Sterol carrier protein-2 expression alters phospholipid content and fatty acyl composition in L-cell fibroblasts

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Abstract **The effects sterol carrier protein-2 (SCP-2) expression on L-cell phospholipid levels and fatty acyl composition was assessed using L-cells transfected with the murine cDNA encoding for either the 15 kDa proSCP-2 or 13.2 kDa SCP-2. Expression of these proteins reduced total phospholipid mass (nmol/mg protein) by 24% and reduced the cholesterol to phospholipid ratio 60 and 28%, respectively. In 15 kDa proSCP-2 expressing cells, individual phospholipid class masses, excluding sphingomyelin (CerPCho), were reduced as follows: phosphatidylinositol (PtdIns) and phosphatidylserine (PtdSer) >> ethanolamine glycerophospholipid (EtnGpl) > choline glycerophospholipid (ChoGpl). Furthermore, ethanolamine plasmalogen mass was decreased 25%, while choline plasmalogen mass was elevated 30% in 15 kDa proSCP-2 expressing cells. In 13.2 kDa SCP-2 expressing cells, phospholipid class mass was decreased as follows: PtdIns and PtdSer >> ChoGpl. These changes in phospholipid mass resulted in altered cellular phospholipid composition. Expression of either protein differentially altered the type of fatty acid esterified onto the phospholipids. These effects included a greater proportion of polyunsaturated fatty acids and a reduction in saturated fatty acids, although 15 kDa proSCP-2 expression had a more robust effect on these parameters than did 13.2 kDa SCP-2 expression. In summary, expression of SCP-2 reduced individual phospholipid class mass, except for CerPCho, and altered the fatty acid composition of each phospholipid class examined. These results clearly demonstrate that SCP-2 expression altered basal phospholipid levels, suggesting that SCP-2 can alter the function of endoplasmic reticulum phospholipid synthetic enzymes.**—Murphy, E. J., T. Stiles, and F. Schroeder. **Sterol carrier protein-2 expression alters phospholipid content and fatty acyl composition in L-cell fibroblasts.** ***J. Lipid Res.*** **2000.** 41: **788–796.**

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Sterol carrier protein-2 (SCP-2) is thought to mediate cholesterol trafficking and metabolism (1–3). SCP-2 is a small ubiquitous protein encoded for by a single gene that contains two initiation sites (4, 5), encoding for the 58 kDa SCP_x and the 15 kDa proSCP-2 (4, 6–8). The 15 kDa proSCP-2 contains an N-terminal 20 amino acid putative mitochondrial targeting sequence (9, 10) as well as a C-terminal peroxisomal targeting sequence (7). However, neither the effect of the N-terminal presequence on intracellular targeting nor the intracellular location in which the N-terminal sequence is cleaved have been clearly delineated. Nevertheless, the N-terminal presequence undergoes rapid post-translational cleavage in most tissues and cells, including L-cell, resulting in the 13.2 kDa form of SCP-2 (9, 10). Because of this rapid post-translational cleavage, the 15 kDa protein is rarely detected by Western blotting (9, 11, 12). For the 13.2 kDa SCP-2, cellular localization studies using immunogold and immunofluorescence methods have indicated that the protein is associated with peroxisomes (13) and endoplasmic reticulum (14) as well as being found in the mitochondria (14, 15) and cytosol (14, 16). The 58 kDa SCP_x, on the other hand, is exclusively localized in the peroxisomal matrix (13, 17), although expression of this protein in L-cell fibroblasts also increases 13.2 kDa SCP-2 levels 2-fold, which may account for the significant extraperoxisomal immunoreactive SCP-2 in these cells (18).

Even though the physiological role for SCP-2 remains elusive, several studies suggest a function in lipid metabolism. SCP-2 not only binds sterol (3, 19), cholesterol (19–21), and phospholipids (20), it more importantly enhances cholesterol transfer between model membranes (20, 22, 23) and L-cell membranes (16, 24). Recently, SCP-2 was shown to increase cholesterol uptake (2) and cholesterol esterification (1) when the 15 kDa proSCP-2 was stably expressed in L-cell fibroblasts. In contrast, expression of the 13.2 kDa SCP-2 did not enhance cholesterol uptake, suggesting that expression of the 15 kDa proSCP-2 containing the N-terminal pre-sequence is essential for proper function in cholesterol uptake.

Abbreviations: EtnGpl, ethanolamine glycerophospholipid; lysoPtdEtn, lysophosphatidylethanolamine; PtdIns, phosphatidylinositol; PtdSer, phosphatidylserine; ChoGpl, choline glycerophospholipid; CerPCho, sphingomyelin; lysoPtdCho, lysophosphatidylcholine; Etn, ethanolamine; Cho, choline; PlsEtn, ethanolamine plasmalogen; PlsCho, choline plasmalogen; SCP-2, sterol carrier protein-2; HPLC, high performance liquid chromatography; GLC, gas–liquid chromatography; TLC, thin-layer chromatography; FAME, fatty acid methyl ester.

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Although many studies in vitro and in transfected L-cells demonstrate a role for SCP-2 in cholesterol metabolism and trafficking, until recently a similar role in fatty acid uptake and trafficking was unrealized. Studies in vitro clearly show that SCP-2 binds fatty acids with an affinity similar to that reported for fatty acid binding proteins (21, 25, 26), although this binding is very sensitive to ethanol (27). In L-cells transfected with SCP-2, both 13.2 kDa SCP-2 and 15 kDa proSCP-2 expression increases uptake and intracellular trafficking of fluorescently labeled fatty acid (28). Furthermore, SCP-2 also binds the activated form of fatty acids, acyl-CoA (21, 29), suggesting a role in fatty acid targeting. A similar study also demonstrated high affinity binding of long-chain fatty acyl-CoA to SCP-2 (30). Expression of the 58 kDa SCP_x in L-cells stimulates not only an increase in 13.2 kDa SCP-2 levels, but also increases the esterification of oleic acid onto cholesterol, further implicating a role in fatty acid trafficking (18). In a separate study, the SCP-2 gene encoding the 58 kDa SCPx,15 kDa proSCP-2, and thereby the 13.2 kDa SCP-2 was knocked out, resulting in a 2-fold decrease in liver glycerides and cholesteryl esters (31), suggesting that these animals have altered phospholipid and fatty acid metabolism.

While the 13.2 and 15 kDa forms of SCP-2 increase fatty acid uptake into L-cells and bind both acylCoA and fatty acids in assays in vitro, the role of these proteins on phospholipid composition and mass as well as phospholipid fatty acyl composition is unclear. Because the 13.2 kDa SCP-2 and 15 kDa proSCP-2 are localized to mitochondria, endoplasmic reticulum, and peroxisomes as well as binding acyl-CoA, SCP-2 expression in L-cell fibroblasts may mediate alterations in phospholipid acyl chain composition and phospholipid levels. These possibilities were assessed using cells stably transfected with the murine cDNA encoding for either the 13.2 kDa SCP-2 or 15 kDa proSCP-2. We report for the first time that SCP-2 expression decreased phospholipid levels by nearly 25%, resulting, in part, in a dramatic increase in the cholesterol to phospholipid ratio. In addition, SCP-2 expression differentially altered phospholipid fatty acid composition and increased the proportions of long-chain polyunsaturated fatty acids esterified to the phospholipids.

METHODS

Cells

The cells used in this study are the same murine L-cells (L arpt$^-$tk$^-$) clones that were stably transfected with the cDNA encoding for either the murine 13.2 kDa SCP-2 or 15 kDa proSCP-2 (1, 2). In previous studies, we demonstrated that mock transfected cells did not alter cholesterol uptake, although expression of the 15 kDa pro-SCP-2 did increase cholesterol uptake (2). SCP-2 expression in these clones was equivalent to that previously demonstrated (1, 2). Control and transfected cells were grown in Higuchi medium containing 10% fetal bovine serum (Hyclone, Logan, UT) and were grown to confluency (32).

