Replacement of linoleic acid with α-linolenic acid does not alter blood lipids in normolipidaemic men

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The effect of partial dietary replacement of linoleic acid (18:2*n*-6; linoleic acid-rich diet) with α-linolenic acid (18:3*n*-3; α-linolenic acid-rich diet) on plasma lipids was investigated in twenty-nine healthy young men. After a 2-week stabilization period subjects were randomly assigned to either the α-linolenic acid-rich diet group (*n* 15), receiving a mean of 10·1 g of α-linolenic acid and 12·1 g of linoleic acid/d, or the linoleic acid-rich diet group (*n* 14), receiving a mean of 1·0 g of α-linolenic acid and 21·0 g of linoleic acid/d, for a 6-week test period. Blood samples were taken at the commencement of the stabilization period and at the start (week 0), midpoint (week 3) and endpoint (week 6) of the test period and plasma lipids analysed. The changes occurring on the linoleic acid-rich diet and α-linolenic acid-rich diet were compared but no significant differences in the changes in plasma total cholesterol, LDL-cholesterol, HDL-cholesterol, the subfractions HDL_2 and HDL_3 or triacylglycerols were found. These results indicate that dietary replacement of linoleic acid with α-linolenic acid in the diet of healthy male subjects offers similar cardioprotective benefits with respect to lipid metabolism.

α-Linolenic acid: Linoleic acid: Blood lipids: Linseed oil

It has been well documented that a relationship exists between plasma cholesterol and triacylglycerols and CHD (Rose & Shipley, 1986; Kromhout *et al.* 1988; National Institute of Health Consensus Conference, 1993). LDL-cholesterol is positively associated with risk of CHD (Pekkanen *et al*. 1990) as are triacylglycerols (Criqui *et al*. 1993), while HDL-cholesterol has an inverse relationship (Salonen *et al.* 1991). It has also been shown that the type of dietary fat ingested is a major factor influencing plasma lipid profiles. Saturated fatty acids, except stearic acid (C18:0), have a hypercholesterolaemic effect (Hegsted *et al*. 1965; Grundy & Vega, 1988). Oleic acid (C18:1*n*-9), the predominant dietary monounsaturated fatty acid, results in lowering of LDL-cholesterol (Bonanome & Grundy, 1988) as does linoleic acid (Becker *et al.* 1983; McDonald *et al.* 1989), the predominant polyunsaturated fatty acid (PUFA). Health authorities have recommended that the proportions of both in the diet be preferentially increased at the expense of saturated fat (Truswell *et al*. 1992).

The *n*-6 PUFA linoleic acid has become predominant in Western diets largely because of its presence in margarines and cooking oils. However, interest in increased consumption of the *n*-3 family of PUFA has grown since studies of Greenland Eskimos showed low incidence of CHD (Bang & Dyerberg, 1972; Dyerberg *et al.* 1975), despite high fat intakes, with the very-long-chain *n*-3 fatty acids eicosapentaenoic acid (EPA; C20:5*n*-3) and docosahexaenoic acid (DHA; C22:6*n*-3) comprising a large proportion of fat intake (Bang *et al.* 1976). This population is characterized by low serum cholesterol and triacylglycerols. Consumption of EPA and DHA in the form of fish oil capsules has shown that triacylglycerols are lowered (Sanders *et al*. 1981; von Schacky *et al.* 1985) while effects on LDL- and HDL-cholesterol have been less well defined. In some studies LDL-cholesterol has risen (Zucker *et al.* 1988) and in others it has fallen (Harris *et al*. 1988). von Schacky *et al.* (1985) saw no change in HDL-cholesterol while Sanders *et al.* (1981) found an increase. However, the benefits of the very-long-chain *n*-3 PUFA are likely to be via mechanisms other than cholesterol metabolism.

A number of studies of the effects on blood lipids of the progenitor fatty acid of the *n*-3 family, α-linolenic acid, have been reported. Interpretation of the results of these studies has been difficult because oleic acid has been altered at the same time (McDonald *et al*. 1989; Wardlaw *et al.* 1991) or differences exist in the subjects' demographic (Sanders & Roshanai, 1983) and blood lipid characteristics (Kestin *et al*. 1990; Mantzioris *et al*. 1994). The importance of establishing the precise effects of α-linolenic acid on blood lipids is considerable given the marked reduction in myocardial infarction ascribed to it (de Lorgeril *et al.* 1994).

In the present study the effects of replacement of linoleic

Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acid.

