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Effects of dietary supplementation with sea buckthorn (*Hippophaë rhamnoides*) seed and pulp oils on atopic dermatitis

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*A placebo-controlled, double-blind study was conducted to investigate the effects of seed and pulp oils of sea buckthorn (*Hipphophaë rhamnoides*) on atopic dermatitis. Linoleic (34%), α-linolenic (25%), and oleic (19%) acids were the major fatty acids in the seed oil, whereas palmitic (33%), oleic (26%), and palmitoleic (25%) acids were the major fatty acids in the pulp oil. The study group included 49 atopic dermatitis patients who took 5 g (10 capsules) of seed oil, pulp oil, or paraffin oil daily for 4 months. During follow-up dermatitis improved significantly in the pulp oil (*$P < 0.01$*) and paraffin oil (*$P < 0.001$*) groups, but improvement in the seed oil group was not significant (*$P = 0.11$*). Supplementation of seed oil increased the proportion of α-linolenic acid in plasma neutral lipids (*$P < 0.01$*), and increases of linoleic, α-linolenic, and eicosapentaenoic acids in plasma phospholipids were close to significant (*$0.05 < P < 0.1$*). Pulp oil treatment increased the proportion of palmitoleic acid (*$P < 0.05$*) and lowered the percentage of pentadecanoic acid (*$P < 0.01$*) in both plasma phospholipids and neutral lipids. In the seed oil group, after 1 month of supplementation, positive correlations were found between symptom improvement and the increase in proportions of α-linolenic acid in plasma phospholipids (*$Rs = 0.84$*; $P = 0.001$) and neutral lipids (*$Rs = 0.68$*; $P = 0.02$). No changes in the levels of triacylglycerols, serum total, or specific immunoglobulin E were detected. In the pulp oil group, a significant (*$P < 0.05$*) increase in the level of high density lipoprotein cholesterol, from 1.38 to 1.53 mmol/L was observed*
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Keywords: atopy; *Hippophaë rhamnoides*; plasma fatty acids; plasma lipids; sea buckthorn oils

Introduction

The characteristic signs of atopic dermatitis (AD) include dry, scaly, itchy skin with eczematous inflammation and typical distribution of lesions. Although genetic factors play an important role, disturbed epidermal barrier function, aberrant immune response, and increased production of immunoglobulin E (IgE) are actively involved in the process of disease development.[1] Traditionally, AD therapy includes the liberal use of topical emollients, avoidance of skin irritating factors, topical glucocorticoids, systemically administered antihistamines, and antimicrobials. Because evidence of the side effects of steroids and certain antihistamines is steadily accumulating, numerous efforts have

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Table 1 Major fatty acids in sea buckthorn seed and pulp oil (weight percentages)

	Fatty acids (%)						
Oils	16:0	16:1(n-7)	18:0	18:1(n-9)	18:1(n-7)	18:2(n-6)	18:3(n-3)
Seed oil	11.3	4.4	2.6	18.9	3.2	34.1	24.9
Pulp oil	33.4	24.9	1.0	26.2	7.3	5.1	1.6

gone into the search for treatment alternatives including dietary management of the disease.[2–6]

Polyunsaturated fatty acids (PUFA) are important parts of the sphingolipids that constitute the water barrier of the epidermis.[7] As essential components of cell membranes, PUFA affect the functions of receptors, enzymes, ion channels, and other messenger systems.[8] Essential fatty acids and their metabolites also participate in the regulation of immune reactions and the inflammatory process.[8–10]

Since the observation of abnormal metabolism of essential fatty acids in AD patients, many investigators have administered oils of different origins systemically to patients for treatment. Clinical improvement has been noted by dietary supplementation of both plant seed oils rich in n-6 PUFA and fish oil rich in n-3 PUFA.[2–6,11–13] However, contradictory results also have been reported.[14,15] More clinical investigations are needed to verify the effects of PUFA on AD.

Although the nutritional effects of PUFA have been proved, the role of monounsaturated fatty acids, especially that of palmitoleic acid (16:1n-7) remains unknown. Increased levels of palmitoleic acid have been found in the tissues under special conditions such as deficiency of essential fatty acids and fatty liver.[16–18] The mechanism behind this phenomenon is not clear. In a clinical trial conducted with 14 subjects, a macadamia nut enriched diet containing palmitoleic acid significantly decreased the plasma total cholesterol, low density lipoprotein (LDL) cholesterol, and triacylglycerol concentrations in plasma.[19] Berries of sea buckthorn (*Hippophaë rhamnoides* L.) have been used in Tibetan, Mongolian, and Chinese traditional medicines for the treatment of different diseases for more than 1,000 years.[20] Oil obtained from the seeds of sea buckthorn berries contains linoleic acid (18:2n-6; 34–40%) and α-linolenic acid (18:3n-3; 23–36%) in abundance.[21–23] The flesh/peel press residue of the berries after juice processing is rich in pulp oil, which has an exceptionally high palmitoleic acid content (24–39%).[21,24,25]

