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Ursula S. Schwab
James C. Callaway
Arja T. Erkkilä
Jukka Gynther
Matti I.J. Uusitupa
Tomi Järvinen

Effects of hempseed and flaxseed oils on the profile of serum lipids, serum total and lipoprotein lipid concentrations and haemostatic factors

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U.S. Schwab, PhD (✉) · A.T. Erkkilä
M.I.J. Uusitupa
Dept. of Clinical Nutrition
University of Kuopio
P.O. Box 1627
70211 Kuopio, Finland
Tel.: +358-17/162-791
Fax: +358-17/162-792
E-Mail: ursula.schwab@uku.fi

U.S. Schwab, PhD
Kuopio University Hospital
Kuopio, Finland

J.C. Callaway · J. Gynther · T. Järvinen
Dept. of Pharmaceutical Chemistry
University of Kuopio
Kuopio, Finland

A.T. Erkkilä
Dept. of Public Health and General Practice
University of Kuopio
Kuopio, Finland

Summary *Background* Both hempseed oil (HO) and flaxseed oil (FO) contain high amounts of essential fatty acids (FAs); i.e. linoleic acid (LA, 18:2n-6) and alpha-linolenic acid (ALA, 18:3n-3), but almost in opposite ratios. An excessive intake of one essential FA over the other may interfere with the metabolism of the other while the metabolisms of LA and ALA compete for the same enzymes. It is not known whether there is a difference between n-3 and n-6 FA of plant origin in the effects on serum lipid profile. *Aim of the study* To compare the effects of HO and FO on the profile of serum lipids and fasting concentrations of serum total and lipoprotein lipids, plasma glucose and insulin, and haemostatic factors in healthy humans. *Methods* Fourteen healthy volunteers participated in the study. A randomised, double-blind crossover design was used. The volunteers consumed HO and FO (30 ml/day) for 4 weeks each. The periods were separated by a 4-week washout period. *Results* The HO period resulted in higher proportions of both LA and gamma-linolenic acid in serum cholesteryl esters (CE) and triglycerides (TG) as compared with the FO period ($P < 0.001$), whereas the FO period resulted in a higher proportion of ALA in both serum CE and TG as compared with the HO period ($P < 0.001$). The proportion of arachidonic acid in CE was lower after the FO period than after the HO period ($P < 0.05$). The HO period resulted in a lower total-to-HDL cholesterol ratio compared with the FO period ($P = 0.065$). No significant differences were found between the periods in measured values of fasting serum total or lipoprotein lipids, plasma glucose, insulin or hemostatic factors. *Conclusions* The effects of HO and FO on the profile of serum lipids differed significantly, with only minor effects on concentrations of fasting serum total or lipoprotein lipids, and no significant changes in concentrations of plasma glucose or insulin or in haemostatic factors.

Key words cholesterol – fat – flaxseed oil – glucose – haemostasis – hemp seed oil – insulin

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Introduction

The quality of dietary fat affects serum concentrations of total and lipoprotein lipids [1]. Saturated fatty acids (SFA) are generally considered as increasing factors and unsaturated fatty acids (FA) as decreasing factors of serum cholesterol concentrations [2, 3]. Polyunsaturated fatty acids (PUFA), especially long chain n-3 FA of marine origin, decrease the serum concentration of triglycerides (TG) as well [2, 4]. It is not well known, however, whether or not there is a difference between n-3 and n-6 FA of plant origin in the effects on serum concentrations of total and lipoprotein lipids when the proportions of saturated, monounsaturated and polyunsaturated FA remain unchanged in the diet.

Both hempseed oil and flaxseed oil contain exceptionally high amounts of the two essential FAs (EFAs); i.e. linoleic acid (LA, 18:2n-6) and alpha-linolenic acid (ALA, 18:3n-3), but almost in opposite ratios. In addition, some examples of hempseed oil contain up to 4% gamma-linolenic acid (GLA, 18:3n-6) and about 2% stearidonic acid (SDA, 18:4n-3), the metabolites of LA and ALA, respectively [5]. Such concentrations of GLA and SDA are considerably higher than in any commonly used oil.

