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Black currant seed oil and fish oil supplements differ in their effects on fatty acid profiles of plasma lipids, and concentrations of serum total and lipoprotein lipids, plasma glucose and insulin

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Abstract

European diets provide a suboptimal intake of eicosapentaenoic (20:5n3) and docosahexaenoic (22:6n3) acids, which are derived mainly from fish oils. The present study indicates that black currant seed oil, which contains 14.5% α-linolenic (18:3n3), 12.6% γ-linolenic (18:3n6), 47.5% linoleic (18:2n6) and 2.7% stearidonic (18:4n3) acids, could potentially serve as alternative to fish oil as a n3 fatty acid source. Fifteen healthy females participated in a randomized, double-blind, crossover study including two 4-week periods with either 3 g/day of black currant seed oil or 2.8 g/day of fish oil separated by a 4-week washout period. The results show that black currant seed oil supplementation increased the proportion of 18:3n6 in triacylglycerols (TAG) and cholesteryl esters (CE), and that of dihomo-γ-linolenic (20:3n6) in TAGs, CEs and glycerophospholipids (GPL) ($P<.05$). Proportion of 18:3n6 was higher ($P<.05$) after black currant seed oil than after fish oil in TAGs and CEs, and that of 20:3n6 in TAGs, CEs and GPLs. Black currant seed oil supplementation caused only minor changes in the proportions of 20:5n3 or 22:6n3. Serum levels of LDL cholesterol were lower ($P<.05$) after black currant seed oil compared to fish oil. Plasma glucose concentration decreased during the fish oil supplementation ($P<.05$).

Keywords: Black currant seed oil; Diet; Fish oil; Human; Lipid

1. Introduction

Humans normally survive on a broad intake of n3 and n6 polyunsaturated fatty acids [1,2], but an imbalance in the ratio of these acids can accentuate n3 fatty acid deficiency [3]. The recommended intake of α-linolenic acid (18:3n3) varies from 1 to 2.4 g/day and that of eicosapentenoic acid (20:5n3) and docosahexaenoic acid (22:6n3) from 0.2 to 1.1 g/day [4–7]. Since the fatty acids compete for chain desaturation and elongation [8], the actual need for the n3 fatty acids is dependent on their chain length and the presence of other polyunsaturated fatty acids in the diet. The amount of total fat in the diet may also be important [9].

The major sources of n3 fatty acids in the diet are fish oils, which contain 7–19% 20:5n3 and 6–13% 22:6n3, along with 0.5–2% 18:3n3 and 2–3% stearidonic acid (18:4n3). Fish oils, however, are in short supply and may contain harmful organic pollutants [10]. Consideration must be given to their replacement in the diet by n3 fatty acid containing plant oils. The seed oil of black currant of the genus *Ribes* (Grossulariaceae) contains 18:3n3 and 18:4n3 as do the seed oils of the families Primulaceae and Grossulariaceae. Black currant seed oil contains 10–19% 18:3n3, 2.4–4.3% 18:4n3 and 12–25% γ-linoleic acid (18:3n6) [11,12]. 18:3n6 affects gene regulation [13] and may influence the conversion of 18:3n3 and 18:4n3 into long-chain polyunsaturates.

A detailed study of the effect of dietary black currant seed oil on tissue polyunsaturated fatty acid levels would be most

Abbreviations: CE, cholesteryl ester; GPL, glycerophospholipids; TAG, triacylglycerol; 18:2n6, linoleic acid; 18:3n3, α-linolenic acid; 18:3n6, γ-linoleic acid; 18:4n3, stearidonic acid; 20:3n6, dihomo-γ-linolenic acid; 20:5n3, eicosapentenoic acid; 22:6n3, docosahexaenoic acid.

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appropriate, especially since such studies have not been performed previously. Other work [14,15] has shown that feeding of black currant seed oil causes a decrease in plasma total and LDL cholesterol, and an increase in HDL cholesterol. Furthermore, plasma triacylglycerol (TAG) concentration has been reported to decrease in hemodialyzed patients receiving black currant seed oil supplement [16].

