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MECHANISMS OF CELLULAR UPTAKE OF FREE FATTY ACIDS

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INTRODUCTION

Fatty acids are an important component of living organisms. They are an integral building block of all membranes, and through their properties affect lipid bilayer fluidity. Fatty acids are precursors of essential organic molecules such as prostaglandins, leukotrienes, and thromboxanes, which in turn affect many cell functions. Fatty acids also appear to be involved in cell proliferation and immune response. Finally, they represent a major source of energy because of their high caloric content.

The subject of this review is the cellular uptake of plasma fatty acids [unesterified or free fatty acids (FFA)] by cells in which FFA play particularly important roles and in which transmembrane fluxes of FFA are extensive—namely, hepatocytes, adipocytes, cardiac myocytes, and intestinal epithelial

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cells. After a brief overview of the general disposition and metabolic pathways of plasma FFA, we examine the process by which they enter these cell types. This process may be thought of as consisting of two distinct yet related steps: dissociation from albumin and transport across the membrane. We review evidence that indicates that unbound FFA cross the plasma membrane by a saturable, specific mechanism that does not appear to involve the participation of albumin. Later, we examine the characteristics of the putative fatty acid carrier and its relationship to the mitochondrial glutamic-oxalacetic transaminase. We then discuss the driving forces for the uptake process and outline the most important questions to be addressed by future research.

SOURCES, DISPOSITION, AND UTILIZATION OF FFA

Total plasma FFA concentration in humans varies from 90 to 1200 μM with basal values of 300–500 μM. The lowest concentrations occur with carbohydrate-rich diets, and the highest values appear during fasting, strenuous exercise, or diabetes; concentrations as high as 2500 μM have been reported. Considering the mean albumin concentration is $\sim$ 600 μM, the molar ratio FFA:albumin varies normally between 0.15 and 2 with basal values of 0.5–0.8. This has important implications for the cellular uptake of FFA, as described further below. Plasma FFA derive from different sources depending on nutritional status of the organism and concomitant conditions (Figure 1). During fasting, stress, or exercise, FFA are hydrolyzed from triglycerides stored in adipose tissue, released into plasma where they bind to albumin, and delivered to various tissues. There, they are metabolized with consequent energy production or, to a lesser extent, re-stored as tissue lipids (57). After a meal, most fat is absorbed in the form of triglycerides incorporated into lipoproteins; in the various tissues throughout the body, FFA are hydrolyzed from triglycerides contained in chylomicrons and very low density lipoproteins by the endothelial lipoprotein lipase and directly utilized by the parenchymal cells. A small portion of hydrolyzed FFA may escape, however, and contribute to the general plasma FFA pool (16, 22, 23, 34, 57).

Once inside the cell, FFA may follow different metabolic pathways depending on cell needs and on the function of the particular organ or tissue. Thus, in adipocytes they are primarily esterified to triglycerides and phospholipids and stored as lipid droplets, while in the heart and skeletal muscle, after activation to acyl CoA and transport into mitochondria, they are oxidized with production of acetyl CoA, which then enters the Krebs cycle and results in the production of ATP. The importance of FFA as an energy source is best highlighted by their rapid plasma turnover (Table 1). For an average man of 70 kg with a typical plasma FFA half-life of 2 min, up to 80% of his caloric need could be met from plasma FFA (57). This approximation is excessive,

