Fatty acid transport: the roads taken

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Schaffer, Jean E. Fatty acid transport: the roads taken. Am J Physiol Endocrinol Metab 282: E239–E246, 2002; 10.1152/ajpendo.00462.2001.—Efficient uptake and channeling of long-chain fatty acids (LCFAs) are critical cellular functions. Although spontaneous flip-flop of nonionized LCFAs from one leaflet of a bilayer to the other is rapid, evidence is emerging that proteins are important mediators and/or regulators of trafficking of LCFAs into and within cells. Genetic screens have led to the identification of proteins that are required for fatty acid import and utilization in prokaryotic organisms. In addition, functional screens have elucidated proteins that facilitate fatty acid import into mammalian cells. Although the mechanisms by which these proteins mediate LCFA import are not well understood, studies in both prokaryotic and eukaryotic organisms provide compelling evidence that uptake of LCFAs across cellular membranes is coupled to esterification by acyl-CoA synthetases. This review will summarize results of studies of non-protein-mediated and protein-mediated LCFA transport and discuss how these different mechanisms may contribute to cellular metabolism.

FATTY ACIDS ARE CRITICAL MOLECULES FOR DIVERSE CELLULAR FUNCTIONS

Long-chain fatty acids (LCFAs) are fuels that can be used to generate ATP efficiently primarily through \[\beta\]-oxidation in mitochondria and peroxisomes. The capacity of vertebrate organisms to store this fuel as triglycerides in cytosolic lipid droplets is an important adaptation for survival during periods of nutritional deprivation. In addition, fatty acids are precursors for the biosynthesis of complex membrane lipids. The acyl chain composition of these lipids helps to determine overall membrane structure and function. Fatty acids are also precursors for lipid-signaling molecules and serve as ligands for transcription factors that control cellular metabolic gene expression.

LCFAs can be generated endogenously in cells from de novo biosynthesis, hydrolysis of triglycerides, and recycling of membrane lipids, or they can be imported into cells from exogenous sources. De novo LCFA biosynthesis in prokaryotic and eukaryotic cells is catalyzed by the highly regulated enzymes acyl-CoA carboxylase and fatty acid synthase (FAS). Prokaryotic organisms require exogenous fatty acids under hypoxic conditions and under circumstances in which the activity of FAS is inhibited and fatty acids are supplied as the sole carbon source in the media. Although most eukaryotic cells are capable of de novo fatty acid synthesis, cells with large requirements for fatty acid \[\beta\]-oxidation, such as cardiac myocytes, rely on import of exogenous LCFAs to provide this critical metabolic fuel. Cells that store potential energy in the form of triglyceride, such as adipocytes, have efficient mechanisms for both LCFA import and export.

How cells import LCFAs has been the subject of much investigation. Experimental evidence provides support for two classes of transport mechanisms, non-protein-mediated and protein-mediated permeation. Much of the literature has focused on determination of which single mechanism best describes LCFA transport; however, there is evidence to suggest that both types of transport play important roles, albeit under different physiological or pathophysiological conditions.

FATTY ACIDS CAN FLIP-FLOP RAPIDLY ACROSS MODEL MEMBRANES

Amphiphilic LCFAs are poorly soluble in aqueous solutions. Although these molecules are ionized at
physiological pH, they readily partition into membranes in which they may be protonated (the ionization constant ($pK_a$) in the environment of the phospholipid bilayer is 7.6, whereas the $pK_a$ is 4.5 in an aqueous compartment where the pH is 7.4 (27)). Movement of LCFA anions from one leaflet to the other is slow (25, 55); however, movement of nonionized LCFA from one leaflet to the other (flip-flop) is extremely rapid. Thus it has been proposed that LCFA enters cells by a process involving adsorption to the membrane, protonation, flip-flop within the membrane, and desorption into the cytosolic space (29). This model does not require facilitation by membrane proteins.

