Effect of dietary supplementation with black currant seed oil on the immune response of healthy elderly subjects[1–4]

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ABSTRACT

Background: We have shown that the age-associated increase in prostaglandin E_2 production contributes to the decline in T cell–mediated function with age. Black currant seed oil (BCSO), rich in both γ-linolenic (18:3n−6) and α-linolenic (18:3n−3) acids, has been shown to modulate membrane lipid composition and eicosanoid production.

Objective: Our objectives were to *1*) test whether dietary supplementation with BCSO can improve the immune response of healthy elderly subjects, and *2*) determine whether the altered immune response is mediated by a change in the factors closely associated with T cell activation.

Design: A randomized, double-blind, placebo-controlled (soybean oil) study was conducted to examine the effect of 2 mo of BCSO supplementation on the immune response of 40 healthy subjects aged ≥65 y. In vivo immune function was determined by delayed-type hypersensitivity skin response. Peripheral blood mononuclear cells (PBMCs) were tested for in vitro immune response.

Results: In subjects supplemented with BCSO, the total diameter of induration at 24 h and individual responses to tetanus toxoid and *Trichophyton mentagrophytes* were significantly higher than their baseline values. The change in response to tetanus toxoid was significantly different from that of the placebo group. The BCSO group showed a significant increase in proliferative response of PBMCs to the T cell mitogen phytohemagglutinin that was not significantly different from that observed in the placebo group. BCSO had no effect on concanavalin A–induced mitogenic response, interleukin 2 and -1β production, and PBMC membrane fluidity. Prostaglandin E_2 production was significantly reduced in the BCSO-supplemented group, and this change was significantly different from that of the placebo group.

Conclusion: BCSO has a moderate immune-enhancing effect attributable to its ability to reduce prostaglandin E_2 production. *Am J Clin Nutr* 1999;70:536–43.

KEY WORDS γ-Linolenic acid, fatty acids, black currant seed oil, immune function, aging, prostaglandins, eicosanoids

INTRODUCTION

Immunologic vigor has been shown to decline with age. Immune cells, especially lymphocytes, have high amounts of polyunsaturated fatty acids (PUFAs) in their membrane phospholipids. Numerous studies have shown that dietary fats can influence the fatty acid composition of membrane phospholipids (1). These fatty acid alterations can modulate the function of the immune cells by several mechanisms, including changing the membrane fluidity, changing the activity of the membrane-associated enzymes, and producing immunoregulating eicosanoids.

Black currant seed oil (BCSO) is rich in both the n−6 PUFA γ-linolenic acid (18:3n−6) and the n−3 PUFA α-linolenic acid (18:3n−3). In addition, it contains a small amount of stearidonic acid (18:4n−3). The composition of BCSO reflects the recommended optimal dietary intake of n−3 and n−6 fatty acids, ie, it has a ratio of n−3 to n−6 fatty acids of 1 to 4 or 5 (2). The effect of α-linolenic acid on immune response is not well documented. We showed previously that long-term dietary supplementation with α-linolenic acid has no effect on the cell-mediated immune function of nonhuman primates (3). Stearidonic acid has been reported to inhibit platelet aggregation and arachidonate 5-lipoxygenase activity (4), suggesting that it might have antithrombotic and antiinflammatory properties.

Several reports have indicated that γ-linolenic acid has antiinflammatory and immunomodulating effects. γ-Linolenic acid is present in small amounts in the oils commonly used in Western diets but is found in high concentrations in certain other plant oils such as borage oil (23%), BCSO (18%), and evening primrose oil (9%). It can also be generated in the body through the desaturation of linoleic acid (18:2n−6) by the action of the rate-limiting enzyme linoleoyl-CoA desaturase (Δ^6 desaturase). The activity of linoleoyl-CoA desaturase is under hormonal and

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TABLE 1
Black currant seed oil (BCSO) and soybean oil (SBO) capsule composition

Ingredient	BCSO	SBO
Oil (mg/capsule)	750	750
Gelatin (mg/capsule)	195	210
Glycerol (mg/capsule)	71	77
Water (mg/capsule)	34	33
Fatty acid (%)		
16:0	6.2 (279)[1]	9.8 (441)
18:0	1.6 (72)	3.9 (176)
18:1n−9	12.7 (572)	26.2 (1179)
18:2n−6	45.9 (2052)	53.8 (2421)
18:3n−3	14.5 (653)	5.9 (266)
18:3n−6	15.0 (675)	ND[2]
18:4n−3	2.9 (131)	ND
20:1n−9	1.1 (50)	ND
Others	0.1 (4.5)	0.4 (18)
Vitamin E (ppm)		
α-Tocopherol	1120 (5)	1060 (4.8)
β-Tocopherol	<10 (<0.05)	<10 (<0.05)
γ-Tocopherol	758 (3.4)	422 (1.9)
δ-Tocopherol	24 (0.1)	59 (0.27)

