

Sea buckthorn berry oil inhibits platelet aggregation

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A small-scale preliminary cross-over study was conducted to investigate the effects of supercritical CO₂-extracted sea buckthorn berry oil (SBO) on some risk factors of cardiovascular disease. Special features of the oil are high proportions of palmitic (16:0), oleic (18:1n-9), palmitoleic (16:1n-7), linoleic (18:2n-6), and α-linolenic (18:3n-3) acids as well as vitamin E, carotenoids, and sterols. Twelve healthy normolipidemic men were recruited and each volunteer consumed SBO and fractionated coconut oil (control) 5 g per day for a period of 4 weeks in a random order (wash-out 4–8 weeks). Phospholipid fatty acids, plasma lipids, and glucose were unaffected by SBO supplementation. Instead, a clear decrease in the rate of adenosine-5'-diphosphate-induced platelet aggregation and maximum aggregation were found. This suggested the beneficial effects of SBO on blood clotting, but further studies on the dose-response effects are needed to assess the practical use of SBO supplements. (J. Nutr. Biochem. 11:491–495, 2000) © Elsevier Science Inc. 2000. All rights reserved.

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Introduction

There is a centuries-old tradition in Chinese medicine to utilize sea buckthorn (*Hippophaë rhamnoides*), and beneficial effects of its oil (e.g., in the treatment of cardiovascular disease [CVD]) have been reported.^{1–3} Supplementation of hyperlipidemic subjects with sea buckthorn berry oil (SBO) has been reported to normalize the plasma lipid levels.² Furthermore, sea buckthorn berries and especially the alcohol extracts of the twigs given orally to humans or animals have been reported to inhibit thrombus formation or platelet aggregation.^{1,3,4}

The composition of combined pulp and seed oil of sea buckthorn berry is unusual including nutritionally important fatty acids and sterols, and a high antioxidative capacity including vitamins and carotenoids.^{5,6} Oil of sea buckthorn berries contains high concentrations of palmitoleic acid (>17%) that may have cholesterol- and triglyceride-lowering as well as stroke-suppressing effects.^{7–9} Antioxidant supplements have been found to decrease the adenosine-5'-

diphosphate (ADP)-induced platelet aggregation in humans.¹⁰ Sea buckthorn is a rich source of antioxidant compounds such as tocopherols, carotenoids, and vitamin C.^{5,11,12} In addition, SBO contains about 1% sterols, with sitosterol being the most abundant individual component.^{5,13} Sitosterol has been reported to inhibit platelet aggregation.¹⁴ Phytosterols including sitosterol are also known to reduce plasma total and low-density lipoprotein (LDL) cholesterol levels by mechanisms affecting both the absorption and synthesis of cholesterol.^{15,16} The aim of the present study was to investigate the effects of the combined sea buckthorn pulp and seed oil on plasma lipid and glucose levels as well as on platelet aggregation in normolipidemic subjects.

Materials and methods

Twelve healthy normolipidemic men (aged 20–59 years, body mass index [BMI] 19.6–26.5 kg/m²) were recruited for a double-blind, randomized, and controlled (fractionated coconut oil [CO]) SBO study. A screening blood sample was taken and normal health status and suitability for the study were checked by biochemical laboratory tests and a life-style questionnaire. Exclusion criteria were obesity (BMI > 30), hormonal, renal, hematological, or hepatic dysfunction, a myocardial infarction, treatment with lipid-lowering or nonsteroidal inflammatory drugs, and consumption of dietary supplements. Basal plasma values (mmol/L) of the volun-

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Table 1 Fatty acid (FA) composition (g/100 g) of sea buckthorn berry oil (seed + berry pulp oil) (SBO) and fractionated coconut oil (CO)

	FA									
	8:0	10:0	16:0	16:1n-7	18:0	18:1n-9	18:1n-7	18:2n-6	18:3n-3	Others
SBO			23.4	17.3	1.5	20.5	5.5	17.9	11.4	2.5
CO	55.2	44.3								0.5

teers were 1.36 (SEM 0.14) for triacylglycerols, 4.54 (SEM 0.23) for total cholesterol, 3.41 (SEM 0.26) for LDL, 1.14 (SEM 0.08) for high-density lipoprotein (HDL), and 4.89 (SEM 0.15) for glucose; thus, being within normal limits.

