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Stimulation of the apo AI–high density lipoprotein system by dietary soyabean lecithin in humans

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The aim of this work was to assess, the effect of a dietary supplement of soyabean lecithin on the apoprotein (apo) AI–high density lipoprotein system in humans. Adult outpatients (three women and seven men, aged 52 ± 5 years) were selected on the basis of a major type IIA hypercholesterolemia (>6.5 mmol/L). For each subject, a previous control period of 6 weeks consisted in a daily dietary supplement of 3 g soyabean oil (SO) as placebo distributed in two capsules at three main meals. Subjects then were given a daily dietary supplement of 3 g purified soyabean phosphatidylcholine (SPC) for 6 weeks, also distributed in two capsules at the three main meals. The usual basal diet of each subject was monitored every 4 weeks by a 3-day food recall. Between the end of the SO period and the end of the SPC period, plasma level of apo AI significantly increased (from 1.01 to 1.23 g/L), as did unesterified cholesterol (from 1.80 to 2.00 mmol/L). The esterified cholesterol to total cholesterol ratio and apo E level significantly decreased between the beginning and the end of the experiment. Esterified cholesterol and apo B levels remained unchanged. The levels of apo AI or unesterified cholesterol were not correlated to the alcoholic calories or to total or lipid energy. Dietary soyabean lecithin contributed to stimulation of the reverse cholesterol transport by increasing apo AI system and decreasing apo AII and apo E systems. Thus, soyabean lecithin could be considered an effective nutrient useful in the dietary treatment of mild hypercholesterolemia. (J. Nutr. Biochem. 9:659–664, 1998) © *Elsevier Science Inc. 1998*

Keywords: soyabean lecithin; diet, human hypercholesterolemia; HDL

Introduction

The role of polyunsaturated fatty acids in decreasing cholesterolemia is well established.[1,2] Previous studies have emphasized the role of high density lipoproteins (HDL) in this cholesterol-lowering effect, by promoting the reverse cholesterol transport (RCT).[3] Overexpression of the apoprotein (apo) AI–HDL system largely contributes to this increase, because HDL containing both apo AI and apo AII are relatively ineffective in stimulating the RCT.[4] In addition, dietary phospholipids from various origins, particularly polyunsaturated fatty acid phospholipids, such as those of soyabean lecithin, have a cholesterol-lowering effect in humans.[5,6] However, in most experiments, this hypocholesterolemic effect is often related to design artefacts[7] because the commercial soyabean lecithin is a mixture of different lipids in which phosphatidylcholine represents only 25% of the lipid lecithin. The other major lipids are triacylglycerols (20–25%) and phosphatidylethanolamine (20%). Thus, the results of such experiments cannot be directly indebted to only one of the lecithin components.

In animal models of normolipidemia or hypercholesterolemia, the plasma cholesterol-lowering effect of purified soyabean phosphatidylcholine (SPC) was previously shown to be related to an enhanced bile lipid secretion, most

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Table 1 Composition of the capsules[1]

Capsule	SO	SPC
Volume	0.862 mL	0.862 mL
Total mass	1,221 mg	1,242 mg
Refined SO	766 mg	—
SPC concentrate	—	780 mg
Gelatine	439 mg	454 mg
Ethanol	16 mg	8 mg
Ascorbyl palmitate (% by weight)	1%	1%
Composition of the SPC concentrate	**% by weight**	
SPC	65	
Other phospholipids	1	
Refined soyabean oil	30	
Ethanol	2	
Water	2	

[1]Nutrition et Santé, Revel, France.
SO—soyabean oil, SPC—soyabean phosphatidylcholine.