[1] Daily dose in milligrams (calculated for 6 capsules/d) in parentheses.
[2] Not detectable.

metabolic regulation and can be influenced by certain diseases (5). For example, linoleoyl-CoA desaturase has been shown to be stimulated by insulin and inhibited by epinephrine, cortisol, thyroxine, glucagon, and saturated fat (5–7). In addition, linoleoyl-CoA desaturase activity decreases with age (8). Lower γ-linolenic concentrations have also been reported in patients with inflammatory diseases (9–11), whereas administration of γ-linolenic has been shown to elevate tissue γ-linolenic concentrations and improve some clinical outcome measures of rheumatoid arthritis (12) or experimentally induced arthritis in an animal model (13).

The investigation of botanical oils rich in γ-linolenic has thus far focused mostly on their antiinflammatory potential, but no comprehensive study has evaluated their effect on cell-mediated immune function in humans. Therefore, in this study we investigated the effect of BCSO (15–19% γ-linolenic acid) on the immune response of healthy elderly subjects.

SUBJECTS AND METHODS

Subjects and experimental design

Subjects were recruited via advertisements and telephone interviews from an existing pool of volunteers. Initially acceptable volunteers, ie, those who were nonsmokers; not suffering from acute or serious chronic diseases; not taking prescription medication or nonsteroidal antiinflammatory drugs on a regular basis; not taking vitamin, mineral, or lipid supplements for the past month; and had not been vaccinated for hepatitis B nor received pneumococcal vaccine were invited to the Jean Mayer USDA Human Nutrition Research Center on Aging (HNRCA) at Tufts University for 1 d of screening procedures. After they signed consent forms, subjects were given a physical examination, a psychosocial evaluation, a chest X-ray (if one had not been done within the previous year), and a 12-lead electrocardiogram. A clinical chemistry profile and complete blood cell and differential counts were determined, and a routine urinalysis was also performed. A total of 146 subjects were screened; 40 subjects were admitted to the study. The enrolled subjects were healthy, free-living adults ≥65 y of age of both sexes. The study protocol was approved by the Tufts University/New England Medical Center Human Investigation Review Committee.

In this double-blind, placebo-controlled protocol, subjects were randomly assigned to either the placebo (20 subjects) or the BCSO (20 subjects) group. Each group received 6 placebo or BCSO capsules per day. Each BCSO capsule contained 750 mg BCSO (15% γ-linolenic acid), and each placebo capsule contained 750 mg soybean oil. Both oils contained adequate amounts of α-tocopherol, calculated by using the formula that estimates minimum requirements of vitamin E for fatty acids with varying degrees of unsaturation (14). The fatty acid composition and vitamin E contents of the soybean oil and BCSO capsules are shown in **Table 1**. The total amount of fat provided by either supplement was 4.5 g or 1.46% of total daily energy intake (calculated from 3-d dietary records).

The subjects consumed the capsules daily for 2 mo while continuing their typical food intake, dietary habits, and lifestyle. An adequate number of capsules were given to subjects on days 11 and 49. Compliance was monitored by capsule count and measurement of plasma fatty acids. Subjects were required to abstain from taking any other supplements or any other medications during the study period without consulting the investigators. To ensure that no substantial change in dietary habits had occurred during the study, each subject completed two 3-d dietary records during the first month of the study.

Plasma fatty acids

Plasma samples (50 μL) were extracted with chloroform-methanol, as described previously (15), to isolate the total lipid fraction. The trichloromethane layer was collected and evaporated under nitrogen at 40°C, and the lipid residue was dissolved in 0.25 mL benzene. The fatty acids were methylated by addition of 0.75 mL of a freshly prepared solution of 10% acetyl chloride in methanol (16), followed by heating at 70°C for 2 h. After cooling, 1.2 mL of 7% aqueous NaCl was added, followed by 2 mL hexane to extract the fatty acid methyl esters.

The hexane extract was concentrated to a 100-μL volume and purified by HPLC (model 1100; Hewlett-Packard, Avondale, PA) to eliminate free cholesterol from the extract. We used an amino column (250 × 4.6 mm, particle size 5 μm; Alltech, Deerfield, IL). The initial mobile phase was 98% hexane, 2% isopropanol, with a flow rate of 1.5 mL/min and an 8-min linear ramp to 10% isopropanol. The HPLC retention times for fatty acid methyl esters and for cholesterol were determined by HPLC analysis of authentic standards at 220 nm. Removal of cholesterol avoids artifacts and column damage that can results from injection of cholesterol onto the gas chromatograph.

