*-9 + 20 : 1*n*-9 + 22 : 1*n*-9 + 24 : 1*n*- 9.
§ Sum of *n*-6 LCPUFA = 20 : 2*n*-6 + 20 : 3*n*-6 + 20 : 4*n*-6 + 22 : 4*n*-6 + 22 : 5*n*-6.
‖ Sum of *n*-6 PUFA = sum of *n*-6 LCPUFA + 18 : 2*n*-6 + 18 : 3*n*-6.
¶ Sum of *n*-3 LCPUFA = 20 : 3*n*-3 + 20 : 5*n*-3 + 22 : 5*n*-3 + 22 : 6*n*-3.
‡‡ Sum of *n*-3 PUFA = sum of *n*-3 LCPUFA + 18 : 3*n*-3.

were significantly higher in patients with CD and values of 22 : 6*n*-3 were significantly higher in patients both with UC and CD compared with healthy subjects. In plasma PL and TAG, values of 20 : 5*n*-3, docosapentoic acid (22 : 5*n*-3), the sum of *n*-3 LCPUFA and the sum of *n*-3 PUFA were significantly higher in patients with UC and CD than in healthy controls.

Ratios

In plasma NEFA, the ratios of 20 : 4*n*-6/20 : 3*n*-6 and 20 : 4*n*-6/18 : 2*n*-6 were significantly lower in patients with UC and CD compared with healthy controls (Fig. 1). In plasma NEFA and PL, the ratios of *n*-6/*n*-3 LCPUFA were significantly lower in patients with UC compared with healthy controls (Fig. 2). In plasma PL, the *n*-6/*n*-3 PUFA ratios were significantly lower in patients with UC than in healthy controls (17·83 (SD 8·49) *v.* 20·99 (SD 9·63), $P<0·05$). No significant differences were seen in the ratios in the other three lipid classes investigated.

Discussion

IBD is a condition known to be accompanied by various nutritional disturbances including impaired LCPUFA status. The potentially reduced availibility of certain LCPUFA may be of particular significance because they are known to be integral parts of cell membranes and they are precursors of important eicosanoids, which participate in inflammatory responses. The hypothesis has been put forward that changes in the metabolism of LCPUFA in IBD may be of relevance in maintaining the chronic inflammatory condition (Kuroki *et al.* 1997).

Contradictory findings concerning the availability LCPUFA in CD were reported both in adult patients and children. As far as the paediatric age-group is concerned, Socha *et al.* (2005) recently reported significantly lower values of 18 : 2*n*-6 and 20 : 4*n*-6 in children with IBD compared with healthy controls, while values of 18 : 3*n*-3 were higher in patients than in controls. Levy *et al.* (2000) found significantly lower values of 18 : 2*n*-6 in children with CD compared with healthy controls.

