Membrane transport of long-chain fatty acids: evidence for a facilitated process

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Abstract In mammalian cells, membrane uptake of long-chain fatty acids is mediated by two separate components; a passive component that is a linear function of the concentration of free fatty acid in the extracellular medium and a saturable component that exhibits the characteristics of a protein-facilitated process. This review summarizes the body of work that has accumulated related to the mechanism of fatty acid transport. Evidence in support of a facilitated uptake process is presented with relation to the different cell types or membrane systems where it was collected. The evidence includes saturation kinetics, competition between different substrates, and sensitivity to a variety of inhibitors. Recent knowledge related to membrane proteins thought to be implicated in the uptake process is reviewed. Factors that may modulate uptake or alter the relative contribution of passive versus facilitated components are briefly discussed. These include the molar ratio of fatty acid to its physiological carrier, plasma albumin and the metabolic or hormonal milieu.—Abumrad, N., C. Harmon, and A. Ibrahimi. Membrane transport of long-chain fatty acids: evidence for a facilitated process. J. Lipid Res. 1998. 39: 2309–2318.

Long-chain fatty acids (FA) are very important physiologically. They provide a major energy source for most tissues. For example, heart tissue can derive up to 70% of its energy needs from FA oxidation (1, 2). FA are components of the triglycerides and cholesteryl esters produced by adipocytes, adrenals, hepatocytes, and macrophages, etc. as part of pathways central to general metabolism. FA are precursors for prostaglandins, which have a variety of regulatory effects (3), and building blocks for phospholipids, which are crucial to membrane integrity and function (4). FA-acylation of membrane proteins can influence their localization and biological role (5–7). FA can also directly modulate ion channel activation, enzyme function, and synaptic transmission (8, 9). More recently, FA were shown to regulate expression of genes involved in lipid metabolism and cell differentiation (10–12). As a result of their multiple functions, FA have been implicated in conditions such as inflammation (13, 14), atherosclerosis (15), immune responses (16, 17) cancer (18), and cell differentiation (19, 20).

Most tissues, which rely heavily on FA, have limited or no capacity for FA synthesis and have to take up the needed FA from the circulation. Based on the general importance of FA it would be expected that cells should be able to regulate FA uptake in order to adapt to changes in energy demands (21–23). For example, the transport of other major substrates, such as glucose, is under finely tuned regulation (24, 25). In the case of FA, however, it was thought that no such regulation was needed at the level of the plasma membrane, as FA could transfer passively across the phospholipid bilayer so uptake would be governed by two factors: the molar ratio of FA to albumin in the circulation and cellular FA metabolism (26). As this review will show, this interpretation has been challenged by a wealth of information, obtained using biochemical and biophysical approaches, that argues for the existence of a facilitated component in membrane transport of long-chain FA.

Determinants of FA uptake

Before discussing the data related to the mechanism of membrane FA uptake, it is helpful to briefly outline the determinants of the uptake process. In the extracellular medium, solubilization and efficient transport of FA is made possible by quantitative binding to plasma albumin. FA complexed to albumin are also used in most uptake assays in vitro to overcome problems resulting from poor FA solubility. In the absence of albumin or other FA-binding proteins, the concentrations of long-chain FA that can be added in assays are very low (27) and can be depleted by cells almost instantaneously. As a result, the linear portion of the uptake time course will be fleeting and impossible to measure. Additional complications are FA adsorption to assay tubes and pipette walls, which can represent a significant fraction of added FA, and FA aggregation. When
FA are added complexed with albumin, uptake follows the molar ratio of FA to albumin rather than the total FA concentration. This was first shown in studies with Ehrlich ascites tumor cells (28) and later confirmed with multiple cell types such as adipocytes (29, 30), hepatocytes (31), and myocytes (32, 33) using a wide range of FA:albumin ratios. There is general agreement that unbound FA, ubFA, is the species that determines cellular uptake. However, its dissociation off albumin and its availability for uptake can become limiting if albumin concentration or the molar ratio used are too low. This can be avoided by increasing the concentration of the FA:albumin complex or by maximizing the volume of assay buffer relative to that of cell suspension (29, 30).