The present study compares the effects of dietary black currant seed oil and fish oil on fatty acid composition of plasma glycerophospholipids (GPL), cholesteryl esters (CE), TAGs, as well as on cholesterol, glucose and insulin levels in healthy females.

2. Methods and materials

2.1. Subjects

Fifteen healthy females participated in the study. The baseline characteristics and inclusion criteria of the subjects are presented in Table 1. Further inclusion criteria were blood pressure <140/90 mm Hg, hemoglobin >120 g/L, normal liver, kidney and thyroid functions, and menstrual cycle 25–31 days. The group was very homogenous, as were their eating habits. Seven of the subjects used oral contraceptives, and all subjects were at the same stage of their menstrual cycle at each blood draw. The subjects were asked to keep their medication unchanged during the study. Each subject provided an informed written consent, and they were free to discontinue their participation in the study at any point without explanations. The study plan was approved by the Ethics Committee of the University of Kuopio and Kuopio University Hospital.

2.2. Experimental design and supplements

The trial was a randomized double-blind crossover study. Each of the three periods including the washout period lasted for four weeks. The subjects were advised to stay on their habitual diet and not to change their fish consumption frequency.

The supplements were supercritical CO_2 extracted black currant seed oil (Aromtech Ltd, Tornio, Finland) 3.0 g/days (500-mg capsules, six per day) containing both n3 (17%) and n6 (60%) fatty acids, and fish oil 2.8 g/days (400-mg capsules, seven per day) containing 28% n3 and only 4% n6 fatty acids. The fatty acid composition of the supplements is presented in Table 2. Fish oil supplement contained 4.48 mg/g α-tocopherol and black currant seed oil 1.28 mg/g α-tocopherol, 0.86 mg/g γ-tocopherol and 0.05 mg/g δ-tocopherol. Black currant seed oil contained 1.17 % free and esterified phytosterols, mainly sitosterol. Fasting venous blood samples were drawn at the beginning and at the end of both supplement periods after a 12-h fast.

Table 1
Baseline characteristics of the subjects

Characteristics	Inclusion criteria	Baseline mean±SD
Age, year	18–45	24.1±5.0
Body mass index, kg/m^2	18.5–25	21.2±1.8
Plasma glucose, mmol/L	<6.0	4.98±0.24
Serum cholesterol, mmol/L		
Total	<5.0	4.32±0.45
HDL	0.8–2.0	1.48±0.29
LDL	<3.5	2.40±0.38
Serum triacylglycerols, mmol/L	<2.0	0.98±0.47

Table 2
Fatty acid compositions of black currant seed oil and fish oil supplements and their daily doses

Fatty acid	Current seed oil		Fish oil	
	Mean, %	mg/day	Mean, %	mg/day
14:0	0	0	5.3	148
16:0	5.6	169	13.2	369
16:1	0.1	2.2	7.4	207
18:0	1.4	42.1	2.8	77.7
18:1(n-9)	13.3	398	12.9	361
18:1(n-7)	0.7	22.0	3.1	85.8
18:2(n-6)	47.5	1426	2.8	79.5
18:3(n-6)	12.6	378	0.3	9.0
18:3(n-3)	14.5	436	0.9	26.4
18:4(n-3)	2.7	82.1	2.4	67.7
20:1(n-9)	0.8	25.5	4.2	117
20:3(n-3)	0	0	1.4	40.1
20:5(n-3)	0	0	13.2	370
22:0	0	0	4.4	122
24:0	0	0	3.4	96.4
22:6(n-3)	0.02	0.6	9.8	274
Others	0.6	19.2	12.5	349
Total	100	3000	100	2800

All subjects were asked to record their dietary intakes on seven consecutive days during the experimental periods of the trial. During the washout period the subjects kept a 4-day food record. Food records were analyzed using the Micronutrica software (version 2.5). The software is based on Finnish food analyses and international food composition tables [17].