Lipid extraction

Cellular lipids were extracted from confluent control and transfected cells using n-hexane–2-propanol 3:2 (v/v) (33, 34). Prior to extraction, cell culture medium was removed and the cells were washed twice with ice-cold phosphate-buffered saline. After removal of the last wash, the cell plate was floated on liquid N_2 to minimize lipid breakdown during cell removal (35). After removal from the liquid N_2, 2 ml of 2-propanol was added to the cell plate and the cells were removed by scraping. The 2-propanol aliquot containing the cells was transferred to a tube containing 6 ml of n-hexane. The cell plate was washed with another 2 ml aliquot of 2-propanol which was also transferred to the tube containing the n-hexane, resulting in n-hexane–2-propanol 3:2 (v/v).

Cell extracts were centrifuged at 2,500 rpm to pellet the denatured protein and other cellular debris. The lipid-containing organic liquid was decanted and saved until analysis. The residual protein pellet was dried overnight at room temperature. All lipid extracts were stored under a N_2 atmosphere at −80°C.

Phospholipid separation

Before high performance liquid chromatography (HPLC), the sample volume was reduced under a stream of nitrogen and the samples were filtered through a nylon 66 0.2 μm filter (Ranin, Emeryville, CA). The filtered sample was then dried to completeness and redissolved in a known volume of HPLC grade n-hexane–2-propanol 3:2 (v/v).

The HPLC system consisted of a Beckman 125 pump module, a Beckman 166 UV/Vis detector (Fullerton, CA), a column heater (Jones Chromatography, Littleton, CO) containing a Phenomenex Selectosil column (5 μm, 4.6 × 250 mm, Torrance, CA) maintained at 34°C. The eluent absorbance was monitored at 205 nm.

The phospholipid classes were separated using a binary gradient of (A) n-hexane–2-propanol 3:2 (v/v) and (B) n-hexane–2-propanol–water 56.7:37.8:5.5 (v/v/v). Initial solvent conditions were 65% A / 35% B with a step gradient to 100% B over 75 min. This method separates all of the major phospholipid classes (36). The ethanolamine glycerophospholipid (EtnGpl) and choline glycerophospholipid (ChoGpl) fractions were quantitatively divided in two equal parts, one of which was used to quantify phospholipid mass by assaying lipid phosphorus (37) and the other was dried under nitrogen and exposed to HCl vapor for 15 min to cleave the vinyl ether linkage of the plasmalogen subclasses (38). The latter fractions were re-separated by HPLC and the glycerophospholipid and lysophospholipid fractions were collected and quantified by assaying for lipid phosphorus (37). All other phospholipid fractions were also quantified by analysis of lipid phosphorus (37).

Thin-layer chromatography

Phospholipid fatty acid composition was analyzed in individual phospholipids separated by thin-layer chromatography (TLC). Silica gel G plates (Analtech, Newark, DE) were heat activated at 110°C for 1 h and samples were streaked onto the plates. The developing solvent was chloroform–methanol–water 65:25:4 (v/v/v). This solvent system separates the phosphatidylinositol (PtdIns) from the phosphatidylserine (PtdSer) as well as the ChoGpl from the sphingomyelin (CerPCho) (39). Bands were visualized using 1 mm 6-p-toluidino-2-naphthalene sulfonic acid dissolved in 50 mm Tris (pH 7.4) (40). Bands corresponding to authentic lipid standards were scraped into screw-top test tubes and subjected to base catalyzed transesterification.

Transesterification

Methanol was added to the individual phospholipid fractions and they were subjected to base-catalyzed transesterification, converting the phospholipid acyl chains to fatty acid methyl esters (FAME) (41). FAME were extracted from the methanol using

2 ml of n-hexane and the n-hexane upper-phase containing the FAME was removed. The lower phase was re-extracted two more times with 2-ml aliquots of n-hexane and these washes were combined with the original aliquot.

Gas–liquid chromatography

FAME were separated by gas–liquid chromatography (GLC) and quantified using flame ionization detection. Individual fatty acids were identified using FAME standards (NuChek Prep, Elysian, MN). Relative correction factors for fatty acids were established using standards and based upon a set concentration of 17:0 added prior to analysis. Detector response was linear within the sample concentration range for all of the fatty acids.

The GLC system consisted of a GLC-14A (Shimadzu, Kyoto, Japan) equipped with an SP-2330 capillary column (0.32 mm ID × 30 m length, Supelco, Bellfontaine, PA). Column temperature was maintained at 185°C, with the injector and detector temperature set at 220°C. The split ratio was 40:1. Peak area data were collected using a Dionex UI-120 analytical to digital interface and converted to peak area using Dionex PeakNet software (Dionex, Sunnyvale, CA).

Protein assay

Proteins were measured using a modified dye-binding assay (42). The dried protein residue from the extracts was digested overnight in 0.2 m KOH at 65°C. After digestion, aliquots were used to measure the protein concentration by converting absorbances to concentrations using a bovine serum albumin standard curve.

Statistics

All groups were compared using one-way ANOVA and Tukey-Kramer multiple comparisons post-test using Instat II (GraphPad, San Diego, CA). All values are expressed as means ± SD. Statistical significance was defined as $P < 0.05$. The n is defined as the number of cultures used to determine each data point.

RESULTS

Total phospholipid mass

The effect of 13.2 kDa SCP-2 and 15 kDa proSCP-2 expression on total cellular phospholipid mass (nmol/mg protein) was determined. The total phospholipid mass was decreased from 299 ± 33 to 229 ± 25, and 225 ± 39 nmol/mg protein in 13.2 kDa SCP-2 and 15 kDa proSCP-2 expressing cells, respectively. Hence expression of either the 13.2 or 15 kDa form of SCP-2 decreased phospholipid mass by nearly 25%. This large change in total cellular phospholipid content suggests that the masses of one or more of the individual phospholipid classes may also be decreased.

Endoplasmic reticulum synthesized individual phospholipid class mass

Changes in total cellular phospholipid content do not necessarily reflect similar changes in all phospholipid classes. Therefore, the phospholipid classes were resolved by HPLC and the individual phospholipid class masses were determined. The largest decrease in phospholipid mass was found in phosphatidylinositol (PtdIns) and phosphatidylserine (PtdSer) (**Table 1A**). In 15 kDa proSCP-2 expressing cells, PtdIns and PtdSer were both markedly reduced 83% each, whereas in 13.2 kDa SCP-2 expressing cells, PtdIns and PtdSer were decreased 67 and 86%, respectively. Choline glycerophospholipid (ChoGpl) mass was also reduced in both cell lines, although the reduction was much less than that observed for the other phospholipids (17 and 12% for the 13.2 kDa SCP-2 and 15 kDa proSCP-2 expressing cells, respectively). Ethanolamine glycerophospholipid (EtnGpl) content was significantly decreased in only the 15 kDa proSCP-2 expressing cells, with the mass reduced by 28% compared to control. In neither cell line was the sphingomyelin (CerPCho) mass altered, indicating that, unlike the other phospholipids, there was no derangement of the CerPCho biosynthesis. As shown above, 13.2 kDa SCP-2 and 15 kDa proSCP-2 expression did not decrease the content of all the phospholipid classes equally. In fact, the content of CerPCho and the lysophospholipids were unaffected (Table 1A)

TABLE 1. Effects of SCP-2 expression on L-cell phospholipid mass and composition

Phospholipid Class	A: Phospholipid Mass		
	Control	13.2 kDa SCP-2	15 kDa proSCP-2
	nmol/mg protein		
EtnGpl	68.5 ± 10.6	59.4 ± 2.7	49.1 ± 0.5[a,b]
lysoPtdEtn	7.6 ± 2.8	4.6 ± 0.9	3.1 ± 1.7
PtdIns	9.0 ± 5.4	3.0 ± 2.7[a]	1.5 ± 0.4[a]
PtdSer	7.0 ± 1.5	0.9 ± 0.9[a]	1.2 ± 1.2[a]
ChoGpl	153.4 ± 13.9	127.1 ± 6.4[a]	135.8 ± 2.9[a]
CerPCho	31.7 ± 7.1	31.5 ± 5.1	32.8 ± 4.2
lysoPtdCho	5.3 ± 3.2	2.8 ± 2.6	2.5 ± 1.3
	B: Phospholipid Composition		
	mole %		
EtnGpl	24.2 ± 2.9	25.9 ± 1.2	21.8 ± 0.2[a,b]
lysoPtdEtn	2.7 ± 0.6	2.0 ± 0.4	1.3 ± 0.8
PtdIns	3.1 ± 1.7	1.5 ± 1.0[a]	0.7 ± 0.2[a]
PtdSer	2.5 ± 0.7	0.4 ± 0.4[a]	0.5 ± 0.5[a]
ChoGpl	54.5 ± 3.3	55.5 ± 2.8	60.4 ± 1.7[a,b]
CerPCho	11.2 ± 2.1	13.8 ± 2.2	14.2 ± 0.8[a]
lysoPtdCho	1.9 ± 1.2	0.9 ± 1.2	1.1 ± 0.6

Values are represent means ± SD, n = 4.
[a] Indicates significant from control, $P < 0.05$.
[b] Indicates significant from 13.2 kDa SCP-2 expressing cells, $P < 0.05$.

