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Major human plasma lipid classes determined by quantitative high-performance liquid chromatography, their variation and associations with phospholipid fatty acids

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Abstract

An HPLC method with evaporative light-scattering detection (ELSD) was optimized and validated for the simultaneous quantitation of cholesteryl esters (CEs), triacylglycerols (TGs), free cholesterol (FC) and phosphatidylcholine (PC) in human plasma. The separation of CEs from TGs, the most variable plasma lipid class, was improved by speeding up the gradient steps and by increasing the re-equilibration time between runs. The calibrations were made at levels of 0.14–14 μg lipid/injection. The intra- and inter-day precision values of the method ranged between 1.9 and 4.5 and 2.3–7.2% (RSD, $n=6$), respectively, including determinations at two concentration levels. In comparison to other lipid classes, quantitation of PC proved to be equally repeatable despite its lowest detector response. The relative recoveries varied from 97.0 to 110.3%, showing good accuracy of the method. The methodological variation of the lipid classes covered 0.6–3.1% of their total variation in the study population ($n=48$). The CE/FC ratio showed an even closer relationship with phospholipid linoleic acid (18:2$n-6$; $r=0.65$, $P<0.001$) than with serum cholesterol levels, while eicosapentaenoic acid (20:5$n-3$) was significantly associated with PC ($r=0.41$, $P<0.01$). The CE/FC ratio increased ($P<0.01$) during soyabean oil substitution and the level of PC increased ($P<0.01$) during cold-pressed rapeseed oil substitution. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Evaporative light-scattering detection; Cholesteryl esters; Triacylglycerols; Free cholesterol; Phosphatidylcholine; Phospholipid fatty acids

1. Introduction

In biological tissues, lipids occur as complex mixtures that include neutral and polar lipids from several subclasses [1,2]. The great variability of lipid structures and polarities usually requires fractionation of the sample before analysis. Thin-layer chro-

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matography (TLC) combined with densitometry has been used for screening serum lipid constituents [3]. High-performance liquid chromatography (HPLC) with UV or fluorescence detection has been used for the determination of, for example, neutral lipids [4–6] and phospholipids (PLs) [7,8]. An evaporative light-scattering (''mass'') detector (ELSD) is well suited for evaluating the lipid profile of animal tissue and plasma extracts containing both neutral lipids and phospholipids [9–12]. Proper resolution provides gradient elution and careful re-equilibration of the chromatographic system [9–11]. For quantitation, it is necessary to calibrate every lipid class separately, since there are considerable differences in their response [9,11] and slight nonlinearity appears at low concentrations, in particular [13].

In human plasma, the cholesterol-containing lipid classes are dominant in the low-density lipoproteins (LDLs). Cholesteryl esters (CEs) are carried in the core of LDL particles, while free cholesterol (FC) and PLs are constituents of their outer monolayer [14,15]. A high proportion of phosphatidylcholine (PC) is characteristic of the high-density lipoprotein (HDL) fraction [1], which is anti-atherogenic [16]. PC is rich in polyunsaturated fatty acids (PUFAs) and its *sn*-2 position is most often occupied by linoleic acid (LA, $18{:}2n-6$), which is utilized by the HDL-associated lecithin–cholesterol acyl transferase (LCAT) to esterify FC to CE [14,15,17]. Most of the plasma cholesterol exists in an esterified form, representing about 70% of total cholesterol [18].

Neutral and polar lipid classes in human plasma have been quantified by combining different isolation and detection methods, like TLC and phosphorus determinations [18]. Simultaneous HPLC analysis with an ELSD has been used in the identification of human metabolic diseases [19], for example. Although good applicability of the ELSD technique is often stated, no validated method for studying the variation of lipid classes at population level has been reported.

In this study, we optimized and validated a quantitative HPLC technique with ELSD at a concentration level suitable for routine analysis of the major lipid classes in human plasma. Variation in the lipid classes and their relationships with enzymatically determined serum lipids and with PL fatty acid composition were studied. In addition, the method was applied in a fat replacement study in order to examine the effect of vegetable oil substitutions on plasma lipid classes.

