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THE METABOLIC SIGNIFICANCE OF MAMMALIAN FATTY-ACID-BINDING PROTEINS: Abundant Proteins in Search of a Function

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CONTENTS

INTRODUCTION

Although the fatty-acid-binding proteins (FABPs) were first described in 1969 (51), their cellular function remains unclear. Originally identified on the basis

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of their ability to bind organic anions, these small (14–15 kDa) cytoplasmic proteins were independently isolated based on their ability to bind fatty acids (69). The heterogeneity of the FABPs and their endogenous ligands were only slowly discovered (29, 79). Numerous roles have been proposed for the fatty-acid-binding proteins. They include (*a*) enhancing cellular fatty acid uptake, (*b*) enhancing the transport and targeting of fatty acids to intracellular organelles, (*c*) targeting different types of fatty acids to different metabolic pathways, (*d*) stimulating the activities of enzymes involved in fatty acid metabolism through direct interactions, and (*e*) protecting cellular enzymes and membranes from the detrimental effects of long-chain fatty acids and their acyl CoA derivatives. However, despite the fact that a wide variety of physiological, biochemical, and molecular biological techniques have been applied to study their structure and function, the physiological role(s) played by these proteins remains incompletely understood.

A number of review articles concerning the mammalian fatty-acid-binding proteins have appeared over the past several years (e.g. 7, 32, 35). The purpose of this paper is to provide an overview of what is known about the metabolic significance of these extraordinarily abundant cellular proteins. In order to do so, a summary of cellular long-chain fatty acid metabolism, as well as fatty-acid-binding protein structure, is provided. Evidence for their roles in the uptake, intracellular trafficking, and metabolic compartmentalization of long-chain fatty acids is examined. Finally, potential directions for future research are discussed.

REVIEW OF EUKARYOTIC LONG-CHAIN FATTY ACID METABOLISM

To identify potential physiological roles for FABPs, it is first necessary to understand the metabolism of long-chain fatty acids in different cell types (see Figures 1 and 2; for a detailed overview, see Ref. 95). The utilization of fatty acids is a function of hormonal and nutritional status, cellular energy and synthetic requirements, as well as tissue type. In eukaryotic cells, fatty acids may undergo β-oxidization for energy, may be incorporated into phospholipids and other complex lipids as structural components of membranes, or may be stored as triglycerides for future energy needs (Figure 1). In addition, arachidonate, a long-chain fatty acid known to be bound by some FABPs, serves as a precursor for a large number of locally acting hormones, including prostaglandins, thromboxanes, and leukotrienes. The fatty acids palmitate and myristate have also been identified as being covalently bound to specific cellular proteins (72). These last two roles for fatty acids are not discussed further here.

Utilization of long-chain fatty acids for either anabolism or catabolism

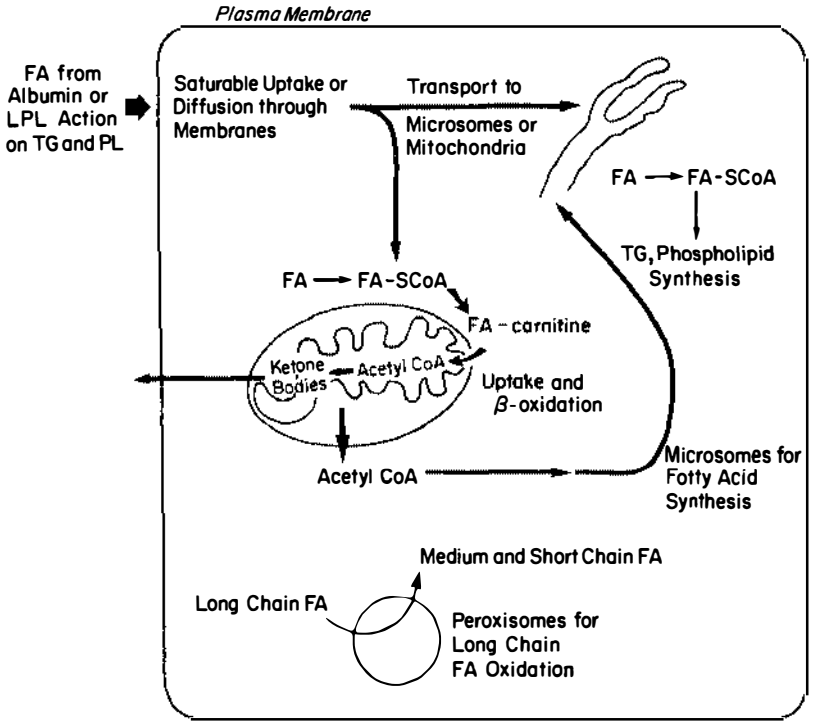

Figure 1 Scheme for fatty acid metabolism in a prototypic cell. Fatty acids are β-oxidized to acetyl CoA in mitochondria and resynthesized into more complex lipids in microsomes. For either of these processes to occur, fatty acids must be absorbed at the plasma membrane, travel through the aqueous cytosolic environment, and be converted to their acyl Coenzyme A derivatives. FA, fatty acid; LPL, lipoprotein lipase; TG, triacylglycerol; PL, phospholipid; FA-SCoA, fatty acyl CoA.

generally requires the conversion of free fatty acids to their acyl CoA derivatives. Long-chain acyl CoA synthetases are located in the endoplasmic reticulum, the outer mitochondrial membrane, and peroxisomes. Once free fatty acids have crossed a cell's plasma membrane, they must be transported through the aqueous cytoplasmic environment to these intracellular organelles for metabolic activation. Enzymes that incorporate fatty acids into more complex lipids use fatty acyl CoAs directly. The inner mitochondrial membrane, however, is impermeable to CoA and its derivatives. To enter the mitochondria for β-oxidation, acyl chains of long-chain fatty acyl CoAs are transferred to carnitine by carnitine palmitoyl transferase I and are translocated into the mitochondrial matrix by carnitine:acylcarnitine translocase. The translocated acyl chain is then reconverted to fatty acyl CoA by carnitine palmitoyl transferase II (Figure 1).

