Nutr Metab Cardiovasc Dis (1996) 6: 65–71

ORIGINAL ARTICLE

The effect of 26 years of habitual fish consumption on serum lipid and lipoprotein levels (The Zutphen Study)

D. Kromhout*, M.B. Katan**, L. Havekes***, A. Groener****, G. Hornstra*****, and C. de Lezenne Coulander*

*Division of Public Health Research, National Institute of Public Health and the Environment, Bilthoven, **Department of Human Nutrition, Agricultural University of Wageningen, ***TNO Institute for Prevention and Health, Gaubius Laboratory, Leiden, ****Department of Pediatrics, University of Leiden, Leiden and *****Department of Human Biology, University of Limburg, Maastricht, The Netherlands

Abstract

An inverse association was found between fish consumption and coronary heart disease in the Zutphen Study. In the present study the long-term effects of a small amount of fish on serum lipids and lipoproteins was investigated. Forty healthy, elderly men whose fish consumption pattern over the previous 26-year period was known, were selected for the present study. The 15 men in the control group consumed, on average, about 2 g of fish per day, and the 25 habitual fish consumers ate, on average, about 33 g of fish per day during the study period. The cholesterol content of the different lipoprotein fractions did not differ between the two groups. The average total triglyceride level and the average triglyceride level of the atherogenic IDL fraction were respectively 26% and 38% ($p < 0.05$) lower in the habitual fish consumers than in the controls. These results suggest that a habitual fish consumption of about 30 g per day for more than 25 years is an effective measure for lowering serum triglyceride levels.

Introduction

In the Zutphen Study we found an inverse relation between fish consumption and 20-year mortality from coronary heart disease (1). Two hypotheses were proposed to explain this relation (2). Fish consumption may influence serum lipids and lipoproteins and therefore atherosclerosis. The second hypothesis states that fish consumption is related to coronary heart disease through an effect on haemostatic parameters such as bleeding time and platelet aggregation. We investigated the effects of long-term fish consumption on serum lipids and lipoproteins and on haemostatic parameters.

Dietary data were collected in the Zutphen Study in 1960, 1965, 1970 and 1985. This made it possible to select two groups of men; one group who ate very little fish during this 25-year period and one group who ate fish once or twice a week. These two groups were invited to take part in a study on the effects of the long-term fish consumption on serum lipids, lipoproteins and some haemostatic parameters. We have already reported that long-term fish consumption was not associated with platelet function (3). In this paper the long-term effects of fish consumption on serum lipids and lipoproteins will be reported.

Methods

Since 1960, a longitudinal investigation of the relations between diet and other risk factors to chronic diseases has been carried out on middle-aged men from the town of Zutphen in The Netherlands. The Zutphen Study is the Dutch contribution to the Seven Countries Study (4-6). Zutphen is an old industrial town in the eastern part of The Netherlands. In 1960 there were 25,000 inhabitants. For this study a random sample of 1088 men aged 40-59 was

Key words: Cholesterol, habitual fish consumption, IDL, lipids, lipoproteins, triglycerides.

Correspondence to: Prof. D. Kromhout, Division of Public Health Research, National Institute of Public Health and the Environment, P.O.Box 1, 3720 BA Bilthoven, The Netherlands.

Received: 23 September 1994; *accepted:* 15 November 1995.

selected, and 872 took part in both the medical examination and the dietary survey. The medical examinations were repeated yearly between 1960 and 1973, in 1977-78 and in 1985. The dietary surveys were carried out in 1960, 1965, 1970 and 1985.

In 1960, 1965 and 1970 data on food intake were collected by the cross-check dietary history method (7) adapted to the Dutch situation (8). This method provides information about the usual pattern of food consumption in the six to 12 months before the interview. Details of this method have been described (9). The cross-check dietary history was also used in 1985 but the information about the usual food consumption pattern was then limited to the month preceding the interview, due to the more diverse food consumption pattern in 1985 compared to that in the 60s. The interviews were carried out by experienced dietitians who had undergone an intensive training course before the fieldwork started. The dietary surveys were always carried out in the spring and early summer. Quantities of foods were estimated by the dietitians, and the quantities that were difficult to estimate were measured on portable scales. The dietary data were coded by the dietitians according to the Uniform Food Encoding (UFE) system developed in The Netherlands (10). These data were converted into energy and nutrient values by means of the UFE food table, an extended, computerized version of the Netherlands food table that contains the energy values and nutrient composition of foods.