Topical applications of the two oils on burned, scaled, wounded, and radioactively damaged skins of both humans and experimental animals have shown healing and anti-inflammatory effects.[26–29] Sea buckthorn seed oil is known to increase the specific and nonspecific immune functions of experimental animals.[30–32] Dietary supplementation with seed oil also has been shown to have a protective effect on cell membranes against lipid peroxidation in animal experimental models.[33,34]

Sea buckthorn seed oil has been administered to both humans and experimental animals with hyperlipemia, indicating lowering of the high levels of total cholesterol and triacylglycerol in plasma.[35] An increasing effect of the oil on high density lipoprotein (HDL) has been reported in the experimental model of hyperlipemia in chickens.[35]

Because of the fatty acid compositions and the reported effects of the two oils on skin and the immune system, we designed a double-blind, placebo-controlled clinical trial to test the effects of sea buckthorn seed oil and pulp oil on AD. The severity and change of AD symptoms, fatty acid compositions of plasma lipid fractions, levels of cholesterol, triacylglycerols, serum total, and specific IgE were examined simultaneously.

Materials and methods

The study was approved by the Ethical Committee of Turku University Central Hospital. The purpose of the study was explained to all patients and written consent were given by all subjects.

Oils

The seeds and soft parts (berry flesh and peel) were separated from the dried press residue of sea buckthorn juice processing. Seed oil was extracted from seeds and pulp oil from the soft parts by aseptic supercritical carbon dioxide process.[36] The fatty acid compositions of the two oils analyzed as methyl esters with gas chromatography are shown in *Table 1*. The oils were encapsulated in soft gelatine capsules each containing 500 mg oil and camouflaged with red and black iron paste (E172). Paraffin oil as placebo was encapsulated analogously. D-α-tocopherol 0.5 mg was added to each capsule. The capsules were sealed in plastic jars, coded randomly, and kept at 4°C until used.

Study design and subjects

The study was designed as a placebo-controlled, parallel, randomized, double-blind experiment and was carried out during the period of 1996 to 1998 at the Department of Dermatology, University of Turku and The Finnish Student Health Service, Turku, Finland. All 78 participating patients had a history of AD from childhood with persistent symptoms during the last 6 months. The participants were randomly divided into three groups receiving sea buckthorn seed oil, sea buckthorn pulp oil, or paraffin oil. Ten oil capsules per day were prescribed, and the whole treatment period lasted 4 months. Patients were asked to follow their normal diet throughout the trial and were allowed to use emollients, hydrocortisone cream, and peroral antihistamine as needed. The severity of AD was evaluated based on the extent and severity of AD symptoms. For evaluation, the SCORAD system[37] was adopted in adult measures with a range in score from 0 (no symptoms) to 3 (extensive severe symptoms). Pruritus and sleep loss were evaluated separately. The maximum total SCORAD value was 103. Patients were clinically examined at the beginning of the trial, after 1 month, and at 4 months, which was the end of the trial. During each visit, consumption of the capsules was controlled using oral communication, and any unconsumed capsules were returned by the patients at the end of the trial. During

Table 2 Symptom SCORAD values of atopic dermatitis in the three groups at different time points during the administration period

Groups	*n*	A		B		C	
		mean	±SD	mean	±SD	mean	±SD
Sea buckthorn seed oil	11	28.0	15.5	22.7	17.1	22.0	17.1
Sea buckthorn pulp oil	16	37.2	17.7	29.2*	20.8	26.8*	19.8
Placebo	18	40.3	16.6	28.4*	14.2	20.6*	16.7

*Significant ($P < 0.01$) compared with point A.
A–at the starting point of the trial. B–after 1 month of treatment. C–after 4 months of treatment.

all visits, plasma and serum samples were taken from the patients for analysis of the fatty acids of plasma phospholipids and neutral lipids and levels of cholesterol, triacylglycerols, serum total, and specific IgE.

Analysis of fatty acid compositions of plasma phospholipids and neutral lipids

Lipids were extracted from 2 g of plasma with 12 mL chloroform-methanol (2:1, v/v) using a modified Folch procedure.[38,39] The lipids were fractionated on silica Sep-Pak columns by eluting with 10 mL chloroform (neutral lipids) and 20 mL methanol (phospholipids). The glycerophospholipids and neutral lipids were transesterified by sodium methoxide catalysis.[40] The fatty acid methyl esters (FAME) were analyzed with a Perkin Elmer AutoSystem Gas Chromatograph equipped with programmed split/splitless injector and flame ionization detector and controlled with the Turbochrom Navigator 4 (Perkin Elmer, San Jose, CA USA). Silica capillary gas chromatography (GC) column NB-351 (L = 25 m, inner diameter = 0.32 mm, $d_f = 0.2$ μm) was used for GC analysis (HNU-Nordion Ltd, Helsinki, Finland). Flow rate of the carrier gas helium was 1.7 mL/min, and the split valve with a split ratio of 1:40 was opened after 1 minute. The temperature program was 120°C held for 2 minutes, increased at a rate of 3°C/min to 230°C, and held for 20 minutes. The injector temperature was programmed from 170°C to 250°C at a rate of 200°C/min. The detector temperature was 270°C. FAME were identified by comparison with a standard mixture of known composition (68D, NuChek Prep, Elysian, MN USA) and the fatty acid composition was expressed as weight percentage of the total fatty acids. The relative contents of fatty acids of the standard mixture agreed with the values stated by the manufacturer, with deviations of less than 5%.

