Liver and Intestinal Fatty Acid-Binding Protein Expression Increases Phospholipid Content and Alters Phospholipid Fatty Acid Composition in L-Cell Fibroblasts

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ABSTRACT: Although fatty acid-binding proteins (FABP) differentially affect fatty acid uptake, nothing is known regarding their role(s) in determining cellular phospholipid levels and phospholipid fatty acid composition. The effects of liver (L)- and intestinal (I)-FABP expression on these parameters were determined using stably transfected L-cells. Expression of L- and I-FABP increased cellular total phospholipid mass (nmol/mg protein) 1.7- and 1.3-fold relative to controls, respectively. L-FABP expression increased the masses of choline glycerophospholipids (ChoGpl) 1.5-fold, phosphatidylserine (PtdSer) 5.6-fold, ethanolamine glycerophospholipids 1.4-fold, sphingomyelin 1.7-fold, and phosphatidylinositol 2.6-fold. In contrast, I-FABP expression only increased the masses of ChoGpl and PtdSer, 1.2- and 3.1-fold, respectively. Surprisingly, both L- and I-FABP expression increased ethanolamine plasmalogen mass 1.6- and 1.1-fold, respectively, while choline plasmalogen mass was increased 2.3- and 1.7-fold, respectively. The increase in phospholipid levels resulted in dramatic 48 and 33% decreases in the cholesterol-to-phospholipid ratio in L- and I-FABP expressing cells, respectively. L-FABP expression generally increased polyunsaturated fatty acids, primarily by increasing 20:4n-6 and 22:6n-3, while decreasing 18:1n-9 and 16:1n-7. I-FABP expression generally increased only 20:4n-6 proportions. Hence, expression of both I- and L-FABP differentially affected phospholipid mass, class composition, and acyl chain composition. Although both proteins enhanced phospholipid synthesis, the effect of L-FABP was much greater, consistent with previous work suggesting that these two FABP differentially affect lipid metabolism.

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The cytosolic fatty acid-binding protein (FABP) superfamily is comprised of immunologically distinct proteins that share similar structures, overlapping ligand specificity, and considerable sequence homology. Why some tissues and cell types contain multiple FABP is not known. For example, intestinal-FABP (I-FABP), a 15.1 kDa cytosolic protein, is found in the columnar epithelial cells of the small intestine, where it constitutes 2–4% of the total cytosolic protein (1). I-FABP expression also varies depending upon developmental stage, diet, and position of the cells along the longitudinal axis of the intestinal tract (2). Liver-FABP (L-FABP), a 14.2 kDa protein, is also found in intestinal enterocytes, where it accounts for 2% of the total cytosolic protein, as well as in the liver, where it accounts for 3–5% of the total cytosolic protein (2–4). The N-terminus of both proteins is blocked by acetylation consistent with the intracellular localization (5). I- and L-FABP have a similar affinity for fatty acids (3,6,7), although some studies suggest that L-FABP has a greater affinity for polyunsaturated fatty acids (PUFA), such as arachidonic acid, than I-FABP (8). This is consistent with our finding that endogenous L-FABP isolated from rat liver contains a substantial quantity of PUFA (9). Furthermore, I- and L-FABP not only bind fatty acids but also are thought to bind long-chain fatty acyl CoA with a high affinity (10–12). In addition, L-FABP binds a number of lipophilic ligands including prostaglandins (13), hydroperoxy- and hydroxyeicosatetraenoic acids (14), heme (15), and warfarin (16). Although suggestive of function, ligand binding alone has not allowed discrimination of the respective physiological role(s) for the different FABP in altering cellular lipid metabolism.

To better assess the physiological function of these FABP in cells, L-FABP (17,18) and I-FABP (19) were stably expressed in L-cell fibroblasts. In these cells, L-FABP (18,20) but not I-FABP expression (19–21) increased fatty acid uptake, whereas both proteins increased the apparent fatty acid cytoplasmic diffusion coefficient (20). A recent study in murine stem cells suggests that the cellular differentiation state affects I-FABP-induced fatty acid uptake, with I-FABP expression enhancing fatty acid uptake only in undifferentiated cells (22). Furthermore, expression of L-FABP or I-FABP dif-

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Abbreviations: CerPCho, sphingomyelin; ChoGpl, choline glycerophospholipid; ER, endoplasmic reticulum; EtnGpl, ethanolamine glycerophospholipid; FABP, fatty acid binding protein; FAME, fatty acid methyl ester; GLC, gas–liquid chromatography; HPLC, high-performance liquid chromatography; I-FABP, intestinal-FABP; L-FABP, liver-FABP; MUFA, monounsaturated fatty acid; PlsCho, choline plasmalogen; PlsEtn, ethanolamine plasmalogen; PtdEtn, phosphatidylethanolamine; PtdIns, phosphatidylinositol; PtdOH, phosphatidic acid; PtdSer, phosphatidylserine; PUFA, polyunsaturated fatty acid; TLC, thin-layer chromatography.

ferentially affected targeting of exogenous fatty acids for esterification into specific lipid pools (18,19,21). This enhancement of fatty acid targeting is supported by studies showing that both L-FABP (10,23,24) and I-FABP (24) stimulate phosphatidic acid (PtdOH) synthesis *in vitro*, although L-FABP has a more robust effect on synthesis than I-FABP (24). In other studies, expression of adipocyte-FABP in Chinese hamster ovary cells enhanced fatty acid uptake and esterification into cellular lipids 1.5- and 2.0-fold, respectively, compared to nontransfected cells (25). Expression of heart-FABP in a human breast cancer cell line increased fatty acid uptake nearly 1.7-fold compared to control cells but did not increase the targeting of fatty acids to either neutral lipids or phospholipids (26). Thus, studies using transfected cell lines suggest that FABP differentially affect fatty acid uptake, cytoplasmic diffusion, and targeting (18–22).