Table 2 Lipid and fatty acid composition of the capsules

	mol/100 mol fatty acids	
Fatty acids	SO	SPC
16:0	10.6	12.0
16:1	0.3	—
18:0	4.0	4.0
18:1	24.3	12.0
18:2	53.4	64.0
18:3	7.4	8.0
Phospholipid classes of SPC	**(mol/100 mol phospholipids)**	
Lysophosphatidylcholine	<3	
Phosphatidylcholine	93	
Phosphatidylinositol	Traces	
Phosphatidylserine	Traces	
Phosphatidylethanolamine	Traces	
Phosphatidic acid	Traces	
Sphingomyelin	—	
Soyabean sterols	<0.5	
Other lipids	—	
Moisture and residual solvent	<1.5	

SO—soyabean oil. SPC—soyabean phosphatidylcholine.

notably bile cholesterol and bile salts.[8] These bile components are provided in increased amounts by the metabolism of plasma cholesterol, especially HDL cholesterol.[8]

Thus, the goal of this work was to assess the effect of a dietary supplement consisting of purified soyabean lecithin on the HDL and low density lipoprotein (LDL) systems in hypercholesterolemic humans.

Materials and methods

Subjects

Ten healthy outpatients (three women and seven men, aged 52 ± 5 years) were selected on the basis of a major type IIA hypercholesterolemia (cholesterol level >6.5 mmol/L; triacylglycerol level <2.3 mmol/L). The subjects gave written informed consent to the study protocol, which was approved by the local Ethics Committee. It was verified by previous clinical investigations that none of the subjects had pathology other than hypercholesterolemia. Patients continued to take their usual pharmacologic treatments (seven were receiving no treatment, one was being treated for hyperlipidemia, and two were being treated for hypercholesterolemia).

Experimental design and diet

The duration of the protocol was 12 weeks. The subjects were instructed not to deviate from regular habits during the study period, and especially to avoid excess alcohol consumption or exercise because these activities reportedly influence lipid metabolism.[9] Each subject consumed his or her usual diet during the 12 weeks.

During the first 6 weeks, which were considered a reference period, a daily dietary supplement of 4.60 g refined soyabean oil (SO) was distributed in two capsules at three main meals. For the following 6 weeks, the subjects received a daily dietary supplement of 3.04 g purified SPC, also distributed in two capsules at three main meals. Compositions of SO and SPC capsules were chosen in order to provide equal quantities of the main polyunsaturated (n-6) linoleic acid (2.2 g daily), in the form of phosphatidylcholine (SPC capsules) or triacylglycerol (SO capsules) (*Tables 1 and 2*). The previous SO period was carried out to obtain a counterbalanced diet period before SPC administration. We observed no change in the subjects' dietary habits during the protocol. Thus, each subject served as his or her own control.

The usual basal diet of each subject was monitored by 3 day food recall during the first week and every 4 weeks thereafter. Calculations were made with the GENI software package (Micro 6, Nancy, France) on a Macintosh microcomputer (Apple Corporation Inc., Cupertino, CA USA). The food database used in this program was established from nutrient tables of Paul and Southgate[10] and Feinberg et al.[11] The subjects relied on a typical Western diet with a moderate energy consumption (8,588 ± 260 kJ/d), in which proteins, carbohydrates, and fat accounted for 17.5 ± 0.6%, 35.5 ± 1.5%, and 42.8 ± 1.5% of energy, respectively. Dietary fiber intake was 17.3 ± 0.6 g/d. Consumption of ethanol, mostly red wine, was 366 ± 53 kJ/d. The polyunsaturated to saturated fatty acid ratio was 0.47 ± 0.03. It was not significantly changed by the SO or SPC supplements. Daily cholesterol intake was 367 ± 23 mg. No noticeable changes in food consumption were recorded during the experiment, except the following: There was a significantly higher carbohydrate consumption with SO capsules (3,530 kJ/d) than with SPC capsules (2870 kJ/d), and there were significant decreases in cholesterol consumption (300 vs. 438 mg) and fiber consumption (16 vs. 19 g/d) at the end of the SPC period compared with the preceding SPC period.

Plasma analyses

Blood samples were taken after an overnight fast. Total[12] and unesterified cholesterol,[13] triacylglycerols,[14] and phospholipids[15] were measured by enzymatic procedures. Total plasma apo AI,[16] apo B,[17] apo AII,[18] and apo E[18] were assayed by immunonephelometry.