Fatty acid compositions of the study oils are presented in *Table 1*. Each of the 12 men consumed each oil (5 g per day) for a period of 4 weeks in random order, separated by a 4–8 week washout. The oils were administered orally as 500-mg capsules (10 capsules per day) and subjects were advised to take them in groups of two to three with plenty of water. Fasting (12 hr) venous blood samples of about 55 mL were taken before and after each supplementation period, making a total of four blood samplings. The volunteers were asked to continue their normal eating and exercise habits throughout the study. After the study, the subjects returned the volunteer feedback information sheets in which the compliance to the study, possible weight changes, and other experiences during the trial were asked.

The study protocol was approved by the Norwich District Ethics Committee. All the subjects were explained the purpose of the study and asked to read and sign a written consent form. Subjects were free to withdraw from the study at any time without having to give a reason for withdrawing. Eleven of 12 subjects completed the whole trial. All blood samplings were carried out by a nurse in the phlebotomy room of the Human Nutrition Unit of the

Institute of Food Research (IFR). Study oils were produced by industrial-scale supercritical CO₂ extraction,¹⁷ which guarantees the solvent residue-free and aseptic oil product especially designed for clinical trials. No harmful effects have been reported to be associated with the consumption of SBO.^{18,19}

Blood was taken into sodium citrate and centrifuged within 1 hr to isolate platelet-rich plasma (PRP). Platelet aggregation in response to eight final concentrations of two agonists—ADP (Bio/Data Corporation, Hatboro, PA USA; 5, 10, 12.5, 15, 17.5, 20, 25, and 30 μ mol/L diluted with 9.0 g/L saline) and arachidonic acid (AA; Bio/Data Corporation, Hatboro, PA USA; 250, 500, 1,000, 1,500, 2,000, 2,500, 3,000, and 4,000 μ g/mL diluted with 9.0 g/L saline)—was measured on PRP by a PAP-4 platelet aggregation profiler (Bio/Data Corporation, Hatboro, PA USA) within 3 hr from blood sampling. Dose-response curves of agonist concentration against aggregation (maximum aggregation at 4 min, % aggregation; slope of aggregation curve, % aggregation/min) were constructed. Platelet count of the PRP was not adjusted in this trial, as the study of Calzada et al.¹⁰, for example, showed that within-individual changes in platelet count along with time are negligible. We used each subject as his own control when calculating the results to minimize the effects of between-individual variation in platelet responses. In 90% of the subjects, we were able to construct a full aggregation curve that reached maximal

Table 2 Effects of oil supplementation on the fatty acid compositions of plasma and platelet phospholipids: A, before supplementation; B, after supplementation. Results are mean (g/100 g) values of the 11 subjects

