# Effect of Omega-3 Fatty Acids in Diet of Type I Diabetic Subjects on Lipid Values and Hemorheological Parameters

EDDY G. RILLAERTS, GUY J. ENGELMANN, KRISTIEN M. VAN CAMP, AND IVO DE LEEUW

**Twelve type I (insulin-dependent) diabetic subjects in stable metabolic control for at least 3 mo received a controlled diet containing 50% carbohydrate, 35% fat, and 15% protein. Calorie intake varied from 1800 to 2200 calories, depending on individual needs. Part of the polyunsaturated omega-6 fatty acids (ω6FAs) were isocalorically exchanged with ω3FAs (2.7 g/day provided by fish oil concentrates) for 10 wk. Subject selection was based on the fact that the atherogenic index (total cholesterol/high-density lipoprotein cholesterol [HDL-chol]) remained >5. Total cholesterol did not change, but HDL-chol ($P < .05$) increased significantly, and the mean ± SD atherogenic index decreased from 5.9 ± 1.1 to 5.1 ± 1.3. Plasma triglyceride levels also decreased ($P < .05$). There was a small (~2%) but significant ($P < .05$) decrease of whole-blood viscosity at low shear rate because of a similarly small (~2%) decrease ($P < .05$) of plasma viscosity. Erythrocyte viscosity values and the erythrocyte transit time, measured with the St. George's filtrometer, remained unchanged during fish oil intake. Four weeks after stopping the ω3FA administration, the triglyceride level was again increased ($P < .05$) and was even higher than the starting value ($P < .05$). Plasma and whole-blood viscosity also increased to the starting levels, demonstrating that lipid alterations are accompanied with blood viscosity changes in the presence of a stable metabolic control. *Diabetes* 38:1412–16, 1989**

Vascular disease is the major cause of morbidity and mortality in patients with diabetes mellitus (1,2). Diabetic subjects have a premature form of atherosclerosis and a distinct type of microangiopathy in different organs. Diabetes is associated with lipid and hemorheological disturbances (3–7). These conditions, together with other abnormalities such as changes in platelet function and clotting factor levels, hyperglycemia, and endothelial cell injury (8,9), are thought to contribute to the development of vascular disease.

Epidemiological studies suggest that populations consuming a diet rich in fish oils have a lower risk of cardiovascular disease (10,11). Ingestion of some marine oils or especially their polyunsaturated omega-3 fatty acids (ω3FAs) has been reported to produce beneficial effects on plasma lipids, platelet aggregation, and blood viscosity in healthy subjects and subjects with vasculopathy (12–15).

These findings suggest that polyunsaturated ω3FAs could offer an attractive additional dietary intervention aimed at improving risk ractors for atherosclerosis in diabetic subjects. Little is known about the effects of ω3FAs in these subjects. Potentially beneficial effects of fish oils have been shown in type I (insulin-dependent) diabetic subjects (16), but their use has been questioned or even discouraged in type II (non-insulin-dependent) diabetic patients (17,18). We studied the effect of ω3FAs in the diet of type I diabetic subjects on lipid values and hemorheological parameters.

## RESEARCH DESIGN AND METHODS

Twelve type I diabetic subjects (6 men, 6 women; mean ± SD age 42 ± 11 yr; mean ± SD duration of diabetes 10 ± 5 yr) in stable metabolic control for at least 3 mo (mean ± SD total glycosylated hemoglobin [$HbA_1$] 9.9 ± 1.5%) received a diet containing 50% carbohydrate, 35% fat (polyunsaturated/saturated [P/S] ratio 1.2), and 15% protein. Calorie intake varied from 1800 to 2200 kcal, depending on individual needs. The average cholesterol intake was 250 mg/day, and the average fiber content was 41 g/day.

From the Laboratory for Endocrinology and Nutrition, University of Antwerp, Wilrijk; the Laboratory of Organic Chemistry, University of Antwerp, Antwerp; and the Nutrition Team, University Hospital Antwerp, Edegem, Belgium.