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Table 1. Demographics and plasma lipid concentrations of subjects at the commencement of the 2-week stabilization period

(Mean values with standard deviations)

	α-Linolenic acid-rich diet (*n* 15)		Linoleic acid-rich diet (*n* 14)	
Variable	Mean	SD	Mean	SD
Age (years)	25·0	4·1	24·0	4·5
Weight (kg)	72·0	9·0	72·5	8·0
BMI (kg/m^2)	22·1	2·0	22·7	1·6
Triacylglycerols (mmol/l)	0·8	0·1	0·7	0·1
Total cholesterol (mmol/l)	4·2	0·2	4·1	0·2

Table 2. The nutrient intakes of the subjects on the stabilization diet and on the 6-week test diets

(Mean values with standard deviations)

	Stabilization diet (*n* 29)		α-Linolenic acid-rich diet (*n* 15)		Linoleic acid-rich diet (*n* 14)	
Nutrient*	Mean	SD	Mean	SD	Mean	SD
Energy (kJ)	12 824	981	13 157	714	12 941	549
% total energy						
Protein	15·4	0·2	15·5	0·1	15·5	0·0
Fat	39·3	0·8	31·2	0·3	31·4	0·3
Carbohydrate	43·2	1·0	49·1	0·4	49·1	0·3
Saturated fatty acids	16·3	0·2	9·7	0·3	9·8	0·2
Monounsaturated fatty acids	14·8	0·6	11·6	0·1	11·8	0·4
Polyunsaturated fatty acids	6·0	0·2	6·7	0·3	6·9	0·0
α-Linolenic acid	0·2	0·0	3·5	0·5	0·1	0·0
Linoleic acid	5·6	0·3	3·1	0·2	6·7	0·0

* Calculated from The Composition of Foods Australia: 6 (Lewis *et al.* 1992).

acid with α-linolenic acid on blood lipids were tested. Subjects were selected to be as homogenous as possible and only two fatty acids were manipulated, α-linolenic acid and linoleic acid, by replacing safflower oil with linseed oil.

Materials and methods

Subjects

Twenty-nine male subjects between the ages of 18–35 years were selected from volunteers responding to advertisements within the University. Following the initial screening of respondents, those found to be free from chronic illness, with a BMI < 29 kg/m^2, total cholesterol < 6·8 mmol/l, triacylglycerols < 2·8 mmol/l, and not taking any medications were included as subjects. Table 1 outlines the subject characteristics. The study was approved by the Human Ethics Committee of the University of Sydney.

Study design

Diets were designed to provide approximately 12 MJ using the Composition of Foods Australia (Lewis *et al.* 1992) for calculation of nutrient composition. All food was supplied to the subjects throughout the duration of the study. Breakfast and dinner were consumed under the supervision of study staff in a Metabolic Unit on weekdays and a boxed lunch was taken away. Weekend meals were packed and labelled to be prepared and consumed as indicated. During a 2-week stabilization period before the study, body weight was measured daily and energy intakes were altered as required to ensure that weight was maintained to within 1 kg. The stabilization diet was designed to supply 39·5 % energy as fat with 16·3 % energy as saturated fat, and 5·8 % energy as polyunsaturated fatty acids (5·5 % energy as linoleic acid and 0·3 % energy as α-linolenic acid).

After the stabilization period subjects were randomly allocated to either the α-linolenic acid-rich group (*n* 15) or the linoleic acid-rich group (*n* 14) and consumed the appropriate diet for 6 weeks. The test diets were designed to have 31·6 % energy as fat with 9·9 % energy as saturated fat and 6·6 % energy as polyunsaturated fat, and an α-linolenic acid : linoleic acid ratio of either 1 : 0·9 (α-linolenic acid-rich) or 1 : 66 (linoleic acid-rich). All subjects maintained a daily food diary. Food items not consumed, or consumed in addition to that which was provided, were recorded in the diary and accounted for in the final computer analysis. After accounting for any deletions or additions by subjects to the prescribed menus, the percentage energy from nutrients were in general agreement with the calculated intakes and are shown in Table 2. No fish or seafood products were included in any of the diets.

Subjects were instructed to maintain normal lifestyle patterns with respect to exercise, and to keep alcohol ingestion to a minimum. Subjects were unaware of which diet they received. All food items consumed during the test period were identical except for muffins, which were made with either safflower or linseed oil. Vitamin E contents of both oils were determined and the α-linolenic acid-rich diet supplemented to bring it to the same concentration as the safflower oil.