The metabolisms of LA and ALA compete for the same enzymes involved in the elongation and desaturation processes, particularly the initial transformation of these FA to GLA and SDA, respectively, by delta-6-desaturase. It has been proposed that an excessive intake of one essential FA (e.g. LA in the Western diet) over the other may interfere with the metabolism of the other [6], thus indicating some optimal dietary balance between n-6 and n-3 FA. The n-6/n-3 ratio differs between hempseed and flaxseed oils; in hempseed oil it is approximately 2.4:1 [1] and in flaxseed oil it is 0.3:1.

Both the amount of fat and the composition of a diet may affect haemostatic factors as well [7]. Fish oils rich in long chain n-3 FA, and other PUFAs have been shown to affect plasma fibrinogen concentration [8], factor VII coagulant activity (FVIIa) [9] and plasminogen activator inhibitor-1 (PAI-1) activity [8] in humans.

The aim of the present study was to compare the effects of dietary hempseed oil and flaxseed oil on the profile of serum lipids, fasting serum total and lipoprotein lipid concentrations, plasma glucose and insulin concentrations and haemostatic factors in healthy volunteers.

Materials and methods

Subjects

Sixteen healthy volunteers (eight males, eight females), without any chronic disease and having normal liver, kidney and thyroid functions, were recruited for the study. Two females dropped out during the first week of the study due to a lack of motivation. Therefore, the final number of volunteers was 14 (eight males, six females). The inclusion criteria were a body mass index (BMI) < 30 kg/m^2, age 25–60 years, fasting serum concentrations of total TG < 3.5 mmol/l, total cholesterol 5.0–7.5 mmol/l and plasma glucose < 6.0 mmol/l. Potential volunteers who were taking lipid-lowering medication were excluded, and none of the participants were on antihypertensive medication. The volunteers were instructed to continue any other medication unchanged and to maintain their normal level of physical activity throughout the study. Volunteers were not allowed to use nutrient supplements during the study, or within 1 month before the onset of the study. The baseline characteristics of the volunteers are presented in Table 1. All participants gave their written informed consent, and the study protocol was approved by the Ethics Committee of the University of Kuopio and Kuopio University Hospital.

Oils

The hempseed was of the Finola variety and the flaxseed was of the Helmi variety. Seeds were cold-pressed under a nitrogen atmosphere, oil bottled in brown glass, and stored at +5°C until use. No antioxidants or other adulterants were added. The peroxide values were below 5 meqv/l. The analysed [5] profiles of oils revealed that the proportions of oleic acid (18:1n-9), LA and ALA were 9, 54 and 22% in the hemp seed oil and 20, 13 and 53% in the flaxseed oil, respectively.

Study design

This was a controlled, randomised double-blind cross over study. The two intervention periods were 4 weeks in duration, as was the wash out period be-

Table 1 Baseline characteristics of the volunteers (mean ± SD)

Males / females	8/6
Age (year)	45 ± 7
Weight (kg)	70.9 ± 9.2
BMI (kg/m^2)	24.54 ± 2.22
RR systolic (mmHg)	114 ± 12
RR diastolic (mmHg)	74 ± 8
fP-glucose (mmol/l)	5.4 ± 0.4
fS-cholesterol (mmol/l)	5.63 ± 0.55
fS-HDL-cholesterol (mmol/l)	1.50 ± 0.28
fS-LDL-cholesterol (mmol/l)	3.63 ± 0.63
fS-triglycerides (mmol/l)	1.12 ± 0.37

BMI, body mass index; RR, blood pressure; fP, fasting plasma; fS, fasting serum; HDL, high density lipoprotein; LDL, low density lipoprotein

tween the intervention periods. The study was carried out during the fall, a period in Finland without any special festivals affecting the food intake. The volunteers visited the research unit at the beginning and end of both intervention periods. A clinical nutritionist gave information regarding the reduction of dietary saturated fats, in order to avoid an increase in the consumption of fat during the intervention periods. The volunteers were also given instructions for incorporating the hempseed and flaxseed oils in their diet, e.g. by incorporating them in food (porridge, yoghurt etc.) or use them as a salad dressing. Frying of the oils was not allowed. The amount of oil consumed was 30 mL/d.