Address correspondence and reprint requests to Eddy Rillaerts, Laboratory for Endocrinology and Nutrition, University of Antwerp (UIA), Universiteitsplein 1 (T4), B-2610 Wilrijk, Belgium.

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The average daily ω3FA consumption from fish in the habitual diet was 0.05 g/day. The subjects showed no clinical signs of major diabetic complications. Only subjects without retinopathy or with background retinopathy and microalbuminuria <30 μg/ml were considered for this study. Subject selection was based on the fact that on the usual diabetic diet, the atherogenic index (total cholesterol/high-density lipoprotein cholesterol [HDL-chol] ratio) remained consistently >5.

To investigate the effects of incorporation of fish oils in the diabetic diet, part of the polyunsaturated ω6FAs were isocalorically exchanged during a 10-wk period with ω3FAs. To do this, 9 g vegetable oil (safflower, corn, or sunflower seed oil) was replaced by 9 g fish oil. Because the P/S ratios of the different vegetable oils were higher than the P/S ratio of the fish oil, the whole amount of omitted ω6FAs was not replaced by ω3FAs. On average, ~5.6 g ω6FAs was exchanged with 2.7 g ω3FAs. Each day, the patients received nine capsules (Maxepa, Thompson, Carson, CA) of a fish oil concentrate providing 1.8 g eicosapentaenoic acid (EPA) and 0.9 g docosahexaenoic acid (DHA). Because every capsule contained 6 mg cholesterol, this resulted in an additional daily intake of 54 mg cholesterol, whereas the fiber content was not altered.

Fasting blood was taken from the antecubital vein before and after 10 wk of fish oil consumption and after a 4-wk washout period. Dietary compliance of fish oil intake was checked by erythrocyte fatty acid determinations.

**Rheological measurements.** Rheological measurements were performed on fasting venous blood, anticoagulated with $K_3$-EDTA. The hematocrit (Hct) was determined by the microhematocrit centrifugation method. All viscosity measurements were performed in duplicate at 37°C with a Contraves Low Shear 30 viscosimeter (Zürich, Switzerland) according to the guidelines of the International Committee for Standardization in Haemorheology expert panel on blood rheology (19). This rotational viscosimeter allows viscosity measurements in a broad range of shear rates (~0.1 to ~130 $s^{-1}$). At low shear rates, whole-blood viscosity is mainly determined by erythrocyte aggregation (20), whereas at high shear rates, blood viscosity is influenced more by erythrocyte deformability (21). Erythrocyte deformability is the property of an erythrocyte to pass through capillaries smaller than its own diameter. Because erythrocyte number is the most important factor determining in vitro blood viscosity, the Hct must be normalized to make comparisons about erythrocyte aggregration and deformability possible. Plasma viscosity influences whole-blood viscosity at all shear rates. Whole-blood viscosity was measured from high (128.5 $s^{-1}$) to low (0.945 $s^{-1}$) shear rate at a normalized Hct of 45%. Plasma viscosity was measured at 69.5 $s^{-1}$.

From a second sample (10 ml), plasma and erythrocytes were separated after centrifugation (15 min, 3000 rpm). Most of the leukocytes were removed by gentle aspiration of the buffy coat. The erythrocytes were washed twice in phosphate-buffered saline (PBS) then finally washed and resuspended in the same buffer containing 5.5 mM glucose and 10 g/L (300 mosmol/kg) bovine serum albumin (pH 7.4). Erythrocyte viscosity was measured at high (128.5 $s^{-1}$) and low (0.945 $s^{-1}$) shear rates at a normalized Hct of 70% as previously described (22). Erythrocyte viscosity measurements at a standardized Hct provide an indirect measure of erythrocyte deformability in the absence of plasma aggregation-promoting factors (23,24).

