review

# Cellular uptake and intracellular trafficking of long chain fatty acids

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**Abstract While aspects of cellular fatty acid uptake have been studied as early as 50 years ago, recent developments in this rapidly evolving field have yielded new functional insights on the individual mechanistic steps in this process. The extremely low aqueous solubility of long chain fatty acids (LCFA) together with the very high affinity of serum albumin and cytoplasmic fatty acid binding proteins for LCFA have challenged the limits of technology in resolving the individual steps of this process. To date no single mechanism alone accounts for regulation of cellular LCFA uptake. Key regulatory points in cellular uptake of LCFA include: the aqueous solubility of the LCFA; the driving force(s) for LCFA entry into the cell membrane; the relative roles of diffusional and protein mediated LCFA translocation across the plasma membrane; cytoplasmic LCFA binding protein-mediated uptake and/or intracellular diffusion; the activity of LCFA-CoA synthetase; and cytoplasmic protein mediated targeting of LCFA or LCFA-CoAs toward specific metabolic pathways. The emerging picture is that the cell has multiple, overlapping mechanisms that assure adequate uptake and directed intracellular movement of LCFA required for maintenance of physiological functions. The upcoming challenge is to take advantage of new advances in this field to elucidate the differential interactions between these pathways in intact cells and in tissues.**—McArthur, M. J., B. P. Atshaves, A. Frolov, W. D. Foxworth, A. B. Kier, and F. Schroeder. **Cellular uptake and intracellular trafficking of long chain fatty acids.** ***J. Lipid Res.*** **1999.** 40: **1371–1383.**

**Supplementary key words** fatty acid • uptake • plasma membrane • binding protein • trafficking • fluorescence

It has been appreciated that long chain fatty acids (LCFA) are important to the cell as a source of metabolic energy and as substrates for membrane biogenesis (phospholipid) and storage of metabolic energy (triglycerides and cholesterol esters). More recent data show that LCFA and their CoA derivatives directly or indirectly regulate the activity of many cellular processes including: membrane receptors, enzymes, ion channels, cell differentiation, cellular development, and gene expression (rev. in refs. 1–3). LCFA, through their metabolites (e.g., phero-mones, prostaglandins, leukotrienes, thromboxanes, platelet activating factor, etc.), serve as intracellular signaling molecules. Because of these important functional roles, it is important to understand the mechanism(s) that cells have evolved for selective uptake and retention of LCFA. The purpose of this review is to provide the reader with an overall appreciation for some of the key steps in this process that occur in most cells and to focus in depth on select aspects and mechanisms of LCFA uptake. Because space limitations prevent adequate coverage of many highly meritorious topics, the reader is referred to earlier reviews focusing on selected aspects of cellular LCFA uptake (4–15).

For the sake of simplicity we have broken cellular LCFA uptake down into several essential, albeit not all inclusive, key processes (**Fig. 1**). Any one of these processes may be rate-limiting in: a specific subcellular compartment or metabolic process; a particular tissue or cell type; a specific dietary, endocrine, or pathological condition. Although it is recognized that some of the regulatory points or processes identified below actually represent "pools" (e.g., extracellular unbound LCFA), for the sake of simplicity each such regulatory point or process is designated as a "step."

Abbreviations: LPL, lipoprotein lipase; FABP, fatty acid binding protein; $FABP_c$, cytosolic FABP; I-FABP, intestinal FABP; L-FABP, liver FABP; H-FABP, heart FABP; A-FABP, adipocyte FABP; ADIFAB, acrylodated I-FABP; $FABP_{pm}$, plasma membrane FABP; FATP, fatty acid transport protein; FAT, fatty acid translocase; BSA, bovine serum albumin; SCP-2, sterol carrier protein 2; SCP-x, sterol carrier protein x; SCP-y, sterol carrier protein y; ACBP, long chain acyl coenzyme A binding protein; LCFACS, long chain fatty acyl CoA synthetase; GPAT, glycerophosphoryl acyl transferase; ES, embryonic stem cells; L-cell, fibroblast cell line; 3T3, adipocyte cell line; O617PY, adipocyte cell line; COS7, adipocyte cell line; HBRIE 380I, intestinal cell line; CHO, chinese hamster ovary cells; CACO-2, colon carcinoma 2 cell line; H9c2, heart muscle cell line; AOFA, anthroyloxy fatty acid; LCFA, long chain fatty acid; CoA, coenzyme A; SH, thiol; NBD-stearic acid, 12-(N-methyl)-N-[(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-octadecanoic acid; BODIPY, 4,4-difluoro-5-methyl-4-bora-3α,4α-diaza-3-indacene-3-dodecanoic acid.

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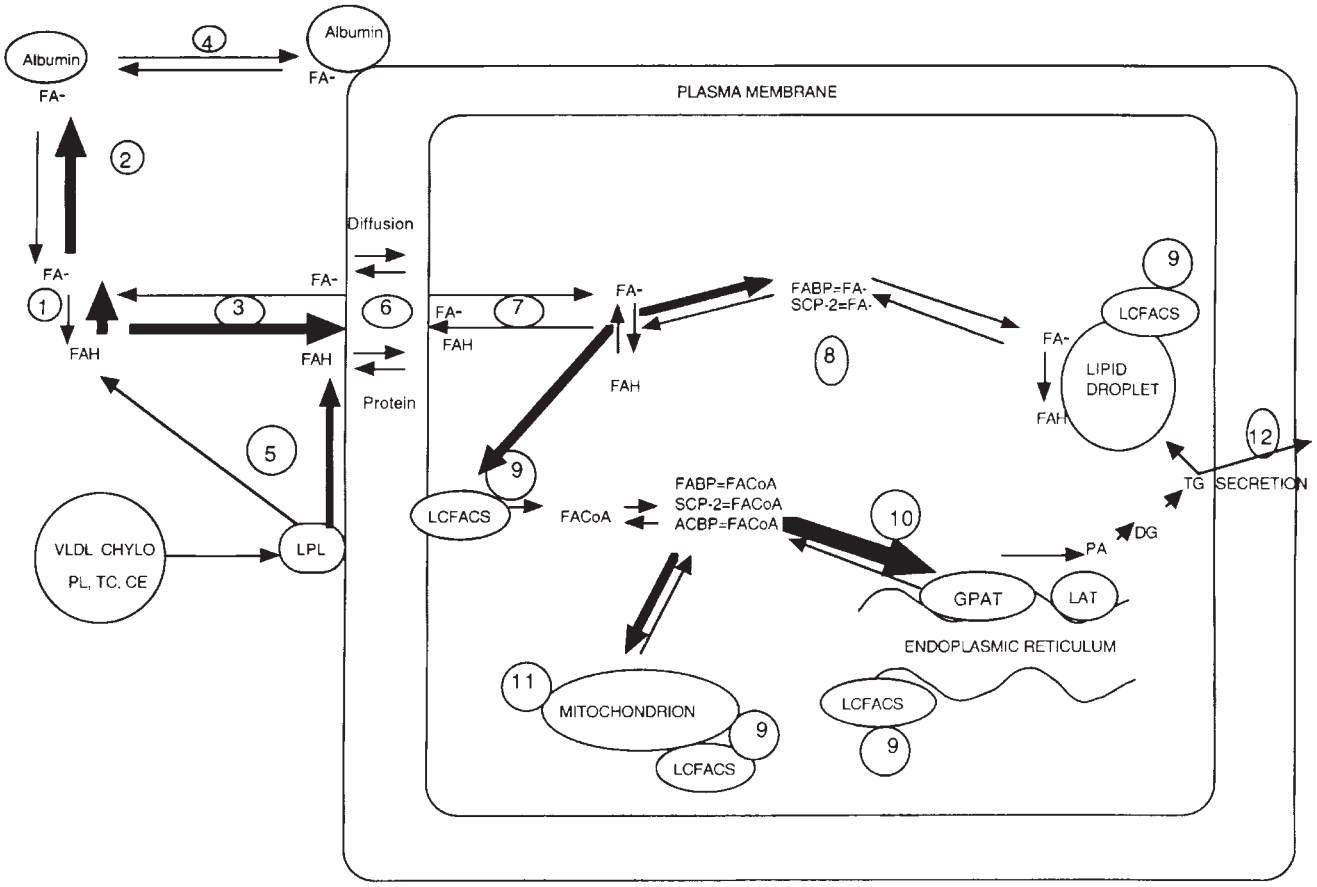