2. Experimental

2.1. Analytical methods

2.1.1. Chemicals

The solvents (hexane, tetrahydrofurane, 2-propanol, chloroform, methanol) were of HPLC grade (Rathburn, Walkerburn, UK) and were degassed at reduced pressure. Deionized water was purified using a Milli-Q Plus system (Millipore, Bedford, MA, USA). Cholesterol (Sigma, St. Louis, MO, USA), cholesteryl oleate, triolein and L-α-distearoylphosphatidylcholine (Nu Chec Prep, Elysian, MN, USA) were used as standards for FC, CEs, TGs and PC, respectively.

2.1.2. Instrumentation and chromatography

The HPLC system consisted of a Kontron HPLC pump 420 and a Gradient Former 425 (Kontron Instruments, Zürich, Switzerland), a Rheodyne 7125 injector with a 10-μl loop (Rheodyne, Cotati, CA, USA) and a Cunow DDL21 evaporative light scattering detector (ELSD; Cunow, Cergy, St. Christophe, France). Separation of the lipid classes was carried out on a Spherisorb S3W column (3 μm, 100×4.6 mm I.D., Phase Separations Ltd., UK). The multistep gradient system (Fig. 1), using hexane–tetrahydrofurane (99:1, v/v), 2-propanol–chloroform (4:1) and 2-propanol–water (1:1) as eluents, was modified from that described previously [9,10]. The flow-rate was 2 ml/min and the total run time was 32 min. The temperature of the ELSD was 40°C and air flow was 27 p.s.i. The peak areas were registered using a Shimadzu C-R5A Chromatopac integrator.

2.1.3. Quantitation of lipid classes

Stock solutions containing 2 mg/ml of CE, FC, TG and PC were prepared in chloroform. Dilutions containing 0.02, 0.10, 0.25, 0.50, 0.75, 1.00 and 1.50 mg/ml of each lipid were made according to their expected levels in plasma. Injections (7 μl) contained 0.14–14.0 μg of lipid. Calibration curves

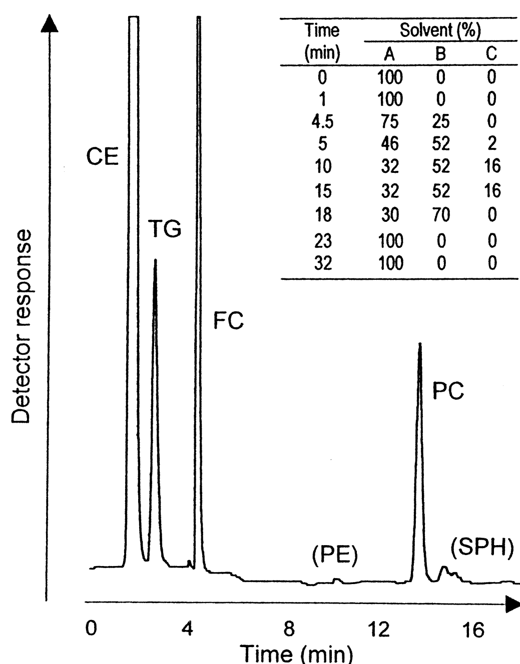

Fig. 1. HPLC-chromatogram of human plasma lipid classes obtained by ELS detection. The gradient program is shown in the figure. Solvents: (A) hexane–tetrahydrofurane (99:1, v/v); (B) 2-propanol–chloroform (4:1, v/v); (C) 2-propanol–water (1:1, v/v). Column: Spherisorb S3W (3 μm, 100×4.6 mm I.D.). Flow-rate, 2 ml/min; amount injected, 7 μl. ELSD, temperature 40°C; air flow, 27 p.s.i. CE, cholesteryl esters, TG, triacylglycerols, FC, free cholesterol, PE, phosphatidylethanolamine, PC, phosphatidylcholine, SPH, sphingomyeline.