Despite these findings, whether L-FABP and I-FABP expression differentially affects phospholipid mass, class composition, or phospholipid fatty acid composition is not known. We report that L-FABP, and to a lesser extent I-FABP, expression differentially increased L-cell phospholipid mass, including plasmalogen mass, resulting in a dramatic decrease in the cholesterol-to-phospholipid ratio. Likewise, L-FABP, and to a lesser extent I-FABP, expression altered phospholipid fatty acid composition, suggesting that these proteins not only enhance phospholipid synthesis but also can modulate fatty acyl chain composition.

MATERIALS AND METHODS

Cells. Murine L-cell fibroblasts (L arpt⁻tk⁻) were stably transfected with the cDNA encoding for either I-FABP (19) or L-FABP (17,18). The transfected cells express these proteins at similar levels as determined by quantitative Western blotting (17–19). Control and transfected cells were grown to confluency in Higuchi medium containing 10% fetal bovine serum (Hyclone, Logan, UT) (27).

Lipid extraction. Lipids were extracted from confluent control and transfected cells using *n*-hexane/2-propanol (3:2 vol/vol) (28,29). Before extraction, the cell culture medium was removed and the cells were washed twice with ice-cold phosphate-buffered saline. Following removal of the last wash, the cell plate was floated on liquid N_2 to minimize acylhydrolase activity during cell removal (30). The cell plate was removed from the liquid N_2, 2 mL of 2-propanol was added, and the cells were removed from the plate by scraping. The 2-propanol 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 transferred to the tube containing the *n*-hexane, resulting in *n*-hexane/2-propanol (3:2 vol/vol).

Cell extracts were centrifuged at 2,500 rpm to pellet the denatured protein and other cellular debris. The lipid-containing organic phase was decanted and stored under a $N_{2(g)}$ atmosphere at –80°C until analysis. These storage conditions limit oxidation of lipids, including PUFA, as demonstrated previously (29,31–33). The residual protein pellet was dried overnight at room temperature.

Phospholipid separation. Before separating the phospholipid classes by high-performance liquid chromatography (HPLC), the sample volume was reduced under a stream of $N_{2(g)}$ 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 vol/vol).

The HPLC system consisted of a Beckman 125 pump module, a Beckman 166 UV/Vis detector (Fullerton, CA), and 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 eluant absorbance was monitored at 205 nm.

The phospholipids were separated using a binary gradient of (A) *n*-hexane/2-propanol (3:2 vol/vol) and (B) *n*-hexane/2-propanol/water(56.7:37.8:5.5 by vol). 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 phospholipids including phosphatidylserine (PtdSer) and phosphatidylinositol (PtdIns) (34). The ethanolamine glycerophospholipid (EtnGpl) and choline glycerophospholipid (ChoGpl) fractions were quantitatively divided into two equal parts, one of which was used to quantify phospholipid mass by assaying lipid phosphorus (35). The other half was dried under $N_{2(g)}$ and exposed to HCl vapor for 15 min to cleave the vinyl ether linkage of the plasmalogen subclasses (36). The latter fractions were re-separated by HPLC and the glycerophospholipid and lysophospholipid fractions collected and quantified by assaying for lipid phosphorus (35). All other phospholipid fractions were also quantified by analysis of lipid phosphorus (35).

The neutral lipid fraction from the phospholipid separation was saved and separated using a binary solvent system consisting of *n*-hexane/2-propanol/acetic acid (98.7:1.2:0.1) and *n*-hexane (18,37). The column used was a Selectosil column (5 μm, 4.6 × 250 mm) maintained at 55°C. Data were collected using the Dionex UI-120 (Sunnyvale, CA) analog-to-digital interface. The unesterified cholesterol mass was calculated by converting absorbance at 205 nm from peak area to mass using a standard curve.

Thin-layer chromatography (TLC). Phospholipid fatty acid composition was analyzed in individual phospholipids separated by TLC. Silica gel G plates (Analtech, Newark, DE) were heat-activated at 110°C for 1 h and samples streaked onto the plates. The developing solvent was chloroform/methanol/water (65:25:4 by vol). This solvent system separates the PtdIns from the PtdSer as well as the ChoGpl from the sphingomyelin (CerPCho) (38). Bands were visualized using 1 mM 6-*p*-toluidino-2-naphthalene sulfonic acid dissolved in 50 mM Tris (pH 7.4) (39). 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, which were subjected to base-catalyzed transesterification, converting the phospholipid acyl chains to fatty acid methyl esters (FAME) (40). This method

of transesterification avoids formation of dimethylacetals and oxidative side reactions common with acid-catalyzed methods. FAME were extracted from the methanol by using 2 mL of *n*-hexane, and the *n*-hexane 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 (GLC). FAME were separated by GLC and quantified using flame-ionization detection. Individual fatty acids were identified using FAME standards (Nu-Chek-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 i.d. × 30 m length, Supelco, Bellefonte, 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.

Protein assay. Proteins were measured using a modified dye-binding assay (41). The dried protein residue from the extracts was digested overnight in 0.2 M KOH at 65°C. Following 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 by a one-way analysis of variance and Tukey-Kramer multiple comparisons posttest 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 L- and I-FABP expression on total cellular phospholipid mass (nmol/mg protein) was determined in L-cells expressing L-FABP and I-FABP at similar levels. Total phospholipid mass was increased from 266 ± 53 in control cells to 452 ± 26 and 343 ± 23 for L-FABP- and I-FABP-expressing cells, respectively. Values represent means ± SD, $n = 4$. I-FABP expression increased total cellular phospholipid mass 1.3-fold compared to control cells, but this increase was significantly less than the 1.7-fold increase observed with L-FABP expression. Thus, FABP expression differentially affected phospholipid mass in L-cells.

