Transport and utilization of fatty acids (FA) in cells is a multistep process that includes adsorption to and movement across the plasma membrane and binding to intracellular fatty acid binding proteins (FABP) in the cytosol. We monitored the transbilayer movement of several polyunsaturated FA and oxidation products (13-hydroxy octadecadienoic acid (HODE) and 15-hydroxytetraenoic acid (HETE)) in unilamellar protein-free phospholipid vesicles containing a fluorescent pH probe. All FA diffused rapidly by the flip-flop mechanism across the model membrane, as revealed by pH changes inside the vesicle. This result suggests that FA oxidation products generated in the cell could cross the plasma or nuclear membrane spontaneously without a membrane transporter. To illuminate features of extra- and intracellular transport, the partitioning of unsaturated FA and oxidized FA between phospholipid vesicles and albumin or FABP was studied by the pyranin assay. These experiments showed that all polyunsaturated FA and oxidized FA (13-HODE and 15-HETE) desorbed rapidly from the phospholipid bilayer to bind to bovine serum albumin, which showed a slight preference for the unsaturated FA over the oxidized FA. FABP rapidly bound FA in the presence of phospholipid bilayers, with a preference of 13-HODE over the unsaturated FA and with a specificity depending on the type of FABP. Liver FABP was significantly more effective than intestinal FABP in binding 13-HODE in the presence of vesicles. The more effective binding of the FA metabolite, 13-HODE, than its precursor 18:2 by FABP may help protect cellular membranes from potential damage by monohydroxy fatty acids and may contribute a pathway for entry of 13-HODE into the nucleus.

Inside mammalian cells, fatty acids (FA) are activated by esterification to coenzyme A and used for energy (β-oxidation), remodeling phospholipids, or storage of fat. Polyunsaturated FA (PUFA) may also be oxidized to products that are biologically active and act, for example, as potent signal agents. Intracellular fatty acid binding proteins (FABP) may deliver FA to sites of utilization and influence intracellular metabolism and storage (1). FABP may also deliver oxidized FA to the nucleus where they bind to receptors and modulate gene transcription (2). Another possible function of FABP is to bind and protect PUFA from oxidation, which may in turn regulate PUFA metabolism (3). Moreover, the toxic effects of oxidative intermediates of lipid peroxidation might be reduced by this interaction (3).

In general it is considered important to minimize the possibility of oxidation of polyunsaturated FA to undesirable monohydroxy FA. Such oxidative products are 13-hydroxy octadecadienoic acid (13-HODE) from linoleic acid (18:2) and 15-hydroxy eicosatetraenoic acid (15-HETE) from arachidonic acid (20:4). These metabolites are formed by free radical processes that include the formation of intermediate lipid hydroperoxides, which may have adverse effects on the functions of cellular membranes (10).

In type II diabetes and atherosclerosis increased amounts of peroxidized FA have been detected (4), and such metabolic changes seem to be functionally important for the outcome of cardiovascular disease (5). Eicosanoids and lipid peroxidation products derived from PUFA in oxidized low density lipoprotein are activators of peroxisomal proliferator-activated receptor γ (PPARγ) and may thus act as downstream regulators of foam cell formation (6, 7). 13-HODE is a particularly effective activator of PPARγ (T), and lipoxigenase metabolites of arachidonic acid and peroxidation products of PUFA, such as 15-15-hydroperoxyeicosatetraenoic acid, bind with higher affinity to liver FABP (L-FABP) than oleic acid (8). Prostaglandin E₁ also binds specifically and rapidly to L-FABP (9).

The mechanism(s) by which FA cross the plasma membrane of mammalian cells are the subject of active investigation and debate (11, 12). A key question for understanding the cellular effects of oxidized FA is whether they can partition into a membrane and cross the membrane without a specialized mechanism. If FA metabolites can quickly exit the cell, they could bind to serum albumin, which would facilitate their removal from cells. Inside the cell, binding to FABP could sequester some of the oxidized FA, also protecting the cell membrane and possibly transporting them to nuclear targets. Therefore, we investigated whether FA metabolites were able to diffuse across a phospholipid bilayer membrane and how effectively albumin and FABP can bind these molecules in the presence of model membranes.