The lipoprotein classes [very low density lipoprotein (VLDL): $d < 1.006$; LDL: $1.006 < d < 1.060$; HDL: $1.060 < d < 1.21$] were separated from 1.5 mL plasma by ultracentrifugation (38,000 rpm at 15°C for 24 hours in a Beckman SW 41 rotor) on a KBr discontinuous density gradient as previously described.[19] Total and unesterified cholesterol, triacylglycerols, and phospholipids were assessed in each lipoprotein class using the enzymatic procedures mentioned above.

Table 3 Plasma cholesterol content

	Beginning of the experiment	SO period			SPC period		
		1	2	3	1	2	3
UC mmol/L	1.76 ± 0.13–a	1.81 ± 0.14–b	1.86 ± 0.13	1.80 ± 0.17–c	1.82 ± 0.13–d	1.84 ± 0.19–e	2.00 ± 0.18
EC/TC mol/mol	0.739 ± 0.006–a	0.733 ± 0.006	0.725 ± 0.009	0.726 ± 0.004	0.732 ± 0.010	0.728 ± 0.007	0.720 ± 0.010

a: Beginning vs. 3° SPC; b: 1° SO vs. 3° SPC; c: 3° SO vs. 3° SPC; d: 1° SPC vs. 3° SPC; e: 2° SPC vs. 3° SPC.
SO—soyabean oil. SPC—soyabean phosphatidylcholines. UC—unesterified cholesterol. EC—esterified cholesterol. TC—total cholesterol.

Liver function was estimated by alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities,[20] alkaline phosphatase,[21] and gamma glutamyl transferase (GGT).[22] Total and conjugated bilirubins were estimated by the method of Gambino and Schreiber.[23]

Lecithin cholesterol acyl transferase (LCAT) activity was measured in the plasma of overnight fasted patients by in vitro assessment of esterification of proteoliposome unesterified [^{14}C] cholesterol[24] after separation of esterified and unesterified cholesterol by chromatography on a silica gel column.[25]

Statistical analyses

Results were expressed as the arithmetical means of each group with their standard errors. We previously verified the homogeneity of the data in all conditions. Thus, differences between the beginning of the experiment and the end of the successive 2-week periods of SO or SPC sequences were compared using analysis of variance (ANOVA) for repeated values. The significant differences were determined by Fisher's post-hoc least significant difference test and/or the Scheffe F-test at a probability value of 95%. However, we restricted the statistical test to comparisons with only one variable. Correlation coefficients between plasma parameters and the food ingesta were obtained from linear regression analyses. All calculations were performed using Stat View™ SE + graphics (Abacus Concepts, Inc, CA USA) on a Macintosh computer.

Results

Fasting plasma lipids and LCAT activity

At the end of the lecithin period, there was a significant increase in the level of plasma unesterified cholesterol compared with the end of the SO period and with the two preceding SPC periods. The level of esterified cholesterol was unchanged throughout the experiment. Thus, it resulted in a significant decrease in the esterified to total cholesterol ratio between the beginning and the end of the experiment (*Table 3*).

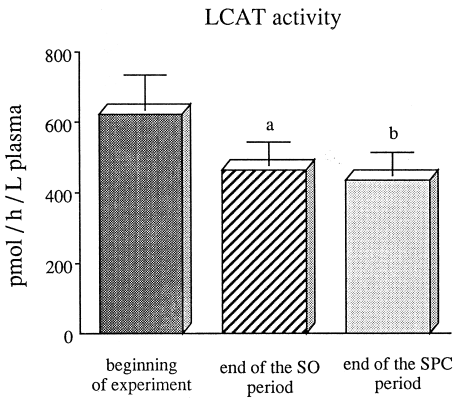

Figure 1 Lecithin cholesterol acyl transferase (LCAT) activity. Values are means with their standard errors ($N = 9$). Differences were assessed by analysis of variance (ANOVA) for repeated values at a probability of 95% by Fisher's post-hoc least significant difference test. a: Beginning of the experiment versus the end of the soyabean oil (SO) period; b: beginning of the experiment versus the end of the soyabean phosphatidylcholine (SPC) period.