Purified fatty acid methyl esters were transferred to 50 μL dichloromethane and 1 μL was analyzed by gas chromatography (model 5890; Hewlett-Packard) on an apparatus equipped with a flame ionization detector. Separation was accomplished by using a 30-m AT-Wax capillary column with a 0.32-mm internal diameter and 0.5-μm film thickness (Alltech). The initial column temperature of 100°C was increased by 10°C/min to 250°C. Injections were made without split, and the split flow was turned on after 30 s. Fatty acid methyl esters in the samples

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were identified with authentic standards (Sigma Chemical Co, St Louis). Chromatograms were recorded digitally and peak areas were determined with GRAMS-386 software (Galactic Industries, Salem, NH).

Isolation of mononuclear cells

Forty milliliters of blood was collected into heparin-containing Vacutainers (Becton Dickinson, Rutherford, NJ) after subjects had fasted for 14 h. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by using Ficoll-Paque (Pharmacia Biotech, Piscataway, NJ) density-gradient centrifugation. The buffy layer was collected and washed twice in RPMI 1640 medium (Sigma) supplemented with 100 000 U penicillin/L, 100 mg streptomycin/L (Gibco Laboratories, Grand Island, NY), 2 mmol L-glutamine/L (Gibco), and 25 mmol HEPES/L (Sigma) (complete RPMI). Cells were counted under a light microscope. Cell viability was assessed by using trypan blue exclusion. Cells were suspended in complete RPMI containing heat-inactivated autologous plasma at appropriate concentrations for different cultures.

Lymphocyte proliferation

Lymphocyte proliferation was measured by [^{3}H]thymidine incorporation after stimulation with T cell mitogens. PBMCs at 1×10^5 cells/well (0.2 mL) in complete RPMI with 5% autologous plasma were cultured in 96-well flat-bottom plates (Becton Dickinson Labware, Lincoln Park, NJ) in the presence or absence of the T cell mitogens concanavalin A (ConA; Sigma) or phytohemagglutinin (PHA; Difco Laboratories, Detroit) at 0.5, 5, and 50 mg/L for 72 h at 37 °C in an atmosphere of 5% CO_2 and 95% humidity. Cultures were pulsed with 18.5 μBq of [^{3}H]thymidine (specific radioactivity of 247.9 GBq/mol; DuPont NEN Products, Boston) during the final 4 h of incubation. Cells were harvested onto glass microtiter filter paper by using a cell harvester (PHD, Cambridge, MA) and counted in a liquid scintillation counter (Beckman Instruments, Palo Alto, CA). The counter had an efficiency of 50% for tritium. The results are reported as corrected counts per minute (ccpm: cpm of mitogen-stimulated cultures minus the cpm of cultures without mitogen).

Interleukin 1β production

PBMCs, at 2.5×10^6 cells/well (1 mL) in complete RPMI with 1% autologous plasma, were cultured in 24-well flat-bottom plates (Becton Dickinson Labware) in the presence or absence of 1 μg lipopolysaccharide (LPS)/L (*Escherichia coli* 0111:B4; Sigma) or heat-killed *Staphylococcus epidermis* at 20 organisms per PBMC for 24 h. The plates were stored at −20 °C. To measure total interleukin 1β (IL-1β; cell-associated and secreted), the samples were exposed to 3 freeze-thaw cycles before IL-1β concentration was determined by radioimmunoassay (17). Antibody to IL-1β was purchased from Cistron Biotechnology (Pine Brook, NJ). Recombinant IL-1β was purchased from Genzyme (Cambridge, MA). [^{125}I]IL-1β was purchased from DuPont NEN.

Interleukin 2 and prostaglandin E_2 production

PBMCs, at 1×10^6 cells/well (1 mL) in complete RPMI with 9% autologous plasma, were cultured in 24-well flat-bottom plates (Becton Dickinson Labware) in the presence or absence of ConA or PHA at 50 mg/L for 48 h. Cell-free supernates were collected and stored at −70 °C for later analysis of interleukin 2 (IL-2) and prostaglandin E_2 (PGE_2). IL-2 activity was measured by using the bioassay method described by Gillis et al (18). Briefly, the samples, in graded dilutions, were cultured with cytotoxic T lymphocyte line 2 (CTLL-2) cells for 24 h. Cultures were pulsed by adding 18.5 μBq [^{3}H]thymidine during the last 6 h before they were harvested. Recombinant IL-2 (Genzyme) was used as the standard. One unit per milliliter is defined as the amount of recombinant IL-2 that causes a half-maximal incorporation of [^{3}H]thymidine into 5×10^3 CTLL-2 cells. PGE_2 was measured by radioimmunoassay as described by McCosh et al (19). The PGE_2 antibody was provided by J Dupont of Florida State University, Tallahassee, and M Mathias of the Agricultural Research Service in Washington, DC. The antibody has a cross-reactivity of 19% with PGE_1; its specificity and cross-reactivity were described previously (20).

