

Fish Oil–Induced Changes in Apolipoproteins in IDDM Subjects

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The aim of this study was to examine the effect of Max EPA (a commercially available fish oil preparation) on serum cholesterol lipoproteins and apolipoproteins in insulin-dependent diabetic (IDDM) men with dosages that were likely to be acceptable to patients. Twenty-two male IDDM patients aged 20–41 yr, 6 of whom had retinopathy, were recruited from the Royal Perth Hospital diabetic clinic. After screening, subjects were divided into three groups. Six of the subjects without retinopathy were randomly selected and allocated to a control group. The remaining 16 patients (10 without and 6 with retinopathy) received a fish oil supplement. All subjects were advised to maintain their usual dietary patterns. Sixteen patients, including the 6 with retinopathy, were instructed to take 15 Max EPA fish oil capsules/day with meals. Patients in the control group did not take Max EPA. Three weeks of Max EPA supplementation without other dietary modification led to a significant rise in total cholesterol ($P < 0.01$), which could be accounted for by increases in low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol. The increase in HDL cholesterol was explained by a 33% rise ($P < 0.001$) in its HDL₂ subclass. Changes in apolipoproteins were examined and showed that the level of apolipoprotein A-I increased after ingestion of fish oil and correlated significantly ($P < 0.05$) with the rise in HDL cholesterol. Apolipoprotein A-II showed a significant fall at the end of Max EPA intake in a subgroup of patients with retinopathy, and this correlated significantly ($P < 0.05$) with the fall in HDL₃ cholesterol observed at this time. A significant rise in apolipoprotein B ($P < 0.05$) was

correlated with the rise in LDL cholesterol. Possible adverse effects of the increase in both total and LDL cholesterol after 15 g/day Max EPA may be compensated for by a rise in the protective HDL₂ subclass. However, in view of this hypercholesterolemic effect and evidence that suggests that LDL apolipoprotein B may be a risk factor for coronary heart disease, these findings raise questions regarding the safety of fish oils in patients with IDDM. *Diabetes Care* 13:725–32, 1990

Epidemiological observations and numerous human feeding trials have provided evidence that suggests that diets high in fatty fish may be of benefit in preventing cardiovascular disease (1,2). This beneficial effect has been attributed to the presence of a relatively high proportion of long-chain polyunsaturated fatty acids of the ω -3 series in fish oil. Eicosapentaenoic acid (EPA), one of the major fatty acids in fish oil, has received considerable attention, although other fatty acids with variable biological activities are also present. It has been postulated that a diet rich in these fatty acids may confer protection through effects on platelet function, platelet-endothelial interactions, and the inflammatory response. Previous trials have indicated that ω -3 fatty acid (ω 3FA) supplementation also results in altered lipid profiles, predominantly a lowering of triglycerides in healthy and hypertriglyceridemic subjects (3,4). The effect on total serum cholesterol is variable and appears to depend on the initial cholesterol level and on the quantity of fish oil taken (1,2). Changes in high-density lipoprotein cholesterol (HDL-cholesterol) and low-density lipoprotein (LDL)-cholesterol have also varied depending on the amount of fish oil provided.

Diabetic subjects are prone to hyperlipidemia and

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Received for publication 20 July 1989 and accepted in revised form 6 February 1990.

vascular disease, the latter being the leading cause of morbidity and mortality in this disorder. Therefore, it was envisaged that dietary supplementation with fish oil could be of benefit in patients with diabetes mellitus. In a preliminary study we described that 15 g Max EPA, a commercially available fish oil preparation, given daily for 3 wk significantly increased serum total cholesterol and decreased serum triglycerides in insulin-dependent diabetic (IDDM) men (5). The increase in total cholesterol was accounted for by a rise in both HDL-chol and LDL-chol. Of interest was the finding of a selective increase in the HDL₂ subfraction, with the HDL₃ fraction either falling slightly or remaining unchanged. This effect did not appear to be more pronounced in a group of patients with retinopathy. Herein, we report the changes in apolipoproteins in relation to the alterations in lipoproteins.

RESEARCH DESIGN AND METHODS

Twenty-two male IDDM patients aged 20–41 yr who were nonsmokers were recruited from the Royal Perth Hospital diabetic clinic. All patients were screened, during which measurements were taken for serum total cholesterol and triglycerides, plasma creatinine, glucose, and glycosylated hemoglobin concentrations. All patients had a serum cholesterol of <6.5 mM. Six patients had retinopathy, but none were hypertensive or had clinical evidence of peripheral vascular disease or renal impairment. They were advised to maintain their usual dietary patterns and physical activities and to avoid any medications known to interfere with lipid metabolism. Weekly records of dietary alterations, medications taken, and alcohol consumption were kept. Approval for the study was obtained from the hospital's human rights committee, and all participants were informed of the purpose of the study and gave written consent.