This paper is available on line at http://www.jbc.org
Materials and Methods

Materials—Lauric acid (12:0), palmitic acid (16:0), oleic acid (18:1), linoleic acid (18:2), arachidonic acid (20:4), 13-ODE, 15(s)-HETE (active form), pentaenoic acid (20:5), docosapentaenoic acid (22:5), and docosahexaenoic acid (22:6) were obtained from Cayman Chemicals (Ann Arbor, MI). Sephadex G25 was from Amersham Pharmacia Biotech. The fluorescence pH probe, pyranin, was from Eastman Kodak Co.. BSA (FA-free, fraction V) was from Sigma. Distilled water was further treated with a Millipore purification apparatus. Molecular weights used for calculations are as follows: BSA, 66,200; I-FABP, 15,200; rat t-FABP, 14,200; and rat cellular retinoic acid binding protein II, 15,000 (13, 14).

Preparation of Delipidated Rat FABP from Escherichia coli—I-FABP, L-FABP, and cellular retinoic acid binding protein II were recombinant rat proteins. The proteins were expressed and purified in E. coli (MG 1655 strain) (14, 15) and delipidated by passage through hydroxylalkoxy-propyldextran (Lipidx 1000; Sigma) using a previously described protocol (14).

Preparation of Vesicles—Small (~25 nm) unilamellar vesicles (SUV) were prepared from 45 mg of egg phosphatidylcholine (PC) (Avanti Polar Lipids, Pelham, AL) containing the fluorescence probe (pH indicator) pyranin (16–18). 100 mM (pH 7.4) Hepes/KOH and 10 mM pyranin were added to a dry film of PC. After the suspension was soaked overnight in the cold room, it was vortexed and sonicated for 60 min with a microtip Branson sonicator (power level 3, 30% duty cycle, kept on ice under nitrogen). After low speed centrifugation, SUV were separated from the extravesicular pyranin on a Sephadex G25 column (4.3 × 30 cm, medium grade, Amersham Pharmacia Biotech). Large (~100 nm) unilamellar vesicles (LUV) were prepared by extrusion (18). Chemical analysis of such vesicle preparations shows a low content of FA (<1.0 mol%) with respect to PC.

Measurement of Fatty Acid Flip-Flop in SUV and LUV—Fluorescence measurements were performed with a Hitachi F2000 fluorimeter using a cuvette with a magnetic stirrer at room temperature. The suspension of PC vesicles (0.51 mM/sUv sample; 3.5 mM/mLUV) with entrapped pyranin was diluted to 2.0 mL in 100 mM Hepes/KOH buffer (pH 7.4). External pH was determined with a pH minielectrode (Micro-electrode, Londonderry, NH), and internal pH (pHin) was determined by pyranin fluorescence (excitation wavelength, 455 nm; emission wavelength, 509 nm). Aliquots of 0.5 μL of FA in ethanol were added to buffer containing PC vesicles. Eight volumes of 0.5-μL ethanol added at the same time intervals showed no effect on the SUV fluorescence. The relationship between pHin and pyranin fluorescence was calibrated as described (16).

Calculations—The effectiveness of proteins to bind FA was measured as the decrease in the amount of FA that partitions into the phospholipid bilayer and is detected by pyranin after it undergoes transbilayer movement (TRBM). Quantitatively, this was expressed by a TRBM50 value that was calculated from the Schild equation, log (DR1/2) = log [B] - log TRBM (DR = dose ratio, B = concentration of BSA), where DR = [IC50 (BSA)IC50 (control)]. By plotting log DR = 1 versus the log concentration of BSA, each shift of the curve to the right following the addition of one BSA concentration can be determined. The relationship between the four BSA concentrations was plotted, and the linear regression line was extrapolated to the x axis. The intercept shows the TRBM50 value, which is the DR at which 50% of the FA was bound to BSA and therefore represents a calculation performed at the equilibrium concentration between FA and BSA. A high TRBM50 indicates that more BSA was used to reduce transmembrane transport of FA, which reflects weaker binding of the FA to BSA.