Initial evidence for this non-protein-mediated mechanism of LCFA transport came from in vitro studies showing rapid rates of flip-flop of nonionized LCFA in unilamellar phosphatidylcholine (PC) membrane vesicles. Flip-flop can be monitored by measuring the quenching of a pH-sensitive fluorescent probe, pyranin, that is trapped on the inside of the vesicles. When free LCFA are added to the aqueous compartment, they rapidly adsorb to the membranes (not rate limiting), and changes in fluorescence represent ionization of the LCFA after its appearance at the inner leaflet. In large unilamellar vesicles (LUVs, diameter 100 nm), addition of high concentrations of free LCFA leads to flip-flop within milliseconds, with a pseudomolecular rate constant for flip-flop ($K_{FAH}$) of 15 s$^{-1}$ and a halftime ($t_{1/2}$) of 23 ms (35). Studies of flip-flop in model membranes have been extended by use of intracellular pH indicators to measure intracellular acidification upon flip-flop of fatty acids across the plasma membrane of pancreatic β-cells (28) and adipocytes (13). In β-cells, the rate of acidification upon addition of extracellular LCFA is slower than for LUVs by several orders of magnitude, with a $t_{1/2}$ of 60 s. Both in LUVs and in cells, the lack of dependence of the rate of acidification on fatty acid chain length or structure (fatty acid dimers or alkylamines have similarly rapid rates) suggests a non-protein-mediated mechanism.

Monitoring intravesicular or intracellular pH changes provides a means for real-time measurements of LCFA flip-flop. Nonetheless, several caveats to these interpretations should be noted. Most of these studies have been performed using supraphysiological free LCFA concentrations that may contribute to the high rates observed. The volume in LUVs (diameter 100 nm) is less than that of a cell (average diameter 20 μm) by a factor of 10$^4$; thus rates of flip-flop measured by changes in concentration (pH) rather than the number of molecules will be higher in smaller volume compartments. Synthetic PC vesicles may not reproduce with fidelity the behavior of cellular membranes that contain a mixture of complex lipids and proteins (38). Although intracellular acidification is consistent with increases in intracellular LCFAs, and intracellular alkalinization is consistent with decreases in intracellular LCFAs (13), these measurements are indirect measures of the movement of the LCFA themselves. Finally, flip-flop of nonionized LCFA within the membrane and subsequent ionization are not necessarily the equivalent of transport of LCFA from the extracellular aqueous space to the intracellular aqueous space for metabolic utilization. Desorption of LCFA (particularly those of longer chain lengths and higher degrees of saturation) from phospholipid bilayers may become rate limiting (75).

Some of these caveats have been addressed in studies using the soluble fluorescent free LCFA indicator ADIFAB to measure the appearance of LCFA in the aqueous phase of the interior of vesicles (38) and red blood cell ghosts (37). These experiments revealed that LCFA were added to LUVs (diameter 100 nm) or giant unilamellar vesicles (GUVs, diameter 200 nm) or to red blood cell ghosts by use of LCFA-BSA complexes with unbound (free) LCFA concentrations in the 14- to 190-nm range. By measuring the changes in fluorescence of ADIFAB trapped within ghosts, the investigators measured the appearance of LCFA in the internal aqueous environment. As predicted, increase in the diameter of PC/cholesterol vesicles from 100 nm (LUVs) to 200 nm (GUVs) decreases the rate of flip-flop by an order of magnitude (38). Nonetheless, rapid rates of flip-flop are observed in red blood cell ghosts, with a constant for flip-flop ($K_{FA}$) ranging from 0.3$^{-1}$ to 3.0 $s^{-1}$ and without evidence of saturation or protease sensitivity (37). This study shows that LCFA permeation across naturally occurring cellular membranes can be extremely rapid and may not occur through protein-mediated mechanisms. This study does not rule out the possibility that alternate or additional mechanisms may be used for LCFA transport for cells with high LCFA metabolic requirements (38), particularly in settings of low physiological unbound LCFA concentrations (low nanomolar range).