All participants kept a 7-day food record (consecutive days) during the third week of both intervention periods, and a 4-day food record (consecutive days, 3 weekdays, 1 weekend day) during the third week of the wash out period. Dietary intake of energy and nutrients were calculated using the Micro-Nutrica® dietary analysis software (version 2.5), based on Finnish food analyses and international food composition tables [10].

Laboratory methods

Body weight was measured using a calibrated electronic scale (Seca Delta, Model 707, Seca, Germany). Blood pressure was manually measured from the right arm after 5 min of rest in a sitting position using a mercury sphygmomanometer. The mean of two measurements performed 5 min apart was used for further analyses.

Venous blood samples were drawn from an antecubital vein after a 12-h overnight fast. For analysis of the FA profile of serum lipids, lipids were extracted from the serum sample with chloroform–methanol (2:1) according to Ågren et al. [11]. Briefly, lipid fractions (cholesteryl esters (CE) and TG) were separated on an aminopropyl-column and the recovered FA in each fraction were transmethylated with 14% borontrifluoride in methanol at 95°C. FA methyl esters were analysed by capillary gas chromatography (Hewlett-Packard 5890 series II, Hewlett-Packard Company, Waldbronn, Germany). Heptanoic acid (17:0) was used as an internal standard.

For the analysis of serum total and lipoprotein lipid concentrations, enzymatic colorimetric methods with commercial kits (Cholesterol CHOD-PAP and Triglycerides GPO-PAP; Roche Diagnostics GmbH, Mannheim, Germany) and an automated instrument (Kone Pro, Thermo Clinical Labsystems, Konelab, Espoo, Finland) were used. High density lipoprotein (HDL) in the infranate was separated from low density lipoprotein (LDL) by precipitation of LDL with dextran sulphate and magnesium chloride [12] and analysed as total cholesterol. LDL cholesterol concentration was calculated using Friedewald's formula. The CV% for the analyses of total and HDL cholesterol concentrations were 1.9% and 1.4% within the batches, respectively. Between batches, the CV% for total cholesterol was 1.4% at 3.55 mmol/l and 1.6% at 7.43 mmol/l. The respective CV% for HDL cholesterol was 3.2% at 1.42 mmol/l and 2.1% at 3.94 mmol/l. The CV% for serum total TG was 1.7% within batches and 1.8% (at 1.41 mmol/l) and 2.4% (at 4.0 mmol/l) between the batches.

The plasma glucose concentration was analysed by the glucose dehydrogenase method (Granutest 250, Diagnostica Merck, Darmstadt, Germany) using an automated instrument (Kone Pro). The CV% within batches was 2% and between batches 2% (at 2.1 mmol/l) and 1.7% (at 5.1 mmol/l). Plasma insulin concentration was analysed by a radioimmunoassay (RIA) method (Phadeseph Insulin RIA 100, Pharmacia & Upjohn Diagnostics, Uppsala, Sweden). The CV% between the batches was 3.5% (at 11.8 mU/L) and 5.5% (at 101.8 mU/L).

Concentrations of serum apolipoproteins (apo) A-I and B were analysed at 340 nm by an immunoprecipitation method enhanced by polyethylene glycol [13]. The Kone Pro, and Apo A-I and Apo B reagents were used (Thermo Clinical Labsystems, Konelab, Espoo, Finland). The CV% within the batches was 1.39% (at 1.39 g/l) and 6.5% (at 2.81 g/l) for Apo A-I and 2.1% (at 0.72 g/l) and 0.9% (at 1.29 g/l) for Apo B. The respective values between the batches were 2.2% (at 1.1 g/l) and 3.8% (at 1.9 g/l) for Apo A-I and 2.4% (at 0.9 g/l) and 1.7% (at 2.1 g/l) for Apo B.