2.3. Laboratory methods

For the fatty acid composition studies, plasma lipids were extracted, and TAGs, GPLs and CEs were separated from the extracted lipid mixture with commercial silica columns (Sep-Pak Silica Cartridges, Waters, Milford, MA) [18,19]. Fatty acid methyl esters were prepared from TAG, GPL and CE with the sodium methoxide method [20]. The fatty acid methyl esters were dissolved in hexane and analyzed twice by gas chromatography (Perkin Elmer AutoSystem, Norwalk, CT) with H-P InnoWax column (30 m×0.32 mm i.d., 0.25 μm film thickness).

Serum cholesterol and TAG were determined by enzymatic colorimetric methods using commercial kits (Cholesterol CHOD-PAP and Triglyceride GPO-PAP; Roche Diagnostics GmbH, Mannheim, Germany) and an automatic analyzer (Kone Specific Pro, Kone Instruments, Espoo, Finland). Low-density lipoprotein was precipitated by dextransulphate-$MgCl_2$ [21], and HDL concentration in the infranatant was determined as above. The concentration of

LDL cholesterol was calculated using the Friedewald's formula [22].

Plasma glucose concentration was analyzed by the glucose dehydrogenase method (Gluc-DH kit, Merck, Darmstadt, Germany, and Kone Specific Pro). Plasma insulin concentration was analyzed with a radioimmunoassay method (Phadeseph Insulin RIA 100, Pharmacia & Upjohn Diagnostics, Uppsala, Sweden). Analyses of apolipoproteins A-I and B were based on a measurement of immunoprecipitation enhanced by polyethylene glycol at 340 nm [23]. The Kone Specific Pro, and apolipoprotein A-I and B reagents (Orion Diagnostica, Espoo, Finland) were used.

2.4. Statistical methods

SPSS-PC+ statistical package version 10 (SPSS Inc., Chicago, IL) and SAS System for Windows Release 8.02 (SAS Institute Inc., Cary, NC) were used. Normal distribution of the data was checked with Shapiro–Wilks's test. General linear model (GLM) for repeated measurements was used to examine the overall changes in variables. Paired samples *t*-test was used for further analysis. Wilcoxon matched pairs signed ranks test was used to analyze data not normally distributed after log-transformation. The data are expressed as mean±SD.

3. Results

The subjects maintained their habitual diet during the study periods and no changes in the intake of energy and energy nutrients were seen (Table 3). Neither was there any change in the body weight of the subjects during the study.

In plasma TAGs, the black currant seed oil supplementation resulted in higher ($P<.01$) proportions of 18:3n6 and 20:3n6, and lower proportions of 20:5n3 and 22:6n3 compared with the fish oil supplementation (Table 4). Within-treatment black currant seed oil increased the proportions of 18:3n6 and 20:3n6, and decreased the proportions of 18:4n3 and 22:6n3. In CEs (Table 5) significant between-treatment differences were found in 18:3n6, 20:3n6, 20:5n3 and 22:6n3. In addition, black currant seed oil increased the proportions of 18:3n6 and 20:3n6 and decreased that of 22:6n3 within treatment. Correspondingly, fish oil increased the proportions of 20:5n3 and 22:6n3, and decreased those of 18:2n6, 18:3n6, 20:3n6. In plasma GPLs, the black currant seed oil supplementation resulted in higher ($P<.05$) proportion of 20:3n6 and lower proportions of 20:5n3 and 22:6n3 compared with the fish oil supplementation. The proportion of 20:3n6 increased during black currant supplementation, while the fish oil supplementation increased 20:5n3 and 22:6n3 proportions, and decreased those of 18:2n6 and 20:3n6 (Table 6). Although black currant seed oil contained both 18:3n3 and 18:4n3, the proportions of these fatty acids or the proportions of the long-chain metabolites of these fatty acids, 20:5n3 and 22:6n3, did not increase in any studied lipid fraction during the black currant seed oil period.