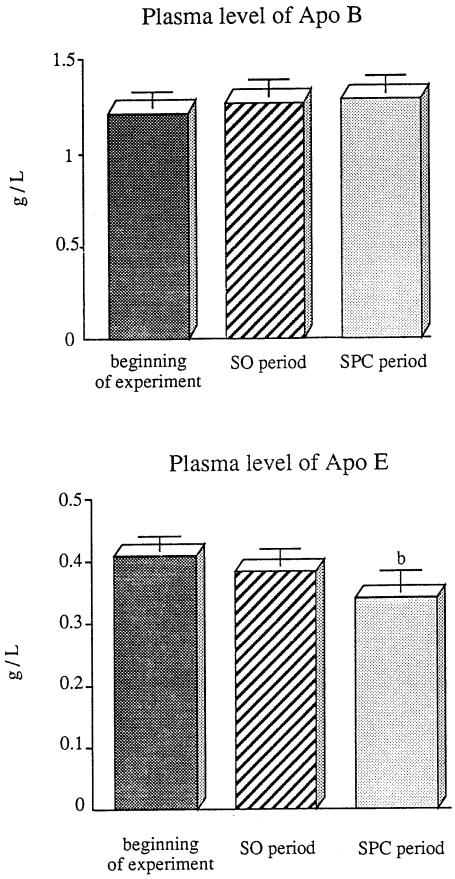

Figure 2 Plasma levels of Apo B and Apo E. Values are means with their standard errors (apo B: $N = 10$; apo E: $N = 9$). Differences were assessed by analysis of variance (ANOVA) for repeated values at a probability of 95% by Fisher's post-hoc least significant difference test. b: Beginning of the experiment versus the mean value of the soyabean phosphatidylcholine (SPC) period.

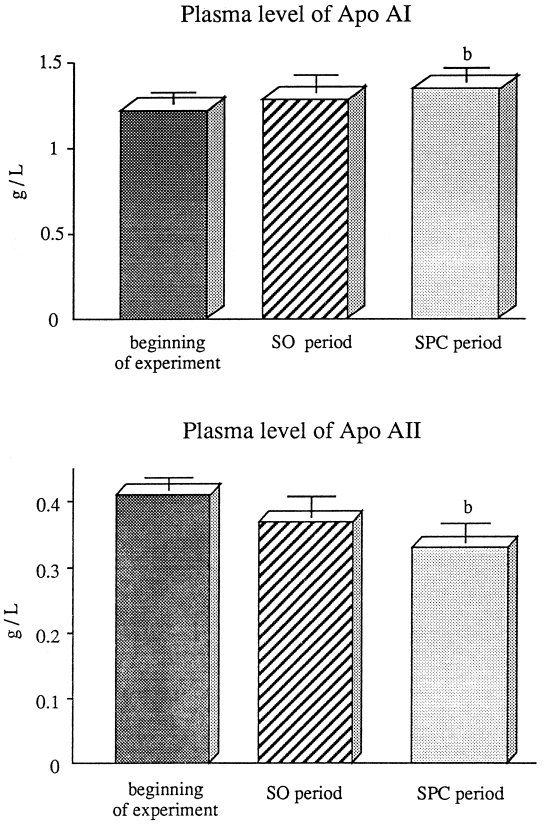

Figure 3 Plasma levels of apo AI and apo AII. Values are means with their standard errors (apo AI: $N = 10$; apo AII: $N = 9$). Differences were assessed by analysis of variance (ANOVA) for repeated values at a probability of 95% by Fisher's post-hoc least significant difference test. b: Beginning of the experiment versus the mean value of the soyabean phosphatidlycholine (SPC) period.

