Supplementary Treatment of Atopic Dermatitis Patients by Choosing Foods to Lower the *N*– 6/*N*– 3 Ratio of Fatty Acids

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In- and outpatients with atopic dermatitis and their families were advised to lower the $n-6/n-3$ ratio of fatty acids of patients' foods throughout one year. Basic nutritional recommendations were to eat traditional Japanese foods, that is, more seafood than meat, and to omit all kinds of high-linoleic acid ($n-6$) vegetable oils and their products (including fried cookies); use of perilla oil as a cooking oil with a very low $n-6/n-3$ ratio was advised. Topical steroidal anti-inflammatory drugs were used at the same time but were decreased toward the 3rd month of treatment. The $n-6/n-3$ ratio of serum lipids decreased significantly, atopic dermatitis area and severity index (ADASI) decreased dramatically and blood eosinophil counts decreased significantly, but the levels of serum IgE, total protein, total cholesterol, hemoglobin, calcium and iron were relatively unchanged. Kampo medicines were also used for some patients with weak constitution, but beneficial effects have been neither proved nor disproved during the year of treatment. Infants were more susceptible to this treatment than adults. Although a longer-term follow-up is necessary, the method was found to be promising and safe for the treatment of atopic dermatitis.

Key words —— atopic dermatitis, $n-6/n-3$ ratio, perilla oil, eicosanoid, α-linolenic acid, arachidonic acid

INTRODUCTION

The number of allergic patients has increased tremendously in the past several decades in Japan.[1,2] Although accurate incidence has not been determined because of a lack of diagnostic standardization, one out of 3 to 4 primary school students in Nagoya had been diagnosed as atopic (unpublished observations). The problem of increasing population with allergic hyper-reactivity in the past several decades appears to be common among the industrialized countries.[3]

Increased allergens have been proposed as causes for the increased number of patients, *e.g.*, tick (mite), fungi, pollen plus diesel particles, air pollutants and food additives which are associated with changed environments in Japan. However, increased allergens alone do not appear to account for the increase in number of allergic hyper-reactive patients. For example, the number of allergic patients has been increasing along with improvements in air pollution in the eastern part of Germany.[3] Patients suffering from a food allergy to rice in Japan and to wheat in Western countries have increased despite significant decreases in consumption of these foods. One effective therapeutic method for food allergy is to eliminate foods with antigenicity. Continuing to have selected foods without antigenicity, the subjects often begin to exhibit an allergy to the eliminated foods. All these observations lead to the suggestion that allergic reactivity of the human body has been enhanced somehow and that people in the industrialized countries have become more susceptible to allergens.[4] Although allergic reactivity of the body is very likely to be associated with genetic factors, the related genes would not have changed much within the past several decades during which period the number of allergic patients has increased dramatically.

One of us (Y.N.) recognized that atopic patients had been rare several decades ago and that patients were recommended to eat traditional Japanese foods, and found them very effective.[5] Linoleic acid ($n-6$) is converted to arachidonic acid ($n-6$), which is

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a precursor of allergic, inflammatory mediators such as leukotrienes, thromboxane and prostaglandins.[6–10] Platelet-activating factor (PAF), another inflammatory mediator, is produced effectively from arachidonic acid (AA)-containing precursor lipids.[11–14] Lipid mediators are also produced from eicosapentaenoic acid (EPA, $n-3$), but EPA is a poorer substrate than arachidonic acid for cyclooxygenases,[15] and lipoxygenase products of EPA have much lower biological activities than those derived from AA.[16–19] Furthermore, α-linolenic acid ($n-3$), EPA ($n-3$) and docosahexaenoic acid (DHA, $n-3$) competitively inhibit the $n-6$ fatty acid metabolism leading to allergic, inflammatory reactions[4]: at the elongation and desaturation step,[20,21] acyltransferase step[22,23] and eicosanoid synthetic step[24,25] as well as at the steps of receptors.[26,27] Therefore, we interpreted this as indicating that the increased intake of linoleic acid and elevated $n-6/n-3$ ratio of ingested foods saturated membrane phospholipids with AA, leading to persistent inflammation and allergic hyper-reactivity through over production of inflammatory lipid mediators.[4] Lipid mediators and inflammatory cytokines are likely to form an amplification cascade of inflammation. Dietary $n-3$ fatty acids also suppress the production and synthetic enzyme expression of these mediators.[28–36] In support of this interpretation, many of the anti-allergic drugs have been known to exert their effects by inhibiting the release from phospholipids and conversion to lipid mediators of AA as well as by inhibiting the release and action of lipid mediators.[4]

Based on this background, we adopted traditional Japanese foods and a selected cooking oil with special emphasis on lowering the $n-6/n-3$ ratio of ingested foods for the treatment of atopic dermatitis, so as to minimize and eliminate the use of steroidal anti-inflammatory drugs.