The fatty acid composition of the diets were determined by lipid extraction (Folch *et al*. 1957) from daily menu

Table 3. The fatty acid composition of the stabilization and test diets as analysed from diet composites (Mean values with standard deviations)

	Stabilization diet (%)		α-Linolenic acid-rich diet (%)		Linoleic acid-rich diet (%)	
Fatty acid*	Mean	SD	Mean	SD	Mean	SD
C10:0	1·7	0·0	0·6	0·0	0·6	0·0
C12:0	2·0	0·0	2·6	0·0	2·6	0·0
C14:0	5·3	0·0	3·5	0·0	3·5	0·0
C15:0	0·6	0·0	0·4	0·0	0·5	0·0
C16:0	22·0	0·0	20·0	0·3	20·4	0·2
C18:0	8·7	0·0	7·1	0·2	7·0	0·3
C14:1	0·3	0·0	0·3	0·0	0·3	0·0
C16:1	1·4	0·0	1·3	0·3	1·3	0·2
C18:1(*n*-9)	39·4	0·0	40·6	2·1	40·5	1·6
C18:1(*n*-7)	1·7	0·0	1·2	0·6	1·3	0·6
C18:2	15·5	0·0	12·1	0·6	21·0	0·8
C18:3	1·4	0·0	10·1	0·6	1·0	0·1

* Fatty acid value expressed as percentage of total fatty acids.

Table 4. Body weights (kg) of the subjects at the commencement and end of test diets (Mean values with their standard deviations)

	α-Linolenic acid-rich diet (*n* 15)		Linoleic acid-rich diet (*n* 14)	
Time	Mean	SD	Mean	SD
Week 0	71·6	2·3	71·6	2·0
Week 6	71·3	2·2	71·3	1·9

composites and quantified with GLC of the fatty acid methyl esters (Lepage & Roy, 1986).

Blood collection and sample preparation

Blood was sampled at the start of the stabilization period, at week 0, which was the end of the stabilization period just before starting the test diet, then at week 3 (midpoint) and week 6 (endpoint) of the test diets. At each sampling point blood was taken twice, separated by a period of 4 d, to account for biological variation in cholesterol concentrations. Before blood sampling, subjects were required to lie in a supine position for 15 min. Venous blood was collected after a 12 h overnight fast into a plastic syringe and 10 ml was dispensed into lithium-heparin tubes and kept on ice until processing ready for cholesterol and triacylglycerol analysis. The blood was centrifuged at 1200 ***g*** for 10 min at 5 °, and the resultant plasma was removed and stored at −20 ° until analysis. On the day of analysis, plasma samples were thawed at room temperature and centrifuged at 1200 ***g*** to remove fibrin clots.

Plasma lipids

Plasma cholesterol and triacylglycerols were measured on the Cobas Fara autoanalyser (Roche Products Pty Ltd., Dee Why, NSW, Australia). Total plasma cholesterol was determined using the enzymic cholesterol CHOD-PAP kit method (Boehringer Manheim, Germany). Standards and controls used were purchased from Abbott Laboratories, Abbott Park, IL, USA. The precipitation method of Warnick *et al*. (1982) using increasing concentrations of a dextran sulfate/$MgCl_2$ solution was used to isolate total HDL- and HDL_3-cholesterol which were measured using the CHOD-PAP method. HDL_2-cholesterol was calculated by difference. Plasma triacylglycerols were measured using the Unimate 5 TRIG diagnostic reagent system (Roche Products Pty Ltd.).The concentration of LDL-cholesterol was calculated using the Friedewald equation (Friedewald *et al.* 1972) and converted to SI units once total- and HDL-cholesterol, and triacylglycerol concentrations had been determined.

Statistical analyses

The differences between lipid concentrations at week 0 and week 3, and at week 0 and week 6 of the test diets were calculated for each group and the changes produced after 6 weeks on the α-linolenic acid-rich and linoleic acid-rich diets compared using the unpaired *t* test. The level of significance was taken as $P < 0{\cdot}05$. The mean differences in changes between the two groups and 95 % CI were also calculated.

Results

The α-linolenic acid-rich diet supplied a mean of 10·1 g of α-linolenic acid and 12·1 g of linoleic acid/d, while the linoleic acid-rich diet supplied 1·0 g of α-linolenic acid and 21·0 g of linoleic acid/d. The individual fatty acid composition of each diet period and group is shown in Table 3. Fatty acids less than C10:0 were not included in the calculations as they were not resolved by the GC program. The diets did not include any fish or seafood hence EPA and DHA constituted < 0·01 % of total fatty acids on all of the diets.