Because ubFA is the species important for cellular uptake its concentration should be known and is generally computed based on FA:albumin binding constants. Estimates obtained using the constants provided by the detailed studies of Goodman (34) and Spector, John, and Fletcher (35) are in the low micromolar range. These have been revised downward based on recent work using novel approaches, which included equilibrium studies of FA between albumin and red cell ghosts (36) and the use of a fluorescent fatty acid binding protein (ADIFAB), (37). The ADIFAB method is especially convenient as ADIFAB is included at low concentration in a solution of protein-bound FA and the amount of ubFA present is evaluated directly from the shift in absorbance for ADIFAB. Current estimates of ubFA levels at circulating FA:albumin ratios are believed to be in the 5–50 nanomolar range (37).

On the intracellular side of the plasma membrane, one or more types of cytosolic FA-binding proteins are present in high amount and are thought to function in solubilizing or buffering intracellular FA and possibly in shuttling them to different cellular compartments (reviewed in 38–40). Although the FA-binding characteristics of cytosolic FA-binding proteins have been studied in detail, the fraction of intracellular FA that is sequestered by these proteins remains unknown. Studies in which intracellular dilution of tritiated oleate was followed do not support a direct role for cytosolic FA-binding proteins in FA uptake as it appears that most of the FA taken up is esterified without being diluted by intracellular FA (41). A role for these proteins in the transfer of lipolyzed FA, for example in adipocytes, is likely to be important and is supported by evidence documenting a collisional mechanism for transfer of the bound FA to membrane (42, 43).

In general, the presence of FA-binding proteins on both sides of the plasma membrane significantly complicates interpretation of FA uptake studies and does not allow the detailed characterization accomplished with other uptake systems. However, despite these limitations, significant progress has been accomplished related to the mechanism of FA uptake. A recent review by Hamilton (44) presented the concept that FA uptake is a simple diffusion process governed by the relative affinities of albumin and bilayer lipid for the FA. It suggested that membrane proteins may only play indirect roles in the process. This review will present the concept that a significant component of FA permeation is protein-facilitated and that the contribution of passive versus facilitated processes to FA uptake will depend on several factors that include FA concentration, cell type, etc. First, we will summarize the extensive biochemical data that argue for the existence of facilitated FA transport. We will then discuss biophysical measurements for the rate of long-chain FA flip-flop across lipid membranes and ask whether flip-flop is fast enough to accommodate FA uptake by all cell types. Finally, a discussion of the proteins that have been identified and implicated in FA transport will be included.

Biochemical evidence for facilitated FA transport

The first argument for facilitated FA transport derived from studies made with isolated rat adipocytes, where two important experimental variables were controlled (29, 30). First, the FA was not esterified by the cell so permeation could be distinguished from metabolism. Second, supply of ubFA by albumin was shown not to limit uptake under the experimental conditions used. FA permeation exhibited non-linear saturation kinetics as a function of the concentration of ubFA. When data from these studies are re-plotted based on the revised (37) ubFA estimates (Fig. 1), the transport $K_m$ obtained is in the low nanomolar range (7 nM). This low value is in line with the expectation that the $K_m$ for carrier-mediated transfer of a particular substrate should be within the physiological concentrations of this substrate. Circulating concentrations of ubFA are in the low nanomolar range (7.5 nm ± 2.5 nm) as ratios of FA to albumin rarely exceed 1 (37). At physiological ratios, the saturable component of FA permeation would account for virtually all cellular uptake. A linear, diffusion-like component becomes more significant as FA:albumin ratios are increased above 1 (29) (Fig. 1). The diffusion component would be expected to contribute more to uptake under conditions where blood FA are increased such as with active intravascular lipolysis of lipoprotein triglycerides. This component is likely to become more significant after exposure to lipolytic agonists that induce FA release from hydrolysis of intracellular triglycerides in adipose tissue. It is also conceivable that diffusion could account for a variable fraction of uptake by cells depending on the density of FA receptors or carriers that are present on the cell membrane.

