Effects of Omega-3 Polyunsaturated Fatty Acids on Inflammatory Markers in COPD*

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Background: **COPD, the fifth-leading cause of death worldwide, is characterized by chronic inflammation. However, no available agent can effectively cure this inflammation. A dietary supplement containing omega-3 polyunsaturated fatty acids (PUFAs) has anti-inflammatory effects. In this study, we hypothesized that nutritional support with omega-3 PUFA-rich diets may be useful for treating COPD, and we compared the clinical features and inflammatory mediator levels between the COPD patients who received an omega-3 PUFA-rich supplement and those who received a nonrich supplement.**

Methods: **Sixty-four COPD patients received 400 kilocalories per day of an omega-3 PUFA-rich supplement (n-3 group) or an omega-3 PUFA-nonrich supplement (n-6 group) for 2 years. We prospectively investigated the clinical features of these patients and measured the levels of inflammatory mediators.**

Results: **In 6-min walk testing, the dyspnea Borg scale and decrease of arterial oxygen saturation measured by pulse oximetry significantly improved in the n-3 group. Leukotriene B_4 levels in serum and sputum and tumor necrosis factor-α and interleukin-8 levels in sputum decreased significantly in the n-3 group, while there was no significant change in the n-6 group. Two patients in the n-3 group and three patients in the n-6 group had mild diarrhea, and three patients in the n-3 group and three patients in the n-6 group had nausea; however, their symptoms were controllable and they improved with treatment. With multiple regression analysis, it was proved that the omega-3 PUFA-rich diet significantly contributed to the change in cytokine levels in this study.**

Conclusion: **We suggest nutritional support with an omega-3 PUFA-rich diet as a safe and practical method for treating COPD.** ***(CHEST 2005; 128:3817–3827)***

Key words: exacerbation; interleukin-8; leukotriene B_4; tumor necrosis factor-α

Abbreviations: AA = arachidonic acid; ALA = αlinolenic acid; ANOVA = analysis of variance; BALF = BAL fluid; BMI = body mass index; EPA = eicosapentaenoic acid; IL = interleukin; LA = linoleic acid; LTB_4 = leukotriene B_4; PUFA = polyunsaturated fatty acid; SGRQ = St. George Respiratory Questionnaire; Spo_2 = arterial oxygen saturation measured by pulse oximetry; TNF = tumor necrosis factor

COPD is characterized by reduced airflow on expiration due to airway obstruction that is not completely reversible.[1] Currently, COPD is a major cause of chronic morbidity and mortality and is a substantial economic and social burden throughout the world. COPD is the fifth-leading cause of death worldwide, and in the next few decades its prevalence and mortality rates are expected to increase.[2] However, none of the currently available agents are capable of slowing the relentless progression of this disease.[1] Therefore, the development of a new strategy for treating COPD is required.

Pathologically, COPD is characterized by chronic inflammation in small airways and lung parenchyma accompanied by infiltration of neutrophils and macrophages.[3] This inflammation is associated with fibrosis and narrowing of the small airways and with lung parenchymal destruction,[4] and this chronic

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inflammation continues even in patients who have given up smoking.[5] This inflammation is considered to mediate excess mucus production, fibrosis, and proteolysis via neutrophil recruitment.[1] Immunologically, several neutrophil chemotactic factors have been reported to be associated with inflammation in COPD, including interleukin (IL)-8, tumor necrosis factor (TNF)-α, and leukotriene B_4 (LTB_4).[1] Therefore, the current strategy of COPD treatment is to control these inflammatory mediators.

The clinical importance of weight loss, however, a common feature in COPD patients,[6] has been shown to adversely affect physical well-being and quality of life.[7] Moreover, weight loss is an unfavorable prognostic factor in survival, independent of lung function,[8] and induces cytokine productions in COPD patients.[9] Therefore, several studies that focused on the importance of nutrition for the treatment of COPD were conducted,[10] and high-fat not high-carbohydrate meals were recommended for nutritional treatment of COPD. This is because high-fat, low-carbohydrate meals were less likely to affect the work performance of COPD patients than low-fat, high-carbohydrate meals.[11]

Fatty acids are classified into omega-6 or omega-3 polyunsaturated fatty acids (PUFAs) series depending on the position at which the first double bond from the methyl end occurs. The omega-6 series fatty acids include linoleic acid (LA) and arachidonic acid (AA), and the omega-3 fatty acids include α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid. Since both fatty series are metabolized by common enzymes (elongases and desaturases), a competitive interaction exists between the fatty acids: the omega-3 PUFA family suppresses the metabolism of the omega-6 PUFA family, and *vice versa*, although the suppression in the latter case is to a lesser extent.[12] Two potent inflammatory eicosanoids, prostaglandin E_2 and LTB_4, are produced from AA (omega-6 PUFA products) and, as described above, the metabolism of EPA and docosahexaenoic acid (omega-3 PUFA products) decreases production of prostaglandin E_2 and LTB_4.[12] In addition, omega-3 PUFAs reduce the ability of leukocytes to produce IL-1, IL-6, and TNF-α in healthy subjects and rheumatoid patients,[13] and in animals it confers protection against the lethal effects of endotoxins, burn injuries, and bacterial infections.[14] Thus, omega-3 PUFAs have anti-inflammatory effects and can decrease cytokine production associated with COPD pathogenesis. Further, omega-3 PUFAs can reduce alveolar inflammatory mediators such as IL-8 in ARDS.[15,16] In this study, we hypothesized that nutritional support with omega-3 PUFA-rich diets may be useful for treating COPD, and we compared the clinical features and the levels of inflammatory mediators between the COPD patients who received the omega-3 PUFA-rich supplement and those who received a nonrich supplement. We found that in comparison with the omega-3 PUFA-nonrich diet, nutritional support with the omega-3 PUFA-rich diet could reduce the levels of inflammatory cytokine in serum and sputum and improve exercise tolerance.

