Dietary Intake, Neutrophil Fatty Acid Profile, 
Serum Antioxidant Vitamins and Oxygen Radical Absorbance 
Capacity in Patients with Ulcerative Colitis

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Summary Nutrition may play an important role in the pathogenesis and treatment of ulcerative colitis. Several studies suggest an association between dietary factors and the onset of ulcerative colitis; however, only few studies have examined the relationship between dietary intake and relapse of ulcerative colitis. The aim of this study was to assess the dietary intake and antioxidative capacity of ulcerative colitis patients and to elucidate the efficacy of dietary therapy for ulcerative colitis. Dietary intake, fatty acid composition of phospholipids in plasma and neutrophils, serum fat-soluble vitamin levels, and oxygen radical absorbance capacity were analyzed in 29 ulcerative colitis patients (7 males and 22 females), who were treated at the Department of Gastroenterology, 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 age- and sex-matched controls. In the neutrophil phospholipids of ulcerative colitis patients, significantly higher levels of linoleic acid and arachidonic acid and a lower level of eicosapentaenoic 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 retinol (r=0.567, p=0.0031), α-tocopherol (r=0.560, p=0.0036) and β-carotene (r=0.440, p=0.0279) concentrations were observed in the ulcerative colitis patients. A diet restricting the intake of linoleic acid and supplemented with eicosapentaenoic acid and antioxidative vitamins may be recommendable for the nutritional management of ulcerative colitis patients.

Key Words ulcerative colitis, diet, antioxidative capacity, neutrophil fatty acid
remission of the disease. In this study, therefore, to elucidate the efficacy of dietary therapy for UC, 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-nine UC patients were recruited in the present study. They were all outpatients treated at the Department of Gastroenterology, Okayama University Hospital. Of the patients, 8 had left-sided colitis and 21 had pancolitis. The diagnosis of UC was based on accepted clinical, radiographic, endoscopic, and histologic criteria (11). Disease severity was estimated according to Truelove and Witts’ classification (12). Of the 29 patients, disease severity of the patients was mild in 10, moderate disease in 11, and severe disease in 8. Eighteen patients were medicated with 5-aminosalicylic acid (5-ASA), 7 with salazosulaphyridine (SASP), 10 with azathioprin and 8 with prednisolone during the previous week. Energy, major nutrients and the frequency and the quantity of food stuffs consumed were evaluated.

Subjects were age- and sex-matched healthy volunteers, 7 males and 22 females (39.7 ± 16.5 y). The BMI was within the normal range for all UC patients. Although WBC counts were significantly lower in the patients than the controls, the lymphocyte ratio was also within the normal range, the lymphocyte ratio was 95 ± 5 and 94.5 ± 5.9%, respectively, were drawn off into another tube. The lymphocytes and neutrophils were washed 3 times with ice-cold saline, and stored at −80°C prior to use.

Total lipid was extracted from plasma and blood cells suspended in 0.5 mL of saline according to the method of Bligh and Dyer (14). Total phospholipid was separated by one-dimensional thin-layer chromatography and the fatty acid composition of total phospholipid was analyzed according to a method described previously (15).

Analysis of fat-soluble vitamins in plasma. Retinol, α-tocopherol and carotenoids were extracted from plasma and analyzed using HPLC system according to the method reported by Milne and Botnen (16).

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 and Prior (17). One run of the ORAC assay comprised one blank, one standard and 6 serum samples. Phosphate buffer (1.75 mL) and R-phycocerythin (R-PE, 3.73 mg/L; 100 μL, Sigma, St. Louis, MO, US) were added to each of 8 fluorimeter cuvettes. The cuvettes were preincubated for 15 min at 37°C. A volume of 100 μL of buffer (blank), 20 μL Trolox standard (Aldrich Chemical Co., Milwaukee, WI) or diluted serum (sample) was then added. The reaction was started by the addition of 320 μM 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 575 nm, excitation 495 nm). The cuvettes were incubated at 37°C during measurements and the fluorescence was recorded every 2 min until 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 5 pg/mL.

Statistical analysis. Results are expressed as means±SD. The significance of differences was determined with an unpaired t-test (two-tailed) and 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.

