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Fatty Acid Compositions of Plasma Lipids in Young Atopic Patients

KEIKO SAKAI,[a] KOHJI UENO,[a] YUNOSUKE OGAWA[b]
and HARUMI OKUYAMA*,[a]

Faculty of Pharmaceutical Sciences, Nagoya City University,[a] 3–1 Tanabedori, Mizuhoku, Nagoya 467, Japan and Department of Pediatrics, Saitama Medical Center,[b] 1981 Kamoda, Kawagoe, Saitama 350, Japan

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The fatty acid compositions of plasma phosphatidylcholine, cholesterol ester, triacylglycerol and free fatty acid fractions were determined in young atopic patients and age-matched controls. No difference was observed in the lipid contents or the fatty acid compositions between the atopic patients and controls. When compared with adult controls, plasma lipids from young children had significantly different fatty acid patterns; the proportions of total linoleate (n-6) series fatty acids and the linoleate/arachidonate ratio were not statistically different, but the proportions of alpha-linolenate (n-3) series fatty acids were much less, particularly in the phosphatidylcholine, triacylglycerol and cholesterol ester fractions, in young children. These results do not provide rationale for the use of gamma-linolenic acid for atopic therapy. The possible usefulness of providing alpha-linolenate series fatty acid supplementation for children is discussed.

Keywords——atopy; plasma lipid; fatty acid; linoleic acid; alpha-linolenic acid; phosphatidylcholine; cholesterol ester

In the past 30 years, the number of atopic patients is said to have increased several-fold in Japan. Atopy is closely related to immunological phenomena, with an increase in IgE and decrease in T-lymphocytes.[1] The involvement of unsaturated fatty acids in atopic eczema has been suggested in relation to essential fatty acid deficiency; dermatitis or skin lesion, one of major symptoms of the deficiency, is prevented by unsaturated fatty acids. Very recently, Horrobin and coworkers reported[2,3] that in atopic patients the proportion of linoleate is elevated with a concomitant decrease in the proportions of its elongation and desaturation products, leading to the hypothesis that Δ^6-desaturase, the presumed rate-limiting step in the formation of dihomo-γ-linolenic acid (20 : 3 n-6)[4] and arachidonic acid (20 : 4 n-6), may be impaired in such patients. This appears to be the basis for proposing γ-linolenic acid (18 : 3 n-6) supplementation for the therapy of atopic eczema.[2,3,5] Evening primrose oil containing γ-linolenic acid has been used, and was reported to improve the eczema significantly.[2,3,5,6] However, the administration of γ-linolenic acid would increase the proportion of arachidonic acid in the long run and its metabolites, leukotrienes, are known to cause bronchoconstriction, a typical symptom of atopy-related asthma. Thus, the supplementation of n-6 series fatty acids may not be a radical cure for atopy. In this study, we compared the fatty acid compositions of plasma lipids in young atopic patients, young controls and adult controls, and failed to find any rational basis for the use of γ-linolenic acid for atopic therapy. Instead, the possible usefulness of alpha-linolenic acid series fatty acid supplementation for children in general is discussed.

Materials and Methods

Six atopic patients, 5 to 40 months of age, 4 males and 2 females, were outpatients of Nagoya City University

Hospital. They were in good health, except for atopic dermatitis, and were receiving topical treatment with steroid hormone-containing ointment with or without oral antihistamines. Normal children were 8 to 36 months old, 5 males and 2 females. Five normal male adults were 40±7 years old. Four females (47±2 years old) with elevated plasma cholesterol levels, 235 to 344 mg/dl, but without overt disease were also examined. Adult subjects were fasted overnight before collection of blood samples.