During the black currant seed oil period the proportions of 18:3n6 and its elongation product, 20:3n6, increased in plasma TAGs (Table 4), CEs (Table 5) and GPLs (Table 6). The proportion of the other elongation product, 20:4n6, did not change. Consequently, the ratios of the mean concentrations of 20:3n6 and 20:4n6 increased from 0.16 to 0.30 in plasma TAGs, from 0.12 to 0.16 in plasma CEs and from 0.39 to 0.52 in plasma GPLs, respectively.

Black currant seed oil supplementation resulted in a decrease in serum LDL cholesterol concentration compared with fish supplementation ($P=.025$). During the black currant seed oil period, serum apolipoprotein A-I concentration increased ($P=.012$) and serum LDL cholesterol concentration tended to decrease (Wilcoxon, $P=.057$). HDL/LDL ratio increased during the black currant seed

Table 3
Intake (mean±SD) of energy, energy nutrients, cholesterol and fiber from diet (supplements not included) during the study periods

	BCO period[a]	FO period[b]	Washout period[c]
Energy, kJ	7646±1212	7538±988	7118±889
Protein, E%	13.9±2.4	14.6±2.1	15.7±3.0
Carbohydrates, E%	49.3±4.4	50.1±3.1	49.3±6.6
Fats, E%	29.4±5.5	27.9±4.6	27.5±7.7
SFA, E%	10.4±1.7	9.7±1.9	10.6±2.5
MUFA, E%	10.2±3.6	9.7±2.7	8.6±3.5
PUFA, E%	6.3±2.2	6.1±1.9	5.5±2.5
Alcohol, E%	2.6±3.0	2.5±2.3	2.6±2.8
Fiber, g	24.1±6.3	25.0±7.4	24.2±7.2
Cholesterol, mg	173±63	159±44	155±47

E%, percentage of daily energy.
[a] Black currant seed oil supplement period, values based on a 7-day food record.
[b] Fish oil supplement period, values based on a 7-day food record.
[c] Values based on a 4-day food record.

Table 4
Fatty acid composition of plasma triacylglycerols before and after black currant seed oil and fish oil periods

Fatty acid	Black currant seed oil period		Fish oil period		GLM
	Before	After	Before	After	*P*
16:0	23.7±2.9	24.9±2.9	24.1±2.1	24.3±3.0	NS
16:1	5.0±1.3	5.0±0.8	5.0±0.9	5.0±0.9	NS
18:0	3.0±0.9	3.0±0.7	3.0±0.5	3.2±0.7	–
18:1n9	38.0±3.1	37.4±2.6	38.3±2.5	37.2±1.9	NS
18:1n7	3.4±0.8	3.2±0.5	3.5±0.8	3.3±0.5	NS
18:2n6	13.6±2.1	14.0±2.5	13.9±1.9	13.8±1.9	–
18:3n6	0.3±0.2	0.4±0.2[a]	0.3±0.2	0.2±0.1[b]	0.01
18:3n3	1.7±0.5	1.8±0.4	1.7±0.4	1.6±0.3	NS
18:4n3	0.8±0.4	0.6±0.3[a]	0.8±0.3	0.6±0.2[a]	–
20:1n9	0.5±0.2	0.4±0.2	0.4±0.2	0.4±0.2	NS
20:3n6	0.2±0.2	0.3±0.2[a]	0.1±0.2	0.1±0.1[b]	–
20:4n6	0.9±0.3	1.0±0.3	1.0±0.3	1.0±0.2	NS
20:5n3	0.2±0.5	0.2±0.3	0.1±0.3	0.6±0.3[a,b]	–
22:6n3	1.7±1.9	1.0±0.6[a]	1.0±0.6	1.8±0.5[a,b]	–

Values are expressed as mean±standard deviation.
[a] Significant difference within a period $P < .05$.
[b] Significant difference between periods $P < .01$.