RESULTS

The body mass index (BMI) and the findings of clinical parameters in the controls and the patients with UC are shown in Table 1. The BMI was within the normal range for all UC patients. Although WBC counts were also within the normal range, the lymphocyte ratio was significantly lower in the patients than the controls. Serum total protein, albumin and hemoglobin concentrations were also significantly lower in the UC patients. The serum sialic acid level was significantly higher than in the controls. There were no significant differences in serum TNF-α (20.2±10.6, 22.3±22.8 pg/mL), zinc...
Lymphocytes (\text{mg/dL}) 59.0
Albumin (\text{g/dL}) 4.8
WBC (\text{\texttimes }10^9/\text{L}) 15.8
Hemoglobin (\text{g/dL}) M 15.8
BMI (kg/m^2) 21.5
Hemoglobin (g/dL) F 15.8
Sialic acid (mg/dL) 59.0
Lymphocyte ratio (%) 38.1
Total protein (g/dL) 7.6
Fat energy ratio (%) 29.0
Retinoid (\text{\mu}g/d) 1,221
Fat (g/kg IBW) 1.0
Vitamin C (mg/d) 94
Carbohydrates (g/kg IBW) 4.0
Protein (g/kg IBW) 1.0
Soluble dietary fiber (g/d) 2.9
Insoluble dietary fiber (g/d) 8.6

Data are the mean\pm SD. **p<0.001, *p<0.01, *p<0.05, compared with controls.
BMI: body mass index.

### Table 2. Energy and nutrient intakes in controls and patients with ulcerative colitis.

<table>
<thead>
<tr>
<th>(\text{\texttimes }10^9/\text{L})</th>
<th>Controls (n=29)</th>
<th>Patients (n=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal/kg IBW)</td>
<td>30\pm 7</td>
<td>33\pm 7</td>
</tr>
<tr>
<td>Fat (g/kg IBW)</td>
<td>1.0\pm 0.4</td>
<td>0.8\pm 0.3*</td>
</tr>
<tr>
<td>Fat energy ratio (%)</td>
<td>29.0\pm 6.1</td>
<td>22.1\pm 4.4*</td>
</tr>
<tr>
<td>Protein (g/kg IBW)</td>
<td>1.0\pm 0.4</td>
<td>1.3\pm 0.3**</td>
</tr>
<tr>
<td>Carbohydrates (g/kg IBW)</td>
<td>4.0\pm 0.9</td>
<td>5.0\pm 1.0**</td>
</tr>
<tr>
<td>Retinoid (\text{\mu}g/d)</td>
<td>1,221\pm 1,764</td>
<td>1,245\pm 571</td>
</tr>
<tr>
<td>Vitamin C (mg/d)</td>
<td>94\pm 54</td>
<td>118\pm 49</td>
</tr>
<tr>
<td>Vitamin E (mg/d)</td>
<td>8.3\pm 2.7</td>
<td>8.0\pm 2.2</td>
</tr>
<tr>
<td>Zinc (mg/d)</td>
<td>7.5\pm 2.4</td>
<td>7.8\pm 2.1</td>
</tr>
<tr>
<td>Copper (mg/d)</td>
<td>1.0\pm 0.3</td>
<td>1.1\pm 0.3</td>
</tr>
<tr>
<td>Soluble dietary fiber (g/d)</td>
<td>2.9\pm 1.2</td>
<td>3.1\pm 1.0</td>
</tr>
<tr>
<td>Insoluble dietary fiber (g/d)</td>
<td>8.6\pm 3.0</td>
<td>9.9\pm 2.7</td>
</tr>
</tbody>
</table>

Data are mean\pm SD. **p<0.01, *p<0.05, compared with controls. †p<0.05, compared with active patients.
IBW: ideal body weight.