Peroxisomes are an additional intracellular compartment where fatty acid

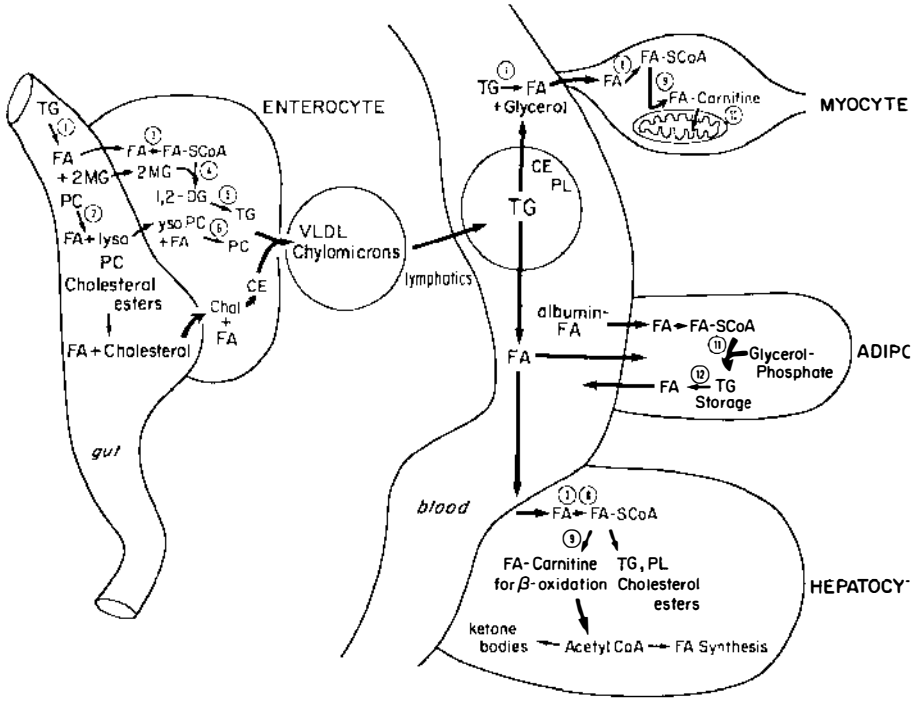

Figure 2 Overall scheme for fatty acid metabolism. Ingested triacylglycerol (TG), and phospholipids (PL) such as phosphatidylcholine (PC) are degraded in the intestinal lumen by pancreatic lipase ① and phospholipase A_2 ② to generate free fatty acids (FA), 2-monoacylglycerol (2-MG), and lysophosphatidyl choline (lyso-PC) before being absorbed by the intestinal lining cell (enterocyte). Cholesterol esters (CE) are also degraded to free fatty acids and cholesterol (Chol) before absorption. Within the enterocyte, fatty acids are converted to their coenzyme A derivatives by microsomal fatty acyl CoA synthetase ③ . Monoacylglycerol acyltransferase ④ and diacylglycerol acyltransferase ⑤ re-esterify these acyl CoA derivatives of fatty acids into triglyceride. Lysophosphatidylcholine acyltransferase ⑥ re-esterifies fatty acyl CoAs and lyso-PC to generate phosphatidylcholine. Cholesterol and fatty acyl CoAs are also re-esterified into cholesterol esters. Triglycerides, phospholipids, and cholesterol esters are packaged into chylomicrons and VLDL, released into the extracellular space and carried to the blood by lymphatics.

Lipoprotein lipase ⑦ on the vascular endothelium degrades lipoprotein-bound triglycerides to fatty acids and glycerol. Free fatty acids are also transported by serum albumin. Fatty acids, when absorbed by myocytes are principally esterified by mitochondrial fatty acyl-CoA synthetase ⑧ to fatty acyl CoA. To enter the mitochondria for β-oxidation, fatty acyl CoAs are converted to fatty acyl carnitine by carnitine palmitoyl transferase I ⑨ , transported into the mitochondria by carnitine:acylcarnitine translocase ⑩ , and then converted back to fatty acyl CoA inside the mitochondria by carnitine palmitoyl transferase II.

Fatty acids absorbed by adipocytes are primarily esterified into triacylglycerols for storage after conversion to fatty acyl CoA. Glycerolphosphate acyltransferase ⑪ combines acyl CoA and sn-glycerol-3-phosphate to form lysophosphatidate. Monoacylglycerolphosphate (lysophosphatidate) acyltransferase ⑪ adds a second fatty acyl CoA to generate phosphatidate, which is then

β-oxidation is known to occur. However, peroxisomal degradation of fatty acids differs from mitochondrial β-oxidation in several respects. The enzymes of peroxisomal β-oxidation generate hydrogen peroxide, those of the mitochondrial system do not. Moreover, the best substrates for peroxisomal β-oxidation are long-chain fatty acyl CoAs (C_{10}–C_{22}); isolated liver (peroxisomal) fatty acid oxidase and thiolase (enzymes of β-oxidation) have minimal activity on fatty acyl chains shorter than C_8. Two carnitine acyltransferases have been identified in peroxisomes: carnitine acetyltransferase and a medium-chain (e.g. C_8) carnitine acyltransferase. The medium length acyl chains produced in peroxisomes are believed to be further degraded in mitochondria. While the exact role of peroxisomes in fatty acid metabolism remains unclear, their importance is indicated by peroxisomal diseases of fatty acid metabolism like Zellweger syndrome (82) and adrenal leukocystrophy (41), where long-chain fatty acids accumulate in tissues.

Animals obtain fatty acids either from the diet or by de novo synthesis from acetyl CoA. Most dietary fatty acids are ingested as triglycerides, with lesser amounts in the form of phospholipids and cholesterol esters. In the small intestine, pancreatic lipase degrades triglycerides to free fatty acids and 2-monoacylglyceride (Figure 2). Phospholipids and cholesterol esters are hydrolyzed to form free fatty acids: lysophospholipids and cholesterol, respectively. Free fatty acid, 2-monoglyceride, lysophospholipid, and cholesterol are generally assumed to diffuse into the small intestinal epithelial cell (enterocyte), although a saturable transport mechanism for long-chain fatty acids has also been implicated.