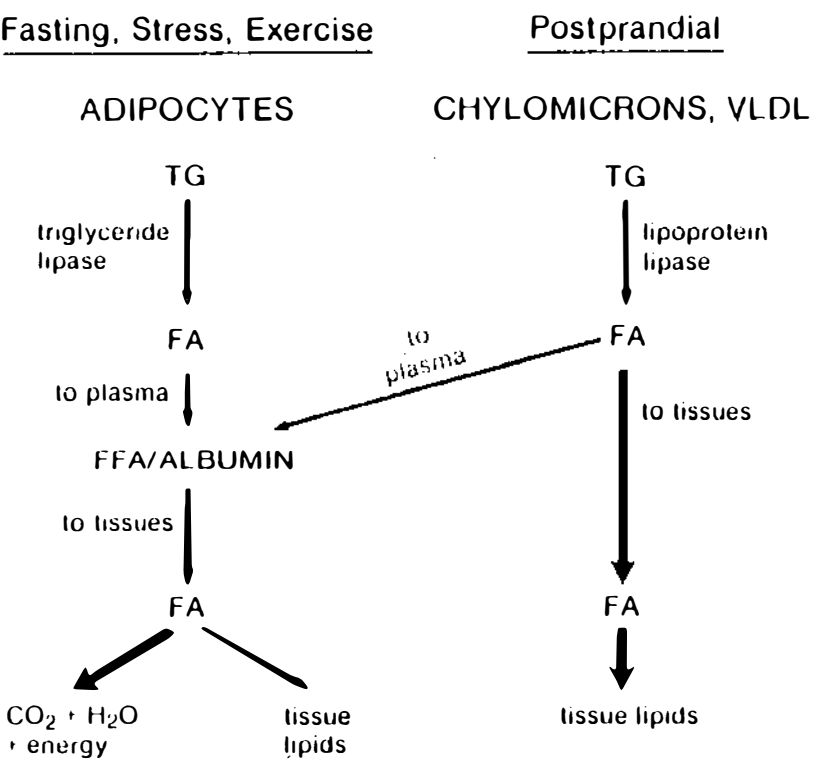

Figure 1 Sources of plasma free fatty acids (FFA) during fasting, stress, exercise, after a meal. TG = triglycerides, FA = fatty acids. [Reproduced from Spector (57) with permission of the author and publisher].

since part of the FFA is used for processes other than energy production, including membrane turnover and production of prostaglandins and other molecules. However, plasma FFA clearly represent a major source of energy under normal conditions. Some organs such as the heart utilize FFA almost exclusively to meet their metabolic requirements.

CELLULAR UPTAKE OF FATTY ACIDS

Dissociation From Albumin

Like many other compounds that are poorly soluble in water, FFA circulate in plasma tightly bound to albumin. Only a very small fraction of FFA (~0.1%) is actually dissolved in the plasmatic water, and this fraction is assumed to be in equilibrium with the albumin-bound fraction. The binding of FFA to albumin thus allows a de facto increase in FFA water solubility and makes FFA a readily available substrate. Binding with albumin also minimizes

Table 1 Estimate of the contribution of plasma free fatty acid to energy metabolism in humans[a]

Parameter	Value
Weight	70 kg
Plasma volume	3 l
Plasma FFA concentration (average value)	0.5 mmol/l
Average FFA molecular weight	280
t ½ of plasma FFA	1–2 min
Turnover of plasma FFA	10 mmol/l/hour
(Assuming t ½ = 2 min)	720 mmol/day
	201.6 g/day
Caloric value of turnover (9 cal/g)	1818 cal/day
Caloric requirement (moderate activity)	2350 cal/day
Maximum contribution from plasma FFA turnover to caloric requirement[b]	77%

[a] Reproduced from Spector (57) with permission of the author and publisher.

[b] This estimate assumes that all of the FFA turnover is utilized for fatty acid oxidation.

the potentially toxic effects of high FFA concentrations. Finally, binding to albumin adds enormous complexity to the study of FFA transport.

It has long been thought that FFA and other albumin-bound ligands are taken up by cells in their unbound form and that the bound fraction rapidly equilibrates to provide unbound substrates for later uptake. Thus, investigators thought that uptake was driven only by the unbound fraction and that albumin participated in the process only as a passive reservoir of ligand (5, 14, 26). This belief stems from the observation that albumin is not internalized during the uptake process (or is internalized orders of magnitude more slowly than ligand) (9, 24, 52); therefore the albumin-ligand complex must dissociate prior to ligand uptake. The main factor in the availability of FFA for uptake was thought to be the FFA:albumin molar ratio, since the estimated unbound concentration of FFA depends principally on the FFA:albumin molar ratio and is independent of the total concentrations of protein and ligand (56).

Recent kinetic data obtained in the perfused rat liver have, however, suggested that the dissociation of ligand-albumin complexes may not be spontaneous but rather may be catalyzed by the cell surface, either unspecifically or by an albumin receptor (19, 72). Four basic observations were considered inconsistent with the traditional model of spontaneous dissociation–rapid equilibration outlined above. First, when albumin and FFA concentrations were increased in parallel, keeping their molar ratio (and the consequent unbound ligand concentrations) constant, FFA uptake appeared

saturable. By contrast, when only the ligand concentration was increased, keeping albumin constant, ligand uptake appeared to be nonsaturable and linearly related to total ligand concentration. The apparent saturation observed at a constant molar ratio occurred at uptake velocities well below those achieved without saturation in studies keeping albumin constant (72). Second, addition of "uncomplexed" albumin to solutions of albumin-ligand complexes was reported to inhibit ligand uptake competitively (72). Third, uptake appeared to correlate with the total rather than with the unbound ligand concentration. Thus, a decrease in unbound ligand concentration was not followed by a parallel decrease in uptake rate (19). Fourth, for many ligands, reported albumin-ligand dissociation rates as measured in vitro appeared too slow to account for the uptake rates of the same ligands (73).

