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Effect of ω3 fatty acid on plasma lipids, cholesterol and lipoprotein fatty acid content in NIDDM patients

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Summary This study was conducted to examine the effect of ω3 fatty acid supplementation on plasma lipid, cholesterol and lipoprotein fatty acid content of non-insulin-dependent diabetic individuals consuming a higher (0.65, $n = 10$) or lower (0.44, $n = 18$) ratio of dietary polyunsaturated to saturated fatty acid (P/S). The participants were initially given an olive oil supplement (placebo) equivalent to 35 mg of 18:1 · kg body weight^{-1} · day^{-1} for 3 months. This was followed by two ω3 supplement periods in a randomized crossover. In these 3-month periods, participants were given a linseed oil supplement equivalent to 35 mg of 18:3ω3 · kg body weight^{-1} · day^{-1} or a fish oil supplement equivalent to 35 mg of 20:5ω3 + 22:6ω3 · kg body weight^{-1} · day^{-1}. At the end of each supplement period, a blood sample was drawn from each participant for lipid, lipoprotein, insulin, glucagon and C-peptide analyses. At the end of each 3-month period a 7-day dietary record was completed to calculate dietary fat intake and P/S ratio. Results indicate that fish oil significantly reduced plasma triacylglycerol level ($p < 0.05$) and increased 20:5ω3 and 22:6ω3 content of all lipoprotein lipid classes. Linolenic acid supplementation had no effect on plasma triacylglycerol level, but it increased 18:3ω3 content of lipoprotein cholesterol ester fractions ($p < 0.05$). A slight increase in 20:5ω3, but not 22:6ω3, content was noted in lipoprotein lipid classes as a result of 18:3ω3 supplementation. LDL and HDL cholesterol, insulin, glucagon and C-peptide levels were not affected by either ω3 supplement. It is concluded that a modest intake of ω3 fatty acids, such as could be obtained from consuming fish regularly, will reduce plasma triglyceride level without affecting LDL or HDL cholesterol levels. [Diabetologia (1997) 40: 45–52]

Keywords Diet, diabetes, lipoprotein, fatty acid, human.

Results from animal studies indicate that the type of dietary fat consumed has a profound effect on insulin action in tissues. Feeding a semipurified diet high in polyunsaturated to saturated fatty acid (P/S) ratio to insulin-dependent diabetic rats as compared to feeding them a diet low in P/S ratio increased insulin binding and insulin action in adipocytes [1] and skeletal muscle [2, 3]. Alteration in the fatty acid composition of plasma membrane phospholipid by dietary P/S ratio has been suggested to be a cause of these changes in insulin action. Beneficial effects of fish oil in preventing the development of insulin resistance in rats caused by a high intake of saturated fatty acids have also been reported [4, 5].

Individuals with insulin-dependent diabetes mellitus are prone to develop disorders in lipid and lipoprotein metabolism, of which hyperlipidaemia is the most common [6, 7]. Elevated plasma triacylglycerol and reduced HDL cholesterol also increase the risk

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Abbreviations: ANOVA, Analysis of variance; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GLC, gas-liquid chromatography; NIDDM, non-insulin-dependent diabetic; P/S, polyunsaturated to saturated fatty acid ratio.

of coronary heart disease, a major cause of morbidity and mortality in non-insulin-dependent diabetic (NIDDM) patients. In NIDDM patients, there is evidence that an elevated plasma lipid level is the direct result of a defect in triacylglycerol removal, and in some patients, there is evidence that it results from an overproduction of VLDL-triacylglycerol [6, 7]. A slight increase in LDL cholesterol may also occur in inadequately controlled insulin-dependent diabetic and NIDDM patients. Other abnormalities noted in lipoprotein metabolism include an increase in apolipoprotein (apo)-B LDL turnover, which may be linked to alterations in LDL composition [8]. Overproduction of VLDL and an increase in LDL apo-B production may be associated with premature coronary artery disease, even in patients without hyperlipidaemia [8].

A role for dietary ω3 fatty acids from marine sources in reducing the risk of coronary heart disease, regardless of total fat and cholesterol intake, has been suggested for many different populations [9, 10]. The incidence of diabetes is low in the Eskimo population. These findings, suggestive of the protective effects of marine food sources against heart disease, have prompted the study of mechanisms for the hypotriacylglyceridaemic action of ω3 fatty acid [11–13] attributed to eicosapentaenoic acid (EPA) (20:5ω3) and docosahexaenoic acid (DHA) (22:6ω3) [14]. Hyperlipidaemic patients consuming high intakes of a marine source of ω3 fatty acids demonstrate a reduction in their plasma and VLDL triacylglycerol level [15]. The effect of ω3 fatty acid on plasma lipid is dose dependent [16] and reversible [17], and several mechanisms have been proposed to explain the triacylglycerol-lowering effect of 20:5ω3 and 22:6ω3 [18–20].