Plasmas obtained by brief centrifugation (1500 rpm for 5 min) were kept frozen at −20 °C. Total lipids were extracted from 0.3 ml of plasma according to the method of Bligh and Dyer.[7] For the separation of individual lipids, Silica gel 60 (Merck) plates (prewashed with developing solvents) were used. Lipids were separated first with petroleum ether–diethyl ether–acetic acid (80 : 30 : 1, v/v) as the solvent. Cholesterol ester, triacylglycerol, free fatty acid and phospholipid fractions were located by spraying with Rhodamin 6G. The spot corresponding to each lipid was scraped off the plate, and lipids were extracted three times with chloroform–methanol (2 : 1). The phospholipid fraction was rechromatographed with chloroform–methanol–water (70 : 30 : 5) as the solvent and the spot corresponding to phosphatidylcholine was treated as described above. A defined amount of heptadecanoic acid was added to each fraction as an internal standard. Fatty acids were converted to methyl esters by using 5% HCl in methanol and analyzed by gas chromatography with a column of 10% EGSS-X on Chromosorb W at 190 °C.

Results

Plasma lipids were separated by silica gel thin-layer chromatography and the fatty acids of each individual lipid were quantitated by gas chromatography with heptadecanoic acid as an internal standard.

When young atopic patients and age-matched controls were compared, no difference was observed in the lipid contents or the fatty acid compositions as shown in Tables I—IV. No tendency for elevation of the linoleate/(dihomo-γ-linolenic acid plus arachidonic acid, poly n-

Table I. Fatty Acids of Plasma Phosphatidylcholine

Fatty acids	Adult, male $n=5$ (%)	Adult, female $n=4$ (%)	Young, normal $n=7$ (%)	Young, atopic $n=6$ (%)
14:0	0.4±0	0.1±0	0.2±0.1	0.3±0
16:0	31.5±0.5	32.5±0.7	31.1±1.3	32.5±1.1
16:1	0.3±0	0.3±0.1	0.7±0.4	0.7±0.2
18:0	17.1±0.7	16.8±0.9	13.3±0.8	15.3±0.8
18:1	8.3±0.6	7.0±0.9	15.0±0.4	13.8±1.4[d]
18:2 n-6	20.0±0.9	20.1±0.9	25.6±1.7	23.9±1.8
18:3 n-6	tr	0.1±0	tr	tr
18:3 n-3	0.1±0	0.1±0	0.1±0	0.2±0
20:1	tr	0.1±0	tr	0.2±0
20:3 n-9	tr	0.3±0.1	0.2±0.1	0.3±0
20:3 n-6	1.7±0.4	2.1±0.5	2.1±0.2	2.3±0.3
20:4 n-6	9.4±0.8	9.1±0.3	6.3±0.3	6.1±0.8[e]
20:5 n-3	3.4±1.0	2.8±0.4	0.3±0.2	tr[d]
22:4 n-6	tr	0.3±0	0.1±0.1	0.2±0.1
22:5 n-3	0.6±0.1	0.6±0.1	0.3±0.1	tr
22:6 n-3	7.1±0.3	7.3±0.6	3.8±0.9	3.6±1.3[e]
Lipid content (mg/dl)	30.3±4.0	66.5±1.4	81.7±8.8	68.8±3.8[c]
Saturated acids	49.0±0.7	49.4±0.4	44.7±1.4	48.1±0.5
Unsaturated acids	51.0±0.7	50.1±0.4	55.1±1.5	51.8±0.5
S/U[a]	0.96±0.02	0.99±0.02	0.82±0.05	0.93±0.02
Total n-3 acids	13.0±1.0	10.8±1.0	4.4±1.1	3.8±1.2[c]
Total n-6 acids	29.4±1.0	31.6±0.8	34.1±1.6	32.4±1.1
(n-3)/(n-6)	0.45±1.00	0.35±0.04	0.13±0.03	0.12±0.04[c]
18:2/poly(n-6)[b]	2.19±0.20	1.77±0.13	3.27±0.49	3.12±0.61

a) Saturated/unsaturated ratio. *b*) Poly(n-6) includes 18:3 n-6, 20:3 n-6, 20:4 n-6 and 22:4 n-6. Significantly different from adult, male: *c*) $p<0.001$; *d*) $p<0.01$; and *e*) $p<0.05$.