After excluding the patients with retinopathy, 6 patients were randomly selected and allocated to a control group not taking Max EPA. After all patients were seen for baseline measurements, the remaining 16 patients, including the 6 with retinopathy, were instructed to take 5 Max EPA capsules 3 times/day with meals for 3 wk. This supplement provided a daily intake of 2.7 g EPA, 1.7 g docosahexaenoic acid (DHA), and a total of 4.9 g ω 3FAs. Blood samples for measurement of plasma creatinine, plasma glucose, glycosylated hemoglobin, and serum lipids were taken ~2 h after a light breakfast. Patients were seen at weekly intervals during the 3 wk of supplementation, and measurements for serum lipids were taken at the end of this period. Six weeks after ceasing Max EPA, further measurements were taken. Therefore, patients were their own controls, in addition to the group described earlier who were not given the supplement. Compliance was assessed by capsule count at each weekly visit and by measurement of changes in

the fatty acid composition of platelet phospholipids. Body weight was recorded at each visit.

Max EPA capsules were obtained from Scherer (Melbourne, Australia) and contained the following fatty acids: 14:0 (7.54%), 16:0 (16.26%), 16:1 (8.86%), 18:0 (3.30%), 18:1 (14.06%), 18:2 (1.35%), 20:4 (1.05%), 20:5 (17.82%), 22:5 (2.78%), and 22:6 (11.49%), as verified by gas liquid chromatography (6). The amounts of vitamins A and E, as determined by high-performance liquid chromatography, were 92 and 526 μ g/g, respectively (6). Cholesterol content was analyzed by gas liquid chromatography and found to be 3.8 mg/g (6).

Analytical methods. Blood (20 ml) from unfasted patients was collected into EDTA (1 mg/ml) and centrifuged at $110 \times g$ for 15 min at room temperature. The platelet-rich plasma was removed and centrifuged at $1000 \times g$ for 15 min, then the platelet plug was resuspended in EDTA/NaCl buffer (pH 7) and recentrifuged, and the lipids were extracted with $\text{CHCl}_3/\text{CH}_3\text{OH}$ (2:1, 5 ml). The phospholipid fraction was obtained after separation of total lipid extracts by thin layer chromatography with a solvent system of hexane/diethyl ether/acetic acid/methanol (170:40:4:4, vol/vol) on silica gel 60 F₂₅₄ precoated aluminium sheets (Merck Art.5554, Rahway, NJ). Methyl esters were prepared by treatment of samples with 4% H_2SO_4 in methanol at 90°C for 20 min and analyzed by gas liquid chromatography with a BP 20 vitreous silica column (0.53 mm \times 12 m) (Scientific Glass Engineering, Melbourne, Australia) temperature programmed from 180 to 220°C at 5°C/min with hydrogen as carrier gas and a split ratio of 50:1. Peaks were identified by comparison with a known standard mixture of fatty acid methyl esters.

Cholesterol and triglycerides were determined enzymatically on an Abbott ABA-100 bichromatic analyzer with reagents (Pasadena, CA) and standardized with serum-based calibrators obtained from the Australian Lipids Standards Program with values traceable to the Centers for Disease Control (Atlanta, GA). The coefficients of variation (C.V.s) were 1.5 and 2.0%, respectively.

Serum HDL-chol was assayed on a heparinmanganese chloride supernatant (7). HDL₂ and HDL₃ were measured by the double precipitation method of Gidez et al. (8). Statistics calculated on 42 separate runs with a subject's aliquoted frozen (-70°C) serum were mean \pm SD total HDL-chol 1.61 ± 0.057 mM (C.V. 3.5%), mean HDL₃-chol 0.734 ± 0.063 mM (C.V. 8.6%), and mean HDL₂-chol 0.874 ± 0.057 mM (C.V. 6.5%). LDL-chol was calculated from the Friedewald formula (9).

Apolipoproteins (apo) A-I, A-II, and B were measured by single radial immunodiffusion with reagents from Immuno AG (Vienna). The apoB standard was prepared in our laboratory. Serum was snap frozen in liquid nitrogen and stored at -80°C until assayed, and samples were assayed in subject batches. C.V.s for apoAI, AII, and B were 5.2, 5.9, and 6.1%, respectively.

Statistics. Results are expressed as means \pm SE. Two-way analysis of variance for repeated measures was used to test for variations between visits with respect to treatment. Simple main effects analysis was used to examine the between-visit effect within each treatment group. Differences between baseline and any other visit were assessed by the Newman-Keuls test. Pearson product moment correlations were calculated for the relationship between changes in lipoproteins and apolipoproteins at baseline and completion of the supplement.

