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Effect of dietary supplementation with sea buckthorn (*Hippophaë rhamnoides*) seed and pulp oils on the fatty acid composition of skin glycerophospholipids of patients with atopic dermatitis

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Sea buckthorn (Hippophaë rhamnoides) *seed and pulp oils have traditionally been used for treating skin diseases in China and Russia, but are not widely used in other countries. A placebo-controlled, parallel study was carried out to investigate the effects of these oils on the fatty acid composition of skin glycerophospholipids of patients with atopic dermatitis. Sixteen patients ate 5 g of sea buckthorn seed oil, pulp oil, or paraffin oil daily for 4 months. Skin fatty acids were analyzed with gas chromatography before and after treatment. The seed oil slightly increased the proportion of docosapentaenoic acid (22:5n-3) and decreased the proportion of palmitic acid (16:0) in skin glycerophospholipids ($0.05 < P < 0.1$). The levels of the other fatty acids remained stable. The results show that the fatty acid composition of skin glycerophospholipids is well buffered against short-term dietary modification.* (J. Nutr. Biochem. 11:338–340, 2000)

Keywords: atopic dermatitis; *Hippophaë rhamnoides*; skin fatty acids; sea buckthorn oils

Introduction

The inflammatory skin disease atopic dermatitis (AD) is characterized by dry, itchy, and lichenous skin. Impaired epidermal barrier function and abnormal synthesis of eicosanoids, the mediators of epidermal inflammation and hyperproliferation, are involved in these skin changes in AD. Acylceramides rich in linoleic acid (18:2n-6) are essential components of the epidermal barrier system. The epidermal glycerophospholipids carry the polyunsaturated fatty acids (PUFA) that are precursors of eicosanoids after being released by the activity of phospholipases, especially of phospholipase A_2. Glycerophospholipids also form a source of linoleic acid for the synthesis of acylceramides and 13-hydroxyoctadecadienoic acid (13-HODE), the latter attenuating epidermal hyperproliferation.[1] PUFA in phospholipids are also components essential to maintaining the proper fluidity of cell membranes, which is important for signal transduction and substance transportation. Lowered levels of n-6 PUFA, especially of arachidonic acid (20:4n-6), and elevated levels of monounsaturated fatty acids have been recognized in epidermal phosphatidylcholine and phosphatidylethanolamine in AD patients compared with healthy controls.[2] Abnormal metabolism of dietary fatty acids and increased utilization of certain PUFA due to the inflammation process are responsible for these changes.[2,3] Sea buckthorn (*Hippophaë rhamnoides*) seed oil contains high levels of linoleic (30–40%) and α-linolenic (18:3n-3, 23–36%) acids, and oil from the flesh/peel of the berries (pulp oil) is rich in palmitoleic acid (16:1n-7, 24–39%).[4–6] In our earlier study, seed oil treatment increased the level of α-linolenic, linoleic, and eicosapentaenoic acids, whereas pulp oil supplementation increased the proportion of palmitoleic acid in plasma phospholipids of AD patients.[7]

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Table 1 Major fatty acids and contents of sitosterol and β-carotene in sea buckthorn seed and pulp oils (weight percentage)

Oils	Fatty acids (%)							Sitosterol (mg/g oil)	β-Carotene (mg/100 g oil)
	16:0	16:1n–7	18:0	18:1n–9	18:1n–7	18:2n–6	18:3n–3		
Seed oil	11.3	4.4	2.6	18.9	3.2	34.1	24.9	5.6	48.9
Pulp oil	33.4	24.9	1.0	26.2	7.3	5.1	1.6	14.0	6.5

Positive correlations were recognized between AD symptom improvement and the increases in the levels of α-linolenic acid in plasma lipids.[7] Further studies on the effects of the two oils on the fatty acid composition of skin phospholipids should provide valuable information on the essential fatty acid metabolism of AD patients. The goal of the present study was to test the effects of dietary supplemented sea buckthorn seed and pulp oils on the fatty acid composition of the skin glycerophospholipids.

Materials and methods

The Ethical Committee of Turku University Central Hospital approved the study. All participating patients understood the purpose of the study and signed written consents.

Oils

The seeds and flesh/peel were separated from the dried press residue of sea buckthorn juice. Seed oil was extracted from seeds and pulp oil from flesh/peel by an aseptic supercritical carbon dioxide process.[8] The fatty acid compositions and contents of β-carotene and β-sitosterol in the two oils are shown in *Table 1*. The oils were encapsulated in soft gelatine capsules containing 500 mg oil each and camouflaged with red and black iron paste (E172). Paraffin oil as a placebo was encapsulated analogously. 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 at the University of Turku and the Finnish Student Health Service (Turku, Finland). All patients had a history of AD from childhood with persistent symptoms throughout the last 6 months. The 22 participating patients were randomly divided into three groups, taking seed oil, pulp oil, or paraffin oil. Ten oil capsules (5 g oil) were prescribed for each patient per day, and the whole treatment period lasted for 4 months. Patients were asked to follow their normal diet and allowed to use emollients, hydrocortisone cream, and per oral antihistamine as prescribed by doctors. Sixteen patients completed the study. Skin biopsies were taken for fatty acid analysis before and after treatment.