To quantify the distribution of FA between SUV and protein, the partition coefficient was calculated. The relative concentrations of FA bound to the vesicle and to the protein were obtained from pHin in SUV measured in the absence and in the presence of protein. The partition coefficient was calculated as FA(vesicle)/FA(protein) × FA(vesicle)/protein × FA(protein) in SUV (16).

RESULTS

Transbilayer movement of FA across a phospholipid bilayer was investigated by a method based on the fluorescence of the pH dye pyranin trapped inside vesicles (16). Fig. 1 compares results in SUV from three unsaturated FA, 18:1, 20:4, and 22:6. Immediately after addition to SUV all FA gave a rapid decrease in fluorescence that was complete within 1–2 s, the time resolution of the experiment, in accordance with previous results for 18:1 and other FA not studied here (16). The same results were obtained upon addition of 18:1, 20:4, and 22:6 to LUV with low curvature (data not shown).

The decrease in fluorescence is directly proportional to a decrease in pHin; this reflects movement of un-ionized FA to the inner leaflet of the membrane ("flip-flop") after binding of the FA to the external leaflet. The extent of the fluorescence (pHin) decrease was similar for all three FA, reflecting equivalent partitioning of FA from the aqueous phase, where they are poorly soluble, into the vesicles. In a second protocol, aliquots of 1 nmol of FA (18:2) were added to the suspension of vesicles in 10-s intervals for a total time interval of 70 s (Fig. 2A). A decrease in fluorescence (pHin) occurred rapidly following each addition of 18:2, as reported previously (3). As shown in Fig. 2B, the monohydroxyl metabolite of 18:2, 13-ODE, also exhibited flip-flop within the time resolution of the measurement. A similar result was obtained for 15-HETE (not shown). In all experiments, the successive additions caused gradually smaller decreases in fluorescence (pHin), as predicted because of the increasing acidification of the vesicle internal volume (16). All FA in this study, including those examined by the protocols of Figs. 2 and 3, showed rapid flip-flop.

To investigate the ability of albumin to bind oxidized FA in the presence of a model membrane, we added aliquots of aqueous BSA to the suspension of SUV containing the highest amount of added FA. Fig. 2 compares parallel experiments with 18:2 and 13-ODE (similar experiments with 18:2 have been published previously; see Ref. 3). Addition of BSA to the external buffer increased the pH inside the vesicle, as a consequence of desorption of FA from the outer leaflet of the SUV to bind to BSA and replenishment of FA by net movement of FA from the inner to the outer leaflet. The increase in pHin was rapid, reflecting rapid desorption of the FA from the vesicle (17) to albumin binding sites and rapid re-equilibration of un-ionized FA across the bilayer. As observed before (16), BSA binds endogenous FA in SUV causing an overshoot of the pHin. Fig. 2A shows that when a total of 2 nmol of BSA was added, the drop in fluorescence (pHin) was more than recovered. Fig. 2B shows that each addition of 13-ODE produced a smaller drop in fluorescence (pHin) compared with the addition of equivalent amounts of 18:2. This is likely a reflection of weaker partitioning of 13-ODE into the PC bilayer. From the illustrated experiment, binding of 18:2 to BSA appeared to be the same as
To obtain more quantitative data about the effectiveness of BSA in binding FA in the presence of vesicles, we modified the protocol used in Fig. 2. In this case a given amount of BSA was added to the external buffer of the SUV suspension prior to the addition of FA. Then the percentage drop in fluorescence (pH_in) following addition of a given FA, in the presence of different concentrations of BSA, was calculated (Fig. 3). The curve to the left represents the fluorescence (pH_in) drop with addition of FA in the absence of BSA, and the four curves shifted to the right the fluorescence (pH_in) drop with different amounts (1–4 nmol) of BSA. With increasing BSA concentration the percentage drop in fluorescence (pH_in) decreased at a given FA concentration. From a plot of the log of the dose ratio of IC_{50} (BSA)/IC_{50} (control) versus the log of the BSA concentration, the intercept with the abscissa was obtained from the regression line. We have designated this value (see “Methods”) the TRBM_{50} value (Table I).