Peroxisomal and endoplasmic reticulum synthesized plasmalogen mass

Plasmalogens, phospholipids containing a vinyl ether linkage at the *sn*-1 position, are synthesized initially in the peroxisome (43, 44) with the polar head group addition and subsequent desaturation of the ether moiety occurring in the endoplasmic reticulum (45–47). Because these ether-containing phospholipids have a role in lipid-mediated signal transduction (48–51), the effects of SCP-2 expression on the mass of these phospholipids were determined separately. In 15 kDa proSCP-2 expressing cells, ethanolamine plasmalogen (PlsEtn) mass was decreased 25%, while choline plasmalogen (PlsCho) mass was increased nearly 30% (**Table 2A**). In the 13.2 kDa SCP-2 expressing cells, separating out the acid-labile plasmalogen subclass from the acid-stable subclasses revealed a 18% decrease in the acid-stable fraction, predominantly phosphatidylethanolamine (PtdEtn). In the 15 kDa proSCP-2 expressing

TABLE 2. Effects of SCP-2 expression on L-cell plasmalogen composition and mass

Phospholipid Class	Control	13.2 kDa SCP-2	15 kDa proSCP-2
	A: Mass of Glycerophospholipid Subclasses		
	nmol/mg protein		
Etn acid stable	43.7 ± 2.1	36.2 ± 0.4[a]	30.7 ± 1.6[a]
Etn acid labile	24.5 ± 3.7	23.2 ± 0.4	18.4 ± 1.6[a]
Cho acid stable	140.9 ± 3.6	115.7 ± 1.8[a]	119.5 ± 1.7[a,b]
Cho acid labile	12.5 ± 0.3	11.4 ± 1.8	16.3 ± 1.7[a,b]
	B: Composition of Glycerophospholipid Subclass		
	mole%		
Etn acid stable	64.0 ± 2.6	60.9 ± 0.6	62.6 ± 3.1
Etn acid labile	36.0 ± 2.3	39.1 ± 0.6	37.4 ± 3.1
Cho acid stable	91.9 ± 1.5	91.0 ± 1.4	87.9 ± 1.3[a,b]
Cho acid labile	8.1 ± 1.5	9.0 ± 1.4	12.1 ± 1.3[a,b]

Values represent means ± SD, n = 4–6.
[a] Indicates significant from control, $P < 0.05$.
[b] Indicates significant from the 13.2 kDa SCP-2 expressing cells, $P < 0.05$.

cells, the acid-stable fraction, predominantly PtdEtn, was decreased 30%. In the ChoGpl subclasses, the majority of the decrease in either cell line was accounted for by a large decrease in the acid-stable fraction, predominantly the phosphatidylcholine subclass. Hence, in both cell lines PtdEtn was decreased, but ethanolamine and choline plasmalogen mass was only altered in the 15 kDa proSCP-2 expressing cells. Thus, 13.2 kDa SCP-2 and 15 kDa proSCP-2 expression differentially affected plasmalogen levels, suggesting a role for the 15 kDa proSCP-2 in modulating plasmalogen biosynthesis in peroxisomes.

Phospholipid class composition

While 13.2 kDa SCP-2 and 15 kDa proSCP-2 expression dramatically decreased total phospholipid mass and differentially affected the masses of individual phospholipid classes, such data do not provide any information regarding the relative distribution of the individual phospholipid classes. Therefore, the effect of 13.2 kDa SCP-2 or 15 kDa proSCP-2 expression on L-cell phospholipid percent composition was calculated from the phospholipid mass data in Table 1A. In 15 kDa proSCP-2 expressing cells, EtnGpl proportions (mole %) were significantly decreased 10%, while PtdIns and PtdSer proportions were reduced nearly 80% (Table 1B). In the 13.2 kDa SCP-2 expressing cells, a similar decrease in PtdSer and PtdIns proportions was observed. In contrast to the 13.2 kDa SCP-2 expressing cells where the mole % of CerPCho and ChoGpl remained unchanged, these mole percentages were increased 1.3- and 1.1-fold, respectively, in 15 kDa proSCP-2 expressing cells (Table 1B). Similarly, in 15 kDa proSCP-2 expressing cells, the proportion of acid-stable and acid-labile fractions of ChoGpl was altered, while there were no changes in the proportion of these two fractions in the EtnGpl (Table 2B). Hence, the increase in the acid-labile fraction is indicative of changes in PlsCho, while PlsEtn was unchanged. Both plasmalogen fractions remained unchanged in the 13.2 kDa SCP-2 expressing cells. Changes in composition illustrate alterations in one phospholipid relative to the other phospholipids and suggest that large changes in individual phospholipid class mole % indicate specific changes in individual phospholipid class synthesis or catabolism. In both the 13.2 and 15 kDa expressing cells, the most notable changes were the changes in the PtdIns and PtdSer fractions, suggesting altered metabolism of these two phospholipids in the SCP-2 expressing cells. In contrast, the mole % of the other phospholipid classes (EtnGpl, ChoGpl, CerPCho, PlsCho) were only altered in the 15 kDa proSCP-2 expressing cells.

Phospholipid to cholesterol ratio

As earlier studies from this laboratory also showed effects of 15 kDa proSCP-2 expression, but not 13.2 kDa SCP-2 expression, on cholesterol uptake in L-cells (1), it was important to examine the effect of these proteins on the cholesterol to phospholipid ratio. The ratio was 0.24 ± 0.02, 0.32 ± 0.05[a], and 0.40 ± 0.04[a,b] for the control, 13.2 kDa SCP-2, and 15 kDa proSCP-2 expressing cells, respectively. The [a] indicates significantly different from control, $P < 0.05$, and the [b] indicates significantly different from the 13.2 kDa SCP-2 expressing cells, $P < 0.05$. Clearly, the expression of these two proteins differentially affected the cholesterol to phospholipid ratio, with the 15 kDa proSCP-2 expressing cells having a ratio that was nearly 65% larger than that for the control cells, while in the 13.2 kDa SCP-2 expressing cells, this ratio was only increased 33%. Because 13.2 kDa SCP-2 expression had no effect on free cholesterol mass (1), but did decrease the phospholipid mass, the changes in the cholesterol to phospholipid ratio were much smaller. These results suggest that the N-terminal presequence of the 15 kDa proSCP-2 was necessary to dramatically increase the ratio of free cholesterol to phospholipid, which is critical as this ratio is a primary determinant of membrane structure.

Phospholipid fatty acid composition

In addition to the cholesterol to phospholipid ratio and phospholipid composition, the other major determinant of membrane structure is the phospholipid fatty acid composition. The fatty acid composition of the two major phospholipid classes, EtnGpl and ChoGpl, as well as that of the two classes with the largest change, PtdIns and PtdSer, was determined in control, 13.2 kDa SCP-2 and 15 kDa proSCP-2 expressing L-cells.