Endoplasmic reticulum (ER)-synthesized individual phospholipid class mass. Changes in total cellular phospholipid mass are not indicative of changes in the mass of individual phospholipid classes. To assess the effects of I- and L-FABP expression on the mass of individual phospholipids formed in the ER, phospholipids were resolved using HPLC and their individual masses calculated. In the I-FABP-expressing cells, the masses of PtdSer and ChoGpl were elevated 3.1- and 1.2-fold, respectively, compared to the control (Table 1). In contrast to I-FABP-expressing cells, L-FABP expression increased the mass of every major phospholipid class relative to control cells, but the magnitudes of these changes were greater than those for I-FABP-expression. In L-FABP-expressing cells, EtnGpl mass was increased 1.4-fold, ChoGpl mass was elevated 1.5-fold compared to control, whereas PtdIns and PtdSer masses were increased 2.6- and 5.6-fold, respectively, compared to the control. Relative to I-FABP-expressing cells, PtdIns and PtdSer masses were increased 3.3- and 1.8-fold in L-FABP-expressing cells, respectively. Lastly, in L-FABP expressing cells, CerPCho mass was increased 1.7- and 1.5-fold relative to control and I-FABP-expressing cells, respectively. Thus, L-FABP expression increased phospholipid mass in L-cells to a much greater extent than I-FABP expression, indicating that these two FABP had differential effects on phospholipid metabolism.

Peroxisomal and ER-synthesized plasmalogen mass. The effects of L- and I-FABP expression on plasmalogen levels were determined. [Plasmalogens are phospholipids containing a vinyl ether linkage in the *sn*-1 position that are synthesized by steps involving both peroxisomes and ER; they have a role in lipid-mediated signal transduction (42–45)]. In I-FABP-expressing cells, ethanolamine plasmalogen (PlsEtn)

TABLE 1
Effect of I- and L-FABP Expression on Phospholipid Mass and Composition in L-cells[a]

	Phospholipid mass (nmol/mg protein)			Phospholipid composition (mol%)		
Phospholipid class	Control	I-FABP	L-FABP	Control	I-FABP	L-FABP
EtnGpl	72.5 ± 8.3	79.5 ± 11.2	101.1 ± 4.7*,**	25.6 ± 1.5	23.2 ± 3.3	22.3 ± 1.0
LysoPtdEtn	5.1 ± 1.9	7.4 ± 1.6	5.3 ± 2.8	1.8 ± 1.6	1.9 ± 0.8	1.5 ± 0.6
PtdIns	10.9 ± 3.3	8.7 ± 4.7	28.6 ± 3.7*,**	3.8 ± 0.9	2.5 ± 1.4	6.3 ± 0.8*,**
PtdSer	4.4 ± 1.3	13.6 ± 4.0*	24.6 ± 3.8*,**	1.6 ± 0.5	3.9 ± 1.1	5.5 ± 0.8*
ChoGpl	153.9 ± 9.4	193.5 ± 7.9*	231.2 ± 14.3*,**	54.5 ± 0.7	56.4 ± 2.3	51.1 ± 3.2*,**
CerPCho	31.0 ± 2.5	35.7 ± 7.9	53.8 ± 8.8*,**	11.1 ± 1.5	10.4 ± 2.2	11.9 ± 1.9
LysoPtdCho	4.6 ± 1.9	5.0 ± 2.9	6.2 ± 1.4	1.6 ± 0.6	1.5 ± 0.9	1.4 ± 0.3

[a]Values are expressed as means ± SD, $n = 4$. A single asterisk (*) indicates significantly different from the control; a double asterisk (**) indicates significantly different from I-FABP-expressing cells, $P < 0.05$. Abbreviations: I-FABP, intestinal fatty acid-binding protein; L-FABP, liver fatty acid-binding protein; EtnGpl, ethanolamine glycerophospholipid; lysoPtdEtn, lysophosphatidylethanolamine; PtdIns, phosphatidylinositol; PtdSer, phosphatidylserine; ChoGpl, choline glycerophospholipid CerPCho, sphingomyelin; lysoPtdCho, lysophosphatidylcholine.

mass was increased 1.1-fold in these cells (Table 2). In contrast, PlsEtn mass was increased in L-FABP-expressing cells 1.6- and 1.4-fold compared to the control and I-FABP-expressing cells, respectively. Similarly, the choline plasmalogen (PlsCho) levels were also differentially affected by I-FABP and L-FABP expression. In I-FABP-expressing cells, the acid-labile ChoGpl, PlsCho, was increased 1.7-fold relative to the control. However, L-FABP expression increased PlsCho mass 2.3- and 1.3-fold compared to the control and I-FABP-expressing cells, respectively. These data also indicate that L-FABP, and to a lesser extent I-FABP, expression increased both the EtnGpl and ChoGpl acid-stable fractions, which contain mainly the diacyl phosphatidyl fraction. Thus, both I- and L-FABP expression increased PlsEtn and PlsCho levels, although only L-FABP expression appeared specifically to affect PlsEtn formation.

Phospholipid class composition. Although both L- and I-FABP expression dramatically increased total phospholipid mass and differentially affected the masses of individual phospholipid classes, these data do not provide information regarding the relative distribution of the individual phospholipids. Therefore, the effect of L- and I-FABP expression on L-cell phospholipid composition was determined from the phospholipid mass data in Table 1. Expression of I-FABP had no effect on the phospholipid percentage composition compared to control cells (Table 1). In contrast, L-FABP expression produced marked changes in phospholipid percentage composition relative to both control and I-FABP-expressing cells (Table 1). PtdSer and PtdIns proportions were increased 3.4- and 1.7-fold, respectively, compared to the control. Both PtdIns and ChoGpl proportions were altered with respect to I-FABP-expressing cells, with the ChoGpl proportion decreased in L-FABP-expressing cells by 10% and the PtdIns increased 2.5-fold. ChoGpl proportions were decreased 6% in L-FABP expressing cells relative to control.