GENETIC SCREENS IDENTIFY PROTEINS REQUIRED FOR FATTY ACID UPTAKE IN ESCHERICHIA COLI

The first proteins involved in LCFA transport were identified by genetic studies in the gram-negative bacteria *E. coli*. These simple organisms transport LCFA across an outer membrane consisting of lipopolysaccharide and phospholipid, a layer of peptidoglycan associated with the inner leaflet of the outer membrane, the periplasmic space, and a cytoplasmic or inner membrane. This envelope is normally impermeable to LCFA. Transport across this barrier is necessary under growth conditions in which de novo LCFA biosynthesis is inhibited by cerulenin and LCFA are provided as the sole carbon source. Screens of mutant cells grown under such conditions led to identification of two genes required for LCFA transport in *E. coli* (reviewed in Ref. 8). The *fadL* gene encodes an outer membrane-bound fatty acid transport protein (FATP) that contains separable regions involved in fatty acid binding and transport (40, 50). The *fadD* gene product is an inner membrane-associated acyl-CoA synthetase (ACS) (9, 36). The observation that LCFA transport for utilization in metabolic pathways is intimately linked to esterification led Klein et al. (36) to propose a mechanism termed “vectorial acylation.” Esterification of
imported LCFA s at the inner aspect of the membrane concomitant with transport would effectively deplete intracellular concentrations of free LCFA s and render the permeation step irreversible. The action of ACS may be the limiting step in LCFA permeation, although ACS may function together with other membrane proteins to transport LCFA s across the membrane.

KINETIC EVIDENCE FOR PROTEIN-MEDIATED 
TRANSPORT IN EUKARYOTIC CELLS

Kinetic analyses of LCFA transport in mammalian cells by use of radiolabeled substrates have also suggested a role for proteins in mediating this cellular function. Initial rates of LCFA transport into isolated rat adipocytes are maximal and linear for 15 s, are a function of concentration of unbound LCFA in the medium, and demonstrate both saturable and nonsaturable components (3, 66, 67). The saturable components are observed primarily at LCFA-to-BSA (LCFA/BSA) ratios <3.0, consistent with the physiological range. In normal human serum, the average LCFA-to-albumin (LCFA/albumin) ratio is 0.74 (53). The nonsaturable components of LCFA uptake are observed at LCFA/BSA ratios ≥3.0. Although such ratios exceed the physiological range, LCFA/albumin ratios as high as 7.5 have been reported in pathophysiological states (39). The rate constant for saturable transmembrane influx in adipocytes (Kₚ = 2.9 s⁻¹) is 10- to 30-fold higher than the rate constant for nonsaturable uptake (Kₑ = 0.26–0.10 s⁻¹, with t₁/₂ = 2.7–6.6 s, depending on LCFA-BSA binding constants used to calculate free fatty acid concentrations) (66). The saturable component is specific for LCFA s but not for medium-chain fatty acids, and competition for transport among a variety of LCFA s and LCFA analogs has been demonstrated (2). Studies in which saturable LCFA uptake is inhibited by prior protease treatment of the cell surface suggest that, in higher organisms, LCFA s are transported by a protein-mediated mechanism (2, 3, 45, 61). Such a mechanism would potentially provide a means for efficient LCFA import, when fatty acid concentrations are in the physiological low nanomolar range, and a means for regulation of LCFA permeation.

PROTEINS THAT BIND FATTY ACIDS AT THE CELL 
SURFACE FACILITATE LCFA TRANSPORT

To identify specific proteins that facilitate LCFA transport in mammalian cells, initial efforts took advantage of the observation that many transport proteins bind their substrates specifically with high affinity. A plasma membrane fatty acid binding protein (FABPpm) was purified by oleate-agarose affinity chromatography from hepatocytes, adipocytes, jejunal enterocytes, and cardiac myocytes and was proposed to play a role in fatty acid import (51, 60, 62). This protein shares amino acid identity with mitochondrial asparagine aminotransferase (mAspAT) (68), a protein synthesized on free polyosomes in the cytosol, with an amino-terminal mitochondrial targeting sequence that is clipped after mitochondrial translocation. Expression of the cDNA encoding mAspAT in fibroblasts increases saturable LCFA uptake (34). Antibodies directed against FABPpm bind the plasma membrane of cells and diminish fatty acid import (63, 76). These studies are consistent with a model in which FABPpm is targeted to the cell surface, where it facilitates LCFA transport; although the mechanism by which this globular protein is targeted to and tethered at the plasma membrane after synthesis on free polyosomes in the cytoplasm remains unclear.