The effect of consuming a large dose of fish oil on the total plasma or lipoprotein cholesterol level is not clear [21]. Normolipidaemic individuals fed more than 10 g of ω3 fatty acids per day display reductions in LDL cholesterol compared with individuals who receive 3 g of ω3 per day over a period ranging from 2–12 weeks [18]. It appears that the hypocholesterolaemic effect of fish oil depends on the presence of 20:5ω3 and 22:6ω3 and also on the overall changes occurring in the individual's consumption of other fatty acids of both the saturated and unsaturated type. Feeding a large dose of fish oil enriched in ω3 fatty acids would result in a change in the dietary polyunsaturated and saturated fatty acid content. Study of the relationship between marine ω3 fatty acids fed at physiological levels and other dietary fatty acids suggests that an optimal dietary P/S ratio may produce the maximum hypolipidaemic effect when fish oil is consumed [22–24].

The favourable effect of fish oil in the reduction of the plasma lipid level in normal subjects has not been clearly established in the treatment of NIDDM hyperlipidaemia. Research results suggest that insulin sensitivity is increased after the addition of 3 g of ω3 fatty acid to the diet of NIDDM patients for 8 weeks [25]. In other NIDDM patients, ingesting 8 g/day of ω3 fatty acids reduced VLDL cholesterol and plasma triacylglycerol levels by 56 and 42 %, respectively [26]. The LDL and HDL cholesterol level was not affected; however, the ω3 fatty acid supplementation increased both the fasting and postprandial plasma glucose level. Feeding fish oil capsules to NIDDM patients without hyperlipidaemia does not change the serum triacylglycerol or the total, LDL and HDL cholesterol level [27]. In these studies, NIDDM patients were fed ω3 fatty acids for short periods when the composition of dietary fat intake was not controlled or assessed.

Studies in rats indicate that the efficacy of ω3 fatty acids in lowering plasma triacylglycerol, cholesterol and arachidonate (20:4ω6) depends on the relative amounts of linoleic acid and saturated fat in the diet [28] and suggest that ω3 fatty acid supplementation may be more effective when the dietary P/S ratio is low. To investigate this hypothesis, the present study was initiated with NIDDM patients in order to determine the effect on the plasma triacylglycerol and lipoprotein cholesterol level after consumption of 20:5ω3 and 22:6ω3 or 18:3ω3 in physiological amounts that could be achieved by the consumption of normal foods. The essential fatty acid pool size of the major lipid classes, including phospholipid, triacylglycerol and cholesterol ester of each plasma lipoprotein, was also assessed.

Patients and methods

The study protocol was approved by the human ethics review committee of the Faculty of Medicine, University of Alberta. The olive, fish and linseed oils used were provided by Lipid-Teknik Ltd., Stockholm, Sweden, as chromatographically pure triglyceride. The oils were artificially flavoured prior to encapsulation, as described earlier [29], to enable blinding of the participants to the supplement. Instructions on conditions for storage of oil capsules during the study were provided to all participants. Oil stability during storage was checked periodically by peroxide analysis using a lipid peroxide Determiner Kit (Kyowa Medex Co., Tokyo, Japan). Fatty acid composition of oil supplements is illustrated (Table 1).

Design and subjects. Twenty-eight patients treated for NIDDM were recruited from the outpatient Metabolic Clinic at the University of Alberta Hospital. Patients currently on lipid-lowering drugs were excluded from the study; however, patients on other non-lipid-lowering medications were included and were instructed to continue with their normal medication during the study. The subjects had been treated for NIDDM for at least several years and did not have cardiac complications.

Patients were assigned to a high P/S group (> 0.5, $n = 10$) or a low P/S group (< 0.5, $n = 18$), based on a 7-day dietary record collected by a dietitian documenting all food and drink

Table 1. ω 3 Fatty acid composition of oil supplements

Oil treatment Fatty acid (% w/w)	Olive	Fish	Linseed
18 : 3 ω 3	0.9	1.3	57.5
20 : 5 ω 3	–	20.8	–
22 : 5 ω 3	–	1.6	–
22 : 6 ω 3	–	12.6	–

The complete fatty acid composition of these oil supplements has been reported elsewhere [29]

Table 2. Descriptive data for subjects on entry

Dietary P/S group:	Low (n = 18)	High (n = 10)
Physical data		
Age (years)	56.2 ± 1.8	59.7 ± 3.0
Ideal body mass	130.6 ± 4.6	120.9 ± 6.5
Dietary data		
Energy (kcal)	1639.1 ± 95	1684.6 ± 115
Protein (% E)	20.3 ± 0.8	19.8 ± 0.8
Carbohydrate (% E)	50.1 ± 2.0	54.5 ± 1.6
Fat (% E)	29.1 ± 1.7	26.1 ± 1.8
Saturated	10.8 ± 0.6	10.9 ± 2.4
Oleic acid	8.1 ± 0.6	7.1 ± 0.6
Linoleic acid	4.4 ± 0.3	5.8 ± 0.6
Cholesterol (mg)	292.7 ± 22.8	306.1 ± 31.0
Polyunsaturated/saturated fatty acid ratio	0.44 ± 0.04[a]	0.65 ± 0.04[b]
Clinical data		
Triglyceride 0.6–2.3 mmol/l	2.41 ± 0.33	2.05 ± 0.48
Total cholesterol 3.2–5.2 mmol/l	5.90 ± 0.22	5.47 ± 0.33
LDL_c 1.7–3.4 mmol/l	3.82 ± 0.21	3.48 ± 0.27
HDL_c 0.9–2.20 mmol/l	1.04 ± 0.05	1.19 ± 0.10
Glucose 3.5–6.4 mmol/l	9.07 ± 0.71[a]	7.04 ± 0.54[b]
HbA_{1c} 0.040–0.063 %	0.06 ± 0.003	0.06 ± 0.003
Insulin 5–20 μU/ml	15.18 ± 1.98	10.20 ± 2.21
C-peptide 0.5–3.0 ng/ml	2.29 ± 0.16[a]	1.67 ± 0.19[b]
Glucagon > 60 ng/l	67.53 ± 3.30	64.30 ± 7.39