TABLE II. Fatty Acids of Plasma Cholesteryl Esters

Fatty acids	Adult, male $n=5$ (%)	Adult, female $n=4$ (%)	Young, normal $n=7$ (%)	Young, atopic $n=6$ (%)
14:0	0.6±0	0.5±0.1	0.9±0.4	0.9±0.1
16:0	12.9±0.4	10.8±0.5	10.8±0.6	12.7±0.7
16:1	3.1±0.5	4.7±1.5	3.5±0.5	2.9±0.5
18:0	0.8±0.2	0.5±0.1	0.8±0.2	0.8±0.1
18:1	15.7±0.4	16.7±2.1	21.4±1.5	23.4±2.5[a]
18:2n-6	55.3±1.7	51.1±4.6	56.2±3.0	52.8±3.2
18:3n-6	tr	0.1±0.1	0.1±0.1	0.3±0.1
18:3n-3	0.3±0	0.4±0.1	0.2±0.1	0.3±0.1
20:1	tr	0.1±0	tr	tr
20:3n-9	tr	tr	0.1±0	0.1±0.1
20:3n-6	0.1±0.1	0.5±0.2	0.3±0.1	0.4±0.1
20:4n-6	7.3±1.1	7.4±0.6	4.4±0.3	4.3±0.7[a]
20:5n-3	2.9±0.5	3.2±0.7	0.3±0.2	tr[b]
22:4n-6	tr	0.1±0	tr	tr
22:5n-3	tr	0.1±0	0.1±0	tr
22:6n-3	1.1±0.2	2.6±0.5	0.4±0.2	0.4±0.2
Lipid content (mg/dl)	63.8±11.0	81.5±9.0	69.5±1.7	67.6±5.4
Saturated acids	14.3±0.4	11.8±0.6	12.5±1.2	14.3±0.7
Unsaturated acids	85.7±0.4	86.9±1.1	87.4±1.2	85.5±0.8
S/U	0.17±0.01	0.14±0.01	0.15±0.02	0.17±0.01
Total n-3 acids	4.4±0.5	6.3±0.8	1.0±0.3	0.8±0.3[b]
Total n-6 acids	62.7±1.0	59.1±4.5	61.1±2.7	57.9±2.6
(n-3)/(n-6)	0.07±0.01	0.11±0.01	0.02±0.01	0.01±0.01[b]
18:2/poly(n-6)	8.36±1.31	6.60±0.84	12.37±1.39	12.71±2.66

Significantly different from adult, male: *a*) $p<0.05$; *b*) $p<0.001$.

6) ratio was seen in the atopic patients. Thus, there is no rationale for supplementing γ-linolenic acid (evening primrose oil) to young atopic patients.

Fatty acid compositions of plasma lipids in adults are also shown in Tables I—IV. The male controls and the female subjects with higher plasma cholesterol levels showed quite similar fatty acid patterns of phosphatidylcholine, cholesterol ester and triacylglycerol.

When adults and children were compared, significant differences were observed (Fig. 1). The difference in the proportions of n-6 series fatty acids was relatively small; the linoleate/(dihomo-γ-linolenate plus arachidonate) ratio appeared to be slightly higher in the children than in the adults, but this was not significant statistically. However, the proportions of n-3 series fatty acids were significantly less in young children than in adults. Not only the proportions of n-3 fatty acids, but also the absolute amounts of n-3 fatty acids in plasma were significantly less in the children than in the adults (Fig. 2). In contrast, the plasma concentrations of linoleate and arachidonate were even higher in the children than in the adults.

Discussion

Higher animals cannot synthesize linoleate and alpha-linolenate. When supplied, however, these fatty acids are metabolized to form more elongated and desaturated fatty acids, some of which are known to serve as precursors of autacoid synthesis.

