Published in final edited form as:

Nat Rev Endocrinol. 2015 October ; 11(10): 592–605. doi:10.1038/nrendo.2015.122.

Metabolic functions of FABPs— mechanisms and therapeutic implications

Gökhan S. Hotamisligil and

Department of Genetics and Complex Diseases and Sabri Ülküer Center, Harvard T.H. Chan School of Public Health, 677 Huntington Avenue, Boston, MA 02115, USA

David A. Bernlohr

Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, 321 Church Street SE, Minneapolis, MN 55455, USA

Abstract

Intracellular and extracellular interactions with proteins enables the functional and mechanistic diversity of lipids. Fatty acid-binding proteins (FABPs) were originally described as intracellular proteins that can affect lipid fluxes, metabolism and signalling within cells. As the functions of this protein family have been further elucidated, it has become evident that they are critical mediators of metabolism and inflammatory processes, both locally and systemically, and therefore are potential therapeutic targets for immunometabolic diseases. In particular, genetic deficiency and small molecule-mediated inhibition of FABP4 (also known as aP2) and FABP5 can potentially improve glucose homeostasis and reduce atherosclerosis in mouse models. Further research has shown that in addition to their intracellular roles, some FABPs are found outside the cells, and FABP4 undergoes regulated, vesicular secretion. The circulating form of FABP4 has crucial hormonal functions in systemic metabolism. In this Review we discuss the roles and regulation of both intracellular and extracellular FABP actions, highlighting new insights that might direct drug discovery efforts and opportunities for management of chronic metabolic diseases.

Introduction

Lipids have wide-ranging roles in many different aspects of biology, functioning as structural building blocks or fuel sources, and as intracellular and extracellular signalling molecules. For example, lipids can modify the action or location of proteins, such as kinases or ion channels, signal via proteins such as cell surface G-protein coupled receptors and can serve as ligands for transcription factors.^{1–3} Free fatty acids can also regulate hormone action, for example by inhibiting the insulin-stimulated phosphoinositide 3-kinase pathway^{4,5} and activating inflammatory molecules such as inhibitor of nuclear factor κ B

Correspondence to: G.S.H. ghotamis@hsph.harvard.edu; D.A.B. bernl001@umn.edu.

Author contributions

Both authors researched data for the article, discussed the content, and wrote, reviewed and edited the manuscript before submission.

Supplementary information is linked to the online version of the paper at www.nature.com/nrendo.

Competing interests

G.S.H. receives research funding under a sponsored agreement with Union Chimique Belge. D.A.B. declares no competing interests.

kinase subunit β (IKK- β)⁶ and c-jun N-terminal kinase (JNK),^{7,8} or engage pattern recognition receptors which can contribute to metabolic regulation and disease.⁹

However, given the relative insolubility of these fatty acids and their potential toxicity in free forms,^{10,11} the need for buffering entities has led to the search for noncatalytic binding proteins, including the fatty acid binding proteins (FABPs). The first FABP was originally described as a small molecular weight intracellular protein of ~12 kDa detected in the rat jejunum, owing to its ability to noncovalently bind to long chain fatty acids.¹² Subsequently, other proteins that also bind to long-chain fatty acids were identified in the liver, myocardium, adipose tissue and kidney.^{12,13} However, as the functions of FABPs have been elucidated, these proteins do not seem to simply buffer lipids, but are in fact crucial mediators of metabolic and other biological activities.¹⁴ As we describe in this Review, we now understand that the functional diversity of FABPs is generated via lipid interactions with these chaperone proteins to support systemic homeostatic networks of immunometabolism by facilitating signalling within and between cells and communication between organs. Consequently, interest has grown in the research community for therapeutically targeting this class of proteins in metabolic and immunometabolic diseases.¹⁴ In this Review we discuss the roles and regulation of FABP4 (fatty acid-binding protein, adipocyte; which is often referred to in the literature as aP2) and the closely related FABP5 (fatty acid binding protein, epidermal; also known as mal1), the main FABPs present in adipose tissue. We will also describe the emerging biological framework, and highlight new insights into the functions and mechanisms that might directly influence drug discovery for metabolic diseases.