Materials and Methods

This study was reviewed and approved by the Kagoshima University Faculty of Medicine Committee on Human Research, and all patients included in this study provided written informed consent.

Patients

All patients fulfilled the diagnostic criteria of the British Thoracic Society.[17] The FEV_1 of all participants was $< 60\%$ of the predicted value.

Body height was determined to the nearest 0.5 cm (WM 715; Lameris; Breukelen, the Netherlands) with subjects standing barefoot. Body weight was assessed using a beam scale to the nearest 0.1 kg (SECA; Hamburg, Germany) with subjects standing barefoot and in light clothing. Patients with body mass index (BMI) [body weight/height^2] > 25 kg/m^2 were excluded from this study. We chose this BMI value because it was reported that below the threshold value of 25 kg/m^2 there is an evident increase in mortality risk.[8]

None of the participants had actively smoked for at least 6 months prior to the study, received antibiotics or nonsteroidal anti-inflammatory drugs, or reported any acute exacerbations for at least 4 weeks prior to the start of this study. We excluded patients with respiratory disorders other than COPD, those treated with systemic corticosteroids, or those who had inhaled corticosteroids or antibiotics in the 4 weeks prior to the start of study. We also excluded patients with concomitant confounding diseases such as malignant disorders, GI abnormalities, rheumatoid arthritis, diabetes mellitus, acute or chronic liver disease, immunologic abnormalities that predispose to opportunistic infection, or severe endocrine disorders. We also excluded those who had undergone recent surgery. All patients were ex-smokers.

Dietary Intake

Before the study and every month during the course of the study, patients were asked to register details of their food intake for 4 consecutive days in order to assess dietary intake. The obtained information was coded for computer nutrient analysis. The nutrient database was derived from the Standard Tables of Food Composition (The Science and Technology Agency of Japan, 1982). Caloric intake was calculated by considering the average intake over 4 days as "daily calories." Dietary records were used since this method is more valid than the dietary history method, and it provides a good representation of the actual consumption of calories because the food intake is averaged over 4 consecutive days; hence, day-to-day variations are largely eliminated.[18] Prior to the beginning the study, the patients were interviewed by a well-trained dietitian regarding dietary preferences with respect to omega-3 or omega-6 PUFAs, and patients with PUFA-specific preferences were excluded from this study.

All participants of this study agreed not to consume nutritional

supplements that contained omega-3 or omega-6 PUFAs. The participants were also asked to provide details regarding their nutritional supplement intake after the start of this study. If the patient consumed nutritional supplements, it was counted as 1 "confounding parameter point."

Study Protocol

All participants were randomly classified into two groups receiving nutritional support of 400 kilocalories per day for 24 months: (1) omega-3 PUFA-rich diet (n-3 group) [1.4% ALA, 4.48% caprylic acid, 1.3% palmitic acid, 0.48% stearic acid, 2% oleic acid, 2.14% LA, 32.1% carbohydrate from rice, 36.2% carbohydrate from wheat flour, 13.1% casein protein, and 6.8% soybeans protein (omega-3 PUFAs, 0.6 g in total calories; omega-6 PUFAs, 0.4 g in total calories)]; or (2) omega-3-PUFA-nonrich diet (n-6 group) [0.18% ALA, 4.48% caprylic acid, 1.3% palmitic acid, 0.48% stearic acid, 2% oleic acid, 3.36% LA, 33.1% carbohydrate from rice, 35.2% carbohydrate from wheat flour, 12.1% casein protein, 5.8% soybeans protein (omega-3 PUFAs, 0.07 g in total calories; omega-6 PUFAs, 0.93 g in total calories)]. The results were prospectively analyzed. Nutritional treatment was independently allocated and dispensed by a member of staff in the pharmacy department who was not involved in the study. Both the investigators and the patients were blinded to the treatment allocation. Unblinding of the study did not occur until the final assessment of the last patient was completed. All participants maintained their treatment such as bronchodilators, rehabilitation, and oxygen therapy.

To evaluate compliance, the patients were asked to declare if they had inadvertently missed consuming the nutritional support for the study. In case the patient had missed consuming the nutritional support on 1 day, it was counted as 1 "compliance point." Clinical features, BMI, blood gas analysis, pulmonary function testing, serologic analysis, serum cytokine levels, and cytokine levels in sputum were investigated once every 3 months. To evaluate exercise capacity, a 6-min walk test[19] was performed before the study and every 3 months during the study. For the evaluation of clinical symptoms, we used the St. George Respiratory Questionnaire (SGRQ)[20] before the study and every 3 months during the study.

Identification of Acute Exacerbation

Patients were reviewed in the outpatient section of our hospital every month in order to evaluate whether they had experienced acute exacerbations. Exacerbations were diagnosed according to previously accepted criteria.[21] We diagnosed an acute exacerbation if the patient had the following symptom patterns for at least 2 consecutive days: either two or more of three major symptoms (increase in dyspnea, sputum purulence, or sputum volume) or any one major symptom along with any one of the following minor symptoms: increase in nasal discharge, wheeze, sore throat, cough, or fever.