MATERIALS AND METHODS

Subject —— Atopic dermatitis patients were in- and outpatients of Shimonoseki City Central Hospital, Department of Pediatrics (Shimonoseki City). They were not only Shimonoseki citizens but were from broader areas of western Japan. Subjects and their families were free to follow or not to follow the dietary recommendations made, but were requested to visit the hospital at least every 6 months for diagnosis and blood sampling. Among 115 subjects who passed 1 year-treatment since the entry, complete data (3 times visit) were available on 77 subjects, which are analyzed in this paper (Table 1). No control group was established for this dietary intervention trial. Establishing a control group to eat foods with elevated $n-6/n-3$ fatty acid ratios was ethically unacceptable to us, because $n-6$ fatty acids are precursors of inflammatory mediators, and many kinds of anti-allergic drugs are known to exert their effects by inhibiting the actions of such mediators.

Table 1. A Classified List with Atopic Patients Treated for 1 Year

Group	Age	Number of patients
Group 1	0–2 Years	39
Group 2	3–6 Years	11
Group 3	7–15 Years	11
Group 4	> 16 Years, male	7
Group 5	> 16 Years, female	9

Medicines Used at Entry —— As internal medicines, ketotifen fumarate, hydroxyzine hydrochloride, azelastine hydrochloride, sodium cromoglycate, oxazolam, urinastatin were used but not anti-inflammatory drugs. Medicines used for external application were dexamethazone valerate, hydrocortisone butyrate, chloramphenicol-P and betamethasone valerate. Several kinds of Tsumura Kampo medicines for ethical use were used for those with weak constitution (TJ-17, TJ-19, TJ-22, TJ-32, TJ-43, TJ-51, TJ-54, TJ-80, TJ-86, TJ-99, TJ-134) but some of them (TJ-19, TJ-22, TJ-80 and TJ-86) were found later to have applications for allergic hyper-reactivity. These were Gorei-san (TJ-17), Sho-sei-ryu-to (TJ-19), Shofu-san (TJ-22), Ninjin-to (TJ-32), Rikkunshi-to (TJ-43), Juncho-to (TJ-51), Yoku-kan-san (TJ-54), Saiko-seikan-to (TJ-80), Toki-inshi (TJ-86), Sho-kenchu-to (TJ-99) and Keishi-ka-shakuyaku-daio-to (TJ-134).

Dietary Recommendations —— Adult subjects and family members of young subjects were recommended to eat traditional Japanese foods, which meant more seafood and vegetables and a lower amount of meat cooked less frequently as compared with current Japanese dishes. They were recommended not to eat commonly available vegetable oils or oil products (margarine, mayonnaise, salad dressing, fried foods or fried cookies). Perilla seed oil consisting of saturated fatty acids (7% of the total fatty acids), oleic acid (17%), linoleic acid ($n-6$)

(15%) and α-linolenic acid ($n-3$) (61%) was advised for use as a cooking oil. It has the lowest $n-6/n-3$ ratio (1/4) among the commonly available vegetable oils. Blood samples were obtained from fasting subjects but the dietary conditions were not accurately defined for infant subjects.

Hematological Parameters and Clinical Evaluations —— Serum IgE, eosinophil counts, total protein, total cholesterol, Ca, Fe and hemoglobin (Hb) were determined by clinical tests routinely used in the hospital. The ADASI was determined according to the standardized method of Ikezawa *et al*.[37] Photocopies of patients' dermatitis were taken as recorded data.

Fatty Acid Composition of Total Serum Lipids —— Frozen serum (100 μl) was mixed with chloroform/methanol, and the total lipids together with heptadecanoic acid added as an internal standard were extracted according to Bligh and Dyer's method.[38] After transmethylation with 5% HCl in methanol, fatty acid methylesters were analyzed by a gas-liquid chromatograph equipped with a capillary column (DB225, J&W Scientific, Folsom, CA). The results were fed back to dietary recommendations, taking the levels of EPA and DHA to indicate the measure of seafood intake, and α-linolenic acid as that of perilla oil intake.

Greenlander value was calculated as an indicator of the extent of fatty acid changes toward the typical fatty acids of Greenland natives (Innuits) having a value of 1.81[39] as follows:

$$\text{Greenlander value} = \{0.3 \times \text{C18}n-3\ (\%) + \text{C20}n-3\ (\%) + \text{C22}n-3\ (\%)\}/\{0.3 \times \text{C18}n-6\ (\%) + \text{C20}n-6\ (\%) + \text{C22}n-6\ (\%)\}/1.81$$

in which C represents carbon chain length of fatty acids, and a factor of 0.3 was tentatively used for correcting the possible differences in physiological activities between C18 carbon polyunsaturated fatty acids and C20 as well as C22 carbon polyunsaturated fatty acids. The values increased from zero (very low intake of $n-3$ fatty acids) to 1 (the level of $n-6$ and $n-3$ balance comparable to that of typical Innuits) and over, depending on the increased amounts of $n-3$ fatty acid intake relative to that of $n-6$ intake.