CD36 (also known as fatty acid translocase or FAT) was identified to play a role in fatty acid import on the basis of its ability to bind a sulfosuccinimidyl LCFA derivative that inhibits LCFA import (30). This 88-kDa plasma membrane was cross-linked to radiolabeled sulfosuccinimidyl-oleate, purified, and subsequently cloned (1). When overexpressed in cultured fibroblasts, CD36 increases saturable, high-affinity, phloretin-sensitive LCFA uptake and utilization (33). Mice with CD36 overexpression in muscle under the control of the muscle creatine kinase gene promoter have leaner body mass, enhanced ability to oxidize fatty acids in response to stimulation/contraction, and decreased blood levels of triglycerides and fatty acids (32). Moreover, CD36 knockout mice have increased serum fasting levels of nonesterified free fatty acids and triglycerides and reduced uptake of [³H]oleate in isolated adipocytes, which is most prominent at low oleate-to-BSA ratios, consistent with impairment of the high-affinity component of the LCFA transport system (19). Uptake of LCFA analogs is reduced in heart (50–80%), skeletal muscle (40–70%), and adipose tissue (60–70%) in CD36-null mice (14). Residual fatty acid uptake in these tissues and preserved fatty acid uptake into other tissues such as liver, kidney, lung, and small intestine suggest that, although CD36 activity accounts for a significant fraction of LCFA uptake activity, other transport mechanisms exist.

CD36 was previously identified as a platelet integral membrane glycoprotein that functions as a multiligand scavenger receptor. In addition to binding reversibly to LCFA s (5), CD36 binds thrombospondin-1, modified low-density lipoprotein, retinal photoreceptor outer segments, Plasmodium falciparum malaria-parasitized erythrocytes, sickle erythrocytes, anionic phospholipids, apoptotic cells, and collagen I and IV (20). CD36 has two transmembrane domains with a large glycosylated extracellular loop. Although CD36 is a plasma membrane protein, recent evidence suggests that distribution of CD36 between intracellular membranes and the plasma membrane may play a role in regulation of this protein’s function in LCFA uptake. In skeletal muscle giant sarcosomal vesicles (GSVs), maximal velocity for palmitate uptake is increased by short-term (30-min) electrical stimulation of muscle contraction before harvest of GSVs. The increased palmitate uptake is associated with increased expression of CD36 in GSVs. Moreover, subcellular fractionation of rat skeletal muscles shows redistribution of CD36 from intracellular membranes to the sarco-
lemma with electrical stimulation, suggesting that localization may play an important role in activity and regulation of CD36-mediated transport (10).

Loss of function of CD36 has been implicated in pathophysiological states. CD36 deficiency has been linked to insulin resistance and abnormal fatty acid metabolism in the spontaneously hypertensive rat (SHR) model (4, 26, 52), although the findings are not consistent between different SHR strains (22). In humans, CD36 deficiency has been reported in individuals with heart muscle hypertrophy or dysfunction (69), a finding that is particularly intriguing given the importance of fatty acid metabolism in normal heart function and its known perturbation in hypertrophy and heart failure. Given the high frequency of CD36 deficiency in the Japanese population studied (74) and the known decreases in fatty acid oxidation in hearts affected by hypertrophy and heart failure (7), it will be important to carry out prospective epidemiological studies to document that the incidence of these common heart diseases is increased in individuals with CD36 deficiency compared with individuals who have normal CD36 expression.