Determination of plasma lipid and IgE levels

Levels of total cholesterol and triacylglycerols in plasma were determined with the reagents CHOD-PAP and GPO-PAP (Boehringer Mannheim, Mannheim, Germany), respectively, and analysed with Hitachi 704 and 717 automatic analyzers (Hitachi Ltd, Tokyo, Japan). HDL cholesterol was determined with Hitachi 704 and 717 automatic analyzers after PEG-6000 precipitation according to Izzo et al.[41] LDL cholesterol levels in plasma were calculated with the formula of Friedewald et al.[42] The radioimmunoassay method[43] was used for the determination of serum total IgE level. The IgE antibodies specific to *Saccharomyces cerevisiae*, *Candida albicans*, and *Pityrosporon orbiculare* were determined with CAP-RAST methods.[44]

Statistical analysis

Data analysis was carried out by statistical program packages Statistic/W version 4.5 (Stat Soft Inc., Tulsa, OK USA) and SPSS 7.5 (SPSS Inc., Chicago, IL USA). Repeated measures analysis of variance (ANOVA) were used to calculate significance of group effect, time effect, and group time interaction effect. Observed significance levels of less than 0.05 were considered statistically significant; levels of less than 0.1 were considered nearly significant. Exact *P*-values are reported in the text. Correlations between changes in SCORAD values and changes in proportions of fatty acids in plasma lipids were calculated with the Spearman correlation coefficient. To perform an overall test for the correlations between symptom improvement and fatty acid changes in plasma lipids, the correlation coefficients were tested using the Wilcoxon Signed Rank Test by SAS 6.12 Univariate Procedure.

Results

Patients and power of the study

During follow-up, 29 patients were excluded due to irregular (or incomplete) use of the capsules, unscheduled plasma sampling, or clinical examination. The remaining 49 patients, (16 male and 33 female) were included in the data analyses. Adequate sample sizes were calculated using SOLO Power Analysis software (BMDP Statistical Software Inc., Los Angeles, CA USA, 1991). The minimum sample size in repeated measure ANOVA tests was 5 subjects (in levels of antigen-specific IgEs in the seed oil group), and the power of detecting interaction effect was 0.71. There were usually at least 12 subjects in each group, and the power of testing interaction effect was over 0.9.

Effects on symptom severity

The mean SCORAD values are shown in *Table 2*. Compared with baseline values, the mean SCORAD values representing symptom severity were significantly lower after 1-month and 4-month administration in the pulp oil and placebo groups. In the seed oil group, the decrease in the value was not significant ($P = 0.11$).

Effects on fatty acid composition of plasma phospholipids

Twenty-one fatty acids were identified in the plasma phospholipids, and the results are shown in *Table 3*. The proportion of α-linolenic acid in the seed oil group increased after 1 month of administration ($P = 0.08$), and remained stable for the next 3 months. The proportions of linoleic acid and eicosapentaenoic acid (20:5n-3) were increased by seed oil treatment almost significantly ($0.05 < P < 0.1$). Supplementation for 1 month with pulp oil clearly increased the proportion of palmitoleic acid of phospholipid fatty acids ($P < 0.05$). The percentage of

Table 3 Weight percentage of the main fatty acids in the plasma phospholipids of patients in the three groups at different time points during administration