TABLE III. Fatty Acids of Plasma Triacylglycerol

Fatty acids	Adult, male $n=5$ (%)	Adult, female $n=4$ (%)	Young, normal $n=7$ (%)	Young, atopic $n=6$ (%)
14:0	1.9±0.3	1.2±0.3	3.1±1.0	2.5±0.4
16:0	26.7±3.8	22.4±1.5	26.2±2.0	28.9±2.1
16:1	5.1±0.4	7.6±1.5	3.7±0.7	3.2±0.4
18:0	3.1±0.6	2.2±0.3	4.7±0.7	4.4±0.5
18:1	33.1±2.0	33.4±1.0	39.8±1.9	40.5±2.7
18:2 n-6	20.8±1.8	23.0±3.8	19.9±2.9	19.3±1.3
18:3 n-6	tr	0.1±0	0.1±0	tr
18:3 n-3	1.4±0.3	1.3±0.3	0.5±0.1	0.8±0.2
20:1	0.4±0.2	0.1±0.1	0.3±0.1	0.2±0.1
20:3 n-9	0.1±0.1	0.1±0	tr	0.5±0.4
20:3 n-6	tr	0.2±0.1	tr	0.2±0.2
20:4 n-6	1.7±0.1	1.3±0.2	0.5±0.1	0.7±0.2[a]
20:5 n-3	1.5±0.6	1.3±0.4	tr	0.1±0.1
22:4 n-6	tr	0.1±0.1	tr	tr
22:5 n-3	0.9±0.3	0.3±0.1	tr	tr
22:6 n-3	3.4±0.6	4.5±1.6	0.2±0.1	0.7±0.5[b]
Lipid content (mg/dl)	42.9±11.0	52.6±6.7	83.2±14.3	60.6±9.3
Saturated acids	31.7±3.9	25.9±2.0	34.0±3.3	35.7±2.7
Unsaturated acids	68.3±3.9	73.3±1.7	65.5±3.3	64.4±2.7
S/U	0.49±0.01	0.36±0.04	0.54±0.1	0.57±0.02
Total n-3 acids	7.2±1.7	7.4±1.8	0.8±0.2	1.7±0.6[a]
Total n-6 acids	22.6±1.8	24.7±3.9	20.5±2.8	20.4±2.0
(n-3)/(n-6)	0.32±0.06	0.33±0.09	0.06±0.03	0.08±0.03[a]
18:2/poly(n-6)	12.40±1.17	14.35±2.75	31.44±11.44	28.79±7.86

Significantly different from adult, male: *a*) $p<0.01$; *b*) $p<0.001$.

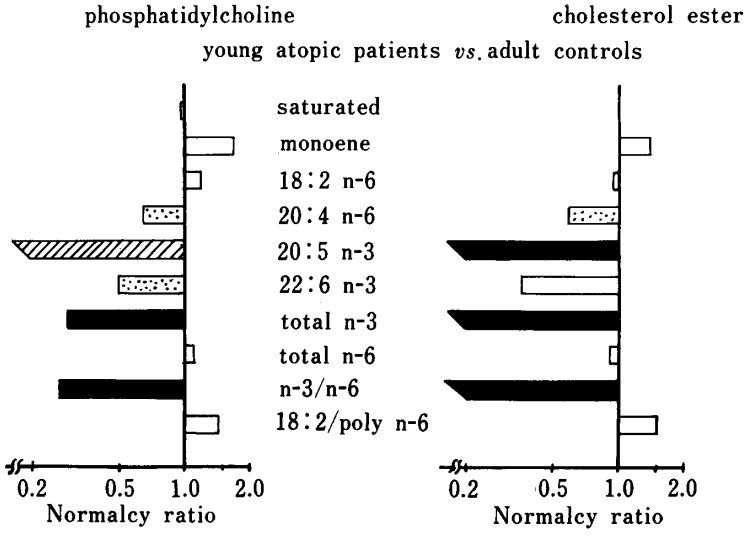

Fig. 1. Profiles of Fatty Acids in Plasma Phosphatidylcholine and Cholesterol Ester