Once converted to fatty acyl CoAs in the enterocyte, triglycerides, phospholipids and cholesterol esters can be resynthesized for export in chylomicrons and VLDL (Figure 2). Monoacylglycerol acyltransferase in the enterocyte converts 2-monoacylglycerol and fatty acyl CoA to diacylglycerol and CoA. An additional acyl chain is added by diacylglycerol acyltransferase to form triacylglycerol. Lysophosphatidylcholine acyltransferase resynthesizes phosphatidylcholine from lysophosphatidylcholine and fatty acyl CoA. These lipids are then complexed with specific proteins (apolipoproteins) to form

converted to 1,2-diacylglycerol by phosphatidate phosphohydrolase. 1,2-Diacylglycerol is the precursor for triglycerides, phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine. Diacylglycerol acyltransferase (11) adds the final acyl CoA unit to create triacylglycerol. Hormone-sensitive lipase (12) releases free fatty acids stored as triacylglycerol when activated by cAMP-dependent phosphorylation.

In the hepatocyte, fatty acids may be synthesized, degraded, or incorporated into more complex lipids depending on the nutritional state of the organism. Acetyl CoA generated from β-oxidation of fatty acids during starvation is also converted to ketone bodies, which the brain may use as an alternate energy source.

lipoproteins (chylomicrons and VLDL), which are released by the enterocyte into the intercellular space. They are carried by lacteals to the lymphatic system and enter the blood through the thoracic duct. Lipoprotein lipase, an enzyme found on the luminal surface of endothelial cells (primarily in muscle and adipose tissue), releases free fatty acids from triacylglycerol and phospholipids so that they may be utilized by the underlying cells.

Fatty acid metabolism in hepatocytes and adipocytes is determined by the nutritional state of the animal (Figure 2). In the fed state, fatty acids are actively synthesized in the liver from acetyl CoA, incorporated into triglyceride, and then exported for storage in adipose tissue. Adipocytes also make triglycerides but primarily utilize the products of local lipoprotein degradation as substrates instead of de novo synthesis of fatty acid. In the fasted state, fatty acid synthesis is inhibited while triglycerides stored in adipose tissue are broken down into free fatty acids and glycerol for release into the bloodstream. In the liver, increased β-oxidation of free fatty acids provides acetyl CoA for the synthesis of ketone bodies, while glycerol from triacylglycerides is used for gluconeogenesis. Free fatty acids and ketone bodies are used as energy sources by many tissues in the fasted state.

These changes occur as a result of hormonal regulation and substrate concentration effects on key enzymatic reactions. After a period of fasting, insulin levels are low while levels of lipolytic hormones, such as glucagon and epinephrine, are high. When glucagon binds to its receptor on the hepatocyte, adenylate cyclase is stimulated. The increased intracellular cyclic AMP concentration stimulates cAMP-dependent protein kinase, which in turn phosphorylates and thus inactivates acetyl CoA carboxylase. Acetyl CoA carboxylase uses acetyl CoA and bicarbonate to synthesize malonyl CoA, a key substrate used by fatty acid synthase for the production of long-chain fatty acids. Inactivation (phosphorylation) of acetyl CoA carboxylase reduces the levels of malonyl CoA and thus decreases the rate of hepatic fatty acid synthesis. Since malonyl CoA is also an inhibitor of carnitine palmitoyl transferase I, the inhibition of acetyl CoA carboxylase by phosphorylation increases transport of long-chain fatty acids into mitochondria. Thus, during fasting, fatty acid synthesis is inhibited while β-oxidation is stimulated. In adipose tissue, glucagon also stimulates cAMP-dependent protein kinase. This leads to phosphorylation and activation of hormone-sensitive lipase, which releases free fatty acids from stored triglycerides. The free fatty acids released are transported through the blood bound to albumin to the liver, where they undergo β-oxidation and ketone body formation.

After a carbohydrate-rich meal, insulin levels rise while glucagon levels fall. Decreased hepatic intracellular cAMP levels lead to increased malonyl CoA synthesis by acetyl CoA carboxylase. This not only provides substrates for fatty acid synthase, but also inhibits carnitine palmitoyl transferase I (CPT I). [Physiological changes in malonyl CoA concentrations are not sufficient in

themselves to account for the observed changes in CPT I activity. Phosphorylation of CPT I regulates its sensitivity to inhibition by malonyl CoA (26, 40).] Phosphatidate phosphohydrolase converts phosphatidate (diacylglycerolphosphate) to diacylglycerol for the synthesis of triglycerides. This enzyme appears to be activated by translocation from a cytoplasmic pool to a membrane-associated form by the increased availability of free fatty acid. Decreased cAMP concentration increases the sensitivity of phosphatidate phosphohydrolase to fatty-acid-induced translocation. Thus, β-oxidation is inhibited, while fatty acid and triglyceride synthesis are stimulated. Insulin also inhibits hormone-sensitive lipase in adipose tissue and increases lipoprotein lipase activity. Together, these actions lead to the efficient synthesis and storage of fatty acids at times of excess caloric intake.

In skeletal and especially in cardiac muscle, long-chain fatty acids are a major energy source. Eighty percent of cardiac energy demands at rest may be supplied by long-chain fatty acid oxidation. Uptake of free fatty acids depends on plasma-free fatty acid concentration and the energy demands of the tissue. At times when uptake exceeds demand, triglycerides may also be synthesized and stored in myocytes for future use.

In addition to their metabolic roles in energy production and membrane biosynthesis, fatty acids and fatty acyl CoAs are known to modulate the activity of a large number of cellular enzymes. Acetyl CoA carboxylase (55), mitochondrial ATP/ADP translocase (5, 74), mitochondrial or microsomal acyl CoA synthase (13, 67, 73, 96), microsomal methyl sterol oxidase (38), hydroxymethylglutaryl CoA reductase (38), acyl CoA:cholesterol acyltransferase (38), phosphofructokinase as well as synaptasomal Na^+-dependent amino acid uptake (see 76, 77) are all inhibited by long-chain fatty acids or fatty acyl CoAs.

EVIDENCE THAT THE MAMMALIAN FABPs MAY PLAY DISTINCT ROLES IN DIFFERENT TISSUES

There are several lines of evidence supporting the notion that the three mammalian fatty-acid-binding proteins characterized to date serve distinct biological functions: (*a*) structural analysis of these proteins indicates that certain regions contained in all the fatty-acid-binding proteins have been highly conserved throughout evolution while others have diverged considerably, (*b*) these proteins have distinct ligand-binding characteristics, and (*c*) the FABP genes have different patterns of tissue-specific expression.