From a third blood sample, the same washing procedure and buffer solutions for viscosity measurements were used, but after every wash, the upper 10% of the packed erythrocytes was discarded. By this technique, residual leukocytes could be reduced to $<0.1 \times 10^9$/L. Leukocyte removal is necessary in filtration experiments because leukocytes strongly influence erythrocyte filtration times (19,25). All buffer solutions were prefiltered through a 0.5-μm pore diameter filter. Erythrocyte-filterability experiments were performed with the St. George's filtrometer (Carri-med, Dorking, UK; 26). Erythrocyte suspensions (Hct 10%) were filtered at 25°C under a 4-cm$H_2O$ negative-pressure head through a vertical polycarbonate filter (Nucleopore, Pleasanton, CA; 5-μm pore diameter, 13-mm filter diameter, batch 54B6A3). Instructions for use and all calculations were performed by microcomputer. The results are expressed as erythrocyte (red cell) transit time (RCTT)

$$RCTT = \frac{1}{Hct}\left(\frac{1 - Fi}{Fi}\right) + 1$$

with Fi/s initial relative filtration rate (26). Thrombin-clottable fibrinogen was determined by the thrombin time test (Ortho Diagnostic, Raritan, NJ).

**Erythrocyte membrane fatty acids.** The erythrocytes from heparinized blood samples were separated and washed three times with PBS before extraction. Erythrocyte lipids were extracted with hexane-isopropanol (3:2) in the presence of butylated hydroxytoluene according to Radin (27). Fifteen milliliters of the extraction solvent hexane-isopropanol was put into a 50-ml extraction tube. Margaric acid was added to the extraction solvent as internal standard. Packed erythrocytes (1.5 ml) were then added to the extraction tube. The sample was extracted over 30 min during continuous shaking at 4°C. The hexane-isopropanol layer was collected, and the residue was extracted twice more with 15 and 10 ml hexane-isopropanol, respectively. The hexane-isopropanol layers were collected, and 20 ml of the total volume was dried under $N_2$. The fatty acids obtained were hydrolyzed and derivatized to *p*-bromophenacyl esters to make them UV visible for high-performance liquid chromatography (28). The *p*-bromophenacyl esters were separated on a 10 RP-8 column (25 × 4.6-mm ID; Chrompack, Middelburg, The Netherlands) with $CH_3CN$/0.01 M $HCOONH_4$ (85:15) as mobile phase (1.7 ml/min; column temperature 40°C; detection at 254 nm).

Varian Vista 5000 liquid chromatograph equipment with a 10-μl loop injector and a Varian UV variable-wavelength detector were used.

**Other methods.** The percentage of total $HbA_1$ was calculated after separation on cation-exchange microcolumns (Boehringer, Mannheim, FRG) at 25°C in a water bath after elimination of the labile aldimin fraction. Serum lipids were measured by standard clinical laboratory methods. Very-low-density lipoprotein cholesterol (VLDL-chol) and low-density

lipoprotein cholesterol (LDL-chol) were calculated by Friedenwald's formula

$$\text{LDL-chol} = \text{TC} - \text{HDL-chol} - \text{VLDL-chol},$$

$$\text{whereas VLDL-chol} = \text{triglycerides}/5$$

where TC is total cholesterol.

This was permitted because no patient had a triglyceride level >3.4 mM. Apoproteins A1 (apoA1) and B were measured by an immunoturbidimetric method. The antiserums for the different apoproteins were obtained from Behring (Marburg, FRG).

Statistical analysis was performed by means of Wilcoxon's test with the SPSS/PC+ statistics package.

## RESULTS

Dietary compliance was confirmed by the fact that in all subjects the ω3FA content in the erythrocytes significantly increased during fish oil administration (mean ± SD baseline EPA, 1.03 ± 0.41; after fish oil, 1.86 ± 0.74 mol% [% wt expressed by mol], $P < .01$; baseline DHA, 3.98 ± 0.66; after fish oil, 5.27 ± 0.90 mol%, $P < .01$). After the washout period, there was a significant decrease of both EPA (after fish oil, 1.86 ± 0.74 mol%; after washout, 1.35 ± 0.34 mol%, $P < .01$) and DHA (after fish oil, 5.27 ± 0.90 mol%; after washout, 4.39 ± 0.89 mol%, $P < .05$) in the erythrocyte membrane.