Delayed-type hypersensitivity skin response

Delayed-type hypersensitivity (DTH) was assessed with a Multi-Test CMI (Merieux Institute Inc, Miami), a single-use, disposable applicator of acrylic resin with 8 heads loaded with a glycerin control and the following 7 recall antigens: tetanus toxoid, diphtheria toxoid, streptococcus (group C), *Mycobacterium tuberculosis*, *Candida albicans*, *Trichophyton mentagrophytes*, and *Proteus mirabilis*, as previously described. The diameter of positive reactions was measured 24 and 48 h after administration of the test. The detailed procedure was described previously (21, 22).

Hematology and lymphocyte subsets

Whole blood was examined with a Baker 9000 Hematology Analyzer for red blood cell and white blood cell counts; a blood smear was used to estimate white blood cell differential counts by microscopy. Lymphocyte subsets were determined by flow cytometry as described previously (21). Briefly, 5×10^5 PBMCs were stained for 30 min on ice with fluorescein- or phycoerythrin-conjugated monoclonal antibodies (Becton Dickinson, San Jose, CA) to total T cells (Leu 4, Anti-CD3), T-helper cells (Leu 3, Anti-CD4), T-cytotoxic/suppressor cells (Leu 2, Anti-CD8), and B cells (Leu 12, Anti-CD19). Cytometric analyses were conducted by using a flow cytometer (FACScan, Becton Dickinson).

Membrane fluidity

Membrane fluidity was measured by using 1,6-diphenyl-1,3,5-hexatriene (DPH; Sigma) as a fluorescence polarization probe (23, 24). Isolated PBMCs were washed twice with phosphate-buffered saline and 8×10^6 cells were incubated with 4 μmol DPH/L for 45 min at 37 °C. Fluorescence intensity was measured by using a Beckman LS-5 spectrofluorometer equipped with a thermostat-controlled cell holder and a polarization accessory. The steady state fluorescence polarization was measured at 37 °C by exciting the cells at 360 nm and recording the emission at 430 nm at 4 combinations of polarizer position. The fluorescence polarization was calculated by using the following equation:

$$P = (I_{vv} - I_{hv}/I_{hh} \times I_{vh})/(I_{vv} + I_{hv}/I_{hh} \times I_{vh}) \quad (1)$$

where P is polarization, I is the measured fluorescence intensity, the first and second subscripts represent the plane of polarization for the excitation and emission beams, respectively, and v and h denote the vertical and horizontal positions, respectively. The fluorescence polarization is inversely related to the fluidity of the membrane.

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TABLE 2
Baseline subject characteristics[1]

Group and sex	Age	BMI
	y	*kg/m²*
Placebo		
Male (*n* = 7)	72.1 ± 2.4	27.6 ± 2.1
Female (*n* = 7)	68.9 ± 1.6	24.4 ± 1.2
BCSO		
Male (*n* = 9)	67.7 ± 0.6	25.7 ± 1.1
Female (*n* = 6)	72.0 ± 2.8	23.5 ± 1.1

[1] $\bar{x}$ ± SEM; BCSO, black currant seed oil.

Statistical analysis

Sample size was calculated on the basis of our previous supplementation studies in which subjects with similar characteristics were evaluated by using the same immunologic methods as those used in the current study (21, 25). On this basis, a sample size of 12 is needed to detect a difference ≥30% at $P = 0.05$ with 80% power.

Data were analyzed by using the SYSTAT statistical package (SYSTAT 7.0, 1997; SYSTAT, Inc, Evanston, IL). Data within each group were analyzed by paired Student's *t* test or the nonparametric, paired Wilcoxon signed ranks test when the data were not normally distributed. The comparison of change between groups was conducted by using Student's *t* test or Kruskal-Wallis one-way analysis of variance when the distribution was not normal.

RESULTS

Six subjects in the placebo group and 5 subjects in the BCSO group dropped out before completion of the study for various reasons, including schedule conflicts, the desire to receive an influenza shot during the study, and modest anemia. There was no significant change in body mass index in either group (**Table 2**). Hematology, blood and urine chemistry, and blood lipid profiles were normal and did not change significantly after 2 mo of supplementation with BCSO (data not shown).

Plasma fatty acid composition

There was no difference in fatty acid profile between the 2 groups at baseline (**Table 3**). By the end of the 2-mo supplementation period, plasma fatty acids in the placebo group did not show a significant difference from values at baseline. However, BCSO supplementation significantly increased the plasma concentrations of γ-linolenic acid, α-linolenic acid, stearidonic acid, and dihomo-γ-linolenic acid (20:3n−6) compared with baseline. When the changes over 2 mo of supplementation were compared between the BCSO and placebo groups, a significantly larger increase in the concentrations of γ-linolenic acid, dihomo-γ-linolenic acid, and stearidonic acid, but an increase only marginally larger ($P = 0.08$) in α-linolenic acid, was observed in the BCSO group.