For the analysis of plasma PAI-1 activity, fibrinogen and D-dimer evacuated tubes (Diatube® H, Diagnostica Stago, Asnières-sur-Seine, France) containing sodium citrate, citric acid and inhibitors for platelet aggregation were used for blood collection. PAI-1 activity was measured by a chromogenic method using the Coatest® PAI kit (Chromogenix—Instrumentation Laboratory SpA, Milan, Italy). The diagnostic plates were read by a Multiscan MCC/340 P EIA plate reader v. 2.33 (Labsystems, Helsinki, Finland) at a wavelength of 492 nm. Quantitative determination of plasma fibrinogen concentration was performed using the Fibri-Prest®Automate kit (Diagnostica Stago) based on a clotting method. The within-run CV% was 2.9% at 1.1 g/l and 1.9% at 8.5 g/l. The between-run CV% was 7.0% at 0.7 g/l and 6.0% at 3.1 g/l. Plasma D-dimer concentration was analysed by an immunological optical ELISA method using the Asserachrom® D-Di kit (Diagnostica Stago). For analysing FVIIa an optical clotting method was used with the reagent system of STA® Neoplastine® Cl and FVII depleted human plasma (STA® deficient

VII, Diagnostica Stago). The Thrombolyzer Compact XR equipment (Behnk Elektronik, Germany) was used to analyse fibrinogen, D-dimer and FVIIa. Sensitive C-reactive protein (CRP) was analysed by an immunoenzymometric assay using chemiluminometric detection (Diagnostic Products Corporation, Los Angeles, CA).

Statistical analyses

Data were analysed by the SPSS statistical software (V10.0, SPSS; Chicago, IL). Before further analyses, the normal distribution of variables was checked by the Shapiro–Wilk's test. Variables with abnormal distributions were log-transformed. The general linear model (GLM) for repeated measures was used to test interactions between time and treatment and changes within time. A paired *t*-test was used for further two-tailed comparisons. Variables that did not reach normal distribution after arithmetic procedures were analysed using the Wilcoxon matched pairs' signed ranks test. All data are expressed as means ± SD. A *P*-level < 0.05 was considered as statistically significant.

Results

Body weight and diets during the study

The body weight of the volunteers increased during the intervention periods, but there were no significant differences between the intervention periods; 71.0 ± 9.4 vs. 71.6 ± 9.6 kg ($P < 0.01$), beginning vs. end of the hempseed oil period, respectively, 71.1 ± 9.3 vs. 71.6 ± 9.4 kg ($P < 0.05$), beginning vs. end of the flaxseed oil period, respectively.

The composition of the diets during the study is presented in Table 2. The energy intake was about 400 kJ (100 kcal) higher during the intervention periods compared with the wash out period. The proportion of energy nutrients and the intake of saturated, monounsaturated or polyunsaturated FA remained fairly constant throughout the study.

Table 2 Intake of energy, energy nutrients, dietary fiber and cholesterol during the hempseed oil, flaxseed oil and wash out periods (mean ± SD)[1]

Nutrient	Hempseed oil period	Flaxseed oil period	Wash out period
Energy (kJ)	8555 ± 1755	8554 ± 1551	8137 ± 1440
Fat (E%)	32.4 ± 7.1	33.8 ± 5.0	31.6 ± 4.8
SFA	12.1 ± 3.2	13.2 ± 2.7	12.5 ± 2.2
MUFA	11.1 ± 3.1	11.4 ± 2.4	10.5 ± 2.2
PUFA	5.3 ± 1.0	5.3 ± 1.0	5.3 ± 1.1
Proteins (E%)	15.7 ± 2.1	15.5 ± 2.3	16.2 ± 2.6
Carbohydrates (E%)	43.6 ± 8.5	42.8 ± 4.7	43.8 ± 5.9
Fiber (g/MJ)	2.9 ± 1.1	2.9 ± 0.9	2.8 ± 1.1
Cholesterol (mg/MJ)	28 ± 7	27 ± 5	30 ± 6
Alcohol (E%)	10.5 ± 8.9	8.8 ± 7.8	10.1 ± 9.9

