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Enterocyte fatty acid-binding proteins (FABPs): Different functions of liver and intestinal FABPs in the intestine ☆

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ABSTRACT

Fatty acid-binding proteins (FABP) are highly abundant cytosolic proteins that are expressed in most mammalian tissues. In the intestinal enterocyte, both liver- (LFABP; FABP1) and intestinal FABPs (IFABP; FABP2) are expressed. These proteins display high-affinity binding for long-chain fatty acids (FA) and other hydrophobic ligands; thus, they are believed to be involved with uptake and trafficking of lipids in the intestine. In vitro studies have identified differences in ligand-binding stoichiometry and specificity, and in mechanisms of FA transfer to membranes, and it has been hypothesized that LFABP and IFABP have different functions in the enterocyte. Studies directly comparing LFABP- and IFABP-null mice have revealed markedly different phenotypes, indicating that these proteins indeed have different functions in intestinal lipid metabolism and whole body energy homeostasis. In this review, we discuss the evolving knowledge of the functions of LFABP and IFABP in the intestinal enterocyte.

1. Fatty acid-binding proteins

The fatty acid-binding protein (FABP) family comprises 14–15 kD size intracellular proteins, which were discovered beginning in the 1970s [1,2]. Presently, there are nine known FABPs that are present in high abundance (1–5%) in the cytosol of most tissues, plus the related cellular retinoid-binding proteins [3,4]. The names for the proteins came from the tissue in which they were initially identified, but as some FABPs are expressed in multiple tissues, a numeric nomenclature is in use as well [5]. The FABPs display high affinity for binding to long chain (> 14 C) fatty acids (FA). Although these proteins have been studied for over 40 years, it remains uncertain why they are so highly expressed, and why there are so many different FABPs; their specific functions are still being elucidated [3].

The FABPs have a highly conserved tertiary structure, consisting of two antiparallel β-sheets with five strands each, forming a "clam shell" or β-barrel in which the ligands are bound. In the barrel interior, positively charged amino acids interact with the carboxylate anion of the FA [6]. A short helix-turn-helix motif connects β-strands A and B and has been shown to be important for the mechanism of ligand transfer [8,10]. Despite the tertiary structural similarities between these proteins, they have only about 20–70% amino acid homology [9,10], suggesting the possibility of functional specificities.

Recently, several FABPs were identified as carriers of anandamide (arachidinoylethanolamide or AEA) [11]. AEA is an n-acylethanolamine (NAE), a group of lipids that are formed by the hydrolysis of n-acylphosphatidylethanolamines (NAPEs) by NAPE-phophospholipase D, and have been shown to regulate food intake [12]. AEA was the first endocannabinoid identified in the NAE family of lipid signaling molecules. It was initially discovered in pig brain and found to have properties similar to Δ^9-tetrahydrocannabinol, the active ingredient in cannabis, hence "ananda," bliss in Sanskrit, was used for the name of this lipid [13]. AEA regulates food intake by acting as an agonist of cannabinoid receptors 1 and 2 (CB_1 and CB_2) on the plasma membrane. Similarly to Δ^9-tetrahydrocannabinol, increased concentrations of AEA result in an acute increase in food consumption [11]. Additionally, 2-arachidonoylglycerol (2-AG), a monoacylglycerol (MG), has been identified as an endocannabinoid, with increased brain levels associated with greater food

Abbreviations: 2-AG, 2-arachidonoylglycerol; AEA, arachidinoylethanolamide; CB_1, cannabinoid receptor 1; CB_2, cannabinoid receptor 2; FA, fatty acid; FXR, farnesoid X receptor; IFABP, intestinal fatty acid-binding protein; ILBP, ileal lipid-binding protein; KO, knockout; LFABP, liver fatty acid-binding protein; MG, monoacylglycerol; NAE, n-acylethanolamine; NAPE, n-acylphosphatidylethanolamines; PPAR, peroxisome proliferator-activated receptor; PCTV, pre-chylomicron transport vesicle; PL, phospholipid; RER, respiratory exchange ratio; TG, triacyglycerol; VLDL, very-low density lipoproteins

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intake levels [14,15]. Moreover, Liver FABP has been recently identified as a carrier of MGs [16]. Hence, it has been hypothesized that the FABPs are involved with transport of NAEs and 2-AG, perhaps to the ER where they are hydrolyzed by fatty acid amide hydrolase or MG lipase, respectively, a critical step in the regulation of these lipids. Because the NAEs and 2-AG are known to play important roles in ingestive behavior, these observations raise the possibility that the FABPs may play important roles in the regulation of food intake.

2. FABPs in the intestinal enterocyte

The intestinal enterocytes are responsible for processing the hydrolysis products of dietary lipids. Intestinal absorption of lipids is highly efficient, with greater than 95% of dietary lipid taken up [17]. It is generally thought that the presence of lipid-binding proteins is critical for this capacity, particularly because levels of FABPs are very high in cytosol [3]. In the intestinal enterocyte, two FABPs are present, largely in the absorptive intestinal villus cells, but not in crypt cells [18]. Liver-type FABP (LFABP; FABP1) was the first protein identified in the FABP family. As the name suggests, it was initially discovered in the liver, but was later found in the intestine and also to a lesser extent in the kidney [3]. Intestinal FABP (IFABP; FABP2) is also present in the enterocyte and is solely expressed in this tissue [3]. Although these proteins share only about 29% amino acid sequence homology, their tertiary structures are quite similar. Humans express more LFABP than IFABP in the intestine [19,20]; however, mice have similar levels of both [19–21]. Expression of both proteins in the mouse occurs as early as 13 weeks of gestation [19].