Sampling and fatty acid analysis of skin biopsies

Punch biopsies (diameter, 4 mm; thickness, 6 mm) were taken from a lesion-free area of skin on the shoulder region of the upper extremity, flushed with a saline solution, frozen with liquid nitrogen, and stored at −70°C. A layer with a thickness of 0.5 mm (containing mainly the epidermis, no blood capillaries included) was taken from each sample for fatty acid analysis.

Lipids were extracted from biopsies by repeating homogenization of the sample on ice in chloroform: methanol (2:1, v/v) in the presence of BHT (2,[6]-Di-tert-Butyl-p-cresol as antioxidant; 50 mg/L solvent). The total volume of the solvent used was 6 mL. The homogenate was collected and transferred to a 10 mL glass tube, vortexed for 1 min, and centrifuged at 3,500 rpm for 5 min. The supernatant was collected, the solvents evaporated, and the lipids dissolved in 0.5 mL chloroform. Lipids were fractionated into neutral lipids and phospholipids on silica Sep-Pak columns (Waters Corp., Milford, MA USA).[7] The glycerophospholipids in the phospholipid fraction were transesterified with sodium methoxide catalysis.[9] The fatty acid methyl esters were analyzed with a Perkin Elmer AutoSystem Gas Chromatograph (Perkin Elmer, San Jose, CA USA) and calculated as weight percentages of the total fatty acids.[7]

Statistical analysis

Data analysis was carried out by statistical program package SPSS 7.5. Paired-samples *t*-test was used to calculate the differences in the proportions of each fatty acid before and after treatment. Observed significance levels less than 0.05 were considered to be statistically significant; levels of less than 0.1 were considered as almost significant.

Results and discussion

The fatty acid composition of skin glycerophospholipids during follow-up is shown in *Table 2*. Seed oil treatment increased the proportion of docosapentaenoic acid (22:5n-3; $P = 0.07$) and decreased the proportion of palmitic acid (16:0; $P = 0.09$) in skin glycerophospholipids almost significantly. Pulp oil treatment slightly increased the proportion of stearic acid ($0.05 < P < 0.1$). A small increase in the proportion of linoleic acid and stearic acid was also observed in the placebo group ($0.05 < P < 0.1$).

Although the effects of PUFA on AD have been discussed for almost a century, most of the investigations have concentrated on the changes in AD symptoms and fatty acid levels in plasma lipids. Very few experiments have investigated the effects of dietary supplemented PUFA on the fatty acid composition of epidermal phospholipids, especially in humans. Miller and Ziboh[10] fed guinea pigs with borage oil containing 25% γ-linolenic acid (18:3n-6) for 8 weeks. The treatment resulted in a 10-, 4-, and 1.3-fold elevation of the level of γ-linolenic acid, dihomo-γ-linolenic acid, and arachidonic acid, respectively, and a 2-fold reduction in linoleic acid level in the epidermal phospholipids.[10] Schäfer and Kragballe[11] investigated the effect of oral supplementation with evening primrose oil at three different doses (2, 4, or 6 g/day), containing 72% linoleic acid and 10% γ-linolenic acid, on the epidermal phospholipids. The 10-week supplementation did not lead to any significant change in the level of linoleic acid in epidermal phospholipids. A moderate increase was recognized in the proportion of dihomo-γ-linolenic acid (20:3n-6) and arachidonic acid in epidermal phosphatidylcholine. No significant change in the fatty acid level was observed in the epidermal phosphatidylethanolamine.