PROTEINS IDENTIFIED IN A FUNCTIONAL SCREEN FOR LCFA UPTAKE

An expression cloning approach identified two adipocyte proteins that facilitate the import of LCFA when expressed in mammalian cells (54). The first of these proteins, fatty acid transport protein 1 (FATP1), is a 646-amino acid integral plasma membrane protein expressed in cells and tissues with high-level fatty acid import for metabolism or storage. Expression of FATP1 in mammalian cells increases import of LCFA and very long-chain fatty acids (VLCA), but not medium-chain substrates. FATP1 has a distinct membrane topology (42) with a long hydrophobic amino-terminal region of 190 amino acids containing three stretches of sequence independently capable of directing integral membrane association of reporter sequences. The extreme amino terminus of FATP1 faces the extracellular/lumenal space, residues 1–190 contain at least one transmembrane domain, and the carboxyl terminus of FATP1 faces the cytosolic space. Residues 191–257 are not membrane associated, likely face the cytosol, and contain a motif that is implicated in interactions with ATP.

FATP1 is a member of a large family of related proteins from diverse organisms that increase fatty acid import when expressed in cultured cells (31). In mammalian organisms, FATP isoforms 1–5 have distinct tissue-specific distributions of expression. The importance of FATP proteins in LCFA transport is underscored by experiments in murine enterocytes in which antisense depletion of FATP4 significantly decreases LCFA import (59). Similarly, disruption of the yeast Saccharomyces cerevisiae FATP1 homolog fat1p results in impaired LCFA uptake and impaired growth under conditions in which de novo LCFA biosynthesis is inhibited and LCFA are supplied as the sole carbon source (16, 18). Although no human diseases have been identified as resulting from FATP1 mutations, an intronic polymorphism in the FATP1 gene is associated with increased plasma triglyceride levels in women (47).

The second protein identified in this functional screen for proteins that facilitate import of LCFA is long-chain acyl-CoA synthetase (ACS1). ACS1 catalyzes esterification of LCFA (C14–C18) at the 1-C position with CoA (72), a required activation step for most anabolic and catabolic pathways in which fatty acids are used. The identification of ACS1 in the screen for proteins that increase LCFA uptake suggests that esterification of LCFA is coupled with transport in mammalian cells. As has been proposed for LCFA import in E. coli, ACS may facilitate import through vectorial acylation. Consistent with this model, overexpression of FATP1 and ACS1 in fibroblasts has synergistic effects on LCFA uptake (21). Furthermore, ACS1 is an integral membrane protein that localizes to several cellular membranes of adipocytes, including the plasma membrane where it codistributes with FATP1.

Additional evidence for the importance of esterification in LCFA uptake in eukaryotes comes from experiments in yeast and in mice. Disruption of the yeast ACS1 orthologs FAA1 and FAA4 severely restricts uptake and metabolic utilization of exogenous fatty acids (12, 17). On the other hand, a transgenic mouse model of increased ACS1 expression in the heart (MHC-ACS) shows opposite results (11). MHC-ACS mice demonstrate marked cardiac myocyte triglyceride accumulation in the setting of a low-fat diet and normal serum lipid levels. Lipid accumulation in the heart results in initial cardiac hypertrophy, followed by the development of left ventricular dysfunction and premature death. This lipotoxic response to constitutive ACS overexpression underlines the importance of regulated cellular LCFA transport.

Although ACS1 is a well characterized enzyme that likely contributes to LCFA uptake through vectorial acylation, the mechanism of action of FATP1 is less clear. The observation of 40% amino acid sequence identity between FATP1 and very long chain acyl-CoA synthetase (VLACS) sequence has led to the hypothesis that this protein represents a plasma membrane ACS with specificity for VLCA. The homology between FATP1 and VLACS includes the 11-amino acid motif (IVYTGTGXPXP) that is highly conserved in enzymes known or predicted to form adenylated reaction intermediates, such as ACSs. FATP1 can be specifically cross-linked to [α-32P]azidoATP, and mutations within this motif abrogate ATP binding and LCFA transport (64, 65). Disruption of the yeast FATP1 ortholog fat1p yields a strain with impaired LCFA import and utilization (18), normal long-chain ACS activity (18, 73), diminished VLACS activity, and increased concentrations of free very long chain fatty acids (VLCA) (12, 16, 73). Studies in which overexpression of FATP1 in Cos7 cells leads to increased VLACS activity that cofractionates with FATP1 also...
suggest that FATP1 contains VLACS activity or closely associates with a VLACS protein (15).