Table 5
Fatty acid composition of plasma cholesteryl esters before and after black currant seed oil and fish oil periods

Fatty acid	Black currant seed oil period		Fish oil period		GLM
	Before	After	Before	After	*P*
16:0	10.9±0.7	11.0±0.6	10.8±0.6	11.3±0.8[a]	<0.01
16:1	3.8±1.6	3.4±1.3	3.5±1.2	3.5±1.1	NS
18:0	0.7±0.1	0.6±0.1	0.7±0.1	0.7±0.1	NS
18:1n9	19.9±1.4	19.4±1.8	19.9±1.6	19.9±1.5	NS
18:1n7	1.4±0.2	1.4±0.2	1.4±0.2	1.5±0.3	NS
18:2n6	49.8±3.5	49.6±3.5	50.5±2.9	48.2±3.7[a]	<0.01
18:3n6	0.8±0.1	1.4±0.3[a]	0.8±0.1	0.7±0.2[a,b]	<0.01
18:3n3	1.0±0.2	1.0±0.2	1.0±0.2	0.9±0.2	NS
18:4n3	0.2±0.1	0.2±0.1	0.2±0.1	0.2±0.1	NS
20:3n6	0.7±0.2	1.0±0.2[a]	0.7±0.2	0.6±0.1[a,b]	<0.01
20:4n6	5.7±1.2	6.1±1.2	5.8±1.0	5.6±0.7	<0.05
20:5n3	1.3±0.6	1.3±0.7	1.1±0.5	2.8±0.9[a,b]	–
22:6n3	0.7±0.2	0.7±1.2[a]	0.7±0.2	0.8±0.1[a,b]	<0.01

Values are expressed as mean±standard deviation.
[a] Significant difference within a period $P < .05$.
[b] Significant difference between periods $P < .01$.

oil period (P=.018). Fish oil did not affect these parameters (Table 7). Serum apolipoprotein B concentration did not change during the study. The mean change of serum LDL cholesterol concentration during the intervention periods differed significantly between the periods (−0.13±0.21 vs. 0.10±0.29 mmol/L, P=.027).

Fish oil supplementation decreased significantly fasting plasma glucose concentration (P<.001) compared with black currant seed oil supplementation (Table 7). The mean change in plasma insulin concentration during the intervention periods differed significantly between the periods (P<.0001). There were no changes in fasting plasma glucose or insulin concentrations during the black currant seed oil period.

Table 6
Fatty acid composition of plasma phospholipids before and after black currant seed oil and fish oil periods

Fatty acid	Black currant seed oil period		Fish oil period		GLM
	Before	After	Before	After	*P*
16:0	27.7±2.5	28.1±2.4	27.6±2.5	28.1±2.6	NS
16:1	0.8±0.3	0.8±0.2	0.8±0.2	0.8±0.2	NS
18:0	11.5±2.2	11.6±1.8	11.4±1.9	11.5±1.9	NS
18:1n9	10.3±0.8	10.1±1.4	10.1±0.9	9.9±0.7	NS
18:1n7	2.0±0.3	1.9±0.3	2.0±0.2	2.0±0.2	NS
18:2n6	20.6±2.1	20.0±2.3	21.5±2.3	19.7±1.9[a]	<0.01
18:3n6	4.7±0.7	4.7±0.8	4.8±0.7	4.8±0.6	NS
18:3n3	0.3±0.1	0.4±0.1	0.4±0.1	0.3±0.1	NS
18:4n3	0.2±0.1	0.2±0.0	0.2±0.0	0.2±0.0	NS
20:1n9	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	NS
20:2n6	0.4±0.1	0.4±0.1	0.4±0.1	0.4±0.1	NS
20:3n6	3.2±0.7	4.4±0.9[a]	3.2±0.8	2.7±0.6[a,b]	<0.01
20:4n6	8.1±1.5	8.4±1.4	8.5±1.2	7.8±0.8	NS
20:5n3	1.6±0.8	1.3±0.6	1.2±0.5	2.9±0.7[a,b]	<0.01
22:6n3	4.9±1.1	4.4±0.9	4.5±0.9	5.5±0.6[a,b]	<0.01

Values are expressed as mean±standard deviation.
[a] Significant difference within a period $P < .05$.
[b] Significant difference between periods $P < .01$.

