Effects of Low-Dose EPA-E on Glycemic Control, Lipid Profile, Lipoprotein(a), Platelet Aggregation, Viscosity, and Platelet and Vessel Wall Interaction in NIDDM

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OBJECTIVE — To assess the effects of low-dose eicosapentaenoic acid-ethyl-ester on diabetes regulation, lipid metabolism, blood rheology, and platelet reactivity.

RESEARCH DESIGN AND METHODS — In a double-blind, randomized, placebo-controlled study, 24 NIDDM subjects received 1800 mg of EPA-E, 900 mg of EPA-E, or a placebo (1656 mg olive oil) daily for 8 wk.

RESULTS — The EPA:arachidonic acid plasma ratio increased over an 8-wk period, then declined after a 4-wk wash-out period in the fish-oil groups in a dose-dependent way. Platelet-activating factor–induced platelet aggregation decreased from 75 ± 7% at wk 0 to 35 ± 21% at wk 8 in the 900-mg group ($P = 0.016$) and from 72 ± 11 to 40 ± 30% in the 1800-mg group ($P = 0.039$), but did not change in the placebo group. No effects on ADP- or collagen-induced aggregation could be attributed to EPA-E. In the 1800-mg group low-density-lipoprotein cholesterol increased significantly, without concomitant rise in apolipoprotein B. Triglycerides, glycemic control, lipoprotein (a), blood and plasma viscosity, erythrocyte deformability, and platelet adhesion to and aggregate formation on extracellular endothelial cell matrix were not significantly influenced.

CONCLUSIONS — Purified EPA-E in doses of 900 and 1800 mg reduces Platelet-activating factor–induced platelet aggregation without negatively affecting glycemic control. Low-density-lipoprotein cholesterol was elevated in the 1800-mg group.

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RECEIVED FOR PUBLICATION 7 APRIL 1992 AND ACCEPTED IN REVISED FORM 17 DECEMBER 1992.

EPA-E, EICOSAPENTAENOIC ACID-ETHYL-ESTER; NIDDM, NON-INSULIN-DEPENDENT DIABETES MELLITUS; AA, ARACHIDONIC ACID; PAF, PLATELET-ACTIVATING FACTOR; LDL, LOW-DENSITY LIPOPROTEIN; Lp(a), LIPOPROTEIN (a); apoB, APOLIPOPROTEIN B; TG, TRIGLYCERIDE; ECM, ENDOTHELIAL CELL MATRIX; CHD, CORONARY HEART DISEASE; DHA, DOCOSAHEXENOIC ACID; CVD, CARDIOVASCULAR DISEASE; HDL, HIGH-DENSITY LIPOPROTEIN; VLDL, VERY-LOW-DENSITY LIPOPROTEIN; ANOVA, ANALYSIS OF VARIANCE; TXB, THROMBOXANE; BMI, BODY MASS INDEX.

Consumption of fish containing n-3 polyunsaturated fatty acids reduced the incidence of CHD in epidemiological studies (1). N-3 fatty acids, such as EPA and DHA, can favorably alter the risk profile for atherosclerotic vascular disease by lowering plasma TG (2–7) and decreasing platelet reactivity (8–14) and blood viscosity (14–17). Patients with diabetes mellitus, who are at high risk for cardiovascular complications, therefore may benefit from long-term consumption of fish oil.

Fish-oil consumption, however, can deteriorate glycemic control (4,21, 22) and elevate LDL-cholesterol and apoB levels (6,23–25) in NIDDM subjects. These adverse effects appear to be dose dependent and have been described in studies using different mixtures of EPA and DHA. Our study was designed to search for a low dose of highly purified EPA-E that would have favorable effects on platelet function and rheological properties of blood without deleterious effects on diabetes regulation and lipid metabolism in NIDDM subjects.

RESEARCH DESIGN AND METHODS

— We selected 24 subjects with NIDDM, as defined by WHO criteria, from the outpatient clinic of the University Hospital Utrecht, according to the following inclusion criteria: no manifestations of hepatic, renal, gastrointestinal, or hematological disease; and no manifestations of CVD in the previous 3 mo. The use of drugs likely to modify plasma lipids or platelet function, especially aspirin, was not allowed. The metabolic status of the subjects, monitored twice over a 1-mo prestudy observation period had to be stable. Table 1 summarizes patient characteristics at the onset of the study.