[1]7-day food record during the intervention periods, 4-day food record during the wash out period
E%, % of energy; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids

FA composition of serum lipids

The proportion of LA was higher in serum CE and TG at the end of the hempseed oil period compared with the flaxseed oil period ($P < 0.001$) (Tables 3 and 4). The proportion of GLA increased significantly in both CE and TG during the hempseed oil period ($P < 0.001$) and decreased significantly during the flaxseed oil period ($P < 0.01$). This difference was highly significant between the periods ($P < 0.001$). The flaxseed oil period resulted in a higher proportion of ALA in serum CE and TG compared with the hempseed oil period ($P < 0.001$). However, the proportion of ALA increased significantly during both periods ($P < 0.001$). The flaxseed oil period resulted in a lower proportion of arachidonic acid (AA) compared with the hempseed oil period ($P < 0.05$) in serum CE. Regarding the long chain n-3 FA, eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-6, DHA), the flaxseed oil period resulted in a slightly higher proportions of EPA in serum TG compared with the hempseed oil period ($P < 0.05$).

Serum total and lipoprotein lipids, apo A-I and B, and blood pressure

Fasting concentrations of serum total TG decreased significantly during the flaxseed oil period ($P < 0.05$), whereas the decrease during the hempseed oil period approached statistical significance ($P = 0.099$) (Table 5). Serum total or lipoprotein cholesterol concentrations did not change during the study, but during the hempseed oil period the total-to-HDL cholesterol ratio decreased significantly ($P < 0.05$) with a significant difference compared with the flaxseed oil period ($P = 0.065$). The apo B concentration decreased during both periods ($P < 0.05$) without a difference between the periods.

Systolic and diastolic blood pressures did not change during the study. At the end of the intervention periods systolic blood pressure was 115 ± 14 vs. 112 ± 12 mmHg for hempseed oil vs. flaxseed oil, respectively, and diastolic blood pressure was 75 ± 8 vs. 75 ± 10 mmHg for hempseed oil vs. flaxseed oil, respectively.

Table 3 The fatty acid (FA) composition of serum cholesteryl esters (mol-% of total fatty acids) at the beginning and end of the intervention periods (means ± SD)

	Hempseed oil period		Flaxseed oil period		
Fatty acid	0 week	4 week	0 week	4 week	P^1
Myristic (14:0)[2]	1.91 ± 0.33	1.92 ± 0.37	2.04 ± 0.27	1.83 ± 0.27[3]	0.016
Palmitic (16:0)	13.58 ± 1.08	12.94 ± 1.12	13.71 ± 0.93	12.76 ± 0.85	NS
Palmitoleic (16:1n-7) [2]	3.23 ± 1.45	2.24 ± 0.97	3.41 ± 1.20	2.53 ± 1.00	NS
Stearic (18:0)	0.92 ± 0.23	0.95 ± 0.14	0.95 ± 0.18	0.90 ± 0.13	NS
Oleic (18:1n-9)	21.78 ± 2.30	16.54 ± 1.63[4]	21.58 ± 1.22	18.53 ± 1.26[4,5]	NA
Linoleic (18:2n-6)	50.90 ± 4.61	56.65 ± 3.34[6]	49.89 ± 3.07	52.90 ± 3.48[7,8]	0.053
γ-Linolenic (18:3n-6)	0.57 ± 0.27	1.19 ± 0.19[6]	0.65 ± 0.29	0.43 ± 0.18[7,8]	0.0001
α-Linolenic (18:3n-3)	0.98 ± 0.21	1.30 ± 0.16[6]	0.96 ± 0.19	4.13 ± 0.46[6,8]	0.0001
Dihomo-γ-linolenic (20:3n-6)	0.53 ± 0.17	0.75 ± 0.25[9]	0.53 ± 0.12	0.42 ± 0.15[5,9]	NA
Arachidonic (20:4n-6)	4.01 ± 0.85	4.10 ± 1.13	4.38 ± 0.87	3.52 ± 0.70[6,10]	0.011
Eicosapentaenoic (20:5n-3) [2]	1.22 ± 0.55	1.10 ± 0.43	1.46 ± 0.76	1.72 ± 0.59	NS
Docosahexaenoic (22:6n-3) [2]	0.37 ± 0.10	0.33 ± 0.13	0.44 ± 0.15	0.34 ± 0.12[3]	0.020