Table 7
Body weight, concentrations of plasma glucose, insulin and serum total and lipoprotein lipids before and after black currant seed oil and fish oil periods

	Black currant seed oil period		Fish oil period		GLM
	Before	After	Before	After	*P*
Weight, kg	58.7±6.7	58.3±6.7	58.6±6.7	58.6±6.8	NS
Glucose, mmol/L	4.9±0.4	5.0±0.2	5.0±0.3	4.8±0.2[a]	<0.001
Insulin, mU/L	7.4±3.2	8.2±2.9	9.3±3.4	7.4±2.6	NS
Cholesterol, mmol/L					
Total	4.55±0.4	4.56±0.4	4.54±0.5	4.65±0.6	NS
HDL	1.50±0.3	1.58±0.4	1.55±0.4	1.57±0.4	NS
LDL	2.58±0.4	2.46±0.4	2.53±0.3	2.62±0.5[b]	–
HDL/LDL	0.60±0.2	0.67±0.2	0.63±0.2	0.63±0.2[b]	<0.1
Triacylglycerols, mmol/L	1.03±0.4	1.15±0.6	1.03±0.4	1.00±0.4	–
Apolipoprotein A-1, g/L	1.84±0.3	1.94±0.3[a]	1.97±0.4	1.93±0.3[b]	<0.01
Apolipoprotein B, g/L	0.78±0.2	0.78±0.2	0.77±0.2	0.79±0.2	NS

[a] Significant difference within a period $P < .05$.
[b] Significant difference between periods $P < .05$.

4. Discussion

Conversion of linoleic acid (18:2n6) and 18:3n3 starts by Δ-6-desaturation and continues by elongation and Δ-5-desaturation yielding 20:4n6 and 20:5n3. Eicosapentaenoic acid can be further converted to 22:6n3 by two successive elongations followed by another Δ-6-desaturation and peroxisomal β-oxidation. The presence of Δ-4-desaturase has never been demonstrated in mammals [24]. Desaturases are expressed in several tissues, their activity varies in different tissues and species, and they may be differently regulated at the various sites [25]. In addition to physiological conditions, diet also regulates the conversion. When intake of 18:2n6 and 18:3n3 and/or their longer derivatives is high in the diet, the activity of Δ-6- and Δ-5-desaturase genes are low [26]. 18:2n6 and 18:3n3 and their derivatives affect each others metabolism in several sites. In rat liver, for instance, dietary 18:3n6 activates Δ-6-desaturation [27]. Specific transportation systems exist for directing eicosanoid precursors efficiently to target tissues such as the brain [25].

The present results show that black currant seed oil supplementation had very little effect on the proportions of 18:3n3, 18:4n3, 20:5n3 or 22:6n3 in plasma. These results are at variance with earlier findings based on larger intakes of black currant seed oil or individual n3 fatty acids. Thus, dietary intervention studies with several grams per day of 18:3n3 (compared to our 440 mg/day) had mostly [28–31], but not always [32], led to a positive change in the proportion of 18:3n3, 20:5n3 and 22:6n3 in serum lipids. Wu et al. [33] found an increase in 18:4n3 in plasma total

fatty acids with 4.5 g/day of black currant seed oil supplements containing 0.131 g/day of 18:4n3. However, in a study providing 0.08 g/day of 18:4n3, which is similar to our supplement (0.105 g/day), a decrease was found in the proportion of 18:4n3 in plasma GPLs [15]. Larger daily doses of 18:4n3 (0.75 and 1.5 g) increased 20:5n3 and 22:5n3 concentrations in erythrocyte and plasma lipids without affecting the 22:6n3 proportion. It was estimated that an 18:4n3 intake of 1.5 g/day would meet the 20:5n3 intake requirement of 300 mg/day [34]. According to Brenna [35], 18:3n3 supplementation increases the proportion of 22:6n3 in neural and retinal tissues, although no changes may be seen in plasma lipid fractions. As already mentioned, eicosanoid precursors are efficiently delivered to target tissues.