RESULTS

Group A was comprised of Max EPA patients without retinopathy. Results are presented separately for the Max EPA patients with retinopathy (group B). The baseline characteristics showed that the three groups were comparable for age, body mass index, kidney function, and degree of diabetic control (Table 1). Group B patients, however, were slightly lighter (67 kg) compared with the other groups (77 and 76 kg) and not surprisingly had a longer history of diabetes. Although some of the patients taking Max EPA showed a slight weight gain, the group means did not change significantly. The control group (group C), who were not given supplemental oil, lost weight (76.2 ± 4.7 vs. 74.8 ± 4.5 kg, $P < 0.05$ by paired t test). The supplement did not lead to any changes in plasma creatinine (group A, 86 ± 2 vs. 87 ± 3 μ M; group B, 87 ± 6 vs. 88 ± 7 μ M), plasma glucose (group A, 11 ± 2 vs. 11 ± 3 mM; group B, 9 ± 1 vs. 12 ± 4 mM), glycosylated hemoglobin (group A, 10.5 ± 1.1 vs. $11.4 \pm 0.6\%$; group B, 10.9 ± 1.8 vs. $10.7 \pm 0.4\%$), or insulin requirements. That is, none of the parameters associated with diabetic control were altered. All subjects complied with the protocol and reported no major changes in diet or life-style.

Compliance with the Max EPA regimen was confirmed by significant incorporation of EPA ($P < 0.001$) and concomitant replacement of arachidonic acid ($P < 0.001$) in platelet phospholipids in both Max EPA-sup-

plemented groups (Table 2). In addition, the levels of docosapentaenoic acid (22:5 ω 3) and DHA (22:6 ω 3), the two carbon elongated and desaturated metabolites of EPA, were significantly increased in both groups. The control group showed no alteration in the composition of these fatty acids.

The changes in plasma lipid levels are shown in Table 3. These indicate highly significant increases in total cholesterol and in LDL- and HDL-chol after Max EPA supplementation in patients both with and without retinopathy. The increase in HDL-chol was accounted for by a significant rise of $\sim 33\%$ in its HDL₂ subfraction. There was a corresponding decrease in HDL₃-chol but this was significant only in patients with retinopathy. Total cholesterol and LDL-chol remained elevated above basal values 6 wk after Max EPA was stopped. This was significant in patients without retinopathy. Slight elevations in HDL₂-chol also persisted at this time, particularly in patients with retinopathy. Serum triglycerides decreased significantly after Max EPA, with the fall being maintained at 6 wk. A fall in triglycerides was also observed in the control group, who were not given fish oil, and is most likely explained by weight loss in these patients.

There were no significant differences between the three groups at baseline, as assessed by analysis of variance. Furthermore, two-way analysis of variance for repeated measures showed no significant treatment or treatment-time interaction between the two Max EPA-supplemented groups. Both groups, however, showed a significant treatment-time interaction ($P < 0.05$) when compared with the control group. The changes observed in both Max EPA groups were not significantly different from one another, but both groups differed significantly from the control group. The changes in groups A and B can therefore be attributed to the fish oil intervention.

The changes in apoA1, apoAII, and apoB are shown in Table 4, and those for group A are shown in Fig 1. Although there was considerable variation between subjects, there were no significant differences in baseline values between the three groups. There was an increase

TABLE 1
Clinical and biochemical characteristics of patients

Characteristics	Max EPA groups		Control group
	No retinopathy	Retinopathy	
<i>n</i>	10	6	6
Age (yr)	33 ± 2	33 ± 2	30 ± 3
Weight (kg)	77 ± 3	67 ± 1	76 ± 5
History (yr)	8.5 ± 1.8	20.5 ± 2.9	6.6 ± 1.5
Insulin (U/day)	49 ± 6	45 ± 4	46 ± 9
Plasma creatinine (μ M)	86 ± 2	87 ± 6	82 ± 3
Plasma glucose (mM)	11 ± 2	9 ± 1	14 ± 5
Glycosylated hemoglobin (%)	10.5 ± 1.1	10.9 ± 1.8	11.0 ± 1.0

Values are means \pm SE. There were no significant differences for any variables at baseline between the 3 groups.

TABLE 2
Percentage incorporation of arachidonic acid and major ω -3 fatty acids into platelet phospholipids