Saturability of FA permeation was demonstrated in many cell types other than adipocytes. The most comprehensive work was carried out with hepatocytes and cardiac myocytes by Stremmel and Berk (45). Other studies documented saturable uptake in Caco2 human intestinal cells (46) and in myocytes (32, 47). Palmitate transport across giant sarcolemmal membrane vesicles prepared from rat hindlimb skeletal muscles and containing FA-binding protein as a FA sink but no mitochondria was recently studied by Bonen and his collaborators (48). Saturable uptake was demonstrated under conditions where all the FA was recovered unesterified inside the vesicles. The $K_m$ for palmitate was estimated to be about 6 nm and was found to be the same whether vesicles were prepared from red or...
was used for transport assays (29). Irreversible inhibition in completely blocking membrane flux of the FA and stop solution containing phloretin was shown to be effective in completely blocking glucose and anion transport systems. A stop solution containing phloretin was shown to be effective in completely blocking membrane flux of the FA and was used for transport assays (29). Irreversible inhibition in white muscles. On the other hand, the $V_{\text{max}}$ was 1.8-fold greater in red as compared with white vesicles, suggesting that the higher capacity of red muscle to metabolize FA may be related, in part, to a higher transport capacity at the membrane level (48).

In line with saturability of the permeation system, competition between different FA for uptake was demonstrated in many cell systems. In our studies with isolated adipocytes (30), albumin was kept constant, a tracer amount of one FA was used, and the concentration of the same FA or of another competing FA was increased. The curves were compared to determine the respective $K_m$ and $K_i$. Competition was demonstrated in the case of several long-chain FA but not in the case of short-chain FA (less than 8 carbons). In more recent work, Sorrentino et al. (49) carried out rigorous competition studies where they estimated the $ubFA$ concentrations of oleate and palmi-
tate present simultaneously on albumin. The study documented competition between the two FA using isolated hepatocytes and the perfused liver (49). In studies with cultured 3T3-F442A adipocytes, where the movement of fluorescent (and more soluble) FA added at low micromolar concentrations in the absence of albumin was examined, oleic acid inhibited the transfer of the 18C fluorescent FA derivative but was without effect on the 11C analog (50). As greater than 87% of the fluorescent FA remained unesterified inside the cells, competition could not be explained by metabolism. Similar competitive behavior of long- but not short-chain FA was reported with Caco-2 cells (46).

The second biochemical argument supporting the existence of carrier-mediated membrane permeation of FA is sensitivity of the process to protein-modifying agents (Table 1). In studies with rat (29, 30) and human (51) adipocytes, the process was inhibited reversibly by phloretin, an inhibitor of glucose and anion transport systems. A stop solution containing phloretin was shown to be effective in completely blocking membrane flux of the FA and was used for transport assays (29). Irreversible inhibition was observed by reacting adipocytes with diisothiodisul-
fonic acid (DIDS) (51), and with reactive sulfosuccinimidyld derivatives of oleate and myristate, SSO and SSM (52, 53) (Fig. 2). DIDS also inhibited the transfer of fluorescent FA derivatives by cultured adipocytes (50). Palmitate uptake into rat renal basolateral membrane vesicles (BLMV) exhibited a component that was responsive to an inwardly directed proton gradient and was significantly inhibited by DIDS (54). SSO was reported to inhibit FA uptake by the perfused rat heart (53, 55). In isolated rat my-
ocyes (47) and in sarcolemmal vesicles from red and white skeletal muscle (48), phloretin and SSO were inhibitory at concentrations similar to those reported for iso-
olated adipocytes (Table 1). In recent work, (56) photoaf-
finity labeling of hepatocytes with the 11-azistearate derivative irreversibly inhibited FA uptake without affect-
ing transport of cholyltaurine and diffusion of 5 β-
choles-
tane-3α, 7α, 12 α-triol. Azistearate was not metabolized and it labeled less than 1% of intracellular FA-binding protein so it was concluded that inhibition was not medi-
ated by effects on intracellular FA-binding or metabolism.

FA transport was generally found to be less sensitive to proteases. Pronase but not trypsin was effective after a prolonged digestion in adipocytes (30) and 0.25% trypsin (but not 0.05%) was 50% inhibitory in myocytes (47). Pro-
teases were without effect in Caco-2 cells (46).

An overview of the inhibitors found to be effective on FA transport and listed in Table 1 indicates that a majority of these compounds were known anion inhibitors. This would suggest that the putative FA carrier may share functional similarities with the anion carriers. Two additional points are apparent from Table 1. First, inhibition magnitude for the same agent or for agents with very similar mechanisms of action differed substantially between studies. This could suggest that the contribution of the protein-facilitated component of FA uptake varied with the cell type or membrane system used and reflected the den-
sity of membrane FA-binding proteins present. However, other factors might have been equally if not more impor-

![Fig. 1. Saturable and nonsaturable components of oleate uptake. The data shown are for isolated rat adipocytes (29) with unbound FA concentrations calculated as described in ref. (80). Adipocytes were held in KRH buffer containing 1% gelatin in order not to disturb medium fatty acid:BSA ratio which ranged from 0.25 to 2.0. Top curve represents total uptake while the bottom curve reflects the estimated diffusion component present at the various ratios.](image-url)
tant. For example, the presence or absence of albumin during the incubation with inhibitor and the FA:albumin ratio used for transport assays could also have important effects. Second, it is significant, that despite the variability mentioned above, the contribution of facilitated uptake always exceeded 54% of total uptake.