### Table 3. Dietary fatty acid intakes in controls and patients with ulcerative colitis.

<table>
<thead>
<tr>
<th>Fatty acid (g/d)</th>
<th>Controls (n=29)</th>
<th>Patients (n=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated fatty acid</td>
<td>15.41\pm 6.21</td>
<td>12.29\pm 5.11</td>
</tr>
<tr>
<td>Monounsaturated fatty acid</td>
<td>17.79\pm 7.84</td>
<td>13.05\pm 5.45**</td>
</tr>
<tr>
<td>Polyunsaturated fatty acid</td>
<td>11.32\pm 4.34</td>
<td>9.66\pm 3.09</td>
</tr>
<tr>
<td>Total n-6 PUFA</td>
<td>9.07\pm 3.42</td>
<td>7.51\pm 2.64***</td>
</tr>
<tr>
<td>Linoleic acid (18:2n-6)</td>
<td>8.92\pm 3.37</td>
<td>7.35\pm 2.59***</td>
</tr>
<tr>
<td>Arachidonic acid (20:4n-6)</td>
<td>0.11\pm 0.05</td>
<td>0.10\pm 0.06</td>
</tr>
<tr>
<td>Total n-3 PUFA</td>
<td>2.24\pm 1.30</td>
<td>2.36\pm 0.77</td>
</tr>
<tr>
<td>α-Linolenic acid (18:3n-3)</td>
<td>1.51\pm 0.68</td>
<td>1.20\pm 0.46</td>
</tr>
<tr>
<td>EPA (20:5n-3)</td>
<td>0.21\pm 0.27</td>
<td>0.34\pm 0.16***</td>
</tr>
<tr>
<td>DHA (22:6n-3)</td>
<td>0.40\pm 0.42</td>
<td>0.61\pm 0.27***</td>
</tr>
<tr>
<td>n-6/n-3 PUFA ratio</td>
<td>4.7\pm 1.6</td>
<td>3.2\pm 0.7***</td>
</tr>
</tbody>
</table>

Data are mean\pm SD. ***p<0.001, **p<0.01, compared with controls. †p<0.05, compared with active patients.
PUFA: polyunsaturated fatty acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid.
compared with the controls. Among n-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels were significantly higher in the UC patients than the controls. Therefore, the n-6/n-3 PUFAs ratio was significantly lower in the UC patients. There was a significant difference in saturated fatty acid intake between the patients in active and remission stages.

Fatty acid profiles of plasma and neutrophils in the controls and the UC patients are shown in Table 4. In the neutrophil phospholipids, an abnormal fatty acid profile was observed in the UC patients, that is, significantly lower levels of saturated fatty acid and EPA and higher levels of PUFAs, especially n-6 PUFAs, including both linoleic acid and arachidonic acid and also DHA in n-3 PUFAs compared with levels in the controls. In contrast, significantly higher levels of saturated fatty acid and lower levels of n-6 PUFAs were observed in the plasma phospholipids of the UC patients. Comparing the UC patients in remission and active stages, significantly high DHA and total n-3 PUFAs levels in neutrophils were observed in the patients in the remission stage. In the present study, we could not compare the difference between the medicated and nonmedicated patients, because only two patients were nonmedicated. In mononuclear cells, mainly lymphocytes, there was no significant difference in the fatty acid composition between the controls and the patients (data not shown). No significant differences were observed in the fatty acid profiles of plasma and neutrophils between the patients treated with and without prednisolone. 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 UC patients than the controls (Table 5). The serum ORAC value was also significantly lower in the patients. However, no significant difference was observed in the serum α-tocopherol level between the controls and the UC patients. Comparing the patients in remission and active stages, no significant differences were observed in serum vitamin concentrations or ORAC values. Significant correlations between the serum ORAC value and retinol ($r=0.567$, $p=0.0031$), α-tocopherol ($r=0.560$, $p=0.0036$) and β-carotene ($r=0.440$, $p=0.0279$) concentrations were observed in the UC patients.
DISCUSSION

In the present UC patients, serum total protein and albumin levels were significantly lower than the levels in controls; however, they remained within the normal range and there were no patients classified as malnourished. The UC patients were instructed to consume a diet rich in energy and protein, but restricted in fat, and the patients in the active stage followed well their dietitian’s advice; however, the patients in the remission stage could not abide a fat-restricted diet, as shown in Table 2.

Arachidonic acid is generally not abundant in natural foods and is mostly synthesized from linoleic acid by desaturation and chain elongation in the liver. Linoleic acid intake was lower in the UC patients than the controls, and as a result, linoleic acid and arachidonic acid molar percentages in the plasma phospholipids of the patients were reduced. Contrary to the plasma phospholipids, significant increases in the levels of linoleic acid and arachidonic acid in neutrophil phospholipids were observed in the UC patients. The fatty acid composition, low in palmitic acid and high in arachidonic acid, was suggested to activate the O2-forming system in the neutrophils migrating into inflammatory exudates (18). The patients were medicated with 5-ASA, SASP and/or azathioprin with or without prednisolone. 5-ASA and SASP provide anti-inflammatory and immuno-suppressing effects by inhibiting the synthesis of leukotriene (LT) B4 and prostagrandin (PG) E2 from arachidonic acid by the 5-lipoxygenase and cyclooxygenase pathways (19, 20). Prednisolone inhibits arachidonic acid and other PUFA release from membrane phospholipids by phospholipase A2.