These data were interpreted by Weisiger et al (72, 73) as evidence for the presence of a specific albumin receptor on the hepatocellular surface, postulated to be capable of accelerating the dissociation of the albumin-ligand complex, thereby making more ligand available for uptake. The albumin receptor model was proposed to explain the four observations outlined above in that increasing concentrations of albumin would saturate (and competitively inhibit) the receptor. On the other hand, FFA transport across the membrane was supposed to be purely passive (lack of saturation with increasing total concentrations of FFA). This novel theory was also considered capable of explaining why uptake rates appear related to bound rather than unbound FFA and why spontaneous dissociation rates of the FFA-albumin complex would be slower than FFA uptake rates (44, 73). It was even proposed that a difference in albumin receptors could explain a sex-related difference in FFA sequestration (71); we now know that this difference is more likely due to FFA membrane transport rates (51). The albumin receptor model found some biochemical support in albumin-membrane binding studies (13, 72). Concomitantly, Forker & Luxon proposed a related but less specific mechanism, the interaction between the albumin-ligand complex and the cell surface, to account for what they believed was accelerated surface-mediated dissociation of the complex (19–21). Similar concepts were also proposed more than 20 years ago by Baker & Bradley (4), who found an unexpected discrepancy between in vivo bromsulphthalein (BSP) uptake rates and the minuscule concentrations of unbound BSP, based on the newly determined in vitro equilibrium binding constants of albumin-BSP complexes; and also by Bloomer et al (11) more than a decade ago, based on studies of bilirubin uptake. Given the important potential implications of these kinetic observations and the theories proposed to explain them, a major effort has been made to clarify the issue.

What has emerged so far can be summarized as follows (53):

1. The hepatocellular surface does not appear to bear a specific albumin

receptor. Albumin binding to hepatocyte plasma membranes does not show features (such as high affinity, loss of binding upon denaturation, or temperature dependence) that are typical of a receptor type of interaction, and no membrane protein with high affinity for albumin has been isolated from plasma membranes (64). Studies of various types and in several laboratories have led to similar conclusions (31, 59, 75).

2. Uptake kinetics identical to those described above can be reproduced (*a*) with different ligand-binding proteins such as ligandin and β-lactoglobulin, indicating that they are not albumin specific (36, 59); (*b*) using isolated hepatocyte suspensions, monolayer cultures, and subcellular preparations, indicating that their nature is not linked to an intact hepatic lobular architecture (6, 10, 18, 36, 47); and (*c*) using diverse cell types such as adipocytes and cardiac myocytes, indicating that they are not even liver specific (40, 50).

3. In retrospect, many of the apparent paradoxes reported in the earlier studies cited above can be explained satisfactorily (53) without invoking cell-mediated dissociation or an albumin receptor. First, the hyperbolic relationship between uptake and the absolute concentration of FFA-albumin complexes observed at a fixed FFA:albumin ratio does not reflect true saturation either of an uptake system or of an albumin receptor. Using [^{3}H]oleate as a representative FFA and suspensions of isolated hepatocytes, adipocytes, and cardiac myocytes, we have shown that in all cell types in this experimental setting the "K_m"[1] of this pseudosaturation curve depends on substrate availability, as determined by the volume of substrate solution added to a fixed volume of cells, while its "V_{max}" is independent of this variable. Thus, in this context, "V_{max}" reflects the capacity of the cells to take up oleate at a given oleate:albumin ratio and consequent oleate unbound concentration, under conditions in which substrate availability is not rate limiting. By contrast, "K_m" is a function of substrate availability; it decreases with increasing volumes of substrate when a fixed volume of cells is used and with decreasing volumes of cells when a fixed volume of substrate is used (49). That the hyperbolic curve in question does not reflect true saturation is also evidenced by the fact that the "V_{max}" values observed in this setting do correlate with the FFA:albumin ratios (and consequent unbound FFA concentrations) used. The nonlinear nature of this correlation in turn indicates that FFA transport across the plasma membrane is a saturable process (see next paragraph) (48, 49, 53). The latter conclusion could have been inferred even from the original data in the publication proposing an albumin receptor

[1]Throughout this review, when K_m and V_{max} are enclosed in quotation marks ("K_m", "V_{max}"), they do not have the usual meaning of these kinetic parameters. Rather, they denote the total concentration of albumin ("K_m") at which ligand uptake is half of its maximal value ("V_{max}") at any fixed ligand:albumin molar ratio.