The same experimental protocols as in Figs. 2 and 3 were performed for several additional FA including lauric (12:0), palmitic (16:0), oleic (18:1), linoleic (18:2), arachidonic (20:4), pentaenoic (20:5), and docosapentaenoic acid (22:5) (Table I). To compare the results of different FA, the TRBM_{50} value was calculated for each FA. A higher TRBM_{50} value corresponds to a lower partitioning of FA to the protein. The TRBM_{50} value generally decreased with increasing FA carbon chain length (Table I) but was not distinguishable for the 18- and 20-carbon FA studied. For the two monohydroxy FA, 15-HETE and 13-HODE, the calculated TRBM_{50} value was about twice that of their respective FA. Thus, twice as much BSA was added to bind 13-HODE and 15-HETE as compared with the respective FA, 20:4 and 18:2, indicating weaker binding of the two monohydroxy FA to BSA (Table I).

As a further analysis of our data, we calculated the partition coefficient (see “Methods”) for FA between BSA and SUV. The data for 18:2 can be analyzed as two regions, one with a small slope at low mole ratios and the other with a larger slope at high mole ratios. The intersection of the two linear portions occurs at ~3.0 mol of 18:2/BSA and is interpreted, as before (16), as the number of high affinity sites for 18:2. After the high
affinity sites are filled, the partitioning to SUV increases at a higher rate. By comparison, there is no detectable break in the plot for 13-HODE, and the slope of the single line is about the same as for 18:2. This predicts that there are fewer high affinity sites for 13-HODE than for 18:2 and other long chain FA. Furthermore, partitioning of 13-HODE compared with 18:2 favors the SUV at all FA/BSA ratios except \(0.5\), where the scatter in the data are too great to distinguish the two FA.

Oxidized FA generated inside the cell could bind to membranes and FABP. To determine the partitioning of FA between a phospholipid bilayer and FABP, experiments were done as in Fig. 2, and the fluorescence (pHin) was used to monitor the

| Fatty Acid                   | TRBM50 \(\mu M\) |
|------------------------------|------------------|
| Lauric acid (12:0)           | 6.5 (1)          |
| Palmitic acid (16:0)         | 6.2 (1)          |
| Oleic acid (18:1, n-6)       | 2.0 (2)          |
| Linoleic acid (18:2, n-6)    | 2.0 \(\pm 0.07\) (3) |
| Arachidonic acid (20:4, n-6) | 2.0 (2)          |
| Pentaenoic acid (20:5, n-3)  | 2.0 (2)          |
| Docosapentaenoic acid (22:5, n-6) | 1.6 (2) |
| Monohydroxy fatty acid       |                  |
| 13-HODE (18:2-OH, n-6)       | 4.0 (2)          |
| 15-HETE (20:4-OH, n-6)       | 4.0 (1)          |

Table I

Partitioning of FA between BSA and phospholipid vesicles; effect of BSA on the transbilayer movement constant of various FA

The inhibitory transbilayer movement constant, TRBM50 value, of each fatty acid to BSA was determined from experiments as illustrated in Fig. 2 for 18:2. Following the addition of several concentrations of BSA the TRBM50 value was estimated from a Schild analysis (see "Methods"). Each value was determined from addition of 4–6 concentrations of BSA. Values within brackets represent number of experiments. Mean values \(\pm\) S.D.