FABP gene expression

Understanding of the regulated expression of genes encoding FABPs and their potential regulatory and metabolic effects began to emerge after their initial discovery. For example, levels of intestinal FABP were found to increase in abundance in response to fasting and a high fat diet,¹³ and this protein can enhance fatty-acid CoA ligase activity.¹⁵ Fatty-acid CoA ligase is involved in the generation of substrates for β -oxidation, complex lipids and signalling molecules.¹⁶ FABPs were subsequently isolated and cloned from other tissues including the brain, heart and lung.^{17–25} FABP4 was identified as a cytosolic protein strongly upregulated during differentiation of preadipocytes into adipocytes.^{26,27} In fact, this protein is one the most abundant proteins ever found in mature adipocytes and adipose tissue.²⁶ Protein preparations from rat and human adipose tissue suggest that FABP4 might be involved in the esterification of long-chain fatty acids,²⁸ and after cloning and sequencing this protein was found to be highly similar to FABP1 (fatty acid-binding protein, liver), FABP2 (fatty acid binding protein, intestinal) and the myelin P2 protein.²⁷ We now know that *FABP4* is a member of a large gene family of mammalian *FABPs* each containing an evolutionarily conserved four exon and three intron organization.²⁹ Mammalian FABPs are part of a large class of intracellular and extracellular lipid proteins that bind a variety of hydrophobic ligands, including fatty acids, haeme, retinoids and different vitamins.³⁰ Moreover, FABPs have sequence and structural similarity to lipocalins from insects, nematodes and fish.²⁹

In initial expression analyses, *FABP4* mRNA was detected at low levels in multiple tissues such as the heart and kidney,³¹ but the protein itself was only detectable in adipose tissue.³² Indeed, the major site of *FABP4* expression is white and brown adipose tissue, and in differentiated adipocytes, this FABP is estimated to account for between 0.5% and 6% of total protein.^{26,33} As a marker for adipocyte differentiation, *FABP4* expression is specifically induced by insulin and/or IGF-1,³⁴ dexamethasone and fatty acids,^{35–37} and occurs as a downstream target of peroxisome proliferator-activated receptor γ (PPAR γ) activation.³⁸ Notably, *FABP4* expression is also readily detected in other cell types such as macrophages, particularly upon inflammatory activation,³⁹ and, under special circumstances, can be found elsewhere, such as the bronchial epithelium where it occurs independently of PPAR γ .⁴⁰

FABP intracellular functions

Studies in cellular and biochemical systems

Substantial effort in determining the role of FABPs has focused on defining their binding affinities and lipid cargos. Using radiolabelled or fluorescent fatty acid analogues, it was shown that FABP4 and FABP3 (fatty acid-binding protein, heart) each have one binding site per molecule, and that the binding site of FABP3 might be more hydrophobic than that of FABP4, which suggests that their intracellular functions might differ.⁴¹ Analysis of the FABP4 crystal structure has revealed that the protein binds to saturated and unsaturated fatty acids in the same conformation.⁴² Furthermore, in a comparison of FABP binding affinities to different ligands, FABP4 was unique among FABPs for its high affinity for both saturated and unsaturated fatty acids.⁴³ The ability of FABP4 to transfer fatty acids to membranes increases with fatty acid chain length but is not altered by relative saturation, which suggests that FABP4-mediated transport might not be dependent on fatty acid solubility and might occur via collision with membranes.⁴⁴ Interaction between FABP4 and phospholipid membranes was confirmed with the use of fourier transform infrared spectroscopy,⁴⁵ and shown to be strongly regulated by positively charged lysine residues.⁴⁶

The basic structure of the FABP fold is a 10-stranded antiparallel β -barrel, in which each strand forms hydrogen bonds with the following strand in an up-and-down manner (Figure 1).²⁹ The first five and the second five strands each form sheets that are orthogonal to each other. The two sheets form a flattened barrel closed at one end with a helix-turn-helix domain between β -strands 1 and 2 closing the opposite end. The interior cavity of FABP4 is $\sim 650 \text{ \AA}^3$, which is large enough to accommodate more than one fatty acid.⁴⁷ Indeed, the cavity within FABPs is $\sim 2\text{--}3$ times the volume required for fatty acid binding and at least one FABP (FABP1) can bind multiple lipids simultaneously.⁴⁷ Interestingly, the charge distribution of side chains within the FABP4 cavity is not appreciably different from that on the surface of the protein, implying that the cavity is not hydrophobic but highly charged and polar.⁴⁸ Evaluation of the binding characteristics using titration microcalorimetry indicates that the binding energy is divided relatively equally between enthalpic and entropic terms,⁴⁹ which indicates that interactions at both the head-group and along the acyl chain determine binding affinity and specificity. Indeed, in both NMR and X-ray crystallographic analyses of natural and pharmacologic ligands, ligand binding seems to occur on one face of

the interior cavity and is dominated by polar and non-polar interactions.⁴⁸ The interior cavity of FABP4 contains ~12 crystallographically ordered water molecules in addition to ~100 disordered waters. Ligand binding displaces some, but not all, of the ordered waters that are in close contact to the bound fatty acids, which suggests that water interactions might be critical for fatty acid association.⁵⁰ Notably, other molecules might also occupy the ligand binding pocket of FABPs in the presence or absence of lipids. For example, FABP1 has been shown to bind to haeme,⁵¹ although the physiological consequences of these interactions are not well understood.