Fatty acid	Baseline	End of intervention	Postintervention
Arachidonic (20:4 ω 6)			
A	39.75 \pm 2.43	29.32 \pm 1.10*	38.00 \pm 2.71
B	43.40 \pm 0.96	30.45 \pm 1.57†	41.65 \pm 2.96
C	50.08 \pm 4.24	47.53 \pm 2.14	47.42 \pm 2.97
Eicosapentaenoic (20:5 ω 3)			
A	0.58 \pm 0.21	3.67 \pm 0.46†	0.66 \pm 0.19
B	0.50 \pm 0.05	3.77 \pm 0.35†	0.52 \pm 0.19
C	0.37 \pm 0.10	0.41 \pm 0.07	0.43 \pm 0.07
Docosapentaenoic (22:5 ω 3)			
A	1.06 \pm 0.31	3.13 \pm 0.76†	0.90 \pm 0.29
B	0.47 \pm 0.17	3.12 \pm 0.85†	0.48 \pm 0.14
C	0.62 \pm 0.15	0.65 \pm 0.17	0.58 \pm 0.13
Docosahexaenoic (22:6 ω 3)			
A	0.74 \pm 0.26	2.92 \pm 0.61†	0.68 \pm 0.24
B	0.49 \pm 0.16	3.13 \pm 0.62†	0.64 \pm 0.23
C	0.48 \pm 0.08	0.54 \pm 0.10	0.46 \pm 0.02

Platelet phospholipid fatty acids in patients without retinopathy (A; $n = 10$) and with retinopathy (B; $n = 6$) during baseline, after 3 wk treatment with Max EPA, and 6 wk after ceasing Max EPA. Patients without retinopathy who were not given Max EPA but observed over the same period are included as control subjects (C; $n = 6$). Values are means \pm SE.

* $P < 0.01$, † $P < 0.001$, vs. significance of difference from baseline (Newman-Keuls). There were no significant differences for any fatty acids at baseline between the 3 groups.

in apoA1 after Max EPA in both groups A and B, although this was not significant (Table 4). Such a rise was observed in 7 of 10 patients in group A (Fig. 1) and 5 of 6 patients in group B. At 6 wk after Max EPA, values in most patients either remained elevated or returned to baseline levels. In Max EPA-treated patients, the rise in apoA1 was correlated with the rise in HDL-cholesterol ($r = 0.52$, $P < 0.05$) and to a lesser extent with that in the HDL₂ subfraction ($r = 0.34$, $P < 0.05$). The apoAII levels were depressed by Max EPA (Fig. 1), although the changes were small and significant only in group B (Table 4). However, the change in apoAII in the Max EPA groups was significantly correlated with the decrease in HDL₃-cholesterol ($r = 0.49$, $P < 0.05$). ApoB increased significantly during Max EPA intake in group A patients (Fig. 1, Table 4) but only marginally in group B patients (Table 4). There was a significant correlation between the rise in apoB and the change in LDL-cholesterol ($r = 0.61$, $P < 0.01$). Group C showed a gradual decrease in apoB levels during the study. Two-way analysis of variance for repeated measures showed a significant treatment-time interaction ($P < 0.05$) for group A compared with group C. Groups A and B were not different by two-way analysis of variance for repeated measures.

The increase in total cholesterol observed with Max EPA was significantly related to the increase in both apoA1 ($r = 0.57$, $P < 0.05$) and apoB ($r = 0.57$, $P < 0.05$). Generally, apoA1 was more closely associated with HDL₂-cholesterol, apoAII with HDL₃-cholesterol, and apoB with LDL-cholesterol.

DISCUSSION

We previously reported that Max EPA taken 15 g/day significantly increased total cholesterol in normolipidemic IDDM subjects (5). This was accounted for by a rise in both HDL- and LDL-cholesterol. The increase in total HDL-cholesterol could be further categorized by an increase in the HDL₂-cholesterol subfraction. Serum triglycerides were significantly reduced. In this study, we present additional data on these subjects, demonstrating that these changes are associated with alterations in apolipoprotein levels.

Patients taking Max EPA were separated into groups with and without retinopathy, a decision that was made before commencement of the study. The reason for this was that patients with retinopathy clearly have diabetic microvascular disease, which therefore indicates a more progressive disease. The subgroup with more aggressive or advanced vascular disease could reasonably be expected to have a more adverse lipid profile and may respond differently to fish oils. However, the results of this study suggest that this effect does not appear to be more pronounced when diabetes is complicated by retinopathy.

To our knowledge, these results represent the first comprehensive analysis of the effect of fish oils on serum lipids in IDDM subjects. This is despite numerous studies in which fish oil supplementation has been examined in both non-insulin-dependent diabetic (NIDDM; 10–13) and IDDM subjects (14–17). The most consistent

TABLE 3
Effect of Max EPA on lipid profiles in insulin-dependent diabetic subjects