In addition to LFABP and IFABP, the ileal lipid or bile acid-binding protein (ILBP or BABP; FABP6) is present in the distal region of the small intestine and has a high affinity for binding bile acids. ILBP can bind FA, but with lower affinity than for bile acids [22]. ILBP has K_d values for FA in the micromolar range, which stands in contrast to LFABP and IFABP, which have K_d values in the nanomolar range [23,24]. Expression of ILBP is increased in response to the presence of bile acids [25–27]. The gene for ILBP has a Farnesoid *X* receptor response element for binding to the Farnesoid *x* receptor (FXR) transcription factor, of which bile acids serve as ligand activators. Mice null for ILBP have increased fecal excretion of bile acids and did not have any changes in protein expression of LFABP in the intestine [28], suggesting an absence of functional overlap; IFABP levels were not reported.

3. Liver fatty acid-binding protein

LFABP is a highly abundant intracellular protein in the intestinal enterocyte. It is present throughout the intestine, but is most highly expressed in the duodenum and jejunum [29]. In vitro studies have shown that LFABP binds FA with high affinity, with K_d values in the nanomolar range [30,31]. LFABP is also unique among the FABPs in that it has two FA-binding sites, and thus has a higher binding capacity than other FABPs. It has also been shown to bind to other lipid species, including lysophospholipids, monoacylglycerols (MG), fatty acyl CoAs, and prostaglandins [3]. LFABP is also unusual in that, unlike most proteins in the FABP family, it transfers FA to membranes through a diffusional mechanism rather than by a direct “collisional” interaction with membranes [32,33]. The subcellular distribution of LFABP in the enterocyte seems to be dependent on the feeding status, as LFABP is found throughout the cytosol of the enterocyte during times of FA availability, but the fasted state results in a more apical localization of this protein [34]. LFABP is also proposed to play an important role in the budding of prechylomicron transport vesicles (PCTVs) from the endoplasmic reticulum [35]. These PCTVs are further processed in the Golgi to mature chylomicrons that leave the enterocyte and enter the lymph and subsequently the general circulation, for delivery of dietary lipid to other tissues.

The promoter region of the LFABP gene has a peroxisome proliferator receptor element and, thus, its expression is regulated by peroxisome proliferator-activated receptor transcription factors (PPARs) [36]. In particular, PPARα is present in the liver and intestine and when bound to ligands such as LCFA and fibrates, will increase transcription of genes involved with oxidation of lipids [37]. Indeed, administration of PPARα agonists such as bezafibrate and clofibrate results in increased transcription of LFABP mRNA in mouse intestine and liver [21,38]. Interestingly, several studies have investigated a role for LFABP in the regulation of genes involved with lipid metabolism, including FA oxidation, via delivery of FA to transcription factors. LFABP has been demonstrated to interact with PPARα, whose target genes include those encoding FA oxidation enzymes, and more recently to interact with HNF4α, a steroid hormone receptor known to promote transcription of genes involved with inflammation in the liver and intestine [39–42]. This putative role of LFABP in the regulation of gene expression is further supported by evidence that oxidation of FA is reduced in the absence of LFABP in the liver [40,43] and in the intestine [44,45]. LFABP expression is also regulated by increased dietary intake of lipids, as high-fat feeding provides increased levels of FAs to the intestine and liver while also increasing expression of LFABP in these tissues [1,38,46–49]. Female rats express higher levels of LFABP in hepatocytes than male rats, indicating that gender also influences expression of the protein [21,50].

3.1. Polymorphism of LFABP

There have been no reported cases of deletion of the LFABP gene in humans; however, a human polymorphism has been described in several recent reports [51,52]. A substitution of alanine for threonine at position 94 (T94A) of LFABP has been identified as a polymorphism that is associated with increased plasma TG and FFA levels [52,53]. Chang liver cells transfected with this variant form of LFABP have reduced uptake of FA, but additional accumulation of cholesterol relative to cells transfected with the wild-type (WT) protein [54]. Interestingly, human subjects who carry the A94 allele of LFABP had a blunted response in plasma TG levels relative to carriers of the T94 allele when they were treated with fenofibrate, an activator of PPARα; therefore, it is hypothesized that the variant form of LFABP may be insufficient for delivery of fibrates to PPARα [52]. Indeed, human hepatocytes that express the A94 form of LFABP show reduced expression of PPARα-regulated genes in response to fenofibrate than those expressing the WT T94 form [55], providing further evidence that LFABP is involved with PPARα-mediated lipid metabolic pathways.

4. Intestinal fatty acid-binding protein

Unlike LFABP, IFABP is solely expressed in intestinal enterocytes of mammals. While IFABP is present throughout the small intestine, its highest expression is in the jejunum [56]. Like LFABP, IFABP is found widely distributed throughout the cytosol of the enterocyte during the fed state, but is localized toward the apical side of the cell in the fasted state [34]. IFABP has not been found to be involved with chylomicron formation [35,57]; hence, IFABP has been proposed to be involved with uptake of FA from