**Fig. 1.** Cellular fatty acid uptake and intracellular trafficking. The following steps are illustrated: Step 1, extracellular concentration of unbound LCFA; Step 2, extracellular concentration of albumin bound LCFA; Step 3, LCFA entry into the cell plasma membrane (PM); Step 4, cell surface albumin receptors; Step 5, local lipoprotein lipase (LPL)-mediated LCFA release from membrane bound lipoprotein; Step 6, LCFA translocation (diffusional or protein-mediated) across the membrane; Step 7, LCFA desorption from the cytofacial leaflet of the plasma membrane; Step 8, LCFA cytoplasmic (spontaneous vs. FABP facilitated) diffusion; Step 9, fatty acyl CoA synthetase; Step 10, cytoplasmic LCFA-CoA binding protein stimulated LCFA-CoA esterification to phospholipids (PL), triacylglycerols (TG), and cholesteryl esters (CE) in endoplasmic reticulum (ER) or mitochondria (MITO); Step 11, cytoplasmic LCFA-CoA binding protein-stimulated LCFA oxidation in MITO and peroxisomes (PER; Step 12, LCFA/LCFA-CoA binding protein-stimulated secretion of esterified LCFA in lipoproteins.

### Step 1. The extracellular concentration of non-esterified, unbound LCFA: aqueous monomeric LCFA concentration

Central to understanding many of the issues in cellular LCFA uptake and intracellular trafficking are the physical properties of LCFA in aqueous solution (rev. in ref. 12). Briefly, at physiological pH 7.4 in aqueous solution, LCFA exist in two forms: 99.6% anions, 0.4% protonated. Protonation and deprotonation are very fast and not rate limiting for the considerations of cellular LCFA uptake and trafficking. Although LCFA are amphiphilic molecules with a tendency to self-associate, there is little or no direct evidence for formation of LCFA aggregates in serum to date. The monomeric LCFA concentrations [defined as LCFA not complexed with either itself, protein (e.g., albumin in blood), bile salt (intestinal lumen), membranes, or other components (e.g., triglyceride droplets, ribosomes, glycogen, etc.] have been determined multiple times over the past half-century with a wide range of values reported. It should be noted that while the unbound monomeric LCFA "concentration" defined above is dependent on the concentration of other LCFA binding molecules present in biological fluids, the monomeric aqueous LCFA "solubility" is defined as the maximum concentration of unbound LCFA that can be dissolved in a solution of defined composition (pH, ionic strength, calcium concentration, etc.) without formation of aggregates. The latter value is theoretically independent of the presence or absence of other LCFA binding species provided they are at equilibrium with the unbound concentration.

### Step 2. The extracellular LCFA concentration: effect of serum albumin

Serum total LCFA (nonesterified) concentrations range from 200 to 600 $\mu$m under normal conditions and up to 4-fold higher in diabetes, cancer, and sepsis (16). Although normal LCFA serum levels are about 7000-fold higher than the monomeric solubility of LCFA (17), serum LCFA levels are normally in the same concentration range as the serum fatty acid binding protein albumin, 624–789 $\mu$m. As it is not yet clear whether the driving force of LCFA uptake is the unbound LCFA (Step 3 below) or the total

TABLE 1. Binding of long chain fatty acids to human (HSA) and bovine (BSA) albumin

| Method | Protein | $K_d$ | Reference |
|---|---|---|---|
| | | *nM* | |
| Heptane partitioning | HSA | 67 | 110 |
| Heptane partitioning | BSA | 2 | 111 |
| Polyethylene dialysis | BSA | 10 | 112 |
| Fluorescence binding | BSA | 10 | 31 |
| Fluorescence displacement | BSA | 8 | 113 |
| Fluorescence displacement | HSA | 7 | 113 |
| Erythrocyte partition | BSA | 11 | 114 |
| Erythrocyte ghost partition | HSA | 2 | 20 |
| Affinity chromatography | BSA | 2 | 115 |

LCFA (Step 4 below), determination of the concentration of unbound monomeric LCFA in serum has become a crucial undertaking significant to resolving the relative importance of several proposed mechanism(s) whereby LCFA translocate across the cell membrane. Measurements of the affinity of albumin for LCFA by the many different methods vary more than 30-fold, $K_d$ = 2–67 nm (**Table 1**). There is considerable debate over which of these values represents the physiologically correct $K_d$. Unfortunately, none of the reported binding measurements were made in the presence of physiological levels of the other LCFA binding sites to which serum or interstitial fluid is normally exposed (lipoproteins, blood cells, platelets, and cells of the vessel wall or tissue). Because of the importance of this issue it is relevant to review some of the basic approaches and to illustrate the difficulty in defining the serum unbound LCFA concentration:

*Competition methods involving partitioning of LCFA between organic (solvent or polyethylene) and aqueous phases.* As compared to the other methods, these are the only methods sensitive enough to determine the unbound monomeric LCFA using physiological concentrations of albumin (rev. in ref. 18). Their disadvantage is that they assume LCFA are uniformly present as fatty acid monomers and that radiochemical impurities present in the assays always reduce the apparent affinity (first stepwise binding constant) of albumin for LCFA. However, extrapolation of the impurity level to zero yields affinity constants similar to that obtained by the red-cell partition and the ADIFAB assays described below (rev. in ref. 19). Finally, a difficulty common to this as well as other methods (see below) is the presence of multiple (3 or more) LCFA binding sites in albumin.

*Competition method involving partitioning of LCFA between erythrocyte and aqueous phases.* The main advantage of this method is that it reflects binding of LCFA by specific sites in the erythrocyte membrane, not just general partitioning into the erythrocyte membrane (20). The major problem of the method, like the organic solvent partition method, is that it is difficult to define the extremely low unbound LCFA monomer concentration (rev. in ref. 20).