In addition to protein-modifying agents, FA transport was shown to be partially inhibited by an antibody raised against a membrane-associated protein, which was isolated by oleate affinity chromatography (see last section). This protein was later shown to be identical to aspartate aminotransferase (AspAT). Antibody against AspAT inhibited FA permeation by hepatocytes and adipocytes (57, 58). An antibody against CD36, a membrane glycoprotein implicated in the binding/transport of long-chain FA, inhibited arachidonic acid uptake by platelets (59).

FA transfer may limit intracellular FA metabolism

A transport step is likely to be an important site of regulation if it rate-limits cellular metabolism of the transported substrate. For example, in isolated adipocytes exposed to a wide range of glucose concentrations, it can be shown that intracellular glucose stays at a value that is only 20% of extracellular glucose (60) indicating that the metabolic capacity for glucose exceeds its transport capacity.

### TABLE 1. Agents that inhibit FA transport and cell types where they are effective

<table>
<thead>
<tr>
<th>Agent, Concentration</th>
<th>Inhibition</th>
<th>Type</th>
<th>Cell Type</th>
<th>Inhibitor</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phloretin 0.2 mM</td>
<td>65 reversible</td>
<td>adipocytes</td>
<td>glucose, anion inhibitor</td>
<td>29, 30</td>
<td></td>
</tr>
<tr>
<td>0.2 mM</td>
<td>60 myocytes</td>
<td></td>
<td></td>
<td>47</td>
<td></td>
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<tr>
<td>0.4 mM</td>
<td>78 cardiomyocytes</td>
<td></td>
<td></td>
<td>47</td>
<td></td>
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<tr>
<td>0.5 mM</td>
<td>90 neutrophil vesicles</td>
<td></td>
<td></td>
<td>104</td>
<td></td>
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<tr>
<td>sarcolemmal vesicles</td>
<td></td>
<td></td>
<td></td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>DIDS 0.4 mM</td>
<td>90 irreversible</td>
<td>rat adipocytes</td>
<td>anion inhibitor</td>
<td>51–53</td>
<td></td>
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<tr>
<td>0.4 mM</td>
<td>75 human adipocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F442A adipocytes</td>
<td></td>
<td></td>
<td>51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>basolateral vesicles</td>
<td></td>
<td></td>
<td>55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSO 0.4 mM</td>
<td>80 irreversible</td>
<td>rat adipocytes</td>
<td>FA derivative</td>
<td>51–53</td>
<td></td>
</tr>
<tr>
<td>0.5 mM</td>
<td>90 human adipocytes</td>
<td></td>
<td></td>
<td>Fig. 2</td>
<td></td>
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<tr>
<td>perfused heart</td>
<td></td>
<td></td>
<td>55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cardiomyocytes</td>
<td></td>
<td></td>
<td>47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sarcolemmal vesicles</td>
<td></td>
<td></td>
<td>48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Azistearate</td>
<td>irreversible</td>
<td>hepatocytes</td>
<td>FA derivative</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>Pentachlorophenol 0.4 mM</td>
<td>98 reversible</td>
<td>neutrophil vesicles</td>
<td>anion inhibitor</td>
<td>103</td>
<td></td>
</tr>
<tr>
<td>Diisosalicylate 0.5 mM</td>
<td>89 reversible</td>
<td>neutrophil vesicles</td>
<td>anion inhibitor</td>
<td>103</td>
<td></td>
</tr>
<tr>
<td>Quercetin 1.0 mM</td>
<td>88 reversible</td>
<td>neutrophil vesicles</td>
<td>anion inhibitor</td>
<td>103</td>
<td></td>
</tr>
<tr>
<td>Pronase 1 mg/ml</td>
<td>54 rat adipocytes</td>
<td>proteolytic</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mg/ml</td>
<td>87 neutrophil vesicles</td>
<td>enzyme</td>
<td>103</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trypsin 2.5 mg/ml</td>
<td>94 neutrophil vesicles</td>
<td>proteolytic</td>
<td>103</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5 mg/ml</td>
<td>78 cardiomyocytes</td>
<td>enzyme</td>
<td>47</td>
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</tbody>
</table>