Statistical Analysis ——Changes in atopic dermatitis area and severity index (ADASI) and other clinical parameters as well as changes in fatty acid compositions during the 1-year intervention were analyzed by a repeated measures analysis of variance (ANOVA) and paired t-test using Statview 5 (SAS Institute Inc., CA), and $p < 0.05$ was judged statistically significant. Student's t-test was used for comparisons of two groups.

RESULTS

Fatty Acid Composition of Serum Lipids

Detailed fatty acid compositions of serum lipids during the 1-year intervention are presented only for Group 1 (0–2 years old) in Table 2. Despite dietary recommendations to reduce the intake of linoleic acid ($18:2n-6$), the average proportion of linoleic acid in serum lipids was relatively unchanged and that of arachidonic acid tended to increase. However, the proportions of EPA ($20:5n-3$) and DHA ($22:6n-3$) increased greatly, hence the EPA/AA ratio, DHA/AA ratio and Greenlander value were elevated significantly by the intervention. Some changes in the proportions of saturated and monounsaturated fatty acids were also noted.

The number of subjects in other groups was relatively small ($n = 7$–11) and detailed fatty acid compositions are not shown, but similar changes in the $n-6/n-3$ ratios were noted, except in Group 2 in which the change in the $n-6/n-3$ ratio was not statistically significant (Fig. 1). In Groups 3 and 5, the proportion of linoleic acid decreased after 1 year of intervention from 28.6 to 25.1 and from 28.4 to 25.4% of the total, respectively; the proportion of AA decreased from 6.1 to 4.5 and from 5.5 to 5.0%, respectively. However, such changes were not apparent in the other groups. The average proportion of α-linolenic acid increased relatively little in all the groups, although significant increases were noted in some individuals (data not shown).

Serum total fatty acid content decreased significantly in Group 1 (from 308 to 239 mg/dl) but was relatively unchanged in Group 2 (240 to 230). It increased significantly in Group 3 (245 to 328) but was unchanged in Group 4 (238 to 239) and Group 5 (267 to 270).

Clinical Parameters

ADASI decreased dramatically even after 6 months of the intervention (Fig. 2). The treatment tended to be more effective for younger generations than for adults (Groups 4 and 5).

The eosinophil counts decreased significantly in Group 1, and tended to decrease in other groups as well (Fig. 3). In contrast, serum IgE level was rela-

Table 2. Fatty Acid Composition of Serum Lipids from Group 1 (0–2 y) (% of Total Fatty Acids)