In 1985, 79 men aged 66-81 were selected, based on their fish consumption pattern in the period 1960-1985. The fish consumers ate on average 30.4±1.3 (SEM) g of fish per day (range 11±107 g) during the period 1960-1985. The average fish consumption of the controls was 3.6±0.4 g of fish per day (range 0±14 g) during that period. Of the potentially available 79 men, 14 did not participate in the present study. Two had died after the examination in 1985, four were suffering from severe chronic diseases, e.g. myocardial infarction, cancer, rheumatoid arthritis and mental illness, four used drugs known to have an influence on platelet function and four refused to participate. Fasting blood samples were obtained from 64 of the 65 selected men. Twenty-one of these 64 men showed an inconsistent fish consumption pattern compared with the period 1960 - 1985. This group consisted of controls who ate more than 14 grams of fish per day in 1986 or of fish consumers who ate less than 11 g of fish per day in 1986. These men were excluded from the analyses. Also three men who drank alcoholic beverages on the day before blood was drawn, were excluded. This resulted in a habitual fish consumption group of 25 individuals with a steady fish consumption of more than 10 grams of fish per day over 26 years. The control group consisted of 15 men with a steady fish consumption of less than 15 grams of fish per day over 26 years.

The present study was carried out in April and May 1986 and consisted of four parts. A dietary investigation took place at home and was carried out in the same way as in 1985 by three dietitians who had also interviewed the participants in 1985. The men were instructed not to use drugs containing salicylates for the three weeks preceding the physical examination, not to drink alcoholic beverages on the day before blood sampling, not to take any food after 10 p.m. on that day, and not to smoke on the morning before blood was drawn. Blood samples were collected at the homes of the participants between 8.00 and 9.30 a.m.; a physical examination (including the measurement of height and weight) was carried out in the Nieuwe Spittaal Hospital, according to a standardized protocol by one internist (4). A haemostatic investigation was also carried out, the results of which have been published separately (3).

Three 10-ml tubes of fasting blood were drawn from an antecubital vein via a 19-gauge butterfly needle. Subjects were in a sitting position and the tourniquet was released before the blood was drawn. Serum was prepared by low speed centrifugation. Lipid and lipoprotein analyses were carried out at the standardized lipid laboratory of the Department of Human Nutrition, Agricultural University Wageningen. Whole serum and lipoprotein fractions were stored at -20°C until analysis, except for ultracentrifugation for which the sera were stored for a maximum of three days at +4°C. Serum total cholesterol was determined enzymatically with the CHOD-PAP Monotest kit (11, 12) and serum total triglycerides with the GPO-PAP kit from Boehringer Mannheim (13). This method is specific for triacylglycerols. Free glycerol is removed enzymatically in a pre-incubation step. HDL cholesterol was determined after magnesium-dextran sulfate precipitation of apo B and apo E containing lipoproteins (VLDL, LDL and HDL 1) (14). All the samples were analyzed in one run. The within-run coefficient of variation was 0.9% for total and 1.8% for HDL cholesterol and 1.0% for triglycerides.

The lipoproteins were separated by density gradient ultracentrifugation. After centrifugation, the lipoprotein fraction was harvested by aspiration on the basis of the known density gradient in centrifuge tubes (15). The following density classes (d in g/ml) were isolated: VLDL

TABLE 1
Habitual fish consumption (g/day) among two groups of men aged 41-56 in 1960 during the period 1960-1986.

	Controls	Habitual fish consumers
Year	n = 15 m ± SD	n = 25 m ± SD
1960	3.3 ± 3.2	29.4 ± 20.9
1965	1.2 ± 2.2	34.4 ± 13.5
1970	0.9 ± 2.1	31.7 ± 15.8
1985	1.5 ± 2.3	37.4 ± 24.0
1986	2.3 ± 3.8	30.8 ± 14.0

TABLE 2
Habitual fish consumption in relation to daily energy and macronutrient intake among men aged 67-82 in 1986.