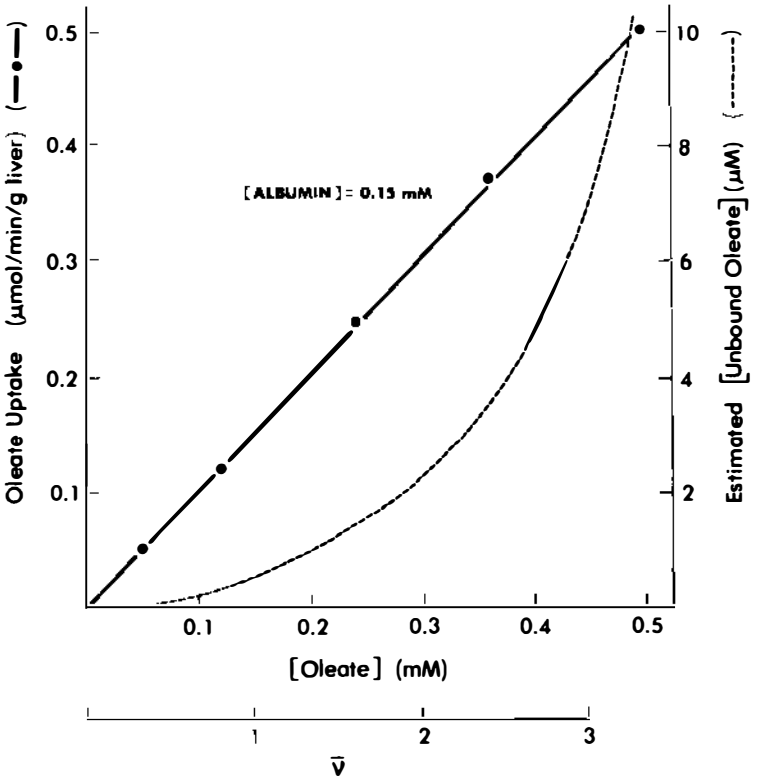

Figure 2 Oleate uptake by perfused rat liver as a function of different oleate:albumin ratios ($\bar{\nu}$). This plot is the original figure as in Ref. 72 [reproduced from Weisiger et al (72) with permission of the author and publisher]. Uptake data plotted against total oleate concentrations yield a straight line.

(72) if the uptake values obtained at different oleate:albumin ratios had been plotted versus the *unbound* rather than the total oleate concentration (see Figures 2 & 3). Elegant competition studies by Goresky and colleagues (25) also indicate that FFA uptake in vivo is a function of unbound rather than bound FFA concentrations.

The second observation that led to the hypothesis of an albumin receptor could be explained obviously and easily (53) if uptake is assumed to depend on the FFA:albumin ratio and consequent unbound FFA concentration. In fact, the addition of a fixed amount of "uncomplexed" albumin to a 1:1 oleate-albumin complex changes the oleate:albumin molar ratio and consequent unbound oleate concentration; for a given amount of uncomplexed albumin added, this effect is more pronounced at low initial concentrations of the oleate-albumin complex. Thus, addition of 66-μM albumin to a 22-μM

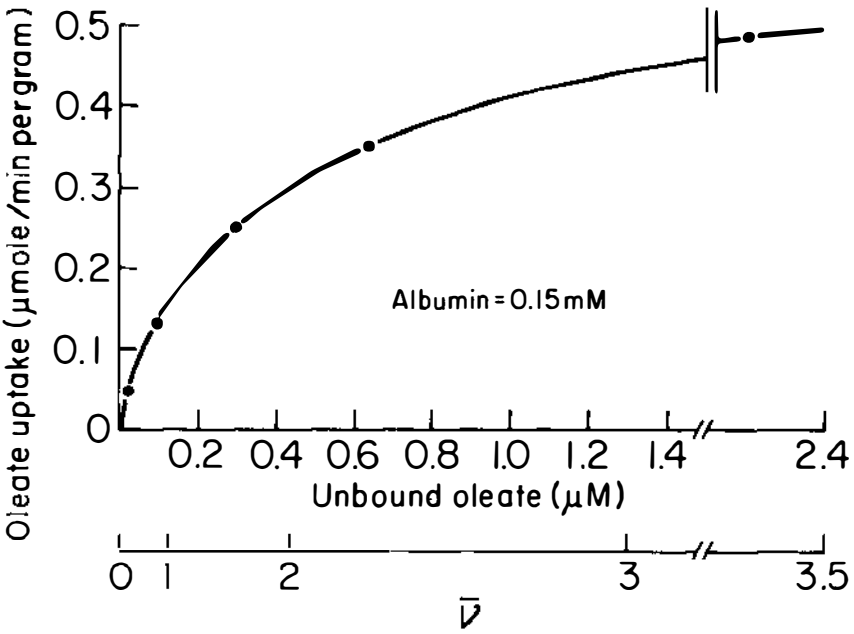

Figure 3 When the same oleate uptake data from Figure 2 are plotted against the correct (12) *unbound* oleate concentrations, a hyperbolic curve is obtained, which indicates that oleate uptake is a saturable function of unbound oleate concentrations.

solution of oleate-albumin complex decreases the oleate:albumin ratio by 75% (1:1 to 0.25:1) and the consequent unbound oleate concentration by 85% (0.12 to 0.018 μM), with a corresponding large decrement in oleate uptake velocity. However, addition of 66-μM albumin to a 600-μM solution of oleate-albumin complex changes the molar ratio by 10%, (1:1 to 0.9:1), with a correspondingly small reduction in concentration of unbound oleate and in oleate uptake velocity. When the results of a series of such studies using a wide range of initial oleate:albumin concentrations is expressed as a double-reciprocal (e.g. Lineweaver-Burk) plot, the resulting graph suggests superficially that the uncomplexed albumin has competitively inhibited oleate uptake from the original oleate-albumin complexes. However, resulting increased "K_m" and unchanged "V_{max}" values are in fact explained (53) by differences in the effects of the added albumin on the resulting unbound concentration, without postulating competitive inhibition for a surface receptor between uncomplexed albumin and albumin-oleate complexes.