The effect of ω3FA exchange in the diet of 12 type I diabetic subjects on body weight, metabolic control, plasma lipids, and apoproteins is shown in Table 1. Glycemic control and body weight did not change throughout the study.

Total cholesterol did not change, but HDL-chol increased significantly ($P < .05$) and the atherogenic index decreased significantly ($P < .05$) during ω3FA intake. This effect also remained after the washout period.

Triglycerides and calculated VLDL-chol decreased significantly ($P < .05$) during ω3FA administration. Calculated LDL-chol, apoA1, apoB, and apoA1/apoB ratio did not change significantly throughout the study. Four weeks after stopping the ω3FA consumption, the triglyceride levels increased again ($P < .05$) and became even higher than the starting values ($P < .05$). Phospholipids were significantly increased after the washout period compared with the fish oil period ($P < .05$). The effects of the exchange of ω3FAs in the diabetic diet on blood rheology are shown in Table 2.

Plasma viscosity ($P < .05$) and whole-blood viscosity at low shear rate ($P < .05$) significantly decreased during fish oil administration and rose again significantly (both $P < .05$) after the washout period. Plasma fibrinogen did not change throughout the study. There was no effect of ω3FA consumption on erythrocyte viscosity values and the RCTT.

## DISCUSSION

Glycemic control parameters did not change throughout the study, indicating that isocaloric exchange with 2.7 g ω3FAs did not alter overall metabolic control in the type I diabetic subjects. In contrast, deterioration of glycemic control was shown in type II diabetic subjects (17,18).

A fall in serum triglycerides caused by ω3FAs is the principal finding in dietary-intervention studies with fish or fish oils (14–16, 29–32). Also in this study, triglyceride levels fell during ω3FA exchange and rose again after the washout period. A similar decrease of triglycerides by fish oil supplementation in type I diabetic subjects was demonstrated by Miller et al. (16). Fish oils are also known to lower the levels of triglycerides in subjects with hypertriglyceridemia (33).

In nondiabetic subjects, the effect of consumption of ω3FAs on serum cholesterol and HDL-chol is less consistent. Some studies reported a decrease of total cholesterol (29,31), an increase of HDL-chol (34), or both (32), whereas others found no difference in total cholesterol (34), HDL-chol (29,31), or both (14,30). Different daily amounts of ω3FAs and other dietary factors may have influenced these discrepancies. In this study, we found an increase of HDL-chol, although total cholesterol remained unchanged, resulting in a decreased atherogenic index in the diabetic subjects. The effect remained even after the washout period and was not associated with any change of the apoA1/apoB ratio. No such increase of HDL-chol or decrease of atherogenic index

TABLE 1
Effect of fish oil consumption on body weight, metabolic control, plasma lipids, and apoproteins

| | Baseline | Fish oil | Washout |
|---|---|---|---|
| Body mass index (kg/m²) | 24.2 ± 0.5 | 24.3 ± 0.4 | 24.0 ± 1.7 |
| $HbA_1$ (%) | 9.9 ± 0.4 | 10.2 ± 0.5 | 9.8 ± 0.5 |
| Fasting blood glucose (mM) | 12.2 ± 1.6 | 9.4 ± 1.6 | 10.6 ± 1.6 |
| Glycosuria (mmol/day) | 116 ± 38 | 127 ± 37 | 127 ± 37 |
| Triglycerides (mM) | 1.41 ± 0.16 | 1.14 ± 0.13* | 1.66 ± 0.23*† |
| Total cholesterol (mM) | 6.27 ± 0.17 | 6.19 ± 0.17 | 6.42 ± 0.15 |
| VLDL-chol (mM) | 0.65 ± 0.08 | 0.52 ± 0.06* | 0.75 ± 0.10† |
| LDL-chol (mM) | 4.53 ± 0.18 | 4.40 ± 0.16 | 4.40 ± 0.16 |
| HDL-chol (mM) | 1.09 ± 0.05 | 1.27 ± 0.08* | 1.27 ± 0.08* |
| Phospholipids (mM) | 3.17 ± 0.10 | 3.06 ± 0.12 | 3.35 ± 0.14* |
| Atherogenic index | 5.9 ± 0.3 | 5.1 ± 0.4* | 5.4 ± 0.4* |
| Apoprotein (mg/dl) | | | |
| A1 | 141 ± 5 | 145 ± 8 | 144 ± 8 |
| B | 158 ± 7 | 158 ± 7 | 166 ± 7 |
| A1/B | 0.91 ± 0.06 | 0.95 ± 0.07 | 0.88 ± 0.05 |