In the EtnGpl, the most marked change was a 1.8-fold increase in 22:6 n–3 and 22:4 n–6 proportions (mole %) in both cell lines relative to control (**Table 3**). Despite this change, there was no significant increase in the overall proportion of polyunsaturated fatty acids (PUFA), primarily due to a large increase, approximately 1.3-fold, in 18:0 proportions which comprised 25–28% of the total fatty acid in the transfected cells. This led to a significant decrease in the unsaturated/saturated index in the 15 kDa expressing cells. However, the n–3/n–6 and PUFA/saturated fatty acid indices were unaffected by either 13.2 or 15 kDa SCP-2 expression. Hence, in the EtnGpl, the most dramatic change was an increase in 22:4 n–6, 22:6 n–3,

TABLE 3. Effect of SCP-2 expression on ethanolamine glycerophospholipid fatty acid composition

Fatty Acid	Control	13.2 kDa SCP-2	15 kDa proSCP-2
16:0	5.94 ± 0.55	5.78 ± 1.71	6.05 ± 0.45
16:1	1.68 ± 0.17	1.62 ± 0.43	1.22 ± 0.32
18:0	20.65 ± 2.70	25.39 ± 0.91[a]	27.82 ± 1.38[a]
18:1 n−9	43.83 ± 3.78	39.23 ± 1.02	38.27 ± 1.47[a]
18:2 n−6	2.45 ± 0.17	3.03 ± 0.18	2.66 ± 0.53
18:3 n−6	0.36 ± 0.20	0.19 ± 0.06	0.23 ± 0.15
18:3 n−3	0.39 ± 0.04	0.33 ± 0.06	0.16 ± 0.03
20:0	0.50 ± 0.09	0.48 ± 0.11	0.58 ± 0.12
20:1	4.04 ± 0.65	2.68 ± 0.07[a]	2.05 ± 0.40[a]
20:2 n−6	0.62 ± 0.47	0.14 ± 0.08	0.28 ± 0.08
20:3 n−6	0.75 ± 0.11	0.81 ± 0.11	0.59 ± 0.17
20:4 n−6	8.73 ± 1.65	10.96 ± 0.53	10.52 ± 1.21
22:0	BLD	0.35 ± 0.15	0.43 ± 0.17
22:1	BLD	0.36 ± 0.17	0.25 ± 0.21
22:3 n−3	1.25 ± 0.18	BLD	BLD
22:4 n−6	1.43 ± 0.52	2.56 ± 0.34[a]	2.62 ± 0.42[a]
22:6 n−3	3.55 ± 1.45	5.59 ± 0.55[a]	5.89 ± 0.27[a]
24:0	1.38 ± 0.54	0.51 ± 0.12	0.37 ± 0.15
Saturated	29.21 ± 2.26	32.51 ± 0.75	35.26 ± 1.90[a]
MUFA	47.87 ± 5.70	43.88 ± 1.27	41.79 ± 1.80
PUFA	21.18 ± 5.53	23.61 ± 1.69	22.95 ± 1.40
n−6	15.08 ± 2.81	17.69 ± 1.13	16.90 ± 1.23
n−3	6.10 ± 1.89	5.92 ± 0.60	6.05 ± 0.30
MUFA/Sat	1.63 ± 0.31	1.35 ± 0.04	1.19 ± 0.11[a]
PUFA/Sat	0.72 ± 0.11	0.73 ± 0.06	0.65 ± 0.06
Unsat/Sat	2.40 ± 0.24	2.08 ± 0.07	1.84 ± 0.15[a]
n−3/n−6	0.40 ± 0.10	0.33 ± 0.02	0.36 ± 0.03
PUFA/MUFA	0.44 ± 0.12	0.54 ± 0.05	0.55 ± 0.05

Values are mole % and represent means ± SD, n ⩾ 3; BLD, below the limit of detection.

[a] Indicates significant difference form control, $P < 0.05$.

TABLE 4. Effect of SCP-2 expression on choline glycerophospholipid fatty acid composition

Fatty Acid	Control	13.2 kDa SCP-2	15 kDa proSCP-2
16:0	23.27 ± 0.67	24.26 ± 0.65	18.25 ± 1.54[a,b]
16:1	6.37 ± 0.21	7.04 ± 0.35	4.45 ± 0.90[a,b]
18:0	15.21 ± 3.03	11.47 ± 0.05	13.22 ± 0.88
18:1 n−9	48.18 ± 1.64	50.50 ± 0.25	56.06 ± 1.34[a,b]
18:2 n−6	1.83 ± 0.08	1.92 ± 0.13	2.07 ± 0.10
18:3 n−6	0.08 ± 0.36	0.08 ± 0.01	0.11 ± 0.07
18:3 n−3	0.04 ± 0.03	0.02 ± 0.04	0.03 ± 0.04
20:0	0.31 ± 0.04	0.35 ± 0.04	0.39 ± 0.09
20:1	1.12 ± 0.22	1.26 ± 0.25	1.29 ± 0.42
20:2 n−6	0.14 ± 0.02	0.17 ± 0.02	0.24 ± 0.11
20:3 n−6	0.32 ± 0.02	0.28 ± 0.01	0.36 ± 0.10
20:4 n−6	1.04 ± 0.20	1.32 ± 0.10	1.99 ± 0.29[a,b]
22:0	0.09 ± 0.10	0.18 ± 0.03	0.22 ± 0.08
22:3 n−3	0.24 ± 0.29	BLD	BLD
22:4 n−6	0.51 ± 0.29	0.26 ± 0.08	0.38 ± 0.10
22:6 n−3	0.35 ± 0.06	0.54 ± 0.06	0.75 ± 0.20[a]
22:5 n−6	0.43 ± 0.06	0.23 ± 0.20	0.06 ± 0.08[a]
24:1	0.11 ± 0.01	BLD	BLD
Saturated	38.68 ± 2.29	36.48 ± 0.52	32.14 ± 0.94[a,b]
MUFA	55.78 ± 1.84	58.93 ± 0.17[a]	61.94 ± 0.93[a,b]
PUFA	5.53 ± 0.51	4.58 ± 0.37[a]	5.91 ± 0.51[b]
n−6	4.70 ± 0.75	4.02 ± 0.28	5.13 ± 0.44
n−3	0.83 ± 0.60	0.56 ± 0.09	0.78 ± 0.16
MUFA/Sat	1.44 ± 0.13	1.62 ± 0.03[a]	1.93 ± 0.08[a,b]
PUFA/Sat	0.14 ± 0.02	0.13 ± 0.01	0.18 ± 0.02[a,b]
Unsat/Sat	1.59 ± 0.15	1.74 ± 0.04	2.11 ± 0.09[a,b]
n−3/n−6	0.18 ± 0.09	0.14 ± 0.01	0.15 ± 0.03
PUFA/MUFA	0.10 ± 0.01	0.08 ± 0.01[a]	0.10 ± 0.01[b]

Values are mole % and represent means ± SD, n ⩾ 3; BLD, below the limit of detection.

[a] Indicates significant differences from control, $P < 0.05$.

[b] Indicates significant difference from 13.2 kDa expressing cells, $P < 0.05$.

and 18:0 proportions, although the increase in 18:0 was sufficient to minimize the effect of the elevated PUFA in the various indices.

In the ChoGpl, the majority of the changes in fatty acid composition were limited to the 15 kDa proSCP-2 expressing cells (**Table 4**). Similar to EtnGpl, the 15 kDa expressing cells had a 2.1-fold increase in 22:6 n−3 proportions, although in this phospholipid class 20:4 n−6 proportions were elevated 1.9-fold, instead of increased 22:4 n−6 proportions. The proportions of 16:0 and 16:1 were decreased 20 and 30%, respectively, while 18:1 n−9 proportions increased 1.2-fold. Because of these changes, the amount of saturated fatty acids in the 15 kDa expressing cells were significantly lower relative to control, resulting in an increase in the PUFA/saturated fatty acid and unsaturated/saturated fatty acid indices. As previously stated, the changes in the 13.2 kDa expressing cells were limited to an overall decrease in PUFA and an increased proportion of monounsaturated fatty acids (MUFA). Thus, in the ChoGpl, the changes in fatty acid composition were limited for the most part to the 15 kDa expressing cells. These changes predominantly involved an elevation in MUFA proportions and a decrease in saturated fatty acids.