The third observation—namely, the higher-than-expected uptake values observed upon shifting from a given FFA:albumin ratio to a lower one—has puzzled investigators more than any other observation (17, 76). Were the uptake dependent on the FFA:albumin ratio and consequent unbound oleate

concentration, one would expect a parallel decrease in uptake upon decreasing the unbound oleate concentration. We now know that this discrepancy is heavily dependent on the experimental conditions employed (48, 49, 53). Whenever this issue is studied it is critically important to use a range of albumin:ligand ratios and consequent unbound ligand concentrations that are well below the K_m of the intrinsic membrane transport system for the ligand in question. In fact, any change in albumin:ligand ratio and unbound concentration under true saturating conditions will obviously yield a nonlinear relationship between uptake and unbound concentrations (42, 53). When this simple yet important notion is taken into account, the discrepancy between observed uptake velocities and those predicted on the basis of the equilibrium concentrations of unbound ligand can be shown to occur only at low, nonphysiologic concentrations of albumin-ligand complexes (49). As these concentrations increase and approach physiologic values, the discrepancy is progressively reduced until, at ligand-albumin concentrations yielding maximal uptake velocities ("V_{max}") for the molar ratios in question, the observed and predicted uptake velocities coincide. While the uptake kinetics at very low concentrations of ligand-albumin complexes deviate from the expected kinetics and must be explained by alternative theories, the studies just summarized indicate, overall, that at physiologic albumin concentrations ($\sim$ 600 μM) oleate uptake into hepatocytes, adipocytes, and cardiac myocytes is a saturable function of the equilibrium unbound oleate concentrations (48–50, 53).

Finally, the discrepancy between ligand-albumin dissociation rates and ligand uptake rates relies on the assumption that in vitro dissociation rates reported a decade ago are identical to those in vivo. While reliable in vivo estimates are still not available, recent measurements in the perfused liver, as well as new in vitro estimates (74), suggest that ligand-albumin dissociation rates may be appreciably faster than those reported earlier.

In conclusion, alternative theories such as cell-mediated dissociation of albumin-ligand complexes (20, 21, 28), the presence of a large unstirred water layer surrounding the cell surface (7), or dissociation-limited availability of substrate (32, 67, 68) may explain deviations of the uptake kinetics from the conventional theory when very low concentrations of albumin are used (or in the absence of albumin). However, a growing preponderance of evidence suggests that FFA uptake in vivo is a saturable function of unbound FFA concentrations and that the conventional theory thus applies in vivo (48, 53).

Membrane Transport

Following dissociation from albumin, FFA cross cell membranes to be either stored or oxidized for energy production. FFA flux through the plasma

membrane has long been considered a passive process that occurred either by simple diffusion or by partitioning into the lipid bilayer (15, 27, 58), after which the binding of these FFA to the cytosolic fatty acid binding protein ($FABP_C$) or their further movement along internal membrane pathways provide for intracellular disposition (46). The hypothesis that FFA cross cell membranes by lipid partitioning mainly derives from the notion that FFA are lipids themselves and basic components of membranes. At physiologic pH, however, FFA exist in plasma to a considerable extent in ionized form and not as a neutral species; therefore, their negative charge may significantly affect their lipid partition (57). This simple theoretical consideration thus raises the possibility of mechanisms of FFA transport more specific (and efficient) than simple diffusion or lipid partitioning. The following paragraphs review the experimental evidence that suggests that FFA transport across the plasma membrane of various cell types is indeed carrier mediated. The evidence stems from binding and kinetic data, from the isolation of a plasma membrane fatty acid binding protein ($FABP_{PM}$), and from the finding that an antibody against $FABP_{PM}$ selectively inhibits FFA uptake into various cell types.

