CHUGOKUGAKUEN J. 2007
Vol. 6, pp. 11-16

CHUGOKUGAKUEN Journal
http://www.cjc.ac.jp/

Original Article

Dietary Intake, Fatty Acid Profile in Plasma and Neutrophil Phospholipids, and Serum Antioxidant Levels in Patients with Crohn's Disease

Yuko Kawakami[1] and Hiroyuki Okada[2]

[1]*Department of Human Nutrition, Chugokugakuen University, Okayama 701-0197, Japan*
[2]*Department of Medicine and Medical Science, Okayama University Graduate School of Medicine and Dentistry, Okayama 700-8558, Japan*

To elucidate the efficacy of dietary therapy for Crohn's disease, we analyzed dietary intake, fatty acid composition of phospholipids in plasma and neutrophils, serum fat-soluble vitamin levels, and oxygen radical absorbance capacity in twenty Crohn's disease patients (15 males and 5 females, 11 with ileitis and 9 with ileocolitis, 30.6±8.2 year), who were treated at the Department of Gastroenterology of Okayama University Hospital. Total fat intake, fat energy ratio and linoleic acid intake were significantly lower, while protein and carbohydrate intakes were significantly higher, in the patients than in age and sex-matched controls. In the neutrophil phospholipids of Crohn's disease patients, significantly higher levels of total n-6 polyunsaturated fatty acid and lower levels of docosahexaenoic acid were observed. The concentrations of serum retinol and β-carotene but not α-tocopherol were significantly lower and serum oxygen radical absorbance capacity was also lower than in the controls. Significant correlations between serum oxygen radical absorbance capacity and zinc ($r=0.797$, $p<0.001$) concentrations were observed in the Crohn's disease patients. A diet restricting the intake of n-6 polyunsaturated fatty acid and antioxidative trace minerals and vitamins may be recommended for the nutritional management of Crohn's disease patients.

Key Words: Crohn's disease, diet, fatty acid, trace mineral, fat-soluble vitamin, neutrophil fatty acid

Introduction

Nutrition may play an important role in the pathogenesis and treatment of inflammatory bowel diseases (IBD) [1, 2, 3]. Several studies suggest an association between dietary factors and the onset of Crohn's disease (CD), [3] however, few have examined the relationship between dietary intake and relapse of CD. A low intake of dietary fiber [4] or high intake of meat, or protein [5] may be implicated in the relapse of CD. Although the pathogenesis of inflammatory bowel diseases is not fully understood, many suspect that diet and various dietary factors may play a modulating role in the disease process [6].

Various epidemiological, experimental and clinical data suggest that the immune response may be sensitive to changes in dietary fatty acid composition [7] because long chain polyunsaturated fatty acids (PUFA) are the precursors of eicosanoids, which participate in the regulation of immunological and inflammatory responses. The balance between oxidant and antioxidant systems is suggested to be

[1]Corresponding author.
Yuko kawakami
Department of Human Nutrition, Faculty of Contemporary Life Science, Chugokugakuen University, 83 Niwase Okayama 701-0197, Japan
Tel & Fax: +81 86 293 1074

important in the pathogenesis and maintenance of tissue injury in CD. Reduced plasma antioxidant concentrations and increased oxidative DNA damage in patients with Crohn's disease and Ulcerative Colitis (UC) were reported [8].

Adequate nutritional management of CD as a moderator of intestinal inflammation may be required for remission of the disease. In this study, therefore, to elucidate the efficacy of dietary therapy for CD, we analyzed the qualitative and quantitative dietary intake, fatty acid composition of phospholipids in plasma and neutrophils, serum levels of fat-soluble vitamins, retinol, β-carotene and α-tocopherol, and Oxygen Radical Absorbance Capacity (ORAC), and evaluated the effects of nutritional factors on the clinical parameters.

Methods

Subjects

Twenty CD patients were examined in the present study. They were all outpatients treated at the Department of Gastroenterology of Okayama University Hospital. Of the patients, 11 had ileitis and 9 had ileocolitis. The diagnosis of CD was based on accepted clinical, radiographic, endoscopic, and histologic criteria [9]. All patients were in remission. Nineteen patients were medicated with 5-aminosalicylic acid (5-ASA), 2 with azathioprin and 15 with an elemental diet at the time of the study. Five patients consumed regular diets. Control subjects were age and sex-matched healthy volunteers, 12 males and 5 females (29.4 ± 13.1 year).