[1]GLM, *P* for the interaction of time and treatment. NS; not significant. NA; GLM not performed due to an abnormal distribution of the variable after arithmetic procedures. [2]Log-transformed. [3,6,7] Difference within a period, [3]$P < 0.05$, [6]$P < 0.001$, [7]$P < 0.01$; paired samples *t*-test. [4,5,9]Wilcoxon matched pairs signed ranks test; [4]$P \leq 0.001$ within a period; [5,9]between periods, [5]$P \leq 0.001$, [9]$P < 0.05$. [8,10]Difference between periods, [8]$P < 0.001$, [10]$P < 0.05$; paired samples *t*-test

Plasma glucose and insulin concentrations, and haemostatic factors

The fasting plasma glucose concentrations did not differ significantly between the end of the intervention periods (5.5 ± 0.5 vs. 5.6 ± 0.4 mmol/l for hempseed oil vs. flaxseed oil, respectively). Neither was there any significant difference in insulin concentrations between the intervention periods (8.2 ± 4.1 vs. 8.8 ± 5.0 mU/L for hempseed oil vs. flaxseed oil, respectively). No significant differences were found in haemostatic factors (D-dimer, fibrinogen, FVIIa and PAI-1 activity) or sensitive CRP concentrations (data not shown) between the intervention periods.

Discussion

Hempseed oil is rich in LA, whereas the major FA in flaxseed oil is ALA. Both of these FAs are essential to human health, as the human body cannot synthesise them from other FAs in the diet. Both of these EFAs are metabolised by the human body to longer and more unsaturated FAs. Increasing intake of ALA, for example, increases the proportion of EPA in plasma lipid fractions and cell phospholipids [14, 15]. However, the efficiency of these complex elongation and desaturation processes is not well established.

The ratio of n-6/n-3 FA in the human diet has been proposed to have metabolic significance, but results

Table 4 The fatty acid (FA) composition of serum triglycerides (mol-% of total fatty acids) at the beginning and at the end of the intervention periods (means ± SD)

	Hempseed oil period		Flaxseed oil period		
Fatty acid	0 week	4 week	0 week	4 week	P^1
Myristic (14:0) [2]	2.57 ± 0.96	2.38 ± 0.72	3.22 ± 1.20	2.41 ± 0.80	NS
Palmitic (16:0) [2]	25.36 ± 4.24	23.33 ± 4.35	27.63 ± 5.39	22.50 ± 3.52	NS
Palmitoleic (16:1n-7)	3.94 ± 1.27	3.02 ± 1.06	3.96 ± 1.18	3.18 ± 0.79	NS
Stearic (18:0)	3.09 ± 0.67	3.16 ± 0.51	3.49 ± 0.86	3.16 ± 0.67	NS
Oleic (18:1n-9)	36.98 ± 3.98	31.54 ± 3.53[3]	36.55 ± 2.75	33.17 ± 3.73[3]	NA
Linoleic (18:2n-6)	17.99 ± 3.43	25.54 ± 5.52[4]	17.03 ± 4.21	19.46 ± 4.38[5,6]	0.016
γ-Linolenic (18:3n-6) [2]	0.43 ± 0.17	1.01 ± 0.34[4]	0.47 ± 0.23	0.36 ± 0.18[5,6]	0.0001
α-Linolenic (18:3n-3)	2.05 ± 0.57	3.76 ± 1.05[4]	1.71 ± 0.39	9.66 ± 3.35[4,6]	0.0001
Dihomo-γ-linolenic (20:3n-6)[2]	0.38 ± 0.20	0.55 ± 0.17	0.34 ± 0.13	0.38 ± 0.29	0.050
Arachidonic (20:4n-6)	2.20 ± 0.98	2.33 ± 0.96	2.12 ± 0.72	1.85 ± 0.65	NS
Eicosapentaenoic (20:5n-3)	2.64 ± 3.80	1.59 ± 0.88	1.45 ± 0.80	2.00 ± 1.00[7,8]	NA
Docosahexaenoic (22:6n-3)	1.97 ± 1.71	1.41 ± 0.70	1.68 ± 0.80	1.45 ± 0.64	NS