| Fatty Acid | 15-HODE | 18:2 | 18:1 |
|------------|---------|------|------|
| I-FABP     | 1.3 \(\pm\) 0.09 | 1.5 \(\pm\) 0.4 | 2.2 \(\pm\) 0.7 |
| L-FABP     | 0.4 \(\pm\) 0.2  | 1.0 \(\pm\) 0.3 | 0.9 \(\pm\) 0.06 |
| BSA        | 0.3 \(\pm\) 0.1  | 0.2 \(\pm\) 0.02 | 0.2 \(\pm\) 0.03 |

Table II

Partitioning of FA between proteins and phospholipid vesicles: the amount of protein (nmol) required to reduce flip-flop by 50% in the presence of a constant FA concentration (1 nmol)

Mean values \(\pm\) S.D., \(n = 2\).

Fig. 4. Partitioning of FA between SUV and BSA. Plot of the partition coefficient (see "Methods") versus the ratio between the total amount of added FA and the BSA concentration (1–4 nmol) for 18:2 (\(\times\)) and 13-HODE (\(\bullet\)). Mean values from two to three experiments are shown. Linear regression of 18:2, second part, showed a multiple correlation coefficient of \(R^2 = 0.89\), and for 13-HODE, \(R^2 = 0.88\).

Fig. 5. Partitioning of FA between SUV and L-FABP. The decrease in fluorescence (pHin) after single additions of 0.5 nmol of 18:2 (A) and 0.66 nmol of 13-HODE (B) as indicated, and the reversal of pHin after single additions of 1 nmol of L-FABP, are shown. The total concentration of 18:2 added to SUV was 3 nmol, which was completely recovered by the addition of \(-5\) nmol of L-FABP (1.7 mol of FABP/mol of FA). The total amount of 13-HODE added was 4 nmol, which was completely reversed by the addition of \(-2\) nmol (0.5 mol of FABP/mol of FA). Table II shows the mean values from two experiments with L-FABP, I-FABP, and BSA.
amount of FA bound to SUV. Because only limited amounts of FABP were available as compared with BSA, a lower concentration of FA was used. The values shown in Table II therefore represent TRBM rather than the TRBM₅₀ determined from multiple experiments with BSA (Table I). In this case the amount of protein required to inhibit TRBM by 50% of 1 nmol FA across SUV was calculated (Table II).

In comparing the flip-flop of the FA in SUV in the presence of BSA, the value for 13-HODE revealed a weaker affinity compared with 18:1 and 18:2, as shown also by the data from Fig. 4. The flip-flop (Table II) for 13-HODE was only slightly lower for BSA as compared for L-FABP.

Fig. 5 compares the pHₐᵢ changes after addition of 18:2 and 13-HODE followed by addition of L-FABP. About 5 nmol L-FABP was used to completely recover the drop in fluorescence (pHᵢₐ) achieved by addition of 3 nmol 18:2 (Fig. 5A), but only 2 nmol of L-FABP was used to recover the effect of 4 nmol 13-HODE (Fig. 5B). This comparison demonstrates a higher affinity of L-FABP for 13-HODE than for 18:2 in the presence of a model membrane. Similar experiments were performed with I-FABP. The amounts of L-FABP, I-FABP, and BSA used to decrease the binding and transport in SUV by 50% for a specific amount of FA (1 nmol) are shown in Table II. We found a higher value for I-FABP and a smaller difference between 18:2 and 13-HODE compared with L-FABP. BSA had significantly lower values for 18:1 and 18:2 compared with both FABP.

The cellular retinoic acid binding protein II had no effect on flip-flop with any of the FA (data not shown). Addition of BSA or FABP protein to the SUV preparation resulted in a small increase in fluorescence, indicating binding of contaminating free FA in the SUV.