	Sea buckthorn seed oil							Sea buckthorn pulp oil							Placebo						
		A		B		C			A		B		C			A		B		C	
Fatty acids	*n*	Mean	±SD	Mean	±SD	Mean	±SD	*n*	Mean	±SD	Mean	±SD	Mean	±SD	*n*	Mean	±SD	Mean	±SD	Mean	±SD
14:0	12	0.37	0.43	0.26	0.08	0.76	1.69	16	0.30	0.11	0.29	0.10	0.31	0.12	21	0.26	0.06	0.28	0.11	0.28	0.08
15:0	11	0.17	0.00	0.16	0.03	0.16	0.04	16	0.18	0.03	0.15‡	0.00	0.16	0.03	21	0.16	0.05	0.18	0.04	0.18	0.05
16:0	12	26.58	1.79	26.00	2.31	25.53	2.43	16	26.38	2.51	25.38	2.52	26.80	1.73	21	25.24	2.26	25.79	1.06	25.73	1.62
16:1 (n-7)	12	1.08	1.75	0.70	0.33	0.60	0.19	16	0.67	0.04	0.86†	0.25	0.81*	0.22	21	0.53	0.19	0.54	0.17	0.57	0.13
18:0	12	12.13	1.43	12.51	1.33	12.45	1.16	16	11.75	1.49	10.91	1.47	11.74	1.22	21	12.46	1.32	12.39	1.31	12.46	1.33
18:1 (n-9)	12	10.17	0.90	10.90	1.28	10.54	1.44	16	11.25	1.25	11.74	2.04	11.79	2.12	21	10.30	1.30	10.60	1.25	10.78	1.31
18:1 (n-7)	12	1.81	0.27	1.75	0.25	1.76	0.23	16	1.93	0.21	1.92	0.25	2.00	0.30	21	1.84	0.22	1.80	0.16	1.80	0.18
18:2 (n-6)	12	22.44	2.55	22.55	2.81	23.89*	2.57	16	21.99	3.15	21.10	2.99	23.21	3.01	21	22.77	2.88	22.61	2.27	21.72	5.42
18:3 (n-6)	6	0.09	0.09	0.10	0.09	0.11	0.11	8	0.06	0.03	0.05	0.03	0.08	0.03	12	0.05	0.01	0.06	0.02	0.06	0.03
18:3 (n-3)	12	0.29	0.07	0.37*	0.14	0.37*	0.14	16	0.31	0.13	0.31	0.14	0.34	0.13	21	0.32	0.15	0.32	0.12	0.30	0.08
18:4 (n-3)	12	0.17	0.04	0.17	0.05	0.15	0.04	16	0.20	0.06	0.20	0.06	0.18	0.05	21	0.16	0.05	0.17	0.04	0.16	0.05
20:0	10	0.04	0.01	0.05	0.01	0.05	0.01	11	0.04	0.01	0.04	0.01	0.05	0.01	16	0.05	0.01	0.04	0.01	0.05	0.01
20:1 (n-9)	12	0.16	0.04	0.15	0.04	0.16	0.04	12	0.16	0.03	0.15	0.03	0.15	0.05	21	0.17	0.06	0.15	0.03	0.16	0.03
20:2 (n-6)	12	0.37	0.08	0.34	0.05	0.36	0.07	16	0.38	0.09	0.38	0.01	0.38	0.12	21	0.35	0.08	0.35	0.10	0.38	0.11
20:3 (n-6)	12	3.65	0.91	3.45	0.67	3.29	0.77	16	3.12	0.76	3.03	0.63	3.10	0.56	21	2.99	0.66	3.00	0.68	3.01	0.81
20:4 (n-6)	12	9.76	2.50	9.45	1.93	9.52	2.16	16	9.01	2.03	9.01	1.79	8.72	1.49	21	9.74	1.43	9.85	1.58	9.78	1.77
20:5 (n-3)	12	1.19	0.40	1.41*	0.5	1.30	0.59	16	1.18	0.62	1.16	0.71	1.21	0.56	21	1.61	1.60	1.38	0.40	1.48	0.88
22:4 (n-6)	12	0.28	0.07	0.27	0.07	0.26	0.06	16	0.27	0.08	0.30	0.08	0.27	0.07	20	0.27	0.06	0.26	0.05	0.26	0.05
22:5 (n-6)	12	0.19	0.06	0.18	0.06	0.17	0.06	16	0.19	0.12	0.19	0.09	0.18	0.07	21	0.17	0.07	0.18	0.09	0.19	0.10
22:5 (n-3)	12	0.92	0.23	0.93	0.25	0.92	0.26	16	0.89	0.27	0.85	0.08	0.88	0.26	21	0.88	0.20	0.95	0.28	0.88	0.21
22:6 (n-3)	12	4.26	1.71	4.13	1.97	3.92	2.81	16	4.29	1.35	3.91	1.50	3.88	1.56	21	4.66	1.56	4.81	1.62	4.70	1.61

*Almost significant ($0.05 < P < 0.1$) compared with point A.
†Significant ($P < 0.05$) compared with point A.
‡Significant ($P < 0.01$) compared with point A.
A–at the starting point of the trial. B–after 1 month of treatment. C–after 4 months of treatment.