	0 month	6 month	12 month	Repeated-measures
Fatty acids	mean ± S.D.	mean ± S.D.	mean ± S.D.	ANOVA
14 : 0	1.75 ± 1.13	1.93 ± 1.75	1.43 ± 0.75 b	N.S.
14 : 1	0.14 ± 0.23	0.04 ± 0.06 a	0.04 ± 0.04 a	$p < 0.01$
16 : 0 DMA	0.55 ± 0.37	0.57 ± 0.62	0.44 ± 0.27	N.S.
16 : 0	22.41 ± 2.30	22.71 ± 2.09	23.39 ± 2.36	N.S.
16 : 1	1.96 ± 0.55	1.84 ± 0.56	2.29 ± 0.74 ab	$p < 0.01$
18 : 0 DMA	0.22 ± 0.30	0.20 ± 0.18	0.15 ± 0.16	N.S.
18 : 1 DMA	0.77 ± 1.25	0.32 ± 0.55	0.18 ± 0.38*a*	$p < 0.01$
18 : 0	8.75 ± 1.31	8.64 ± 4.37	7.97 ± 1.30 a	N.S.
18 : 1	21.39 ± 4.37	15.81 ± 3.31 a	17.90 ± 3.69 ab	$p < 0.001$
18 : 2 $n-6$	24.15 ± 4.08	25.50 ± 5.40	24.39 ± 4.71	N.S.
18 : 3 $n-6$	0.58 ± 0.72	0.37 ± 0.60	0.42 ± 0.47	N.S.
18 : 3 $n-3$	0.91 ± 0.45	0.90 ± 0.29	1.04 ± 0.44 b	N.S.
20 : 0	0.30 ± 0.10	0.26 ± 0.08	0.26 ± 0.06	N.S.
20 : 1	0.27 ± 0.25	0.48 ± 1.25	0.25 ± 0.21	N.S.
20 : 3 $n-6$	1.04 ± 0.34	0.94 ± 0.29	1.04 ± 0.33 b	N.S.
20 : 4 $n-6$	5.14 ± 1.41	5.81 ± 0.99 a	5.49 ± 1.15	$p < 0.05$
20 : 5 $n-3$	1.96 ± 1.50	3.94 ± 2.32 a	3.37 ± 2.48 a	$p < 0.001$
22 : 0	0.63 ± 0.44	0.49 ± 0.13	0.47 ± 0.10 a	$p < 0.05$
22 : 1	0.48 ± 1.10	0.07 ± 0.24 a	0.00 ± 0.00 a	$p < 0.01$
22 : 4 $n-6$	0.05 ± 0.10	0.01 ± 0.03 a	0.03 ± 0.06 b	$p < 0.05$
22 : 5 $n-6$	0.19 ± 0.22	0.12 ± 0.13	0.15 ± 0.17	N.S.
22 : 5 $n-3$	0.58 ± 0.26	0.89 ± 0.32 a	1.03 ± 0.34 ab	$p < 0.001$
22 : 6 $n-3$	4.27 ± 1.67	6.48 ± 1.96 a	6.64 ± 1.74 a	$p < 0.001$
24 : 0	0.34 ± 0.23	0.39 ± 0.20 a	0.39 ± 0.19	N.S.
24 : 1	1.19 ± 0.38	1.33 ± 0.33	1.25 ± 0.35	N.S.
EPA / AA	0.39 ± 0.31	0.67 ± 0.38 a	0.62 ± 0.43 a	$p < 0.001$
DHA / AA	0.84 ± 0.31	1.13 ± 0.34 a	1.25 ± 0.41 ab	$p < 0.001$
$n-6/n-3$	5.26 ± 4.41	3.14 ± 1.58 a	3.03 ± 1.38 a	$p < 0.001$
Greenlander value	0.29 ± 0.14	0.46 ± 0.21 a	0.46 ± 0.22 a	$p < 0.001$
Total FA (mg/dl)	313.28 ± 74.84	266.04 ± 60.30 a	242.92 ± 44.74 ab	$p < 0.01$

Values are means ± S.D. for 39 atopic patients. Values in a row with different superscripts differ significantly at $p < 0.05$. (N.S., not significant). Fatty acids are designated by the carbon chain length: the number of double bonds, and the position of the first double bond numbered from methyl terminus as $n-6$ or $n-3$. DMA denotes dimethylacetal derived from plasmalogens.

tively unchanged during the 1-year intervention in all the groups (Fig. 4).

In Group 1, possible correlation between the ADASI and IgE level as well as that between ADASI and eosinophil counts was examined (Fig. 5) and correlation was found to be good in both areas at 0 month. Later on, ADASI and eosinophil counts decreased significantly and the correlation was not apparent. At 6 and 12 months, there was a case with an exceptionally high IgE level, indicating that the correlation between ADASI and IgE is relatively unreliable. For other groups, the number of subjects was too small to analyze the correlation among these parameters.

Medicines Prescribed

Atopic dermatitis was generally treated first with steroidal anti-inflammatory drugs (SAID, strong), shifted to SAID (medium strength) at around 2 months of the treatment and then to a weak type of SAID. Most infants (0–2 years old) no longer required SAID after 6 months of the treatment or thereafter, while it took longer for aged groups.

At entry, 45% and 31% of the patients received internal medicines and Kampo medicines as described in Materials and Methods, respectively (Fig. 6). However, the number of those receiving medicines and the number of prescribed medicines decreased significantly during the 1 year of the dietary intervention. Similar changes in the prescribed

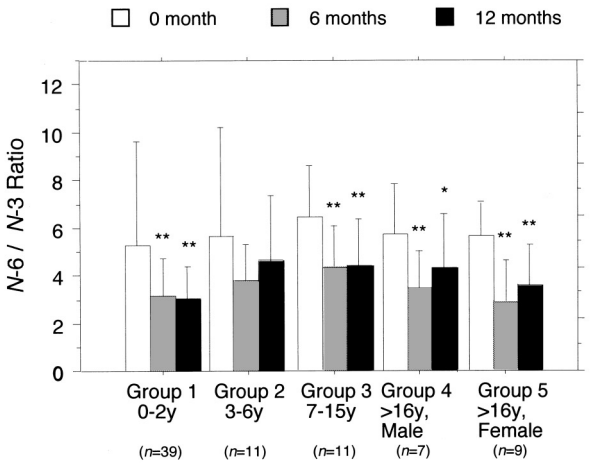

Fig. 1. Changes in the $n-6/n-3$ Ratios of Serum Lipids during 1 Year Intervention

Each column with a bar represents mean ± S.D. Statistic analysis was performed with repeated-measures one-way ANOVA and Student's *t*-test. * $p < 0.05$, ** $p < 0.01$ *vs.* 0 month.