*C. Harmon and N. Abumrad, unpublished observations.*

**Fig. 2.** Effect of sulfosuccinimidyl-oleate (SSO) on the time course of the oleate transport in human adipocytes. Isolated human adipocytes were pre-incubated with SSO at various concentrations for 25 min at 37°C. The cells were then washed three times to remove all unreacted SSO. Subsequently, [³H]oleate uptake was measured at 23°C as described in ref. (80). Final concentrations of [³H]oleate and BSA in the assay mixture were, respectively, 50 μM and 100 μM.
The situation is reversed by treatment with insulin which produces a 30-fold increase in transport capacity and equilibration between extracellular and intracellular glucose. Similar measurements relating FA uptake and metabolism are difficult to obtain as they are complicated by binding of the FA outside and inside the cell. The concentration of uBF can be estimated for the extracellular medium, as the total amounts of FA and binding protein are known, but it cannot be done for the intracellular space because distribution of intracellular FA between binding proteins and intracellular membranes is not known. However, there is indication that transport of FA may rate-limit cellular metabolism and may be subject to modulation.

First, extracellular FA do not appear to equilibrate with intracellular FA, which would suggest that the exogenous FA is being metabolized as fast as it enters the cell (41). Two phases can be distinguished in the initial time course of FA uptake by adipocytes (29, 41). The early one, which would represent approach of intracellular FA to isotopic steady state, is very short (0.2 min). If all measured cell FA exchanged with extracellular FA, isotopic equilibration would have required a much longer time (estimated at 5 min). In addition, dilution of exogenous label inside the cell by intracellular unesterified FA is very low (as assessed by [3H]oleate/[14C]glucose incorporation ratios) indicating that the FA exchanges with less than 2% of cell FA.

Second, there is evidence for regulation of FA transport. We have shown that FA influx and efflux in and out of adipocytes were stimulated by norepinephrine and this was mediated by the cAMP-protein kinase A pathway (41). An earlier study by Schimmel and Goodman (61) reported that efflux of preformed FA from adipocytes is accelerated by cAMP and may require energy. In both our studies and those of Schimmel and Goodman (61), assays were carried out in the presence of lipolytic inhibitors to distinguish effects on transport from those on lipolysis. Although localized and undetectable alterations of intracellular free FA concentration cannot be ruled out and may have complicated interpretation, these findings strongly support the possibility that FA transport is regulated by cAMP and that changes in activities of transport and of hormone-sensitive lipase are coordinated to bring about optimal lipolysis and FA release. More recent studies have suggested a similar co-regulation of FA uptake and FA utilization in muscle tissue. Turkicke et al. (62) working with the perfused heart reported that muscle contraction was associated with an increase in the uptake and oxidation of palmitate, which was independent of plasma flow and increased palmitate delivery. The authors concluded that the data were consistent with an effect of contraction on the membrane transport of the FA. This same interpretation was reached by Bonen and collaborators (48, and personal communication) who demonstrated an increase in FA transport \( V_{\text{max}} \) in giant sarcolemmal vesicles prepared from electrically stimulated muscle. In these studies, transport was measured in the vesicles and was not complicated by metabolism of the FA. It remains to be determined whether electrical stimulation/contraction can produce translocation of FA transporters to the plasma membrane, similar to what occurred with insulin stimulation of glucose transport (24, 37), or whether it results in activation of transporters already present in the membrane. At any rate, the above studies advance the concept that FA transport may rate-limit FA metabolism in some tissues, notably muscle, such that its activation is required under conditions where FA utilization is increased. Ongoing studies by several groups of investigators should provide us with more definitive information related to whether this is correct.