In the PtdIns, changes in fatty acid composition were limited in both 13.2 kDa SCP-2 and 15 kDa proSCP-2 expressing cells (**Table 5**). In the 15 kDa proSCP-2 expressing cells, proportions of 16:0 and 18:2 were elevated 1.7- and 3.4-fold, respectively. In both 13.2 kDa SCP-2 and 15 kDa proSCP-2 expressing cells, the PUFA/saturated index was increased 1.4- and 1.6-fold, respectively. This change was due to an increase in PUFA, as opposed to a large decline in saturated fatty acid proportions. The n−3/n−6 ratio was significantly increased in the 15 kDa proSCP-2 expressing cells, mainly due to an increase in the proportion of 22:3 n−3. Hence, the overall change in PtdIns was an increase in the PUFA/saturated fatty acid index.

There were a number of marked changes in PtdSer fatty acid composition in both the 13.2 kDa SCP-2 and 15 kDa proSCP-2 expressing cells (**Table 6**). These changes included increased 20:4 n−6, 22:4 n−6, 22:3 n−3, and 22:6 n−3 proportions in 15 kDa proSCP-2 expressing cells, coupled with a 20 and 33% decrease in 18:0 and 18:1 n−9, respectively. In 13.2 kDa SCP-2 expressing cells there was a 4-fold increase in 20:4 n−6 proportions as well as increased 22:4 n−6 proportions. Similar to the 15 kDa proSCP-2 expressing cells, 18:0 was decreased 23% relative to control in the 13.2 kDa SCP-2 expressing cells. Overall, PUFA increased 2.3- and 3.6-fold in the 13.2 kDa SCP-2 and 15 kDa proSCP-2 expressing cells, coupled with a 15 and 13% reduction in saturated fatty acids, respectively. These changes increased both the PUFA/saturated fatty acid index as well as the unsaturated/saturated fatty acid index. Thus, there was a significant alteration in PtdSer fatty acid composition as the 13.2 kDa SCP-2 and 15 kDa proSCP-2

TABLE 5. Effect of SCP-2 expression on phosphatidylinositol fatty acid composition

Fatty Acid	Control	13.2 kDa SCP-2	15 kDa proSCP-2
16:0	5.02 ± 1.61	5.47 ± 0.35	8.66 ± 0.90[a,b]
16:1	0.51 ± 0.15	1.07 ± 0.81	1.83 ± 1.66
18:0	31.76 ± 2.12	29.51 ± 1.64	26.53 ± 1.06[a]
18:1 n−9	41.48 ± 6.14	43.37 ± 2.90	40.26 ± 0.81
18:2 n−6	1.02 ± 0.56	2.39 ± 1.18	3.50 ± 0.83[a]
18:3 n−6	0.26 ± 0.10	0.34 ± 0.33	BLD
18:3 n−3	0.07 ± 0.09	0.27 ± 0.47	BLD
20:0	BLD	0.66 ± 0.25	BLD
20:1	1.51 ± 0.55	1.18 ± 0.76	1.12 ± 0.25
20:2 n−6	0.38 ± 0.13	0.06 ± 0.13[a]	BLD
20:3 n−6	1.06 ± 0.16	0.98 ± 0.09	0.92 ± 0.45
20:4 n−6	8.02 ± 0.68	9.91 ± 1.53	8.28 ± 0.49
22:3 n−3	BLD	BLD	4.65 ± 0.29
22:4 n−6	1.39 ± 0.53	1.36 ± 0.73	0.73 ± 0.12
22:6 n−3	1.12 ± 0.75	1.65 ± 0.20	1.03 ± 0.10
Saturated	36.87 ± 1.17	35.63 ± 1.63	35.19 ± 2.24
MUFA	43.49 ± 6.57	45.61 ± 1.95	43.21 ± 2.02
PUFA	11.97 ± 1.52	16.95 ± 1.44[a]	19.11 ± 1.14[a]
n−6	10.84 ± 1.18	15.04 ± 1.83[a]	13.43 ± 0.95
n−3	1.12 ± 0.74	1.91 ± 0.49	5.68 ± 0.35[a,b]
MUFA/Sat	1.18 ± 0.14	1.28 ± 0.11	1.20 ± 0.12
PUFA/Sat	0.33 ± 0.04	0.48 ± 0.05[a]	0.52 ± 0.05[a]
Unsat/Sat	1.50 ± 0.12	1.76 ± 0.12	1.72 ± 0.16
n−3/n−6	0.10 ± 0.07	0.13 ± 0.05	0.42 ± 0.03[a,b]
PUFA/MUFA	0.28 ± 0.07	0.37 ± 0.04	0.43 ± 0.03[a]

Values are mole % and represent means ± SD, n ⩾ 3; BLD, below the limit of detection.

[a] Indicates significant difference from control, $P < 0.05$.

[b] Indicates significant difference from 13.2 kDa expressing cells, $P < 0.05$.

TABLE 6. Effect of SCP-2 expression on phosphatidylserine fatty acid composition

Fatty Acid	Control	13.2 kDa SCP-2	15 kDa proSCP-2
16:0	4.20 ± 0.66	6.15 ± 1.18	5.79 ± 1.95
16:1	1.20 ± 0.17	2.02 ± 1.38	1.74 ± 0.25
18:0	51.63 ± 1.12	39.94 ± 3.03[a]	41.02 ± 3.59[a]
18:1 n−9	36.52 ± 0.64	35.20 ± 2.21	24.53 ± 5.93[a,b]
18:2 n−6	1.82 ± 0.11	5.82 ± 1.93[a]	2.97 ± 0.87[b]
18:3 n−6	1.93 ± 0.16	1.05 ± 0.41[a]	0.42 ± 0.18[a,b]
20:0	0.60 ± 0.05	0.59 ± 0.54	1.31 ± 1.04
20:1	BLD	1.09 ± 0.27[a]	1.45 ± 0.46[a]
20:3 n−6	0.81 ± 0.06	BLD	0.61 ± 0.42[b]
20:4 n−6	1.26 ± 0.19	5.05 ± 0.87[a]	3.16 ± 0.55[b]
22:0	BLD	1.44 ± 0.60[a]	1.39 ± 0.90[a]
22:3 n−3	BLD	BLD	7.15 ± 2.15[a]
22:4 n−6	BLD	1.64 ± 0.24[a]	3.27 ± 0.29[a]
22:6 n−3	BLD	BLD	3.35 ± 0.60[a]
Saturated	56.44 ± 0.48	48.13 ± 3.08[a]	49.51 ± 3.72[a]
MUFA	37.73 ± 0.63	38.30 ± 1.95	27.72 ± 5.29[a,b]
PUFA	5.82 ± 0.17	13.57 ± 2.48[a]	20.92 ± 2.57[a,b]
n−6	5.82 ± 0.17	13.57 ± 2.48[a]	10.43 ± 0.79[a]
n−3	BLD	BLD	10.50 ± 2.33[a,b]
MUFA/Sat	0.67 ± 0.02	0.80 ± 0.08	0.56 ± 0.15[b]
PUFA/Sat	0.10 ± 0.01	0.29 ± 0.07[a]	0.42 ± 0.65[a,b]
Unsat/Sat	0.77 ± 0.02	1.08 ± 0.13[a]	0.98 ± 0.15
n−3/n−6	BLD	BLD	1.01 ± 0.22[a,b]
PUFA/MUFA	0.15 ± 0.01	0.36 ± 0.07	0.78 ± 0.23[a,b]

Values are mole % and represent means ± SD, n ⩾ 3; BLD, below the limit of detection.

[a] Indicates significant difference from control, $P < 0.05$.

[b] Indicates significant difference from 13.2 kDa expressing cells, $P < 0.05$.

expressing cells became more unsaturated as proportions of PUFA increased.

Overall, expression of 15 kDa proSCP-2, and to a lesser extent 13.2 kDa SCP-2, resulted in an increase in PUFA in the four major phospholipids. This resulted in a general increase in the PUFA/saturated fatty acid index as well as the unsaturated/saturated fatty acid index. Proportions of 22:6 n−3, 22:3 n−3, 22:4 n−6, and 20:4 n−6 were increased, to some degree or another.