Values are means ± SEM of baseline measurements. Normal ranges for clinical data according to University of Alberta Hospital standards are given in parentheses. Values within rows without a common superscript are significantly different ($p < 0.05$). Significant differences between high and low P/S participants were determined by a two-tailed t-test

consumed on a daily basis. Dietary intake, including energy, total fat, saturated and unsaturated fatty acids as well as cholesterol, was also calculated. A fasting blood sample was drawn from each participant and analysed for the total plasma triacylglycerol, total plasma and lipoprotein cholesterol level. The plasma glucose and glycated haemoglobin levels were also determined. The physical, dietary and clinical data for both groups of NIDDM patients on entry to the study are summarised (Table 2).

The study was designed as a double-blind crossover comparison between the effect of fish oil compared to linseed oil supplementation. All participants were given capsules of olive oil in an initial "placebo" period of 3 months. The olive oil was provided at a dose equivalent to 35 mg of oleic acid (18:1) per kg body weight per day. This was followed by either fish oil or linseed oil supplementation in a random order. After a 3-month period consuming the first ω3 fatty acid supplement (fish oil or linseed oil), the participants consumed the second ω3 treatment for a further 3 months. Participants who had previously received the fish oil treatment were switched to linseed oil and vice versa. A 7-day diet record was collected in the last week of each 3-month supplement period, and the dietary P/S ratio and dietary nutrient intake was calculated. A fasting blood sample was collected from each participant at the end of each treatment period. The ω3 fatty acid supplement provided 35 mg of ω3 fatty acid per kg of body weight per day, similar to levels previously described [27]. Compliance was assessed by pill counts and periodic telephone or personal contact. During the course of the study, participants were instructed to continue their usual dietary intake.

Analytical procedures. Blood was drawn into tubes containing disodium EDTA from each participant after he or she had fasted for 12–14 h. Total triacylglycerol, total plasma, HDL and LDL cholesterol, blood glucose and haemoglobin level was analysed by the University of Alberta Hospitals Clinical Laboratory and compared to hospital reference values. The plasma insulin, glucagon and C-peptide content of plasma was determined using radioimmunoassay techniques by the Muttart Diabetes Research Center, University of Alberta. Detailed lipid analysis was performed as described earlier [29]. Briefly, plasma was separated from whole blood and then transferred to a clear tube and ultracentrifuged in a Beckman Benchtop Ultracentrifuge (Model TL-100) to separate the chylomicron fraction. Since the participants fasted overnight prior to the blood collection, only a small amount of chylomicron was present in the plasma. Following chylomicron removal, plasma, VLDL, LDL and HDL fractions were separated by sequential ultracentrifugation at 100 000 rev/min at 20 °C in a Beckman TLA100.2 angle head rotor [29, 30].

Lipoprotein lipids were extracted by a modified Folch method [29, 31], with chloroform:methanol (2:1 v/v) solution containing 0.01 % (v/v) ethoxyquin as an antioxidant. Phospholipid, triacylglycerol and cholesterol ester fractions were separated and the fatty acids quantitated as described earlier [29].

Fatty acid analysis. Fatty acid methyl esters were prepared and separated by automated gas-liquid chromatography (GLC) (Varian Instruments, Georgetown, Ontario, Canada) as previously described [32]. These conditions are capable of separating methyl esters of saturated, cis-monounsaturated and cis-polyunsaturated fatty acids from 14 to 24 carbon chains in length. Fatty acid content was calculated from the known amount of standard added.

Statistical analysis

Data collected from the two groups of participants when they entered the study were initially compared by t-test at the $p < 0.05$ level. The effect of ω3 fatty acid supplementation on plasma triacylglycerol and total, HDL and LDL cholesterol was analysed by repeated measures analysis of variance (ANOVA) using SAS statistical software (SAS Inc., Cary, N.C., USA). Significant differences among means as revealed by the F-test of ANOVA on the P/S ratio or type of oil supplement were further compared by a Duncan Multiple Range Test [33] at the $p < 0.05$ level. Quantitative data of major fatty acids present in the three lipid classes (phospholipid, triacylglycerol and cholesteryl ester) of each lipoprotein fraction were analysed statistically using the same linear model, with the type of ω3 fatty acid supplement treated as a repeated

Table 3. Plasma triacylglycerol and LDL cholesterol levels in diabetic individuals for each oil supplement period

Fraction	Diet P/S	Olive	Fish	Linseed
Triglycerol	Low	2.13 ± 0.31^{a}	1.52 ± 0.19^{b}	2.18 ± 0.31^{a}
	High	1.94 ± 0.54^{a}	1.36 ± 0.46^{b}	2.33 ± 0.77^{a}
LDLc	Low	3.79 ± 0.19	4.08 ± 0.23^{p}	3.98 ± 0.31^{p}
	High	3.33 ± 0.21	3.35 ± 0.23^{q}	3.20 ± 0.20^{q}