Sputum Processing

In all participants, sputum was induced via inhalation of a hypertonic saline solution aerosol generated by an ultrasonic nebulizer (Ultraneb 2000; DeVilbiss; Somerset, PA). The viscid portions of the expectorated sample were separated from the sputum and processed within 15 min after termination of the induction. Sputum plugs were collected every 3 months in the outpatient section of Kagoshima University. For verification of the methodology, a pool of mucopurulent and mucoid spontaneous sputum (characterized according to a 9-point color chart[22]) sol phase was obtained. The sputum was closely examined by light microscopy to ascertain the least possible involvement of sputum with squamous cells. An appropriate sample was then filled into a 1-mL Eppendorf tube, weighed, and mixed with an equal volume of 0.1% dithiothreitol (Calbiochem; Schwalbach, Germany) in phosphate-buffered saline solution. Sputum was gently vortex mixed and placed into a water bath at 37°C for 15 min to allow homogenization of the sample. This procedure diluted the sputum and dithiothreitol concentration by twofold. Samples were centrifuged for 10 min, and the supernatant was aspirated and recentrifuged for 5 min to completely remove cellular components, and the supernatants were immediately frozen at − 80°C. The cell pellet was resuspended in phosphate-buffered saline solution to a concentration of to 0.5×10^6 cells/mL, and 75 μL was suspended in cytocentrifuge cups and centrifuged at 450 revolutions per minute for 6 min in a cytocentrifuge (Shandon II; Shandon; Runcorn, Cheshire, UK). The suspension was air-dried and stained (Hemacolor; Merck; Darmstadt, Germany), and a differential cell count was obtained by counting > 400 nonsquamous cells. Cell counting was performed by an experienced observer blinded to the clinical characteristics of subjects. Only the supernatant of sputum samples with squamous cell contamination of < 20% was used for further analysis.

Cytokine Measurement

LTB_4, IL-8, and TNF-α levels were measured using a commercial enzyme-linked immunosorbent assay kit (R&D Systems; Minneapolis, MN) in accordance with the protocol of the manufacturer. Optical density at 450 nm was measured on a plate reader (Titertek Multiskan MC; Flow Laboratories; Helsinki, Finland), and cytokine concentrations were determined by linear regression from a standard curve using software (GraphPad; Flow Laboratories; Helsinki, Finland) for analysis.

Statistical Analysis

Prior to the start of study, parameters between the two groups were statistically analyzed using the Mann-Whitney *U* test. The changes in the levels of cytokines and other parameters during the study were statistically analyzed using two-way analysis of variance (ANOVA) on the change from baseline as the dependent measure.

Multiple regression analysis was used to identify the factors that were most strongly related to serum and sputum cytokine concentrations. We designated nonnumeric factors as follows: n-3 group = 1, n-6 group = 0; side effect yes = 1, side effect no = 0; theophylline yes = 1, theophylline no = 0; and acute exacerbation yes = 1, acute exacerbation no = 0. The following factors were used as independent variables: n-3 or n-6 group, age, smoking index, daily energy intake, BMI prior to the start of study, BMI after the study, side effects, compliance points, confounding parameter points, theophylline usage, and existence of acute exacerbation. At first, we performed all possible subset regression analysis and then performed forward stepwise regression analysis. Data were analyzed according to the guidelines of Altman and coworkers[23] using statistical software (Statistical Package for the Social Sciences, version 6.0 for Windows; SPSS; Chicago, IL). Values were expressed as mean ± SD; $p < 0.05$ was considered significant.

Results

Clinical Features and Serologic Factors

In total, 64 COPD patients (n-3 group, n = 32; n-6 group, n = 32) participated in this study. Prior to the

start of therapy, there was no significant difference between the two groups in the male/female ratio (n-3 group, 29/3; n-6 group, 28/4), age (n-3 group, 65.8 ± 23.1 years, n-6 group, 66.2 ± 24 years), smoking index (29 patients in the n-3 group were ex-smokers [smoking index, 982 ± 672]; 28 patients in the n-6 group were ex-smokers [smoking index, 997 ± 678]), BMI, blood gas analysis, pulmonary function testing, daily caloric intake, investigated serologic factors, serum fatty acid concentrations, serum cytokine levels, and sputum cytokine levels (Table 1). Four patients in the n-6 group had an acute exacerbation during the observation period; however, no patients in the n-3 group had an acute exacerbation ($p = 0.057$, Fisher Exact Test). In the n-6 group, four patients were hospitalized because of acute exacerbation and one patient was hospitalized because of injuries sustained in a traffic accident that occurred during the study. In the n-3 group, one patient was hospitalized because of a traffic accident-related injury that occurred during the study. BMI, serum protein levels, and serum albumin levels significantly increased in both groups (Table 1; $p < 0.05$, two-way ANOVA); however, there was no significant difference between the two groups ($p = 0.89$, two-way ANOVA). In each group, there was no significant change in the blood gas analysis data at rest and in the pulmonary function test results (Table 1). However, in the 6-min walk test, dyspnea Borg scale points and decrease in arterial oxygen saturation measured by pulse oximetry (Spo_2) significantly improved in the n-3 group (Table 2; $p < 0.05$, two-way ANOVA). In the n-3 group, dyspnea Borg scale points and decrease in Spo_2 were significantly improved 21 months and 24 months after the study in comparison with the results obtained prior to the start of the study (Fig 1, Table 2; $p < 0.05$, two-way ANOVA). SGRQ scores, particularly symptoms and activity domains, improved in n-3 group patients in comparison with n-6 group patients. However, this difference was not statistically significant (symptoms score, $p = 0.051$; activity score, $p = 0.052$). In the n-3 group, the sputum neutrophil percentage decreased after the study, while there was almost no change in sputum cell populations in the n-6 group (Table 2; $p = 0.0517$). In the n-3 group, serum EPA levels significantly increased and AA levels were significantly decreased after the study ($p < 0.05$, two-way ANOVA); however, there was no significant change in the n-6 group (Fig 2, Table 1). In the n-3 group, the serum EPA levels 6 months after the start of the study and the AA levels 12 months after the start of study significantly differed from those prior to the start of the study (Fig 2, Table 1; $p < 0.01$, two-way ANOVA).