L- and I-FABP expression also differentially affected ChoGpl and EtnGpl subclass composition (Table 2). L-FABP expression increased the proportion of acid-labile EtnGpl, PlsEtn, relative to the acid-stable fraction consisting primarily of phosphatidylethanolamine (PtdEtn) (Table 2). As such, the proportion of the acid-stable fraction was also significantly reduced compared to either control or I-FABP-expressing cells. In contrast, I-FABP and L-FABP expression increased the proportion of acid-labile ChoGpl, PlsCho, compared to the control. Hence, L-FABP expression differentially affected phospholipid composition in L-cells relative to I-FABP and control cells, suggesting a specific increase in PtdSer and PtdIns relative to the other phospholipids.

Free cholesterol to phospholipid ratio. Earlier studies from this laboratory demonstrated a change in the biophysical membrane dynamics in L-FABP-expressing cells (17,20,46), although such data are limited for I-FABP expressing cells (20). Because of the large changes in phospholipid mass in these FABP-expressing cells, the cholesterol-to-phospholipid ratio was determined. Unesterified (free) cholesterol values (nmol/mg protein) were 73 ± 5, 69 ± 11, and 65 ± 6 for control, L-FABP-, and I-FABP-expressing cells, respectively. The cholesterol- to-phospholipid ratio was 0.28 ± 0.02, 0.15 ± 0.02, and 0.19 ± 0.02 for the control, L-FABP-, and I-FABP-expressing cells, respectively. In I-FABP-expressing cells, the cholesterol-to-phospholipid ratio was significantly decreased, 32% compared to the control, whereas in L-FABP-expressing cells this significant reduction was nearly 50%. Furthermore, similar to the effects on phospholipid metabolism, the extent of the decrease in the cholesterol-to-phospholipid ratio was significantly greater in L-FABP-expressing cells than I-FABP-expressing cells. Because there was little change in the free cholesterol levels, the change in the cholesterol-to-phospholipid ratio was primarily the result of increased phospholipid mass. Nonetheless, these results indicate that this ratio, which is a major determinant of membrane structure, was decreased in L- and I-FABP-expressing cells. These results are consistent with previous reports of increased membrane fluidity in L-FABP-expressing cells (17,20,46).

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 effect of L- and I-FABP expression on the phospholipid fatty acid composition was determined for the EtnGpl, ChoGpl, PtdIns, and PtdSer (Tables 3–6) in L-FABP-expressing, I-FABP-expressing, and control L-cells.

For EtnGpl, expression of either protein increased the mole percentage of 18:0 and 20:3n-6 1.3- and 1.9-fold, respectively, whereas only L-FABP expression increased the mole percentage of 22:6n-3 (Table 3). Because of the limited increase in PUFA, there was no significant change in the PUFA/saturated fatty acid index, and there was a decrease in the unsaturated/saturated fatty acid index in both L- and I-FABP-expressing cells. Hence, the net result of either L- or I-

TABLE 2
Effect of I- and L-FABP Expression on Plasmalogen Composition and Mass in L-Cells[a]

Phospholipid class		Composition of glycerophospholipid class (mol%)			Mass of glycerophospholipid subclass (nmol/mg protein)		
		Control	I-FABP	L-FABP	Control	I-FABP	L-FABP
Etn	acid stable	64.4 ± 0.6	62.7 ± 1.1	59.3 ± 1.8*,**	41.2 ± 0.4	49.9 ± 0.9*	60.0 ± 1.1*,**
	acid labile	35.6 ± 0.6	37.3 ± 1.1	40.7 ± 1.8*,**	22.7 ± 0.4	29.6 ± 0.9*	41.1 ± 1.1*,**
Cho	acid stable	91.3 ± 0.9	88.5 ± 2.1*	87.6 ± 1.6*	135.9 ± 1.6	171.1 ± 3.7*	200.9 ± 5.2*,**
	acid labile	8.6 ± 0.9	11.6 ± 1.9*	12.4 ± 1.6*	13.0 ± 1.6	22.4 ± 3.7*	30.3 ± 5.3*,**

[a]Values are expressed as means ± SD, $n = 4$. A single asterisk (*) indicates significantly different from the control; a double asterisk (**) indicates significantly different from I-FABP-expressing cells, $P < 0.05$. Etn, ethanolamine; Cho, choline; for other abbreviations see Table 1.

TABLE 3
Effect of I- and L-FABP Expression on EtnGpl Fatty Acid Composition

Fatty acid	Control	L-FABP expressors	I-FABP expressors
16:0	5.78 ± 0.55	4.65 ± 0.74	6.51 ± 0.66
16:1	1.58 ± 0.09	0.34 ± 0.12*	0.63 ± 0.43*
18:0	22.25 ± 3.69	28.71 ± 1.02*	28.21 ± 1.93*
18:1n-9	42.25 ± 5.72	37.08 ± 1.23	39.49 ± 1.26
18:2n-6	2.85 ± 0.59	2.90 ± 0.39	3.34 ± 0.38
18:3n-6	0.35 ± 0.19	0.11 ± 0.13	0.30 ± 0.25
18:3n-3	0.39 ± 0.04	0.25 ± 0.11	0.12 ± 0.14
20:0	0.50 ± 0.09	0.67 ± 0.13	0.61 ± 0.28
20:1	3.67 ± 0.72	2.14 ± 0.47*	1.41 ± 0.35*
20:2n-6	0.37 ± 0.06	0.21 ± 0.07	0.19 ± 0.16
20:3n-6	0.80 ± 0.03	1.48 ± 0.13*	1.60 ± 0.18*
20:4n-6	9.12 ± 1.80	11.25 ± 0.51	11.11 ± 1.67
22:3n-3	1.33 ± 1.02	0.45 ± 1.01	BLD
22:4n-6	1.74 ± 0.78	1.90 ± 0.14	1.28 ± 0.31
22:6n-3	4.09 ± 1.80	6.56 ± 0.63*	4.73 ± 0.76
24:0	1.09 ± 0.55	0.29 ± 0.40	BLD
Saturated	29.21 ± 2.26	34.92 ± 0.92*	35.72 ± 2.14*
MUFA	47.50 ± 5.70	39.91 ± 1.61*	41.58 ± 2.02
PUFA	21.56 ± 5.53	25.17 ± 0.99	22.67 ± 1.19
n-6	15.08 ± 2.81	17.91 ± 0.36	17.82 ± 1.74
n-3	6.08 ± 2.25	7.26 ± 0.83	4.85 ± 0.70
MUFA/saturated	1.63 ± 0.31	1.14 ± 0.08*	1.17 ± 0.12*
PUFA/saturated	0.74 ± 0.11	0.72 ± 0.03	0.64 ± 0.06
Unsat/saturated	2.40 ± 0.24	0.87 ± 0.08*	1.80 ± 0.16*
n-3/n-6	0.40 ± 0.14	0.41 ± 0.05	0.28 ± 0.06
PUFA/MUFA	0.45 ± 0.18	0.63 ± 0.05	0.55 ± 0.04