Values reported are means ± SEM for variables that were significantly altered by oil treatment. Significant oil treatment effects were determined by repeated measures analysis of variance. Values within rows without a common superscript are significantly different ($p < 0.05$). Values within columns with a different superscript (p, q) are significantly different ($p < 0.05$)

factor. To compare the effects of feeding 18:3ω3 (linseed oil) 20:5ω3 and 22:6ω3 (fish oil) on the arachidonate and ω3 polyunsaturated fatty acid content in the lipoproteins, the change in each fatty acid content was also calculated for both dietary P/S groups by subtracting the fatty acid levels of the olive oil treatment period from the fatty acid levels of the fish oil and linseed oil treatment. This difference was compared by Student's paired *t*-test ($p < 0.05$). The Pearson correlation coefficient was used to determine the presence of any overall correlations between clinical data and selected dietary fat intake variables.

Results

The difference between the mean dietary P/S ratios for the two groups of participants selected at the beginning the study was statistically significant. Individuals in the high P/S group had significantly lower plasma glucose and C-peptide levels than individuals in the low P/S group, which may be suggestive of better diabetes control (Table 2).

Fasting glucose, insulin, and glucagon levels were not influenced by the type of ω3 fatty acid (data not illustrated); however, plasma glucose, insulin, and glucagon levels were lower in NIDDM patients consuming the high P/S diet (Table 2). Dietary total fat intake, P/S ratio, monounsaturated to saturated fatty acid ratio, daily total and saturated fat intake as well as a daily intake of 14:0, 16:0, 18:0, 18:1 and 18:2 were calculated (data not shown). These intake variables were not correlated with the plasma triacylglycerol or cholesterol level.

Total triacylglycerol, total plasma and lipoprotein cholesterol. The plasma triacylglycerol level was influenced by the type of ω3 fatty acid supplementation provided in both the high and low P/S groups ($p < 0.05$). The plasma triacylglycerol level was lowest when NIDDM patients consumed the fish oil supplement (Table 3). The total plasma cholesterol level was not significantly affected by the type of ω3 fatty acid ingested. The mean LDL cholesterol level was lower in participants consuming a high P/S diet, and this difference was significant when participants consumed fish oil or linseed oil, but not when they consumed olive oil. The type of oil supplement or the P/S ratio of the diet consumed did not affect the HDL cholesterol level.

Fatty acid content of lipoproteins. The fatty acid content of the VLDL, LDL and HDL triacylglycerol fractions indicates that the effect of the ω3 fatty acid supplement was most pronounced in the VLDL fraction (Table 4). Consumption of fish oil by diabetic individuals decreased the level of 18:2ω6 (Table 4) and oleic acid (data not shown) content in the triacylglycerol fraction of the VLDL. Linoleic and arachidonic acid content decreased; whereas, 20:5ω3 and 22:6ω3 increased compared to when participants consumed the olive oil supplement. The VLDL triacylglycerol fraction contained a higher level of linolenic acid after the linseed oil supplement period compared to the other supplement periods. The 20:5ω3 and 22:6ω3 content in the LDL triacylglycerol was higher when participants consumed fish oil in both the high and low P/S groups; whereas, 18:3ω3 content was elevated when participants consumed the 18:3ω3 supplement ($p < 0.05$). Very little change in fatty acid content was noted in the HDL triacylglycerol. In general, the reduced fatty acid content in the VLDL fraction suggests a reduction of total plasma triacylglycerol when consuming fish oil.

The content of major fatty acids in the lipoprotein phospholipid fraction (Table 5) indicates that the effect of fish oil or linseed oil treatment on fatty acid content of the phospholipid fraction was less noticeable than in the triacylglycerol fraction. Consuming fish oil increased the 20:5ω3 content in the phospholipid of all lipoprotein fractions; whereas, 22:6ω3 increased significantly only in the LDL phospholipid. The linseed oil supplement increased the 18:3ω3 in phospholipid of all lipoprotein fractions. After fish oil was consumed 18:2ω6 and 20:4ω6 decreased in the VLDL and HDL fractions.

The fatty acid content of the cholesteryl ester from each lipoprotein indicates similar trends in the fatty acid content described for the triglyceride and phospholipid fraction (Table 6). Consumption of fish oil increased the 20:5ω3 content in the cholesteryl ester of all lipoproteins, but 22:6ω3 increased only in the LDL and HDL. The VLDL cholesterol ester content of 18:2ω6 and 20:4ω6 decreased as a result of consuming fish oil. 18:3ω3 was consistently higher in all lipoprotein cholesterol ester fractions when the participants consumed linseed oil.