Table 1—*Clinical Data Before and 24 Months After the Start of the Study

Variables	n-3 Group (n = 32)		n-6 Group (n = 32)	
	Before	After	Before	After
Weight, kg	51.2 ± 11.2	55.8 ± 5.9	51.6 ± 11.8	55.6 ± 6.8
BMI, kg/m^2	19.1 ± 3.8	21.1 ± 2.9	19.3 ± 3.9	20.9 ± 3.1
Vital capacity, % of predicted value	78.9 ± 22.3	79.2 ± 23.1	77.6 ± 23.7	76.8 ± 25.1
FEV_1, % of predicted value	50.1 ± 10.2	51.2 ± 13.1	51.2 ± 9.8	47.6 ± 13.3
Pao_2 on room air, mm Hg	52.1 ± 10.1	53.2 ± 11.2	51.8 ± 12.1	50.1 ± 15.2
$Paco_2$ on room air, mm Hg	45.1 ± 15.2	44.2 ± 15.1	44.2 ± 16.1	46.3 ± 18.2
Daily caloric intake	1,732 ± 411	2,137 ± 432	1,765 ± 422	2,165 ± 433
Compliance points, No.		34.3 ± 21.1		34.2 ± 24.1
Confounding parameter points, No.		43.2 ± 32.1		43.3 ± 32.7
Use of theophylline, No./total		17/32		18/32
WBC count, /μL	6,782 ± 3,221	6,744 ± 3,321	6,897 ± 3,456	6,901 ± 3,544
Neutrophils, %	60.6 ± 24.2	61.2 ± 23.1	61.2 ± 25.2	61.8 ± 26.7
Lymphocytes, %	35.1 ± 12.1	34.9 ± 13.1	36.2 ± 13.9	34.9 ± 14.3
CD4, %	34.3 ± 16.2	34.2 ± 17.1	35.6 ± 17.2	33.4 ± 16.1
CD8, %	25.1 ± 14.1	24.8 ± 14.4	24.9 ± 15.2	25.1 ± 15.2
Human leukocyte antigen-DR, %	25.3 ± 18.1	26.7 ± 17.8	26.1 ± 18.6	25.8 ± 17.7
Natural killer cells, %	22 ± 18.2	21.8 ± 19.1	22.1 ± 17.9	21.3 ± 19.2
Hemoglobin, g/dL	13.4 ± 3.2	13.3 ± 3.3	13.3 ± 3.5	13.3 ± 3.4
Platelets, $\times 10^4/\mu L$	20.1 ± 4.5	20.2 ± 4.4	21.1 ± 4.7	23.1 ± 4.7
Total protein, g/dL	6.9 ± 1.7	7.1 ± 1.9	6.9 ± 1.8	7.09 ± 1.9
Albumin, g/dL	4.0 ± 1.2	4.2 ± 1.3	4.1 ± 1.3	4.19 ± 1.29
Free fatty acid, mEq/L	0.56 ± 0.28	0.57 ± 0.29	0.57 ± 0.29	0.57 ± 0.33
AA, μg/mL	154.9 ± 43.2	121.1 ± 44.1	154.1 ± 49.4	154.7 ± 43.1
EPA, μg/mL	83.9 ± 23.9	130.5 ± 22.1	88.8 ± 16.7	82.7 ± 16.2

*Data are presented as mean ± SD unless otherwise indicated.

Table 2—*Results of 6-min Walk Test, SGRQ Scores, Serum Cytokine Concentrations, Sputum Cell Population, and Sputum Cytokine Concentrations Before and 24 Months After the Start of the Study