*Competition assay using fluorescence displacement.* Recently a new method, referred to as the ADIFAB assay, was used to determine unbound LCFA concentration in serum (21). In this application, the ADIFAB assay utilizes a three-way competition between LCFA, albumin, and acrylodan covalently bound within the I-FABP LCFA binding pocket (i.e., ADIFAB). In addition, the ADIFAB assay requires that LCFA displaces a covalently attached acrylodan moiety from the ligand binding pocket of ADIFAB. For example, when acrylodan is located in the binding pocket it exhibits a fluorescence emission maximum near 432 nm [denoted by (−) in **Fig. 2A**]. In contrast, in the presence of LCFA such as oleic acid [denoted by (+) in Fig. 2A] the acrylodan is displaced from the binding pocket, the fluorescence emission at 432 nm decreases markedly, and a new fluorescence emission maximum appears near 505 nm. The proposed advantages of the ADIFAB assay are that it does not require separation of bound from free LCFA and that ADIFAB specifically binds only LCFA. However, the latter assumption is not supported by the literature (22, 23). ADIFAB binds LCFA-CoA such as oleoyl CoA (Fig. 2B) and exhibits spectral changes even larger than those obtained with LCFA. Furthermore, ADIFAB also binds acetyl CoA (Fig. 2C) and CoASH (Fig. 2D). All these ligands decrease the ADIFAB fluorescence emission at 432 nm and exhibit saturation binding curves over the same concentration range as LCFA (insets in Fig. 2A–D). Furthermore, to our knowledge the ADIFAB assay has only been applied to one LCFA binding protein, other than albumin, with multiple binding sites, i.e., L-FABP. In the case of L-FABP, the ADIFAB displacement assay yields a 20- to 30-fold lower $K_d$ than $K_d$s obtained with direct LCFA-binding assays, i.e., naturally occurring fluorescent LCFA and titration microcalorimetry (25–28). For both L-FABP and albumin, the unbound LCFA concentration will be dependent on the $K_d$. In summary, these concerns suggest caution in interpretation of results from application of the ADIFAB assay to determine unbound LCFA in heterogeneous biological fluids (blood, serum, interstitial fluid, cytosol, etc.) containing proteins with multiple LCFA binding sites.

*Direct binding method using fluorescent fatty acids.* The binding of naturally occurring fluorescent LCFA such as *cis*- or *trans*-parinaric acid to albumin represents a direct LCFA binding assay. In contrast to the ADIFAB fluorescence displacement assay, a direct fluorescence binding assay is defined as an assay that does not require displacement of a bound fluorescent ligand (e.g., covalently bound acrylodan or non-covalently bound fluorescent LCFA) and does not require competition with other binding proteins, membranes, Lipidex beads, etc. The validity of the use of parinaric acids in direct fluorescence binding assays for determination of the $K_d$s of fatty acid binding proteins with both single and multiple LCFA binding sites has been confirmed by comparison to other methods (25–27, 29–31). Despite the above advantages of the direct parinaric acid binding assay, the complexity of resolving five different $K_d$s, one for each BSA binding site, makes it very difficult to accurately determine the individual affinities (31). Furthermore, the assay can only be used below physiological concentrations of albumin and cannot be used with serum or cells due to parinaric acid binding, as well as flu-

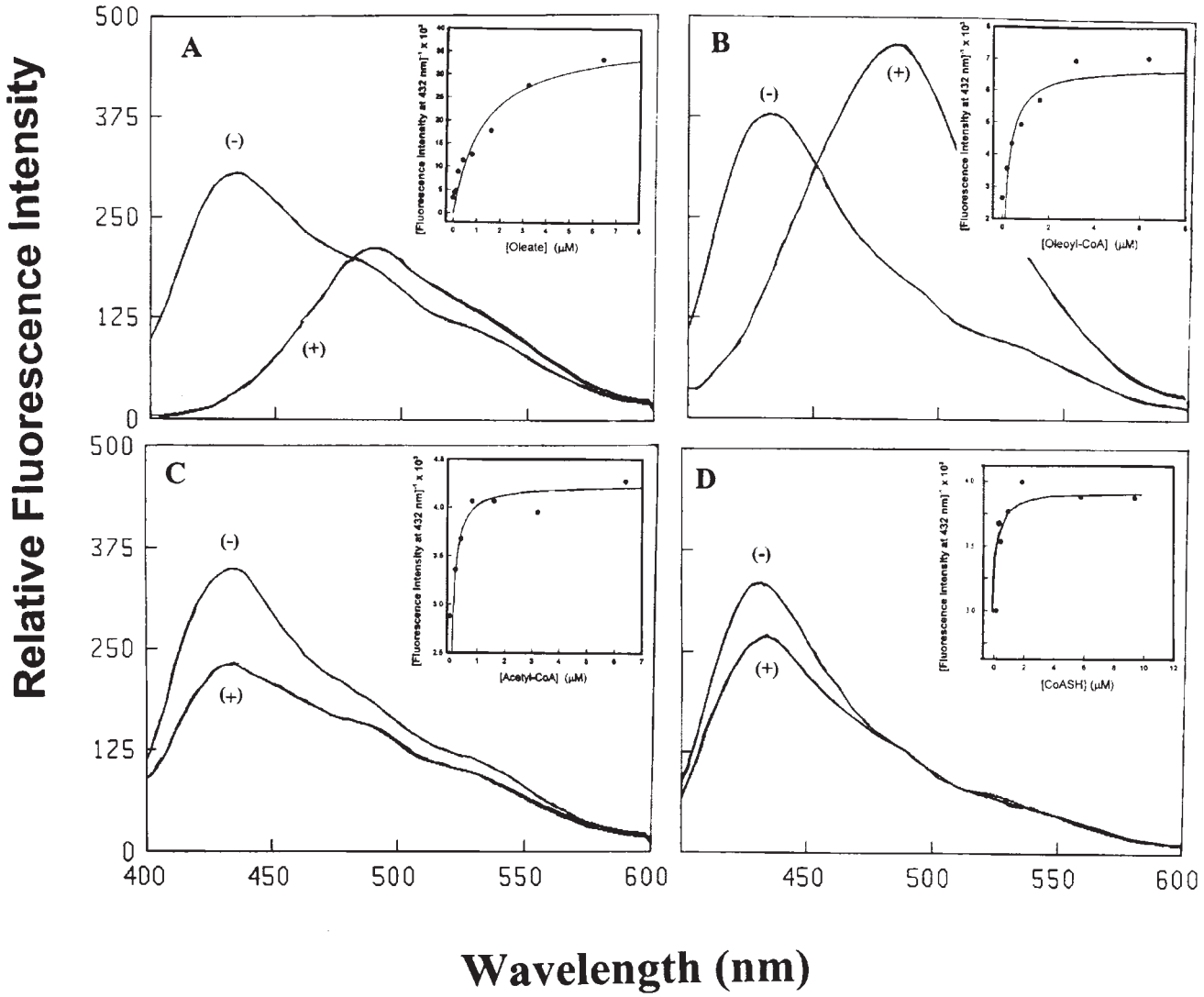


**Fig. 2.** Effect of ligands on ADIFAB fluorescence emission spectra. ADIFAB fluorescence emission spectra were obtained as described earlier (22). ADIFAB (0.1 μm) was excited at 390 nm and emission spectra were obtained in the absence (−) or presence (+) of added ligand. The (+) spectra are representative ADIFAB emission spectra in the presence of either 6.4 μm oleic acid (panel A), 9.6 μm oleoyl-CoA (panel B), 6.4 μm acetyl CoA (panel C), or 3.2 μm CoASH (panel D). Insets in each panel show titration curves for ADIFAB with the respective ligands plotted as a function of the [fluorescence intensity at 432 nm]$^{-1} \times 10^3$.

orescing, when bound to serum lipoproteins or membranes. Finally, this type of assay cannot be used with nonfluorescent LCFA, unless it is applied as a displacement assay.