During the black currant seed oil period, there was a significant increase in the proportion of 20:3n6 in all studied fractions. At the same time the content of 20:4n6 remained unchanged. These results were similar to those obtained by others [14,15,33,36,37], who had shown that black currant seed oil increased the proportion of both 18:3n6 and/or 20:3n6, but did not have any effect on the proportion of 20:4n6, leading to a beneficial increase in the ratio of 20:3n6/20:4n6. Johansson [15] had found an increase in 18:3n6 in the plasma GPL fraction of male subjects, while in the present study in females a significant increase in 18:3n6 proportion was found only in plasma TAG and CE fractions. Our studies are also in line with other human studies, in which 18:3n6 did not augment the proportion of long-chain n3 fatty acids in plasma lipids [38,39]. In these experiments, changes in tissue proportions were not studied.

Compared to black currant seed oil the fish oil supplement decreased the proportion of 20:3n6. This may lead to alterations in the eicosanoid profile. Grimsgaard et al. [40] and Mori et al. [41] had found similar changes in plasma GPLs in studies with purified 20:5n3 and 22:6n3. The increase in 20:3n6 in GPLs with black currant seed oil supplementation and the increase in 20:5n3 with fish oil supplementation may have led to comparable changes in the balance of eicosanoid derived effects, e.g., stimulation of the immune system and gene regulation. It is known that 18:4n3 inhibits in a dose-dependent manner 5-lipoxygenase, leading to reduced formation of 20:4n6 in vitro, which would suggest the possibility of immunosuppression in vivo [42].

The observed lack of effect of black current seed oil supplementation on the proportions of the long-chain n3 fatty acids in plasma lipids may partly have been due to a high baseline proportion of 18:3n3, 20:5n3 and 22:6n3 in our subjects. Raatz et al. [9] and Mantzioris et al. [29] have reported the proportion of 18:3n3 in GPLs to be 0.18–0.21% compared to our estimate of 0.3% (GPLs) and 1.7% (TAGs). According to Brenna [35], dietary increases in 18:3n3 and 18:2n6 should generally result in a decrease in the conversion of 18:3n3 to 20:5n3 and 22:6n3. One reason for the high baseline proportion of n3 fatty acids in our subjects may have been the common use in the Finnish population of rapeseed oil, which contains about 11% 18:3n3 [17] and which at an intake level as low as 5 g/day would have provided >500 mg/day n3 fatty acids.

Likewise, the low total fat intake may have had a significant effect on the fatty acid metabolism and on the effect of individual unsaturated dietary fatty acids. In the present study the ad libitum fat intake of the subjects was at the recommended (30% or less of total energy intake) level [43]. Raatz et al. [9] showed that high total fat intake (45% of total energy intake) increases n6 fatty acids and low fat intake (20 E% of total energy intake) increases n3 fatty acids in plasma GPLs and CEs. Thus, the low total fat intake may have affected the baseline fatty acid proportion of GPLs in our subjects. The combination of the relatively low doses of 18:3n3 and 18:4n3 in black currant seed oil with the high baseline n3 fatty acids and the low fat intake may have prevented the detection of the expected changes in fatty acid profile. It is also possible that the smaller amounts of long-chain metabolites produced may have been preferentially utilized by other tissues than plasma (e.g., retinal and neural tissues, which were not assayed in the present study).