DISCUSSION

We studied the transport of several polyunsaturated FA and oxidized FA across phospholipid vesicles with a single bilayer encapsulating a fluorescent pH dye. Fluorescence measurements quantitated the effectiveness of FABP and BSA in recovering the FA-induced decrease in pHᵢₐ. Because the FA diffused rapidly across the bilayer and desorbed rapidly from the phospholipid membrane, the extent of the recovery of pHᵢₐ after addition of protein was related directly to the ability of the protein to bind each FA. Although our study did not mimic the full complexity of cell membranes, it is more relevant to cell physiology than an assay in aqueous media, because the lipophilic FA molecules will bind extensively to the lipid bilayer in biological membranes.

Adsorption and Transmembrane Movement of Polyunsaturated FA and Oxidized FA in Phospholipid Bilayer—Previously we described a diffusion pathway in simple model membranes and in cells based on pH effects associated with FA flip-flop (18). In the present study we examined the transmembrane movement of several FA not previously studied with the pyranin assay, 20:4, 20:5, 22:5, and 22:6 and the peroxidation products, 13-HODE and 15-HETE. Although these FA have increased water solubility compared with their saturated counterparts, they are still highly hydrophobic molecules and bind mainly to the lipid (membrane) phase in an aqueous suspension of phospholipid vesicles, as shown by the decrease in pHᵢₐ. The PUFA caused the same decrease in pHᵢₐ as oleic acid, when equal amounts of each FA were added to vesicles, indicating equivalent partitioning. Among the FA studied, a significant decrease in partitioning into vesicles in buffer (without added protein) was observed only for 13-HODE and 15-HETE.

All FA studied caused an immediate and rapid decrease in pHᵢₐ after their addition to vesicles (SUV or LUV). Thus, the PUFA precursors for oxidized FA and the 13-HODE and 15-HETE can likely enter (and exit) a cell by simple diffusion without a transporter protein. This could represent a mechanism whereby peroxides of FA exit the cell spontaneously and rapidly to act as inducers for low density lipoprotein oxidation in the extracellular matrix. Although a recent study suggested that certain monohydroxy saturated FA cannot flip-flop (19), our results for 13-HODE and 15-HETE are in accord with NMR (20) and fluorescence spectroscopy (16) experiments that showed fast flip-flop of mono- and dihydroxy bile acids across phospholipid membranes. The rapid flip-flop rates of polyunsaturated FA are consistent with the interpretations of the kinetics of transfer of polyunsaturated FA from phospholipid vesicles (21).

Partitioning between Albumin and Phospholipid Bilayers—To determine the effectiveness of albumin in removing FA from membranes and to provide comparative data for FABP experiments, we used BSA as an acceptor of FA in bilayer membranes. BSA was able to remove all FA species examined from membranes with a relative effectiveness quantified as the TRBM₅₀ (Table I). The TRBM₅₀ was the same or nearly so for all unsaturated FA, indicating an equivalent partitioning of these FA between albumin and membranes; i.e. the ratio of binding constants for each FA with BSA or vesicles alone is the same. The equilibrium constants for binding of saturated FA to phospholipid bilayers (22) or to albumin alone (23) decrease with decreasing chain length. Our results reflect a relatively greater decrease in the binding to BSA, shifting the partition coefficient more in favor of the membrane. The oxidized FA showed an even greater partitioning to the membrane and less effective binding to albumin compared with the PUFA. Differential binding of 18:2 and its metabolite 13-HODE was revealed by the data analysis of Fig. 4, which showed higher partitioning of 18:2 to BSA at all mole ratios of FA except possibly at low mole ratios (<0.5), where the data were indeterminate.

Partitioning between FABP and Phospholipid Bilayers—FABP are small (<15 kDa) intracellular proteins with a single binding site for FA, except for L-FABP, which has two binding sites for FA (24). As shown in Table II, 13-HODE partitioned more favorably than 18:1 or 18:2 to I-FABP and L-FABP. All three ligands showed better binding to L-FABP compared with I-FABP. The results for 18:2 and 13-HODE provide evidence that a metabolite of a FA can bind better to cytosolic FABP than its precursor FA. On the basis of displacement of oleic acid from FABP, Veerkamp and Maatman (25) found that certain prostaglandins, which are more polar than 15-HETE and 13-HODE, bound to both I-FABP and L-FABP with about the same affinity as FA; L-FABP was much more effective in binding prostaglandins than muscle FABP, which is more closely related to I-FABP.