The subjects were selected randomly to receive 1800 mg of EPA-E, 900 mg of EPA-E, or a placebo (1656 mg olive oil) in a double-blind fashion. All subjects received two capsules three times/day for 8 wk. This was followed by

Table 1— *Clinical characteristics of study subjects at baseline*

	Study subjects		
	1800 mg	900 mg	Placebo
Age (yr)*	56.5 (37–71)	51 (39–62)	62 (55–68)
Sex (M/F)	5/3	5/3	5/3
Weight* (kg)	66.3 (56–83)	78.9 (70–90)	77.7 (73–84)
BMI (kg/m^2)*	23.5 (20–30)	26.8 (21–29)	28.6 (25–32)
Duration of diabetes (yr)*	6.5 (2–15)	4 (1–12)	10 (2–21)
P/S*†	0.55 (0.2–1)	0.4 (0.2–0.7)	0.3 (0.1–1)
Treatment of diabetes			
Sulphonylurea derivatives	3	4	4
Diet	4	3	3
Tolbutamide		1	
Insulin	1		
apoE phenotype	5× 3/3;	7× 3/3	6× 3/3
	2× 4/3; 1× 4/4	1× 3/2	1× 3/2
Alcohol consumption (10-g units/day)*	0.3 (0–2)	0.3 (0–2)	0.6 (0–3)
Cholesterol (mM)‡	6.3 ± 1 (5.2–8.0)	6.1 ± 1.1 (5.0–8.6)	6.5 ± 1.1 (4.5–8.2)
TG (mM)‡	1.7 ± 1 (0.6–3.6)	2.45 ± 1.9 (0.9–6)	2 ± 0.25 (1.7–2.2)
HbA_1 (%)‡	8.6 ± 2.7 (4.9–13)	7.6 ± 2.9 (5.2–12.8)	9.2 ± 2.7 (6.1–13.2)

*Data are median (range).
†Ratio of polyunsaturated to saturated fatty acids in the diet.
‡Data are means ± SD (range).

a 4-wk wash-out period. For the group receiving 1800 mg, all six capsules contained 300 mg of 93.6% highly purified EPA-E (MND 21, Rhône Poulenc Santé, Antony Cedex, France). For the placebo group all six capsules contained 0.3 ml (276 mg) of olive oil. The 900-mg group received one capsule of the 300-mg EPA-E and one of the olive-oil placebos three times/day. All capsules were gelatin coated and indiscernible.

Each patient provided a dietary history before the study and was instructed not to change diet, alcohol intake (expressed as 10-g units), smoking habits, or other therapies throughout the study. Subjects were told not to use alcohol during the 24 h before each visit.

They came to the clinic at wk 0, 2, 4, 8, and plus 4 wk for a review of side effects, a capsule count, a physical examination, and for collection of fasting blood samples.

Hematological parameters

We determined bleeding time with a Simplate-II device. Plasma fibrinogen level was determined according to Clauss (28). We performed platelet aggregations in a PAP-4 aggregometer on platelet-rich (250,000) plasma from citrate anticoagulated blood, in response to equine collagen (1 and 4 μg/ml), ADP (2.5 and 5 μM), and PAF (0.1, 0.2, 0.5, and 5 μM). The maximal aggregation achieved within 5 min after the addition of collagen, ADP, or PAF was expressed as a percentage of 100% light transmission (39). We evaluated only those subjects with a concentration of PAF, ADP, or collagen-inducing aggregation at wk 0 of >50% (13). We did not evaluate subjects with aggregation responses <50% at wk 0.

Lipid profile

Lipoprotein classes were fractionated by one-step preparative ultracentrifugation, as described previously (29). Cholesterol and TG were determined enzymatically in total plasma and in fractions of HDL, LDL, and VLDL. Plasma Lp(a) was determined with immunoradiometric assay (30). Plasma apoA and apoB were determined nephelometrically (31).