DISCUSSION

Although SCP-2 is known to bind many different lipids, ligand binding alone does not necessarily indicate a physiological function. Expression of the 15 kDa, but not the 13.2 kDa, form of SCP-2 not only facilitates cholesterol uptake (2) and esterification (1), but also appears to enhance trafficking of cholesterol (1) and fatty acids (28) within cells. Such studies support results of experiments in vitro showing that SCP-2 stimulates cholesterol exchange between membranes (16, 20, 22–24, 52). Recent results show that SCP-2 expression in L-cells stimulates fatty acid esterification (1) and fatty acid trafficking (26), while studies in vitro show fatty acid (21, 25–27) and acyl-CoA (21, 29, 30) binding as well as an alteration in fatty acid metabolism in gene targeted animals (31). Consequently, the effect of SCP-2 expression on phospholipid mass and phospholipid fatty acid composition was determined in L-cells stably transfected with the murine cDNA encoding for the 13.2 kDa SCP-2 or 15 kDa proSCP-2.

In L-cells, SCP-2 expression altered both the phospholipid composition and mass (Table 1) as well as increasing the cholesterol to phospholipid ratio. Expression of either form of SCP-2 decreased total phospholipid mass 24%, with the largest quantitative decrease (nmol/mg protein) in the ChoGpl, although this decrease only represented 12–17% of the total ChoGpl mass (Table 1A). Surprisingly, there was a specific reduction in both PtdIns and PtdSer as illustrated by the large decrease in their respective mole % as well as a large reduction in mass (Table 1B). Similar to the decrease in total phospholipid mass, both proteins had nearly the same effect on the content of PtdIns and PtdSer. In contrast, only expression of the 15 kDa proSCP-2 influenced cholesterol uptake (1, 2), suggesting that expression of the protein containing the N-terminal pre-sequence (9, 10) is necessary for this function. A graded effect in fatty acid uptake was seen in L-cells expressing either the 13.2 kDa SCP-2 or 15 kDa proSCP-2 (28), although only the 15 kDa proSCP-2 expressing cells had an increase in the apparent fatty acid intracellular diffusion rate. Nonetheless, in the results presented herein, both the 13.2 kDa SCP-2 and 15 kDa proSCP-2 expressing cells had similar effects on total phospholipid mass, even though there were some differences between the two transfected cell lines suggesting that expression of the 15 kDa form of SCP-2 had a more robust effect.

In general, phospholipid biosynthesis proceeds through

a number of different specific enzymes steps which are localized in the endoplasmic reticulum and comprise what is called the Kennedy pathway (53–57). Surprisingly, CerPCho levels and composition were not decreased by SCP-2 expression. In 15 kDa proSCP-2 expressing cells, the proportion (mole %) of CerPCho was actually increased relative to the other lipids. Because CerPCho synthesis goes through a pathway independent of the Kennedy pathway (53, 54), these data support an alteration specifically in the Kennedy pathway affecting the other phospholipids. The lack of an increase in lysophospholipids indicate that an increase in phospholipid catabolism by various acylhydrolase pathways does not appear to be operative in the transfected L-cells. The observed results are consistent with the localization of SCP-2 to the endoplasmic reticulum (14, 18), although previous results suggested that the effect of SCP-2 was limited to the transfer and esterification of cholesterol (1, 18). Hence, we speculate that SCP-2 expression in L-cells alters endoplasmic reticulum-localized phospholipid formation by specifically decreasing the activities of key enzymes in the Kennedy pathway.

While the majority of synthetic enzymes for phospholipid biosynthesis are located in the endoplasmic reticulum, a site where SCP-2 is significantly detected (14, 18), plasmalogen synthesis requires both peroxisomal (43, 44) and microsomal steps (45–47). The formation of the 1-*O*-alkyl, 2-acyl-glycerophosphatidic acid is in the peroxisome (43, 44) and the desaturation of the 1-*O*-alkyl moiety to form the plasmalogen occurs in the microsomes (45–47). In the 15 kDa proSCP-2, but not the 13.2 kDa SCP-2 cells, ethanolamine plasmalogen levels were decreased 20% and choline plasmalogen levels were increased 30% (Table 2A). With the known association of SCP-2 with the cytosolic side of the peroxisomal membrane (13) and the presence of the C-terminal peroxisomal targeting sequence (7), it is easy to speculate that the expression of the 15 kDa proSCP-2 targeted at least some of the SCP-2 to the peroxisome where it affected peroxisomal function in L-cells. However, because 22:6 n–3 formation is dependent on a peroxisomal step (58), it is unlikely that peroxisomal function is compromised in the 15 kDa proSCP-2 expressing cells because 22:6 n–3 proportions were elevated. An equally plausible explanation for a decrease in ethanolamine plasmalogen levels would be a decrease in the formation of ethanolamine plasmalogen because of altered desaturase activity, consistent with our proposed alteration in the Kennedy pathway. Alternatively, the conversion of ethanolamine plasmalogen to choline plasmalogen may account for this difference, although this decrease in ethanolamine plasmalogen is not equal to the increase in choline plasmalogen (Table 2). However, because the pathway for the conversion of ethanolamine to choline plasmalogen is controversial, primarily a phospholipase C- or D-mediated mechanism (59) or a methylation process (49, 60), the lack of a one to one molar stoichiometry may be valid. As such, the decrease in ethanolamine plasmalogen levels could be the result of increased choline plasmalogen levels.

Lastly, expression of either the 13.2 kDa SCP-2 or 15 kDa proSCP-2 caused significant changes in the phospholipid fatty acid composition (Tables 3, 4, 5, and 6). In general, the effect of SCP-2 expression on phospholipid fatty acids was a decrease in saturated fatty acids and an increase in PUFA. This included increased proportions (mole %) of 20:4 n–6, 22:4 n–6, and 22:6 n–3. These results were consistent with the known ability of SCP-2 to bind both fatty acids (21, 25, 26) and fatty acyl-CoA (21, 29, 30) in that SCP-2 may facilitate interactions of fatty acids and fatty acyl-CoA with CoA-dependent and CoA-independent acyltransferases (61). Within the cellular milieu, SCP-2 may exhibit preferential binding for PUFA over saturated fatty acids. Indeed, comparison of 18 carbon fatty acyl CoA indicates that SCP-2 preferentially binds unsaturated over saturated fatty acyl CoA (29). Further, SCP-2 could also facilitate the delivery of fatty acids to the elongases / desaturases that function to form PUFA from n–3 and n–6 precursors (62). Hence, SCP-2 expression increased the proportions of PUFA and decreased the proportions of saturated fatty acids esterified onto the major phospholipids.

In summary, we report for the first time that expression of either the 13.2 or 15 kDa forms of SCP-2 markedly reduced phospholipid mass in L-cell fibroblasts. We speculate that the underlying mechanisms for this reduction were specific changes in Kennedy pathway enzymes. The lack of a change in CerPCho supports this possibility as well as the lack of an increase in lysophospholipids which suggest that an increase in acylhydrolase activity did not occur. The alteration in plasmalogen levels could be the result of either altered peroxisomal or microsomal function; however, the increased proportions of 22:6 n–3 in the phospholipids suggest that abolition of peroxisomal activity did not occur. More than likely this change in ethanolamine plasmalogen levels was accounted for by an increase in choline plasmalogen levels. The general increase in PUFA and decrease in saturated fatty acid esterified onto the phospholipids may be the result of SCP-2 facilitating the acylation of PUFA or elongation / desaturation of long-chain PUFA precursors. Finally, SCP-2 expression dramatically altered the content of PtdIns, plasmalogens, and PUFA, suggesting that this protein may mediate multiple aspects of lipid metabolism which ultimately affect cellular lipid-mediated signaling pathways. Future experiments will be needed to resolve these points.

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REFERENCES

1. Murphy, E. J., and F. Schroeder. 1997. Sterol carrier protein-2-mediated cholesterol esterification in transfected L-cell fibroblasts. *Biochim. Biophys. Acta.* **1345:** 283–292.
2. Moncecchi, D., E. J. Murphy, D. R. Prows, and F. Schroeder. 1996. Sterol carrier protein-2 expression in mouse L-cell fibroblasts alters cholesterol uptake. *Biochim. Biophys. Acta.* **1302:** 110–116.
3. Schroeder, F., A. Frolov, J. Schoer, A. Gallegos, B. P. Atshaves, N. J.