The concentrations of triacylglycerols, esterified and unesterified cholesterol, and phosphatidylcholines remained unchanged in the different lipoproteins (VLDL, LDL, HDL) (results given only in the text).

LCAT activity was significantly decreased in both SO and SPC periods compared with the beginning of the experiment. There was no change between the SO and SPC periods (*Figure 1*).

Plasma Apoproteins

In the SO or SPC groups, all significations were calculated from means of the three values obtained each 2-week period within the groups.

The level of apo B was unchanged throughout the experiment (*Figure 2*). The level of apo E was significantly decreased after SPC compared with the value obtained at the beginning of the experiment (*Figure 2*).

In the SPC period, the level of apo AI was significantly increased and the level of apo AII was significantly decreased compared with the values at the beginning of the experiment (*Figure 3*). It resulted in a significant increase in the apo AI to apo AII ratio under SPC compared with this ratio value at the beginning of the experiment (*Figure 4*).

No correlation was found between plasma apo AI and the alcoholic energy consumption or diet fiber level. The apo AI

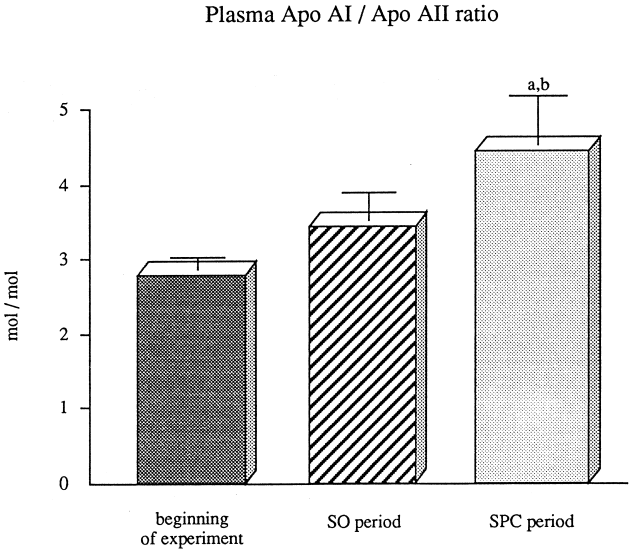

Figure 4 Plasma apo AI to apo AII ratio. Values are means with their standard errors ($N = 9$). Differences were assessed by analysis of variance (ANOVA) for repeated values at a probability of 95% by Fisher's post-hoc least significant difference test or the Scheffe F-test. a: Significant difference as assessed by Fisher's test; b: significant difference by both tests [beginning of the experiment versus the mean value of the soyabean phosphatidylcholine (SPC) period].

and apo B levels were not correlated to the total ingested energy, lipid energy, or polyunsaturated to saturated fatty acid ratio of the ingesta.

Hepatic functions

The two transaminase activities (ASAT and ALAT), as well as activities of alkaline phosphatases and GGT, remained within the normal range values during the protocol. Total and conjugated bilirubin levels also did not increase in the SO or SPC periods and remained at normal values. However, SPC led to a significant decrease in the total bilirubin level compared with the SO period or the beginning of the experiment (results given only in the text).

Discussion

Previous studies have shown that the hypocholesterolemic effect of dietary lecithin could be due to the presence of the polyunsaturated linoleic acid (18:2 n-6).[7] In addition, other minor components of lecithin, such as phytosterols, polyphenol molecules, and other phospholipid components (e.g., phosphatidylethanolamine, phosphatidylinositol) could be involved in the cholesterol-lowering effect.[26] Thus, in our work, we chose a purified soyabean lecithin that did not contain molecules other than phosphatidylcholine.

We did not observe an hypocholesterolemic effect of this purified SPC. Indeed, administration of lecithin consisted only of a dietary supplement of 3 g per day, which represented 5% of the lipid daily energy intake. This low lecithin consumption was chosen to avoid a lipid overload of the ingesta. Such supplementation was selected to favor the HDL system and to slow the LDL system, as described previously,[27] when the polyunsaturated (n-6) fatty acid

supplement represents no more than 11% of the total energy intake. In fact, our objective was to test the importance of the vehicle—triacylglycerols or phospholipids—of polyunsaturated (n-6) fatty acids on the plasma lipid and apoprotein changes.