Indomethacin, which has no efficacy in the treatment of patients with inflammatory bowel disease, on the other hand, selectively inhibited PGE2 formation in inflamed colonic mucosa (20) without reducing synthesis of LTs. These findings may suggest that LT is a more important mediator in inflammatory bowel disease than is PG. Neutrophils prominently produce LTBs, but lymphocytes reportedly produce small amounts of 5-lipoxygenase and also cyclooxygenase products (21). Therefore, inhibition of the metabolism of arachidonic acid by 5-ASA and SASP may induce such an accumulation of arachidonic acid specifically in neutrophil phospholipids and this may be followed by an increase in linoleic acid. To confirm this speculation, further study may be needed in medicated and nonmedicated patients. Although the intake of EPA was high in the UC patients, the molar percentage of EPA in neutrophil phospholipids was very low. The increase in arachidonic acid may induce such a decrease because EPA is incorporated into the second position of glycerol in the membrane phospholipids competing with arachidonic acid. Preventing such an abnormal arachidonic acid accumulation in neutrophil phospholipids, supplementation of EPA or strict restriction of dietary linoleic acid may be useful to maintain the remission.

Arachidonic acid up-regulated phospholipase A2 and caused rapid increase in the surface expression of TNF receptors on human neutrophils (22). An increase in the arachidonic acid concentration of phospholipids may be accompanied by a rise in phospholipase A2 activity within the intestinal mucosa (23). A significant increase of arachidonic acid reacylation in the stimulated neutrophils induced a dramatic increase of production of LTs (24). LTB4 is a chemoattractant factor that facilitates the accumulation of neutrophils with the consequent production of reactive oxygen species causing damage from oxidative stress (25). EPA competitively inhibits the cyclooxygenase and lipoxygenase pathways and reduces the production of LTB4 (26) which is increased in the rectal mucosa of patients with active UC (27). An n-3 fatty acid-rich diet prevented an early response to elevated levels of interleukin (IL)-6 compared with an n-6 fatty acid-rich diet in trinitrobenzene sulfonic acid-induced enteritis (28). A decrease in EPA and increase in arachidonic acid in neutrophils may not be beneficial to UC patients.

The present UC patients had higher protein diets than the controls. Jowett et al. (6) suggested that a high meat diet rich in sulphur and sulphate may be implicated in relapse of UC. Amino acids containing sulphur are abundant in animal proteins but not in soy protein. Soy products such as soy-curd and soy-milk may be recommended as a source of protein for UC patients.

The rate of relapse in patients with quiescent UC treated with soluble dietary fiber was not higher than that of patients given 5-ASA at the usual maintenance dose (5). Although the intake of dietary fiber, both soluble and insoluble, in the present UC patients was the same as that in the controls, the total fiber intake was less than that considered adequate for adult Japanese (29). A therapeutic approach using dietary fiber, especially soluble fibers acting as prebiotics, may also be possible in UC.

Retinol and \( \beta \)-carotene are fat-soluble vitamins; therefore, the low fat diets may be an explanation for the low serum levels of fat-soluble vitamins observed in the present UC patients. An \( \alpha \)-tocopherol deficiency was suggested in IBD (30). Serum \( \alpha \)-tocopherol level correlates with the serum cholesterol concentration. The absence of a significant decrease in \( \alpha \)-tocopherol in the present UC patients may reflect a serum total cholesterol level (196 ± 30 mg/dL) similar to controls (200 ± 42 mg/dL).

The significant decrease in the serum ORAC value and significant correlations between the serum ORAC value and serum retinol, \( \alpha \)-tocopherol and \( \beta \)-carotene levels observed in the present patients may indicate a deficiency of antioxidative vitamins. The antioxidative vitamins and ORAC capacity in the active patients were slightly but not significantly lower than in remission. This may be an explanation for the significantly low levels of DHA and total n-3 fatty acid observed in the neutrophils of active state patients, but the exact reason was unclear. Reactive oxygen metabolites such as superoxide and hydroxyl radicals are produced by activated neutrophils and macrophages and are considered...
to contribute to the tissue damage seen in IBD (31). Increases in the concentrations of reactive oxygen species have been found in the plasma and mucosa of patients with UC (32). Supplementation of antioxidant vitamins may be beneficial for UC patients.

From the present observations, diets restricting the intake of vegetable oils rich in linoleic acid and providing EPA from fish and antioxidative vitamins may be recommended for the nutritional management of UC patients. These recommendations, however, should be tested with further examination.

REFERENCES


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