Biochemical tests

We performed biochemical tests on a Hitachi 717 autoanalyzer. HbA_1 was measured with boronate affinity chromatography using Pierce reagents as described previously (32). The GHb reference value in our laboratory is 3.5–6.5%.

Viscosity

Whole blood and platelet-poor plasma viscosity were determined with a Couette-type viscosimeter. Erythrocyte deformability was measured with laser diffractometry, as described previously (33).

Perfusion experiments

Platelet adhesion to and aggregate formation on extracellular ECM was measured in a perfusion model with recirculating blood, as described previously (34,36).

Table 2—*The influence of dietary supplementation with 1800-mg EPA-E, 900-mg EPA-E, or placebo during 8 wk on PAF-, ADP-, and collagen-induced platelet aggregation*

Platelet aggregation	Wk 0	Wk 8	P value
PAF			
1800 mg	72 ± 11	40 ± 30	0.039*
900 mg	75 ± 7	35 ± 21	0.016*
Placebo	78 ± 7	68 ± 29	>0.5
ADP			
1800 mg	79 ± 9	56 ± 23	0.031*
900 mg	63 ± 10	58 ± 27	>0.5
Placebo	66 ± 13	59 ± 21	>0.5
Collagen			
1800 mg	78 ± 3	79 ± 7	>0.5
900 mg	77 ± 6	75 ± 13	>0.5
Placebo	80 ± 5	74 ± 17	>0.5

Data are means ± SD. Wilcoxon signed-rank tests were used to analyze platelet aggregation responses. PAF-, ADP-, and collagen-induced aggregations were expressed as the percentage of maximal light transmission (100%).
*$P < 0.05$ between wk 0 and wk 8.

Statistical analysis

We used repeated measures ANOVA to test for significant differences among groups. $P < 0.05$ was considered significant. Wilcoxon signed-rank tests analyzed the platelet-aggregation responses and Lp(a) levels, which are not normally distributed. The bleeding times were analyzed with Student's *t* tests for paired samples.

RESULTS— As shown in Table 1, 24 NIDDM subjects completed the study. None had a history of CVD, and no adverse effects were recorded. Capsule counts and EPA-E plasma concentrations proved good compliance. Body weights did not change from wk 0 to wk 4 to wk 8 to plus 4 wk, and median values were 66.3 (1800 mg), 78.9 (900 mg), and 77.7 kg (placebo) at baseline. The mean body weight in the 1800-mg group was significantly lower than in the 900-mg and placebo groups ($P = 0.006$).

EPA-E plasma concentrations

The EPA-E plasma concentrations, expressed as the EPA:AA ratio, increased from 0.124 ± 0.07 (wk 0) to 0.334 ± 0.10 (wk 4) to 0.308 ± 0.10 (wk 8) in the 900-mg group and from 0.132 ± 0.13 (wk 0) to 0.613 ± 0.23 (wk 4) to 0.675 ± 0.25 (wk 8) in the 1800-mg group. At plus 4 wk, the plasma levels had declined to 0.187 ± 0.13 in the 900-mg group and to 0.126 ± 0.05 in the 1800-mg group.

Glycemic control and lipids

Glucose and HbA_1 levels showed no significant difference between the groups during the study (Table 1). This remained the case after correction for weight and Quetelet index. The increase in glucose values between wk 0 and plus 4 wk in each group was not statistically significant. The HbA_1 values (mean ± SD) showed no significant differences within each group from wk 0 to wk 8: 8.2 ± 2.8 to 7.9 ± 2.1 in the 1800-mg group ($P = 0.3$); 7.6 ± 2.9 to 8.1 ± 2.8 in the 900-mg group ($P = 0.37$); 9.2 ± 2.7 to 9.3 ± 3 in the placebo group ($P = 0.9$).

The lipid variables did not demonstrate statistically different trends among groups with repeated measures ANOVA. Performing Student's *t* tests separately by treatment group showed a slight but significant increase in LDL-cholesterol level in the 1800-mg group: 4.2 ± 0.8 (wk 0); 4.5 ± 0.9 (wk 4); 4.8 ± 1.1 (wk 8). *P* values were 0.04 for wk 0 versus wk 4 and 0.014 for wk 0 versus wk 8.