During the black currant seed oil period, serum LDL cholesterol tended to decrease, while there was no significant change in serum HDL cholesterol. However, the serum apoprotein A1 concentration increased significantly. A decrease in LDL cholesterol following black currant seed oil feeding had been previously reported by Spielmann et al. [14]. Fish oil supplementation (2.8 g/day) did not affect serum HDL- or LDL-cholesterol levels in our subjects. This finding agrees with earlier studies showing that moderate amounts of 20:5n3 and 22:6n3 have only minor influence on serum cholesterol levels [44–48]. The greater decrease in plasma LDL cholesterol during black currant seed oil supplementation compared to fish oil supplementation was most probably due to the greater intake of 18-carbon polyunsaturated fatty acids. Thus, there is evidence that 18:3n6 and 20:3n6 are more potent than 18:2n6 in lowering plasma cholesterol indicating that 18:2n6 needs to be converted to 20:3n6 and probably beyond to exert the hypocholesterolemic effect [49]. According to Chan et al. [50], 18:3n3 and 18:2n6 are equally effective in lowering blood cholesterol in normolipemic subjects. The protective effect of 18:3n3 on cardiovascular disease has been reported in both primary and secondary prevention studies [31,51,52], but conflicting results have also been published [53]. James et al. [34] did not find significant changes in plasma lipid when giving either 18:3n3, 18:4n3 or 20:5n3 to healthy human subjects as 0.75 or 1.5 g daily supplements for 3 weeks. In a recent population study (97 male subjects), Tremblay et al. [54] found a positive correlation between HDL2-cholesterol and the proportion of 18:3n6 in plasma TAGs.

No significant changes were detected in serum TAG or apoprotein B levels, probably due to the moderate dietary doses of the n3 fatty acids, or to the low baseline TAG levels in our subjects, or both. Sanchez-Muniz et al. [48] found a decrease in the total number of VLDL, LDL and HDL

particles but no change in serum TAG concentration with a supplement dose of only 0.82 g/day of 20:5n3 and 22:6n3. Doses of 2–5 g/day of 20:5n3 and 22:6n3 have been reported to significantly lower serum TAG [55–57], but even lower intakes may be beneficial [58]. Other work [40,41] has shown that 22:6n3 decreases serum TAG levels more efficiently than 20:5n3. A negative correlation has been found between the proportion of 18:3n6 in plasma TAGs and plasma TAG content in a population study [54].

In the current study, plasma glucose and insulin levels were measured as indicators of the general effects of feeding black current oil in comparison to fish oil. Fish oil supplementation significantly decreased plasma glucose concentration. Tremblay et al. [54] have found a strong negative correlation ($P<0.001$) between the proportion of 18:3n6 in plasma TAG fraction and plasma fasting insulin. There are very little data on the effects of n3 fatty acids on plasma glucose and insulin levels in healthy subjects. The data for type II diabetics are controversial [59]. In mildly hypercholesterolemic men, 20:5n3 supplementation (4 g/day), but not that of 22:6n3, tended to increase fasting glucose [41]. According to Hu et al. [60], long-chain n3 fatty acids have beneficial effects in type II diabetics. These discrepancies in earlier studies can be partly attributed to differences in doses of supplementation, fatty acid composition and 20:5n3/22:6n3 ratios of fish oils, and possibly by differences in the overall fat intake. The effect of 18:4n3 supplementation on plasma glucose and insulin levels has not been previously investigated.

In conclusion, during the black currant seed oil supplementation the proportion of long-chain n3 fatty acids did not increase in plasma lipids. However, their proportion did not decrease either although the intake of n6 fatty acids increased. The increased intake of n6 fatty acids was reflected in the fatty acid composition of plasma lipids by increased proportions of 18:3n6 and 20:3n6. During the fish oil supplementation the proportions of 20:5n3 and 22:6n3 increased. Black currant seed oil had a beneficial effect on serum lipid profile whereas fish oil affected beneficially fasting plasma glucose concentration without having an effect on the concentration of serum total TAGs or lipoprotein lipid profile in healthy normolipidemic females.

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