The subjects' body weights remained unchanged throughout the study (Table 4).

Table 5 shows the plasma lipids for the α-linolenic acid-rich and the linoleic acid-rich diet groups. There was no significant difference between the two groups for any of the changes occurring in the plasma lipid fractions after 6 weeks on the diets.

Table 5. Changes in plasma lipid concentrations after 3 and 6 weeks on the α-linolenic acid- and the linoleic acid-rich diets*

Plasma lipid (mmol/l)	α-Linolenic acid-rich diet (*n* 15)						Linoleic acid-rich diet (*n* 14)						Difference between the changes at week 6 for α-linolenic *v.* linoleic acid-rich diet	
	Stabilization	Week 0	Change at week 3		Change at week 6		Stabilization	Week 0	Change at week 3		Change at week 6			
	Mean	Mean	Mean	SEM	Mean	SEM	Mean	Mean	Mean	SEM	Mean	SEM	Mean	95 % CI
Total cholesterol	4·2	3·8	–0·03	0·14	–0·11	0·13	4·1	3·9	–0·17	0·14	–0·25	0·10	0·14	–0·19, 0·47
LDL-cholesterol	3·1	2·3	0·04	0·16	–0·05	0·11	3·1	2·5	–0·16	0·12	–0·22	0·10	0·17	–0·13, 0·46
HDL-cholesterol	1·2	1·1	–0·12	0·05	–0·07	0·05	1·2	1·1	–0·06	0·04	–0·10	0·05	0·03	–0·11, 0·17
HDL_2-cholesterol	0·4	0·4	–0·02	0·04	–0·01	0·04	0·4	0·4	–0·03	0·03	–0·04	0·03	0·03	–0·08, 0·14
HDL_3-cholesterol	0·8	0·7	–0·10	0·02	–0·06	0·03	0·7	0·7	–0·02	0·03	–0·06	0·04	0·00	–0·08, 0·08
LDL : HDL	2·7	2·3	0·32	0·36	0·02	0·17	2·9	2·4	–0·07	0·17	0·12	0·26	–0·10	–0·72, 0·52
Triacylglycerols	0·8	0·8	0·11	0·05	0·03	0·05	0·7	0·7	0·11	0·05	0·15	0·06	–0·12	–0·28, 0·04

* For details of diets and procedures, see pp. 164–165.

Discussion

The present study revealed no variation in plasma cholesterol when half the *n*-6 PUFA, as linoleic acid, were replaced by the *n*-3 fatty acid, α-linolenic acid. The diets were identical except for the replacement of safflower oil with linseed oil. Previous studies using linseed oil as a dietary supplement in normolipidaemic subjects have similarly shown no effects on serum cholesterol, but interpretation is complicated because diets have either been unmonitored (Sanders & Roshani, 1983) or more than one dietary variable has been changed (Kelley *et al.* 1993). Valsta *et al* (1995) studied forty subjects using a crossover experiment with two 6-week periods on high monounsaturated fat diets with either a high (low erucic acid-rapeseed oil) or low (TRISUN® sunflower oil) α-linolenic acid content and linoleic acid the only other variable. They reported similar declines in total and LDL-cholesterol and triacylglycerols on both diets. Mantzioris *et al.* (1994) instructed subjects to follow a low-fat diet and replace oils and margarines with either linseed or sunflower products. Dietary intakes were monitored throughout the study but after 4 weeks no effect on LDL-cholesterol or HDL-cholesterol was found for either diet. These results together with those of our strictly controlled study indicate that α-linolenic acid has similar effects to linoleic acid on blood lipids in normolipidaemic subjects.

The present findings should not be extrapolated to hyperlipidaemic subjects but the effects are likely to be similar. Kestin *et al*. (1990) and Singer *et al.* (1990) have studied patients with hyperlipidaemias comparing safflower or sunflower, linseed and fish oils and reported no change in serum total cholesterol, LDL-cholesterol, or HDL-cholesterol with linseed oil. Harris (1997), in a review of human studies, concluded that α-linolenic acid was equivalent to *n*-6-rich oils for lipid and lipoprotein effects.