[a]Values are mole percentage and represent means ± standard deviation, $n \geq 3$. A single asterisk (*) indicates significantly different from the control, $P < 0.05$. BLD, below limit of detection, MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; for other abbreviations see Table 1.

TABLE 4
Effect of I- and L-FABP Expression on ChoGpl Fatty Acid Composition

Fatty acid	Control	L-FABP expressors	I-FABP expressors
16:0	23.45 ± 1.05	23.36 ± 1.95	23.72 ± 1.28
16:1	6.33 ± 0.29	3.85 ± 0.67*	4.37 ± 0.69*
18:0	15.40 ± 2.21	17.33 ± 0.68	17.62 ± 1.10
18:1n-9	47.41 ± 0.67	44.72 ± 1.24*	44.69 ± 0.60*
18:2n-6	1.85 ± 0.03	2.97 ± 0.13*	2.81 ± 0.22*
18:3n-6	0.25 ± 0.14	0.24 ± 0.12	0.08 ± 0.10
18:3n-3	0.04 ± 0.03	0.09 ± 0.08	BLD
20:0	0.27 ± 0.06	0.34 ± 0.04	0.39 ± 0.10
20:1	1.10 ± 0.22	1.44 ± 0.32	1.48 ± 0.08
20:2n-6	0.12 ± 0.01	0.29 ± 0.03*	0.26 ± 0.07*
20:3n-6	0.35 ± 0.04	0.76 ± 0.05	1.16 ± 0.84
20:4n-6	1.03 ± 0.26	2.52 ± 0.24*,**	1.87 ± 0.40*
22:0	0.19 ± 0.05	0.22 ± 0.03	0.35 ± 0.14
22:3n-3	0.42 ± 0.28	0.15 ± 0.34	BLD
22:4n-6	0.57 ± 0.19	0.32 ± 0.02	0.40 ± 0.29
22:6n-3	0.39 ± 0.24	0.98 ± 0.09*	0.70 ± 0.16
22:5	0.51 ± 0.06	0.26 ± 0.15	BLD
24:1	0.11 ± 0.08	0.03 ± 0.05	BLD
Saturated	39.27 ± 1.22	41.51 ± 1.16*	42.08 ± 0.54*
MUFA	54.96 ± 0.38	50.17 ± 0.67*	50.64 ± 1.21*
PUFA	5.77 ± 0.86	8.32 ± 0.76*	7.28 ± 1.21
n-6	4.71 ± 0.79	7.25 ± 0.63*	6.58 ± 1.08*
n-3	1.06 ± 0.17	1.06 ± 0.17	0.70 ± 0.16
MUFA/saturated	1.40 ± 0.05	1.21 ± 0.05*	1.20 ± 0.04*
PUFA/saturated	0.15 ± 0.02	0.20 ± 0.02*	0.17 ± 0.03
Unsat/saturated	1.55 ± 0.08	1.41 ± 0.07*	1.38 ± 0.03*
n-3/n-6	0.22 ± 0.05	0.15 ± 0.02*	0.11 ± 0.01*
PUFA/MUFA	0.10 ± 0.01	0.17 ± 0.01*	0.14 ± 0.03*

[a]Values are mole percentages and represent means ± standard deviation, $n \geq 3$. A single asterisk (*) indicates significantly different from the control; double asterisk (**) indicates significantly different from I-FABP-expressing cells, $P < 0.05$. For abbreviations see Table 1 and 3.

FABP expression on the EtnGpl fatty acid composition was an increase in saturated fatty acids despite a small, but significant increase in specific PUFA.

Changes observed in ChoGpl were similar to those observed in EtnGpl (Table 4). Of the PUFA, 20:4n-6 and 22:6n-3 mole percentages were increased 2.4- and 2.5-fold in L-FABP- expressing cells, but only the 20:4n-6 mole percentage was elevated in I-FABP-expressing cells. Both proteins significantly elevated the proportion of the n-6 fatty acids relative to the control. Although the percentages of these PUFA were changed in I-FABP-expressing cells, only L-FABP expression significantly elevated PUFA levels and led to an increase in the PUFA/saturated fatty acid index. Because both proteins decreased the mole percentage of 18:1n-9 and 16:1n-7, there was a net decrease in monounsaturated fatty acids (MUFA), resulting in an overall decrease in the unsaturated/saturated fatty acid index. Thus, L- and I-FABP expression altered ChoGpl fatty acid composition, although L-FABP expression appeared to elicit a larger effect.

For PtdSer, expression of either protein had dramatic effects on the fatty acid composition (Table 5). In L-FABP-expressing cells, several PUFA were increased including 2.7-and 1.4-fold increases in 20:3n-6 and 20:4n-6, respectively, relative to the control. These changes, including an increase in 22:6n-3 above the level of detection in L-FABP-expressing cells, resulted in a 2.3-fold increase in PUFA and a 2.4-fold increase in the PUFA/saturated fatty acid index. Even though there is a large decrease in the proportions of 18:1n-9, 16:1n-7, and 18:0 in L-FABP-expressing cells, and to a lesser extent in I-FABP-expressing cells, the 16:0 proportion increased twofold in L-FABP-expressing cells, leaving the overall proportion of saturated fatty acids unchanged in L-FABP-expressing cells relative to control and I-FABP-expressing cells. Thus, in the PtdSer fraction, L-FABP-increased the amount of PUFA, thereby increasing the PUFA/saturated fatty acid index; however, the marked decrease in MUFA left the unsaturated/saturated fatty acid index unchanged. I-FABP expression, on the other hand, had limited effects on PtdSer fatty acid composition, although there was a significant decrease in MUFA/saturated fatty acid index.