Table 4 Weight percentage of main fatty acids in the plasma neutral lipids of the patients in the three groups at different time points during administration

	Sea buckthorn seed oil							Sea buckthorn pulp oil							Placebo						
		A		B		C			A		B		C			A		B		C		
Fatty acids	*n*	Mean	±SD	Mean	±SD	Mean	±SD	*n*	Mean	±SD	Mean	±SD	Mean	±SD	*n*	Mean	±SD	Mean	±SD	Mean	±SD
14:0	12	1.77	1.21	1.57	0.91	1.72	0.66	16	1.62	0.81	1.64	0.68	1.47	0.61	21	1.39	0.50	1.40	0.46	1.49	0.63
14:1 (n-5)	12	0.15	0.16	0.12	0.09	0.12	0.06	15	0.16	0.11	0.15	0.09	0.13	0.08	18	0.10	0.06	0.11	0.05	0.12	0.06
15:0	12	0.24	0.08	0.23	0.08	0.23	0.06	16	0.24	0.05	0.21*	0.04	0.20*	0.04	21	0.23	0.05	0.22	0.06	0.23	0.06
16:0	12	19.11	5.39	18.17	4.35	19.05	3.49	16	17.70	2.36	18.51	3.31	17.80	2.73	21	16.69	2.66	16.90	2.09	16.93	2.78
16:1 (n-7)	12	3.51	1.18	3.59	1.10	3.13	0.80	16	3.69	1.11	4.45*	1.28	4.12	1.28	21	3.19	1.00	3.26	0.83	3.18	0.82
18:0	12	2.61	1.18	2.43	0.97	2.92	1.23	16	2.37	1.17	2.40	1.32	2.16	0.67	21	2.18	1.06	1.95	0.47	2.19	0.93
18:1 (n-9)	12	30.52	3.24	30.55	3.28	32.06	5.87	16	30.63	3.28	29.59	2.78	29.95	3.05	21	28.75	3.55	29.49	2.73	29.15	3.98
18:1 (n-7)	12	2.19	0.38	2.21	0.37	2.41	0.55	16	2.21	0.28	2.34	0.37	2.18	0.47	21	2.02	0.40	1.95	0.30	1.97	0.39
18:2 (n-6)	12	27.12	9.12	29.07	9.19	26.39	10.09	16	29.39	6.78	29.44	7.63	31.22	6.62	21	32.42	7.10	32.96	4.91	31.96	7.20
18:3 (n-3)	12	1.31	0.63	1.67*	0.63	1.86*	0.57	16	1.24	0.50	1.14	0.34	1.24	0.48	21	1.30	0.39	1.30	0.31	1.24	0.33
18:3 (n-6)	12	0.38	0.13	0.46	0.24	0.39	0.32	16	0.41	0.22	0.43	0.16	0.46	0.24	21	0.42	0.13	0.45	0.18	0.46	0.15
18:4 (n-3)	12	0.36	0.09	0.33	0.10	0.30	0.08	16	0.37	0.08	0.35	0.09	0.32	0.08	21	0.32	0.10	0.31	0.08	0.31	0.10
20:1 (n-9)	12	0.23	0.09	0.23	0.11	0.32	0.24	16	0.22	0.08	0.21	0.08	0.20	0.09	21	0.20	0.10	0.18	0.07	0.21	0.08
20:2 (n-6)	12	0.11	0.04	0.11	0.04	0.13	0.05	16	0.10	0.03	0.11	0.06	0.10	0.05	21	0.11	0.05	0.09	0.03	0.11	0.06
20:3 (n-6)	12	0.49	0.23	0.42	0.09	0.37	0.16	16	0.41	0.12	0.46	0.22	0.41	0.08	21	0.47	0.13	0.44	0.07	0.43	0.10
20:4 (n-6)	12	3.26	1.30	3.13	1.09	2.71	1.49	16	3.33	1.01	3.40	0.99	3.28	1.08	21	3.91	1.17	3.99	0.99	3.75	1.13
20:5 (n-3)	12	0.58	0.22	0.67	0.32	0.61	0.39	16	0.64	0.32	0.69	0.55	0.64	0.36	21	1.05	1.25	0.89	0.49	0.88	0.63
22:4 (n-6)	12	0.08	0.22	0.07	0.02	0.08	0.02	12	0.06	0.01	0.06	0.02	0.06	0.02	18	0.06	0.02	0.06	0.02	0.06	0.03
22:5 (n-6)	12	0.08	0.04	0.06	0.02	0.09	0.08	11	0.06	0.03	0.06	0.02	0.06	0.03	15	0.06	0.02	0.06	0.03	0.08	0.05
22:5 (n-3)	12	0.24	0.10	0.25	0.12	0.26	0.13	16	0.20	0.08	0.20	0.09	0.20	0.12	20	0.22	0.11	0.20	0.09	0.22	0.12
22:6 (n-3)	12	0.83	0.43	0.71	0.43	0.70	0.50	16	0.79	0.44	0.79	0.51	0.73	0.47	20	1.11	0.94	0.95	0.50	1.01	0.57

*Significant ($P < 0.01$) compared with point A.
A–at the starting point of the trial. B–after 1 month of treatment. C–after 4 months of treatment.