	SBO supplementation				Coconut oil supplementation			
	Plasma		Platelets		Plasma		Platelets	
	A	B	A	B	A	B	A	B
16:0	26.6	26.6	12.7	13.5	26.3	26.2	12.1	12.7
16:1n-7	0.50	0.53	0.26	0.27	0.46	0.51	0.17	0.18
18:0	13.3	13.2	20.8	20.2	13.6	13.7	20.8	20.9
18:1n-9	9.36	9.69	15.9	15.8	9.33	9.67	15.5	15.7
18:1n-7	1.83	1.91	1.67	1.66	1.80	1.73	1.63	1.56
18:2n-6	26.2	26.5	6.56	6.66	26.7	26.4	6.54	6.66
18:3n-6	0.06	0.07	0.05	0.04	0.05	0.07	0.03	0.03
18:3n-3	0.19	0.27	0	0	0.21	0.21	0	0
18:4n-3	0.20	0.19	0.05	0.02	0.19	0.20	0.01	0.03
20:0	0.05	0.04	1.21	1.11	0.04	0.05	1.21	1.21
20:1n-9	0.16	0.15	1.26	1.07	0.15	0.15	1.17	1.13
20:2n-6	0.35	0.34	0.43	0.46	0.35	0.37	0.41	0.43
20:3n-6	3.02	2.97	1.88	1.82	3.01	3.25	1.88	1.91
20:4n-6	10.0	9.78	28.5	28.5	9.74	9.47	29.1	28.9
20:5n-3	1.01	0.94	0.53	0.52	0.87	0.96	0.49	0.47
22:4n-6	0.34	0.33	2.87	2.77	0.33	0.35	2.90	2.84
22:5n-3	1.15	1.13	2.16	2.15	1.11	1.06	2.26	2.13
22:6n-3*	3.47	3.31	1.68	1.74	3.59	3.45	1.93	1.82
Others	2.26	2.15	1.50	1.68	2.20	2.25	1.86	1.40
n-6	40.0	40.0	40.3	40.3	41.89	39.9	40.9	40.8
n-3	6.02	5.84	4.42	4.43	5.81	5.88	4.69	4.45
n-6/n-3	6.64	6.84	9.11	9.09	7.21	6.78	8.72	9.17

*Sum of the proportions of 22:6n-3 and 24:1n-9.
SBO—sea buckthorn berry oil.