L- or I-FABP expression had limited effects on PtdIns fatty acid composition (Table 6). L- and I-FABP expression increased 16:0 proportions nearly 1.8-fold compared to the control. L-FABP-expressing cells had a decreased mole percentage of 18:1n-9; whereas I-FABP-expressing cells had an increase in 18:2n-6 mole percentage. Although the effects by either protein were limited, in both, the overall amount of saturated fatty acids increased, causing a significant decrease in the unsaturated/saturated fatty acid index relative to control.

TABLE 5
Effect of I- and L-FABP Expression on PtdSer Fatty Acid Composition

Fatty acid	Control	L-FABP expressors	I-FABP expressors
16:0	5.02 ± 1.57	10.14 ± 1.07*,**	6.56 ± 0.68
16:1	1.94 ± 0.81	BLD	0.47 ± 0.27*
18:0	50.41 ± 1.86	41.48 ± 2.16*,**	48.27 ± 1.30
18:1n-9	35.97 ± 0.88	27.70 ± 0.54*	30.12 ± 2.30*
18:2n-6	1.93 ± 0.21	3.39 ± 1.30	2.25 ± 0.21
18:3n-6	2.16 ± 0.12	1.27 ± 1.12	0.04 ± 0.10*
20:0	0.50 ± 0.10	1.44 ± 0.23	0.13 ± 0.19
20:1	BLD	1.86 ± 0.33	0.84 ± 0.09
20:2n-6	BLD	1.17 ± 0.30	1.58 ± 1.09
20:3n-6	0.69 ± 0.14	1.90 ± 0.69*,**	0.51 ± 0.24
20:4n-6	1.47 ± 0.51	2.05 ± 0.13**	1.40 ± 0.14**
22:0	BLD	2.69 ± 0.78*	1.75 ± 0.34*
22:1	BLD	0.31 ± 0.30*,**	2.97 ± 0.59*
22:4n-6	BLD	1.38 ± 0.16*,**	BLD
22:6n-3	BLD	3.20 ± 0.30*	3.10 ± 0.49*
Saturated	55.93 ± 0.82	55.75 ± 2.05	58.64 ± 1.61
MUFA	37.71 ± 0.94	29.87 ± 0.26*	32.18 ± 1.99*
PUFA	6.36 ± 0.67	14.38 ± 2.31*,**	9.18 ± 0.86
n-6	6.36 ± 0.67	11.17 ± 2.53*,**	5.94 ± 0.35
n-3	0	3.20 ± 0.30*	3.24 ± 0.56*
MUFA/saturated	0.67 ± 0.02	0.54 ± 0.02*	0.55 ± 0.05*
PUFA/saturated	0.11 ± 0.02	0.26 ± 0.05*,**	0.16 ± 0.01
Unsat/saturated	0.79 ± 0.02	0.80 ± 0.07	0.71 ± 0.05
n-3/n-6	0.00 ± 0.00	0.30 ± 0.10*,**	0.54 ± 0.07*
PUFA/MUFA	0.17 ± 0.02	0.48 ± 0.08*,**	0.29 ± 0.04

[a]Values are mole percentages and represent means ± standard deviation, $n \geq 3$. A single asterisk (*) indicates significantly different from the control; double asterisk (**) indicates significantly different from I-FABP-expressing cells, $P < 0.05$. For abbreviations see Table 1 and 3.

TABLE 6
Effect of I- and L-FABP Expression on PtdIns Fatty Acid Composition

Fatty acid	Control	L-FABP expressors	I-FABP expressors
16:0	4.00 ± 0.69	7.71 ± 1.65*	7.20 ± 0.90*
16:1	0.81 ± 0.52	1.02 ± 0.33	0.67 ± 0.26
18:0	33.94 ± 0.65	34.86 ± 2.20	34.10 ± 1.82
18:1n-9	43.06 ± 4.06	35.28 ± 2.78*	40.93 ± 2.86
18:2n-6	0.92 ± 0.11	1.74 ± 0.50	2.17 ± 0.58*
18:3n-6	0.74 ± 0.68	0.40 ± 0.29	BLD
18:3n-3	0.07 ± 0.09	BLD	BLD
20:0	0.16 ± 0.03	0.48 ± 0.06	0.39 ± 0.10
20:1	1.38 ± 0.28	1.42 ± 0.81	0.96 ± 0.66
20:2n-6	0.37 ± 0.06	0.11 ± 0.21	0.49 ± 0.21
20:3n-6	1.13 ± 0.14	1.77 ± 0.88	1.47 ± 0.10
20:4n-6	9.69 ± 1.17	11.30 ± 1.45	8.22 ± 1.75
22:4n-6	0.69 ± 0.78	0.68 ± 0.65	1.12 ± 0.54
22:6n-3	1.42 ± 0.88	1.84 ± 0.96	1.47 ± 0.22
Saturated	36.78 ± 2.79	43.04 ± 2.17*	41.68 ± 1.11*
MUFA	45.26 ± 4.05	37.90 ± 2.28*	42.56 ± 2.96
PUFA	13.57 ± 1.95	17.84 ± 1.06*	14.95 ± 2.04
n-6	12.12 ± 1.19	16.00 ± 1.83	13.48 ± 2.10
n-3	1.45 ± 0.86	1.84 ± 0.96	1.47 ± 0.22
MUFA/saturated	1.24 ± 0.15	0.88 ± 0.09*	1.02 ± 0.09
PUFA/saturated	0.37 ± 0.05	0.41 ± 0.03	0.36 ± 0.04
Unsat/saturated	1.61 ± 0.12	1.29 ± 0.11*	1.38 ± 0.06*
n-3/n-6	0.12 ± 0.05	0.12 ± 0.07	0.11 ± 0.03
PUFA/MUFA	0.30 ± 0.06	0.47 ± 0.04*	0.36 ± 0.07

[a]Values are mole percentages and represent means ± standard deviation, $n \geq 3$. A single asterisk (*) indicates significantly different from the control; double asterisk (**) indicates significantly different from I-FABP-expressing cells, $P < 0.05$. For abbreviations see Table 1 and 3.