Six atopic patients were compared to 5 adult controls. The normalcy ratio, according to Holman *et al.*,[11] is the ratio of the proportion of each fatty acid in the patient to that of the control. Significance is indicated by shading: open bars, not significant; ▨, $p<0.05$; ▨, $p<0.01$; ■, $p<0.001$. Pointed bars extend beyond the scale. It should be noted that no significant difference was observed between normal children and atopic children, as shown in Table I—IV.

linoleic acid (n-6) ⟶ γ-linolenic acid ⟶ dihomo-γ-linolenic acid ⟶ arachidonic acid

alpha-linolenic acid (n-3) ⟶ ⟶ eicosapentaenoic acid ⟶ ⟶ docosahexaenoic acid

These polyenoic acids are not necessary for the growth and division of animal cells in culture. However, deprivation of these fatty acids causes essential fatty acid deficiency. Supplementation of n-6 series fatty acids such as linoleic and arachidonic acids apparently ameliorates the essential fatty acid deficiency symptom, but alpha-linolenate is partially effective. Furthermore, rats could be raised without overt deficiency symptom through three generations by supplementing only linoleate.[8] Thus, it has long been recognized that linoleate is essential for higher animals but alpha-linolenate is not. The essential requirement for linoleate has been supported by the finding that various arachidonate metabolites have physiological activities.

In contrast to rats, in which linoleate and alpha-linolenate are rapidly desaturated and elongated, it appears that in humans a longer time is necessary for these fatty acids to be metabolized. Furthermore, impairment of the desaturase and elongation systems appears to occur in fatty liver or in cirrhosis,[9] since the linoleate/dihomo-γ-linolenate plus arachidonate) ratio is elevated under such pathologic conditions. Horrobin and coworkers[2,3] proposed that Δ^6-desaturase may be impaired in human atopic patients. If this were the case, the supplementation of γ-linolenic acid or evening primrose oil containing γ-linolenic acid would

TABLE IV. Fatty Acids of Plasma Free Fatty Acid

Fatty acids	Adult, male $n=5$ (%)	Adult, female $n=4$ (%)	Young, normal $n=7$ (%)	Young, atopic $n=6$ (%)
14:0	1.8±0.2	1.4±0.1	1.7±0.4	2.3±0.2
16:0	30.1±2.8	25.8±0.5	25.8±2.0	27.3±1.0
16:1	3.8±0.6	6.0±1.1	2.3±0.7	3.0±0.8
18:0	10.5±0.7	5.8±0.5	13.0±1.5	12.8±1.0
18:1	34.2±2.7	34.6±0.8	37.7±2.3	34.1±2.6
18:2 n-6	15.1±0.8	17.6±2.0	14.9±2.1	13.4±1.7
18:3 n-6	tr	tr	0.5±0.5	tr
18:3 n-3	1.2±0.1	1.3±0.2	0.3±0.2	0.8±0.2
20:1	0.6±0.1	0.1±0.1	0.3±0.2	0.3±0.1
20:3 n-9	tr	0.1±0.1	0.2±0.1	0.1±0.1
20:3 n-6	tr	0.2±0.1	0.1±0.1	0.1±0
20:4 n-6	1.0±0.1	0.8±0.1	1.0±0.2	1.4±0.6
20:5 n-3	0.1±0.1	0.2±0.1	0.2±0.1	0.5±0.3
22:4 n-6	tr	0.4±0.1	tr	0.3±0.2
22:5 n-3	tr	0.6±0.1	0.1±0	0.1±0.1
22:6 n-3	1.6±0.4	1.1±0.4	0.1±0	0.5±0.3[a]
Lipid content (mg/dl)	8.4±1.3	11.2±2.8	9.8±1.6	10.3±2.5
Saturated acids	42.4±2.3	32.9±0.6	40.6±3.2	42.5±1.4
Unsaturated acids	57.6±2.3	62.7±2.1	58.5±3.3	57.5±1.4
S/U	0.75±0.08	0.53±0.02	0.73±0.10	0.75±0.05
Total n-3 acids	2.9±0.6	3.1±0.5	0.7±0.2	1.9±0.5
Total n-6 acids	16.1±0.9	18.9±1.9	16.5±1.7	16.9±2.5
(n-3)/(n-6)	0.18±0.03	0.17±0.04	0.05±0.02	0.11±0.02
18:2/poly(n-6)	16.30±2.82	13.97±2.79	11.46±4.56	6.98±2.39[a]