Rioux et al.[28] and Iwata et al.[29] found no evidence of any relationship between the hypocholesterolemic effect of lecithin and the degree of polyunsaturation of lecithin fatty acids. Thus, as we have advanced elsewhere,[8] the beneficial effect of dietary lecithin in the plasma could be linked to the hydrophilic nature of the lecithin head. However, in this study, we cannot entirely refute the beneficial effect of polyunsaturated fatty acids, because the most important results concern differences between the beginning of the experiment and the SCP period, the results in the SO period being between them.

Our results demonstrated that soyabean lecithin supplement induced significant changes in plasma apoproteins, notably in HDL. Previous findings have shown that HDL is the main lipoprotein involved in the physiologic transport of phosphatidylcholines[30] and that HDL largely contributes to the transport and exchange of cholesterol between tissues and plasma.[31,32] In the present study, carried out in hypercholesterolemic subjects, an important transfer of cholesterol probably takes place from the endothelial cells through the liver and bile, under soyabean lecithin. Thus, the cholesterol trafficking in plasma is probably increased by the lecithin, which leads to a higher plasma level of unesterified cholesterol, so we cannot observe an hypocholesterolemic effect. However, it resulted in a decrease in the esterified:total cholesterol ratio.

Soyabean lecithin induces an increase in the apo AI level and a decrease in the apo AII level, which in turn contributes to the reinforcement of the RCT by HDL. Overall, the results of in vivo studies appear to be reasonably consistent with predictions based on studies of RCT in vitro.[4] In particular, the special role of prebeta-migrating LpA-I HDL is supported, as is the concept that HDL containing both apo AI and apo AII are relatively ineffective in promoting the RCT.[4] Such effect is not indebted to the alcoholic energy intake or fiber consumption, because no correlation was found between these dietary parameters and the observed apo AI changes. However, we cannot entirely discard the hypothesis that, in comparison to soyabean oil, soyabean lecithin, by leading to a decrease in carbohydrate consumption, could by itself induce an increase in certain HDL subclasses, as described by Williams et al.[33] Such a pattern seemed related to the presence of apo E2 allele,[33] which is rarely expressed. Thus, in our study, the observed decrease in plasma apo E level under soyabean lecithin may particularly concern the apo E4 allele. This allele is described in cases of elevated risk of atherosclerosis,[34] and it is invoked by administration of a diet inducing a LDL cholesterol-lowering effect.[35] However, we did not observe any change on the apo B rich–LDL system, particularly on that apo B level.

In conclusion, our results showed that polyunsaturated soyabean lecithin promotes the plasma apo AI system without promoting apo AII or apo E, which are both decreased. Unesterified plasma cholesterol was increased by soyabean lecithin, which led to a decrease in the intestinal cholesterol absorption and a decrease in the cholesterol new synthesis (personal results). Thus, it could be advanced that the increase in unesterified cholesterol by polyunsaturated lecithin was provided from an expanded extraction of cholesterol from peripheral cells, which in turn increased the RCT by the apo AI-rich HDL system.

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References

1 Connor, S.L., Gustafson, J.R., Artaud-Wild, S.M., Flavell, D.P., Classick-Kohn, C.J., Hatcher, L.F., and Connor, W.E. (1986). The cholesterol saturated fat index: An indication of the hypercholesterolemic and atherogenic potential of food. *Lancet* **1,** 1229–1232

2 Winckler, G., Doring, A., Keil, U., Pietinen, P., Arveiler, D., Cambou, J.P., Nuttens, C., Richard, J.L., Evans, A., and Mc Lean, R. (1992). Comparison of dietary intakes in four selected European populations. *Clin. Invest.* **70,** 889–895