In summary, the $K_d$s in Table 1 can be categorized into three groups [$K_d$ = 2 nm (3 methods), $K_d$s = 7–10 nm (5 methods), and $K_d$ = 67 nm (1 method)]. At present no conclusion can be reached regarding which of the above methods provides the physiologically "correct" value for unbound LCFA in serum or in serum containing other LCFA binding constituents (e.g., lipoproteins, blood cells, endothelial cells, hepatocytes). This complicates selection of a specific $K_d$ for LCFA binding to albumin (Table 1) in order to differentiate the mechanism, diffusional or protein-mediated, whereby LCFA translocate across the cell surface membrane. Nevertheless, it would appear that regardless of the method used to determine serum unbound LCFA, the majority of serum LCFA are found associated with albumin (Table 1).

### Step 3. Entry of extracellular free long chain fatty acid into the cell plasma membrane: role of unbound LCFA

*Role of the albumin-LCFA dissociation rate.* LCFA dissociates very rapidly from LCFA-albumin with a dissociation rate constant of 0.04–0.14 $s^{-1}$ (32–34). Whether or not dissociation of LCFA from albumin is rate limiting to cellular uptake depends not only on the rate of dissociation of the LCFA from albumin, but also on the rate that the unbound pool is removed by cellular processes (35). Dissociation is most likely to be rate limiting in tissues with rapid metabolism of LCFA (e.g., liver, heart). Recent analysis of hepatic LCFA uptake by liver suggests that dissociation is slow enough to partially limit LCFA uptake at steady-state, but that other factors such as membrane and

cytoplasmic transport are more important limiting factors (36).

*Role of the unstirred water layer at the cell surface.* In order to enter the cell membrane, the LCFA must not only dissociate from albumin, but must also cross the unstirred water layer, a physical gradient at the membrane surface that forms a diffusional barrier (37). Codiffusion of bound and unbound LCFA into the unstirred water layer may be one mechanism for crossing the unstirred layer (38). Codiffusion would allow replenishment of unbound LCFA from the bound pool as equilibrium is re-established.

*Form of the LCFA in the cell membrane.* Both protonated and ionized LCFA can bind to membranes (rev. in ref. 12). While in aqueous solution <1% of LCFA is protonated at physiological pH, but when LCFA partition into lipid bilayers their $pK_a$ values shift from 5 to 9, a very rapid process resulting in about 50% of membrane-bound LCFA being protonated (rev. in refs. 4, 12). Once membrane bound, either the protonated LCFA diffuses across the membrane bilayer or the ionized (or possibly the protonated) LCFA may bind to LCFA translocase proteins for transbilayer transport (see Step 6).

### Step 4. Entry of extracellular free long chain fatty acid into the cell membrane: effect of albumin bound LCFA

At present it is unclear whether the driving force for LCFA uptake is the gradient of the total or only of the unbound LCFA (rev. in refs. 39, 40). This will also depend on whether LCFA translocate across the plasma membrane by spontaneous diffusion or by translocase protein(s). Some methods (Table 1) indicate serum unbound LCFA values are up to 7-fold lower than the amount of LCFA calculated to dissociate from albumin in the time required for clearance of LCFA from the circulation, 14 nm (rev. in ref. 41). On this basis it has been suggested that additional processes must be taking place that clear the serum LCFA faster than can be accounted for by albumin–LCFA dissociation rate (41). One such additional factor could be the role of the undissociated albumin–LCFA complex, which itself accounts for the vast majority of serum LCFA regardless of which $K_d$ is used (Table 1), and the presence of "albumin receptor(s)" in the cell surface (rev. in ref. 42). Albumin–LCFA may bind to the cell albumin receptor and thereby deliver LCFA directly to the cell membrane, bypassing the aqueous dissociation of albumin–LCFA to unbound LCFA. Direct interaction of certain cytoplasmic fatty acid binding proteins ($FABP_c$) such as I-FABP (43) and A-FABP (44) with a membrane surface appears to be required for transfer of LCFA to or from some $FABP_c$ (43) (see step 8 below).

### Step 5. Entry of extracellular free long chain fatty acid into the cell membrane: effect of lipoprotein lipase or other components

Serum unbound LCFA and albumin–LCFA complexes are not the only sources of fatty acid for cellular fatty acid uptake. Lipases associated with the vascular endothelium or in the intestinal lumen may play an important role. It has been shown that 90–95% of LCFA released from very low density lipoproteins or chylomicrons by lipoprotein lipase (LPL) bound at the endothelial cell surface directly enters the tissue, while only 5–10% of the released LCFA equilibrates with the plasma (45, 46). Consequently, it must be considered that the local concentration of LCFA released to the endothelial membrane (by LPL acting on endothelial bound very low density lipoproteins or chylomicrons) may be sufficiently high to saturate endothelial plasma membrane translocation processes (see Step 6, below). At present, to our knowledge there is no information on the potential flux of LCFA per plasma membrane LCFA protein translocase molecule. Thus, it is not possible to determine whether the LPL mediated release of LCFA saturates putative protein-mediated LCFA translocase(s) and, if so, would the diffusional LCFA translocation predominate?

### Step 6. Translocation of LCFA across cellular plasma membranes

At present there is no general agreement whether LCFA cross the cell surface by simple diffusion, by plasma membrane protein-mediated translocation, or both.

*(i) Form of the fatty acid translocating across the membrane: protonated or ionized.* It is generally thought that the unionized form of the fatty acid can diffuse spontaneously and freely ($t_{1/2}$ = 20 msec–2 sec) while the ionized fatty acid diffuses slowly ($t_{1/2}$ = min) across the membrane bilayer (rev. in ref. 12). However, several recent reports suggest that ionized lipophilic molecules and LCFAs may also translocate rapidly and spontaneously across the lipid bilayer (47, 48). If so, then LCFA uptake in cells may not necessarily require a protein-mediated translocation across the plasma membrane.

*(ii) Passive diffusional mechanism.* The fact that many naturally occurring (lauric, myristic, palmitic, stearic) as well as synthetic LCFA rapidly translocate ($t_{1/2}$ = 20 msec–2 sec) across model membrane bilayers led to the hypothesis that a protein-mediated translocase process was not obligatory for LCFA transport across the membrane (rev. in ref. 12). Consistent with this hypothesis, diffusional LCFA uptake accounts for LCFA translocation across the inner membrane bilayer of bacteria and across the erythrocyte membrane (4, 49, 50). Various studies with inhibitors of protein-mediated LCFA uptake also are consistent with a significant non-inhibitable (i.e., diffusional?) LCFA uptake in mammalian cells. For additional details on diffusional LCFA uptake, the reader is referred to an excellent recent review focusing on this aspect of LCFA translocation across membranes (12).

*(iii) Protein-mediated mechanism.* Heat denaturation, trypsin treatment, SH reagents, antisera to specific plasma membrane proteins, energy poisons, etc. were originally used to inhibit LCFA uptake, thereby leading to the hypothesis that LCFA uptake is protein-mediated (rev. in refs. 39, 51). Unfortunately, many of the parameters assigned to putative membrane LCFA translocase proteins were not unique to protein as opposed to diffusional translocation mechanisms and are likely due to indirect effects (rev. in ref. 51). However, at least one recent paper has showed

that heart FATP, a putative plasma membrane LCFA transporter, contains an ATP binding motif and specifically binds azido-ATP (52). Although this may indicate that this putative LCFA translocase is energy requiring, other data suggest that some putative translocases are long chain fatty acyl CoA synthetases (LCFACS) which also require ATP (53) (see Step 9).