Comparing the Binding of FA and 13-HODE to BSA and FABP—The partitioning experiments summarized in Table II can be discussed in terms of affinities of the proteins for the different ligands, as the membrane component was constant in all cases. The finding that the affinity of BSA for 13-HODE is lower than for 18:1 or 18:2 can be rationalized by reference to the structures of binding sites (30, 31). It is likely that the hydroxylated FA have less stable interactions in the FA high affinity sites and are relegated to lower affinity sites for FA, possibly the flexible site in the middle of albumin that binds diverse ligands such as bile acids and bilirubin with high affinity (31).

In contrast to the α-helical, multiple domain structure of albumin, FABP consists of a β-barrel lined with both hydrophobic and polarcharged amino acids. In I-FABP, which has a
high specificity for FA, a single FA molecule is buried inside the binding cavity (32, 33). L-FABP is capable of binding two FA molecules, as well as other ligands (34). Ideal lipid binding protein, another member of the same family, binds bile acids with one or more OH groups with higher affinity than FA (35, 36). Thus it is predictable that FABP could bind hydroxylated FA such as 15-HETE and 13-HODE as well or better than FA and that L-FABP could show a higher affinity for these ligands than I-FABP. It is interesting to note that the partitioning of 13-HODE to L-FABP is almost indistinguishable from BSA (Table II).

Summary and Implications—Our study yielded the following important findings: (i) PUFA and the hydroxylated products 13-HODE and 15-HETE adsorb rapidly to a phospholipid bilayer, and the un-ionized form of the FA diffuses rapidly across the bilayer by the flip-flop mechanism; (ii) all FA desorb from the phospholipid bilayer rapidly to bind to BSA, which shows a preference for the unsaturated FA over the oxidized FA; (iii) all FA desorb from the phospholipid bilayer to bind to FABP, which shows a preference of oxidized FA over the unsaturated FA and a specificity depending on the type of FABP. The interactions of oxFA such as 13-HODE and 15-HETE (and their FA precursors) with membranes, intracellular FABP, and albumin must therefore be considered to understand their biological effects and metabolism.

Extrapolation of these and previous results with simple model systems to cells show that a complex relationship might exist between FABPs and intracellular oxidative metabolism of FA. Intracellular oxidation of PUFA could be decreased by their binding to FABP (3), and binding protects the product from further degradation and cell membranes from effects of oxidized FA. It is also possible that membrane-associated proteins, including those described as “transporters” (11, 12), could protect membranes by binding and sequestering oxFA.

Binding of 13-HODE to FABP could also influence the interactions of HODE with nuclear targets, such as PPAR. 13-HODE can be formed in the cytosol or can be internalized with membranes, intracellular FABP, and albumin must therefore be considered to understand their biological effects and metabolism.

In considering potential roles of FABP in transporting oxFA, it is important to consider that only a minor fraction of intracellular FABP is complexed with FA under normal cellular conditions; most FA is bound to cell membranes (27). Thus, FABP could bind oxFA without displacement of the highly abundant typical FA. A protective role of FABP in ischemia of intestinal (38), liver (37), and myocardial tissue (28) has been suggested, although precise mechanisms were not established. The role of L-FABP in binding oxFA may be particularly important in cells where it is abundant, which are the liver, stomach, small intestines, and kidney. The roles of FABP in binding oxFA suggested by our study may be important in diseases related to increased lipid peroxidation and altered utilization of FA, such as insulin resistance and atherosclerosis. The intracellular concentration and type of FABP could regulate their binding to membranes and their transport to the nucleus.

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