Structural Analysis

Fatty-acid-binding proteins (FABPs) have been isolated from rat intestine (60, 66, 69), liver (60, 69), heart (22), skeletal muscle (80), adipose tissue (39), and brain (10), as well as from mouse preputial gland (62) and chick neural

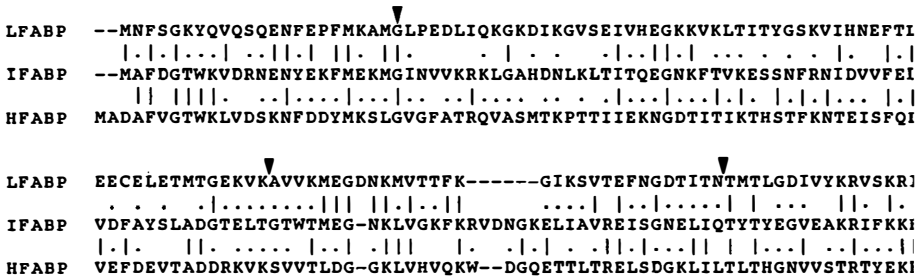

Figure 3 Amino acid sequence comparison of rat L-FABP with rat I-FABP and H-FABP. Identical residues are indicated by a vertical line, while conservative substitutions with similar amino acids [as defined by the 250 PAMs matrix, (6)] are indicated by a dot. Positions of the three introns in the rat L-FABP gene are indicated by arrows (93).

retina (50). The primary structures of three mammalian intracellular FABPs have been fully defined (Figure 3). Liver FABP (L-FABP) [also known as hepatic FABP (hFABP) (7), Z protein (51), or sterol carrier protein 1 (SCP-1) (20)] has been the most extensively characterized. The human and rat proteins contain 127 amino acids (14.3 kDa) (17, 33, 53). Rat intestinal FABP (I-FABP) is also known as gastrointestinal FABP (gFABP) (7). It contains 132 amino acids (15.1 kDa) (2). Rat heart FABP (H-FABP) contains 133 amino acids (15.0 kDa) (78; R. O. Heuckeroth, et al, manuscript in preparation). The nomenclature used for these FABPs is misleading in that it implies a limited tissue distribution. In fact, naming of the FABPs is more closely related to history (i.e. the tissues from which they were first isolated) than to the pattern of expression of their genes in different organs (see below).

The fatty-acid-binding proteins belong to a family[1] of low-molecular-weight (14–16 kDa) cytosolic proteins that bind hydrophobic ligands. Eight members of this family have been identified to date. In addition to L-FABP, I-FABP, and H-FABP, this protein family includes cellular retinol-binding protein (CRBP) (89), cellular retinol-binding protein II (CRBPII) (52), cellular retinoic-acid-binding protein (CRABP) (90), the P2 protein of peripheral

[1]Dayhoff et al (6, 18) defined a protein "family" as a group of proteins that have ≥50% sequence identity. A "superfamily" is composed of proteins whose probability of achieving sequence similarity by chance alone is less than 10^{-6}. In this classification, H-FABP and the 422 and myelin P2 proteins would comprise a family. The family would not include L- or I-FABP. All three FABPs would, however, belong to one superfamily. We prefer a revised definition of these terms proposed by Doolittle (21). According to Doolittle, a protein "family" consists of a group of proteins whose sequences can be shown to be significantly related by statistical methods, regardless of the precise percent identity that they exhibit, while a "superfamily" is composed of two or more families containing a subset of related proteins (21). With this definition all three FABPs would be considered members of a single protein family.

nerve myelin (48), and the 422 (aP2) adipocyte protein (11). The cellular retinol/retinoic-acid-binding proteins are thought to be involved in directly modulating the biological effects of vitamin A on cellular differentiation and/or to participate in the absorption, metabolism, and intracellular compartmentalization of retinoids (91). Retinols delivered to the intestinal epithelium and liver are converted to retinyl esters in much the same way that fatty acids are handled by these tissues (91). Little is known about the endogenous ligand population, substrate specificities, tissue distributions, or biological functions of the P2 and 422 (aP2) proteins. However, these proteins are highly homologous to H-FABP (78). The extreme sequence conservation between H-FABP and the 422 (aP2) protein (overall amino acid identity = 62% to H-FABP) and the P2 protein (59% identity to H-FABP) suggests that these three polypeptides may serve related functions in different cell types and/or may interact with a similar set of ligands.

Comparative sequence analyses have not been able to detect any significant degree of homology between the primary sequences of members of this protein family and serum albumin or several well-characterized intracellular lipid transfer proteins (35). Albumin is the principal carrier of long-chain fatty acids and other organic anions in plasma. Nonspecific lipid transfer protein, also known as sterol carrier protein 2 (SCP-2), and phosphatidylcholine transfer protein are two of several intracellular lipid transfer proteins that transfer phospholipids between membranes (for overview, see 97). SCP-2 can mediate the in vitro transfer of phospholipids, cholesterol, and gangliosides between membranes and may function as a modulator of cholesterol biosynthesis. Phosphatidylcholine transfer protein appears to be a specific carrier for phosphatidylcholine. The lack of homology of the fatty-acid-binding proteins to serum albumin and the lipid transfer proteins indicates that these proteins have evolved independently and are likely to have distinct roles in lipid metabolism (35).

The rat L-FABP, rat CRBPII, and mouse aP2 (422) genes all contain three introns that interrupt the coding sequences at precisely the same positions (18a, 43, 93). This *identical* genomic organization strongly suggests that they all evolved from a common primordial gene. Chan et al recently proposed an evolutionary tree for I-FABP, L-FABP, P2, and the 422 protein based on an analysis of mRNA and protein sequences (17). They calculated that approximately 10^9 years ago a primordial sequence diverged giving rise to a precursor of the liver and intestinal FABPs and a precursor of the 422 and myelin P2 proteins. Their analysis indicated that the liver and intestinal FABPs diverged 650–690 million years ago, whereas the 422/P2 precursor diverged ~490 million years ago (17).