First, long-chain FFA such as oleate bind in a saturable fashion to freshly isolated nondenatured liver plasma membranes. Kinetic analysis of oleate binding data suggested the existence of a single class of high-affinity binding sites with a $K_d \sim 10^{-8}$M; binding of FFA to heat-denatured plasma membranes is greatly decreased (66). In cellular uptake studies, the use of a rapid filtration technique and a "stop solution" consisting of albumin/phloretin effectively blocks oleate influx and efflux and permits accurate quantification of material actually internalized rather than merely membrane bound (1, 2, 55, 62). In addition, metabolism (i.e. esterification and oxidation) has been shown not to be significant at early times in cellular uptake studies. As a result, the initial portion of the cumulative FFA curve thus measured closely approximates FFA flux across the plasma membrane and into the cell (1, 2, 55, 62). In several different cell types, oleate uptake velocity has now been shown to be a function of the unbound oleate concentrations in the presence of physiologic or quasiphysiologic concentrations of albumin. Thus, the system fulfills the basic criteria of a carrier-mediated transport system (1, 2, 45, 55, 60, 65), namely, saturation, selective competitive inhibition, and counter-transport-transtimulation (35, 43, 48) (Figure 4). For all cell types studied (hepatocytes, cardiac myocytes, adipocytes, and jejunal enterocytes), saturation of the transport system occurs within the physiologic range of FFA:albumin ratios and consequent unbound FFA concentrations, which suggests that under normal conditions FFA uptake may be principally dependent upon a single transport mechanism (1, 2, 45, 55, 60, 65). Necessary but not sufficient to establish the existence of a carrier-mediated process, these studies prompted a search for a plasma membrane protein with high affinity for FFA.

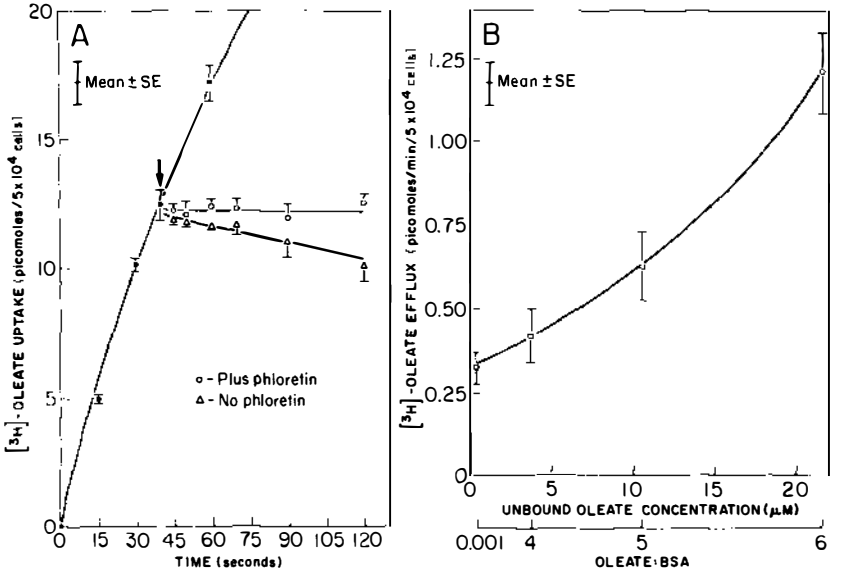

Figure 4 Countertransport and *trans*-stimulation of [^{3}H]oleate efflux in isolated canine cardiomyocytes. (*A*) 50 μl of [^{3}H]oleate-albumin (5:50 μM) was added to 50 μl of cells, and cell-associated radioactivity was determined at various times. Control cells were incubated for 120 sec (*closed circles*). In the experimental incubations, 2.5 ml of unlabeled oleate-BSA (1,500:250 μM), with (*open circles*) or without (*triangles*) 200 μM phloretin, was added to the cells after 40-sec incubation with the isotope (*arrow*), and the cell-associated radioactivity was monitored over the next 80 sec. The figure shows that efflux of [^{3}H]oleate occurs against the prevailing concentration gradient (55), while phloretin abolishes the phenomenon. (*B*) [^{3}H]oleate efflux rates measured under the conditions described for *A* are plotted versus the extracellular unlabeled oleate-albumin molar ratios and consequent unbound oleate concentrations following addition of unlabeled oleate-albumin solutions containing no phloretin. Under these conditions [^{3}H]oleate efflux increases with increasing extracellular unbound oleate. [Reproduced from Sorrentino et al (55) with permission of the author and publisher.]

Isolation of FABP$_{PM}$

After solubilization with triton, plasma membranes from liver and jejunum were loaded onto oleate-agarose columns and eluted with 8M urea. The protein thus isolated from liver and gut, named FABP$_{PM}$, was shown to have a molecular weight of 40,000, to contain no carbohydrates, and to have a *pI* ~ 9 (63, 66).[2] Specific binding of ^{14}C-oleate to FABP$_{PM}$ was demonstrated by cochromatography, which also showed that this protein binds a variety of unesterified long-chain FFA but does not bind fatty acids esterified into more

[2]To avoid confusion with the nomenclature for the cytosolic FABP, we have proposed (45) the subscript suffixes $_{PM}$ or $_C$ to indicate the cellular location (plasma membrane or cytosol) and the prefixes h, a, g, my, or sm to designate origin from hepatic, adipose, gut, myocardial, or skeletal muscle sites, respectively. Thus h-FABP$_{PM}$ denotes the liver FABP from plasma membrane.