(Fig. 2) contained from five to nine points, from two–four replicate runs.

Lipids from plasma (200 μl) were extracted with 800 μl of chloroform–methanol (2:1, v/v) in Eppendorf tubes by vortex-mixing for 0.5 min. After 15 min, the samples were centrifuged at 10 000 rpm for 2 min. The lower layer was separated and a 7-μl aliquot was used for injection. In the fat-substitution study, lipid quantitation was based on duplicate HPLC analyses, with the concentrations calculated using the equations of the calibration curves (Fig. 2) and taking into account a dilution coefficient of 400. Determinations were performed within one month of blood sampling.

2.1.4. Method validation

The repeatability of the injection process was determined from calibration samples, using triplicate injections at three concentration levels, as follows: CE, FC and TG (0.10, 0.25 and 0.50 mg/ml) and PC (0.50, 1.00 and 1.50 mg/ml). For precision studies, two pooled plasma samples, representing high and low lipid concentrations, were prepared. Intra-day precision was determined by analysing six separate samples at both concentration levels (Table 1). For inter-day precision, lipid extraction and HPLC analysis ($n=6$) were performed on six successive days. Accuracy of the measurement was studied by determining the extraction and relative recoveries (Table 2). Each lipid (0.40 mg) was added to pooled plasma samples and water ($n=5$–7), and the amounts recovered were compared.

2.2. Fat-substitution study

2.2.1. Subjects and substitutions

This work forms part of a major study investigating the effect of vegetable-oil substitutions on serum lipid and fibrinogen levels, and on lipid-oxidation parameters. The subjects were working-aged (mean, 38 years) men ($n=9$) and women ($n=39$) with total serum cholesterol levels of 3.3–6.9 mmol/l (127.7–267.0 mg/dl) and an average body mass index of 24.4 (kg/m^2). They either did not consume fish at all, or, at most, had one–two fish meals per week. The subjects used mainly margarines on bread. Since the content of *trans*-fatty acids (2–3%) in the margarines was clearly lower than in our earlier substitution study (8%) [20], their levels in plasma PLs were not monitored. The nutrient data were analysed by the Nutrica program. Blood samples from fasting (12 h) individuals were collected into EDTA-tubes and the plasma samples were stored at −20°C.

The subjects replaced fat on bread by cold-pressed rapeseed oil (CPRSO, $n=26$; Virgino, Kankaisten öljykasvit Oy, Hämeenlinna, Finland), conventional rapeseed oil (RSO, $n=16$) or soya-bean oil (SBO, $n=6$) for a 6-week period. The Canola-type rapeseed oils contained 59, 22 and 12% of oleic acid, linoleic acid (LA) and alpha-linolenic acid (α-LLA), respectively. SBO contained 55% LA and 7% α-LLA. The average amounts of oils used, for example, as salad dressings, were 17, 15 and 16 ml/day for CPRSO, RSO and SBO, respectively.