Triacylglycerols remained unchanged on the α-linolenic acid-rich compared with the linoleic acid-rich diet. Previously it has been reported that conversion of α-linolenic acid to EPA will inhibit the enzymic conversion of diacylglycerol into triacylglycerol (Rustan *et al.* 1988). A number of studies have reported that dietary linseed oil causes a minor reduction in serum triacylglycerol (Singer *et al.* 1990; Kelley *et al.* 1993). In some studies no changes have been detected (Sanders & Roshanai, 1983; Kestin *et al.* 1990; Mantzioris *et al.* 1994). This may be because α-linolenic acid was fed in low amounts, so only a small amount of EPA was produced

A significant reduction in plasma LDL-cholesterol occurred following the 2-week stabilization diet for both subject groups ($P< 0·05$). This suggests that the composition of the subjects' diets was different before they started the study but no attempt was made to quantify accurately their pre-study intakes. A reduction from approximately 16 % to 10 % energy from saturated fat during the test diets did not produce any further reductions in LDL-cholesterol over the 6 weeks. It may be that because these subjects had plasma cholesterol at the lower end of normal and that no real changes were made in total unsaturated fat intake, that the LDL-cholesterol was stable.

In conclusion, the lack of any deleterious effects on lipid metabolism of substituting α-linolenic acid for linoleic acid supports its inclusion in the diet. This means that investigators can explore the benefits of α-linolenic acid on other variables in the cardiovascular disease equation. It is in these other areas such as thrombotic tendency and heart arrhythmias in which α-linolenic acid is most likely to produce benefits.

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References

Bang HO & Dyerberg J (1972) Plasma lipids and lipoproteins in Greenlandic west coast Eskimos. *Acta Medica Scandinavica* **192**, 85–94.

Bang HO, Dyerberg J & Hjørne N (1976) The composition of food consumed by Greenland Eskimos. *Acta Medica Scandinavica* **200**, 69–73.

Becker N, Illingworth DR, Alaupovic P, Connor WE & Sundberg EE (1983) Effects of saturated, monounsaturated, and ω-6 polyunsaturated fatty acids on plasma lipids, lipoproteins, and apoproteins in humans. *American Journal of Clinical Nutrition* **37**, 355–360.

Bonanome A & Grundy SM (1988) Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. *New England Journal of Medicine* **318**, 1244–1248.

Criqui MH, Heiss G, Cohn R, Cowan LD, Suchindran CM, Bangdiwala S, Kritchevsky S, Jacobs DR Jr, O'Grady HK & Davis CE (1993) Plasma triglyceride level and mortality from coronary heart disease. *New England Journal of Medicine* **328**, 1220–1225.

de Logeril M, Renaud S, Mamelle N, Salen P, Martin JL, Monjaud I, Guidollet J, Taboul P & Delaye J (1994) Mediterrenean α-linolenic acid-rich diet in secondary prevention of coronary heart disease. *Lancet* **343**, 1454–1459.

Dyerberg J, Bang HO & Hjørne N (1975) Fatty acid composition of the plasma lipids in Greenland Eskimos. *American Journal of Clinical Nutrition* **28**, 958–966.

Folch J, Lees M & Sloane-Stanley G (1957) A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* **226**, 497–509.

Friedewald WT, Levy RI & Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without the use of the preparative ultracentrifuge. *Clinical Chemistry* **18**, 449–502.

Grundy SM & Vega GL (1988) Plasma cholesterol responsiveness to saturated fatty acids. *American Journal of Clinical Nutrition* **47**, 822-824.

Harris WS (1997) *n*-3 Fatty acids and serum lipoproteins; human studies. *American Journal of Clinical Nutrition* **65**, 1645S–1654S.

Harris WS, Connor WE, Alam N & Illingworth DR (1988) Reduction of postprandial triglyceridemia in humans by dietary *n*-3 fatty acids. *Journal of Lipid Research* **29**, 1451–1460.

Hegsted DN, McGandy RB, Myers SM & Stare FJ (1965) Quantitative effects of dietary fat on blood cholesterol in man. *American Journal of Clinical Nutrition* **14**, 776–787.

Kelley DS, Nelson GJ, Love JE, Branch LB, Taylor PC, Schmidt PC, Mackey BE & Iacono JM (1993) Dietary α-linolenic acid alters tissue fatty acid composition, but not blood lipids, lipoproteins or coagulation status in humans. *Lipids* **28**, 533–537.

Kestin M, Clifton P, Belling GB & Nestel PJ (1990) *n*-3 Fatty acids of marine origin lower systolic blood pressure and triglycerides but raise LDL cholesterol compared with *n*-3 and *n*-6 fatty acids from plants. *American Journal of Clinical Nutrition* **51**, 1028–1034.