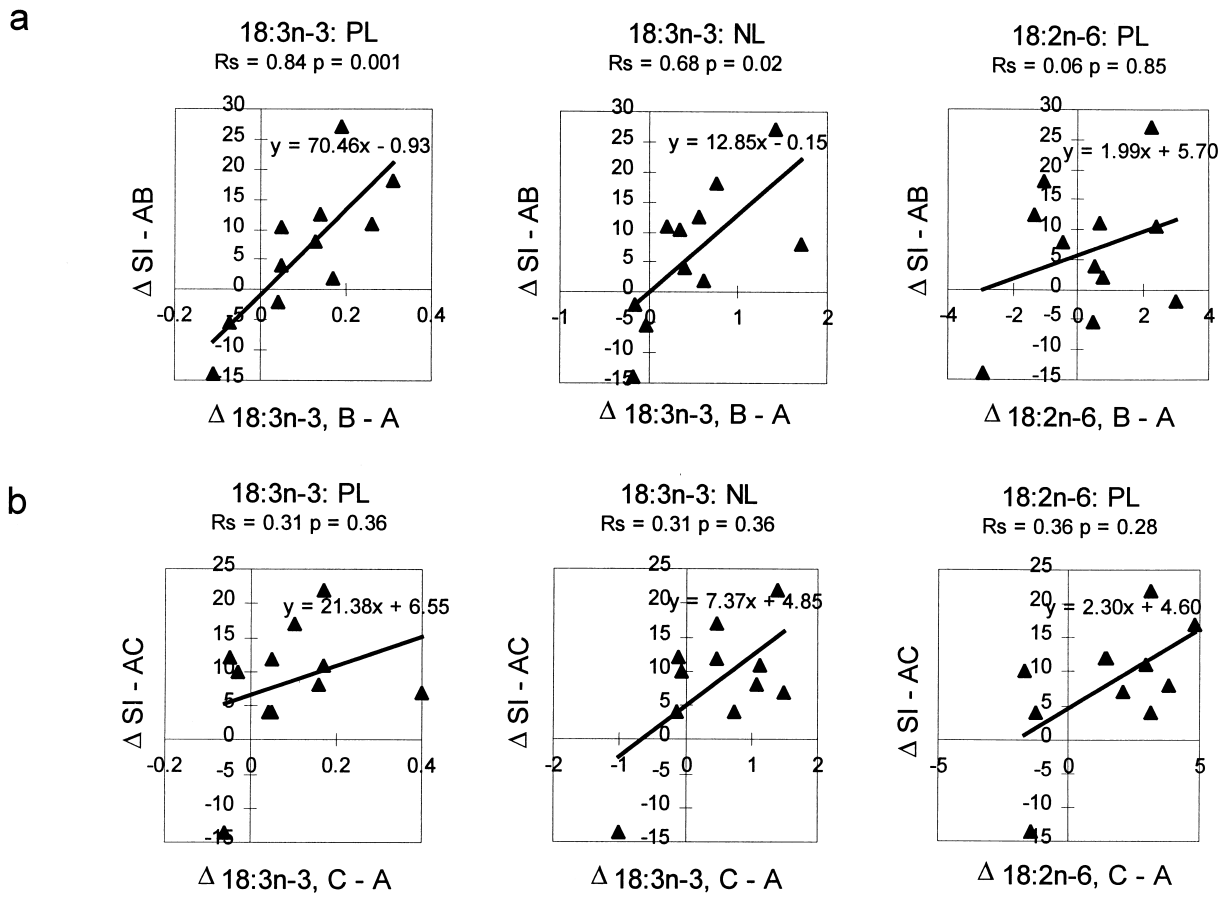

Figure 1 Correlations between symptom improvements and increases in proportions of fatty acids (Δ 18:3n-3 or Δ 18:2n-6) in plasma lipids in the seed oil group (*a*) after 1 month (Δ SI-AB) and (*b*) after 4 months of administration (Δ SI-AC). PL, phospholipids; NL, neutral lipids; A, before treatment; B, after 1 month of treatment; C, after 4 months of treatment.

pentadecanoic acid (15:0) was decreased by the pulp oil treatment ($P < 0.01$). No significant changes were observed in the paraffin oil group during follow-up.

Effects on fatty acid composition of plasma neutral lipids

Twenty-one fatty acids were identified in the plasma neutral lipids and the results are shown in *Table 4*. At the end of the administration, the percentage of α-linolenic acid increased in the seed oil group from 1.31 to 1.86%; this was an increase of over 40%. Pulp oil administration increased the percentage of palmitoleic acid and lowered the percentage of pentadecanoic acid ($P < 0.01$). All the major fatty acid species such as oleic, linoleic, and palmitic acids remained fairly constant in all participants. Paraffin oil supplementation did not cause changes in the fatty acid composition of plasma neutral lipids.

Correlations between symptom improvement and changes in proportions of fatty acids in plasma lipids

In the seed oil group, increases in the proportions of α-linolenic acid in both phospholipids and neutral lipids were positively correlated with symptom improvements, reflected by a decrease in SCORAD values (correlation coefficients and *P*-values shown in Figure 1). A positive correlation was also observed between the increase in the proportion of linoleic acid in plasma phospholipids and AD symptom improvement, but the correlation was not statistically significant (Rs = 0.36, $P = 0.28$). The overall test of the correlation coefficients showed significant positive correlation between symptom improvement and changes in the proportions of α-linolenic and linoleic acids in plasma lipids caused by seed oil supplementation ($P = 0.03$). In the pulp oil group, no correlations were observed between changes in SCORAD values and changes in proportions of palmitoleic acid in plasma phospholipids and neutral lipids during the follow-up.