Delayed-type hypersensitivity skin response

There was no significant difference in baseline DTH response between the 2 groups. As shown in **Table 4**, of the 7 antigens applied with the Multi-Test CMI, the BCSO group showed significant increases in the diameter of induration in response to tetanus toxoid, *T. mentagrophytes*, and the total diameter of induration at 24 h compared with baseline; the placebo group did not show any significant change from baseline. Only the change in the diameter of induration in response to tetanus toxoid was significantly different ($P = 0.02$) from that of the placebo group. There was no significant change in the diameter of induration in response to other antigens, the total diameter of induration at 48 h, or the total number of antigens with positive responses in either of the groups (data not shown).

Lymphocyte proliferation

Neither the BCSO nor the placebo group showed significant changes in unstimulated (background) proliferation or ConA-stimulated proliferation of PBMCs after 2 mo of supplementation (data not shown). The percentage change in PHA-stimulated PBMC proliferation after 2 mo of supplementation compared

TABLE 3
Effect of black currant seed oil (BCSO) supplementation on plasma fatty acid composition of healthy elderly subjects[1]

	Placebo group (*n* = 11)		BCSO group (*n* = 11)	
Fatty acid	Baseline	2 mo	Baseline	2 mo
	% of total fatty acids			
16:0	22.69 ± 0.79	22.16 ± 0.47	22.58 ± 0.58	22.44 ± 0.45
16:1n−7	2.20 ± 0.21	2.18 ± 0.17	2.60 ± 0.23	2.50 ± 0.21
18:0	7.70 ± 0.16	7.55 ± 0.18	7.44 ± 0.16	7.66 ± 0.22
18:1n−9	21.29 ± 0.78	21.25 ± 0.82	22.82 ± 1.84	22.02 ± 1.47
18:2n−6	32.40 ± 1.12	33.23 ± 1.01	31.75 ± 1.04	31.40 ± 0.71
18:3n−6	0.54 ± 0.04	0.53 ± 0.02	0.50 ± 0.04	0.82 ± 0.06[2,3]
18:3n−3	0.71 ± 0.06	0.73 ± 0.06	0.58 ± 0.05	0.69 ± 0.05[4]
18:4n−3	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.08 ± 0.01[4,5]
20:3n−6	1.61 ± 0.12	1.66 ± 0.09	1.53 ± 0.09	2.10 ± 0.11[2,3]
20:4n−6	7.73 ± 0.46	7.86 ± 0.34	7.63 ± 0.34	7.90 ± 0.40
20:5n−3	1.10 ± 0.05	1.05 ± 0.06	0.92 ± 0.08	0.97 ± 0.06
22:6n−3	1.91 ± 0.17	1.78 ± 0.16	1.57 ± 0.20	1.43 ± 0.16

[1] $\bar{x}$ ± SEM.
[2,4] Significantly different from baseline (Student's paired *t* test): [2] $P < 0.001$, [4] $P < 0.05$.
[3,5] Significantly larger change than the placebo group (Student's *t* test): [3] $P < 0.001$, [5] $P < 0.05$.

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TABLE 4
Effect of black currant seed oil (BCSO) supplementation on delayed-type hypersensitivity skin response in healthy elderly subjects[1]

Group and measure	Induration diameter		Change	P^2
	Baseline	2 mo		
	mm		*%*	
Placebo (n = 14)				
Total induration at 24 h	27.3 (19.0, 35.3)[3]	27.5 (23.3, 32.5)	6.4 (−14.2, 76.1)	0.44
Induration to TT	8.8 (5.5, 10.3)	8.1 (4.6, 8.6)	−16.8 (−40.1, 49.9)	0.35
Induration to TM	3.0 (1.9, 4.8)	4.0 (2.2, 6.0)	0 (−37.7, 136.0)	0.21
BCSO (n = 15)				
Total induration at 24 h	17.5 (12.2, 26.3)	21.3 (17.0, 30.1)	23.3 (2.3, 84.7)	0.05
Induration to TT	4.5 (2.3, 6.3)	6.3 (4.2, 7.3)	28.6 (7.2, 189.8)[4]	0.03
Induration to TM	1.0 (1.4, 3.9)	3.25 (2.2, 5.3)	29.4 (17.2, 163.1)	0.05

[1] TT, tetanus toxoid; TM, *Trichophyton mentagrophytes*.
[2] Comparison between values at baseline and 2 mo by Wilcoxon signed-rank test within each group.
[3] Median (lower, upper quartile).
[4] Significantly different from the placebo group, $P < 0.05$.

with baseline is shown in **Figure 1**. Both the BCSO and placebo groups showed a small increase in proliferation in response to all 3 amounts of PHA used, but the increase was significant only in the BCSO group in response to 5 and 50 mg PHA/L. The increase observed in the BCSO group, however, was not significantly different from that of the placebo group.