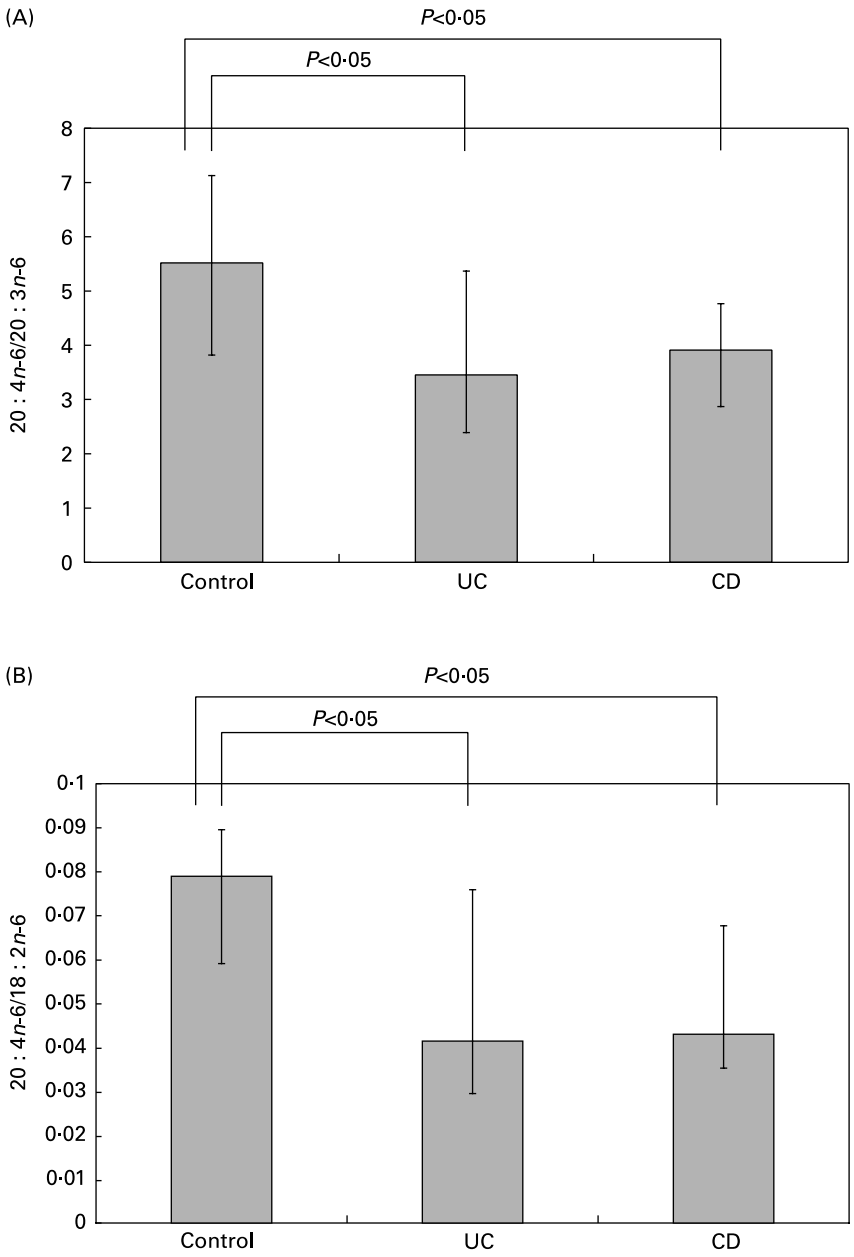

Fig. 1. The ratio of arachidonic acid (20 : 4*n*-6) to dihomo-γ-linolenic acid (20 : 3*n*-6) (A) and the ratio of 20 : 4*n*-6 to linoleic acid (18 : 2*n*-6) (B) in NEFA in patients suffering from ulcerative colitis (UC, *n* 30) or Crohn disease (CD, *n* 24) and in healthy controls (*n* 21). Values are median with the interquartile range depicted by vertical bars.

To complicate matters, Trebble *et al.* (2003) reported lower 18 : 2*n*-6 and 18 : 3*n*-3 values in children with active than in those with inactive CD; unfortunately, the values of patients were not compared with those of healthy children.

As far as adult patients are concerned, Siguel & Lerman (1996) found higher values of SFA and MUFA, but lower values of *n*-3 and *n*-6 PUFA in patients with various chronic intestinal disorders compared with control subjects. Kuroki *et al.* (1997) reported lower serum values of 20 : 4*n*-6, 20 : 5*n*-3 and 22 : 6*n*-3, as well as a significantly increased ratio of *n*-6 PUFA to *n*-3 PUFA in patients with CD when compared to controls.

In contrast, Esteve-Comas *et al.* (1992) reported elevated plasma values of 18 : 3*n*-3 and 22 : 6*n*-3 and decreased values of 20 : 3*n*-6 in active IBD patients compared to controls.

Later on, Esteve-Comas *et al.* (1993) reported significantly increased *n*-3 LCPUFA values in patients both with IBD and CD, together with significantly increased values of *n*-6 LCPUFA in inactive patients with UC compared with controls.

It is to be noted that fatty acid composition of plasma total lipids has been used as indicator of fatty acid status in many previous studies (Esteve-Comas *et al.* 1992, 1993; Siguel &