Net changes in 18:3ω3, 20:5ω3, 22:6ω3 and 20:4ω6 were calculated by subtracting the fatty acid content at the end of each treatment period from the placebo treatment period. These changes are illustrated (Figs. 1–3). Linolenic acid content increased in almost all lipid classes in the three lipoprotein fractions after the fish oil and linseed oil treatment periods (data not

Table 4. Essential fatty acid content of plasma triacylglycerol of LDL, VLDL and HDL after each treatment period

Composition (μg fatty acid/ml plasma) Oil treatment:	Olive		Fish		Linseed	
	Dietary polyunsaturated/saturated fatty acid ratio group					
	Low	High	Low	High	Low	High
VLDL						
18 : 2 ω 6	72.31 ± 9.12	73.61 ± 14.35^{xy}	57.62 ± 8.37	56.91 ± 20.67^{x}	69.46 ± 10.30	92.17 ± 39.05^{y}
18 : 3 ω 3	5.29 ± 0.73^{a}	3.72 ± 0.61^{x}	5.25 ± 0.86^{a}	4.06 ± 1.72^{x}	8.81 ± 1.76^{b}	9.84 ± 3.49^{y}
20 : 4 ω 6	4.50 ± 0.64	3.66 ± 0.49^{xy}	3.70 ± 0.61	2.55 ± 0.44^{x}	4.56 ± 0.74	4.50 ± 1.40^{y}
20 : 5 ω 3	0.92 ± 0.17^{a}	1.05 ± 0.24	3.44 ± 0.81^{b}	2.26 ± 0.59	1.73 ± 0.32^{a}	2.09 ± 0.75
22 : 6 ω 3	2.22 ± 0.40^{a}	2.05 ± 0.54	5.33 ± 1.23^{b}	3.48 ± 0.89	3.05 ± 0.78^{a}	2.61 ± 0.66
LDL						
18 : 2 ω 6	22.37 ± 3.36	25.24 ± 6.72	28.06 ± 6.07	26.19 ± 8.68	24.78 ± 3.80	30.01 ± 13.57
18 : 3 ω 3	1.74 ± 0.32^{a}	1.16 ± 0.22	2.34 ± 0.39^{ab}	1.79 ± 0.58	3.20 ± 0.53^{b}	2.82 ± 1.03
20 : 4 ω 6	2.19 ± 0.25	2.12 ± 0.21	2.79 ± 0.69	1.90 ± 0.27	2.36 ± 0.33	1.98 ± 0.51
20 : 5 ω 3	0.42 ± 0.07^{a}	0.61 ± 0.17	2.48 ± 0.71^{b}	1.69 ± 0.34	0.83 ± 0.13^{a}	0.73 ± 0.14
22 : 6 ω 3	0.62 ± 0.13^{a}	0.77 ± 0.19^{x}	2.32 ± 0.39^{b}	1.67 ± 0.34^{y}	0.85 ± 0.21^{a}	0.69 ± 0.18^{x}
HDL						
18 : 2 ω 6	9.79 ± 1.72	11.06 ± 3.09	9.12 ± 1.73	9.94 ± 2.44	8.68 ± 1.34	10.88 ± 4.62
18 : 3 ω 3	0.74 ± 0.14	0.75 ± 0.19	1.15 ± 0.23	0.78 ± 0.35	1.43 ± 0.42	1.59 ± 0.41
20 : 4 ω 6	1.18 ± 0.28	0.84 ± 0.14	0.81 ± 0.17	0.63 ± 0.09	0.87 ± 0.20	1.04 ± 0.21
20 : 5 ω 3	0.48 ± 0.10	0.32 ± 0.05	0.61 ± 0.14	0.70 ± 0.21	0.78 ± 0.36	0.53 ± 0.15
22 : 6 ω 3	0.26 ± 0.05^{a}	0.21 ± 0.05	0.42 ± 0.08^{b}	0.35 ± 0.05	0.20 ± 0.04^{a}	0.24 ± 0.04

Values are means ± SEM. Significant oil treatment effects were determined by repeated measures ANOVA.

^{abc} Significant oil treatment effects within subjects in the low dietary P/S group ($p < 0.05$)

^{xyz} Significant oil treatment effects within subjects in the high dietary P/S group ($p < 0.05$).

Levels of 14 : 0, 16 : 0, 18 : 0, 18 : 1 in the VLDL triacylglycerol fraction were 7.96, 114.29, 16.58 and 176.15.

Levels of 14 : 0, 16 : 0, 18 : 0, 18 : 1 in the LDL triacylglycerol fraction were 2.64, 42.77, 8.36 and 73.46.

Levels of 14 : 0, 16 : 0, 18 : 0, 18 : 1 in the VLDL triacylglycerol fraction were 1.61, 17.83, 5.04 and 27.38

Table 5. Essential fatty acid content of plasma phospholipid fraction in HDL, LDL, and VLDL after each treatment period