Variables	n-3 Group (n = 32)		n-6 Group (n = 32)	
	Before	After	Before	After
Six-minute walk test				
Heart rate, beats/min				
Baseline	83 ± 8.1	82.1 ± 8.2	81.2 ± 9.2	83.2 ± 9.1
End of test	113 ± 24.1	114 ± 25.2	113 ± 23.1	115 ± 23.7
Dyspnea (Borg scale)				
Baseline	1.89 ± 1.34	1.88 ± 1.44	1.87 ± 1.44	1.87 ± 1.42
End of test	5.88 ± 2.11	4.99 ± 1.99	5.89 ± 2.24	5.88 ± 2.27
Leg fatigue (Borg scale)				
Baseline	0.34 ± 0.13	0.34 ± 0.11	0.31 ± 0.12	0.32 ± 0.11
End of test	3.42 ± 1.89	3.33 ± 1.91	3.44 ± 1.91	3.33 ± 1.89
Fall of Spo_2, %	10.1 ± 2.1	8.2 ± 1.7	10.3 ± 2.22	9.8 ± 2.13
Distance predicted, %	68.2 ± 18.1	73.2 ± 19.2	69.2 ± 17.8	68.2 ± 18.1
SGRQ domain scores				
Symptoms	49.2 ± 22.2	43.2 ± 21.2	49.3 ± 20.1	47.3 ± 21.9
Activity	52.1 ± 25.3	48.1 ± 26.1	53.1 ± 26.2	49.2 ± 27.3
Impact	34.3 ± 25.2	33.2 ± 24.9	35.2 ± 24.8	34.2 ± 25.1
Total	44.2 ± 20.2	40.2 ± 19.1	44.3 ± 22.2	43.2 ± 21.9
Serum cytokine concentrations				
LTB_4, pg/mL	817.7 ± 234.2	557.6 ± 218.5	812.3 ± 192.2	814.7 ± 207.2
TNF-α, pg/mL	27.4 ± 24.5	28.7 ± 23.1	28.8 ± 32.3	28.2 ± 22.2
IL-8, pg/mL	31.1 ± 44.4	32.7 ± 49.5	32.8 ± 39.1	33.3 ± 37.8
Sputum cytokine concentrations				
LTB_4, pg/mL	69.9 ± 38.4	41.5 ± 19.4	72.5 ± 35.9	68.6 ± 36.2
TNF-α, pg/mL	41.7 ± 22.2	21.7 ± 15.3	39.9 ± 16.5	40.1 ± 15.6
IL-8, pg/mL	14.1 ± 9.1	6.4 ± 5.3	12.2 ± 6.4	11.1 ± 8.5
Sputum cell population				
Macrophages, %	56.7 ± 18.2	61.2 ± 17.3	57.1 ± 18.1	57.8 ± 18.9
Neutrophils, %	46.2 ± 18.9	40.8 ± 18.7	45.9 ± 18.8	44.8 ± 17.4

*Data are presented as mean ± SD.

Prior to the start of this study, 14 patients in the n-3 group and 13 patients in the n-6 group required oxygen therapy. In the n-3 group, six patients showed a decrease in the required amount of oxygen after the study. In the n-6 group, three patients showed a decrease in the required amount of oxygen after the study; however, three patients required oxygen therapy after an exacerbation episode. In the n-3 group, 10 patients reported a decreased frequency of short-acting bronchodilator use, while 3 patients in the n-6 group reported a decreased frequency of short-acting bronchodilator use. Two patients in the n-3 group and three patients in the n-6 group had mild diarrhea, and three patients in the n-3 group and three patients in the n-6 group had nausea. However, the symptoms were controllable and improved with treatment.

Cytokine Levels in Serum and Sputum

In the n-3 group, serum LTB_4 levels 15 months after the start of the study were significantly lower than those in the n-6 group (Fig 2, Table 1; $p < 0.05$, two-way ANOVA) and those before the start of the study. Serum levels of IL-8 and TNF-α did not change in both groups (Table 1). The sputum levels of LTB_4, IL-8, and TNF-α significantly decreased after therapy, while there was no significant change in the n-6 group. In the n-3 group, sputum LTB_4, IL-8, and TNF-α concentrations 18 months after the start of therapy were significantly lower than those of the n-6 group ($p < 0.01$, two-way ANOVA, Fig 2). In the n-6 group, there was no apparent change in the serum and sputum cytokine levels during the observation period (Fig 2, 3).

In summary, in the n-3 group, serum EPA levels, serum AA levels, serum LTB_4 levels, and sputum cytokine levels began to change at 6 months, 12 months, 15 months, and 18 months after the start of study, respectively. The dyspnea Borg scales and the decrease in Spo_2 in the n-3 group improved 24 months after the start of therapy.

Multiple Regression Analysis

To evaluate whether the omega-3 PUFA diet contributed to the change in cytokine levels in the n-3 group, we performed multiple regression analy-

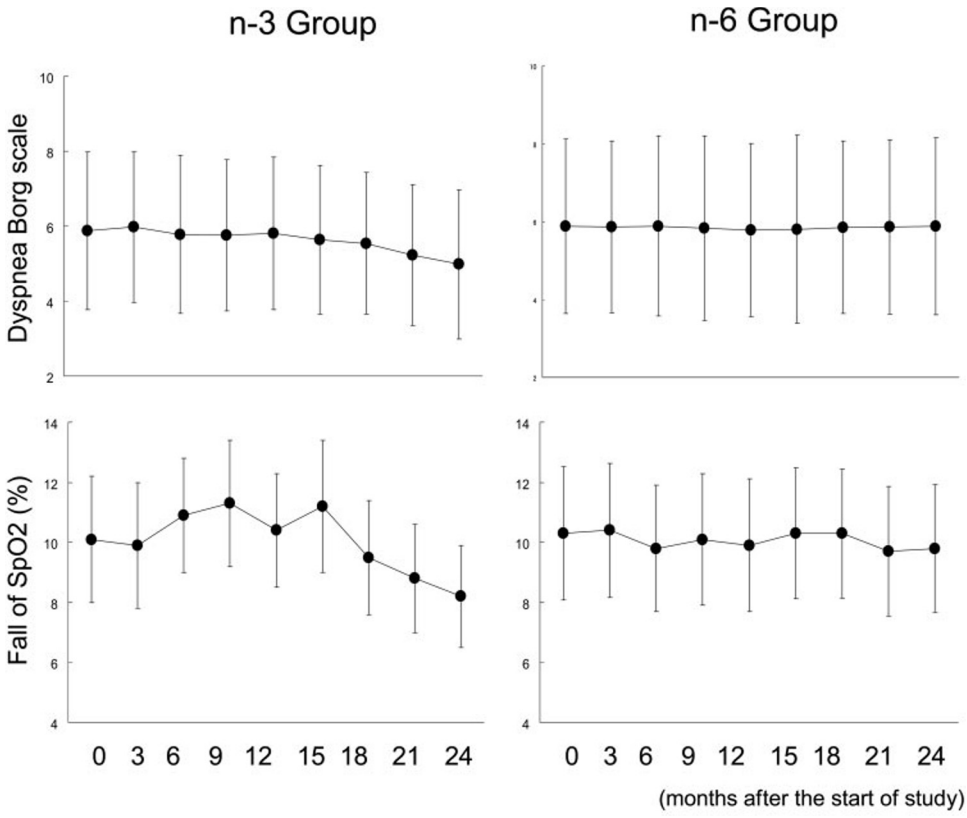

FIGURE 1. Comparison of the two groups in terms of changes in the dyspnea Borg scale and decrease in SpO_2 during the 6-min walk test.