Several studies have revealed that the greatest degree of sequence identity exists among the NH_2-terminal halves of these proteins (reviewed in 93). Of

the four exons contained in the rat L-FABP gene (see Figure 3), only the 23-amino-acid peptide encoded by the first exon exhibits significant homology to *all* the other family members (93). Because exonic boundaries often define functional domains, this observation suggests that the first exon of the L-FABP gene may specify a functional or structural property common to all family members. Sequence divergence in exons 2–4 of the three (defined) genes might reflect the different ligand specificities of their protein products and/or differences in the organelles and enzymes with which they may interact (78, 93).

Comparative Analysis of Ligand Specificities and Affinities

To date there has been no single, detailed, systematic comparison of the ligand specificities, binding affinities, and stoichiometry of all three fatty-acid-binding proteins using the same analytic technique. Although several studies focused on individual FABPs, direct comparison of experimental results is hampered by uncertainty arising from the use of different assays for measurement of these parameters. The most common methods employed for measuring FABP-ligand interactions are based on radiochemical assays. After equilibrium is achieved, labeled free fatty acids are separated from protein-bound fatty acids using dextran-coated charcoal, Lipidex (hydroxyalkoxypropyl dextran), or gel filtration (reviewed in 31). Another procedure used to measure ligand binding involves the transfer of fatty acids from donor liposomes to FABPs, followed by separation of bound and free fatty acids by centrifugation (71). Comparison of values obtained from these different methods is further hindered by the use of different protein purification and delipidation procedures, which could affect protein conformation (32). Finally, many assays have used relatively crude protein preparations which, in some cases, actually contained more than one FABP (e.g. 66).

The ligand-binding properties of L-FABP have been extensively characterized. Up to 60% of the endogenous long-chain fatty acids in liver cytosol are noncovalently bound to this protein (68). From 50 to 70% of the endogenous, bound, fatty acids are unsaturated, consisting primarily of oleate ($C_{18:1}$), linoleate ($C_{18:2}$), and arachidonate ($C_{20:4}$). Palmitate ($C_{16:0}$) and stearate ($C_{18:0}$) are the principal saturated fatty acids associated with L-FABP purified from rat liver (reviewed in 7, 32).

The preferential binding of unsaturated fatty acids to L-FABP is also evident in vitro. L-FABP binds long-chain fatty acids with K_ds of 0.4–0.9 μM (7). Oleic acid is bound with the highest affinity. Acyl CoA derivatives of fatty acids are bound, but with lower affinity than free fatty acids. Water-soluble medium- and short-chain (C_6–C_{12}) fatty acids exhibit little if any affinity for L-FABP (7). In addition to fatty acids, a variety of other ligands have been reported to bind to L-FABP in vitro. These include heme,

cholephilic anions, and carcinogens (reviewed in 7). It is not known whether any of these ligands are associated with L-FABP in vivo.

Human heart FABP may have a slight preference for oleate ($K_d = 0.6\ \mu M$) over palmitate ($K_d = 0.8\ \mu M$). H-FABP differs from L-FABP in that it does not have a higher affinity for unsaturated compared to saturated long-chain fatty acids; arachidonate is bound with somewhat lower affinity ($K_d = 1.4\ \mu M$) than either palmitate (0.8 μM) or oleate (0.6 μM) (30).

There is some controversy concerning the precise stoichiometry of ligand binding in L- and H-FABP. Until recently it has generally been accepted that they all bind one mole of fatty acids in a noncovalent fashion. However, bovine L-FABP may contain two binding sites for oleate (42), and a recent report by Offner et al (71) indicates that both rat L-FABP and rat H-FABP bind two moles of oleic acid or palmitic acid per mole of protein. These workers suggested that previous determinations of FABP concentrations using colorimetric assays were inaccurate and responsible at least in part for these disparate measurements.

The binding affinities and stoichiometries of purified rat I-FABP have not been reported. Previous studies (66, 67) using crude preparations of FABP from intestine were performed before it was realized that both L-FABP and I-FABP are found in high concentrations in the intestine (8, 34). Recently, both rat I-FABP and rat L-FABP have been expressed in *E. coli* (53a, 54). Studies using pure I-FABP and L-FABP will allow direct comparison between the binding characteristics of these two proteins.

In summary, although the ligand-binding properties of these three fatty-acid-binding proteins have not yet been directly compared, it is clear that at least in the case of L-FABP and H-FABP there are differences in ligand specificity.

Tissue-Specific Expression

Further evidence that these FABPs may have discrete functions comes from studies documenting their distinct distributions in adult rat tissues. The tissue distribution of these three FABPs in adult rat tissues has been determined using an immunodiffusion assay (8) and (in the case of L-FABP and I-FABP) RNA blot hybridizations (34). The results of these studies are summarized in Figure 4.

Liver FABP is found primarily in small intestinal epithelium and liver (8, 34, 36). It accounts for 3–5% of the cytosolic protein mass in adult male rat hepatocytes and 2% of cytosolic proteins in jejunal enterocytes (9). Although the protein and its mRNA have been detected in other tissues, their levels are only a few percent of those encountered in liver and small intestine (8, 34).

In contrast to L-FABP, I-FABP is found in significant levels only in the gastrointestinal tract. The protein is found in highest concentrations in the