Values are means ± SE for 12 insulin-dependent diabetic subjects. $HbA_1$, glycosylated hemoglobin; VLDL-chol, LDL-chol, and HDL-chol, very-low-density, low-density, and high-density lipoprotein cholesterol, respectively.
*$P < .05$ vs. baseline; †$P < .05$ vs. fish oil (Wilcoxon's test).

TABLE 2
Effect of fish oil consumption on blood rheology

| | Baseline | Fish oil | Washout |
|---|---|---|---|
| Hematocrit (%) | 43.2 ± 1.1 | 42.4 ± 1.0 | 43.0 ± 0.8 |
| Whole-blood viscosity (mPa · s) with 45% Hct | | | |
| Low shear | 24.4 ± 0.7 | 23.8 ± 0.6* | 25.1 ± 0.6† |
| High shear | 5.1 ± 0.1 | 5.2 ± 0.1 | 5.3 ± 0.1 |
| Plasma viscosity (mPa · s) | 1.35 ± 0.01 | 1.32 ± 0.02* | 1.37 ± 0.01† |
| Erythrocyte viscosity (mPa · s) with 70% Hct | | | |
| Low shear | 28.3 ± 0.5 | 28.8 ± 0.3 | 29.0 ± 0.6* |
| High shear | 8.1 ± 0.1 | 8.2 ± 0.1 | 8.4 ± 0.1*† |
| Erythrocyte transit time‡ | 11.3 ± 0.2 | 11.4 ± 0.2 | 11.5 ± 0.1 |
| Fibrinogen (mg/dl) | 321 ± 33 | 314 ± 13 | 315 ± 22 |

Values are means ± SE for insulin-dependent diabetic subjects. Hct, hematocrit.
*$P < .05$ vs. baseline; †$P < .05$ vs. fish oil (Wilcoxon's test).
‡See RESEARCH DESIGN AND METHODS.

in type I diabetic subjects was demonstrated by Miller (16). Some differences between both studies may have contributed to this discrepancy, such as subject selection (based on atherogenic index in our study), ω3FA exchange instead of supplementation, and other dietary differences.

Fish oils have been shown to lower blood viscosity in healthy subjects (14), subjects with peripheral vascular disease (15), and type I diabetic subjects (16). In these studies, a decrease of plasma viscosity was absent, suggesting an improvement of erythrocyte deformability.

During ω3FA administration, we observed a small but significant decrease of whole-blood viscosity at low shear rate, which is strongly influenced by erythrocyte aggregation. This effect was due to a decrease of plasma viscosity. The fall in plasma and low-shear whole-blood viscosity was not related to a decrease of fibrinogen, probably the major factor influencing plasma viscosity and erythrocyte aggregation in diabetes (35). Because available data suggest that the mechanism of triglyceride lowering by fish oil fatty acids is to inhibit VLDL production (36) and it has been shown that VLDLs have an effect on plasma viscosity, this phenomenon may have contributed to the decrease of plasma viscosity in our study (37). After the washout period, both plasma and the low-shear whole-blood viscosity rose again, supporting the fact that both decreases were indeed related to the fish oil consumption.