[1]GLM, P for the interaction of time and treatment. NS; not significant. NA; GLM not performed due to an abnormal distribution of the variable after arithmetic procedures. [2]Log-transformed. [3,7,8]Wilcoxon matched pairs signed ranks test; [3]$P \leq 0.01$ within period, [7]$P < 0.05$ between periods, [8]$P = 0.056$ within a period. [4,5]Difference within a period, [4]$P < 0.001$, [5]$P < 0.01$; paired samples *t*-test. [6]Difference between periods, $P < 0.001$; paired samples *t*-test

Table 5 Fasting concentrations of serum total and lipoprotein lipids, and apolipoproteins at the beginning and at the end of the intervention periods (means ± SD)

	Hempseed oil period		Flaxseed oil period		
	0 week	4 week	0 week	4 week	P^1
fS-cholesterol (mmol/l)					
total	5.71 ± 0.64	5.57 ± 0.60	5.77 ± 0.70	5.60 ± 0.42	NS
HDL	1.47 ± 0.34	1.53 ± 0.30	1.45 ± 0.23	1.50 ± 0.35	NS
LDL	3.73 ± 0.75	3.58 ± 0.68	3.73 ± 0.73	3.62 ± 0.53	NS
fS-triglycerides (mmol/l)[2]	1.23 ± 0.64	1.03 ± 0.44[3]	1.18 ± 0.32	1.07 ± 0.37[4]	0.019
fS-apolipoproteins (g/l)					
A-I	1.79 ± 0.35	1.86 ± 0.27	1.76 ± 0.20	1.86 ± 0.35	0.063
B	1.06 ± 0.24	1.00 ± 0.25[4]	1.08 ± 0.25	1.02 ± 0.21[5]	0.014
Total-to-HDL cholesterol ratio	4.15 ± 1.44	3.85 ± 1.19[4]	4.12 ± 1.08	3.99 ± 1.21[6]	0.003
LDL cholesterol-to-apolipoprotein B ratio	3.56 ± 0.47	3.64 ± 0.37	3.49 ± 0.34	3.62 ± 0.41	NS

[1]GLM, P for the main effect of time; no interaction of time and treatment. [2]Log-transformed. [3,4,5]Difference within a period [3]$P = 0.099$, [4]$P < 0.05$, [5]$P < 0.01$. [6]Difference between the oil periods, $P = 0.065$

from well-controlled clinical data have been inconclusive. The n-6 and n-3 FAs compete for the same elongase and desaturase enzymes, e.g. delta-5 and delta-6 desaturases, throughout their metabolism to longer, more unsaturated FAs [16]. These enzymes favor certain FAs: e.g. delta-6 desaturase has a higher affinity for ALA compared with LA [17]. Thus, a high n-6/n-3 ratio in a diet may antagonise the in vivo production of longer n-3 FAs [15]. Conversely, high levels of dietary ALA can antagonise the production of n-6 FAs, as seen in the present study as a statistically significant decreases in GLA during the flaxseed oil period in both lipid fractions. An optimal ratio of n-6/n-3 FAs in the diet has been proposed to be between 2–3:1 [18]. This ratio is 2.4:1 for the hempseed oil and 0.3:1 for the flaxseed oil used in this study.