IL-1β, IL-2, and PGE_2 production

As shown in **Table 5**, there was no significant change in either group in ConA- or PHA-stimulated IL-2 production nor in LPS- or *S. epidermis*–stimulated IL-1β production by PBMCs after 2 mo of supplementation. Unstimulated or ConA- and PHA-stimulated PGE_2 production by PBMCs did not change significantly in the placebo group. In the BCSO group, both unstimulated and stimulated PGE_2 production showed a decrease; however, only the decrease in PHA-stimulated PGE_2 production was significant ($P < 0.05$). Furthermore, the percentage change in PHA-stimulated PGE_2 production was significantly different from that of the placebo group ($P < 0.05$).

Lymphocyte subpopulations and membrane fluidity

Neither the BCSO nor the placebo group showed a significant change in the proportion of any lymphocyte subset (**Table 6**) or in PBMC membrane fluidity (**Table 7**).

DISCUSSION

An age-associated decline in T cell–mediated immune function is well documented (26). Nutritional supplementation has been shown to enhance the immune response in elderly humans (25, 27, 28). Change in both the total amount and the type of dietary lipids has been shown to influence the immune response (29–31). In this study, we investigated the immunologic effects of BCSO, an oil rich in γ-linolenic and α-linolenic acids, on the immune response of healthy elderly subjects.

DTH skin tests have been widely used as an in vivo assay to determine cell-mediated immune function. Several investigators have shown that a decrease in DTH is associated with increased morbidity and mortality (32, 33). In this study, BCSO significantly increased the total diameter of induration after 24 h and response to specific antigens (tetanus toxoid and *T. mentagrophytes*), when compared with presupplementation measurements. This increase, however, was significantly different from the change in the placebo group only in response to tetanus toxoid. Few studies have evaluated the effect on DTH of oils containing γ-linolenic acid. Nerad et al (34) showed that consumption of 2 g γ-linolenic acid/d, provided through borage oil for 12 wk, increased the cumulative score (total diameter of induration) in healthy young volunteers. The dose of γ-linolenic acid and the duration of supplementation in their study were larger (2 g γ-linolenic acid/d for

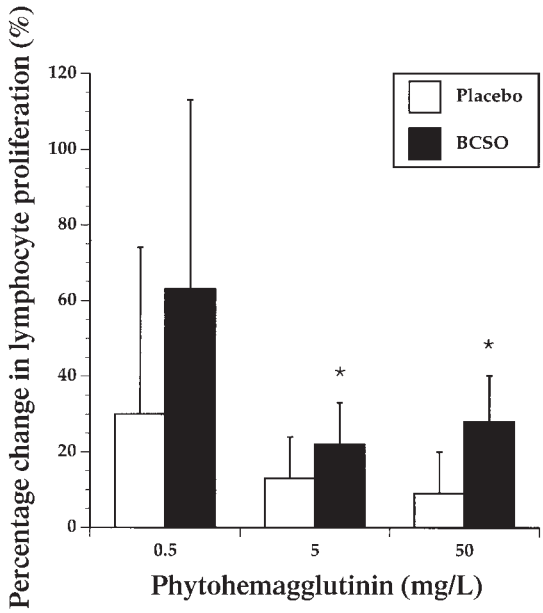

FIGURE 1. Effect of black currant seed oil (BCSO) supplementation on lymphocyte proliferation. Peripheral blood mononuclear cells (1×10^5) were stimulated with phytohemagglutinin at different concentrations for 72 h and pulsed with [^{3}H]thymidine during the last 4 h to determine DNA synthesis. The average baseline proliferative responses to 0.5, 5, and 50 mg phytohemagglutinin/L were 8568, 72 775, and 57 670 corrected counts per minute (ccpm) for the placebo group, and 5696, 70 775, and 56 525 ccpm for the BCSO group, respectively. $\bar{x} \pm$ SEM; n = 14 in the placebo group, n = 15 in BCSO group. *Significantly different from baseline, $P < 0.05$ (Student's paired t test).