This experiment was performed in accordance with the principles of the Declaration of Helsinki of the World Medical Association, and informed consent was obtained from all the subjects. This study was approved by our institutional review board.

Estimation of dietary intake

Dietary intake was assessed using a food-frequency questionnaire. Energy, major nutrients and fatty acid consumption were calculated using a method reported by Suzuki et al [10].

Fatty acid analysis

Fasted blood was drawn into tubes containing disodium EDTA. Plasma was separated after the centrifugation of blood samples at 1,600x g for 15 min at 4℃ and stored at −80℃ until assayed. To obtain neutrophils, a 3.5-ml sample of undiluted blood was layered onto 3ml of Mono Poly Resolving Medium (Dainippon Pharmaceuticals, Osaka, Japan) and centrifuged at 400x g for 20min at 4℃. The neutrophil layer was drawn off into another tube. The neutrophils were washed 3 times with ice-cold saline, and stored at −80℃ prior to use.

Total lipid was extracted from plasma and neutrophils suspended in 0.5ml of saline according to the method of Bligh and Dyer [11]. Total phospholipid was separated by one-dimensional thin-layer chromatography using silica gel plates (Silica Gel 60, Merck, Darmstadt, Germany) and a solvent system of petroleum ether/ethyl ether/acetic acid (80: 20: 1, v/v/v). The spots corresponding to phospholipid were scraped from the plates and transmethylated for 2h at 85℃ with 2ml of acetyl-chloride methanol (5: 50, v/v). The fatty acid composition of total phospholipid was analyzed according to a method described previously [12].

Analysis of fat-soluble vitamins in plasma

Retinol, α-tocopherol and carotenoids were extracted from plasma and analyzed using the method reported by Milne et al. [13]. Briefly, 1ml of methanol was added to each sample (0.2ml of plasma plus 0.8ml of distilled water) and extracted twice with 3ml of dichloromethane and 6ml of n-hexane. After shaking and centrifugation (3,000rpm, 10℃, 15min), the upper layer was collected and evaporated under reduced pressure. The residue was dissolved in 50 μl of acetonitrile-dichloromethane-methanol (70: 20: 10, v/v/v), and a 50-μl aliquot was injected into the HPLC system. The system was composed of a reverse-phase column (YMC s-5 120A ODS, 6×150mm, Yamamura Chemical Co., Kyoto, Japan) and integrator (CR-4A, Shimadzu Co., Kyoto, Japan). The injected sample was eluted isocratically with acetonitrile-dichloromethane-methanol (70: 20: 10, v/v/v), at a flow rate of 1ml/min, with detectors set at 290nm, 350nm and 450nm. Retinol, α-tocopherol, and β-carotene were quantified by determining the peak areas calibrated against known amounts of standards.

Oxygen radical absorbance capacity (ORAC) assay

Serum total antioxidant capacity was measured using the manual version of the oxygen radical absorbance capacity (ORAC) assay as described by Cao et

al. [14]. One run of the ORAC assay comprised one blank, one standard and 6 serum samples. Phosphate buffer (1.75ml) and R-phycoerythrin (R-PE, 3.73mg/l; 100μl, Sigma, St Louis, MO, US) were added to each of 8 fluorimeter cuvettes. The cuvettes were preincubated for 15min at 37℃. A volume of 100μl of buffer (blank), 20μM Trolox (standard, Aldrich Chemical Co., Milwaukee, WI) or diluted serum (sample) was then added. The reaction was started by the addition of 320mM 2,2-azobis (2-amidino-propane) dihydrochloride (AAPH, Wako Pure Chemicals Co., Ltd., Osaka, Japan) solution. The fluorescence was measured using the JASCO FP-6300 fluorescence spectrophotometer (emission wavelength 575nm, excitation 495nm). The cuvettes were incubated at 37℃ during measurements and the fluorescence was recorded every 2min it had diminished to less than 5% of the initial value. The final result was determined by calculating the difference of area under the R-PE decay curve between the blank and a sample, and expressed using Trolox equivalents.

TNF-α assay

Evaluation of serum TNF-α was performed using commercially available enzyme-amplified sensitive immunoassays (IMMUNOTECH, Marseille, France). The minimal detectable concentration of TNF-α was 5pg/ml.

Statistical analysis

All statistical calculations were performed using SPSS software. Results are expressed as means ± standard deviations. The significance of differences was determined with an unpaired two-tailed t-test and a Mann-Whitney U-test. Correlation coefficients were calculated by Spearman's rank-correlation analysis when appropriate. Two-sided p values less than 0.05 were considered significant.