Other data in support for a protein-mediated mechanism come from LCFA probe molecules [e.g., bipolar fatty acids and 12-(9-anthroyloxyl)stearic acid] that exhibit slow ($t_{1/2}$ = minutes) spontaneous flip-flop across the membrane (rev. in res. 12, 54, 55). However, it is possible that the large anthroyloxy moiety may significantly affect the behavior of LCFAs in the bilayer as binding of anthroyloxy-labeled LCFA to cytosolic fatty acid binding proteins ($FABP_c$) (56) does not accurately reflect the interaction of naturally occurring LCFA with these proteins (25–27, 57, 58).

LCFA affinity labeling has also been used to identify a number of LCFA-binding proteins/putative LCFA translocases (molecular masses near 22, 43, 60, 63, and 88 kDa) in cell plasma membranes. However, several of these putative plasma membrane LCFA translocases appear to be multifunctional proteins: 43 kDa $FABP_{pm}$ is identical to mitochondrial matrix aspartate aminotransferase; 88 kDa FAT appears to be a homologue of CD36, a lipoprotein receptor (59); the yeast *FAT-1* gene product is a very long chain fatty acyl CoA synthetase (53); FATP has distinct homology to LCFA-CoA synthetase (39); a 22 kDa protein is caveolin (60). Furthermore, no single photoaffinity LCFA simultaneously labeled all of the above putative translocases in the same cell membrane. The basis for these contrasting observations is not known, but may be related to differences in the reactive groups available to each type of reagent within the protein binding site of each translocase or perhaps to possible tissue specificity introduced by possible differences in glycosylation. This has not been studied, however. The finding that some putative LCFA transporters, 88 kDa FAT/CD36 and 22 kDa caveolin, colocalize to caveolae brings up the possibility that the putative LCFA translocases may mediate LCFA uptake indirectly through interactions with other proteins concentrated within the caveolae (rev. in ref. 4). For example, the calcium binding proteins MRP8 and MRP14 individually do not bind fatty acids. However, a noncovalent heterodimer of MRP8 with MRP14 binds oleic and arachidonic acid with high affinity, near 0.1 μm. If similar dimer or heterodimer formation of the putative plasma membrane translocases with other membrane proteins (e.g., caveolin) could occur, then indirect regulation of the LCFA uptake process through such proteins may be considered.

One of the difficulties in resolving whether expression of a specific membrane protein is involved in LCFA uptake is that multiple families of proteins that bind LCFA appear to be coordinately up-regulated. For example, intestinal microvillar membrane FAT and cytosolic fatty acid binding proteins (I-FABP and L-FABP) are responsive to induction by dietary content of LCFA (62, 63). Likewise, FATP and LCFA-CoA synthetase are coordinately regulated (64). Similarly, LCFA uptake studies in adipocytes of diabetic rats showed that regulation of fatty acid uptake (increased) may be the consequence not only of increased levels of two particular plasma membrane transporters ($FABP_{pm}$ and FATP, but not FAT) (65) but also of altered plasma membrane fluidity, plasma membrane lipid composition, and $FABP_c$ (66). $FABP_{pm}$ and $FABP_c$ are also co-expressed in mammary (67) and muscle (68, 69). Finally, drugs that activate peroxisome proliferator receptor (PPAR) α and γ upregulate $FABP_c$ (rev. in ref. 70) as well as the putative plasma membrane LCFA translocases FAT and FATP, but not $FABP_{pm}$ (71). Thus, these findings complicate resolution of a role(s) for the putative plasma membrane LCFA translocases in LCFA uptake. Finally, the existence of multiple plasma membrane transporter proteins that may be independently or coordinately regulated within a single cell makes resolution of the diffusional component from each protein-mediated component(s) of plasma membrane LCFA translocation difficult.

To date there is very little functional data confirming a role for plasma membrane fatty acid binding proteins/translocases in LCFA uptake. While one report with transfected COS cells overexpressing putative LCFA translocases suggested 15- to 90-fold enhancement of BODIPY–fatty acid uptake (72), most studies with naturally occurring LCFA (oleic, palmitic, or arachidonic acid) demonstrated that overexpression of LCFA translocases enhanced uptake of these LCFA 0- to 4-fold (**Table 2**).

In summary, it is difficult to resolve a specific, unique, and exclusive role for putative plasma membrane LCFA translocases in the transfer of LCFA across the plasma membrane. This is due not only to concomitant LCFA spontaneous diffusion, but also to observations that *i*) many of the putative LCFA translocases are multifunctional proteins, and *ii*) co-expression and co-regulation of the plasma membrane translocases with the cytosolic $FABP_c$ and/or LCFA-CoA synthetase enzymes occurs in tissues or cells. Giant vesicles may be powerful tools to resolve these issues (69).

### Step 6. Desorption of fatty acids from the plasma membrane into the cytoplasm: a role for cytosolic fatty acid binding proteins ($FABP_c$)

Once LCFA translocate across the plasma membrane by passive diffusion and/or by protein-translocase-facilitated diffusion, the LCFA desorb from the cytofacial leaflet into the cytoplasm. The fact that cytosolic fatty acid binding proteins ($FABP_c$) such as L-FABP increase oleic acid flux 3-fold through a model lipid–water interface (38) suggests that $FABP_c$ may accelerate LCFA uptake at this step in several ways.

First, $FABP_c$ may increase the rate of desorption of LCFA from the cytosolic leaflet of the plasma membrane (7) and model membranes (73). However, at least in model membranes, desorption of LCFA is spontaneous, rapid, and apparently not rate limiting as compared to LCFA translocation across the bilayer (rev. in ref. 12).

Second, the high cytosolic concentrations of $FABP_c$,

TABLE 2. Fatty acid uptake in transfected cells overexpressing specific LCFA binding proteins

| Protein Intracellular Location | Protein | Cell Type | Fatty Acid | LCFA Uptake | Reference |
|---|---|---|---|---|---|
| | | | | *fold-increase* | |
| Plasma membrane | $FABP_{pm}$ | 3T3 | oleic | 3.5 | 116 |
| | $FABP_{pm}$ | *Xenopus laevis* | oleic | 2 | 13 |
| | FATP | 3T3 | oleic | 2.6 | 96 |
| | FATP | 3T3 | palmitic | 2.6 | 96 |
| | FATP | O617PY | arachidonic | 2.5 | 96 |
| | FATP | 3T3 | oleic | 2–4 | 117 |
| | FAT | H9c2 | palmitic | 0 | 118 |
| | FAT | O617PY | palmitic | 2–4 | 117 |
| Cytoplasm | A-FABP | CHO | oleic | 2 | 57 |
| | A-FABP | COS7 | LCFA | 0 | 39 |
| | A-FABP | L6 myoblast | palmitic | 0 | 93 |
| | L-FABP | L-cell | *cis*-parinaric | 1.2–1.5 | 89, 119, 120 |
| | L-FABP | L-cell | oleic | 1.8 | 89 |
| | I-FABP | L-cell | *cis*-parinaric | 0 | 102, 120 |
| | I-FABP | L-cell | oleic | 0 | 90, 102 |
| | I-FABP | ES | NBD-stearic | 1.7 | 86 |
| | I-FABP | HBRIE 380i | oleic | 5 | 103 |
| | I-FABP | CACO-2 | oleic | 2 | 92 |
| | I-FABP | CACO-2 | palmitic | 2 | 92 |
| | H-FABP | L6 myoblast | palmitic | 0 | 93 |
| | H-FABP | yeast | palmitic | 1.5 | 94 |
| Membranes | LCFACS | COS7 | BODIPY | increase | 56, 96 |