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TABLE 5
Effect of black currant seed oil (BCSO) supplementation on interleukin (IL) 1β, IL-2, and prostaglandin (PG) E_2 production[1]

	Placebo group (n = 14)		BCSO group (n = 15)	
Mitogen	Baseline	2 mo	Baseline	2 mo
IL-1β (ng/L)				
LPS at 1 mg/L	8.8 ± 0.9	9.3 ± 1.4	10.0 ± 1.1	9.3 ± 1.2
S. epidermis at 20/cell[2]	19.7 ± 3.8	20.4 ± 2.8	21.3 ± 3.8	20.1 ± 2.9
IL-2 (10^3 U/L)				
ConA at 10 mg/L	1.7 ± 0.6	1.0 ± 0.8	1.4 ± 0.3	1.2 ± 0.3
PHA at 10 mg/L	11.0 ± 2.1	11.1 ± 2.4	8.7 ± 1.6	9.4 ± 2.1
PGE_2 (ng/L)				
Medium (no mitogen)	39 ± 4	31 ± 7	22 ± 7	16 ± 5
ConA at 10 mg/L	97 ± 27	103 ± 19	99 ± 18	76 ± 16
PHA at 10 mg/L	319 ± 63	367 ± 63	488 ± 154	258 ± 74[3,4]

[1] $\bar{x}$ ± SEM. LPS, lipopolysaccharide; ConA, concanavalin A; PHA, phytohemagglutinin. There was no significant change in IL-1β or IL-2 production in either group.
[2] 20 heated-killed *Staphylococcus epidermis* cells applied per peripheral blood mononuclear cell in culture.
[3] Significantly different from baseline, $P < 0.05$ (Wilcoxon signed-rank test).
[4] Change in the BCSO group was significantly different from that of the placebo group, $P < 0.05$ (Kruskal-Wallis one-way ANOVA).

3 mo) than that in the current study (0.675 g γ-linolenic acid/d for 2 mo). In an animal study (35), however, the intravenous injection of 0.05 or 0.5 mL 10% dihomo-γ-linolenic acid (immediate metabolic product of γ-linolenic acid) emulsion suppressed DTH in mice. The suppressive effect observed in that study could have been due to the much higher amount of dihomo-γ-linolenic acid administered. High fatty acid intakes, regardless of the degree of unsaturation of the fatty acids, have been shown to suppress immune response (36).

In this study, BCSO supplementation also caused a modest, but significant, increase in proliferative response of PBMCs to the T cell mitogen PHA. No significant effect was observed on the production of the proinflammatory cytokine IL-1β or the T cell growth factor IL-2.

Devi and Das (37) examined the in vitro effect of several PUFAs (linoleic acid, α-linolenic acid, γ-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid) on human lymphocytes and on the leukemic cell line Molt-2; they found that all PUFAs dose-dependently inhibited lymphocyte proliferation, with γ-linolenic acid being the most inhibitory. In addition, they noted that all PUFAs reduced IL-2 production in human lymphocytes but elevated IL-2 production in Molt-2 cells (37). Madhavi et al (38) confirmed in vitro inhibition of lymphocyte proliferation by PUFAs, including γ-linolenic acid at 40 mg/L. In contrast, Kelly and Parker (39) reported that linoleic acid, γ-linolenic acid, dihomo-γ-linolenic acid, and arachidonic acid stimulated at low concentrations (0.1–1.0 mg/L), but inhibited at high concentrations (>10 mg/L), the proliferative response of human lymphocytes to mitogens. The differences between the in vitro and in vivo studies as well as within the in vitro studies themselves could be due to the differences in the final concentration of γ-linolenic acid to which the immune cells were exposed.

Dietary supplementation with γ-linolenic acid has been shown to suppress lymphocyte proliferation but increase IL-2 production by PBMCs from healthy young subjects (34), and to enhance the ConA-induced proliferative response of rat splenocytes (40). In this study, the moderate enhancement of cell-mediated immune function in the BCSO group was not accompanied by a change in production of IL-1 or IL-2, both of which are involved in the regulation of T cell activation. Nor was there any change in the lymphocyte subpopulations. This agrees with the study reported by Harbige et al (40) in which oral γ-linolenic acid administration to Lewis rats enhanced splenocyte proliferation but had no effect on the proportion of splenic $CD4^+$ and $CD8^+$ lymphocytes. A more recent study, however, showed that Brown-Norway rats fed a diet containing evening primrose oil (6.5% γ-linolenic acid) had a higher percentage of $CD4^+$ T cells than did those fed diets containing safflower oil or pine seed oil (neither of which has a

TABLE 6
Effect of black currant seed oil (BCSO) supplementation on lymphocyte subpopulations[1]

	Placebo group (n = 12)		BCSO group (n = 13)	
Lymphocyte	Baseline	2 mo	Baseline	2 mo
		%		
T cells	68.5 ± 3.9	71.4 ± 2.8	71.2 ± 2.5	72.0 ± 3.0
$CD4^+$	50.1 ± 3.9	48.2 ± 3.7	48.5 ± 3.2	49.6 ± 3.1
$CD8^+$	16.9 ± 2.8	14.6 ± 2.3	17.3 ± 1.9	16.6 ± 2.5
$CD4^+$:$CD8^+$	4.1 ± 0.8	4.8 ± 1.2	3.1 ± 0.4	3.4 ± 0.5
B cells	9.5 ± 1.5	9.1 ± 1.1	8.5 ± 0.7	8.7 ± 0.7

[1] $\bar{x}$ ± SEM. There was no significant change in any cell types measured in either diet group.