Stolwich, A. I. Scott, and A. B. Kier. 1998. Intracellular cholesterol binding proteins, cholesterol transport, and membrane domains. *In* Intracellular Cholesterol Trafficking. D. Freeman and T. Y. Chang, editors. Kluwer Academic Publishers, Boston. 231–234.
4. Ohba, T., H. Rennert, S. M. Pfeifer, Z. He, R. Yamamoto, J. A. Holt, J. T. Billheimer, and J. F. Strauss III. 1994. The structure of the human sterol carrier protein x/sterol carrier protein 2 gene (SCP2). *Genomics.* **24:** 370–374.
5. Seedorf, U., and G. Assmann. 1991. Cloning, expression, and nucleotide sequence of rat liver sterol carrier protein 2 cDNAs. *J. Biol. Chem.* **266:** 630–636.
6. Moncecchi, D., A. Pastuszyn, and T. J. Scallen. 1991. cDNA sequence and bacterial expression of mouse liver sterol carrier protein-2. *J. Biol. Chem.* **266:** 9885–9892.
7. Yamamoto, R., C. B. Kallen, G. O. Babalola, H. Rennert, J. T. Billheimer, and J. F. Strauss III. 1991. Cloning and expression of a cDNA encoding human sterol carrier protein 2. *Proc. Natl. Acad. Sci. USA.* **88:** 463–467.
8. Ossendorp, B. C., G. P. H. van Heusden, and K. W. A. Wirtz. 1990. The amino acid sequence of rat liver non-specific lipid transfer protein (sterol carrier protein 2) is present in a high molecular weight protein: evidence from cDNA analysis. *Biochem. Biophys. Res. Commun.* **168:** 631–636.
9. Matsuura, J. E., H. J. George, N. Ramachandran, J. G. Alvarez, J. F. Strauss III, and J. T. Billheimer. 1993. Expression of the mature and the pro-form of human sterol carrier protein 2 in *Escherichia coli* alters bacterial lipids. *Biochemistry.* **32:** 567–572.
10. Seedorf, U., S. Scheek, T. Engel, C. Steif, H-J. Hinz, and G. Assmann. 1994. Structure–activity studies on human sterol carrier protein 2. *J. Biol. Chem.* **269:** 2613–2618.
11. van Heusden, G. P. H., J. Souren, M. J. H. Geelen, and K. W. A. Wirtz. 1985. The synthesis and esterification of cholesterol by hepatocytes and H35 hepatoma cells are independent of the level of nonspecific lipid transfer protein. *Biochim. Biophys. Acta.* **846:** 21–25.
12. Johnson, W. J., and M. P. Reinhart. 1994. Lack of requirement for sterol carrier protein-2 in the intracellular trafficking of lysosomal cholesterol. *J. Lipid Res.* **35:** 563–573.
13. van Heusden, G. P. H., K. Bos, C. R. H. Raetz, and K. W. A. Wirtz. 1990. Chinese hamster ovary cells deficient in peroxisomes lack the nonspecific lipid transfer protein (sterol carrier protein 2). *J. Biol. Chem.* **265:** 4105–4110.
14. Keller, G. A., T. J. Scallen, D. Clarke, P. A. Maher, S. K. Krisans, and S. J. Singer. 1989. Subcellular localization of sterol carrier protein-2 in rat hepatocytes: its primary localization to peroxisomes. *J. Cell Biol.* **108:** 1353–1361.
15. Conneely, O. M., D. R. Headon, C. D. Olson, F. Ungar, and M. E. Dempsey. 1984. Intramitochondrial movement of adrenal sterol carrier protein with cholesterol in response to corticotropin. *Proc. Natl. Acad. Sci. USA.* **81:** 2970–2974.
16. Vahouny, G. V., P. Dennis, R. Chanderbhan, G. Fiskum, B. J. Noland, and T. J. Scallen. 1984. Sterol carrier protein 2 (SCP2)-mediated transfer of cholesterol to mitochondrial inner membranes. *Biochem. Biophys. Res. Commun.* **122:** 509–515.
17. Van der Krift, T. P., J. Leunissen, T. Teerlink, G. P. H. van Heusden, A. J. Verkleij, and K. W. A. Wirtz. 1985. Ultrastructural localization of a peroxisomal protein in rat liver using the specific antibody against the non-specific lipid transfer protein (sterol carrier protein 2). *Biochim. Biophys. Acta.* **812:** 387–392.
18. Atshaves, B. P., A. D. Petrescu, O. Starodub, J. B. Roths, A. B. Kier, and F. Schroeder. 1999. Expression and intracellular processing of the 58 kDa sterol carrier protein-2/3-oxoacyl-CoA thiolase in transfected mouse L-cell fibroblasts. *J. Lipid Res.* **40:** 610–622.
19. Colles, S. M., J. K. Woodford, D. Moncecchi, S. C. Myers-Payne, L. R. McLean, J. T. Billheimer, and F. Schroeder. 1995. Cholesterol interaction with recombinant human sterol carrier protein-2. *Lipids.* **30:** 795–803.
20. Gadella, T. W. J., Jr., P. I. H. Bastiaens, A. J. W. G. Visser, and K. W. A. Wirtz. 1991. Shape and lipid-binding site of the nonspecific lipid-transfer protein (sterol carrier protein 2): a steady-state and time-resolved fluorescence study. *Biochemistry.* **30:** 5555–5564.
21. Stolwich, N. J., A. Frolov, A. Petrescu, A. I. Scott, J. T. Billheimer, and F. Schroeder. 1999. Holo-sterol carrier protein-2: ^{13}C-NMR investigation of cholesterol and fatty acid binding sites. *J. Biol. Chem.* **274:** 35425–35433.
22. van Amerongen, A., R. A. Demel, J. Westerman, and K. W. A. Wirtz. 1989. Transfer of cholesterol and oxysterol derivatives by the nonspecific lipid transfer protein (sterol carrier protein 2): a study on its mode of action. *Biochim. Biophys. Acta.* **1004:** 36–43.
23. Butko, P., I. Hapala, T. J. Scallen, and F. Schroeder. 1990. Acidic phospholipids strikingly potentiate sterol carrier protein 2 mediated intermembrane sterol transfer. *Biochemistry.* **29:** 4070–4077.
24. Frolov, A., J. K. Woodford, E. J. Murphy, J. T. Billheimer, and F. Schroeder. 1996. Spontaneous and protein-mediated sterol transfer between intracellular membranes. *J. Biol. Chem.* **271:** 16075–16083.
25. Schroeder, F., S. C. Myers-Payne, J. T. Billheimer, and W. G. Wood. 1995. Probing the ligand binding sites of fatty acid and sterol carrier proteins: effects of ethanol. *Biochemistry.* **34:** 11919–11927.
26. Stolwich, N. J., A. Frolov, B. Atshaves, E. J. Murphy, C. A. Jolly, J. T. Billheimer, A. I. Scott, and F. Schroeder. 1997. The sterol carrier protein-2 fatty acid binding site: an NMR, circular dichroic and fluorescence spectroscopic determination. *Biochemistry.* **36:** 1719–1729.
27. Avdulov, N. A., S. V. Chochina, U. Igbavboa, C. S. Warden, F. Schroeder, and W. G. Wood. 1999. Lipid binding to sterol carrier protein-2 is inhibited by ethanol. *Biochim. Biophys. Acta.* **1437:** 37–45.
28. Murphy, E. J. 1998. Sterol carrier protein-2 expression increases NBD-stearate uptake and cytoplasmic diffusion in L cells. *Am. J. Physiol.* **275:** G237–G243.
29. Frolov, A., T. H. Cho, J. T. Billheimer, and F. Schroeder. 1996. Sterol carrier protein-2, a new fatty acyl coenzyme A-binding protein. *J. Biol. Chem.* **271:** 31878–31884.
30. Dansen, T. B., J. Westerman, F. S. Wouters, J. R. Wanders, A. van Hoek, T. W. Gadella, Jr., and K. W. Wirtz. 1999. High-affinity binding of very-long-chain fatty acyl-CoA esters to the peroxisomal non-specific lipid-transfer protein (sterol carrier protein-2). *Biochem. J.* **339:** 193–199.
31. Seedorf, U., M. Raabe, P. Ellinghaus, F. Kannenberg, M. Fokker, T. Enge, S. Denis, F. Wouters, K. W. A. Wirtz, R. V. A. Warders, N. Maeda, and G. Assmann. 1998. Defective peroxisomal catabolism of branched chain fatty acyl CoA in mice lacking the sterol carrier protein-2/sterol carrier protein-x gene function. *Genes Dev.* **12:** 1189–1201.
32. Higuchi, K. 1970. An improved chemically defined culture medium for strain L mouse cells based on growth responses to graded levels of nutrients including iron and zinc. *J. Cell Physiol.* **75:** 65–72.
33. Hara, A., and N. S. Radin. 1978. Lipid extraction of tissues with a low-toxicity solvent. *Anal. Biochem.* **90:** 420–426.
34. Murphy, E. J., T. A. Rosenberger, and L. A. Horrocks. 1997. Effects of maturation on the phospholipid and phospholipid fatty acid compositions in primary rat cortical astrocyte cell cultures. *Neurochem. Res.* **22:** 1205–1213.
35. Demediuk, P., D. K. Anderson, L. A. Horrocks, and E. D. Means. 1985. Mechanical damage to murine neuronal-enriched cultures during harvesting: effects on free fatty acids, diglycerides, Na^+,K^+-ATPase, and lipid peroxidation. *In Vitro Cell. Dev. Biol.* **21:** 569–574.
36. Dugan, L. L., P. Demediuk, C. E. Pendley II, and L. A. Horrocks. 1986. Separation of phospholipids by high pressure liquid chromatography: all major classes including ethanolamine and choline plasmalogens, and most minor classes, including lysophosphatidylethanolamine. *J. Chromatogr.* **378:** 317–327.
37. Rouser, G., A. Siakotos, and S. Fleischer. 1969. Quantitative analysis of phospholipids by thin-layer chromatography and phosphorus analysis of spots. *Lipids.* **1:** 85–86.
38. Murphy, E. J., R. Stephens, M. Jurkowitz-Alexander, and L. A. Horrocks. 1993. Acidic hydrolysis of plasmalogens followed by high-performance liquid chromatography. *Lipids.* **28:** 565–568.
39. Nakagawa, Y., and K. Waku. 1988. Phospholipids. *In* Neuromethods 7. Lipids and Related Compounds. A. A. Boulton, G. B. Baker, and L. A. Horrocks, editors. Humana Press, Clifton, NJ. 149–178.
40. Jones, M., R. W. Keenan, and P. Horowitz. 1982. Use of 6-p-toluidino-2-naphthalenesulfonic acid to quantitate lipids after thin-layer chromatography. *J. Chromatogr.* **237:** 522–524.
41. Brockerhoff, H. 1975. Determination of the positional distribution of fatty acids in glycerolipids. *Methods Enzymol.* **35**: 315–325.
42. Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:** 248–254.
43. Hajra, A. K., C. L. Burke, and C. L. Jones. 1979. Subcellular localization of acyl coenzyme A: dihydroxyacetone phosphate acyltrans-