	Baseline (mM)	End of intervention (mM)	Postintervention (mM)
Total cholesterol			
A	4.48 ± 0.20	4.90 ± 0.23*	4.74 ± 0.16†
B	4.77 ± 0.39	5.13 ± 0.48*	4.92 ± 0.30
C	5.18 ± 0.33	5.20 ± 0.26	5.08 ± 0.48
Low-density lipoprotein cholesterol			
A	2.44 ± 0.14	2.95 ± 0.18‡	2.84 ± 0.11‡
B	2.92 ± 0.36	3.27 ± 0.44*	3.12 ± 0.34
C	2.88 ± 0.18	3.08 ± 0.13	2.92 ± 0.35
HDL cholesterol			
A	1.38 ± 0.08	1.56 ± 0.08*	1.39 ± 0.09
B	1.26 ± 0.12	1.38 ± 0.15†	1.33 ± 0.17
C	1.41 ± 0.08	1.45 ± 0.10	1.48 ± 0.12
HDL ₂ cholesterol			
A	0.66 ± 0.07	0.88 ± 0.08‡	0.71 ± 0.08
B	0.59 ± 0.10	0.79 ± 0.13‡	0.69 ± 0.15†
C	0.79 ± 0.06	0.81 ± 0.08	0.78 ± 0.11
HDL ₁ cholesterol			
A	0.72 ± 0.03	0.68 ± 0.03	0.68 ± 0.03
B	0.68 ± 0.05	0.58 ± 0.04‡	0.65 ± 0.03
C	0.68 ± 0.03	0.70 ± 0.03	0.70 ± 0.05
Triglycerides			
A	1.44 ± 0.14	0.85 ± 0.08*	1.10 ± 0.11†
B	1.27 ± 0.20	1.07 ± 0.11†	1.02 ± 0.15†
C	1.93 ± 0.50	1.45 ± 0.47*	1.50 ± 0.38*

Serum lipids in patients without retinopathy (A; *n* = 10) and with retinopathy (B; *n* = 6) during baseline, after 3 wk treatment with Max EPA, and 6 wk after ceasing Max EPA. Patients without retinopathy not given Max EPA were observed over the same period as control subjects (C; *n* = 6). Values are means ± SE. HDL, high-density lipoprotein.

**P* < 0.01, †*P* < 0.05, ‡*P* < 0.001, vs. significance of difference from baseline (Newman-Keuls). There were no significant differences for any variables at baseline between the 3 groups.

finding has been a fall in triglycerides both in NIDDM subjects, with either normal or elevated lipids (10–12), and in IDDM subjects (17). The most likely mechanism

for this fall in triglycerides is suppression of very-low-density lipoprotein (VLDL) synthesis in the liver or increased VLDL removal (18). However, a study in IDDM

TABLE 4
Effect of Max EPA on apolipoproteins (apo) A-I, A-II, and B in insulin-dependent diabetic subjects

	Baseline (g/L)	End of intervention (g/L)	Postintervention (g/L)
ApoAI			
A	1.35 ± 0.06	1.46 ± 0.05	1.43 ± 0.06
B	1.31 ± 0.08	1.38 ± 0.10	1.40 ± 0.12
C	1.52 ± 0.04	1.48 ± 0.07	1.47 ± 0.08
ApoAII			
A	0.49 ± 0.03	0.48 ± 0.02	0.49 ± 0.02
B	0.49 ± 0.02	0.44 ± 0.02*	0.47 ± 0.03
C	0.55 ± 0.04	0.55 ± 0.04	0.55 ± 0.05
ApoB			
A	0.76 ± 0.04	0.82 ± 0.05†	0.75 ± 0.03
B	0.89 ± 0.09	0.90 ± 0.10	0.82 ± 0.07
C	0.92 ± 0.07	0.87 ± 0.06	0.76 ± 0.09

ApoAI, AII, and B in patients without retinopathy (A; *n* = 10) and with retinopathy (B; *n* = 6) during baseline, after 3 wk treatment with Max EPA, and 6 wk after ceasing Max EPA. Patients without retinopathy who were not given Max EPA but observed over the same period are included as control subjects (C; *n* = 6). Values are means ± SE.

**P* < 0.01, †*P* < 0.05, vs. significance of difference from baseline (Newman-Keuls). There were no significant differences for any variables at baseline between the 3 groups.