Composition (μg fatty acid/ml plasma) Oil treatment:	Olive		Fish		Linseed	
	Dietary polyunsaturated/saturated fatty acid ratio group					
	Low	High	Low	High	Low	High
VLDL						
18 : 2 ω 6	25.54 ± 5.38	24.37 ± 9.80	17.18 ± 3.51	13.06 ± 3.64	26.15 ± 3.58	23.09 ± 6.12
18 : 3 ω 3	0.36 ± 0.09^{a}	0.34 ± 0.12	0.64 ± 0.17^{ab}	0.42 ± 0.14	1.02 ± 0.23^{b}	0.64 ± 0.20
20 : 4 ω 6	10.83 ± 1.92^{ab}	10.72 ± 4.22^{y}	6.66 ± 1.38^{a}	4.06 ± 1.32^{x}	11.97 ± 2.27^{b}	8.69 ± 2.16^{xy}
20 : 5 ω 3	1.23 ± 0.27^{a}	0.75 ± 0.15	2.91 ± 0.59^{b}	1.61 ± 0.50	1.95 ± 0.37^{ab}	1.59 ± 0.30
22 : 6 ω 3	3.55 ± 0.87	2.39 ± 0.56	3.32 ± 0.65	2.07 ± 0.67	3.42 ± 0.76	3.11 ± 0.45
LDL						
18 : 2 ω 6	37.12 ± 4.76	33.57 ± 5.96	35.36 ± 3.55	33.41 ± 4.03	37.95 ± 4.12	38.88 ± 3.29
18 : 3 ω 3	0.39 ± 0.07	2.19 ± 1.75	0.67 ± 0.08	0.59 ± 0.12	1.19 ± 0.17	2.89 ± 1.87
20 : 4 ω 6	16.18 ± 2.26	13.06 ± 2.45	14.58 ± 2.09	12.55 ± 0.96	16.38 ± 2.57	12.36 ± 1.50
20 : 5 ω 3	1.51 ± 0.22^{b}	1.49 ± 0.42^{y}	6.69 ± 0.99^{a}	5.13 ± 0.51^{x}	2.74 ± 0.45^{b}	2.24 ± 0.31^{y}
22 : 6 ω 3	4.65 ± 0.68^{b}	3.26 ± 0.72^{y}	7.65 ± 1.16^{a}	6.90 ± 0.76^{x}	4.81 ± 0.80^{b}	3.31 ± 0.75^{y}
HDL						
18 : 2 ω 6	35.98 ± 5.80^{b}	35.28 ± 4.84	22.56 ± 2.76^{a}	28.01 ± 2.96	29.62 ± 3.01^{ab}	39.51 ± 5.71
18 : 3 ω 3	0.46 ± 0.09^{a}	0.53 ± 0.14^{x}	0.69 ± 0.18^{ab}	0.88 ± 0.20^{x}	1.08 ± 0.17^{b}	1.92 ± 0.53^{y}
20 : 4 ω 6	17.35 ± 3.37	18.08 ± 2.36	10.73 ± 1.47	12.80 ± 1.67	14.92 ± 1.81	16.52 ± 2.67
20 : 5 ω 3	1.65 ± 0.32^{a}	1.67 ± 0.28^{x}	5.20 ± 0.72^{b}	5.46 ± 0.85^{y}	2.58 ± 0.53^{a}	3.13 ± 0.55^{x}
22 : 6 ω 3	4.62 ± 1.01	4.13 ± 0.84	5.43 ± 0.83	5.54 ± 0.99	3.56 ± 0.50	5.71 ± 1.12

Values are means ± SEM. Significant oil treatment effects were determined by repeated measures analysis of variance.

^{abc} Significant oil treatment effects within subjects in the low dietary P/S group ($p < 0.05$)

^{xyz} Significant oil treatment effects within subjects in the high dietary P/S group ($p < 0.05$).

Levels of 14 : 0, 16 : 0, 18 : 0, 18 : 1 in the VLDL phospholipid fraction were 0.55, 32.11, 17.26 and 14.46.

Levels of 14 : 0, 16 : 0, 18 : 0, 18 : 1 in the LDL phospholipid fraction were 0.88, 57.09, 28.60 and 24.35.

Levels of 14 : 0, 16 : 0, 18 : 0, 18 : 1 in the HDL phospholipid fraction were 0.83, 51.21, 26.30 and 22.05

Table 6. Essential fatty acid content of plasma cholesteryl ester fraction in HDL, LDL, and VLDL after each treatment period