The decrease in the mole percentage of 18:1n-9 in L-FABP-expressing cells resulted in a net decrease in MUFA, leading to a decrease in the MUFA/saturated fatty acid index and an increase in the PUFA/MUFA index, illustrating that even though there were no significant changes in individual PUFA, there was nonetheless a net increase in PUFA.

In summary, both L-FABP and I-FABP expression altered phospholipid acyl chain composition, although L-FABP expression appeared to have a greater effect than I-FABP expression. In general, L-FABP expression increased PUFA, predominantly through a 1.5- to 2.5-fold increase in 22:6n-3 and a 1.4- to 2.4-fold increase in 20:4n-6 proportions relative to the control. MUFA was decreased, resulting in a decrease in the unsaturated/saturated fatty acid index but an increase in the PUFA/saturated fatty acid index. In I-FABP-expressing cells, there was a 6–15% decrease in MUFA along with limited changes in PUFA, resulting in a decrease in the unsaturated/saturated fatty acid index. Hence, L-FABP and I-FABP expression differentially affected L-cell phospholipid fatty acid composition.

DISCUSSION

The physiological role(s) proposed for FABP include fatty acid uptake, intracellular metabolism, cellular growth, and differentiation (2,12). Previously, we showed that I- and L-FABP expression in L-cells differentially affects fatty acid uptake (21) and targets exogenous fatty acids for esterification into distinct lipid pools (18,19,21). However, both I- and L-FABP increase the apparent fatty acid intracellular diffusion coefficient (20), indicating both proteins are involved in intracellular fatty acid trafficking, consistent with results showing that FABP stimulate fatty acid transfer between membranes *in vitro* (47,48). Because both I- and L-FABP differentially stimulate an increase in PtdOH synthesis (24), this suggests that both I- and L-FABP expression in L-cells may affect not only phospholipid mass but also the phospholipid acyl chain composition. To determine if I- and L-FABP expression differentially affects these properties, cells expressing either L-FABP or I-FABP and control cells were grown under the same conditions, and the phospholipid acyl chain composition, phospholipid, and cholesterol mass were analyzed.

Phospholipid levels. Prior studies suggested that L-FABP expression, but not I-FABP expression, increased phospholipid mass in L-cells (18,19). In contrast, we show here that L-FABP, and to a lesser extent I-FABP, expression increased total cellular phospholipid mass compared with the control. These data with transfected cells are supported by results showing that L-FABP had a significantly greater effect on PtdOH biosynthesis *in vitro* than I-FABP (24). When the individual phospholipid classes were separated, the effect of I-FABP was limited to increased ChoGpl and PtdSer mass

(Table 1). On the other hand, L-FABP expression increased the mass of all the major phospholipids between 1.4- and 5.6-fold, depending upon the phospholipid class (Table 1). Previous results suggested only CerPCho, ChoGpl, and EtnGpl mass was increased in L-cells expressing L-FABP (18); however, results presented here clearly indicate a robust effect on both PtdIns and PtdSer mass. Thus, L-FABP markedly increased phospholipid mass of all the phospholipid classes, whereas I-FABP expression had a limited effect on total phospholipid mass; and these changes were limited to two phospholipid classes.

Both I- and L-FABP-expressing cells had altered plasmalogen mass and proportions relative to control cells (Table 2). L-FABP expression significantly increased the mass of PlsCho and PlsEtn to a much greater extent than I-FABP expression. Similarly, both proteins differentially increased the mass of the acid-stable fraction. Thus, by analyzing the ChoGpl and EtnGpl subclasses, it became evident that these proteins elevated both the acid-stable and acid-labile fractions relative to the control, indicating I- and L-FABP facilitated not only plasmalogen biosynthesis but also PtdEtn and PtdCho synthesis. Because plasmalogens have a role in lipid-mediated signal transduction (42–44), FABP may support not only the synthesis of PtdIns but also of other phospholipids involved in cell signaling.

Phospholipid composition. In L-cells, expression of L-FABP but not I-FABP significantly altered the phospholipid percentage composition. In L-FABP-expressing cells, total cellular phospholipid composition was dramatically changed, with proportions (mol%) of both PtdIns and PtdSer increased and ChoGpl proportions decreased (Table 1). These results are consistent with data showing plasma membrane phospholipid composition is altered in L-FABP-expressing L-cells (46). We also report an alteration in the composition of ChoGpl and EtnGpl subclasses (Table 2). In L-FABP-expressing cells, PlsEtn proportions, expressed as mole percentage of EtnGpl, were increased. In contrast, I-FABP expression had no effect on the composition of the EtnGpl subclasses. These results indicate that PlsEtn synthesis was increased at the expense of the predominantly diacyl subclass, PtdEtn. In contrast, both I- and L-FABP expression increased PlsCho proportions, once again with a decrease mainly in the PtdCho subclass. Clearly, there was a profound effect on plasmalogen biosynthesis, with the increase in PlsCho proportions suggesting an increase in the utilization of PlsEtn to form the PlsCho (49,50). This increase in PlsCho proportions is important as PlsCho is the active plasmalogen pool involved in signal transduction (42–44,50,51).