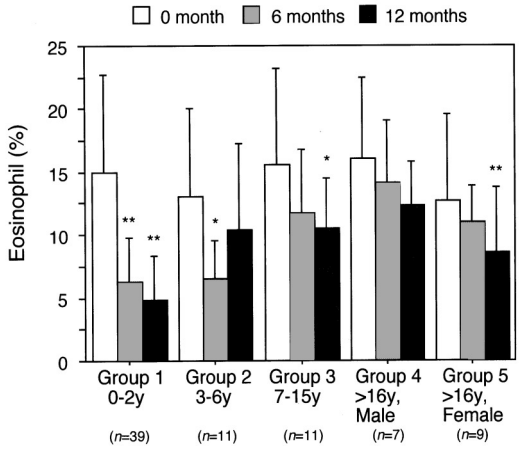

Fig. 3. Changes in Eosinophil Counts during 1 Year of Intervention

Each column with a bar represents mean ± S.D. Statistic analysis was performed with repeated-measures one-way ANOVA and Student's *t*-test. * $p < 0.05$, ** $p < 0.01$ *vs.* 0 month.

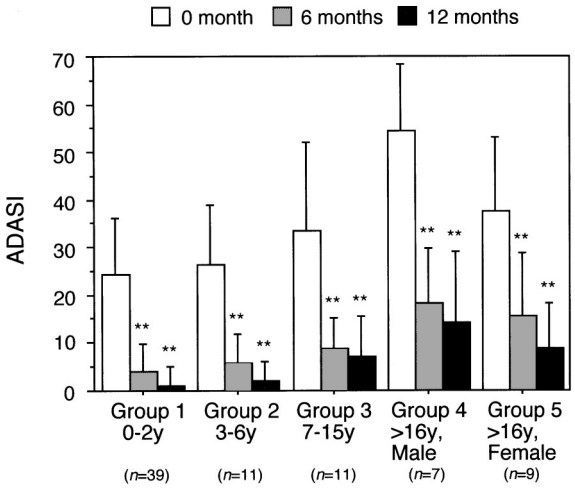

Fig. 2. Changes in the ADASI during 1 Year Intervention

Each column with a bar represents mean ± S.D. Statistic analysis was performed with repeated-measures one-way ANOVA and Student's *t*-test. ** $p < 0.01$ *vs.* 0 month.

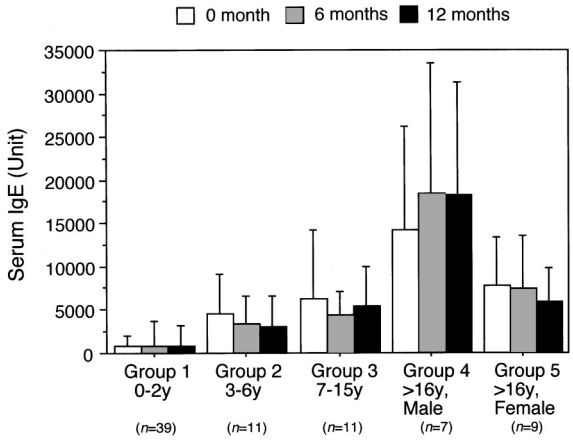

Fig. 4. Effect of Dietary Intervention on Serum IgE Level

Each column with a bar represents mean ± S.D. Statistic analysis was performed with repeated-measures one-way ANOVA and Student's *t*-test.

medicines were observed when Group 1 was analyzed separately (data not shown).

To examine the role of Kampo medicines, Group 1 was classified into 4 groups: (a) Kampo medicines were prescribed throughout the 12 months, (b) they were prescribed at entry but were stopped after 6 months, (c) they were not prescribed at entry but were begun after 6 months of intervention, and (d) they were not prescribed at all during the 12 months. ADASI decreased similarly in all groups, indicating that the Kampo medicines did not significantly affect atopic dermatitis (Fig. 7). Both the $n-6/n-3$ ratios and Greenlander values changed as expected from the dietary recommendations in all the groups except group (c). Interestingly in group (c) with Kampo medicines prescribed from 6 months of intervention, the serum fatty acid composition was relatively unchanged as judged by $n-6/n-3$ ratio and Greenlander values (Fig. 7). This analysis was made after decision by the clinicians at 6 months of intervention, suggesting a possible correlation between the insufficient practice of the recommended nutrition and insufficient improvement in ADASI in this group.