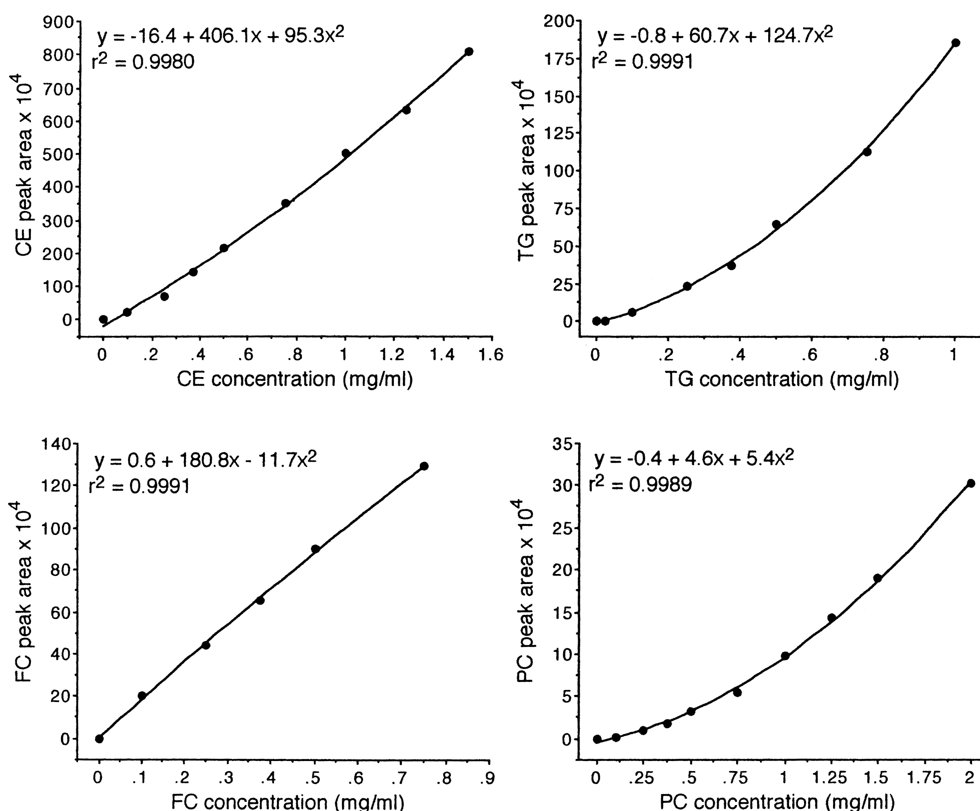

Fig. 2. Calibration curves and their equations for cholesteryl esters (CE), free cholesterol (FC), triacylglycerols (TG) and phosphatidylcholine (PC). Each point represents 2–4 replicate injections. Standards used: Cholesteryl oleate (for CE), cholesterol (for FC), triolein (for TG), distearoylphosphatidylcholine (for PC).

2.2.2. Determination of serum lipids and plasma fatty acids

Serum total cholesterol (S-Chol), HDL-cholesterol (HDL-C) and triacylglycerol (S-Trigl) concentrations were determined by routine enzymatic methods from samples taken before (0 weeks) and at the end (6 weeks) of substitution. Low-density lipoprotein cholesterol (LDL-C) levels were calculated accord-

Table 1
Intra- and inter-day precision of the method at high (H) and low (L) concentration levels, expressed as relative standard deviations (RSD, %)

Lipid class[a]	High/low concentration	Intra-day precision			Inter-day precision		
		Mean (mg/dl)	(SD)	RSD (%)	Mean (mg/dl)	(SD)	RSD (%)
CE	H	212.9	(9.4)	4.4	219.2	(7.4)	3.4
	L	139.8	(5.4)	3.9	138.0	(5.7)	4.1
FC	H	57.7	(2.6)	4.5	57.1	(2.8)	4.9
	L	40.5	(1.6)	4.1	37.7	(3.0)	7.2
TG	H	81.6	(3.1)	3.8	83.0	(3.2)	3.8
	L	59.7	(2.3)	3.9	58.4	(4.0)	6.8
PC	H	448.9	(8.9)	1.9	451.6	(10.6)	2.3
	L	289.8	(12.2)	4.2	271.6	(14.6)	5.4

[a] CE, cholesteryl esters, FC, free cholesterol, TG, triacylglycerols, PC, phosphatidylcholine.