3 Oram, J.F. and Yokoyama, S. (1996). Apolipoprotein-mediated removal of cellular cholesterol and phospholipids. *J. Lipid Res.* **37,** 2473–2491

4 Fielding, C.J. and Fielding, P.E. (1995). Molecular physiology of reverse cholesterol transport. *J. Lipid Res.* **36,** 211–228

5 Tompkins, R. and Parkin, L.G. (1980). Effects of long-term ingestion of soya phospholipids on serum lipids in humans. *Am. J. Surg.* **140,** 360–364

6 O'Brien, B. and Andrews, V.G. (1993). Influence of dietary egg and soybean phospholipid and triacylglycerols on human serum lipoproteins. *Lipids* **28,** 7–12

7 Knuiman, J.T., Beynen, A.C., and Katan, M.B. (1989). Lecithin intake and serum cholesterol. *Am. J. Clin. Nutr.* **49,** 266–268

8 Polichetti, E., Diaconescu, N., Lechène de la Porte, P., Malli, L., Portugal, H., Pauli, A.M., Lafont, H., Tuchweber, B., Yousef, I., and Chanussot, F. (1996). Cholesterol-lowering effect of soyabean lecithin in normolipidaemic rats by stimulation of biliary lipid secretion. *Br. J. Nutr.* **75,** 471–481

9 Cohen, J.C., Noakes, T.D., and Spinner Benade, A.J. (1989). Postprandial lipemia and chylomicron clearance in athletes and in sedentary men. *Am. J. Clin. Nutr.* **49,** 443–447

10 Paul, A.A. and Southgate, D.A.T. (1978). *The Composition of Foods.* Elsevier Science Publishers, Amsterdam

11 Feinberg, M., Favier, J.C., and Ireland-Ripert, J. (1987). *Répertoire Général des Aliments. Tome 1: Table de Composition des Corps Gras. Tome 2: Table de Composition des Produits Laitiers,* Techniques et documentation Lavoisier/Ciqual-Régal, Paris

12 Siedel, J., Hägele, E.O., Ziegenhorn, J., and Wahlefeld, A.W. (1983). Reagent for the enzymatic determination of serum total cholesterol with improved lipolytic efficiency. *Clin. Chem.* **29,** 1075–1080

13 Stähler, F., Gruber, W., Stinshoff, K., and Röschlau, P. (1977). A practical enzymatic cholesterol determination. *Med. Lab.* **30,** 29–37

14 Fossati, P. and Prencipe, L. (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin. Chem.* **28,** 2077–2080

15 Takayama, M., Itoh, S., Nagasaki, T., and Tanimizu, I. (1977). A new enzymatic method for choline containing phospholipids. *Clin. Chim. Acta* **79,** 93–98

16 Sievet-Desrumeaux, C., Dedomder-Decoopman, E., Fruchart, J.C., Dewailly, P., Sézille, G., and Jaillard, J. (1980). Immunochemical determination of human apolipoprotein AI by laser nephelometry. *Clin. Chim. Acta* **107,** 145–148

17 Sievet-Desrumeaux, C., Dedomder-Decoopman, E., Fruchart, J.C., Dewailly, P., and Sézille, G. (1979). Immunochemical determination of human apolipoprotein B by laser nephelometry. *Clin. Chim. Acta* **95,** 405–408