No significant change in whole-blood viscosity at high shear rate was found throughout the study. Because high-shear whole-blood viscosity is influenced by erythrocyte deformability, this suggested a lack of effect of fish oils on the latter parameter. This was confirmed by the erythrocyte-viscosity and filtration measurements, two experimental techniques to assess erythrocyte deformability. This is in contrast with a previous report claiming an improved erythrocyte flexibility by fish oil supplementation (3.6 g EPA/day) in nondiabetic subjects (14). However, in that study, the whole-blood–filtration technique, which is strongly influenced by platelet aggregates and leukocytes, was used (19). With erythrocyte-viscosity measurements, Popp-Snijders et al. (38) found no effect of fish oil supplementation (3 g EPA + DHA/day) on erythrocyte deformability in nondiabetic subjects.

We conclude that isocaloric exchange of ω3FAs may improve some lipid and hemorheological parameters in type I diabetic subjects. Further long-term prospective studies deserve attention to evaluate the possible usefulness of fish oils in the prevention of vascular disease in these subjects.

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**REFERENCES**

1. Steiner G: Atherosclerosis: the major complication of diabetes. *Adv Exp Med Biol* 189:277–97, 1985
2. Krolewski A, Kosinski E, Warram J, Leland S, Busick E, Asmal C, Rand L, Christlieb R, Bradley R, Kahn R: Magnitude and determinants of coronary artery disease in juvenile-onset insulin-dependent diabetes mellitus. *Am J Cardiol* 59:750–55, 1987
3. Sosenko J, Breslow J, Miettinen O, Gabbay K: Hyperglycemia and plasma lipid levels: a prospective study of young insulin-dependent diabetic patients. *N Engl J Med* 302:650–54, 1980
4. Colwell J, Winocour P, Lopes-Virella M, Haluska P: New concepts about the pathogenesis of atherosclerosis in diabetes mellitus. *Am J Med* 75 (Suppl. 5B):67–80, 1983
5. McMillan DE: Physical factors important in the development of atherosclerosis in diabetes. *Diabetes* 30 (Suppl. 2):97–103, 1981
6. Barnes AJ, Locke P, Scudder PR, Dormandy TL, Dormandy JA, Slack J: Is hyperviscosity a treatable component of diabetic microcirculatory disease? *Lancet* 2:789–91, 1977
7. Schmid-Schönbein H, Volger E: Red cell aggregation and red cell deformability in diabetes. *Diabetes* 25:897–902, 1976
8. Juhan I, Buonocore M, Jouve R, Vague P, Moulin JP, Vialettes B: Abnormalities of erythrocyte deformability and platelet aggregation in insulin-dependent diabetics corrected by insulin in vivo and in vitro. *Lancet* 1:535–38, 1982
9. Brownlee M, Cerami A: The biochemistry of the complications of diabetes mellitus. *Annu Rev Biochem* 50:385–432, 1981
10. Kromhout D, Bosschieter EB, 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
11. Bang HO, Dyerberg J, Sinclair HM: The composition of the Eskimo food in North Western Greenland. *Am J Clin Nutr* 33:2657–61, 1980
12. Leaf A, Weber PC: Cardiovascular effects of n-3 fatty acids. *N Engl J Med* 318:549–57, 1988
13. Hirai A, Hamazaki T, Terano T, Nishikawo T, Tamura Y, Kamagai A, Sajiki S: Eicosapentaenoic acid and platelet function in Japanese. *Lancet* 2:1132–33, 1980
14. Terano T, Hirai A, Hamazaki T, Kobayashi S, Fujita T, Tamura Y, Kumagoi 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
15. Woodcock BE, Smith E, Lambert WH, Morris Jones W, Galloway JH, Greaves M, Preston FE: Beneficial effect of fish oil on blood viscosity in peripheral vascular disease. *Br Med J* 228:592–94, 1984
16. Miller M, Anagnostou A, Ley B, Marschall P, Steiner M: Effect of fish oil concentrates in hemorheological and hemostatic aspects of diabetes mellitus: a preliminary study. *Thromb Res* 47:201–14, 1987
17. Schectman G, Kaul S, Kissebah AH: Effect of fish oil concentrate on