Table 2
Extraction recoveries of the lipid standards added to plasma and water

Lipid class[a]	Added (mg/dl)	n	Recovered						Relative recovery[b] (%)
			Water			Plasma			
			Mean (mg/dl)	(SD)	%	Mean (mg/dl)	(SD)	%	
CE	160.0	7	139.9	(9.8)	87.4	146.2	(12.2)	91.4	104.6
FC	160.0	5	173.0	(17.1)	108.1	171.5	(16.1)	107.0	99.0
TG	160.0	5	157.9	(7.6)	98.7	153.2	(3.9)	95.8	97.0
PC	160.0	5	166.4	(12.5)	104.0	184.1	(29.9)	114.7	110.3

[a] Standards: cholesteryl oleate (CE), cholesterol (FC), triolein (TG) and distearoylphosphatidylcholine (PC).
[b] The amount recovered from plasma/the amount recovered from water (%).

ing to the Friedewald equation [21]. The fatty acid composition of plasma PLs isolated by TLC and of total plasma, including free fatty acids (FFAs), were analysed by gas chromatography (GC) according to the methods used in corresponding substitution studies [22–24].

3. Results and discussion

3.1. Optimization of the HPLC run

The main plasma lipid components (CE, FC, TG and PC) were well separated and eluted as symmetric peaks (Fig. 1). Despite the considerable increase in the polarity of the eluent, the detector baseline remained stable, which is a typical feature of ELS detection [9–12]. The solvent mixtures used were as described previously [9], but the gradient steps did not always give proper separation and precision of the retention times for CE and TG, mainly arising from tenfold differences in plasma TG levels. Therefore, further optimization of the run cycle and re-equilibration of the column were required. The final composition of the eluent mixture was attained faster (in 10 min), and it was maintained for up to 15 min (Fig. 1). The re-equilibration step was longer (17 min) but without any stopping of the solvent flow before the next run. In plasma analyses (n=48), the precision of the retention times was 2.6, 6.7, 9.1 and 1.3% (RSD) for CE, TG, FC and PC, respectively.

When selecting the appropriate concentration level of the assay, the separation between CE and TG was also used as the main criterion. Therefore, minor constituents, such as phosphatidylethanolamine (PE), sphingomyelin (SPH, Fig. 1) and FFAs, could not always be properly detected and integrated, and they were not quantified. FFA can be conveniently analysed by GC [22] and, in the present study (n=48), FFAs accounted for about 6% of total plasma fatty acids.

3.2. Quantitation

The calibration curves for CE and FC were relatively linear, while those for TG and PC were bent, especially at the lowest concentrations (Fig. 2). The average repeatabilities of the injections were 5.2, 3.8, 4.2 and 2.6% (RSD) for authentic samples, respectively. The shape of the curves and the order of the responses (CE>FC>TG>PC) closely resembled those described earlier [11] in the analysis of lipids from animal tissue at about the same concentration levels (<20 μg lipid/injection). The detector responses are usually more linear at higher concentrations [9,13]. The differences in the responses of the lipid classes are marked, but they are, in contrast to UV-detection, practically independent of the degree of unsaturation of the fatty-acid chains [9,25].

3.3. Validation parameters

HPLC analyses of plasma samples showed good repeatability of the method (Table 1). At higher lipid concentrations, the intra-day precision was 4.4, 4.5, 3.8 and 1.9% (RSD, n=6) for CE, FC, TG and PC, respectively. The corresponding inter-day precision values for the four lipid classes were 3.4, 4.9, 3.8 and 2.3%, respectively. Analyses at lower concen-

trations gave intra- and inter-day precision values of 4.2 and 5.4% for PC, indicating that its quantitation is still very repeatable despite having the lowest detector response. The repeatabilities of neutral lipids varied between 3.9 and 7.2% (RSD), which agree well with those obtained previously using the ELSD technique [6].

The accuracy of the method was determined after the addition of authentic, single lipid, components into plasma and water (Table 2). The extraction recoveries from plasma ranged from 91 (CE) to 115% (PC), and the relative recoveries (compared to water) ranged from 97 (TG) to 110% (PC), indicating good accuracy of the method. The relative recovery of 99% shows that FC is equally well extracted from plasma and water. Higher quantitative deviations in other lipid classes are apparently due to the fact that they contain a variety of plasma fatty acids.