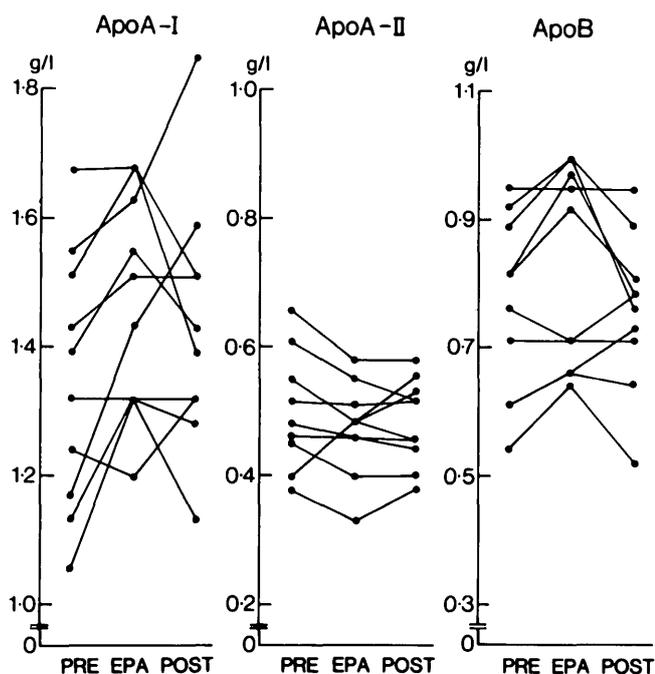


FIG. 1. Individual serum apolipoprotein A-I (ApoA-I), A-II (ApoA-II), and B (ApoB) levels in patients without retinopathy (group A; $n = 10$) during baseline (PRE), after 3 wk treatment with Max EPA (EPA), and 6 wk after ceasing Max EPA (POST).

subjects, with an almost identical Max EPA regimen to that used in this study, failed to show a change in triglyceride levels (16). Similarly, in the results presented herein, a specific effect of Max EPA cannot be claimed, because a similar fall in triglycerides occurred in untreated control subjects, which was possibly due to the weight loss observed in these patients. However, because it was considered undesirable to obtain fasting blood samples, interpretation of changes in triglycerides under these circumstances is difficult. The weight loss in the control group may perhaps be explained by some small dietary modification that was induced by regular patient-investigator contact and compensated for in patients taking fish oil by the extra calorie intake.

Variable changes have been reported in the plasma total cholesterol concentration after consumption of fish oil, with only the highest dosages resulting in a fall (1–3). Similarly, the changes in both HDL- and LDL-cholesterol have been inconsistent and like total cholesterol appear to depend on the quantity of fish oil provided (1,2). Generally, the HDL-cholesterol subfractions HDL₂ and HDL₃ have not been examined in fish oil feeding experiments, although in one study an increase in HDL₃-cholesterol was found in healthy subjects after a moderate Max EPA supplement (19). Changes in apolipoproteins have not, with the exception of apoB, been described (4,11,18,20). Results from studies in which fish oils have been given to diabetic subjects are somewhat varied (10–17). In patients with NIDDM consuming 12 g/day Max EPA for 4 wk, the levels of total cholesterol and LDL-cholesterol were

unaffected, although apoB was increased (11). However, when a greater quantity of ω 3FA was used, there was a decrease in total cholesterol (12). Small quantities of ω 3FA (<1 g EPA/day) had no effect on cholesterol and lipoproteins in both NIDDM and IDDM subjects (13–15).

The rise in total cholesterol observed here is a consequence of the increased cholesterol content of LDL and HDL, particularly the HDL₂ subfraction. In contrast, it was reported that a small but significant increase in total cholesterol in normolipidemic IDDM subjects given an almost identical amount of Max EPA was due to a rise in LDL-cholesterol with no appreciable change in HDL-cholesterol (16). In another study, IDDM subjects receiving 20 g Max EPA/day for 8 wk showed an initial increase in total cholesterol and LDL-cholesterol after 3 wk, which had returned to pretreatment levels at its completion (17). The changes in HDL-cholesterol were not significant. This study was, however, limited in that only five patients were included. Neither of these studies mentioned changes in apolipoprotein concentration (16,17).

In this study, we observed that the increase in apoAII after Max EPA was significantly correlated with the rise in HDL-cholesterol. Generally, apoAII is more closely correlated with HDL₂-cholesterol than the other lipoproteins, and this is supported by the findings here. The significant decrease in apoAII in patients with retinopathy is consistent with the fall in the HDL₃-cholesterol subclass seen in this group. These findings are in accordance with the HDL₂-cholesterol subfraction predominantly containing apoAII, whereas HDL₃-cholesterol contains both apoAII and apoAIII. Consequently, HDL₃-cholesterol is richer in apoAIII.

Of particular interest is the rise in apoB seen in patients without retinopathy and to a lesser extent in those with retinopathy, which was significantly correlated with the increase in LDL-cholesterol. This is consistent with the fact that most of the circulating apoB resides in the LDL class. An increase in LDL-cholesterol, after both high- and low-dose fish oil supplements, was observed in patients with type V hyperlipidemia. (4,21). The same quantity of Max EPA that was used in our study also increased LDL-cholesterol in IDDM subjects, in patients with hypertriglyceridemia, and in healthy subjects (16,20). These changes in LDL-cholesterol have been associated with an increase in apoB (11,18,20).