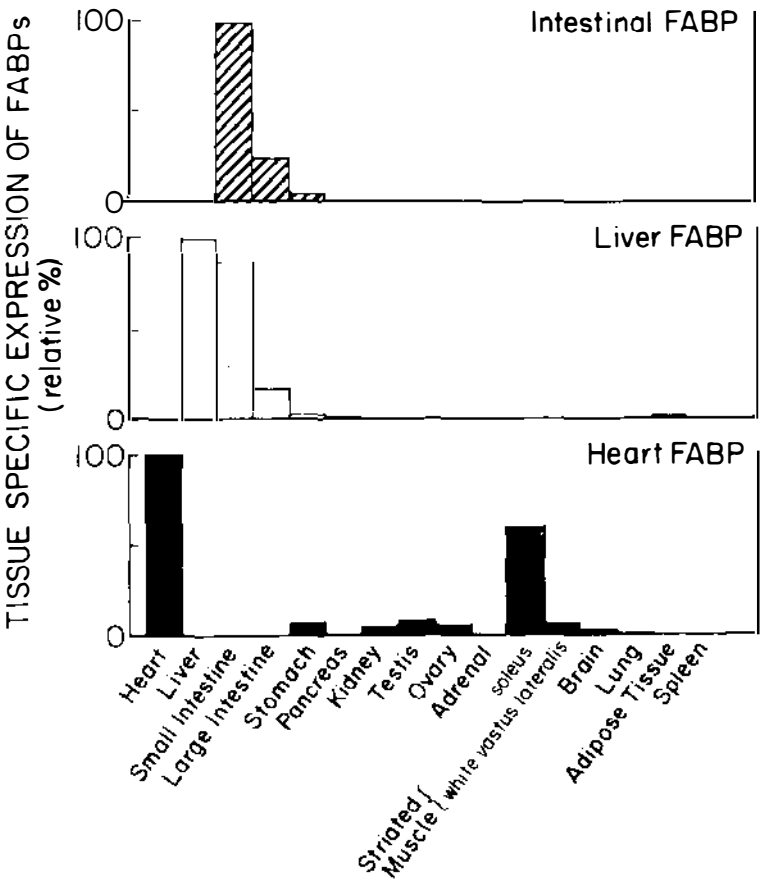

Figure 4 Comparison of the patterns of expression of the rat L-FABP, I-FABP, and H-FABP genes in adult tissues. The data shown are taken from Bass & Manning (8) and Miller et al (58) and represent a survey of several adult rat tissues using an immunodiffusion assay and antibodies specific for each of the three FABPs. The tissue with the highest cytosol concentration of each FABP was assigned a value of 100%. Levels in other tissues were expressed as a percentage of the reference tissue.

small intestine, but is also detectable in the large intestine (at ~25% the level in the small intestine) and stomach (~5% small intestinal levels). It is not detectable in liver (8). Using dot blot hybridizations, I-FABP mRNA has also been detected in a variety of extragastrointestinal tissues at levels that are <3% of those found in small intestine (34). In small intestinal enterocytes, its mRNA represents ~2% of translatable mRNA (36). The mRNAs encoding I- and L-FABP are the most abundant mRNA species that are known to accumulate in the rodent small intestinal mucosa (36).

In addition to their differences in tissue distribution, these two FABPs also exhibit distinct patterns of developmental expression in the rat intestine (34).

Heart FABP is more widely distributed among tissues compared to L- and I-FABP (see Figure 4). H-FABP is most abundant in heart, where it accounts for 4–5% of cytosolic proteins (28). Relatively high levels are also found in skeletal muscle, the content of which is fiber specific. About 60–75% of heart levels are found in soleus and red vastus lateralis, while only 15% of heart levels are found in white vastus lateralis (58). It is not detectable in liver or intestine, but is present in stomach, kidney, brain, testes, ovaries, lung, and adipocytes (8).

The differing structures, ligand affinities, and tissue-specific distributions of these three fatty-acid-binding proteins suggest that these proteins may have evolved to serve distinct functions, perhaps related to differences in lipid metabolism in their tissues of origin.

METABOLIC STUDIES

Identifying definitive roles for FABPs has been complicated by uncertainty as to which enzymatic reactions to examine. Many of the fatty acid metabolic enzymes examined are membrane-bound and thus are difficult to purify. Few studies of fatty-acid-binding protein function have used purified enzymes or purified FABP. Furthermore, in most cases, it is not clear whether the fatty acid substrates for the reactions are delivered from the aqueous medium, are FABP bound, or are dissolved in membranes. For purposes of clarity, we have organized the vast array of studies that focus on the metabolic roles played by the FABPs into four general categories: (*a*) in vivo observations that suggest a role in modulating the intracellular metabolic pathway entered by exogenous long-chain fatty acids; (*b*) in vitro analyses of their effects on specific enzymes involved in fatty acid metabolism; (*c*) studies on the function(s) of heart FABP; and (*d*) considerations of whether the FABPs affect uptake of fatty acids into cells.

In Vivo Studies of FABPs and Their Relationship to Fatty Acid Metabolism

Numerous correlations between cellular FABP levels and metabolic activities provide suggestive evidence for their role(s) in fatty acid metabolism in a variety of organs. Originally, Ockner et al (69) observed that "although saturated and unsaturated fatty acids were taken up by everted jejunal sacs at equal rates, unsaturated fatty acids were esterified more rapidly." This difference was not due to intrinsic microsomal fatty acyl CoA ligase activity. Instead, enhanced binding affinity of FABPs for unsaturated over saturated fatty acids was suggested to account for different rates of substrate delivery to

microsomal enzymes. The role of FABP in intestinal fat absorption is supported by the fact that high-fat diets significantly increase FABP concentrations especially in the middle and distal small bowel (66)[2]. In addition, immunocytochemistry shows L- and I-FABPs to be more abundant in intestinal villus than in crypt cells, in proximal than in distal intestine, and in the apex than in the base of enterocytes (83). It has been suggested (9) that the presence of two distinct, independently regulated fatty-acid-binding proteins in intestine (I-FABP and L-FABP) is related to the uptake by the intestine of fatty acids from two sources. Unesterified fatty acids from the intestinal lumen are primarily re-esterified to triglyceride via 2-monoacylglyceride (presumably I-FABP-mediated), while those fatty acids from plasma are primarily used for energy production and phospholipid biosynthesis (presumably L-FABP-mediated) (27). This hypothesis has not been formally tested.

Correlations between FABP levels and the state of fatty acid metabolism have also been documented in liver. In "female" hepatocytes, the rate of triglyceride synthesis is double that in "male" hepatocytes. However, hepatic long-chain acyl CoA synthetase, phosphatidate phosphohydrolase, and diacylglyceride acyltransferase activities were similar in male and female rats, while glycerol-3-phosphate acyltransferase activity was only slightly higher in microsomes prepared from female compared to male animals. L-FABP concentrations, however, were 44% higher in female than in male hepatocytes. This suggested that FABPs may be, at least in part, responsible for the esterification differences noted by enhancing the rate of intracellular fatty acid transport (64). In intact liver, single-pass oleate uptake as well as esterification into triglycerides is also nearly two-fold higher in livers from females than from males (49). FABP levels and oleate utilization (especially incorporation into triglycerides) are both modulated by sex steroids with strong correlations between FABP levels and oleate utilization rates (65). Refeeding of fasted rats is known to increase hepatic triglyceride synthesis (45). L-FABP levels also significantly increase on refeeding (44).