FA flip-flop may not be fast enough to accommodate cellular uptake

A physical–chemical model for entry of FA and other water-insoluble compounds into cells has been proposed (44, 63), based on the assumption that transfer of FA across lipid bilayers is very rapid. In its most simplified form, the model argues that there is no FA transporter since such a carrier is not needed (44, 63–65). The model proposes that FA transfer easily from albumin to the outer leaflet of the plasma membrane. In the membrane, the extent of FA protonation is increased as a result of an apparent shift in the FA pK\(_a\) from 5 to about 7–8. The protonated and lipid-soluble FA can then flip-flop rapidly to the inner leaflet. This model, although attractive because of its simplicity, does not address important aspects of FA uptake. First, significant transfer of FA from albumin to bilayer lipid was only demonstrated at very high (2–8) FA to albumin ratios (44, 66) and not at more physiologically relevant ones (0.25–1.0) where transfer may be limited by tight FA binding to the high affinity sites on albumin. Second, although a significant fraction of the FA partitioned in the membrane may be protonated, the relative contributions of the protonated and ionized forms to FA uptake remain uncertain. It is worth noting, as apparent from Table 1, that FA uptake in a variety of cells or membrane vesicles is sensitive to a list of known anion inhibitors and that these inhibitors can often block the major part of the uptake process. Finally, the interpretation that the FA partitioned in the outer leaflet of the membrane can flip-flop to the inner leaflet at a rate that is fast enough to support cellular metabolism is still open to question, as recently demonstrated by the work of Kleinfeld, Chu, and Storck (67, 68) and discussed below.

Although there is agreement that short-chain FA can transfer very rapidly across lipid membranes (66, 69), in the case of long-chain FA the rates measured differ considerably. Investigators also disagree on the characteristics and dependences of the transfer process. Zhang, Kamp, and Hamilton (64) and Kamp et al. (65) measured transfer of long-chain FA from the drop in pH across phospholipid vesicles entrapping pyranin and reported flip-flop rates shorter than 20–30 ms (rate constant of 15 s\(^{-1}\)). The rates measured were independent of chain length and comparable for native and fluorescent FA. These findings contrasted with those of Kleinfeld and collaborators (67, 70) who described the transfer of fluorescent FA (AOFFA) between phospholipid vesicles as a bi-exponential process consisting of two components with the slowest and rate-limiting component being flip-flop of the FA between bi
layer leaflets. Using the pyranin methodology and a second technique, where carboxylfluorescein was trapped within lipid vesicles, the authors measured a transfer rate for long-chain fluorescent FA of more than 50 s (67). Transfer of native FA, measured using the pyranin method or ADIFAB entrapped within vesicles, was faster than for fluorescent FA (71, 72) and it decreased with an increase in FA chain length. Both observations suggested that formation of a free volume cavity or defect within the lipid bilayer was involved that limited the process. In the case of giant unilamellar vesicles, oleate flip-flop required more than 3 s. The authors concluded that in cell-size membranes, bilayer transfer of long-chain FA may be limiting to cellular uptake (72). For example, they estimated it could be 1.5- to 50-times slower than the uptake needed to support FA metabolism by isolated myocytes.

It is likely that FA flip-flop rates, derived in vitro, will have to be revised before extrapolation to native cell membranes, as a result of differences related to lipid composition, size, and the presence of protein. Still, the in vitro data cannot be interpreted as a definitive or strong argument against the existence of protein-facilitated FA transfer. On the contrary, as argued by Kleinfeld and collaborators (67, 70), the possibility that flip-flop may limit cellular uptake, at least for cells highly active in FA metabolism, should be considered seriously.

Membrane proteins implicated in FA transport

There are several ways that a membrane protein could facilitate the transport of long-chain FA. The protein could function as a very high affinity FA receptor. Under physiological conditions of low FA:albumin ratios (0.1 to about 1.0), it would be advantageous for cells very active in FA utilization to possess a high affinity mechanism allowing recruitment of needed FA from albumin and the generation of high local concentrations of FA. FA would be similar to other quantitatively important cellular substrates, which are targeted to tissues by tissue-specific receptors or carriers. Alternatively, the protein could function as a specialized carrier. Transport could be facilitated either by decreasing the activation energy required for the transport of the polar FA head group through the lipid bilayer or by destabilizing the FA partitioned in the outer leaflet. For example, the existence of a pore or channel along the protein/lipid interface would provide favorable interactions for the carboxyl group. Alternatively, hydrophobic interactions of the FA chain within the outer leaflet could be destabilized by binding with a protein. FA-binding might also occur within the lipids interfacing with the protein, which are in a more disordered state than the bulk lipid. This last interpretation was suggested by Bojesen and Bojesen (73) who studied the kinetics of red cell membrane binding for various long-chain FA and described specific but independent binding sites for oleic, palmitic, and arachidonic acids.