Effects on the levels of IgE, cholesterol, and triacylglycerols in plasma

The mean levels of plasma IgE, three antigen-specific IgE antibodies, and plasma lipids of the patients in the three groups at baseline, after 1 month, and at the end of administration are shown in *Table 5*. Administration of the three oils did not have significant effects on the levels of

Table 5 The levels of plasma cholesterol, triacylglycerols, serum total, and specific immunoglobulin E of patients in the three groups at different time points during administration

		Sea buckthorn seed oil							Sea buckthorn pulp oil							Placebo						
			A		B		C			A		B		C			A		B		C	
Parameters	Units	*n*	Mean	±SD	Mean	±SD	Mean	±SD	*n*	Mean	±SD	Mean	±SD	Mean	±SD	*n*	Mean	±SD	Mean	±SD	Mean	±SD
Total IgE	IU/L	9	1,767	1,736	1,717	1,789	3,027	5,400	16	2,601	4,724	2,410	4,340	2,672	4,708	18	2,485	2,842	2,125	2,600	1,997	2,566
S. cerevisiae	IU/L	5	9.41	11.19	9.31	12.07	10.57	12.77	8	5.26	9.28	4.34	6.85	4.99	8.21	9	6.64	10.16	4.59	6.70	5.08	6.40
C. albicans	IU/L	5	13.91	17.1	14.83	22.09	13.05	16.54	9	12.49	20.52	7.88	12.16	11.46	21.54	10	18.63	24.10	15.49	21.30	14.67	18.57
P. orbiculare	IU/L	7	26.37	30.8	18.56	20.23	17.35	21.11	10	14.36	11.69	16.51	16.05	13.99	11.29	9	16.12	18.52	14.25	13.28	13.14	14.4
Total cholesterol	mmol/	12	4.34	0.56	4.09	0.64	4.41	0.58	16	4.63	0.88	4.43	0.81	4.80	0.88	20	4.81	0.74	4.93	0.83	4.95	0.86
Triacylglycerols	g/L	12	1.39	1.19	1.52	1.43	1.48	1.17	16	0.96	0.30	1.08	0.39	1.01	0.35	20	1.14	0.70	1.12	0.43	1.14	0.51
HDL cholesterol	mmol/L	12	1.36	0.38	1.27	0.39	1.35	0.43	16	1.38	0.29	1.31	0.28	1.53*	0.35	20	1.40	0.34	1.45	0.39	1.49	0.43
LDL cholesterol	mmol/L	12	2.38	0.58	2.16	0.55	2.38	0.72	16	2.83	0.74	2.63	0.63	2.83	0.71	20	2.91	0.63	2.98	0.70	2.94	0.71
HDL cholesterol	mmol/L	12	32.08	10.35	32.26	11.56	31.85	12.45	16	30.38	7.29	30.13	6.26	32.39	7.54	20	29.60	7.41	29.91	7.61	30.48	8.06

*Significant ($P < 0.05$) compared with point A.
A–at the starting point of the trial. B–after 1 month of treatment. C–after 4 months of treatment.
HDL–high density lipoprotein. LDL–low density lipoprotein.

total IgE, the three specific IgE antibodies, total cholesterol, LDL cholesterol, or triacylglycerols in the plasma of the subjects. A statistically significant, though small increase in HDL cholesterol level from the baseline was recognized in the pulp oil group at the end of the treatment. Seed oil and paraffin oil did not show significant effects on HDL cholesterol level in plasma.

Discussion

In AD patients, deviation in essential fatty acid levels from healthy controls have been reported. A higher proportion of linoleic acid and decreased level of arachidonic acid (20:4n-6) have been recognized in plasma lipids.[45–47] A commonly accepted hypothesis suggests that AD patients have a defect in Δ-6 desaturase, which converts linoleic acid and α-linolenic acid to γ-linolenic acid (18:3n-6) and steridonic acid (18:4n-3), respectively. This results in accumulation of linoleic and α-linolenic acids and in decreased levels of their longer chain desaturated metabolites such as dihomo-γ-linolenic acid (20:3n-6), arachidonic acid, and eicosapentaenoic acid (20:5n-3) in plasma phospholipids.[47] This theory is supported by an improvement of AD symptoms, especially itching, after dietary administration of evening primrose oil rich in γ-linolenic acid.[2] However, Zevenverger and Houtsmuller[48] and Pfeiffer et al.[49] demonstrated that Δ-6 desaturase is not the only enzyme implicated in the tissue levels of the PUFA, and they did not find it to be deficient in AD patients. Thus, more evidence is required to confirm Δ-6 desaturase deficiency in AD patients.