Dietary variables		Controls n = 15 m ± SD	Habitual fish consumers n = 25 m ± SD
Energy	(Kcal)	2043 ± 270	2282 ± 418*
Vegetable protein	(g)	24.7 ± 5.6	23.6 ± 6.1
Animal protein	(g)	52.5 ± 14.0	58.0 ± 11.1
Saturated fat	(g)	39.6 ± 8.8	42.2 ± 9.5
Monounsaturated fat	(g)	35.2 ± 10.1	36.8 ± 9.1
Polyunsaturated fat	(g)	15.2 ± 6.5	19.6 ± 10.8
Linoleic acid	(g)	12.7 ± 6.5	16.9 ± 10.3
N-3 Polyunsaturated fat	(mg) (1)	49 ± 93	245 ± 411***
Mono and disaccharides	(g)	99.9 ± 35.9	117.8 ± 28.2
Polysaccharides	(g)	114.0 ± 21.8	112.4 ± 31.1
Alcohol	(g)	6.3 ± 9.8	15.2 ± 18.0*
Dietary cholesterol	(mg)	292 ± 111	341 ± 104
Dietary fiber	(g)	26.0 ± 4.9	25.4 ± 6.1

(1) N-3 Polyunsaturated fat = eicosapentaenoic acid + docosahexaenoic acid.
*$p<0.05$; ***$p<0.001$

(d<1.010); IDL (1.010<d<1.019); LDL (1.019<d<1.055); HDL 1 including Lp(a) (1.055<d<1.075); HDL 2 (1.075<d<1.110); HDL 3 (1.110<d<1.210); bottom fraction (d<1.210). Customarily, d=1.063 g/ml is taken as the demarcation between LDL and HDL. However, this results in inclusion of appreciable amounts of the atherogenic lipoprotein(a) (sinking pre-β lipoprotein) in HDL. By interposing an extra fraction with d=1.055-1.075, Lp(a), if present, is largely separated from LDL and HDL2. The cholesterol and triglyceride content of the lipoprotein fraction were determined with minor modifications of the same methods as used for the whole serum cholesterol and triglyceride analyses. On average 96±5% (m±SD) of cholesterol and 80±8% of the triglycerides in serum were recovered in the fractions. The lower recovery of the triglycerides was probably due to a minor loss of VLDL during aspiration of the d<1.010 g/ml fraction.

Apo A-I and A-II were measured by radial immunodiffusion (16) as well as apo B (17). The lecithin cholesterol acyltransferase (LCAT) activity was measured according to Stokke and Norum (1971)(18), Pownall et al. (1982)(19) and Chen and Albers (1982)(20) and the cholesterol ester transfer protein (CETP) activity was measured according to Groener, Pelton and Kostner (1986)(21).

The lipid and lipoprotein concentrations of the two groups were tested for statistical significance by means of a two-sample t-test. Because of the skewed distribution of the total triglycerides and the triglyceride content of the different lipoprotein fractions, the natural logarithm of the triglyceride concentrations was used for the t-test. Differences in N-3 polyunsaturated fatty acid and alcohol intake between the two groups were tested for statistical significance by the non-parametric Mann-Whitney test because of the non-Gaussian distribution of the data. Body weight is an important determinant of serum lipid and lipoprotein fractions (22, 23). Therefore the means of the two groups were adjusted for differences in body weight by analysis of covariance. Two-sided probabilities were used for testing statistical significance.

Results

The average fish consumption of the men in the control group varied between 0.9 and 3.3 g per day from 1960 to 1986 (Table 1). The habitual fish consumers ate on average between 29.4 and 37.4 g of fish per day. Of the 2.3 g of fish per day consumed by the controls in 1986, 1.7 g was fatty fish and 0.6 g was lean fish. The habitual fish consumers ate 30.8 g fish per day in 1986, consisting of 4.9 g fatty fish and 26.0 g lean fish. The fatty fish was mainly herring and the lean fish was primarily cod.