LCFACS, long chain fatty acyl CoA synthetase.

coupled with their high affinity for LCFA, suggests that $FABP_c$ can solubilize LCFA from membranes and act as a cytoplasmic 'sink' for LCFA. $FABP_c$ are expressed at levels as high as 2–5% of cytosolic protein (0.2–1.0 mm) in tissues active in LCFA metabolism (e.g., liver, enterocyte, heart, adipose, etc.) (63). $FABP_c$ increase the equilibrium aqueous/membrane partition coefficient of LCFA (rev. in refs. 74, 75). $FABP_c$ increase the equilibrium partitioning of LCFA from model membranes toward the aqueous >25-fold more than does albumin, such that as much as 50% of model membrane bound LCFA becomes aqueous soluble (rev. in refs. 76, 77). Similarly, in microsomes, 95–97% of LCFA is membrane bound (22, 77). In contrast, both L-FABP and I-FABP shift the equilibrium partitioning of LCFA from microsomal membranes to the aqueous such that as much as 38% of microsomal bound LCFA is solubilized as aqueous FABP–LCFA complexes (22, 77, 78). Finally, in intact cells, the soluble fraction of LCFA is determined by the $FABP_c$ concentration in the cytoplasm (42, 79).

Third, $FABP_c$ such as L-FABP enhance LCFA transfer to acceptor membranes by aqueous transfer of LCFA (43) while other $FABP_c$ directly interact with membranes either via ionic interactions between Lys residues in these $FABP_c$ and acidic phospholipids in the acceptor membrane (43, 44, 80, 81) or by interaction with high affinity ($K_d$ = 29 pm) specific membrane receptors (82).

### Step 7. The spontaneous diffusion of LCFA in the cytoplasm

Once the LCFA has desorbed from the cytoplasmic face of the plasma membrane, it moves through the cytoplasm either spontaneously or bound to protein (Fig. 1, steps 7 and 8). Although spontaneous diffusion of LCFA through aqueous buffers is fast, near $0.28 \times 10^{-5}$ $cm^2 \times s^{-1}$ rev. in ref. 83, LCFA diffusion through cytosol is several orders of magnitude slower (rev. in refs. 6, 79). Cytosol is 2- to 6-fold as viscous as water due to the presence of 15–26% cytosolic protein. The major portion of cytoplasmic water is tightly bound to other molecules, further reducing the ability of LCFA to diffuse freely through the cytoplasm. However, because the diffusion of small molecules is much more dependent on solvent viscosity than bulk viscosity of cytoplasm, it is believed that the greater viscosity of cytoplasm is not the most important factor hindering LCFA cytoplasmic diffusion (84). Instead, it is thought that the tortuosity of the diffusional path and the presence of cytoskeleton are primarily responsible for the 2- to 3-orders of magnitude slower LCFA spontaneous diffusion in cytoplasm as compared to simple aqueous buffers (rev. in refs. 6, 79). Tortuosity is due to the presence of nearly 1 $m^2$ of membranes/ml liver cytoplasm that constrains free LCFA diffusional movement (governed by multiple random collisions) along this non-linear path. Equally important, the cell interior contains about 2 $m^2$ of cytoskeletal filaments/ml, representing 16–21% of cell volume. Thus, spontaneous diffusion of LCFA through the cytoplasm is too slow to account for either the rapidity of LCFA intracellular movement or LCFA selective targeting toward specific organelles (see step 9 below).

### Step 8. Cytosolic FABP-mediated diffusion free fatty acids

*Effect of $FABP_c$ on cytoplasmic diffusion in vitro and in intact cells.* It is not intuitively obvious as to how $FABP_c$, with their 70-fold greater mass than typical LCFA, can actually stimulate the cytoplasmic diffusion of LCFA either in aqueous buffers or in cytoplasm (rev. in refs. 6, 83). Two factors are considered to lead to $FABP_c$-enhanced cyto-

plasmic LCFA diffusion. *i*) $FABP_c$ enhance the rate of desorption of LCFA from model and biological membranes (see Step 6). As indicated previously, this is unlikely (12, 80, 85). *ii*) $FABP_c$ increase the aqueous solubility of LCFA in the cytoplasm by binding LCFA, thereby reducing the binding of LCFA to model and biological membranes 8- to 25-fold more so than does albumin (see Step 6) (12, 80, 85). Experimental determinations confirm that $FABP_c$ increase diffusion of LCFA in vitro and in intact cells. For example, I-FABP expression correlates with a 1.8-fold increase in effective cytoplasmic diffusion rate of NBD–stearic acid in undifferentiated, transfected embryonic stem cells (**Table 3**). Differentiation abolished the effect of I-FABP expression in the transfected ES cells (86). The effective cytoplasmic diffusion rate of NBD–stearic acid was 1.7-fold faster ($P < 0.001$) in hepatocytes from female than male animals (Table 3). This correlated well with a 2- to 3-fold higher L-FABP content in the female hepatocytes (79). In liver cells the measured LCFA effective cytoplasmic diffusion rate of NBD–stearic acid was directly proportional to the soluble fraction of LCFA (determined by $FABP_c$) in the cytoplasm (42, 79). Inhibition of NBD–stearic acid binding to the $FABP_c$ in liver hepatocytes reduced the effective cytoplasmic diffusion rate in proportion to the degree of binding inhibition (87). LCFA effective cytoplasmic diffusion can be facilitated not only by $FABP_c$, but also by other cytoplasmic proteins such as sterol carrier protein-2 (SCP-2) which binds LCFA (28, 88). Overexpression of SCP-2 in transfected L-cells increased the effective cytoplasmic diffusion rate of NBD–stearic acid from 1.4-fold in 13 kDa sterol carrier protein-2 expressing cells and 1.5-fold in 15 kDa pro-SCP-2 expressing cells ($P < 0.05$) (Table 3).

It should be noted that the effective cytoplasmic diffusion coefficient in the above measurements is comprised of both membrane (fraction of LCFA in membrane) × (LCFA diffusion coefficient in the membrane) and cytosolic (fraction LCFA in cytosol) × (LCFA diffusion coefficient in cytosol) components that are technically difficult to resolve in intact cells. In the presence of microsomal membranes, L-FABP and I-FABP induced redistribution of LCFA from 2% aqueous to 38% and 26%, respectively, in vitro. If these and other $FABP_c$ induced similar LCFA redistribution in other membranes, this up to 20-fold increase in aqueous LCFA alone would indicate that the actual fold-stimulation of the cytoplasmic diffusion component by the I-FABP and L-FABP is significantly greater than indicated by the effective cytoplasmic diffusion coefficient. Theoretical considerations predict that $FABP_c$ stimulate LCFA diffusion in cytoplasm by an order of magnitude or more (rev. in ref. 83).