Kromhout D, Bosschieter EB, Drijver M & de Lezenne Coulander C (1988) Serum cholesterol and 25-year incidence of and mortality from myocardial infarction and cancer. The Zutphen Study. *Archives of Internal Medicine* **148**, 1051–1055.

Lepage G & Roy CC (1986) Direct transesterification of all classes of lipids in a one-step reaction. *Journal of Lipid Research* **27**, 114–120.

Lewis J, Holt R & English R (1992) *Composition of Foods Australia: 6*. Canberra: Australian Government Publishing Service.

McDonald BE, Gerrard JM, Bruce VM & Corner EJ (1989) Comparison of the effect of canola oil and sunflower oil on plasma lipids and lipoproteins and on in vivo thromboxane A_2 and prostacyclin production in healthy young men. *American Journal of Clinical Nutrition* **50**, 1382–1388.

Mantzioris E, James MJ, Gibson RA & Cleland LG (1994) Dietary substitution with an α-linolenic acid-rich vegetable oil increases eicosapentaenoic acid concentrations in tissues. *American Journal of Clinical Nutrition* **59**, 1304–1309.

National Institute of Health Consensus Conference (1993) Triglyceride, high-density lipoprotein, and coronary heart disease. *Journal of the American Medical Association* **269**, 505–510.

Pekkanen J, Linn S, Heiss G, Suchindran CM, Leon A, Rifkind BM & Tyroler HA (1990) Ten-year mortality from cardiovascular disease in relation to cholesterol level among men with and without preexisting cardiovascular disease. *New England Journal of Medicine* **332**, 1700–1707.

Rose G & Shipley M (1986) Plasma cholesterol concentration and death from coronary heart disease: 10 year results of the Whitehall study. *British Medical Journal* **293**, 306–307.

Rustan AC, Nossen JØ, Christiansen EN & Drevon CA (1988) Eicosapentaenoic acid reduces hepatic synthesis and secretion of triacylglycerol by decreasing the activity of acyl-coenzyme A:1,2-diacylglycerol acyltransferase. *Journal of Lipid Research* **29**, 1417–1426.

Salonen JT, Salonen R, Seppänen K, Rauramaa R & Tuomilehto J (1991) HDL, HDL_2, and HDL_3 subfractions, and the risk of acute myocardial infarction. *Circulation* **84**, 129–139.

Sanders TAB & Roshanai F (1983) The influence of different types of ω3 polyunsaturated fatty acids on blood lipids and platelet function in healthy volunteers. *Clinical Science* **64**, 91–99.

Sanders TAB, Vickers M & Haines AP (1981) Effect of blood lipids and haemostasis of a supplement of cod-liver oil, rich in eicosapentaenoic and docosahexaenoic acids, in healthy young men. *Clinical Science* **61**, 317–324.

Singer P, Wirth M & Berger I (1990) A possible contribution of decrease in free fatty acids to low serum triglyceride levels after diets supplemented with *n*-6 and *n*-3 polyunsaturated fatty acids. *Atherosclerosis* **83**, 167–175.

Truswell AS, Craske JD, English R, Nestel PJ, Sinclair A, Lester IH & Lilburne A (1992) *The Role of Polyunsaturated Fats in the Australian Diet.* Report of the National Health and Medical Research Council Working Party. Canberra: Australian Government Publishing Service.

Valsta LM, Jauhiainen M, Aro A, Salminen I & Mutanen M (1995) The effects on serum lipoprotein levels of two monounsaturated fat rich diets differing in their linoleic and α-linolenic acid contents. *Nutrition and Metabolism in Cardiovascular Disease* **5**, 129–140.

von Schacky C, Fischer S & Weber PC (1985) Long-term effects of dietary marine ω-3 fatty acids upon plasma and cellular lipids, platelet function, and eicosanoid formation in humans. *Journal of Clinical Investigation* **76**, 1626–1631.

Wardlaw GM, Snook JT, Lin MC, Puangco MA & Kwon JS (1991) Serum lipid and apolipoprotein concentrations in healthy men on diets enriched with either canola oil or safflower oil. *American Journal of Clinical Nutrition* **54**, 104–110.

Warnick GR, Benderson J & Albers JJ (1982) Dextran sulfate-Mg^{2+} precipitation procedure for quantitation of high-density-lipoprotein cholesterol. *Clinical Chemistry* **28**, 1379–1388.

Zucker ML, Bilyeu DS, Helmkamp GM, Harris WS & Dujovne CA (1988) Effects of dietary fish oil on platelet function and plasma lipids in hyperlipoproteinemic and normal subjects. *Atherosclerosis* **73**, 13–22.