Clofibrate, a hypolipidemic agent and peroxisome proliferator, increases L-FABP concentration in liver 98% while increasing free fatty acid uptake in isolated perfused livers by 76% (9, 75). Other peroxisome proliferators also increase hepatic FABP concentration. Significant correlation has been demonstrated between FABP content and peroxisomal β-oxidation (46). Feeding rats cholestyramine, a bile acid sequestrant, increases the rate of palmitate and glycerol incorporation into triglyceride in isolated hepatocytes and also increases fatty acid binding to 12,000–15,000-Da cytosolic fractions (47).

[2]A distinction between L- and I-FABP was not made in these studies since their unique identities were not yet appreciated.

Glatz et al (28) have shown diurnal variation of FABP in both rat liver and heart. The two-fold higher levels of L-FABP and H-FABP found in liver and heart, respectively, during the mid-dark phase relative to the mid-light phase correlate with increased palmitate oxidation during the dark period. They suggest these changes may be related to the rat's nocturnal feeding habits. Finally, immunocytochemistry has shown that L-FABP exists in a gradient-like distribution, with highest concentrations near the two compartments intimately involved in fatty acid metabolism: mitochondria and smooth endoplasmic reticulum (14).

A Role in Modulating the Activities of Enzymes Involved in Fatty Acid Metabolism

L-FABP function has been extensively studied in vitro, yet the exact roles and mechanism of action of this FABP remain uncertain. The formation of triglyceride from palmitoyl CoA and diacylglycerol requires the 105,000 × g supernatant of rat liver (56). The supernatant components required are soluble, heat sensitive, and destroyed by trypsin. L-FABP can substitute for 105,000 × g supernatant, enhancing 10-fold the incorporation of palmitoyl CoA into 1,2-diacylglycerol to form triglyceride. By contrast, albumin has no effect (70). L-FABP also stimulates the incorporation of palmitoyl CoA into sn-glycerol-3-phosphate 6–10-fold better than albumin (61). However, this effect depends on the concentration of protein in the assay; both albumin and L-FABP can stimulate long-chain acyl CoA:glycerol-3-phosphate acyltransferase to about the same maximal activity, but at different concentrations (13). Desaturation of stearate to oleate is stimulated by L-FABP, perhaps related to the higher affinity of L-FABP for oleate compared to stearate and the removal of oleate (i.e. product) inhibition of the desaturase (15).

The effect of L-FABP on fatty acyl CoA synthetase is less clear. Ockner & Manning report a greater than two-fold stimulation of fatty acid CoA synthetase activity in intestinal microsomes in the presence of FABP (67). Burnett et al found that both albumin and L-FABP can stimulate mitochondrial and microsomal acyl CoA synthetase activities to similar maximal levels. However, albumin becomes inhibitory at higher protein concentrations, while L-FABP remains stimulatory (13). Wu-Rideout et al (96) reported stimulation of *microsomal* acyl CoA synthetase (140% of control), but inhibition of *mitochondrial* acyl CoA synthetase (60% of control) by L-FABP, while albumin had no effect. Their results suggest that L-FABP may selectively target fatty acids toward microsomal and away from mitochondrial esterification. In contrast, Suzue & Marcel reported the absence of any effect of FABP on membrane-bound acyl CoA synthases (92). Microsomal and mitochondrial membrane fractions were not, however, separated, which makes these results difficult to interpret.

L-FABP (SCP 1) has also been implicated in cholesterol biosynthesis and cholesterol metabolism to bile acids and steroid hormones (19, 84). L-FABP (SCP-1) is reportedly required for the multistep conversion of lanosterol to cholesterol in vitro (19, 86). However, a recent report suggests that this function is performed by nonspecific lipid transfer protein (SCP-2) and that L-FABP may not possess sterol carrier protein activity (81).

Numerous enzymatic reactions are inhibited by long-chain fatty acids and their CoA derivatives. FABP has been shown to modulate the activities of several of these systems. Acetyl CoA carboxylase, a key regulatory enzyme for fatty acid synthesis (see the section on eukaryotic long-chain fatty acid metabolism above), is inhibited 50% by 8-μM palmitoyl CoA. At physiological concentrations, FABP fractions protected against enzyme inhibition by exogenous palmitoyl CoA and enhanced enzyme activity in the absence of added palmitoyl CoA (55). The rate-limiting step of oxidative phosphorylation catalyzed by mitochondrial ADP/ATP translocase is also inhibited by long-chain fatty acids in vitro. FABP reverses this inhibition (5) and may account for the absence of inhibition by long-chain acyl CoA in intact hepatocytes. Increased acyl CoA concentrations resulting from clofibrate treatment also appear *not* to inhibit adenine nucleotide translocase, perhaps because of increased FABP levels (4).

Sodium-dependent synaptosomal amino acid uptake is specifically inhibited by low concentrations of unsaturated long-chain fatty acids (76). Several proteins that bind long-chain fatty acids stimulate sodium-dependent synaptosomal uptake, including L-FABP and BSA (77). Recently, a "brain" FABP has been identified that may perform this role in vivo (10).

Potential Physiologic Roles Played by Heart FABP

Heart FABP has been proposed to carry long-chain fatty acids and their CoA esters to mitochondria for β-oxidation and possibly to other intracellular organelles. Stearate and palmitate bound to H-FABP are good substrates for the mitochondrial β-oxidation system (22, 29).