TABLE 3. Effect of long chain fatty acid binding proteins on the effective cytoplasmic diffusion coefficient, $D_{eff}$, of NBD-stearic acid

| Protein | Cell Type | $D_{eff}$ ($\times 10^{-10}$ $cm^2$/sec) | n |
|---|---|---|---|
| Control[a] | undifferentiated ES cells | 8.3 ± 0.6 | 17 |
| I-FABP[a] | undifferentiated ES cells | 14.9 ± 1.7** | 17 |
| Control[a] | differentiated ES cells | 8.1 ± 0.6 | 18 |
| I-FABP[a] | differentiated ES cells | 6.9 ± 0.4 | 16 |
| Control[b] | L-cells | 8.7 ± 0.75 | 18 |
| 13 kDa SCP-2[b] | L-cells | 12.1 ± 1.7* | 9 |
| 15 kDa pro-SCP-2[b] | L-cells | 13.2 ± 1.3** | 8 |
| L-FABP[c] | male hepatocytes | 30.5 ± 2.1 | 61 |
| L-FABP[c] | female hepatocytes | 50.3 ± 3.7*** | 42 |

[a] Data for intestinal fatty acid binding protein, I-FABP, taken from ref. 86; ** refers to $P < 0.01$ as compared to control.

[b] Transfected L-cells overexpressing either 13 kDa sterol carrier protein, SCP-2, or 15 kDa pro-sterol carrier protein, pro-SCP-2, were as described (121). All measurements were performed as described previously (86). Values represent the mean ± SEM with n as stated in the table; * refers to $P < 0.05$ as compared to control; ** refers to $P < 0.01$ as compared to control.

[c] Data for liver fatty acid binding protein, L-FABP, taken from ref. 79; *** refers to $P < 0.01$ as compared to male hepatocytes.

### Step 7/Step 8. Net effect of $FABP_c$ expression on LCFA uptake in intact cells

As $FABP_c$ may stimulate not only cytoplasmic diffusion (Step 8) but LCFA desorption (Step 7) as well, it would seem likely that $FABP_c$ expression would enhance net LCFA uptake into certain cell types. Because studies with radiolabeled fatty acids are complicated by rapid (<1 min) intracellular metabolism (esterification, oxidation), slowly metabolizable (less than 3% at 30 min) LCFA such as parinaric acid or NBD–stearic acid were used to determine the effect of LCFA binding protein expression on the uptake component. Several studies with transfected cells have for the first time convincingly demonstrated that expression of LCFA binding proteins correlates with enhanced initial rate and/or maximal uptake of LCFA in intact cells (Table 2). In transfected L-cell fibroblasts expressing L-FABP, uptake of [$^3$H]oleic acid (89) and of fluorescent *cis*-parinaric acid (90) was nearly doubled. The effect is due to elevated maximal uptake and, in part, to increased rate of LCFA of uptake. In the presence of albumin, the rate of palmitic acid uptake by hepatocyte monolayers is determined primarily by cytosolic L-FABP (91). The effect of intestinal I-FABP expression on LCFA uptake is highly dependent on cell type: no effect in transfected L-cell fibroblasts (90); 1.6-fold enhancement of both rate and maximal NBD–stearic acid uptake in transfected undifferentiated (but not differentiated) embryonic stem cells (86); immortalized intestinal Caco-2 cells, transfected with two different alleles of I-FABP, demonstrate differential enhancement of uptake and secretion of radiolabeled LCFA (92). The effect of adipocyte A-FABP expression on radiolabeled LCFA uptake is also highly dependent on cell type: 2-fold enhancement in transfected CHO cells (57), but not in transfected COS7 (39) or L6 myoblasts (93). Likewise, expression of heart H-FABP stimulates LCFA uptake 1.5-fold in transfected *S. cerevisiae* (94), but not in transfected L6 myoblasts (95). Taken together, these transfected cell models provide the first functional evidence correlating L-FABP, I-FABP, A-FABP, and H-FABP expression with up to 2-fold enhancement of LCFA uptake, depending on the cell type and differentiation state of the cell. However, it is important to note that in these studies the effects of $FABP_c$ overexpression on up- or down-regulation of putative plasma membrane LCFA

translocases (see Step 6) or LCFACoA synthetases (see Step 9) are not known.

### Step 9. Fatty acid metabolic targeting: esterification by long chain fatty acyl CoA synthetase as a driving force for LCFA uptake

The rapid intracellular removal of fatty acids from the cytofacial side of the plasma membrane and their subsequent esterification occurs with half-times typically <1 min in the cell. Rapid removal of LCFA from the membrane can be accomplished, at least in part, by LCFA esterification to CoASH mediated by long chain fatty acyl CoA synthetases (LCFACS), ubiquitous, multiple, membrane-associated (mitochondria, endoplasmic reticulum, peroxisome, plasma membrane) enzymes within the cell. At least one of the putative LCFA translocase proteins, the *FAT-1* gene product, is a very long chain fatty acyl CoA synthetase (53).

Schaffer and Lodish (96) provided the first functional evidence that LCFACS expression correlated with LCFA uptake. Using cloning strategies, they identified a gene encoding for a LCFACS, which increased LCFA uptake when overexpressed in cultured cells (Table 2). LCFACS mRNA and the plasma membrane fatty acid transport protein FATP mRNA are coordinately regulated through activation of peroxisome proliferator-activated receptors in adipose tissue (64). Some early studies with native liver L-FABP suggested that L-FABP stimulated the enzymatic activity of LCFACS (rev. in ref. 63). However, subsequent studies with recombinant acyl CoA binding protein (ACBP) and recombinant L-FABP (devoid of possibly contaminating ACBP) showed that ACBP (97), but not L-FABP (22, 97), stimulated LCFACS. However, by binding LCFA-CoA all three known cytosolic LCFA-CoA binding proteins ($FABP_c$, ACBP, SCP-2) inhibit LCFA-CoA hydrolase to increase LCFA-CoA pool size (rev. in ref. 98) and indirectly regulate LCFACS activity thereby.

### Step 10. Fatty acid metabolic targeting: esterification to glycerides (phospholipid, triacylglycerol)

The enzymes responsible for acylating glycerol-3-phosphate to lysophosphatidic acid (GPAT) and subsequently to phosphatidic acid (LAT) are localized in both microsomes and mitochondria. Nearly all the cytosolic LCFA-CoA binding proteins modulate the activities of these enzymes, ranging from up to 28-fold enhancement (99) (C. A. Jolly, D. A. Wilton, and F. Schroeder, unpublished results) to 5-fold inhibition depending on the specific organelle, protein, and isoform (101) involved (**Table 4**). What is most striking is that the effects of the LCFA-CoA binding proteins on stimulating GPAT (up to 28-fold) are much greater than their effect on LCFA uptake or the effects of overexpressing LCFACS (Table 2) or plasma membrane LCFA transporters (Table 2) on LCFA uptake. This suggests the possibility that intracellular esterification to glycerides, dramatically stimulated/inhibited LCFA-CoA binding proteins, is one of the driving forces of LCFA uptake.