sis with factors that might affect the result. During the observation period, 17 patients in the n-3 group and 18 patients in the n-6 group used theophylline. First, possible subset regression test, age, BMI, daily caloric intake, side effects, compliance, confounding parameters, use of theophylline, and acute exacerbation did not show a significant p value. Subsequently, on performing forward stepwise regression analysis, the different groups showed significant p values. Therefore, we conclude that the n-3 PUFA group significantly contributed to the cytokine changes observed in this study (Table 3).

DISCUSSION

In this study, we assessed the clinical value and anti-inflammatory effects of omega-3 PUFA-rich nutrition in the treatment of COPD. There is an urgent need to develop new anti-inflammatory treatment because no currently available agent, including corticosteroids,[24] can slow the chronic progressive inflammation of COPD.[1] Hence, certain anti-inflammatory agents such as chemokine antagonists and inhibitors of transcription factors are now under clinical trial; however, some of these agents are not suitable for treatment because of their adverse side effects.[1] The side effects of nutritional treatment with an omega-3 PUFA-rich supplement were mild and controllable as observed in this study. Apart from this, nutritional support with an omega-3 PUFA-rich diet had anti-inflammatory effects and improved exercise tolerance. In patients with COPD, dyspnea is the most common symptom that limits exercise, and it is a better predictor of the 5-year survival rate than airway obstruction.[25] Exercise capacity is significantly correlated with mortality in COPD patients.[26] In this regard, we propose that nutritional treatment with n-3 PUFA-rich diet might be an effective and safe anti-inflammatory practical strategy for treating COPD.

In our study, nutritional support with an omega-3 PUFA-rich diet decreased serum and sputum LTB_4 levels and sputum TNF-α levels and improved symptoms. LTB_4 is one of the most potent activators of granulocytes and macrophages.[27] LTB_4 binds to a specific G protein-coupled receptor,[28] and this receptor has been found to be expressed on granulocytes, monocytes, and T-lymphocytes.[27] Exposure of cells to LTB_4 induces adhesion of granulocytes to endothelial cells, degranulation of the lysosomal