The intake of N-3 polyunsaturated fatty acids, alcohol and energy was significantly higher in 1986 among the habitual fish consumers than in the controls (Table 2). The habitual fish consumers had an intake of five times as much N-3 polyunsaturated fat and twice as much alcohol as the persons in the control group. The percentage of cigarette smokers was 32 per cent among the habitual fish con-

TABLE 3
Habitual fish consumption in relation to age, height, weight, serum lipids, apoproteins and enzymes among men aged 67-82 in 1986

Risk factor		Controls n = 15 m ± SD	Habitual fish Consumers n = 25 m ± SD
Age	(yrs)	73.0 ± 4.4	73.2 ± 4.5
Height	(cm)	173.0 ± 6.6	173.1 ± 6.6
Weight	(kg)	78.5 ± 13.5	77.0 ± 7.6
Total triglycerides	(mmol/l)	1.72 ± 0.70	1.27 ± 0.71*
Total cholesterol	(mmol/l)	6.48 ± 0.93	6.30 ± 0.93
HDL cholesterol	(mmol/l)	1.08 ± 0.16	1.18 ± 0.33
Apoprotein A-I	(mg/dl)	140.5 ± 13.4	145.8 ± 25.7
Apoprotein A-II	(mg/dl)	61.6 ± 7.1	62.2 ± 8.3
Apoprotein B	(mg/dl)	113.1 ± 14.0	108.1 ± 16.6
LCAT	(nmol/ml/h)	154.2 ± 22.4	151.2 ± 28.4
CETP	(nmol/ml/h)	125.4 ± 46.4	134.0 ± 47.3

*$p<0.05$; LCAT = Lecithin cholesterol acyltransferase; CETP = Cholesterol ester transfer protein; Conversion: SI to traditional units; Triglycerides : 1mmol/l = 88.5 mg/dl; Cholesterol: 1mmol/l = 38.7 mg/dl

TABLE 4
Habitual fish consumption in relation to the cholesterol content of different lipoprotein fractions.

Cholesterol		Controls n = 15 m ± SD	Habitual fish Consumers n = 25 m ± SD
VLDL	(mmol/l)	0.609 ± 0.289	0.496 ± 0.382
IDL	(mmol/l)	0.317 ± 0.129	0.289 ± 0.170
LDL	(mmol/l)	3.865 ± 0.887	3.596 ± 0.798
HDL 1	(mmol/l)	0.203 ± 0.090	0.260 ± 0.151
HDL 2	(mmol/l)	0.231 ± 0.105	0.304 ± 0.159
HDL 3	(mmol/l)	0.882 ± 0.135	0.954 ± 0.313

Conversion: SI to traditional units; Cholesterol: 1 mmol/l = 38.7 mg/dl

sumers and 40 per cent among the controls. No difference was observed in age, height and weight between the two groups (Table 3). Average serum triglyceride levels were 26% lower among the habitual fish consumers than in the controls. This difference was statistically significant. Total and HDL cholesterol did not differ between the two groups. Also the concentrations of apoprotein A-I, A-II and B and of the enzymes LCAT and CETP did not differ between the two groups.

No differences were observed in the cholesterol content of the different lipoprotein fractions (Table 4). The average triglyceride content of the IDL fractions was, however, 38% lower ($p<0.05$) among the habitual fish consumers compared with the controls (Table 5). Body weight was more closely associated with total triglycerides ($r=0.26$, $p=0.06$) than with IDL triglycerides ($r=0.19$, $p=0.12$). Energy and alcohol intake were not associated with total triglycerides and with the triglyceride content of the IDL lipoprotein fraction. In order to adjust for the small differences in body weight between the two groups, analyses of covariance were carried out. Also after these analyses, total triglycerides and the triglyceride content of the IDL fraction were significantly ($p<0.05$) lower among the habitual fish consumers than in the controls.

Discussion

The unique feature of the present study is the information about the habitual fish consumption in a free-living population over a 26-year observation period.

For the present study, healthy elderly men were selected who had a constant fish consumption pattern over the last 26 years. This reduced the problem of misclassification so common in observational studies. An advantage of the present study over several intervention trials, is the use of a control group (24, 25). A disadvantage of the present study is the small sample size. This is due to the large variability in the fish consumption pattern of the participants in the Zutphen Study during the 26-year follow-up period.