complex lipids, or taurocholate, BSP, and bilirubin (63, 66). Subsequent studies showed that the protein could be extracted from plasma membranes with 2M salt, and because of its relatively unusual *pI*, could be separated from other proteins by preparative isoelectric focussing and further purified by affinity chromatography and gel permeation HPLC (39, 45, 55). These procedures permitted isolation from adipose tissue and myocardium of $FABP_{PM}$ that showed properties very similar to those of the proteins isolated from the liver and jejunum (39, 45, 55). All $FABP_{PM}$ were shown, by double radial immunodiffusion and by Western blotting, to react against polyclonal rabbit anti–rat liver $FABP_{PM}$ (39, 55). The function of these proteins in cellular FFA uptake was tested by preincubating hepatocytes, adipocytes, and cardiac myocytes with the IgG fraction of the serum of rabbit immunized against liver $FABP_{PM}$. Compared with control cells pretreated with pre-immune serum, antibody significantly inhibited oleate uptake in each of these cell types in a dose-dependent, noncompetitive fashion, while it had virtually no effect on the uptake of other compounds (i.e. BSP and taurocholate in hepatocytes, glucose in cardiomyocytes) (55, 65).

New insights and questions about the function of $FABP_{PM}$ arise from recently acquired amino acid sequence data (8). Unexpectedly, of the first 24 *N*-terminal amino acids of $FABP_{PM}$, all of the 21 thus far identified are identical to those of the mitochondrial isozyme of glutamate-oxaloacetate-transaminase (mGOT), a well-studied enzyme without known relation to FFA transport or metabolism. Aside from amino acid composition, the two proteins appear similar in many other aspects: (*a*) Both are unusually basic (*pI* ~ 9.1). (*b*) Like $FABP_{PM}$, mGOT binds FFA and can be separated from other proteins by oleate-agarose affinity chromatography; conversely, sinusoidal liver plasma membranes have GOT enzymatic activity and purified h-$FABP_{PM}$ and mGOT have identical specific activities of ~0.11 units/μg. (*c*) Both $FABP_{PM}$ and mGOT have similar complex absorption spectra, with peak absorbance at 450 nm under acid conditions, shifting to ~350 nm at alkaline pH. (*d*) On Western blot, mGOT reacts against polyclonal rabbit anti–rat $FABP_{PM}$, while $FABP_{PM}$ reacts against an analogous anti-mGOT. (*e*) Like anti-$FABP_{PM}$, anti-mGOT noncompetitively inhibits oleate uptake by isolated hepatocytes but has no effect on BSP or taurocholate uptake (D. Sorrentino, P. D. Berk, unpublished). With rabbit antisera against either $FABP_{PM}$ or mGOT, immunofluorescence and immunoelectronmicroscopy detect these proteins in plasma membranes and in cytoplasmic sites (presumably in mitochondria). The latter observation seems to exclude a possible artifact in $FABP_{PM}$ isolation caused by contamination of plasma membranes with mitochondria. Thus, these studies collectively indicate that (*a*) in suspended hepatocytes, adipocytes, cardiac myocytes, and jejunal enterocytes, FFA uptake is saturable and involves similar 40-kd $FABP_{PM}$; and (*b*) $FABP_{PM}$ bears a striking resemblance to mGOT both functionally and structurally.

The definitive description of the relationship between these two proteins, however, awaits gene cloning and sequencing, which have been accomplished thus far only for mGOT (12, 29, 30, 33). Conversely, the physiologic role of mGOT and $FABP_{PM}$ in FFA uptake will be evaluated by using in vivo models.

Driving Forces for FFA Uptake

While the available evidence indicates that FFA uptake in various cell types conforms to the criteria of a carrier-mediated transport system, the driving forces for the uptake process need to be further elucidated. Early studies from our laboratory (65) and those of others (60, 61, 70) reported that oleate uptake by isolated hepatocytes is sodium dependent and inhibited by ouabain and metabolic inhibitors. Such results imply that uptake is energy dependent and possibly even similar to other well-characterized Na^+-dependent symport systems (i.e. taurocholate). Studies conducted with plasma membrane vesicles and perfused liver also suggest that FFA uptake is actively driven by the sodium and electrochemical gradient (61, 70). We and others have shown more recently, however, that FFA uptake in a variety of cell types including hepatocytes (41, 48, 54), adipocytes (1, 45), and cardiomyocytes (38, 55) is neither sodium nor energy dependent. These apparently conflicting data may be explained by the different experimental conditions of the respective studies.