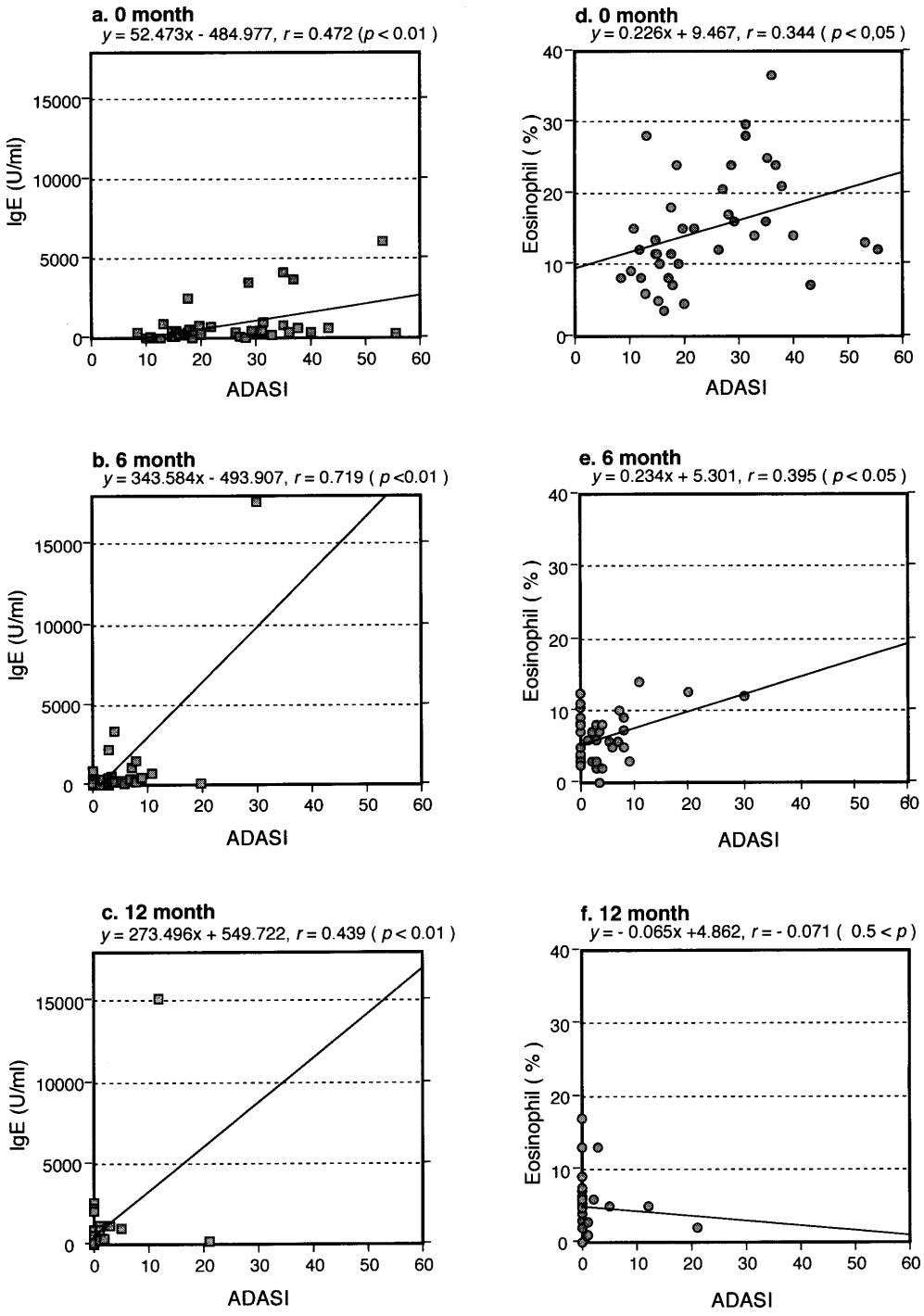

Fig. 5. Correlation between ADASI and IgE or between ADASI and Eosinophil Count in Group 1 (0–2 y)

DISCUSSION

Polyunsaturated fatty acids of the $n-6$ series (*e.g.*, linoleic, γ-linolenic, dihomo-γ-linolenic and AA) and the $n-3$ series (α-linolenic, EPA and DHA) are not synthesized de novo in mammals. Because different foods contain different proportions of $n-6$ and $n-3$ fatty acids, the $n-6/n-3$ balance of cellular lipids can be modified by dietary manipulation, resulting in an altered inflammatory tone and haemodynamics.[4)]

In the present intervention trial, some subjects had Greenlander values much higher than an average Japanese (0.25) at entry, possibly because of available information on beneficial effects of $n-3$ fatty acids on allergic hyper-reactivity.[40–53)] We