Whatever the mechanism of protein-facilitated FA transport, one of its major advantages could be to help channel the FA into specific metabolic sites in line with the concept of functionally specialized membrane domains (74, 75). Future research might elucidate important interactions between membrane and intracellular proteins that would promote directional and more efficient substrate transfer from bilayer to enzymes. For example, it would be tempting to postulate such an interaction between intracellular proteins that bind FA or key metabolic enzymes, such as the acyl-CoA synthetase and membrane FA-binding proteins.

Several groups have devoted much effort towards identifying the FA carrier(s) in mammalian cells. At least three candidate proteins have been cloned and are being characterized. The main findings are discussed in this section and a more detailed discussion will be presented elsewhere (N. Abumrad and A. Ibrahimi, unpublished observations).

Our studies identified an adipocyte 88 kDa membrane protein (FAT/CD36) as a candidate FA carrier by covalent labeling with inhibitors of the FA-transport step (52–54). We synthesized monofunctional esters of the 3H-labeled long-chain FA, myristate and oleate (SSM and SSO) and showed that they irreversibly inhibited FA transport. When binding to membranes was examined, one integral membrane protein was labeled by three inhibitors of FA transport, DIDS, SSM, and SSO. The protein was isolated and then a full coding clone from a fat cell cDNA library was obtained based on its amino-terminal sequence (53). The clone coded for a protein with 85% homology to human platelet CD36. Our data with this clone, summarized below, strongly support a role for CD36 in the transport of long-chain fatty acids.

CD36 distribution favors tissues with a high metabolic capacity for FA (adipose, muscle, heart, intestine) while it is absent from tissues like brain that do not utilize exogenous FA (76). In muscle, expression is much higher in muscles with a predominance of red oxidative fibers and it is up-regulated with electrical stimulation and muscle contraction (48). CD36 overexpression in muscle tissues in mice leads to changes in circulating lipids that are consistent with an accelerated clearance of FA (A. Ibrahimi and N. Abumrad, unpublished observations).

CD36 mRNA is induced by preadipocyte differentiation into adipocytes and induction is paralleled by an increase in the uptake of long-chain FA. The mRNA is also strongly induced by FA in preadipocytes, similar to the mRNA of other genes coding for proteins involved in FA metabolism like acyl CoA synthetase and glycerolphosphate dehydrogenase (77, 78). Induction by FA has also been reported in neonatal cardiomyocytes (79).

Expression of CD36 in fibroblasts lacking this protein induces appearance of a saturable, high affinity, phloretin-sensitive component of FA uptake (80). The transport K_m of this component is within the range of plasma ubFA. The CD36 protein purified from adipose tissue binds long-chain FA but not short chain FA (81).

Finally, regulation of CD36 expression is consistent with its role in FA binding/transport. CD36 is increased in the muscle of diabetic animals (82, 83) and in mice fed a high fat diet (82) and is modulated by metabolic conditions where FA utilization is altered (84). It is expressed and co-regulated with the cytosolic FA-binding protein in mam-
mary tissue (85) and heart muscle (86). Expression of CD36 in the intestine was found to be very high in the jejunum where the bulk of FA absorption occurs and it was up-regulated by a FA load (87).

The reactive FA derivative, SSO, was used to purify the heart tissue CD36 homologue by Kusaka et al. (55) and Tanaka, Sohmiya, and Kawamura (88). The same authors reported an association between deficiency or mutation in CD36 and hypertrophic cardiac myopathy in humans and linked it to impaired uptake of long-chain FA by the myocardium (89). Involvement of CD36 in the uptake of arachidonic acid by platelets and possibly in platelet activation by FA was documented (59).

Plasma membrane FA-binding protein, FABPpm

Stremmel and collaborators (90) presented extensive evidence to support the function in FA transport of a 40 kDa protein (FABPpm) loosely associated with the plasma membrane. The protein was isolated by olate-agarose affinity chromatography from solubilized rat hepatocyte plasma membranes. It was later shown that FABPpm was a plasma membrane-bound form of mitochondrial aspartate aminotransferase (mAspAT) (57).