The purpose of the present study was to investigate the long-term effects of a small amount of fish on lipids and lipoproteins. Therefore the contrast in fish consumption between the two groups was of primary importance. During the 26 years of observation, the difference in fish consumption between the two groups varied between 25 and 35 g/day. In order to study the long-term effects of a small amount of fish, the difference in other exposure variables between the two groups had to be as small as possible. Of the exposure variables measured, only energy and alcohol intake differed significantly between the two groups. These potential confounders were taken into account in further analyses.

The two groups differed significantly in the intake of N-3 polyunsaturated fatty acids (Table 2). This difference was confirmed by the results of the fatty acid analyses of the

TABLE 5
Habitual fish consumption in relation to the triglyceride content of different lipoprotein fractions.

Triglycerides		Controls n = 15 m ± SD	Habitual fish consumers n = 25 m ± SD
VLDL	(mmol/l)	0.864 ± 0.493	0.637 ± 0.472
IDL	(mmol/l)	0.126 ± 0.070	0.078 ± 0.043*
LDL	(mmol/l)	0.255 ± 0.101	0.239 ± 0.057
HDL 1	(mmol/l)	0.019 ± 0.010	0.018 ± 0.008
HDL 2	(mmol/l)	0.022 ± 0.007	0.022 ± 0.010
HDL 3	(mmol/l)	0.091 ± 0.028	0.078 ± 0.030

*$p<0.05$; Conversion: SI to traditional units;
Triglycerides: 1 mmol/l = 88.5 mg/dl

phospholipids. The content of the N-3 polyunsaturated fatty acids, eicosapentaenoic acid and docosahexaenoic acid, of the phospholipids was significantly higher among the habitual fish consumers than in the controls (3). These results confirm that the two groups were significantly different with respect to fish consumption patterns.

In the present study, significantly lower total triglyceride levels were observed among habitual fish consumers compared with controls (Table 3). This difference may be due to the difference in N-3 polyunsaturated fatty acids between the two groups. The association between N-3 polyunsaturated fatty acids and total triglycerides could be confounded by alcohol. The alcohol intake of the habitual fish consumers was twice as high as that of the controls. However, in the present study alcohol intake was not related to serum triglycerides. Also in a study among Eskimos, alcohol consumption was not related to plasma triglycerides (26).

Another confounder in the association between fish consumption and serum triglycerides could be energy intake. Energy intake was significantly higher among the habitual fish consumers compared with the controls. This could mean that the habitual fish consumers were more physically active than the controls. This may explain the 1.5-kg lower body weight of the habitual fish consumers compared with the controls. Energy intake was not associated with total triglycerides and the difference in total triglycerides between the two groups remained significant after adjustment for body weight. It is therefore unlikely that the difference in serum triglycerides between the habitual fish consumers and the controls could be explained by any confounding effects of energy intake and body weight. The results of the present study suggest that the difference in total triglycerides between the long-term habitual fish consumers and the controls is probably due to differences in fish consumption. It cannot however, be definitely ruled out that other confounders may explain this relation.

Eskimo men had about 50% lower average total triglyceride levels than Danish controls (27). The estimated difference in N-3 polyunsaturated fatty acid intake between Eskimos and Danes amounted to 6 grams per day (28). In the present study a 26% difference in total triglycerides was observed between the men who consumed approximately 30 g of fish per day over more than 25 years and the controls. The difference in N-3 polyunsaturated fatty acid intake between these two groups amounted to about 200 mg per day. The difference in total triglyceride levels between the two groups in the present study is comparable with that observed in short-term intervention studies in normolipidemic subjects consuming on average 5 grams of N-3 polyunsaturated fatty acids (29). Eskimo men also had significantly lower total and significantly higher HDL cholesterol levels than the Danish controls (27). In the present study the long-term habitual fish consumers and the controls did not differ in their total and HDL cholesterol. Also in the short-term intervention studies, no significant results were obtained regarding these lipid fractions (29). The results of the present study suggest that a habitual fish consumption of about 30 g/day for more than 25 years is an effective measure for lowering total triglycerides but not for lowering total cholesterol or increasing HDL cholesterol.