Concentrations of apoB were 0.9 ± 0.2 g/L in the 1800-mg group, 0.9 ± 0.3 g/L in the 900-mg group, and 0.9 ± 0.2 g/L in the placebo group. The concentrations did not change significantly, as with the other lipid parameters. The baseline values of Lp(a) were 259 ± 210 mg/L. The Lp(a) changes in the treatment groups were not significant.

Platelet aggregation studies and bleeding time

Platelet aggregation results from 4 subjects were excluded from analysis, because the platelet aggregation responses were <50% at wk 0. As shown in Table 2, PAF-induced platelet aggregation decreased significantly in both EPA-E groups from wk 0 to wk 8: 72 ± 11 to 40 ± 30% in the 1800-mg group ($P = 0.039$); and 75 ± 7 to 35 ± 21% in the 900-mg group ($P = 0.016$). It did not change significantly in the placebo group: 78 ± 7 to 68 ± 29% ($P > 0.5$).

ADP-induced platelet aggregation at wk 0 was significantly higher in the 1800-mg group versus the 900-mg and placebo groups. The ADP aggregation was significantly reduced in the 1800-mg group: 79 ± 9 to 56 ± 23% ($P = 0.031$) and did not change in the 900-mg or placebo groups (Table 2). The collagen-induced platelet aggregation did not change, nor did the Simplate bleeding time.

Platelet and vessel wall interaction

The platelet adhesion, measured as total coverage of ECM with adherent platelets, showed no significant difference between groups. The platelet aggregation, measured as number and size of aggregates, demonstrated no significant difference between groups (data not shown).

Viscosity, plasma protein, and fibrinogen

No significant between-group differences were found in plasma viscosity, whole blood viscosity, erythrocyte deformability, total plasma protein, and fibrinogen (data not shown).

CONCLUSIONS— The purpose of this study was to find a low dose of fish oil with beneficial effects on platelet reactivity and no adverse effects on lipid levels or glycemic control. Highly purified EPA-E was used in daily 900- and 1800-mg doses. Resorption was adequate and dose dependent, according to the EPA-E plasma concentrations.

Reduced platelet reactivity is an important mechanism by which consumption of fish oil contributes to a reduced risk for atherosclerotic vascular disease. The platelet aggregation (platelet-platelet interaction) to PAF was significantly reduced in both EPA-E groups, but not in the placebo group, so this effect is attributable to EPA-E. The likely mechanism is an EPA-E–induced shift from production of platelet TXB-A2 to TXB-A3, the latter causing less aggregation and vasoconstriction.

If the plasma EPA:AA ratio reflects the EPA:AA concentration in the platelet (11), the reduction in TXB-A2 and subsequent reduction in platelet aggregation should be proportional to the dose of EPA-E. Our results show a similar reduction in PAF aggregation in both the 900- and 1800-mg groups. Possible explanations are: *1*) in the 1800-mg group, LDL cholesterol, which enhances platelet aggregation (47), is elevated at wk 8 and counteracts the platelet inhibitory effect of EPA-E; or 2) PAF aggregation is only partly TXB dependent (48). As far as we know, higher body weights or the combination of olive oil and EPA-E in the 900-mg group cannot influence platelet aggregation. The 1800-mg group has higher baseline ADP aggregations, so the significant reduction in ADP aggregation in this group could not be ascribed with certainty to EPA-E.

The Simplate bleeding time, an in vivo test for platelet and vessel wall interaction, did not change, as observed in other studies with diabetic patients (39,50). The perfusion experiments serve as an ex vivo test for platelet-vessel wall and platelet-platelet interaction. Adhesion, measured as total coverage of adherent platelets on ECM, is not influenced by EPA-E. This finding is in agreement with the unaltered Simplate bleeding time, but stands in contrast to results from other studies that found a decrease in total coverage (19) and to observations made in healthy volunteers using mixtures of EPA and DHA (20). In diabetes mellitus, higher doses of purified EPA-E or mixtures of EPA and DHA appear to be required to influence in vivo and ex vivo platelet and vessel wall interaction. The same applies to rheological parameters (14–17).

In the 1800-mg group LDL cholesterol increased during EPA-E supplementation, as found by others (21,49). Concentrations of apoB were unaffected. This unfavorable effect can reflect an increased conversion from VLDL to LDL particles or a decreased clearance of LDL particles.