3.4. Population versus methodological variation

In the study population, the variations in the CE and total cholesterol (S-Chol) concentrations were equal (18.9 and 19.3%, RSD, Table 3), while FC was clearly more variable (30.9%). TGs appeared as the most variable lipid class (51.7%, RSD), with the variation being within the tenfold range. Phosphatidylcholine (PC) levels ranged between 173.4 and 559.6 mg/dl (27.3%, RSD). The proportion of the methodological variation out of population variation ($n=48$), calculated from the variances of inter-day precision values, appeared to be very low, being 3.1, 2.3, 0.6 and 1.4% for CE, FC, TG and PC, respectively.

Table 3
Variation of the plasma lipid classes and serum lipids ($n=48$)

	Mean (mg/dl)	(SD)	Range (mg/dl)	RSD (%)
Lipid class[a]				
CE	221.4	(41.9)	139.1–291.8	18.9
FC	59.4	(18.4)	30.9–116.0	30.9
CE/FC ratio	3.94	(0.94)	2.14–5.86	23.8
TG	82.6	(42.7)	26.0–230.0	51.7
PC	327.0	(89.2)	173.4–559.6	27.3
Serum lipids[b]				
S-Chol	191.4	(37.0)	127.7–267.0	19.3
LDL-C	115.2	(33.5)	50.8–190.4	29.0
S-Trigl	86.0	(43.6)	31.0–247.8	50.7
HDL-C	59.1	(14.8)	32.9–106.4	25.0
HDL-%	31.8	(8.8)	15.8–53.3	27.8

[a] Determined by HPLC. CE, FC, TG and PC are the same as in Table 1.
[b] Determined by enzymatic methods. S-Chol, serum total cholesterol, LDL-C, low-density lipoprotein cholesterol, S-Trigl, serum triacylglycerols, HDL-C, high-density lipoprotein cholesterol, HDL-%, HDL-C/S-Chol (%).

3.5. Associations with serum lipid levels

Both CE and FC had high correlations with the S-Chol concentration ($P<0.001$, Table 4) but not with that of HDL-C. The associations also show that the distribution of cholesterol into esterified and free forms (CE/FC) was not strongly dependent on the S-Chol level ($P<0.05$). TG levels were highly correlated with those obtained by the enzymatic method (S-Trigl; $r=0.96$, $P<0.001$, Table 4) but tended to be slightly lower when quantified by HPLC (Table 3). PC was more closely correlated to HDL-C ($r=0.51$, $P<0.001$) than to LDL-C levels ($r=0.32$, $P<0.05$, Table 4). Thus, elevated PC concentrations tend to reflect higher proportions of HDL-C out of S-Chol (Fig. 3). However, even a low PC level can be linked to a high percentage of HDL-C when the S-Chol level is also low.

3.6. Associations with phospholipid fatty-acid composition

The level of linoleic acid (LA, $18{:}2n-6$) in plasma PL was closely correlated with the level of FC ($r=-0.56$, $P<0.001$) but not with that of CE (Table 4), most probably because that both PL and FC are located in the outer monolayer of lipoproteins and PL LA is preferred as the substrate for the esterification of FC [14,15,17]. The inverse association between LA and FC is also linked to the diet, since higher S-Chol (and FC) levels usually indicate a lower intake of LA [18,26]. LA, rather than serum lipids, also showed the highest correlation with the CE/FC ratio ($r=0.65$, $P<0.001$). The relationships between eicosapentaenoic acid (EPA, $20{:}5n-3$) and FC, PC and the CE/FC ratio were the opposite to those with LA. These fatty acids reflect the competition between $n-6$ and $n-3$ PUFA [27], which can

Table 4
Relationships (r) between the levels of plasma lipid classes, serum lipids and the proportions of phospholipid (PL) fatty acids (n=48)