Result

The body mass index (BMI) and the findings of clinical parameters in the controls and the patients with CD are shown in Table 1. The BMI was within the normal range for 19 patients. The BMI of 1 patient was low (14.1). Although white blood cell (WBC) counts were also within the normal range, the lymphocyte ratio and lymphocytes were significantly lower in the patients than the controls. Serum total protein, albumin and hemoglobin concentrations were also significantly lower in the CD patients. The serum sialic acid level was significantly higher than in the controls. There were no significant differences in serum, TNF-α (21.7 ± 14.9, 20.2 ± 10.6pg/ml), and zinc (76.1 ± 15.6, 82.5 ± 14.6mg/dl) levels between the CD patients and controls. The serum copper (126.0 ± 25.5, 90.2 ± 26.1mg/dl, respectively) levels were significantly higher ($P<0.001$) than in the controls.

Table 2 shows intakes of energy and major nutrients. In the CD patients, fat intake and the fat energy ratio were significantly lower, while protein and carbohydrate intakes were significantly higher.

Table 1 BMI and clinical parameters in controls and patients with Crohn's disease

	Gender	Controls (M=12, F=5)	Patients (M=15, F=5)
Age		29.4 ± 13.1	30.6 ± 8.2
BMI (kg/m²)		21.4 ± 2.5	20.5 ± 2.7
Hemoglobin (g/dl)	M	15.6 ± 0.9	13.0 ± 1.5*
	F	13.5 ± 0.7	11.4 ± 2.2*
WBC (/μl)		5,053 ± 621	5,910 ± 1676
Lymphocyte ratio (%)		42.3 ± 2.6	22.7 ± 11.8***
Lymphocytes (/μl)		2,167 ± 640	1,237 ± 543***
Total protein (g/dl)		7.7 ± 0.4	7.3 ± 0.7*
Albumin (g/dl)		4.8 ± 0.3	4.0 ± 0.5*
Sialic acid (mg/dl)		0.2 ± 0.1	1.3 ± 2.0*

Data are the mean ± standard deviation. ***p<0.001, *p<0.05; compared with controls.
BMI: body mass index

Table 2 Energy and nutrient intakes in controls and patients with Crohn's disease

(/day)	Controls (n=17)	Patients (n=20)
Energy (kcal/kg IBW)	31 ± 9	35 ± 5
Fat (g/kg IBW)	1.1 ± 0.3	0.4 ± 0.3***
Fat energy ratio (%)	29.7 ± 7.8	11.6 ± 7.4***
Protein (g/kg IBW)	1.1 ± 0.3	1.4 ± 0.3***
Carbohydrate (g/kg IBW)	4.1 ± 1.0	6.4 ± 1.4***
Retinoid (μg/day)	1,216 ± 2,017	1,424 ± 380
Vitamin C (mg/day)	85 ± 58	141 ± 80
Vitamin E (mg/day)	9.0 ± 3.5	14.8 ± 4.7***
Zinc (mg/day)	8.0 ± 2.7	10.0 ± 2.6*
Copper (mg/day)	1.0 ± 0.3	1.3 ± 0.2**
Soluble dietary fiber (g/day)	3.1 ± 1.1	2.1 ± 1.4**
Insoluble dietary fiber (g/day)	8.8 ± 3.2	6.8 ± 4.8**

Data are the mean ± standard deviation. ***p<0.001, **p<0.01, *p<0.05; compared with controls
IBW: ideal body weight

In the patients, vitamin E, zinc and copper intakes were significantly higher than the controls. Ingested amounts of dietary fatty acids are summarized in Table 3. In the patients, significantly lower intakes of monounsaturated fatty acid and n-6 polyunsaturated fatty acid (PUFA), especially linoleic acid, were observed compared with the controls. Among n-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels were significantly higher in the CD patients than the controls. Therefore, the n-6/n-3 PUFA ratio was significantly lower in the CD patients.

Fatty acid profiles of plasma and neutrophils in the controls and the CD patients are shown in Table 4.