A consistent effect of fish oil is a reduction of plasma TG. This is ascribed to reduced production of VLDL-TG in the liver (3). The effect is dose dependent and more pronounced when initial TG levels are higher. In this study, baseline TG values were nearly normal (Table 1) and did not change. This has been described before in normolipidemic NIDDM, using 1.6 g of EPA and 1.2 g of DHA (23). Higher doses of fish oils probably are required to reduce TG in diabetes mellitus.

Serum levels of Lp(a) >300 mg/L are considered a risk factor for CHD (26). Some studies have suggested a relationship among Lp(a), diabetes, and fish-oil consumption (27,43–45). Supplementation with EPA-E did not influence Lp(a) in our study. Neither dose of EPA-E influenced glycemic control.

This study demonstrates that both 900- and 1800-mg doses of EPA-E can reduce PAF-induced platelet aggregation without affecting glycemic control. The unaltered lipid variables in the 900-mg group and the slight elevation of LDL cholesterol in the 1800-mg group suggests a 900-mg dose of EPA-E can be administered safely to NIDDM patients.

Acknowledgments—J.C. de Graaf received a grant from The Netherlands Heart Foundation (86072).

We thank Margot Gerrits for technical assistance, Martijn Akveld for determining the Lp(a) concentrations; the patients, nurses, and staff of the diabetes outpatient clinic for their cooperation; Herman Wijnne and Cas Kruitwagen for performing the statistics, and Rhône-Poulenc for providing us with the fish-oil capsules.

This study was presented in part at the XIII Congress of the International Society on Thrombosis and Hemostasis in July 1991.

References

1. Kromhout D, Bosschieter EG, de Lezenne Coulander C: The inverse relation between fish consumption and 20-year mortality from coronary heart disease. *N Engl J Med* 312:1205–209, 1985
2. Green P, Fuchs J, Schoenfeld N, Leibovici L, Lurie Y, Beigel Y, Rotenberg Z, Mamet R, Budowski P: Effects of fish oil ingestion on cardiovascular risk factors in hyperlipidemic subjects in Israel: a randomized, double-blind crossover study. *Am J Clin Nutr* 52:1118–24, 1990
3. Harris WS, Connor WE, Illingworth DR, Rothrock DW, Foster DM: effects of fish oil on VLDL triglyceride kinetics in humans. *J Lip Res* 31:1549–58, 1990
4. Friday KE, Childs MT, Tsunehara CH, Fujimoto WY, Bierman EL, Ensinck JW: Elevated plasma glucose and lowered triglyceride levels from omega-3 fatty acid supplementation in type II diabetes. *Diabetes Care* 12:276- 81, 1989
5. Nestel PJ, Connor 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
6. Schectman G, Kaul S, Kissebah A: Effect of fish oil on plasma lipoproteins and platelet function in NIDDM (Abstract). *Diabetes* (Suppl. 1) 36:45A, 1987
7. Harris WS, Connor WE, Inkeles SB, Illingworth DR: Dietary omega-3 fatty acids prevent carbohydrate-induced hypertriglyceridemia. *Metabolism* 33:1016–19, 1984
8. Braden GA, Knapp HR, Fitzgerald DJ, FitzGerald GA: Dietary fish oil accelerates the response to coronary thrombolysis with tissue-type plasminogen activator. *Circulation* 82:178–87, 1990
9. Brox JH, Killie JE, Osterund B, Holme S, Nordoy A: Effects of cod liver oil on platelets and coagulation in familial hypercholesterolemia (type IIa). *Acta Med*