Possible mechanisms for enhanced phospholipid synthesis. Several mechanisms may account for the observed increases in phospholipid levels. The general increase in phospholipid mass in the L-FABP-expressing cells may be the result of elevated PtdOH biosynthesis, since L-FABP stimulates PtdOH synthesis *in vitro* (10,23,24). This appears plausible as PtdOH is the central and key intermediate for the Kennedy pathway (52,53). The reported difference in the magnitude of stimulation by L-FABP and I-FABP *in vitro* (24) may account, in part, for the reduced effect of I-FABP expression on L-cell phospholipid mass. In addition, L-FABP, and not I-FABP, has been reported to be localized in the ER as well as the cytosol (12). It is also quite possible that L-FABP affected other enzymes in the Kennedy pathway, in particular, the portion of the pathway involved in PtdIns and PtdSer biosynthesis. These two phospholipids were selectively elevated, suggesting L-FABP expression enhanced more than just PtdOH synthesis and may have stimulated activity of key enzymes in the Kennedy pathway.

Both I- and L-FABP expression increased plasmalogen mass in L-cells. Plasmalogen synthesis requires both peroxisomal (54,55) and microsomal (56–58) steps. Formation of the 1-*0*-alkyl linkage occurs in the peroxisome (54,55), whereas the desaturation of the 1-*0*-alkyl moiety occurs in the microsome (56–58). Because L-FABP has been detected in the ER but not in peroxisomes (12), FABP expression more likely stimulates the microsomal as compared to peroxisomal pathways. However, because L-FABP increased 22:6n-3 proportions to a greater extent than I-FABP and because 22:6n-3 formation is peroxisome-dependent (59), increased plasmalogen mass may merely be the result of an overall increase in peroxisomal function, stimulated to a greater degree by L-FABP expression than I-FABP expression. This postulated increase in peroxisomal function may be correlated with increased fatty acid uptake in L-FABP-expressing cells and the enhancement of peroxisome proliferator-activated receptor activity by L-FABP (60).

Alternatively, because the final steps for plasmalogen biosynthesis are microsomal (56–58), I- and L-FABP expression may also increase desaturase activity. Such a mechanism is consistent with the known (10,23,24) and proposed effects on Kennedy pathway enzymes. Furthermore, expression of either protein dramatically increased levels of PlsCho, which is made using PlsEtn as the direct precursor (49,50). Regardless of whether I- and L-FABP expression enhanced either the peroxisomal steps or the microsomal steps of plasmalogen synthesis or both, expression of either protein increased plasmalogen levels. This elevation in plasmalogens may be very important as plasmalogens are active components of several cascades involved in lipid-mediated signal transduction (42–44, 50,51,61).

Free cholesterol to phospholipid ratio. We also report a large decrease in the cholesterol to phospholipid ratio. Similar to all of the other effects of I- and L-FABP expression on lipid metabolism, L-FABP decreased the cholesterol-to-phospholipid ratio to a greater extent than I-FABP. The decrease in this ratio is consistent with another study indicating L-FABP expression decreased this ratio (17). Furthermore, the lateral membrane mobility in L-FABP expressing cells is reduced (20), consistent with a decrease in the cholesterol-to-phospholipid ratio. The reduction in this parameter may account for the observed decrease in acyl chain order in these cells (17,46).

Phospholipid acyl chain composition. Lastly, expression of either L- or I-FABP caused significant changes in the phospholipid fatty acid composition (Tables 3–6). In general, the effect of FABP expression on phospholipid fatty acids was a decrease in MUFA and an increase in PUFA. For L-FABP, this included a 1.5- to 2.5-fold increase in 22:6n-3 and a 1.4- to 2.4-fold increase in 20:4n-6 proportions (mol%) relative to control. In contrast, I-FABP expression produced limited increases in 20:4n-6 proportions along with a 6–15% decrease in the MUFA. These changes are consistent with binding affinity data showing a preferential binding of PUFA to L-FABP compared to I-FABP (8). Because I- and L-FABP bind both fatty acids and fatty acyl-CoA with a high affinity, these proteins may facilitate interactions of fatty acids and fatty acyl-CoA with CoA-dependent and CoA-independent acyltransferases (62). Within the cellular milieu, L-FABP may exhibit preferential binding for PUFA over MUFA, similar to that observed *in vitro* (8), accounting for the differential effects on PUFA composition in I- and L-FABP-expressing cells. Taken in context with the increased levels of particular phospholipids involved in lipid-mediated signal transduction, an increase in the amount of 20:4n-6 in the phospholipids would increase the amount of 20:4n-6 potentially liberated during signal transduction. This increase in the potential availability of 20:4n-6 could profoundly affect cellular function.

In summary, both L- and I-FABP expression increased total cellular phospholipids; however, the extent of this increase was significantly different between L- and I-FABP-expressing cells. L- and I-FABP expression also differentially affected individual phospholipid levels and phospholipid composition. We speculate that both L- and I-FABP stimulated an increase in phospholipid biosynthesis *via* the Kennedy pathway by affecting not only PtdOH biosynthesis, but also specific enzymes in the pathway, thereby accounting for the increased PtdSer and PtdIns mass. Furthermore, L- and I-FABP increased plasmalogen mass, perhaps indicating an increase in peroxisomal function or ER function or both, especially in the L-FABP-expressing cells. The increased phospholipid levels, in the absence of elevated cholesterol mass, resulted in a substantial decrease in the cholesterol to phospholipid ratio. Lastly, both proteins altered the phospholipid fatty acid composition by increasing the mole percentage of PUFA at the expense of MUFA. In conclusion, L- and I-FABP expression in L-cells differentially enhanced phospholipid synthesis and altered phospholipid fatty acid composition. These findings extend the previously reported differential effect of L- and I-FABP expression on fatty acid uptake (18–21) and targeting (18,19,21). However, the dramatic effects on phospholipid pools involved in cell signaling as well as an increase in 20:4n-6 proportions suggest that FABP may have an important role in maintaining lipid pools used in lipid-mediated signal transduction. Our results suggest L-FABP has a greater role in this process than I-FABP.

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