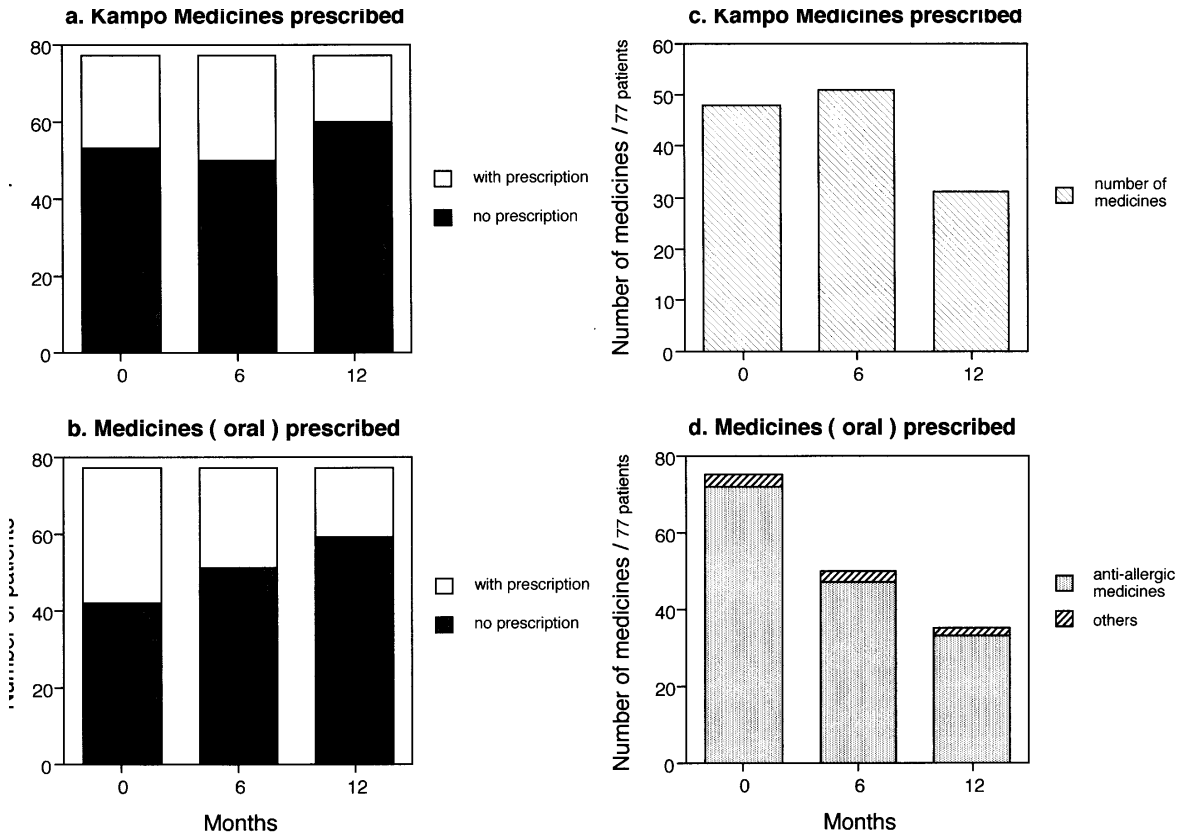

Fig. 6. Number of Patient for Whom Medicines Were Prescribed and the Number of Medicines Prescribed (n = 77)

strongly recommended reducing the intake of $n-6$ fatty acids but decreased proportions of these fatty acids in serum lipids were observed only in Groups 3 and 5. There were large variations among individuals in the responses of serum fatty acids to dietary recommendations. The failure to reduce serum $n-6$ fatty acids in most infants is likely to be due mainly to the fact that many kinds of infant formula and foods for weaning infants contain extraordinarily high amounts of linoleic acid [Hayashi H. *et al.*, Abstract, *J. Lipid Nutr.*, **8**, 78 (1999)]. Another factor is the AA content in seafood. Although this is relatively small (less than several % of the total), it is preferentially incorporated into membrane phospholipids, and therefore decreasing AA in plasma lipids only by fish oil supplement is not easy even in animal experiments, although EPA/AA ratios could be increased significantly. Nevertheless, we emphasize that serum $n-6$ fatty acids could be reduced when their supply was limited to very low levels, *e.g.*, below 3.8 energy % as recommended by the Japan Society for Lipid Nutrition[54] and at an International Workshop.[55]

Total fatty acid content in serum decreased significantly during the 1-year intervention in Group 1, possibly reflecting the dietary changes from high-fat milk (~ 50 energy %) to conventional foods with lower fat contents (~ 25 energy %), and these changes are probably physiological. In contrast, the total fatty acid content increased significantly in Group 3 (7–15 years old) at 1 year of intervention, and we have found no rational explanation for these changes because $n-3$ fatty acids, particularly fish oil EPA and DHA have activities to lower plasma triacylglycerol levels. We suspect that people this age are eating more high-fat foods than other groups are.