Composition (μg fatty acid/ml plasma)						
Oil treatment:	Olive		Fish		Linseed	
	Dietary polyunsaturated/saturated fatty acid ratio group					
	Low	High	Low	High	Low	High
VLDL						
18 : 2 ω 6	44.50 ± 10.83[b]	29.42 ± 7.44	21.87 ± 3.77[a]	18.86 ± 4.75	35.63 ± 7.75[ab]	32.66 ± 9.87
18 : 3 ω 3	0.86 ± 0.35	0.47 ± 0.15	0.57 ± 0.10	1.04 ± 0.25	1.63 ± 0.76	1.40 ± 0.39
20 : 4 ω 6	5.67 ± 0.97[b]	3.57 ± 0.88	2.51 ± 0.58[a]	1.56 ± 0.67	4.44 ± 1.10[b]	3.30 ± 1.00
20 : 5 ω 3	1.15 ± 0.29	0.66 ± 0.15	1.78 ± 0.45	0.84 ± 0.08	1.23 ± 0.36	0.75 ± 0.25
22 : 6 ω 3	0.66 ± 0.17	0.57 ± 0.27	0.43 ± 0.10	0.22 ± 0.66	0.29 ± 0.07	0.25 ± 0.09
LDL						
18 : 2 ω 6	175.86 ± 15.26	157.91 ± 13.55	167.01 ± 17.75	154.41 ± 25.17	177.45 ± 13.69	145.14 ± 72.24
18 : 3 ω 3	2.07 ± 0.75[a]	1.39 ± 0.30[x]	2.15 ± 0.37[a]	1.75 ± 0.38[x]	4.31 ± 0.53[b]	3.42 ± 0.63[y]
20 : 4 ω 6	22.96 ± 2.33	19.90 ± 1.74	22.26 ± 2.71	17.45 ± 1.91	22.49 ± 3.02	16.28 ± 1.91
20 : 5 ω 3	3.15 ± 0.43[a]	2.80 ± 0.59[x]	14.97 ± 2.36[b]	8.64 ± 1.52[y]	4.95 ± 0.85[a]	2.25 ± 0.59[x]
22 : 6 ω 3	1.56 ± 0.23[a]	1.47 ± 0.30[x]	2.49 ± 0.40[b]	2.51 ± 0.42[y]	1.59 ± 0.25[a]	1.28 ± 0.17[x]
HDL						
18 : 3 ω 3	0.82 ± 0.13[a]	0.80 ± 0.16[x]	0.95 ± 0.19[a]	0.86 ± 0.22[x]	1.93 ± 0.29[b]	1.93 ± 0.36
20 : 4 ω 6	9.78 ± 0.96[b]	10.40 ± 1.88	5.89 ± 0.82[a]	9.17 ± 1.18	9.18 ± 1.01[b]	10.08 ± 1.15
20 : 5 ω 3	2.97 ± 1.71	1.47 ± 0.35	3.62 ± 0.59	5.67 ± 0.91	2.05 ± 0.26	2.24 ± 0.43
22 : 6 ω 3	0.57 ± 0.07	0.80 ± 0.16[x]	0.72 ± 0.16	1.26 ± 0.18[y]	0.67 ± 0.05	0.76 ± 0.08[x]

Values are means ± SEM. Significant oil treatment effects were determined by repeated measures analysis of variance.
[abc] Significant oil treatment effects within subjects in the low dietary P/S group ($p < 0.05$)
[xyz] Significant oil treatment effects within subjects in the high dietary P/S group ($p < 0.05$)
Levels of 14 : 0, 16 : 0, 18 : 0, 18 : 1 in the VLDL cholestryl ester fraction were 0.73, 12.97, 3.77 and 19.59.
Levels of 14 : 0, 16 : 0, 18 : 0, 18 : 1 in the LDL phospholipid fraction were 2.82, 45.06, 4.91 and 74.03.
Levels of 14 : 0, 16 : 0, 18 : 0, 18 : 1 in the HDL phospholipid fraction were 1.18, 18.99, 2.78 and 30.26

shown). Fish oil reduced the arachidonic acid (20:4ω6) content (Fig. 1) and reduced the 20:5ω3 content (Fig. 2) in all lipid classes. Change in 20:5ω3 and 22:6ω3 content (Figs. 2, 3) was most obvious in the cholesteryl ester and phospholipid fraction of both LDL and HDL fractions. The slight increase in 20:5ω3 content resulting from 18:3ω3 supplementation appears to indicate only a small increase in the conversion of 18:3ω3 to 20:5ω3 (Fig. 2) in the liver with little or no change occurring in 22:6ω3 levels except when fish oil was consumed (Fig. 3).

Discussion

The effect of 20:5ω3 and 22:6ω3 on the reduction of the plasma triacylglycerol level was clearly demonstrated in this study. With a daily intake of 7–8 g/day of fish oil, the mean reduction in plasma triacylglycerol after 3 months of fish oil consumption was 29.1 and 29.9 % for low and high P/S participants, respectively. This observation is comparable to the reduction in triacylglycerol levels observed after NIDDM patients received 8 g of ω3 fatty acids for 8 weeks [26]. In the present study, ingestion of 18:3ω3 by NIDDM patients did not reduce plasma triacylglycerol levels. Schectman et al. [34] report finding a decrease in the triacylglycerol level when 7.5 g of ω3 fatty acids was given for 4 weeks but not when a lower dose of 4 g per day was provided.

Effects of fish oil supplements on plasma and lipoprotein cholesterol levels are inconsistent [35], and this inconsistency can be explained partly by the variation between study designs and lack of control comparisons. For example, few studies include the monitoring of dietary, polyunsaturated and saturated fat, intake. In the present study, a non-significant increase in the total plasma cholesterol was noted in the NIDDM patients consuming the low P/S ratio diet supplemented with fish oil (5.72 ± 0.22 to 5.81 ± 0.23) or linseed oil (5.72 ± 0.22 to 5.94 ± 0.23), but it remained unchanged in the group consuming the high P/S diet. It has been reported previously that non-significant change occurs in fasting total plasma, total cholesterol, HDL, and LDL levels in individuals fed a typical diabetic diet having a P/S ratio of 0.5 and 10 g/day of fish oil [36]. Others have reported a significant increase in total plasma cholesterol in diabetic patients due mainly to an increase in HDL cholesterol resulting from 20:5ω3 and 22:6ω3 intake. In normal individuals, cholesterol-lowering effects were noted in studies with doses of ω3 fatty acids of up to 30 g/day [10, 33]. Recently, it has been suggested that treatment with 22:6ω3 is more effective in lowering LDL cholesterol than treatment with 20:5ω3 [37], indicating that EPA and DHA may play different roles in reducing plasma cholesterol. Hence, the relative amount of 20:5ω3 and 22:6ω3 in the fish oil supplemented may be important in altering the plasma and lipoprotein lipid pattern observed in this study.