Scand 213:137–44, 1983
10. Benedict CR, Todd GE: Mechanisms of prevention of coronary thrombosis following dietary intake of fish oil (Abstract). 63rd scientific session III-477
11. von Schacky C, Weber PC: Metabolism and effects on platelet function of the purified eicosapentaenoic and docosahexaenoic acids in humans. *J Clin Invest* 76:2446, 1985
12. Goodnight SH, Harris WS, Connor WE: The effects of dietary n-3 fatty acids on platelet composition and function in man. *Blood* 58:880–85, 1981
13. Skeaff CM, Holub BJ: The effect of fish oil consumption on platelet aggregation responses in washed human platelet suspensions. *Thromb Res* 51:105–15, 1988
14. Bach R, Schmidt U, Jung F, Kiesewetter H, Hennen B, Wenzel E, Schieffer H, Bette L, Heyden S: Effects of fish oil capsules in two dosages on blood pressure, platelet functions, haemorheological and clinical chemistry parameters in apparently healthy subjects. *Ann Nutr Metab* 33:359–67, 1989
15. Rillaerts EG, Engelmann GJ, van Camp KM, de Leeuw I: Effect of omega-3 fatty acids in diet of type I diabetic subjects on lipid values and hemorheological parameters. *Diabetes* 38:1412–16, 1989
16. Terano T, Hirai A, Hamazaki T, Kobayashi S, Fujita T Tamura Y, Kumagai A: Effect of oral administration of highly purified eicosapentaenoic acid on platelet function, blood viscosity and red cell deformability in healthy human subjects. *Atherosclerosis* 46:321–31, 1983
17. Woodcock BE, Smith E, Lambert WH, Jones WM, Galloway JH Greaves M, Preston FE: Beneficial effect of fish-oil on blood viscosity in periferal vascular disease. *Br Med J* 288:592–94, 1984
18. Hostmark AT, Bjerkedal T, Kierulf P, Flaten H, Ulshagen K: Fish oil and plasma fibrinogen. *Br Med J* 297:180–81, 1988
19. Li X, Steiner M: Dose response of dietary fish oil supplementations on platelet adhesion. *Arterioscler Thromb* 11:39–46, 1991
20. de Graaf JC, Sixma JJ, Erkelens DW, de Groot PhG, Banga JD: Fish oil consumption reduces platelet aggregate formation under flow conditions: a pilot study. Abstract for the 53rd annual meeting of the European Atherosclerosis Society, Vienna, April 1989
21. Glauber H, Wallace P, Griver K, Brechtel G: Adverse metabolic effect of omega-3 fatty acids in non-insulin-dependent diabetes mellitus. *Ann Int Med* 108:663–68, 1988
22. Vessby B: n-3 fatty acids and blood glucose control in diabetes mellitus. *J Int Med* (Suppl. 1) 225:207–10, 1989
23. Kasim SE, Stern B, Khilnani S, McLin P, Baciorowski S, Jen KLC: Effects of omega-3 fish oils on lipid metabolism, glycemic control and blood pressure in type II diabetic patients. *J Clin Endocrinol Metab* 67:1–5, 1988
24. Sorisky A: Fish oil and diabetes (Editorial). *Diabetes Care* 12:302–304, 1989
25. Axelrod L: Omega-3 fatty acids in diabetes mellitus. *Diabetes* 38:539–43, 1989
26. Hoefler G, Harnoncourt F, Paschke E, Mirtl W, Pfeiffer KP, Kostner GM: Lipoprotein (a)—a risk factor for myocardial infarction. *Arteriosclerosis* 8:398–401, 1988
27. Scanu AM: Lipoprotein (a), twenty-five years of progress. *Arteriosclerosis* 9:565–66, 1989
28. Clauss A: Gerinnungsphysiologische schnelle Methode zur Bestimmung des Fibrinogens. *Acta Haematol* 17:237–46, 1957
29. Department of Health, Education, and Welfare: Lipid Research Clinics program: lipid and lipoprotein analysis. In *Manual of Laboratory Operations*. Vol. 1. 1974, p. 51–59 (NIH publ. no. 75–628)
30. de Bruin TWA, van Barlingen H, Akveld M, van Vuurst de Vries AR, Erkelens DW. Lipoprotein (a) and apolipoprotein B in hyperthyroid, euthyroid and hypothyroid subjects. *J Clin Endocrinol Metab* 76:121–26, 1993
31. Weisweiler P, Schwandt P, Friedl C: Determination of human apolipoprotein A1, B and E by laser nephelometry. *J Clin Chem Biochem* 22:113- 18, 1984
32. Kortlandt W, van Rijn HJM, Hoeke JOO, Thijssen JHH: Comparison of three different assay procedures for the determination of HbA1 with special attention to the influence of pre-HbA1-C, temperature and haemoglobin concentration. *Ann Clin Biochem* 22:261–68, 1985
33. Groner W, Mohandas N, Bessis M: New optical technique for measuring erythrocyt deformability with the ektacytometer. *Clin Chem* 26(9):1435–42, 1980
34. Nievelstein PFEM, de Groot PG, D'Alessio P, Heijnen HFG, Orlando E, Sixma JJ: Platelet adhaesion to vascular cells: the role of exogenous von Willebrand Factor in platelet adhaesion. *Arteriosclerosis* 10:462–69, 1990
35. Houdijk WPM, de Groot PG, Nievelstein PFEM, Sakariassen KS, Sixma JJ: Subendothelial proteins and platelet adhaesion. Von Willebrand factor and fibronectin, not thrombospondin are involved in platelet adhesion to the extracellular matrix of human vascular endothelial cells. *Arteriosclerosis* 6:24–33, 1986
36. Zwaginga JJ, Sixma JJ, de Groot PG: Activation of endothelial cells induces platelet thrombus formation on their matrix. Studies of a new in vitro thrombus model with low molecular weight heparin as anti-coagulant. *Arteriosclerosis* 10: 49–61, 1990
37. Lawson LD, Hughes BG: Absorption of eicosapentaenoic acid and docosahexaenoic acid from fish oil triacylglycerols or fish oil ethyl esters co-ingested with a high-fat meal. *Biochem Biophys Res Commun* 156:960–63, 1988
38. Goodnight SH: Mechanism of the antithrombotic effects of fish oil. In *Bailliere's Clinical Haematology*. Vol. 3, no. 3, 1990, p. 601–23
39. Tilvis RS, Rasi V, Viinikka L, Ylikordala O, Miettinen TA: Effects of purified fish-oil on platelet lipids and function in diabetic women. *Clin Chim Acta* 164: 315–22, 1987
40. Kloprogge E, de Haas GH, Gorter G, Akkerman JWN: Stimulus- response coupling in human platelets. *Thromb Res* 30: 107–12, 1983
41. Banga JD, Sixma JJ: Diabetes Mellitus, vascular disease and thrombosis. *Clin Haematol* 15:465–91, 1986
42. van der Veen EA, Vermes I: Rheological alterations in diabetes. *Tijdschrift Nederlandse Vereniging voor Klinische Chemie* 15:112–16, 1990
43. Gries A, Malle E, Wurm H, Kostner GM:

Influence of dietary fish-oils on plasma LP(a) levels. Letter to the editor-in-chief. *Thromb Res* 58:667–68, 1990
44. Bruckert E, Davidoff P, Grimaldi A, Truffert J, Giral P, Doumith R, Thervet F, De Gennes JL: Increased serum levels of lipoprotein(a) in diabetes mellitus and their reduction with glycemic control. *JAMA* 263:35–36, 1990
45. Joven J, Vilella E: Serum levels of lipoprotein(a) in patients with well controlled non-insulin-dependent diabetes mellitus. *JAMA* 265:1113- 14, 1990
46. Feskens EJM, Bowles CH, Kromhout D: Inverse association between fish intake and risk of glucose intolerance in normoglycemic elderly men and women. *Diabetes Care* 14:935–41, 1991
47. Watanabe J, Wohltmann HJ, Klein RL, Colwell JA, Lopes-Virella MF: Enhancement of platelet aggregation by low-density lipoproteins from IDDM patients. *Diabetes* 37:1652, 1988
48. McCulloch RK, Vandongen R: Mechanisms of platelet activating factor induced aggregation and secretion in human platelets. *Prostaglandins* 39:13, 1990
49. Mori TA, Vandongen R, Masarei JRL: Fish oil induced changes in apolipoproteins in IDDM subjects. *Diabetes Care* 13:725, 1990
50. Haines AP, Sanders TA, Imeson JD, Mahler RF, Martin J, Mistry M, Vickers M, Wallace PG: Effects of a fish oil supplement on platelet function, haemostatic variables and albuminuria in insulin dependent diabetics. *Tr Res* 43:643, 1986