Table 3 Dietary fatty acid intakes in controls and patients with Crohn's disease

Fatty acid (g/day)	Controls (n=17)	Patients (n=20)
Saturated fatty acid	17.65 ± 10.36	6.52 ± 5.35***
Monounsaturated fatty acid	21.12 ± 10.81	7.77 ± 6.16**
Polyunsaturated fatty acid	13.18 ± 5.33	7.20 ± 3.65***
Total n-6 PUFA	9.39 ± 3.40	5.60 ± 3.00***
Linoleic acid (18: 2n-6)	10.55 ± 4.31	5.47 ± 2.95***
Arachidonic acid (20: 4n-6)	0.11 ± 0.05	0.13 ± 0.06
Total n-3 PUFA	2.46 ± 1.18	1.96 ± 1.14***
α-Linolenic acid (18: 3n-3)	1.76 ± 0.78	0.79 ± 0.51***
EPA (20: 5n-3)	0.19 ± 0.18	0.35 ± 0.24***
DHA (22: 6n-3)	0.38 ± 0.30	0.60 ± 0.41***
n-6/n-3 PUFA ratio	4.7 ± 1.3	3.0 ± 1.4***

Data are the mean ± standard deviation. ***p<0.001, **p<0.01; compared with controls.
PUFA; polyunsaturated fatty acid, EPA; eicosapentaenoic acid, DHA; docosahexaenoic acid

In the plasma and neutrophil phospholipids, an abnormal fatty acid profile was observed in the CD patients. In the plasma phospholipids, significantly low levels of n-6 PUFA, especially linoleic aicd was recognized in CD patients compared with control subjects. In the neutrophil phospholipids, saturated fatty acid was significantly lower, and total PUFA, n-6 PUFA, arachidonic acid and DHA were higher in the CD Patients than the control subjects. Additionally, in the plasma and neutrophil phospholipids, the arachidonic acid/ linoleic acid ratio in CD patients was significantly higher than those of control subjects. There was no significant correlation among the fatty acid composition in neutrophils and TNF-α, CRP or sialic acid levels.

Serum retinol and β-carotene concentrations were significantly lower in the CD patients than the controls (Table 5). The serum ORAC value was also

Table 5 Serum fat-soluble vitamin concentrations and ORAC values in controls and patients with Crohn's disease

		Controls (M=12, F=5)	Patients (M=15, F=5)
Retinol	(μmol/L)	2.51 ± 2.17	0.28 ± 0.22*
β-Carotene	(μmol/L)	1.16 ± 2.04	0.14 ± 0.03*
α-Tocopherol	(μmol/L)	23.6 ± 14.9	23.3 ± 12.6
Zinc	(μg/dL)	82.5 ± 14.6	76.1 ± 15.6
Copper	(μg/dL)	90.2 ± 26.1	126.0 ± 25.5***
ORAC value	(μmol/L)	6847 ± 1044	4998 ± 331***

Data are the mean ± standard deviation. ***p<0.001, *p<0.05; compared with controls.
ORAC; oxygen radical absorbance capacity
M; male, F; female

Table 4 Fatty acid composition of phospholipids in plasma and neutrophils of controls and patients with Crohn's disease

	Plasma phospholipids		Neutrophil phospholipids	
	Controls (n=17)	Patients (n=20)	Controls (n=17)	Patients (n=20)
Saturated fatty acid	44.35 ± 6.25	51.63 ± 1.98***	60.83 ± 11.06	48.87 ± 5.21**
Polyunsaturated fatty acid	42.22 ± 6.42	32.19 ± 5.00***	18.51 ± 5.41	25.35 ± 4.29**
Total n-6 PUFA	32.49 ± 4.33	23.91 ± 0.12***	14.54 ± 5.88	20.90 ± 3.93**
Linoleic acid	21.11 ± 2.71	13.60 ± 5.16***	7.33 ± 4.57	7.47 ± 1.95
Arachidonic acid	8.32 ± 2.18	6.93 ± 1.02*	4.32 ± 2.99	9.23 ± 2.10*
Total n-3 PUFA	9.73 ± 3.37	8.28 ± 3.30	3.97 ± 2.58	4.46 ± 1.80
EPA	2.08 ± 1.12	1.91 ± 1.05	1.33 ± 0.99	0.90 ± 0.46
DHA	6.28 ± 2.42	5.37 ± 2.14	1.08 ± 0.70	1.95 ± 1.07*
Arachidonic acid/Linoleic acid	0.40 ± 0.11	0.59 ± 0.25**	0.69 ± 0.37	1.35 ± 0.59***

Data are the mean ± standard deviation. ***p<0.001, **p<0.01, *p<0.05; compared with controls.
PUFA; polyunsaturated fatty acid, EPA; eicosapentaenoic acid, DHA; docosahexaenoic acid