ferase in rat liver peroxisomes (microbodies). *J. Biol. Chem.* **254:** 10896–10900.
44. Singh, H., K. Beckman, and A. Poulos. 1993. Exclusive localization in peroxisomes of dihydroxyacetone phosphate acyltransferase and alkyl-dihydroxyacetone phosphate synthase in rat liver. *J. Lipid Res.* **34:** 467–477.
45. Hajra, A. K., and J. E. Bishop. 1992. Glycerolipid biosynthesis in peroxisomes via the acyl dihydroxyacetone phosphate pathway. *Ann. NY Acad. Sci.* **386:** 170–182.
46. van den Bosch, H., R. B. H. Schutgens, R. J. A. Wanders, and J. M. Tager. 1992. Biochemistry of peroxisomes. *Annu. Rev. Biochem.* **61:** 157–197.
47. van den Bosch, H., G. Schrakamp, D. Hardeman, A. W. M. Zomer, R. J. A. Wanders, and R. B. H. Schutgens. 1993. Ether lipid synthesis and its deficiency in peroxisomal disorders. *Biochimie* **75:** 183–189.
48. Horrocks, L. A., H. W. Harder, R. Mozzi, G. Goracci, E. Francescangeli, S. Porcellati, and G. G. Nenci. 1986. Receptor mediated degradation of choline plasmalogen and glycerophospholipid methylation: a new hypothesis. *In* Enzymes of Lipid Metabolism. Vol. 2. L. Freysz, H. Dreyfus, R. Massarelli, and S. Gatt, editors. Plenum Press, New York. 707–711.
49. Horrocks, L. A., Y. K. Yeo, H. W. Harder, R. Mozzi, and G. Goracci. 1986. Choline plasmalogens, glycerophospholipid methylation, and receptor-mediated activation of adenylate cyclase. *In* Advances in Cyclic Nucleotide Protein Phosphorylation Research. Vol. 20. P. Greengard and G. A. Robinson, editors. Raven Press, New York. 263–292.
50. McHowat, J., and S. Liu. 1997. Interleukin-1β stimulates phospholipase A_2 activity in adult rat ventricular myocytes. *Am. J. Physiol.* **272:** C450–C456.
51. McHowat, J., S. Liu, and M. H. Creer. 1998. Selective hydrolysis of plasmalogen phospholipids by Ca^{2+}-independent PLA_2 in hypoxic ventricular myocytes. *Am. J. Physiol.* **274:** C1727–C1737.
52. Frolov, A. A., J. K. Woodford, E. J. Murphy, J. T. Billheimer, and F. Schroeder. 1996. Fibroblast membrane sterol kinetic domains: modulation by sterol carrier protein-2 and liver fatty acid binding protein. *J. Lipid Res.* **37:** 1862–1874.
53. van den Bosch, H., and D. E. Vance. 1997. Editorial. *Biochim. Biophys. Acta.* **1348:** 1–2.
54. Thompson, G. A., Jr. 1973. Phospholipid metabolism in animal tissues. *In* Form and Function of Phospholipids. (B.B.A. Library, Vol. 3). G. B. Ansell, J. N. Hawthorne, and R. M. C. Dawson, editors. Elsevier Scientific Publishing Co., Amsterdam. 67–96.
55. Kuge, O., and M. Nishijima. 1997. Phosphatidylserine synthase I and II of mammalian cells. *Biochim. Biophys. Acta.* **1348:** 151–156.
56. Antonsson, B. 1997. Phosphatidylinositol synthase from mammalian tissues. *Biochim. Biophys. Acta.* **1348:** 179–186.
57. Heacock, A. M., and B. W. Agranoff. 1997. CDP-diacylglycerol synthase from mammalian tissues. *Biochim. Biophys. Acta* **1348:** 166–172.
58. Sprecher, H., Q. Chen, and F. Q. Yin. 1999. Regulation of the biosynthesis of 22:5 n–6 and 22:6 n–3: a complex intracellular process. *Lipids.* **34:** S153–S156.
59. Radominska-Pyrek, A., J. Strosznajder, Z. Dabrowiecki, G. Goracci, T. Chojnacki, and L. A. Horrocks. 1977. Enzymic synthesis of ether types of choline and ethanolamine phosphoglycerides by microsomal fractions from rat brain and liver. *J. Lipid Res.* **18:** 53–58.
60. Mozzi, R., D. Gramignani, C. Andriamampandr, L. Freysz, and R. Massarelli. 1989. Choline plasmalogen synthesis by the methylation pathway in chick neurons in culture. *Neurochem. Res.* **14:** 579–583.
61. Yamashita, A., T. Sugiura, and K. Waku. 1997. Acyltransferases and transacylases involved in fatty acid remodeling of phospholipids and metabolism of bioactive lipids in mammalian cells. *J. Biochem.* **122:** 1–16.
62. Los, D. A., and N. Murata. 1998. Structure and expression of fatty acid desaturases. *Biochim. Biophys. Acta.* **1394:** 3–15.