Circular dichroism, electron spin resonance, and polyacrylamide gel electrophoretic studies indicate that pig heart FABP undergoes concentration-dependent self-aggregation in vitro to form at least four molecular "states" (23, 25). Binding of spin-labeled fatty acids to H-FABP is competitive with H-FABP aggregation, but the monomeric state is predicted to have low affinity for fatty acids. Because of this self-aggregation property, small concentration changes can significantly alter the binding characteristics of H-FABP and thus modulate membrane-bound enzyme activity. Using spin-labeled fatty acids and mathematical modeling, Fournier & Rahim (24) determined the distribution of fatty acids between aqueous solution, mitochondrial membranes, and FABP as a function of FABP concentration

(24). Comparisons of in vitro β-oxidation rates as a function of FABP concentration indicated that both *solution* phase and *FABP*-bound fatty acids are substrates for mitochondrial β-oxidation, but that membrane-bound fatty acid is not. Peaks of β-oxidation activity occurred around 1–2.5 mg/ml FABP. At these concentrations, the dimer and trimer aggregation states are predicted to predominate. Calculations based on immunoelectron microscopy of heart muscle indicate a gradient-like distribution of FABP with concentrations around mitochondria of 2.77 mg/ml (24). This value should be viewed cautiously, however, as significant concentrations of FABP were also found inside mitochondria, in capillary endothelium, and in intercellular spaces. Also, Offner et al failed to find circular dichroism evidence of rat H-FABP aggregation (71). A single report indicates that H-FABP may be involved in peroxisomal β-oxidation (3).

Relationship Between FABPs and Cellular Fatty Acid Uptake

While FABPs appear to have some role in modulation of long-chain fatty acid metabolism (either by directly enhancing delivery of substrates in a usable form, targeting substrates to particular metabolic fates, or eliminating inhibitory effects of long-chain fatty acids), the ability of FABPs to enhance cellular fatty acid uptake is debated. FABPs can clearly enhance the transfer of fatty acids from microsomes or mitochondria to liposomes (57) and may also remove fatty acid from multilamellar liposomes (12, 16). They thus seem suited for the role of intracellular fatty acid carrier. Theoretically, the fatty acid flux from the bloodstream to some intracellular organelle can be enhanced by an intracellular fatty acid carrier (94). Some theoretical analyses have concluded that the FABP-bound component is insignificant (63), while others calculate that FABP may enhance fatty acid uptake and transport to intracellular organelles by an order of magnitude or more (95). Treatment of isolated hepatocytes with glucose, which enhances oleate esterification and may thus increase cytosolic FABP binding sites, has little effect on oleate uptake (85). Flavaspidic acid-N-methyl-glucaminate and α-bromopalmitate, two agents that competitively inhibit oleate binding to FABP, failed to affect oleate uptake by everted jejunal sacs while they inhibited incorporation into triglyceride ~60% (67). Flavaspidic acid did, however, inhibit oleate uptake by isolated hepatocytes (~20%) (13). In intact liver, indicator dilution studies demonstrate that flavaspidic acid increases palmitate uptake, presumably secondary to displacement of the fatty acid from albumin (37, 59). Palmitate efflux and, in contrast to other studies, metabolic sequestration also increase. Thus, the importance of the cytoplasmic FABPs for fatty acid uptake is uncertain.

Recently a membrane-associated fatty-acid-binding protein has been described in hepatocyte plasma membranes. While it is generally assumed that

long-chain fatty acids diffuse passively through plasma membranes, Stremmel and coworkers present evidence for an additional high-affinity ($K_a = 2.0 \times 10^8\ M^{-1}$) saturable uptake mechanism (86). Binding of [^{14}C] oleate to liver plasma membranes was reduced more than 60% by including 10-fold excess of cold oleate (86). In addition, heating the membranes to 100°C for 5 minutes prior to assay reduced oleate binding by 95%, while trypsin and phospholipase A_2 reduced oleate binding by 51% and 49%, respectively (86). Trypsin treatment of isolated hepatocytes also reduced the rate of oleate uptake by 50% (85). Oleate-agarose affinity chromatography of solubilized membrane proteins led to the purification of a 40-kDa protein constituting no more than 12% of intrinsic membrane proteins by weight (88). The isolated protein specifically binds the long-chain fatty acids tested (C_{16}–C_{20}), but does not bind cholesterol esters, phosphatidylcholine, bilirubin, bromosulphothalein, or taurocholate (87, 88). Specific antibodies to the liver plasma membrane fatty-acid-binding protein (LPM-FABP) inhibit heat-sensitive oleate binding by 41% (87, 88). Immunofluorescence microscopy indicated the presence of LPM-FABP cross-reactive species in liver plasma membranes as well as along the apical brush border of jejunal and ileal villus and crypt cells and in the intercalated discs of cardiac muscle. The antibody did not cross-react with L-FABP (88). Others have shown that long-chain fatty acid uptake in rat adipocytes also occurs by a carrier-mediated process but may involve a different protein of ~85 kDa (1).

FUTURE DIRECTIONS

The FABPs remain abundant sequences whose physiologic roles have yet to be precisely defined. Now that their structures and tissue distributions have been documented, at least one early impediment to meaningful interpretation of their biological significance has been removed. To begin to decipher the functional consequences of their structural similarities and differences, a number of obvious experiments need to be done. Documentation of the endogenous ligand populations and a direct comparison of FABP binding specificities, affinities, and stoichiometry for exogenous ligands should be undertaken. These studies will require pure FABPs and a variety of analytical techniques based on different principles for separating bound from free ligand. Purified fatty-acid-binding proteins could be prepared from their cells of origin or from *E. coli* harboring prokaryotic expression vectors containing cloned FABP cDNAs. Careful analysis of their capacity for self-association and its effect on fatty acid binding as well as definition of their tertiary structures will be important for fully understanding the details of ligand-protein interaction. Isolation of large quantities of these proteins in pure form from a uniform source (e.g. *E. coli*) will allow direct in vitro comparison of

the effects of various FABPs on enzymes involved in long-chain fatty acid metabolism. Differentiation of specific effects (e.g. enzyme activation) from nonspecific effects (e.g. removal of enzyme inhibition by binding of long-chain fatty acids and fatty acyl CoAs) may be possible if these FABPs have unique cellular roles.

Further direct experimental approaches are needed to determine the relationship between the FABPs and fatty acid uptake, intracellular trafficking, compartmentalization, and/or metabolism. Now that several of the FABP genes have been isolated, a number of experimental strategies become possible for addressing these questions. Transfer of the FABP genes to cells that do not normally express them or repression of specific FABP gene expression in cells that normally synthesize these sequences now is possible using "conventional" recombinant DNA techniques. The consequences of such manipulations on fatty acid uptake and metabolism should provide important insights about the functions of these abundant proteins in vivo.

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