In the present study, the FA composition of serum CE and TG were clearly affected by the FA composition of the ingested oils: i.e. the proportion of LA and GLA increased during the hempseed oil period as compared with the flaxseed oil period, where the proportion of ALA increased during both periods (Tables 3 and 4). The proportion of AA, a metabolite of LA, did not differ in TG after either period. In CE, however, the flaxseed oil period resulted in a lower proportion of AA compared with the hempseed oil period. However, during the hempseed oil period the proportion of AA did not increase, which is supported by results from other studies [15, 19]. Previous studies have also shown that the FA composition of plasma lipids, platelets, erythrocytes and adipose tissue is actually quite resistant to supplementation with AA or its precursors [20, 21]. It is likely that increased levels of n-6 FAs in the diet are diverted to produce series 1 prostaglandins from di-homo-GLA and, thus, may not contribute substantially to AA levels in the human matrix [16].

The proportion of DHA did not differ between the periods for either oil in serum TG (Table 4). In serum CE the proportion of DHA decreased during the flaxseed oil period without a corresponding difference during the hempseed oil period (Table 3). Previous findings regarding the conversion of ALA to EPA [14, 15] and further to DHA [22] support the present results. However, ALA has also been reported to be converted inefficiently to EPA [23] and this conversion does not seem to be up-regulated by increasing the substrate, i.e. ALA [24, 25]. On the contrary, ALA conversion seems to be down-regulated by increased availability of conversion products, i.e. EPA and DHA [25]. Gender may partly explain the results of the present study seen in the proportion of DHA in serum CE. It has been suggested that males may have lower capacity for ALA conversion to EPA and DHA than females of childbearing age [22]. This putative gender difference could not be confirmed in the present study probably due to the small gender groups (eight males and six females).

In the present study, serum total TG concentration decreased significantly during the flaxseed oil period (Table 5). During the hempseed oil period this decrease approached statistical significance. The total-to-HDL cholesterol ratio, which has been suggested to better predict the risk of coronary heart disease than LDL cholesterol concentration alone [26, 27] decreased significantly during the hempseed oil period as compared with the flaxseed oil period ($P < 0.05$). The LDL cholesterol-to-apo B ratio did not change significantly during the study indicating no change in the composition of LDL particles. In previous studies regarding plant-derived PUFAs, no significant differences in the effects on serum total or lipoprotein lipid concentrations were found between LA and ALA in healthy young males [28]. In volunteers with hypercholesterolemia and at least two other coronary heart disease risk factors, an intervention with margarines rich in either ALA or LA, ALA resulted in higher serum total-to-HDL cholesterol ratio, lower serum HDL cholesterol concentrations and higher serum TG concentrations than LA [29]. In another study of

hypercholesterolemic volunteers, no differences in concentrations of serum total or LDL cholesterol or TG were found between LA and ALA within the context of a diet low in saturated fat and cholesterol and high in PUFA [30]. While the effects of replacing dietary LA with ALA on serum concentrations of total lipids have been inconsistent, ALA has been shown to be efficient in the secondary prevention of coronary events in the context of a modified Mediterranean diet [31]. ALA has also been reported to be associated with lower risk of fatal ischemic heart disease [32].

FA composition of a diet has been reported to affect haemostatic factors. Dietary ALA has been reported to result in lower plasma fibrinogen concentration compared with LA in volunteers with multiple risk factors for cardiovascular diseases [29]. An increased LA intake may even raise plasma fibrinogen concentrations [33]. No changes in plasma fibrinogen concentrations were observed in this study. Long chain n-3 PUFAs have been reported to result in increased factor VII coagulant activity in the context of a diet low in saturated fat [33]. In a recent study, no significant difference in the effect on haemostatic factors was found among n-3 PUFA of marine origin, plant origin or LA in moderately hyperlipidemic subjects of wide age range [34] which supports the findings of the present study. Freese and Mutanen [35] earlier reported no difference between ALA, EPA and DHA supplemented diets on plasma fibrinogen concentration, FVIIa or PAI-1 activity, which is also in line with the results of the present study.

In conclusion, hempseed oil and flaxseed oil have marked differences in their effects on serum FA composition. However, these differences did not modify their effects on serum concentrations of total or lipoprotein cholesterol, plasma glucose or insulin or haemostatic factors, but both oils resulted in decreases in serum total TG concentration.

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