The evidence supporting involvement of the membrane protein, FABPpm, in FA transport was initially based on the observation that an antibody raised against it partially inhibited FA uptake in a variety of cell types, which included hepatocytes, myocytes, and adipocytes (57, 58). However, more recent supporting data were obtained using a multiplicity of experimental approaches.

Expression of FABPpm in Xenopus laevis oocytes (91) and in 3T3 fibroblasts (58) was associated in each case with an increase in FA uptake rates (58, 91). The increase in 3T3 fibroblasts reflected the addition of a saturable component and could be inhibited by an antibody against the protein.

Regulation of FABPpm during differentiation of 3T3-L1 cells (59) and in adipocytes of Zucker rats with diabetes (84) has been observed. The protein has also been reported to be regulated during endurance training and with fasting (92, 93) consistent with its potentially important role in FA metabolism.

Fatty acid transport protein

A 63 kDa protein has been recently identified by Schaffer and Lodish (94) using expression cloning and fluorescent FA. Screening of COS7 cells for fluorescence after expression of cDNAs from a 3T3-L1 adipocyte cDNA library yielded two types of cDNAs. One of these coded for a novel protein of 71.3 kDa with four to six possible transmembrane domains that was designated as FATP. The other cDNA coded for a protein with a high analogy to liver fatty acyl CoA synthetase (94).

Overexpression of FATP in cells is associated with an increase in the uptake of fluorescent FA (94). Two homologs of FATP were cloned recently. The rat homolog of FATP (95) had a predicted protein sequence that was highly similar (97%) and the yeast (S. cerevisiae) homolog (96) had a 54% amino acid sequence similarity. FATP is ubiquitous with relatively high expression in the brain (95, 97).

FATP has interesting properties. It has sequence similarity with very-long-chain FA-acyl CoA synthetases (98) and has two domains that are specific to members of the firefly luciferase family and that are related to ATP-binding and hydrolysis. The AMP-binding site is highly conserved and appears essential for FA transport activity (J. E. Schaffer, personal communication). The significance of this finding remains uncertain at present as there is little evidence for active transport of FA. The presence of an AMP-binding motif would suggest that FATP has CoA-synthetase activity (99) and that its effect on transport might be indirect and mediated by enhancing metabolism of the FA. The authors did not completely rule out this possibility although they considered it unlikely based on the observation that uptake of a variety of long-chain FA (oleic, palmitic etc.) was increased in FATP-expressing cells despite the fact that only 30% of the FA was recovered esterified. However, the proportion of unesterified FA, determined at 15 sec may not have accurately reflected the situation at the 1-min uptake measurement shown (94).

Recently, Faergeman et al. (96) described a fatty acid transport, FAT1, protein in the yeast (S. cerevisiae) based on its homology to FATP. Disruption of the FAT1 structural gene resulted in a marked decrease in cell growth on medium containing FA and cerulenin. FA uptake and incorporation of exogenous olate into the phospholipid pool were decreased. Acyl-CoA synthetase activity in the wild strain and in the FAT1 defective strain were comparable, while olate and palmitate were substrates. Based on this, the authors concluded that the data were consistent with FAT1 functioning as a FA transporter. Recently Watkins et al. (99) were able to document decreases in very long chain acyl-CoA synthetase after disruption of the FAT1 gene activity and established that FAT1 has acyl-CoA synthetase activity against both very-long- and long-chain FA.

It is possible that the protein may function both as a transporter for long-chain FA and as a CoA-synthetase for very-long-chain FA. For example, its enzymatic activity may be important in the brain which utilizes very-long-chain FA and where FATP is highly expressed (95, 98).

Finally, regulation of FATP mRNA by fasting and by insulin was recently documented (84, 100).

In summary, much compelling biochemical and biophysical evidence supports the existence of two components of FA transport: a simple diffusion component that increases in significance as the concentration of unbound FA is increased, and a protein-mediated component that would be a major contributor at low physiological ubFA concentrations. This evidence has motivated recent work aimed at the identification of the membrane proteins involved in the transfer process. Three such candidates have been characterized and implicated in binding or uptake of the FA, but there is still little knowledge related to the mechanism involved in facilitation of FA uptake by any of these proteins. The precise role of each protein and the
physiological significance of this role are likely to depend on the specific tissues or metabolic conditions considered as tissue distribution and regulation differ for all three proteins (101, 102). Interaction between membrane and intracellular FA-binding proteins is also an area of research where there is little known but where findings could have important metabolic implications.

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REFERENCES