Fatty acid binding proteins differentially target LCFA to phospholipids, triacylglycerols, or cholesteryl esters in intact transfected cells. Studies with transfected cells have provided a wealth of information regarding differential targeting of LCFA to phospholipids, triacylglycerols, and cholesteryl esters. Generally speaking, the LCFA-CoA binding proteins modulated radiolabeled LCFA-CoA incorporation in the transfected cells into lipids ranging from 6- to 10-fold stimulation (90, 102, 103) to 8-fold inhi-

TABLE 4. Fatty acid binding proteins alter glycerolipid synthesis

| Enzyme | Organelle | FABP | Ligand/Substrate | + Stimulation or −Inhibition | Reference |
|---|---|---|---|---|---|
| | | | | *−fold* | |
| GPAT | MICRO | L-FABP[ab] | oleoyl CoA | +14 | 101, 122, 123 |
| | | I-FABP[c] | oleoyl CoA | +7 | 22 |
| | | SCP-2[a] | oleoyl CoA | +13 | [d] |
| | | ACBP[a] | oleoyl CoA | +28 | 99,[c,d] |
| | | ACBP[b] | oleoyl CoA | +4 | 99 |
| | | L-FABP[b] | palmitoyl CoA | −3 | [c] |
| | | L-FABP[b] | palmitoyl CoA | +0 to +4 | 101 |
| | | ACBP[a] | palmitoyl CoA | −3 | [c] |
| | | H-FABP[ab] | palmitoyl CoA | +3 | 124 |
| GPAT | MITO | L-FABP[b] | glycerol-3-$PO_4$ | +3 | 100 |
| | | L-FABP[b] | glycerol-3-$PO_4$ | +5 | 100 |
| | | ACBP[a] | palmitoyl CoA | −4 | 97 |
| LAT | MICRO | L-FABP[b] | palmitoyl CoA | +1.4 | 100 |
| | | ACBP[a] | oleoyl CoA | 0 | 100 |
| | | ACBP[b] | palmitoyl CoA | +2 | 100 |
| | | SCP-2[a] | oleoyl CoA | +2 | [d] |
| | MITO | L-FABP[b] | palmitoyl CoA | −5 | 100 |
| | | H-FABP[a] | palmitoyl CoA | +5 | 124 |

GPAT, glycerol-3-phosphate acyltransferase; LAT, lysophosphatidylphosphate acyltransferase; MICRO, lysophosphaphatidic acid acyltransferase microsomes; MITO, mitochondria.

[a] Recombinant.

[b] Native.

[c] C. A. Jolly, D. A. Wilton, and F. Schroeder, unpublished results.

[d] C. A. Jolly, J. T. Billheimer, and F. Schroeder, unpublished results.

bition (89), depending on the specific lipid class involved (phospholipid or triglycerides or cholesterol esters). These effects were highly specific for type of LCFA binding protein, type of cell, and the differentiation state of the cell. Furthermore, these effects were not just due to enhanced LCFA turnover in specific lipid classes, but were reflected in altered lipid class mass. For example, I-FABP expression in undifferentiated ES-cells increased the mass (mg/mg) of cholesteryl esters, triacylglycerols, and phospholipids by 195-, 7-, and 5-fold, respectively (86). These data clearly demonstrate the differential metabolic targeting ability of the LCFA-CoA binding proteins.

### Step 11. Fatty acid metabolic targeting: mitochondrial and peroxisomal oxidation

Fatty acids are a major source of metabolic energy, especially in the heart (rev. in ref. 104). It is thought that the LCFA/LCFA-CoA binding proteins stimulate fatty acid oxidation by delivering LCFA-CoA to mitochondria or peroxisomes (rev. in 63, 104, 105).

### Step 12. Formation and secretion of chylomicrons and VLDL

The newly synthesized phospholipids, triglycerides, cholesteryl esters are incorporated into chylomicrons and secreted into the mesenteric lymph vessels for transport to peripheral tissues (rev. in ref. 106). Although there is no direct evidence that this process can participate in regulating fatty acid uptake, experiments with transfected CACO-2 intestinal (colon derived) cells suggest that expression of I-FABP may stimulate fatty acid uptake and/or secretion as triglycerides and cholesteryl esters (92).

### Conclusions and future directions

LCFA uptake and intracellular trafficking or metabolism appear to be highly regulated. In contrast to extracellular total LCFA which may vary as much as 13-fold (rev. in ref. 107), intracellular LCFA are maintained relatively constant due to multiple pathways whose net effect is to rapidly internalize LCFA, esterify LCFA, and oxidize or secrete esterified LCFA in the cell (Fig. 1). Key extracellular factors include LCFA binding in serum (albumin, lipoproteins, fetuin), the presence of albumin receptors, and the local release of LCFA by lipoprotein lipase anchored at the cell surface. There is considerable discussion regarding nearly all aspects of these processes, especially the driving forces of LCFA uptake, the role of unbound LCFA versus albumin bound-LCFA, the contribution of albumin receptors, and the mechanism whereby LCFA cross the endothelial cell to enter the interstitial space. With regard to the plasma membrane LCFA translocation step, there is as yet no consensus regarding the relative importance of the diffusional versus protein ($FABP_{pm}$, FATP, FAT)-mediated components of LCFA translocation. Although some evidence suggests that the protein-mediated LCFA translocation may require energy or $Na^+$ cotransport, it appears that these proteins act by facilitated diffusion rather than active transport. Consequently, the intracellular processes play a large part in driving cellular LCFA uptake. Intracellular factors include: the presence of cytosolic LCFA/LCFA-CoA binding proteins ($FABP_c$, SCP-2 family, ACBP); LCFA-CoA synthetases (present in many intracellular membranes); glycerolipid and cholesteryl ester synthesis (microsomes, mitochondria); and LCFA oxidation (peroxisomes and mitochondria). Very important to intracellular targeting of LCFA is the presence of cytosolic LCFA binding ($FABP_c$, SCP-2) and LCFA-CoA binding ($FABP_c$, SCP-2, ACBP) protein families that target specific organelles and/or differentially modulate LCFA incorporation into esterified form (phospholipid, triacylglycerol, cholesteryl ester).

To date there is no convincing evidence demonstrating that any one of the above factors universally represents the single rate limiting step in LCFA uptake and metabolism in all cells, tissues, and organs. Part of the problem lies in the paucity of functional and physiological data. Recent studies with transfected cells overexpressing specific LCFA/LCFA-CoA binding proteins have provided some of the first functional data allowing the construction of a relative scale for stimulation of LCFA uptake or metabolism by specific LCFA/LCFA-CoA binding proteins. First, several processes are stimulated 2- to 5-fold (initial rate of LCFA uptake, total LCFA uptake, LCFA effective cytoplasmic diffusion, esterification to LCFA-CoA). Second, another group of intracellular processes is stimulated slightly more, 5- to 28-fold (esterification to glycerides such as phospholipids or triacylglycerol). Third, some processes are stimulated more than 200-fold (cytosolic LCFA-CoA binding protein-mediated cholesteryl ester formation). In short, the cytoplasmic LCFA and LCFA-CoA binding protein-mediated stimulation of intracellular Steps 7–11 are cumulatively and, in some cases individually, several orders of magnitude larger in transfected cells than those observed in Step 6 (LCFA protein-mediated translocation across the membrane). Furthermore, LCFA and LCFA-CoA also act as messengers to regulate gene expression, and induce the proteins to which they bind and the enzymes involved in their metabolism (rev. in refs. 108, 109). In summary, it is possible that many of the effects on LCFA uptake attributed to putative plasma membrane protein translocases may be explicable in large part by intracellular metabolic reactions stimulated by the cytoplasmic LCFA and LCFA-CoA binding proteins. This is especially likely in view of the apparent co-regulation of putative plasma membrane LCFA translocases and the cytoplasmic LCFA and LCFA-CoA binding proteins in many tissues. A focus for future studies will be to further clarify these issues and establish the complex interrelationships of these multiple factors influencing cellular LCFA uptake, intracellular trafficking, and metabolism.

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