lipoprotein composition in NIDDM. *Diabetes* 37:1567–73, 1988
18. Kasim SE, Stern B, Khilnani S, McLin P, Baciorowski S, Jen CKL: 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
19. ICSH Expert Panel on Blood Rheology: Guidelines for measurement of blood viscosity and erythrocyte deformability. *Clin Hemorheol* 6:439–53, 1986
20. Schmid-Schönbein H, Gaehtgens P, Hirsch H: On the shear rate dependence of red cell aggregation in vitro. *J Clin Invest* 47:1447–54, 1968
21. Schmid-Schönbein H, Wells R, Goldstone J: Influence of deformability of human red cells upon blood viscosity. *Circ Res* 25:131–43, 1969
22. Rillaerts E, Van Camp G, Claeys M, DeLeeuw I: Increased low shear rate erythrocyte viscosity in insulin dependent diabetes mellitus. *Clin Hemorheol* 8:73–80, 1988
23. Chien S: Principles and techniques for assessing erythrocyte deformability. *Blood Cells* 3:71–99, 1977
24. Mohandas N, Phillips WM, Bessis M: Red blood cell deformability and hemolytic anemias. *Semin Hematol* 16:95–114, 1979
25. Chien S, Schmalzer EA, Lee MM, Impelluso T, Skalak R: Role of white blood cells in filtration of blood cell suspensions. *Biorheology* 20:11–27, 1983
26. Dormandy J, Flute P, Matrai A, Bogar L, Mikita J, Lowe G, Anderson J, Chien S, Schmalzer E, Hersenfeld A: The St. George's blood filtrometer. *Clin Hemorheol* 5:483–91, 1985
27. Radin NJ: Extraction of tissue lipids with a solvent of low toxicity. *Methods Enzymol* 72:5–7, 1981
28. Durst HD, Milano H, Kikta EJ, Cannelly SA, Grushka E: Phenacylesters of fatty acids via Crown ether catalysis for enhanced ultraviolet detection in liquid chromatography. *Anal Chem* 47:1797–801, 1975
29. Harris W, Connor W, McMurry M: The comparative reductions of the plasma lipids and lipoproteins by dietary polyunsaturated fats: salmon oil versus vegetable oils. *Metabolism* 32:179–84, 1983
30. Bronsgeest-Schoute HC, van Gent CM, Luten JB, Ruiter A: The effect of various intakes of omega-3 fatty acids on the blood lipid composition in healthy human subjects. *Am J Clin Nutr* 34:1752–57, 1981
31. von Lossonczy TO, Ruiter A, Bronsgeest-Schoute HC, van Gent CM, Hermus RJ: The effect of a fish diet on serum lipids in healthy human subjects. *Am J Clin Nutr* 31:1340–46, 1978
32. Sanders T, Roshanai F: The influence of different types of omega-3 polyunsaturated fatty acids on blood lipids and platelet function in healthy volunteers. *Clin Sci* 64:91–99, 1983
33. Phillipson B, Rothrock D, Connor W, Harris W, Illingworth R: Reduction of plasma lipids, lipoproteins, and apoproteins by dietary fish oils in patients with hypertriglyceridemia. *N Engl J Med* 312:1210–16, 1985
34. Sanders T, Vickers M, Harnes A: Effect on blood lipids and haemostasis of a supplement of cod-liver oil, rich in eicosapentaenoic and docosahexaenoic acids, in healthy young men. *Clin Sci* 61:317–24, 1981
35. Lowe GDO, Lowe JM, Dummond MM, Reith S, Belch JJF, Kesson CM, Wylie A, Foulds WS, Forbes CD, MacCuish AC, Manderson WG: Blood viscosity in young male diabetics with and without retinopathy. *Diabetologia* 18:359–63, 1980
36. Nestel PJ, Connor WE, Reardon MR, 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
37. Seplowitz A, Chien S, Smith FR: Effects of lipoproteins on plasma viscosity. *Atherosclerosis* 38:89–95, 1981
38. Popp-Snijders C, Schouten JA, van Blitterswijk WJ, van der Veen EA: Changes in membrane lipid composition of human erythrocytes after dietary supplementation of (n-3) polyunsaturated fatty acids: maintenance of membrane fluidity. *Biochim Biophys Acta* 854:31–37, 1986