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TABLE 7
Effect of black currant seed oil (BCSO) supplementation on membrane fluidity of peripheral blood mononuclear cells[1]

	DPH fluorescence polarization	
Group	Baseline	2 mo
Placebo (n = 12)	0.274 ± 0.003	0.270 ± 0.004
BCSO (n = 14)	0.274 ± 0.003	0.270 ± 0.003

[1] $\bar{x}$ ± SEM. DPH, 1,6-diphenyl-1,3,5-hexatriene. The fluorescence polarization is inversely related to the fluidity of the membrane. There was no significant change in either diet group.

detectable amount of γ-linolenic acid (41). These inconsistent results may be due to differences in animal models, human subject characteristics (such as age and sex), source and dose of γ-linolenic acid, or the route of administration used in these studies.

Because dietary lipids can modify membrane fatty acid composition, which can in turn influence the function of the immune cells, we hypothesized that a BCSO-induced change in membrane fluidity is a possible mechanism of its effect. There was, however, no effect of BCSO on membrane fluidity. Still, this does not rule out the possibility that BCSO-induced changes in specific lipid domains associated with important membrane proteins might have caused the observed effects.

Another mechanism through which BCSO can enhance cell-mediated immunity is through reduction of PGE_2 production. γ-Linolenic acid can be readily converted to dihomo-γ-linolenic acid. γ-Linolenic acid supplementation increases dihomo-γ-linolenic acid concentrations in tissues but causes little or no change in arachidonic acid concentrations (42–45). In agreement with previous studies (13, 44, 46–48), we found that after dietary BCSO supplementation, plasma γ-linolenic acid and dihomo-γ-linolenic acid concentrations were higher, but the arachidonic acid concentration did not change (possibly because of poor $\triangle^5$ desaturase activity), resulting in an increased ratio of dihomo-γ-linolenic to arachidonic acids. Increased dihomo-γ-linolenic acid, either formed from γ-linolenic acid or directly provided in food, has been shown to increase PGE_1 (46, 49, 50) and suppress PGE_2 and leukotriene B_4 production (13, 48, 50), a consequence of the competition between dihomo-γ-linolenic acid and arachidonic acid for prostaglandin endoperoxide synthase (cyclooxygenase) and arachidonate 5-lipoxygenase. PGE_2 is a well-known suppressor of T cell function (51). Leukotriene B_4 has diverse inflammatory activity and has also been reported to down-regulate lymphocyte proliferation (52). In this study, a significant decrease in PGE_2 production was observed after consumption of BCSO. PGE_2 was measured by radioimmunoassay and the antibody used in the assay had 19% cross-reactivity with PGE_1. Because of sample limitations, we were unable to separate PGE_1 and PGE_2 before analysis by radioimmunoassay. However, as reported previously (46, 49, 50), it is reasonable to assume that increased dihomo-γ-linolenic acid would lead to increased PGE_1 synthesis. Thus, PGE_1 may have contributed to a portion of the PGE_2 reported in the subjects consuming BCSO; ie, the data presented in Table 5 might be an overestimation of PGE_2 concentrations in the BCSO group. Thus, the moderate enhancement of immune response observed after γ-linolenic acid supplementation can, at least in part, be attributed to the decreased PGE_2 production.

We used BCSO as a source of γ-linolenic acid in this study. In addition to n−6 PUFAs, BCSO contains more α-linolenic acid and stearidonic acid (not detectable in soybean oil) than does soybean oil. The plasma fatty acid profiles reflected the increased intake of these 2 fatty acids. Our previous study showed no significant effect of dietary supplementation with 3.5% and 5.3% α-linolenic acid for 28 wk on lymphocyte proliferation, IL-2, and PGE_2 production in nonhuman primates (3). The effect of stearidonic acid on immune function is not known. Guichardant et al (53) observed that stearidonic acid inhibited the synthesis of arachidonate 5-lipoxygenase product of arachidonic acid, leukotriene B_4, and 5-hydroxyeicosatetraenoic acid in an in vitro study using human leukocytes. In addition, preincubation with stearidonic acid was reported to reduce the synthesis of hydroxyheptadecatrienoic acid, a prostaglandin endoperoxide synthase product, from endogenous arachidonic acid in human platelets. This suggests that stearidonic acid may contribute to the BCSO-induced reduction in PGE_2 production. Plasma concentrations of eicosapentaenoic acid and docosahexaenoic acid were unchanged by BCSO consumption despite increased precursor fatty acids α-linolenic acid and stearidonic acid, indicating an inefficient in vivo conversion process.

In conclusion, BCSO consumption does not adversely affect the immune response of healthy elderly subjects and may have a moderate immunoenhancing effect that is, in part, due to its reduction of PGE_2 production.

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