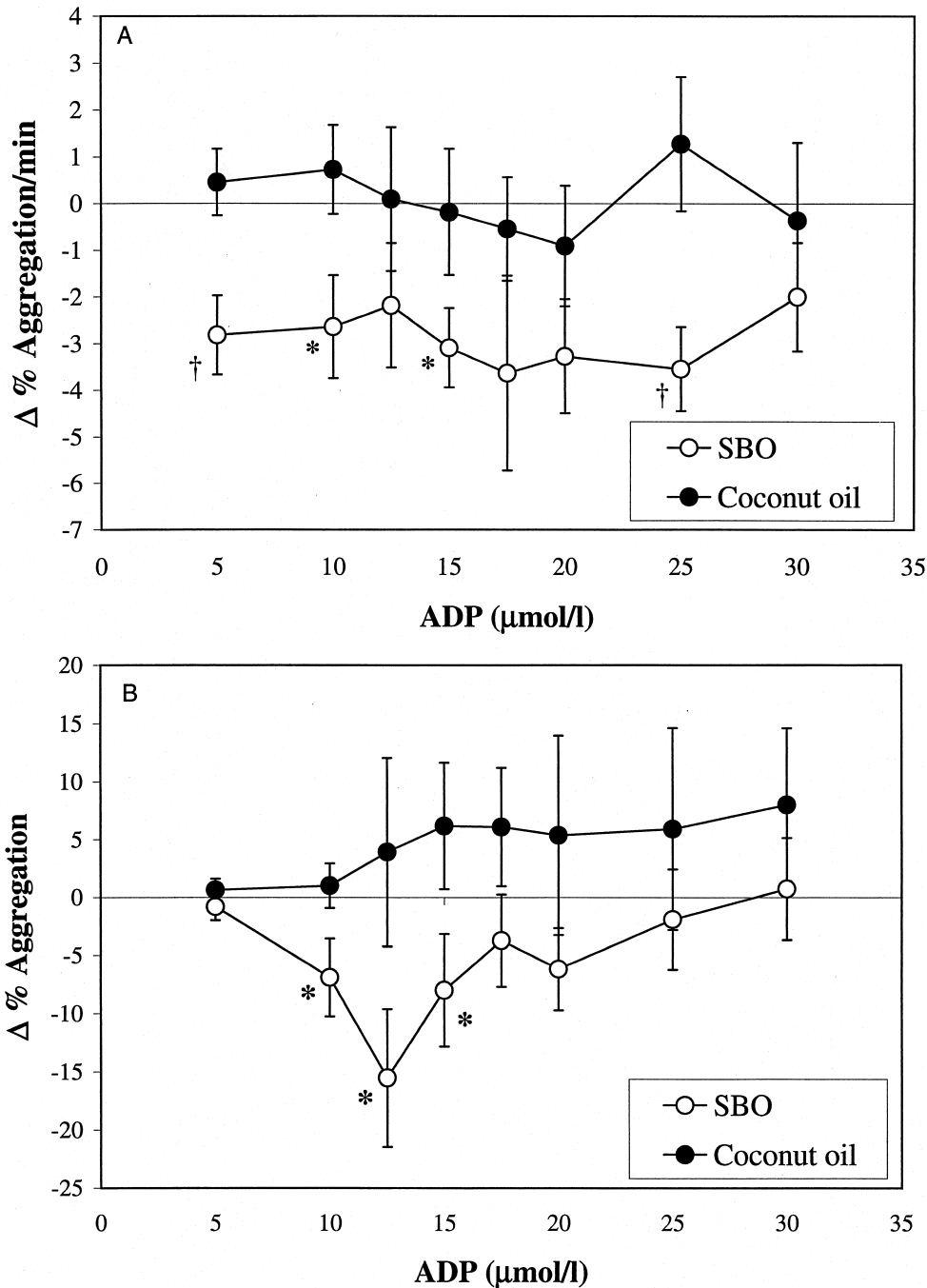


Figure 1 Δ -values (value after – value before supplementation) of the adenosine-5'-diphosphate-induced aggregation. (A) Difference in the rate of aggregation reaction (Δ % aggregation/min). (B) Difference in the maximum aggregation (Δ % aggregation) at 4 min. Results are mean of 11 subjects \pm SEM. Δ -values of sea buckthorn berry oil (SBO) and fractionated coconut oil supplementations differ significantly at * $P < 0.05$ or † $P < 0.01$.

aggregation with the highest agonist concentrations despite not adjusting the platelet count before measurements. The remaining PRP was repeatedly centrifuged and washed to yield platelets for the phospholipid fatty acid analysis.

Blood samples were collected into EDTA and centrifuged to yield plasma for the measurements of total cholesterol, triglycerides, glucose, HDL-cholesterol, and LDL-cholesterol by standard spectrophotometric methods (Unimate 5 Chol, Trig, and Gluc, HDL-reagent, F. Hoffmann-La Roche Ltd., Basel, Switzerland; 353-A LDL-kit, Sigma Diagnostics, St. Louis, MO USA) and for the phospholipid fatty acid analysis. LDL-cholesterol was measured from fresh unfrozen plasma, whereas for other determinations, the plasma was stored at -80°C . Comparison values for measured LDL-cholesterol were calculated by using a traditional

Friedewald equation²⁰ ($\text{LDL-cholesterol} = \text{total cholesterol} - \text{triglycerides} / 2 - \text{HDL-cholesterol}$, mmol/L).

Fatty acid compositions of the study and control oils were determined on a Hewlett Packard 6890 gas chromatograph (column CP Sil-88 FAMES, 100 m \times 0.25 mm i.d., d_f 0.20 μm , Chrompac, Middelburgh, The Netherlands) after methylation of the fatty acids with 2 M KOH in methanol.²¹ Total lipids of plasma and platelets were extracted with chloroform/methanol (2:1, v/v) using a modified Folch procedure^{22,23} and dissolved in chloroform. Phospholipids were isolated on silica Sep-Pak columns by eluting first with 10 mL chloroform to remove neutral lipids and then with 20 mL methanol to yield phospholipids. Fatty acids of phospholipids were methylated by NaOMe-catalyzed transmethylation²⁴ and analyzed on a Perkin-Elmer (Perkin-Elmer Corpora-