18 Labeur, C., Sheperd, J., and Rosseneu, M. (1990). Immunological

assays of apolipoproteins in plasmas. Methods and instrumentation. *Clin. Chem.* **36,** 591–597
19 Lacombe, C., Corraze, G., Nibbelink, M., Boulze, D., Douste-Blazy, P., and Camare, R. (1986). Effects of a low-energy diet associated with egg supplementation on plasma cholesterol and lipoprotein levels in normal subjects: Results of a cross-over study. *Br. J. Nutr.* **56,** 561–575
20 Kessler, G., Morgenstern, S., Snyder, L., and Varady, R. (1975). *Improved point assays for ALT and AST in serum using the Technicon SMAC high speed computer controlled, biochemical analyser to eliminate the common errors found in enzyme analysis.* 9th International Congress for Chemical Chemistry, Toronto
21 Morgenstern, S., Kessler, G., Auerbach, J., Flor, R.V., and Klein, B. (1965). An automated p-nitrophenylphosphate serum alkaline phosphatase procedure for the autoanalyser. *Clin. Chem.* **11,** 876–881
22 Persijn, J.P. and Van der Slik, W. (1976). A new method for the determination of gamma-glutamyl transferase in serum. *J. Clin. Chem. Clin. Biochem.* **14,** 421–427
23 Gambino, R.S. and Schreiber, H. (1963). The measurement and fractionation of bilirubin on the autoanalyzer by the method of Jendrassik and Grof. In *Automation in Analytical Chemistry*. Technicon Symposia, Mediad. Inc., White Plains, NY, USA
24 Chen, C.H. and Albers, J.J. (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
25 Ingalls, S.T., Kriaris, M.S., Xu, Y., DeWulf, D.W., Tserng, K.Y., and Hoppel, C.L. (1993). Method for isolation of non-esterified fatty acids and several other classes of plasma lipids by column chromatography on silica gel. *J. Chromatography* **619,** 9–19
26 Imaizumi, K., Sekihara, K., and Sugano, M. (1991). Hypocholesterolemic action of dietary phosphatidylethanolamine in rats sensitive to exogenous cholesterol. *J. Nutr. Biochem.* **2,** 251–254
27 Iacono, J.M. and Dougherty, R.M. (1991). Lack of effect of linoleic acid on the high density lipoprotein-cholesterol fraction of plasma lipoproteins. *Am. J. Clin. Nutr.* **53,** 660–664
28 Rioux, F., Perea, A., Yousef, I.M., Lévy, E., Malli, E., Carillo, M.C., and Tuchweber, B. (1994). Short-term feeding of a diet enriched in phospholipids increases bile formation and the bile acid transport maximum in rats. *Biochim. Biophys. Acta* **1214,** 193–202
29 Iwata, T., Kimura, Y., Tsutsumi, K., Furukawa, Y., and Kimura, S. (1993). The effect of various phospholipids on plasma lipoproteins and liver lipids in hypercholesterolemic rats. *J. Nutr. Sci. Vitamin.* **39,** 63–71
30 Martins, I.J., Lenzo, N.P., and Redgrave, T.G. (1989). Phosphatidylcholine metabolism after transfer from lipid emulsions injected intravenously in rats. Implications for high-density lipoprotein metabolism. *Biochim. Biophys. Acta* **1005,** 217–224
31 Schwartz, C.C., Zech, L.A., Van den Broek, J., and Cooper, P.S. (1993). Cholesterol kinetics in subjects with bile fistula. Positive relationship between size of the bile acid precursor pool and bile acid synthetic rate. *J. Clin. Invest.* **91,** 923–938
32 Fielding, C.J. and Fielding, PE. (1997). Intracellular cholesterol transport. *J. Lipid Res.* **38,** 1503-1521
33 Williams, P.T., Dreon, D.M., and Krauss, R.M. (1995). Effects of dietary fat on high-density-lipoprotein subclasses are influenced by both apolipoprotein E isoforms and low-density-lipoprotein subclass patterns. *Am. J. Clin. Nutr.* **61,** 1234–1240
34 Davignon, J., Gregg, R.E., and Sing, C.F. (1991). Apolipoprotein E polymorphisms affect atherosclerosis in young males. *Arterioscler. Thromb.* **11,** 1237–1244
35 Lopez-Miranda, J., Ordovas, J.M., Mata, P., Lichtenstein, A.H., Clevidence, B., Judd, J.T., and Schaefer, E.J. (1994). Effect of apolipoprotein E phenotype on diet-induced lowering of plasma low density lipoprotein cholesterol. *J. Lipid Res.* **35,** 1965–1975