Taken together, these findings suggest that FATP1 has esterification activity. FATP1 may be a bifunctional molecule with separable transport and esterification activities (16). Alternatively, changes in the magnitude of LCFA uptake due to changes in FATP1 expression may more indirectly affect esterification. For example, disruption or overexpression of a gene encoding a protein in a metabolic pathway may result in compensatory metabolic changes in the cells. In particular, it is unclear whether some of the observed changes in VLACS activity are the result of alterations in the levels of LCFA ligands for peroxisome proliferator-activated receptors that, in turn, alter the level of expression of the peroxisomal enzyme VLACS. None of the studies to date examined purified protein outside the context in which metabolic compensations are likely to occur. Moreover, there remain unresolved discrepancies in substrate specificity. FATP1 facilitates import of LCFAs and VLCFAs, whereas known VLACS enzymes have no activity toward long-chain substrates.

ARE ANY OF THESE PROTEINS TRANSPORTERS?

Whether CD36, FABPpm, and FATP1 function as transporters for LCFAs remains to be established. Although functional studies suggest that these proteins play a role in LCFA import in mammalian cells, several questions remain. First, the membrane topology for each of these proteins does not resemble that of polytopic membrane transporters for hydrophilic substrates. Many transporters are predicted to have transmembrane domains (4–12 per transporter) consisting primarily of α-helical structures of 17 or more amino acids that span the phospholipid bilayer. The transmembrane domains of well characterized transporters are thought to form a three-dimensional channel through which substrate passes. Second, gain and loss


**Fig. 1.** Model for saturable and nonsaturable long-chain fatty acid (LCFA) import into mammalian cells. At low extracellular unbound free LCFA concentrations (A, B, C), LCFA import is saturable (C) and rapid due to facilitation by proteins that promote dissociation of free LCFA from albumin (e.g., putative albumin (ALB) receptor, ALB-R) or by plasma membrane proteins that bind or transport LCFA (e.g., CD36, plasma membrane FA binding protein (FABPpm), or FA transport protein (FATP)). When import is not coupled to esterification (A), movement of fatty acids may be bidirectional, with net effects on permeation determined by relative concentrations of LCFA in the extracellular and intracellular spaces. When import is coupled to esterification at the inner leaflet of the membrane by acyl-CoA synthetase (ACS; B), transport is vectorial and leads to intracellular accumulation of LCFA esters. At high extracellular free LCFA concentrations (D, E, F), import is unsaturable (F) and occurs by spontaneous flip-flop of nonionized LCFA within the membrane. In the absence of esterification activity associated with the cytosolic aspect of the membrane, movement across the membrane may be bidirectional (D). When import is coupled to esterification at the inner leaflet of the membrane (E), movement into cells is vectorial and enables concentration of LCFA esters within cells.
of function studies are potentially confounded by cellular metabolic compensations. Third, tight coupling of transport and esterification renders measurements of transport alone difficult. Fourth, loss of function studies may be complicated by compensatory upregulation of expression of other highly related protein family members.