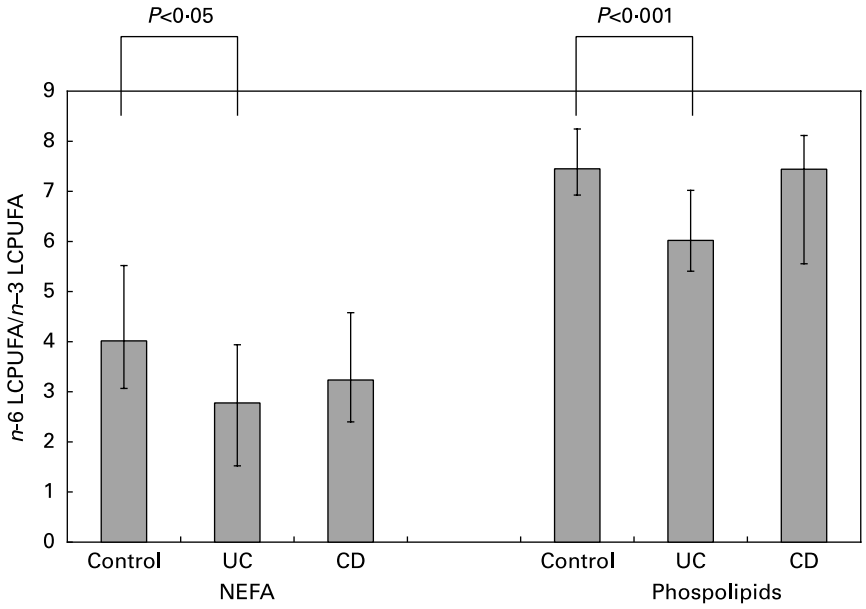

Fig. 2. The ratio of *n*-6 to *n*-3 long-chain PUFA (LCPUFA) in plasma NEFA and phospholipids in patients suffering from ulcerative colitis (UC, *n* 30) or Crohn disease (CD, *n* 24) and in healthy controls (*n* 21). Values are median with the interquartile range depicted by vertical bars.

Lerman, 1996; Kuroki *et al.* 1997; Levy *et al.* 2000), and dyslipidaemia may profoundly influence that parameter. In the present study, we investigated plasma lipid classes separated by TLC. Nevertheless, the results obtained in the present study showed also significant differences in plasma fatty acid patterns in patients with IBD compared to healthy controls. However, somewhat different pictures were seen in lipids with different biological roles. In either plasma PL and TAG, significantly higher values of various *n*-3 and *n*-6 LCPUFA including the eicosanoid precursors (20 : 5*n*-3, 20 : 3*n*-6 and 20 : 4*n*-6), which may play an important role in the pathogenesis of IBD, were seen in patients with either UC or CD as compared to controls. Though a different picture was seen in NEFA, namely significantly lower values of both 20 : 3*n*-6 and 20 : 4*n*-6 in patients either with UC or CD than in controls; however, the contributions of LCPUFA to NEFA is much smaller than to PL. Hence, the data obtained in the present study do not support the concept of the deficiency of PUFA or LCPUFA in patients with IBD.

Previous studies reporting significantly lower values of *n*-3 PUFA in patients with IBD than in controls (Siguel & Lerman, 1996; Kuroki *et al.* 1997) gave rise to dietary interventions with fish oil rich in 20 : 5*n*-3 and 22 : 6*n*-3 (Lorenz *et al.* 1989; Aslan & Triadafilopoulos, 1992; Hawthorne *et al.* 1992; Stenson *et al.* 1992). However, the studies investigating the effects of *n*-3 PUFA supplementation in IBD patients yielded partly contradictory results. On the one hand, the steroid-sparing effect (Aslan & Triadafilopoulos, 1992; Hawthorne *et al.* 1992), significantly improved activity score of the disease (Aslan & Triadafilopoulos, 1992), significant gain in body weight (Stenson *et al.* 1992) indicated clinical benefits of supplementation. On the other hand, no significant steroid-sparing effect (Stenson *et al.* 1992), failure in prevention of clinical relapse (Hawthorne *et al.* 1992) and unchanged clinical activity (Lorenz *et al.* 1989) were also described. The contradictory findings of the various studies reported may be due to methologic differences, the different activity of the disease in the patients studied, or different nutritient intakes of the patients.

Eicosanoid synthesis utilises NEFA, i.e. the NEFA pool of plasma lipids may provide some information on the demand of eicosanoid precursors. In the present study, both 20 : 3*n*-6 and 20 : 4*n*-6 values were significantly lower in patients with IBD than in controls.

Moreover, the *n*-6 to *n*-3 PUFA ratio, thought to be related to the availability of precursors for inflammatory and anti-inflammatory eicosanoid production, was significantly lower in patients with UC than in controls. It is tempting to speculate that patients with IBD utilised more 20 : 4*n*-6 than controls. Indeed the significantly lower ratios of 20 : 4*n*-6 to 20 : 3*n*-6 and 20 : 4*n*-6 to 18 : 2*n*-6 appear to support this concept.

In summary, the data obtained in the present study indicate not reduced but abundant contribution of both *n*-6 and *n*-3 LCPUFA to the major plasma lipid classes together with some indirect sign of enhanced utilisation of *n*-6 LCPUFA in patients with IBD. Hence, reduction of dietary *n*-6 PUFA intakes may be an alternative to *n*-3 LCPUFA supplementation in attempting to modify inflammatory processes in IBD.

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