In our earlier study, dietary supplementation of seed oil

Table 2 Fatty acid composition of skin glycerophospholipids (GPL) and proportions of linoleic and linolenic acids in plasma glycerophospholipids of the patients before and after treatment in the three groups (as weight percentage). A, before treatment; C, after treatment

Fatty acids	Seed oil group						Pulp oil group						Placebo group					
Skin GPL Skin	*n*	A		C		*P*	*n*	A		C		*P*	*n*	A		C		*P*
		Mean	SD	Mean	SD			Mean	SD	Mean	SD			Mean	SD	Mean	SD	
14:0	5	0.48	0.18	0.50	0.25	0.67	7	0.59	0.22	0.46	0.16	0.16	4	0.45	0.29	0.77	0.77	0.29
16:0	5	16.24	2.05	14.31	2.32	0.09[a]	7	14.15	1.87	13.70	1.18	0.11	4	14.74	4.18	15.77	1.37	0.54
16:1n–9	5	2.84	2.27	2.15	1.28	0.43	7	1.85	0.99	1.16	0.51	0.15	4	1.63	1.06	1.52	1.09	0.57
16:1n–7	5	0.59	0.27	0.58	0.23	0.95	7	0.71	0.29	0.66	0.37	0.46	4	0.68	0.14	0.75	0.08	0.15
18:0	5	16.55	2.28	17.58	1.34	0.31	7	16.78	1.21	18.09	2.07	0.07[a]	4	17.80	2.77	18.50	3.65	0.09[a]
18:1n–9	5	19.18	1.91	18.67	1.38	0.67	7	18.85	2.25	17.62	1.87	0.10	4	17.18	1.03	16.37	1.24	0.39
18:1n–7	5	1.95	0.17	1.92	0.20	0.78	7	2.15	0.38	2.16	0.42	0.93	4	2.02	0.14	2.15	0.09	0.27
18:2n–6	5	19.87	2.64	20.29	4.87	0.85	7	21.3	3.00	21.01	2.13	0.95	4	19.80	1.24	21.43	1.66	0.05[a]
20:0	5	0.62	0.22	0.80	0.16	0.18	7	0.62	0.12	0.66	0.13	0.18	4	0.65	0.14	0.58	0.12	0.50
20:1n–9	4	0.35	0.09	0.31	0.09	0.22	5	0.31	0.16	0.28	0.05	0.61	3	0.35	0.07	0.38	0.10	0.77
20:3n–6	5	0.39	0.17	0.29	0.13	0.54	7	2.12	0.35	2.29	0.34	0.38	4	2.30	0.78	2.14	0.51	0.37
20:4n–6	5	12.50	1.33	12.37	2.24	0.83	7	14.10	1.90	14.73	1.50	0.41	3	14.28	1.83	12.78	0.94	0.19
20:5n–3	4	0.35	0.13	0.38	0.14	0.74	5	0.34	0.16	0.33	0.15	0.86	3	0.24	0.08	0.19	0.05	0.36
22:1n–9	5	0.29	0.11	0.35	0.09	0.41	7	0.27	0.06	0.36	0.18	0.27	3	0.41	0.09	0.32	0.06	0.41
22:5n–3	5	1.11	0.16	1.36	0.15	0.07[a]	7	1.39	0.43	1.56	0.39	0.34	4	1.62	0.32	1.12	0.23	0.16
22:6n–3	5	3.21	0.68	1.09	0.49	0.69	7	3.09	0.54	3.33	1.48	0.68	4	3.78	0.80	2.83	0.70	0.10
Plasma GPL																		
18:2n–6	5	22.88	1.91	25.55	3.02	0.02[b]	4	23.17	3.09	21.23	4.97	0.38	5	22.39	0.84	22.94	1.26	0.20
18:3n–3	5	0.33	0.11	0.49	0.14	0.14	4	0.30	0.14	0.22	0.09	0.48	5	0.26	0.10	0.26	0.09	0.99

[a] $0.05 < P < 0.01$, close to significant.
[b] $P < 0.05$, significant.

increased the level of linoleic acid, α-linolenic acid, and eicosapentaenoic acid (20:5n-3) in plasma phospholipids, and the pulp oil treatment increased the proportion of palmitoleic acid.[7] In the present study, a significant increase ($P < 0.05$) in the proportion of linoleic acid was recognized in plasma glycerophospholipids of patients in the seed oil group (*Table 2*). Seed oil treatment slightly increased the proportion of docosapentaenoic acid and decreased the proportion of palmitic acid in skin glycerophospholipids. Docosapentaenoic acid is a metabolite of eicosapentaenoic acid. The slight increase in the level of docosapentaenoic acid may have been due to the increased level of eicosapentaenoic acid in plasma phospholipids. The high levels of linoleic acid in seed oil and palmitoleic acid in pulp oil did not lead to significant increases in the levels of the fatty acids in skin glycerophospholipids. Slight increases in the proportions of linoleic and stearic acids were observed in the placebo group, which may have resulted from the small number of patients in the group. These results, which are consistent with the observations of Schäfer and Kragballe,[11] indicate that the fatty acid composition of the skin phospholipids is well buffered against a short-term dietary change. However, due to the small number of patients the results require confirmation. β-Sitosterol and β-carotene in the oils may also have effected on the symptoms of AD; however, effects of these compounds on the incorporation of fatty acids into plasma and skin lipids are not clear.

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