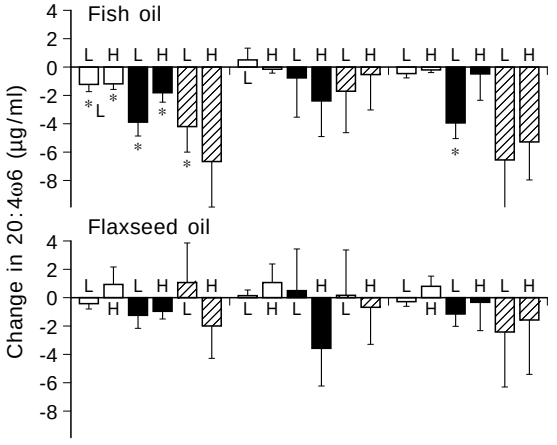

Fig. 1. Effect of ω3 fatty acid treatments on change in 20:4ω6 content in the triacylglycerol □, cholesterol ester ■ and phospholipid ▨ fractions. Values are means ± SEM of differences in fatty acid levels between olive oil and ω3 fatty acid treatments. *Significant effect of oil treatment ($p < 0.05$). L, H indicate low and high dietary P/S groups, respectively

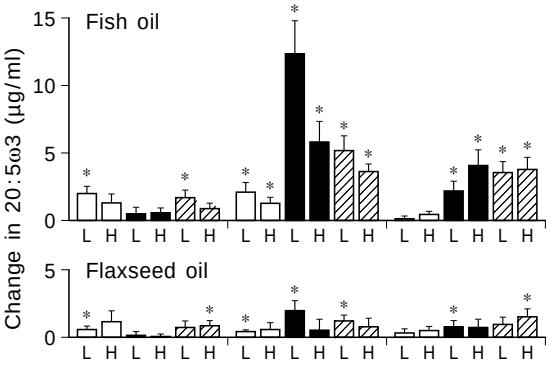

Fig. 2. Effect of ω3 fatty acid treatments on change in 20:5ω3 content in the triacylglycerol □, cholesterol ester ■ and phospholipid ▨ fractions. Values are means ± SEM of differences in fatty acid levels between olive oil and ω3 fatty acid treatments. *Significant effect of oil treatment ($p < 0.05$). L, H indicate low and high dietary P/S groups, respectively

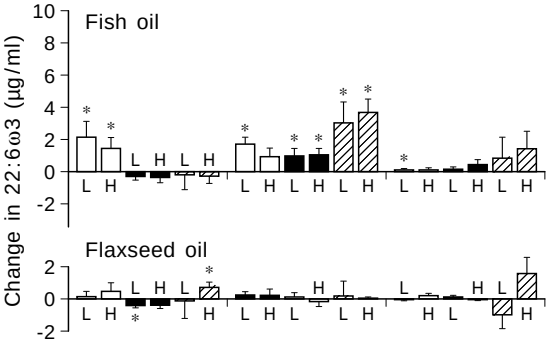

Fig. 3. Effect of ω3 fatty acid treatments on change in 22:6ω3 content in the triacylglycerol □, cholesterol ester ■ and phospholipid ▨ fractions. Values are means ± SEM of differences in fatty acid levels between olive oil and ω3 fatty acid treatments. *Significant effect of oil treatment ($p < 0.05$). L, H indicate low and high dietary P/S groups, respectively

The fatty acid content of lipoprotein fractions clearly demonstrated the influence of diet on the fatty acid content of different plasma lipoprotein components. The incorporation of 20:5ω3, 22:6ω3 and 18:3ω3 varied from one lipid class to another as well as from one lipoprotein fraction to another. Increase in 20:5ω3 and 22:6ω3 content occurred in almost all lipid classes of each lipoprotein after the consumption of fish oil but not after the consumption of linolenic acid. These observations are in agreement with recent observations reported for normal individuals except that the increases observed in the NIDDM patients were of a lower magnitude [29].

The highest level of incorporation of 20:5ω3 was found in the cholesteryl esters of the LDL, and it appears that approximately 1.8-fold more 20:5ω3 was incorporated into this fraction in participants consuming a low P/S diet than in those participants consuming a high P/S diet (14.97 vs 8.64 μg/ml plasma). Accordingly, this would suggest that more 20:5ω3 may be incorporated into body tissue pools of NIDDM patients consuming a low P/S diet. Similarly, it was noted that significantly more 18:3ω3 was incorporated into the LDL cholesteryl ester in the low P/S participant group than in the high P/S participant group. The total fatty acid content of the LDL cholesteryl ester appeared to confirm the observation that the LDL cholesterol level was higher in participants consuming a low P/S diet compared with the level in participants consuming a high P/S diet.

It is concluded that in NIDDM patients' intake of 20:5ω3 and 22:6ω3, at a level that can be achieved by the consumption of normal foods, for example fish that are high in 20:5ω3, will result in a significant reduction in the plasma triacylglycerol level without deleterious changes occurring in other plasma lipid parameters or measures of diabetic control. Thus, regular consumption of specific foods of marine origin providing relatively conservative intakes of 20:5ω3 and 22:6ω3 should help reduce the risk of cardiovascular disease in NIDDM patients.

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