One important factor appears to be the buffer in which cells are resuspended while the uptake process is studied. The reported Na^+ dependence for FFA uptake by isolated hepatocytes and liver plasma membrane vesicles was observed by employing a simple buffer (phosphate-buffered saline) (61, 65). Under these conditions the data are reproducible. When a more physiologic buffer (i.e. Hanks' or Krebs-Ringer) is employed, however, Na^+ dependence is no longer demonstrable in any cell type (D. Sorrentino, P. D. Berk, unpublished; Ref. 54). Various explanations of these observations can be proposed (53): First, the use of a given buffer may significantly affect the cellular transmembrane electrical potential and, as a consequence, the electrochemical driving forces for Na^+ and oleate uptake. Second, the buffer composition may greatly affect the affinity of albumin for FFA and consequently the unbound FFA concentrations. Obviously, were this phenomenon unrecognized, the end result (i.e. increased or decreased uptake) would be attributed erroneously to other mechanisms. Finally, although our own data were compatible with a single oleate transport system, the possibility of two remains. This would be a logical consequence of any incontrovertible demonstration of a sodium-dependent component of oleate uptake, since FFA uptake also displays saturation, antibody inhibition, and other features compatible with carrier-mediated transport under conditions in which Na^+-dependence cannot be demonstrated (53).

Most likely, if there are two systems, the sodium-independent system is the dominant, high-capacity system, while the second system is sodium dependent and low capacity; the expression of the latter would be overwhelmed under physiologic conditions by the former (53). This postulate is consistent with the recent preliminary report of Na^+-dependent oleate uptake in the isolated perfused liver, based on studies at a low, nonphysiologic oleate:albumin ratio (0.006:1) and consequent unbound oleate concentrations (70). More physiologic conditions were not investigated.

In further support of the hypothesis that the major uptake component is not sodium dependent are the observed sex differences in FFA uptake. Were a Na^+-FFA symport a major driving force for FFA uptake, one would expect a smaller FFA uptake in female hepatocytes, since they have a more positive membrane potential (69) (which thereby provides a weaker driving force for Na^+ uptake and for a putative Na^+-FFA cotransport system). FFA uptake, however, is much greater in females (51). Thus, whether FFA uptake involves a true Na^+-FFA cotransport system analogous to that described for taurocholate remains unknown. Likewise, the in vivo significance of a putative Na^+-dependent carrier mechanism needs to be evaluated under physiologic conditions.

Future Perspectives

While FFA uptake by suspensions of cells (hepatocytes, adipocytes, cardiac myocytes) that display high FFA fluxes in vivo appears to be specific and mediated by a 40-kd $FABP_{PM}$, these findings need to be complemented by whole-organ perfusion and in-vivo studies (e.g. indicator-dilution studies). mGOT has been cloned and fully sequenced at the amino acid (12, 30) and cDNA (29, 33) levels. Isolation and cloning of the gene responsible for $FABP_{PM}$ synthesis will lead to a definite understanding of the relationship between these two proteins. Gene structure analysis will help clarify whether the $FABP_{PM}$/mGOT is related to a 44-kd superficial membrane protein isolated in *E. coli* and shown to be part of a protein complex responsible for FFA uptake in this microorganism (37). Purification of large amounts of $FABP_{PM}$ by use of recombinant technology will allow the development of techniques for quantification of its cell-surface expression in various physiologic and pathologic conditions. Protein availability will also permit more detailed physicochemical studies, as well as liposome reconstitution studies. Such studies in turn will clarify whether $FABP_{PM}$ is both necessary and sufficient to carry out the task of FFA uptake or the process requires additional components. Similar studies and electrophysiologic measurements should determine whether the FFA transport system is energy and Na^+ dependent. Regulation of the system will be another important aspect to be investigated. The overall disposition of FFA, once thought to be regulated at the metabolic and intracellular level, may be subject to regulation at the membrane level as

well. For example, studies in adipocytes suggest that epinephrine and insulin may affect FFA influx/efflux by acting at the level of the membrane carrier (3). Likewise, the reported sex- and starvation-related differences in FFA uptake may be dependent upon a change in the structure and/or function of the membrane transport system (51, 52a).

CONCLUSIONS

FFA constitute an important class of endogenous compounds. They are components of membranes, precursors of many biologically active substances, and a main source of energy. This review has dealt primarily with mechanistic aspects of cellular FFA uptake. Albumin appears to participate in the process only as a passive reservoir of FFA. FFA transport across membranes, once thought to be a relatively uninteresting, nonspecific process, now appears to be highly specific and to involve a 40-kd $FABP_{PM}$ isolated from heart, liver, gut, and adipose tissue. $FABP_{PM}$ appears closely related to mGOT. Future research will focus on the relationship between $FABP_{PM}$ and mGOT and their role in FFA uptake in vivo, on the definition of the driving forces for FFA uptake, and on the molecular structure, dynamics, and regulation of the carrier system in normal and pathologic conditions. A thorough understanding of these aspects will greatly enhance our knowledge of the basic biochemistry and physiology of FFA and may open new therapeutic possibilities.

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