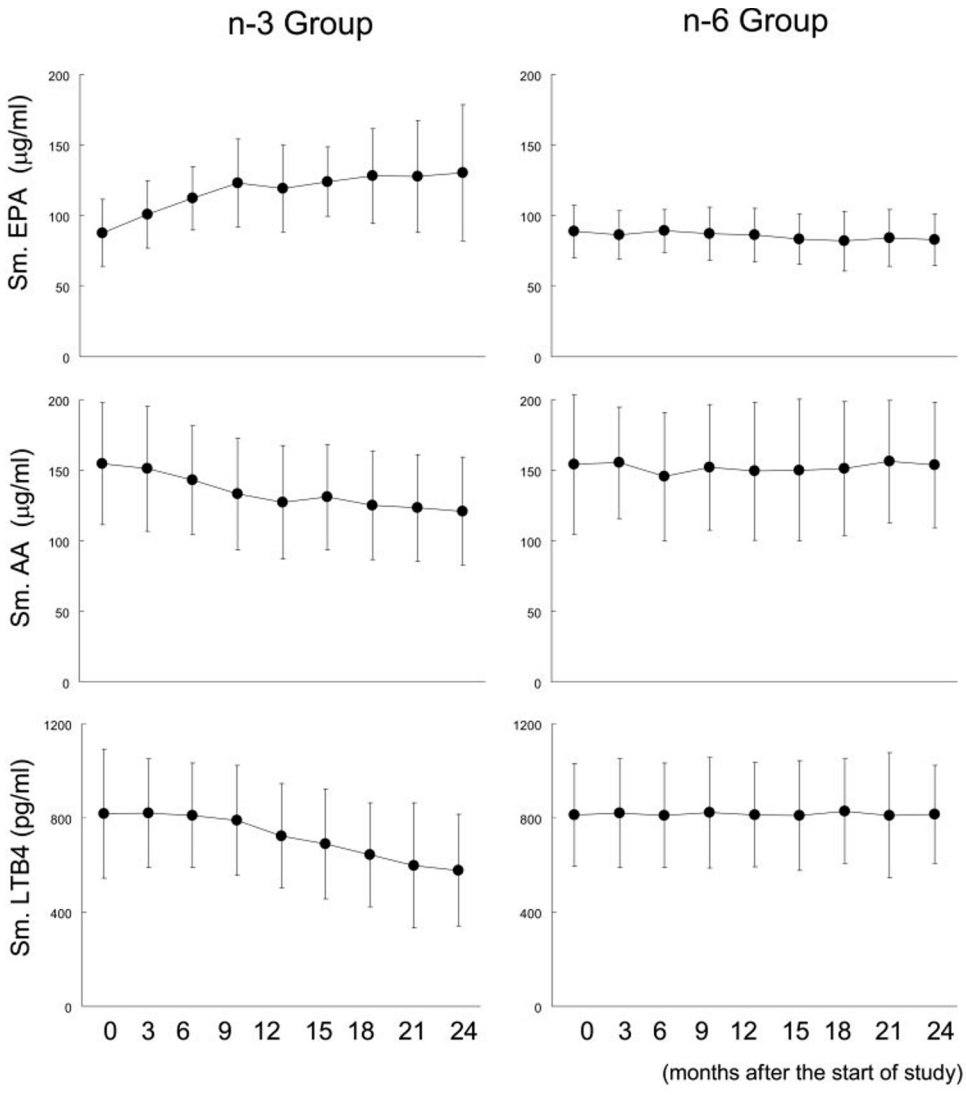

FIGURE 2. Comparison of the two groups in terms of the changes in serum (Sm) EPA levels, AA levels, and LTB_4 concentrations.

enzymes, generation of superoxide, and transmigration of granulocytes.[27] In COPD patients, the concentrations of LTB_4 in the sputum and exhaled breath are higher than those in normal subjects,[29] and alveolar macrophages are considered to be cellular sources of LTB_4.[1] Nutritional support with an omega-3 PUFA-rich diet can suppress the synthesis of LTB_4 by competitive inhibition[12,14] and can also suppress LTB_4-induced neutrophil chemotaxis by inhibition of phosphoinositide formation.[30] Overproduction of LTB_4 is also observed in ulcerative colitis, and an omega-3 PUFA-rich diet could reduce LTB_4 levels in rectal dialysate and improve histologic findings and weight gain.[31] However, TNF-α is a powerful proinflammatory cytokine and a key mediator of inflammation.[32] Endothelial cells exposed to TNF-α up-regulate surface adhesion molecules;[33] this facilitates phagocytic activity of alveolar macrophages in the alveolus.[34] In ARDS, nutrition with omega-3 PUFAs reduced IL-8, LTB_4, and TNF-α levels in BAL fluid (BALF)[16] and resulted in decreased number of neutrophils in BALF.[15] In COPD patients, TNF-α was found to be elevated in BALF and bronchial biopsy specimens, and it was induced in sputum.[35] In rheumatoid arthritis, an omega-3 PUFA-rich diet reduced cytokine production from leukocytes, including production of TNF-α.[12,13] Thus, our findings are compatible with previous reports[12–16,30] regarding the anti-inflammatory effect of an omega-3 PUFA-rich diet on chronic inflammatory diseases.

In our study, nutritional support with an omega-3 PUFA-rich diet decreased sputum IL-8 levels. Concentrations of IL-8 are very high in the sputum of