These results pose the question regarding the possible mechanism by which moderate amounts of fish oils increase LDL-cholesterol and LDL-apoB. It is well established that fish oils lower triglyceride concentration by reducing the synthesis of both triglyceride and apoB in VLDL (18,22). Because LDL-apoB is derived from VLDL-apoB, decreased levels of VLDL-apoB would be expected to result in decreased levels of LDL-apoB (23,24). Results of this and other studies indicate that this is not the case (11,18,20). A possible mechanism may be that fish oils lead to an increased catabolism of VLDL to LDL, although evidence suggests that Max EPA decreases VLDL by impairment of synthesis (18,22). A more likely explanation has been suggested, whereby a moderate in-

take of fish oil decreases hepatic triglyceride synthesis resulting in smaller than normal VLDL particles being secreted (20). These smaller and denser particles are known to be more readily converted to LDL than the larger triglyceride-rich particles (25). It has also been shown that LDL-apoB is mainly derived from these smaller VLDL particles (25). Thus, alterations in VLDL particles secreted by the liver could influence the conversion of VLDL-apoB to LDL-apoB. The reduced availability of triglycerides for incorporation into VLDL after fish oil intake may also determine the size and density of the VLDL particle formed. A third explanation for the observed results is that fish oils may induce a substantial independent and direct secretion of LDL-like particles (18).

It cannot be disregarded that elevated serum LDL-cholesterol is strongly linked to coronary and other atherosclerotic vascular diseases. Furthermore, case-control studies suggest that LDL-apoB concentrations are strongly associated with such risk (26,27). Thus, although a reduction in VLDL synthesis by diets supplemented with fish oils could confer some benefit, the accompanying increase in LDL-cholesterol and LDL-apoB may be deleterious.

In conclusion, we have shown that adding moderate amounts of Max EPA to the diet of IDDM subjects has a hypercholesterolemic effect. Both LDL- and HDL-cholesterol are increased resulting in a rise in total cholesterol. The rise in HDL-cholesterol appears to be due to its HDL₂ subclass, which is considered the major fraction responsible for the protective effect of HDL-cholesterol in vascular disease (28). The changes in apolipoproteins were closely correlated with changes in the lipoproteins, particularly between HDL-cholesterol and apoAI and HDL₃-cholesterol and apoAII, as well as between LDL-cholesterol and apoB. ApoB and apoAI levels were elevated, whereas apoAII levels were decreased after consumption of fish oil.

In view of these results, it would appear that caution should be used before recommending dietary supplementation with fish oils in IDDM subjects, at least in those with relatively normal lipid profiles. However, these findings are not necessarily incompatible with an overall protective effect of fish oils through other mechanisms favorably modifying platelet function, platelet-endothelial interactions, and the inflammatory response.

ACKNOWLEDGMENTS

This work was supported by the Diabetes Research Foundation of Western Australia, the Raine Centre for Cardiovascular Studies, and the National Heart Foundation of Australia. Max EPA capsules were donated by Scherer, Melbourne, Australia.

We thank Dr. Kim Stanton, who kindly provided assistance in the recruitment of diabetic patients from the Royal Perth Hospital diabetic clinic; Didi Dunbar for nursing skills; Lynette Kelly for technical assistance; and

Kevin Dwyer for analysis of the serum lipids. Statistical analysis of the data was performed by Dr. Ian Rouse.

REFERENCES

1. Herold PM, Kinsella JE: Fish oil consumption and decreased risk of cardiovascular disease: a comparison of findings from animal and human feeding trials. *Am J Clin Nutr* 43:566-98, 1986
2. Sanders TAB: Influence of fish oil supplements on man. *Proc Nutr Soc Engl Scot* 44:391-97, 1985
3. Sanders TAB, Roshanai F: The influence of different types of ω 3 fatty acids on blood lipids and platelet function in healthy volunteers. *Clin Sci* 64:91-99, 1983
4. Phillipson BE, Rothrock DW, Connor WE, Harris WS, Illingworth DR: Reduction of plasma lipids, lipoproteins, and apoproteins by dietary fish oils in patients with hypertriglyceridemia. *N Engl J Med* 312:1210-16, 1985
5. Vandongen R, Mori TA, Codde JP, Stanton KG, Masarei JRL: Hypercholesterolaemic effect of fish oil in insulin-dependent diabetics. *Med J Aust* 148:141-43, 1988
6. Mori TA, Codde JP, Vandongen R, Beilin LJ: New findings in the fatty acid composition of individual platelet phospholipids in man after dietary fish oil supplementation. *Lipids* 22:744-50, 1987
7. Warnick GR, Albers JJ: A comprehensive evaluation of the heparin-manganese precipitation procedure for estimating high density lipoprotein cholesterol. *J Lipid Res* 19:65-76, 1978
8. Gidez LI, Miller GJ, Burstein M, Slagle S, Eder HA: Separation and quantitation of subclasses of human plasma high density lipoproteins by a single precipitation procedure. *J Lipid Res* 23:1206-23, 1982
9. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin Chem* 18:499-502, 1972
10. Popp-Snijders C, Schouten JA, Heine RJ, Van der Meer J, Van der Veen EA: Dietary supplementation of omega-3 fatty acids improves insulin sensitivity in non-insulin dependent diabetes mellitus. *Diabetes Res* 4:141-47, 1987
11. Schectman G, Kaul S, Kissebah A: Effects of fish oil on plasma lipoproteins and platelet function in non-insulin-dependent diabetes mellitus (NIDDM) (Abstract). *Diabetes* 36 (Suppl. 1):12A, 1987
12. Friday KE, Childs M, Tsunehara C, Fujimoto WY, Bierman EL, Ensnick JW: Omega 3 fatty acid supplementation has discordant effects on plasma glucose and lipoproteins in type II diabetes (Abstract). *Diabetes* 36 (Suppl. 1):12A, 1987
13. Kamada T, Yamashita T, Baba Y, Kai M, Setoyama S, Chuman Y, Otsuji S: Dietary sardine oil increases erythrocyte membrane fluidity in diabetic patients. *Diabetes* 35:604-11, 1986
14. Velardo B, Lagarde M, Guichardant M, Dechavanne M, Beylot M, Sautot G, Berthezene F: Decrease of platelet activity after intake of small amounts of eicosapentaenoic acid in diabetics. *Thromb Haemostasis* 48:344, 1982
15. Beitz J, Schimke E, Liebaug U, Block H-U, Beitz A, Honigsmann G, Sziegoleit W, Muller G, Mest H-J: Influence of a cod liver oil diet in healthy and insulin-dependent diabetic volunteers on fatty acid pattern, inhibition of