The present experiment was designed to evaluate the effectiveness of dietary manipulation on atopic dermatitis while minimizing the use of internal and external medicines. In fact, prescriptions of these drugs decreased significantly during the 1-year intervention. We used several kinds of Kampo medicines with application for those with weak constitution but some of them had application for allergic hyper-reactivity. Although Kampo medicines did not affect the ADASI or IgE levels (Fig. 7), it should be emphasized that the present results did not disprove the proposed effectiveness of these medicines for allergic hyper-reactivity. Under the current Medi-

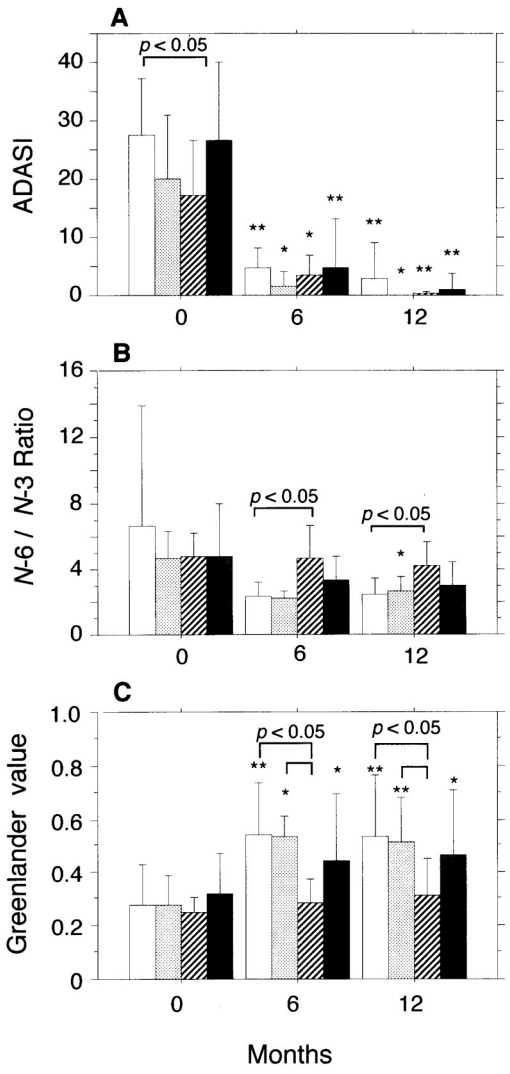

Fig. 7. Effect of Kampo Medicine Prescription on ADASI (A), $n-6/n-3$ Ratio (B) and Greenlander Value (C)

□, Kampo medicines were prescribed throughout the 12 months (n=11);

▨, they were prescribed at entry but were stopped after 6 months (n=5);

▨, they were not prescribed at entry but begun after 6 months (n=7);

■, they were not prescribed at all during the 12 months (n=16).

care System in a public hospital in Japan, it would not be easy to treat patients without using registered ethical medicines. Although some patients continued to use medicines after the 1-year period, the dietary intervention was found to be promising for the treatment of atopic dermatitis using steroidal anti-inflammatory drugs only in the early stages of treatment.

As to the safety of the present dietary recommendations, we emphasize that the essential amount of linoleic acid is 1 energy %, and that those who take enough energy will not suffer from $n-6$ fatty acid deficiency; common food materials such as rice, wheat, meat and egg supply at least 2 en %. In fact, the subjects experienced no health problems associated with the present dietary intervention or with the clinical parameters such as serum protein, cholesterol, Ca, Fe and hemoglobin.

Despite significant improvement in ADASI and eosinophil counts, no significant improvement in serum IgE levels was observed under the treatment conditions. Recently, allergic hyper-reactivity in the airway was found to occur without involving mast cells or IgE; eosinophils and IgG appear to be involved.[56] In animal experiments, IgE production was slightly increased without increase in IgG production by perilla oil with a very low $n-6/n-3$ ratio.[57] The observed 1.5-fold increase in IgE production by perilla oil is much lower than the variations of IgE levels observed in humans (from undetectable level to ~ 10^4 unit). Whether the IgE level could be lowered by longer-term dietary intervention or whether dietary $n-6/n-3$ balance does not affect this level significantly in humans remains to be elucidated. A longer-term follow-up is necessary to ascertain if IgE levels can also be lowered and recurrence does not occur as long as a low tissue $n-6/n-3$ balance is maintained.

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