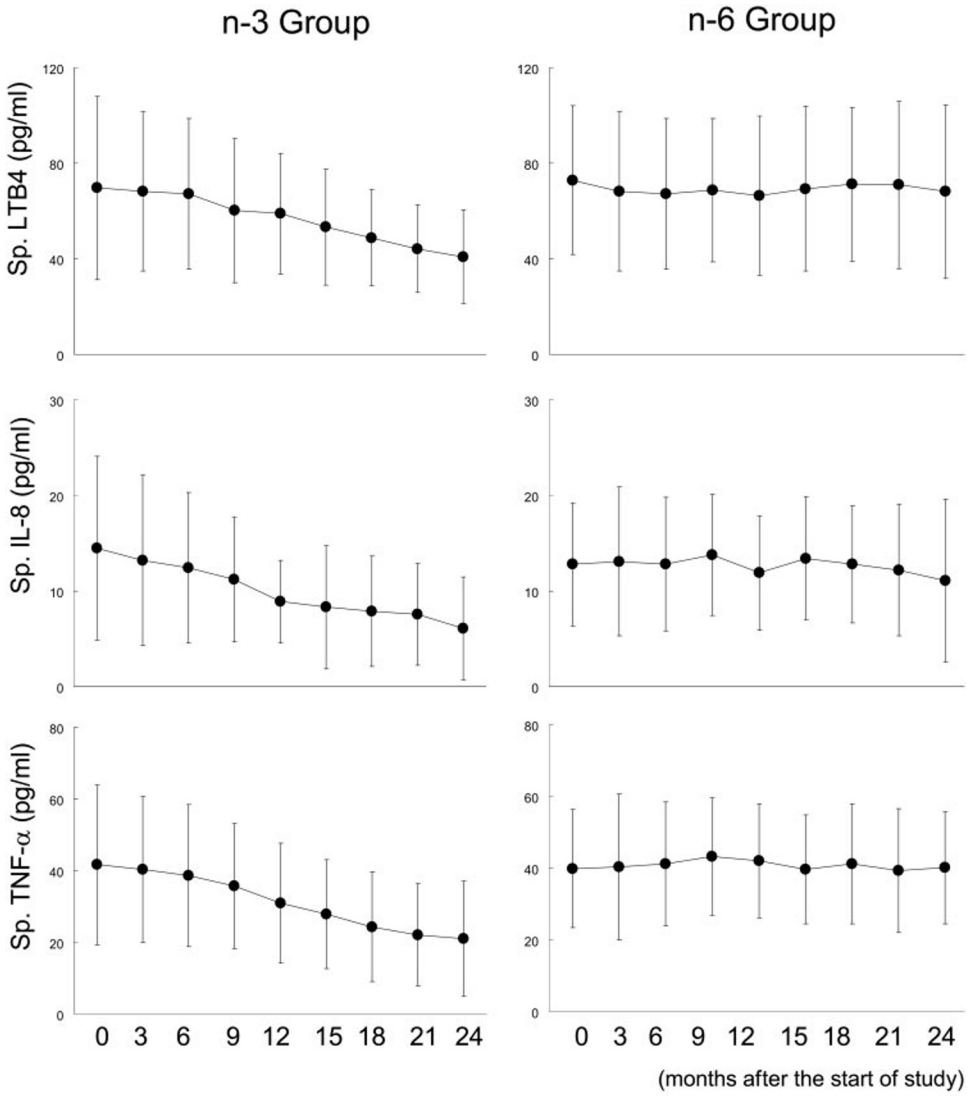

FIGURE 3. Comparison of the two groups in terms of the changes in sputum (Sp) LTB_4, IL-8, and TNF-α levels.

COPD patients and are correlated with disease severity and acute exacerbation.[36] IL-8 is a potent chemoattractant that is required for recruitment and activation of neutrophils,[37] and it is produced and released by a variety of cells including neutrophils, T-lymphocytes, fibroblasts, and epithelial cells, in the lung.[37] Airway epithelial cells are considered to be a particularly important source of this chemoattractant.[38] Furthermore, cigarette smoke is reported to enhance IL-8 release from bronchial epithelial cells.[39] Thus far, there is no evidence that nutritional support with an omega-3 PUFA rich diet can directly down-regulate IL-8 production from bronchial epithelial cells. However, we believe that it can indirectly affect IL-8 production because TNF-α activates bronchial epithelial cells to produce various inflammatory mediators, including IL-8,[40] and nutritional support with an omega-3 PUFA-rich diet can down-regulate TNF-α production from leukocytes.[12,13]

Regarding omega-3 PUFAs, there are reports not only on the effect on prostaglandin-associated pathways but also effects on other cellular signaling and gene expression activities. Lipid rafts are one target for omega-3 PUFAs, and affect membrane function. Lipids rafts are one target for omega-3 PUFA effects on membrane function. Lipid rafts are regions within the exoplasmic leaflet of the plasma membrane that are enriched in cholesterol and sphingolipids.[41] Rafts selectively incorporate proteins and govern protein-protein and protein-lipid interactions. They contribute to the structure and function of caveolae, plasma membrane invaginations, signal transduction, endocytosis, transcytosis, and cholesterol trafficking, as

Table 3—*Multiregression Analysis To Investigate Which Parameter Contributes to the Change of Cytokines*

Variables	Serum LTB_4 p Value	Sputum LTB_4 p Value	Sputum IL-8 p Value	Sputum TNF-α p Value
Group n-3 or n-6 (cumulative R^2)	< 0.05 (0.45)	< 0.05 (0.427)	< 0.05 (0.572)	< 0.05 (0.348)
Age	0.571	0.189	0.153	0.155
Smoking index	0.192	0.877	0.262	0.279
Daily energy intake (daily calories)	0.234	0.133	0.261	0.405
BMI before	0.322	0.231	0.243	0.333
BMI 24 mo after	0.123	0.112	0.141	0.134
Side effects	0.566	0.456	0.654	0.553
Compliance point	0.087	0.518	0.654	0.771
Confounding parameter point	0.153	0.987	0.856	0.971
Theophylline, yes or no	0.153	0.187	0.767	0.358
Acute exacerbation, yes or no	0.233	0.188	0.178	0.165

well as tyrosine kinase and sphingolipid cell signaling.[42] Despite its effect on the lipid raft, dietary omega-3 PUFA suppresses T-cell protein kinase Cθ lipid raft recruitment for IL-2 production,[43] modulates T-cell cytokinetics,[44] alters calcium signaling in T-cells[45] and vascular smooth-muscle cells,[46] and affects G protein-coupled signaling.[47] Furthermore, omega-3 PUFAs affect other inflammatory cytokines. Omega-3 PUFAs can suppress IL-1β gene expression,[48] affect IL-6 secretion,[49] diminish interferon-γ signaling,[50] reduce vascular endothelial growth factor expression, modulate extracellular signal-regulated kinase and hypoxia-inducible-factor 1α,[51] and inhibit cyclooxygenase-2 expression through the modulation of Toll-like receptor-mediated signaling pathways.[52] Moreover, omega-3 PUFAs affect transcriptional factors, inhibit activator protein-1 transcription factor activation by modulating mitogen-activated protein kinase signaling,[53] and alter nuclear factor-κB activity in leukocytes[54] and tumor cells.[55] Thus, omega-3 PUFAs have several effects on intracellular molecules. Therefore, we believe that n-3 PUFA supplementation might have other effects on the molecular mechanism of COPD.

The sample size and the time period used in our study were insufficient for reaching a definitive conclusion. We believe that a study on a larger scale and for a longer period of time should be conducted to assess the effectiveness of omega-3 PUFAs for treating COPD. However, the American Heart Association recommends supplementation of diets with omega-3 PUFAs[56] because they reduces the incidence of the cardiovascular disease incidence,[57] and our results also suggest a similar benefit. Taken together, we recommend nutritional support with omega-3 PUFAs for the nutritional treatment of COPD. The dosage of omega-3 PUFAs in our study is smaller than the standard dose used to treat cardiovascular diseases.[57] There is a strong negative correlation between the dosage of omega-3 PUFAs and the risk for sudden death in cardiovascular disease.[58] Further studies on the effects of high doses of omega-3 PUFAs in the treatment of COPD might be interesting.

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