	Plasma lipid classes[a]									
	CE		FC		CE/FC		TG		PC	
	r[b]	P[c]	r	P	r	P	r	P	r	P
Serum lipids[d]										
S-Chol	0.94	***	0.81	***	−0.34	*	0.47	***	0.55	***
LDL-C	0.86	***	0.74	***	−0.28		0.44	**	0.32	*
HDL-C	0.15		0.16		−0.12		−0.38	**	0.51	***
S-Trigl	0.40	**	0.34	*	−0.17		0.96	***	0.24	
PL fatty acids[e]										
SaFA	−0.11		0.01		−0.15		0.04		0.11	f
MUFA	−0.10		0.17		−0.32	*	−0.14		0.41	**
$n-6$ PUFA	−0.14		−0.43	**	0.50	***	−0.08		−0.55	***
LA, 18:2$n-6$	−0.15		−0.56	***	0.65	***	−0.37	**	−0.48	***
HGLA, 20:3$n-6$	0.01		0.24	*	−0.32	*	0.52	***	0.05	
AA, 20:4$n-6$	0.08		0.31		−0.34	*	0.35	*	0.05	
$n-3$ PUFA	0.48	***	0.51	***	−0.29	*	0.28		0.29	*
EPA, 20:5$n-3$	0.54	***	0.57	***	−0.34	**	0.16		0.41	**
DHA, 22:6$n-3$	0.38	**	0.39	**	−0.19		0.32		0.23	
$n-6/n-3$ PUFA	−0.40		−0.55	***	0.47	***	−0.30	*	−0.39	*

[a] CE, FC, TG and PC are the same as in Table 1.
[b] r=Pearson's correlation coefficient.
[c] Significance level: *$P<0.05$, **$P<0.01$, ***$P<0.001$.
[d] S-Chol, LDL-C, HDL-C and S-Trigl are as in Table 3.
[e] SaFA (saturated fatty acids): 16:0, 18:0; MUFA (monounsaturated fatty acids): 16:1$n-7$, 18:1$n-9$; $n-6$ PUFA (polyunsaturated fatty acids): 18:2$n-6$, 20:3$n-6$, 20:4$n-6$; $n-3$ PUFA: 18:3$n-3$, 18:4$n-3$, 20:5$n-3$, 22:5$n-3$, 22:6$n-3$.
[f] Correlation between 16:0 and PC, r=0.45, $P<0.01$; correlation between 18:0 and PC, r=−0.39, $P<0.01$.

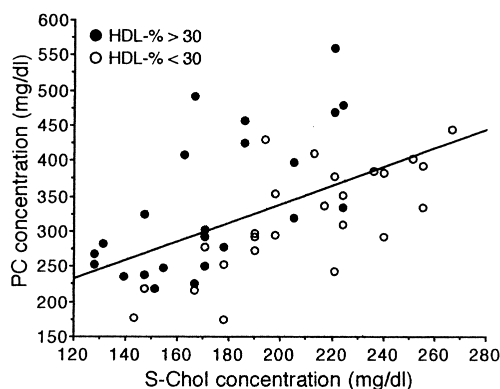

Fig. 3. Association between plasma phosphatidylcholine (PC) and serum total cholesterol (S-Chol) concentrations. Low (<30%) and high (>30%) proportions of high-density lipoprotein cholesterol out of S-Chol (HDL-%) are indicated by open and filled circles, respectively.

also be seen as changes in PL fatty-acid composition during fat substitutions [23,24]. EPA, a precursor of antithrombotic eicosanoids [28], was the only PUFA that had a marked positive correlation with PC (r= 0.41, $P<0.01$).