A number of studies have reported changes in patterns of expression of FATP, FAT, and FABPpm at the level of mRNA (Northern blot) or protein (Western blot) abundance in homogenates of whole tissue. Changes in expression of the various proteins in experimental or pathophysiological states that correlate with changes in the magnitude of LCFA transport are consistent with an important role of these proteins in regulating fatty acid import (6, 23, 46, 49, 71). However, lack of expected changes in mRNA or protein abundance in a total cellular lysate (as shown in Ref. 44) cannot be taken as proof that a particular protein is irrelevant to LCFA import. Such methods may fail to detect changes in posttranslational modification, protein half-life, or subcellular localization. Indeed, recent studies indicate that subcellular localization of CD36 may play an important role in regulation of fatty acid uptake in contracting skeletal muscle (10).

CONCLUSIONS

Experimental evidence exists for both flip-flop and protein-mediated LCFA permeation (Fig. 1). Although flip-flop of nonionized LCFA is sufficiently rapid to allow LCFA uptake, such a mechanism may facilitate LCFA uptake primarily in settings of high extracellular free LCFA concentrations. In vertebrate organisms, free LCFA are generated by lipase-mediated hydrolysis of triglycerides in adipose stores or by hydrolysis of triglycerides from lipoprotein particles in plasma. Within serum, free LCFA are largely bound to albumin, resulting in low nanomolar concentrations (mean 7.5 nm) of free unbound fatty acids under normal physiological conditions (53). In pathophysiological states, however, LCFA/BSA ratios as high as 7.1 have been observed, with corresponding concentrations of unbound fatty acids of 550 nm (39). Under such conditions, membrane permeation of nonionized LCFA may not be facilitated by proteins but rather occur by flip-flop.

On the other hand, proteins are likely to play an important role in LCFA import in settings in which LCFA are ionized and in which FA/BSA ratios of <3.0 result in low unbound LCFA concentrations. Under such conditions, plasma membrane albumin receptors may facilitate LCFA dissociation to increase local concentrations of LCFA at the extracellular face of the membrane. In eukaryotes, proteins such as FABPpm, CD36, or FATP may contribute to the permeation step or its regulation. Changes in expression or function of such proteins may orchestrate the observed developmental and pathophysiological regulation of LCFA utilization in accordance with nutritional and hormonal signals. The mechanism of action of these proteins is not well understood and is the subject of ongoing investigations by a number of groups.

Regardless of the mechanism employed for membrane permeation, studies from diverse organisms provide compelling evidence that esterification of imported LCFA plays an important role in LCFA import. The enzymes that catalyze esterification may affect both the rate and directionality of LCFA movement across membranes. These enzymes may be physically as well as functionally coupled to other proteins that participate in LCFA import as constituents in cell surface transport complexes. Or these enzymes may function at the inner leaflet of the membrane in concert with permeation by flip-flop. In the absence of esterification at the inner leaflet, LCFA permeation may be bidirectional. For cells that demonstrate efficient LCFA efflux as well as influx, regulation of expression, localization, or activity of esterification enzymes may be required to facilitate bidirectional movement of LCFA in response to appropriate stimuli.

Because of the physiological significance of regulated LCFA flux in vivo, understanding the mechanism of action of proteins implicated in LCFA uptake is an important goal. Once transported across the membrane of parenchymal cells, LCFA are targeted to specific metabolic fates. Under normal conditions, adipocytes have the capacity to store excess LCFA as triglyceride in lipid droplets, whereas nonadipose cells generally achieve a balance between fatty acid uptake and utilization. In pathophysiological states such as non-insulin-dependent diabetes mellitus (NIDDM), high serum levels of LCFA may contribute to high-level LCFA import in excess of cellular needs for these molecules (41, 58). Evidence is emerging that accumulation of fatty acids in skeletal muscle and pancreatic β-cells leads to insulin resistance and abnormal insulin secretion, respectively (24, 43, 56). Furthermore, in several animal models, increased fatty acid import into pancreatic β-cells and cardiac myocytes leads to accumulation of triglycerides, induction of programmed cell death, and organ dysfunction manifested as diabetes (57) and heart failure (11, 77). Thus the mechanism of LCFA import and the manner in which it is regulated have relevance to common human diseases. Understanding the mechanism of LCFA permeation may contribute to the design of novel therapies to treat NIDDM or heart failure.

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