Clinical studies have shown linoleic acid, the substrate of Δ-6 desaturase, to have contradictory effects on AD.[6,15,50,51] A recently published study showed a significant improvement of AD after fatty acid supplementation in the form of sunflower oil (63% linoleic acid).[6] As an essential component of the epidermal barrier system, the effect of linoleic acid was positively correlated with an increased level of 13-hydroxyoctadecadienoic acid (13-HODE) in skin.[6] 13-HODE, a direct metabolite of linoleic acid, attenuates epidermal hyperproliferation[6,7,52] and possibly also reduces inflammation.[7,53] Several clinical trials have also been carried out to investigate the effects of eicosapentaenoic and docosahexaenoic acids on AD.[5,11] However, α-linolenic acid has attracted less attention in AD treatment.

Sea buckthorn seed oil contains 34% linoleic acid and 25% α-linolenic acid of its total fatty acids. Supplementation with the oil resulted in a rise in the proportions of these two fatty acids in plasma lipids. No increase in the percentages of their metabolites γ-linolenic, dihomo-γ-linolenic, arachidonic, or stearidonic acids was observed. However, the increase in the proportion of α-linolenic acid was accompanied by a slight elevation in the proportion of eicosapentaenoic acid in plasma phospholipids in the seed oil group, suggesting that α-linolenic acid was more effectively metabolized in the desaturation-elongation cascade than was linoleic acid. The results are consistent with the assumption that Δ-6 desaturation of linoleic acid especially is lowered in AD patients.[47]

Interestingly, in the seed oil group symptom improvements were positively correlated with increase in proportion of α-linolenic acid in plasma lipids caused by seed oil

supplementation. The correlation between symptom improvement and increase in proportion of linoleic acid in plasma phospholipids was not significant. This result suggests positive effects of α-linolenic acid on AD. α-Linolenic acid is the precursor of eicosapentaenoic acid, which is further converted into the 5-series leukotrienes by a 5-lipoxygenase pathway. Eicosapentaenoic acid competitively inhibits the formation of the 4-series leukotrienes from arachidonic acid. The 5-series leukotrienes have less potent proinflammatory and hyperproliferative effects than the 4-series leukotrienes. In our study, supplementation with seed oil increased the proportion of eicosapentaenoic acid in plasma phospholipids. The effects of α-linolenic acid may be due to an elevated synthesis of 5-series leukotrienes and a decreased synthesis of the 4-series leukotrienes, which resulted from the increased level of eicosapentaenoic acid in cell membranes. However, we did not find a statistically significant correlation between the AD improvement and the increase in the proportion of eicosapentaenoic acid in plasma phospholipids, possibly due to the low number of patients. Future studies on the effects of seed oil supplementation on the levels of α-linolenic acid, linoleic acid, and their metabolites in skin lipids should provide valuable information of the function and metabolism of essential fatty acids in AD patients.

Significant improvement also occurred in the paraffin oil group without any changes in plasma fatty acid levels, suggesting the improving effect of the traditional treatments. This may have been due to the better compliance provoked by the regular control visits during the trial. This kind of improvement, the placebo effect, was also highlighted by Morse et al.[2] in the meta-analysis of 9 placebo-controlled studies on the efficacy of evening primrose oil in the treatment of AD. At the starting point of the trial, the general symptom severity in the seed oil group happened to be lower than in the pulp oil and placebo groups. Together with the smaller number of patients, this may explain the less significant symptom improvement in the seed oil group compared with the other two groups.

The high content of palmitoleic acid in the pulp oil resulted in an increase in the proportion of the fatty acid in plasma lipids. The increase neither correlated with the symptom improvement nor led to clear changes in the plasma levels of total cholesterol, LDL cholesterol, or triacylglycerols. The significant improving effect of the pulp oil also may be due to components other than its fatty acids. According to our analysis (unpublished results), pulp oil contains a higher amount of plant sterols (2%) than seed oil (0.8%). In the pulp oil plant sterol fraction, 68% consists of sitosterol and 5% of sitostanol. Both of these are reported to have an anti-inflammatory effect.[54–56] Zak et al.[57] reported that dietary supplementation with sitosterol decreased LDL and slightly increased HDL levels in hypercholesterolemic patients. The slight increase in HDL level discovered after the pulp oil treatment in our study is in agreement with their observations. The high content of carotenoides (1 mg/g oil), especially of β-carotene (0.4 mg/g oil), which is an antioxidant and precursor of vitamin A, in the pulp oil may also have contributed to the significant AD improvement in the pulp oil group.

In conclusion, α-linolenic acid in sea buckthorn seed oil seemed to have a beneficial effect on AD. The improving effect shown by pulp oil treatment is probably not related to the fatty acid composition of the oil alone. These results require confirmation because the number of patients in the seed oil group was so small. Further studies are in progress to discover the effects of the two oil supplementations on the fatty acid composition in skin lipids of AD patients.

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