3.7. Changes during vegetable-oil substitutions

The subjects replaced the fat on bread by cold-pressed or conventional RSO or by SBO, which represented 24% of the daily fat intake, on average. Despite the small size of the SBO group, the use of oil was well reflected as an increase in $n-6$ PUFA (2.3% units, $P<0.01$, Table 5), derived mainly from LA. A reduction in FC ($P<0.05$) and a rise in the CE/FC ratio (from 3.6 to 5.1, $P<0.01$) would indicate more efficient esterification of FC to CE. Such effects could be expected since SBO is an abundant source of LA (~55%). RSO substitutions had no effect on the CE/FC ratio. Instead, they

Table 5
Plasma and serum lipid levels and phospholipid (PL) fatty-acid composition before and at the end of 6-weeks of fat substitution

	Substitution groups[a]								
	CPRSO (n=26)			RSO (n=16)			SBO (n=6)		
	Baseline		Change at 6 weeks[b]	Baseline		Change at 6 weeks	Baseline		Change at 6 weeks
	Mean	(SD)		Mean	(SD)		Mean	(SD)	
Serum lipids (mg/dl)[c]									
S-Chol	200.6	(38.3)	−5.2	180.0	(35.7)	−6.8	181.9	(26.9)	−3.2
S-Trigl	92.8	(47.0)	4.1	81.8	(44.0)	−0.3	67.6	(17.3)	9.7
Plasma lipid classes (mg/dl)[d]									
CE	232.9	(41.4)	−0.9	207.5	(45.3)	−2.7	208.8	(17.4)	8.9
FC	63.0	(20.4)	−2.8	52.9	(14.1)	−1.3	61.3	(16.5)	−18.2*
CE/FC ratio	3.93	(0.92)	0.04	4.08	(0.98)	0.02	3.62	(0.97)	1.51**
TG	90.7	(44.9)	14.7	71.9	(42.7)	13.1	76.0	(29.2)	22.4
PC	329.8	(85.6)	36.2**	316.0	(104.0)	20.5	344.5	(70.6)	10.8
PL fatty acids (%)[e]									
SaFA	43.1	(1.0)	−0.1	45.6	(0.9)	−0.4	44.9	(0.6)	−0.2
MUFA	14.1	(2.0)	0.4	14.3	(1.8)	0.2	14.9	(0.4)	−1.7*
$n-6$ PUFA	36.0	(2.4)	−0.8	34.3	(2.2)	−0.1	34.0	(1.0)	2.3**
$n-3$ PUFA	6.8	(1.6)	0.5*	5.8	(1.0)	0.3*	6.1	(0.5)	−0.4
$n-6/n-3$ PUFA	5.7	(1.6)	−0.4*	6.1	(1.4)	−0.3	5.6	(0.6)	1.0*

[a] Cold-pressed rapeseed oil (CPRSO), conventional rapeseed oil (RSO), soya-bean oil (SBO).
[b] Paired t-test: *$P<0.05$, **$P<0.01$ compared to the baseline.
[c] S-Chol and S-Trigl as in Table 3.
[d] CE, FC, TG and PC as in Table 1.
[e] SaFA, MUFA, $n-6$ and $n-3$ PUFA as in Table 4.

tended to increase $n-3$ PUFA ($P<0.05$, Table 5), which is a result of the metabolism of α-LLA (18:3$n-3$; 11% in RSO). This is a typical change that occurs when replacing margarine or butter by RSO [23,24]. PC was positively correlated with $n-3$ PUFA (Table 4) and its rise during CPRSO substitution ($P<0.01$, Table 5) seemed to occur in parallel with $n-3$ PUFA ($P<0.05$).

4. Conclusions

An HPLC method using ELSD was improved and validated for the simultaneous determination of lipid classes in human plasma samples. Quantitative analyses showed good repeatability and accuracy of the method. The proportion of the methodological variation out of the total variation in a group of 48 subjects remained insignificant (below 4%). The CE/FC ratio and PC concentrations, which were closely correlated with the proportion of PUFA in plasma phospholipids, could be affected by fat substitutions. The CE/FC ratio increased in parallel with levels of $n-6$ PUFA during SBO substitution, while CPRSO increased the levels of PC and $n-3$ PUFA. This method has been shown to be applicable to the quantitation of lipid classes and it gives rise to a new approach, together with fatty-acid data, in evaluating lipid balance in human plasma and, further, the effects derived from dietary fats.

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