- prostacyclin formation by low density lipoprotein (LDL) and platelet thromboxane. *Klin Wochenschr* 64:793–99, 1986
16. Haines AP, Sanders TAB, Imeson JD, Mahler RF, Martin J, Mistry M, Vickers M, Wallace PG: Effects of a fish oil supplement on platelet function, hemostatic variables and albuminuria in insulin-dependent diabetics. *Thromb Res* 43:643–55, 1986
 17. Miller ME, Anagnostou AA, Ley B, Marshall P, Steiner M: Effect of fish oil concentrates on hemorrheological and hemostatic aspects of diabetes mellitus: a preliminary study. *Thromb Res* 47:201–14, 1987
 18. Nestel PJ, Conner WE, Reardon MF, Connor S, Wong S, Boston R: Suppression by diets rich in fish oil of very low density lipoprotein production in man. *J Clin Invest* 74:82–89, 1984
 19. Sanders TAB, Mistry M: Controlled trials of fish oil supplements on plasma lipid concentrations. *Br J Clin Pract* 38:78–81, 1984
 20. Sullivan DR, Sanders TAB, Trayner IM, Thompson GR: Paradoxical elevation of LDL apoprotein B levels in hypertriglyceridemic patients and normal subjects ingesting fish oil. *Atherosclerosis* 61:129–34, 1986
 21. Simons LA, Hickie JB, Balasubramaniam S: On the effects of dietary n-3 fatty acids (max EPA) on plasma lipids and lipoproteins in patients with hyperlipidemia. *Atherosclerosis* 54:75–88, 1985
 22. Sanders TAB, Sullivan DR, Reeve J, Thompson GR: Triglyceride-lowering effect of marine polyunsaturates in patients with hypertriglyceridemia. *Arteriosclerosis* 5:459–65, 1985
 23. Schaefer EJ, Levy RI: Pathogenesis and management of lipoprotein disorders. *N Engl J Med* 31:1300–310, 1985
 24. Sigurdsson G, Nicholl A, Lewis B: Conversion of very low density lipoprotein: a metabolic study of apolipoprotein B kinetics in human subjects. *J Clin Invest* 56:1481–90, 1975
 25. Packard CJ, Munro A, Lorimer AR, Gotto AM, Shepherd J: Metabolism of apolipoprotein B in large triglyceride-rich very low density lipoprotein of normal and hypertriglyceridemic subjects. *J Clin Invest* 74:2178–92, 1984
 26. Sniderman A, Shapiro S, Marpole D, Skinner B, Teng B, Kwiterovich PO: Association of coronary atherosclerosis with hyperapoproteinemia (increased protein but normal cholesterol levels in human low density [beta] lipoproteins). *Proc Natl Acad Sci USA* 77:604–608, 1980
 27. Sniderman AD, Wolfson C, Teng B, Franklin FA, Bachoriok PS, Kwiterovich PO: Association of hyperapoproteinemia with endogenous hypertriglyceridemia and atherosclerosis. *Ann Intern Med* 97:833–39, 1982
 28. Miller NE, Hammett F, Saltissi S, Rao S, van Zeller H, Coltart J, Lewis B: Relation of angiographically defined coronary artery disease to plasma lipoprotein subfractions and apolipoproteins. *Br Med J* 282:1741–44, 1981