Significantly different from adult, male: *a*) $p<0.05$.

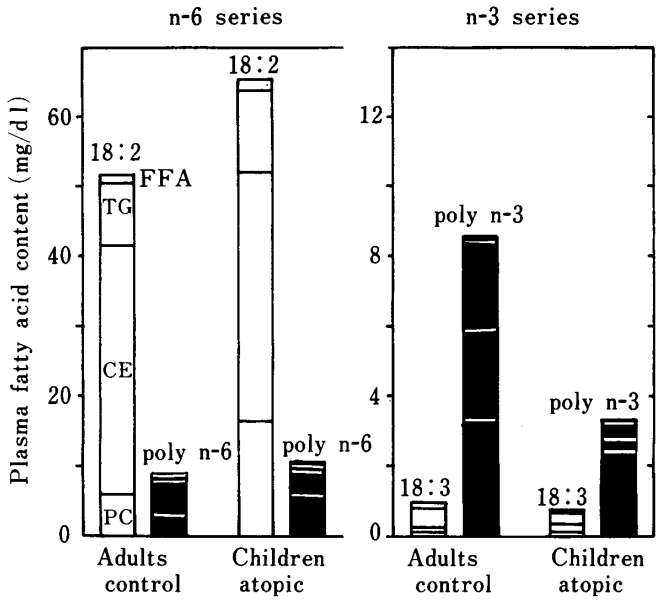

Fig. 2. Fatty Acid Contents in Plasma

Linoleate (n-6) series and alpha-linolenate (n-3) series fatty acids were compared between atopic patients and adult male controls. Poly n-6 includes $\Delta^{8,11,14}$-20:3, $\Delta^{5,8,11,14}$-20:4, and $\Delta^{7,10,13,16}$-22:4, while poly n-3 includes $\Delta^{5,8,11,14,17}$-20:5, $\Delta^{7,10,13,16,19}$-22:5 and $\Delta^{4,7,10,13,16,19}$-22:6. In each column, the amounts of the indicated fatty acids in various lipids are shown from the top in the order of free fatty acid (FFA), triacylglycerol (TG), cholesterol ester (CE) and phosphatidylcholine (PC) fractions. It should be noted that no significant difference was observed between normal children and atopic children, as shown in Tables I—IV.

have therapeutic significance for atopy. However, no sign of lower Δ^6-desaturase activity in atopic patients was observed as compared with age-matched controls (Tables I—IV).

Young children had essentially the same proportions of linoleate and arachidonate as adult controls. However, the proportions of n-3 series fatty acids were significantly lower. Dietary changes in the past 30 years in Japan have tended to decrease the intake of alpha-linolenate series, (increase in alpha-linolenate poor vegetable oil and increase in corn/soybean-fed cattle).

Contrary to the current concept that alpha-linolenate may not be essential for higher animals as described above, several lines of evidence recently presented indicate that it may be required for proper functioning of the nervous system.[10-12] Furthermore, the importance of n-3 series fatty acids in the functioning of T-lymphocytes and macrophages has also been reported.[13] Since the nervous system develops rapidly in young children, the supplementation of n-3 series fatty acids for children may be beneficial.

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