The average triglyceride content of the IDL fraction was 38% lower among the habitual fish consumers than among the controls. No significant difference was found in the cholesterol content of this lipoprotein fraction between the groups. These results suggest that the IDL particles of the fish consumers contain less triglycerides than those of the controls. The triglyceride-poor IDL particles of the fish consumers may be less atherogenic than the triglyceride-rich IDL particles of the controls (30-32).

The results of the present study show that a habitual fish consumption of approximately 30 grams of fish per day over more than 25 years is effective in lowering total and IDL triglycerides. Until now most emphasis in short-term intervention studies has been on using large amounts of fish oil in order to lower serum triglycerides. In future studies more emphasis should be given to the long-term use of small amounts of fish. This may provide an effective alternative.

Acknowledgements

We are indebted to the many people who co-operated in the longitudinal study (the Zutphen men who took part and their wives; the fieldwork team in Zutphen, especially E.B. Bosschieter M.D., Ms. A.H. Thomassen-Gysbers, Ms. D. Haverhals-Post and Ms. B. Sueters; Drs. J.J.P.M. Jaspers, head Clinical Chemistry and Haematology Laboratory, Nieuwe Spittaal Hospital, Zutphen; and the dietitians H.E. Goddijn, P. Schneyder and L. van der Wilt. The lipid and lipoprotein analyses were skilfully performed by Z. Kruyswijk, F.J.M. Schouten and A.E.M.F. Soffers, Department of Human Nutrition, Agricultural University, Wageningen. The lecithin cholesterol acyltransferase activity was measured by Dr. E. Steyrer, Department of Medical Biochemistry, University of Graz, Austria (Head Professor Dr. G.M. Kostner). This research was supported by grants from the Netherlands Heart Foundation and the Netherlands Prevention Foundation.