Table 3 Effects of oil supplementation on the plasma lipid and glucose levels: A, before supplementation; B, after supplementation. Results are mean values (mmol/l) of the 11 subjects (SEM)

	SBO supplementation		Coconut oil supplementation	
	A	B	A	B
Triglycerides	1.37 (0.09)	1.43 (0.12)	1.52 (0.15)	1.61 (0.15)
Total cholesterol	4.66 (0.23)	4.71 (0.20)	4.79 (0.27)	4.85 (0.26)
HDL-cholesterol	1.17 (0.07)	1.13 (0.09)	1.12 (0.08)	1.10 (0.08)
LDL-cholesterol (measured)	3.43 (0.23)	3.51 (0.24)	3.41 (0.27)	3.61 (0.24)
LDL-cholesterol (calculated)	2.80 (0.23)	2.87 (0.21)	2.91 (0.24)	2.94 (0.20)
Glucose	5.00 (0.14)	5.30 (0.16)**	5.11 (0.17)	5.36 (0.13)*

Value B differs significantly from value A at * $P < 0.01$ and ** $P < 0.001$.

SBO—sea buckthorn berry oil. HDL—high-density lipoprotein. LDL—low-density lipoprotein.

tion, Norwalk, CT USA) gas chromatograph (column NB-351, 25 m \times 0.32 mm i.d., d_f 0.20 μ m, HNU Nordion, Helsinki, Finland).

The possible period effects and supplement-period interactions were tested by two-sample two-tailed t -tests.²⁵ Effects of oil supplementations on plasma lipids/glucose, fatty acids, and platelet aggregation (each agonist concentration at a time) were tested by paired two-tailed t -tests. Study oil-control comparisons were performed by two-sample two-tailed t -tests of the subject-specific Δ -values (value after – value before supplementation).

Results and discussion

The present study on the effects of orally administered SBO on plasma lipids and platelet aggregation was the first conducted in a Western country. Sea buckthorn berry fractions have been used in treating cardiovascular disease (CVD) both in animal experiments and clinical investigations in China,^{1–3,26–28} but further experiments should be performed in Western countries because of differences in diet, nutritional status, and genetic factors.

Phospholipid fatty acids of neither plasma nor platelets were influenced by SBO or CO supplementation (Table 2). Despite negligible effects on the fatty acid compositions, clear differences between oil supplements were seen in their influences on platelet aggregation, especially on aggregation induced by ADP. Differences could be easily displayed by plotting the Δ -values (after – before supplementation) of the aggregation parameters in relation to agonist concentration (Figure 1). Both the rate of aggregation reaction (% aggregation/min) and maximum aggregation (% aggregation at 4 min) were reduced by SBO supplementation, whereas fractionated CO was ineffective in regard to platelet reactivity. Differences between the curves reached statistical significance in several points of ADP concentrations in both aggregation parameters. A net reduction in the AA-induced aggregation was observed after SBO supplementation, but the differences between SBO and control were not significant.

SBO supplementation of 4 weeks did not change the plasma lipid profile of healthy normolipidemic men, nor did fractionated CO supplementation (Table 3). Plasma glucose level was raised a little, but significantly, by both SBO and CO, which may reflect increased total fat consumption during the study. However, none of the subjects reported losing or gaining weight during the study period.

In summary, this small-scale intervention study suggested that SBO could be of value when treating persons with increased tendency to blood clotting. The mechanisms of the effects remained unclear, as eicosanoid-sensitive process, platelet aggregation, for example, was influenced by SBO without fatty acid modifications of the platelet membranes. Further studies on the dose-response effects are needed to assess the practical use of SBO as natural adjunctive form of therapy in the primary and secondary prevention of CVD. In addition, more research is needed for identifying the active components of the oil and also for clarifying the respective effect mechanisms in humans.

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