References

1. Kromhout D, Bosschieter EB, De Lezenne de Coulander C (1985) Fish consumption and 20-year mortality form coronary heart disease. N Engl J Med 312: 1205-1209
2. Goodnight SH Jr, Harris WS, Connor WE, Illingworth DR (1982) Polyunsaturated fatty acids, hyperlipidemia, and thrombosis. Arteriosclerosis 2: 87-113
3. Van Houwelingen R, Hornstra G, Kromhout D, De Lezenne Coulander C (1989) Habitual fish consumption, fatty acids of serum phospholipids, and platelet function. Atherosclerosis 75: 157-165
4. Keys A, Aravanis C, Blackburn HW, Van Buchem FSP, Buzina R, Djordjevic BS, Dontas AS, Fidanza F, Karvonen MJ, Kimura N, Lekos D, Monti M, Puddu V, Taylor HL (1967) Epidemiological studies related to coronary heart disease: characteristics of men aged 40-59 in seven countries. Acta Med Scand (Suppl): 460
5. Keys A (1970) Coronary heart disease in seven countries. Circulation 41 (Suppl 1)
6. Keys A (1980) Seven countries: a multivariate analysis of death and coronary heart disease. Cambridge, Mass., Harvard University Press
7. Burke BS (1947) The dietary history as a tool in research. J Am Diet Assoc 23: 1041-1046
8. Den Hartog C, Van Schaik ThFSM, Dalderup LM, Drion EF, Mulder T (1965) The diet of volunteers participating in a long-term epidemiological field survey on coronary heart disease at Zutphen, The Netherlands. Voeding 26: 184-208
9. Kromhout D, De Lezenne Coulander C, Obermann-de Boer GL, Van Kampen-Donker M, Goddijn E, Bloemberg BPM (1990) Changes in food and nutrient intake in middle-aged men during the period 1960-1985 (The Zutphen Study). Am J Clin Nutr 51: 123-129
10. Hautvast JGAJ (1975) Commissie uniforme codering voedingsenquêtes: ontwikkeling van een systeem om gegevens van voedingsenquêtes met behulp van de computer te verwerken. Voeding 36: 356-360
11. Stähler F, Grüber W, Stinshoff K, Röschlau P (1977) Eine praxisgerechte enzymatische Cholesterin-Bestimmung. Med Lab 30: 29-37
12. Seidell J, Schlumberger H, Klose S, Ziegenhorn J, Wahlefeld AW (1981) Improved reagent for the enzymatic determination of serum cholesterol. J Clin Chem Clin Biochem 19: 838-839
13. Sullivan DR, Kruijswijk Z, West CE, Kohlmeier M, Katan MB (1985) Determination of serum triglycerides by an accurate enzymatic method not affected by free glycerol. Clin Chem 31: 1227-1228
14. Warnick GR, Benderson J, Albers JJ (1982) Dextran sulfate-Mg^{2+} precipitation procedure for quantification of high density lipoprotein cholesterol. Clin Chem 28: 1379-1388
15. Terpstra AHM, Woodward CJH, Sanchez-Muniz FG (1981) Improved techniques for the separation of serum lipoprotein by density gradient ultracentrifugation -visualization by prestaining and rapid separation of serum lipoproteins from small volumes of serum. Anal Biochem 111: 149-157
16. Cheung MC, Albers JJ (1977) The measurement of apolipoprotein A-1 and A-II levels in men and women with immunoassay. J Clin Invest 60: 43-50
17. Havekes L, Hemmink J, De Wit E (1981) Low density lipoprotein apoprotein B in plasma as measured by radial immunodiffusion and rocket immunoelectrophoresis. Clin Chem 27: 1829-1833
18. Stokke KT, Norum KR (1971) Determination of lecithin: cholesterol acyltransferase in human blood plasma. Scan J Clin Lab Invest 27: 21-27
19. Pownall HJ, Van Winkle WB, Pao Q, Rohde M, Gotto AM (1982) Action of lecithin: cholesterol acyltransferase on model lipoproteins. Preparation and characterisation of model nascent high density lipoprotein. Biochem Biophys Acta 713: 494-503
20. Chen C, Albers JJ (1982) Characterization of proteoliposomes containing apoprotein A-I: a new substrate for the measurement of lecithin: cholesterol acyltransferase activity. J Lipid Res 23: 680-691
21. Groener JEM, Pelton RW, Kostner GH (1986) Improved estimation of cholesterol ester transfer/exchange activity in serum or plasma. Clin Chem 32: 283-286
22. Kromhout D (1983) Body weight, diet and serum cholesterol in 871 middle aged men during 10 years of follow-up (the Zutphen Study). Am J Clin Nutr 38: 591-598
23. Katan MB (1984) Diet and HDL. In: NE Miller GJ Miller (Eds). Clinical and metabolic aspects of high-density lipoproteins. Elsevier Science Publishers, Amsterdam: 103-131
24. Herold PM, Kinsella JE (1986) Fish oil consumption and decreased risk of cardiovascular disease: a comparison of findings from animal and human feeding trials. Am J Clin Nutr 43: 566-598
25. Hornstra G (1989) The significance of fish and fish-oil enriched food for prevention and therapy of ischaemic cardiovascular disease. In: Vergroesen AJ, Crawford MA (eds), The role of fat in human nutrition II. Academic Press, 152-235

26. De Knijff P, Johansen LG, Rosseneu M, Frants RA, Jaspersen J, Havekes LM (1992) Lipoprotein profile of a Greenland Inuit population. Arterioscler Thromb 12: 1371-1379
27. Bang HO, Dyerberg J, Brondum Nielsen (1971) A Plasma lipid and lipoprotein pattern in Greenlandic west coast Eskimos. Lancet 1: 1143-1146
28. Bang HO, Dyerberg J (1985) Fish consumption and mortality from coronary heart disease. (letter to the editor). N Engl J Med 313: 822-823
29. Harris WS (1989) Fish oils and plasma and lipoprotein metabolism in humans: a critical review.J Lip Res 30:785-807
30. Coniglio JG (1992) How does fish oil lower plasma triglycerides? Nutr Rev 50: 195-197
31. Zilversmit DB (1979) Atherogenesis: a postprandial phenomenon. Circulation 60: 473-485
32. Mahley RW (1985) Atherogenic lipoproteins and coronary artery disease; concepts derived from recent advances in cellular and molecular biology. Circulation 72: 943-948