Ligand access to the interior cavity of FABP4 is likely to occur via a domain referred to as the portal region (Figure 1). The portal region is bounded by amino acids found in the loops that connect β -strand 3 to β -strand 4, β -strand 5 to β -strand 6 and α -helix II.^{29,48} Importantly, the volume of most ligands exceeds that of the opening from the external environment into the cavity,⁴⁷ which suggests that dynamic flexibility on or around the portal region must accompany ligand binding. One plausible consideration is that the entire helix-turn-helix domain dynamically rotates at the hinge region of the connecting β -strands to allow ligand entry and exit.⁵² Bound fatty acids form a salt bridge with Arg106, Arg126 and Tyr128, and mutations in each of these amino acids results in proteins with undetectable fatty acid binding.⁵³ Interestingly, within the cavity, Cys117 is covalently modified by oxidized lipids such as 4-hydroxynonenal in a process known as protein carbonylation.⁵⁰ Indeed, in 3T3-L1 adipocytes as well as in adipose tissue from mice and humans, FABP4 is the major carbonylated cytoplasmic protein,⁵⁴ suggesting that FABP4 might function as an antioxidant, scavenging reactive lipids from the cellular environment.

At the plasma membrane, uptake of fatty acids is mainly regulated by transport proteins such as CD36, plasma membrane-associated FABP, and the fatty acid transport protein family.^{55,56} However, FABPs might also be involved in this uptake, and some evidence suggests that FABPs can interact directly with CD36.⁵⁷ By enabling the uptake of sterol, FABP1 alters the lipid composition of the plasma membrane, which results in increased fluidity.⁵⁸ Further investigations have revealed that FABPs have redundant and overlapping roles in mediating uptake of fatty acids in various tissues, for example, both FABP4 and FABP5 can contribute to the import of fatty acids into the heart.⁵⁹ However, FABP-promotion of fatty acid uptake might also be context or cell-type-specific. For example, in L-cell fibroblasts, overexpression of either *FABP1* or *FABP2* results in increased synthesis of phospholipids, but only FABP1 can increase fatty acid uptake.⁶⁰ Importantly, genetic deficiency of *FABP4* seems to alter adipose tissue fatty acid uptake in a lipid specific manner,⁶¹ has a profound influence on fatty acid efflux,⁶² and regulates *de novo* lipogenesis,^{63–65} which results in increased intracellular levels of fatty acids in FABP4-deficient cells.⁶² Although FABP4 binds and donates its fatty acid ligand via collisional interactions with membranes, it does not form thermodynamically stable complexes with membranes *in vivo* or *in vitro*.⁶⁶ This finding suggests that intracellular fatty acid trafficking between membranes *in vivo* occurs via transient interactions with membranes and/or proteins.

FABPs have also been implicated in lipid sensing and response mechanisms by delivering lipids to nuclear receptors such as PPARs to mediate transcriptional programmes,⁶⁷ although

this effect might be context-dependent and specific to particular FABPs and PPAR isoforms. Using fluorescently labelled fatty acids, FABP1 expression was shown to promote the uptake of free fatty acids into the cytoplasm and mediate their delivery to the nucleus, with an apparent preference for longer chain fatty acids.⁶⁸ Similarly, FABP5 has been implicated in the delivery of retinoic acid to PPAR β/δ with important consequences for neuronal development,⁶⁹ and can also promote the hydrolysis of the endocannabinoid anandamide into a form that functions as a PPAR β/δ ligand and, therefore, regulates hippocampal function.⁷⁰ In some studies that use cell culture systems, FABP4 can translocate to the nucleus and interact with, and potentially deliver ligands to PPAR γ ,^{71–73} but the functional consequences of this interaction are unknown. Alternatively, apo-FABP4 might transiently interact with PPAR γ in the nucleus to extract a ligand from the protein and attenuate nuclear receptor action. As discussed further later in the text, evidence from genetic mouse models suggests that although PPAR γ can directly increase *FABP4* gene expression, FABP4 has a net negative effect on PPAR γ activity⁷⁴ and represents a negative feedback loop to control signalling from this nuclear hormone receptor.

Studies in mouse models

An increasingly comprehensive understanding of FABP functions has emerged with the establishment of genetic mouse models, the first of which was the *Fabp4*^{−/−} mouse.⁷⁵ The initial experience with this mouse model was quite disappointing due to a lack of any phenotype under standard laboratory conditions. This result was, at least in part, attributable to a compensatory upregulation of FABP5 in adipose tissue.^{75,76} However, a number of fascinating phenotypes have emerged as these models have been examined in the context of metabolic stress. In the setting of both diet-induced and genetic obesity, compensation by FABP5 is not sufficient to completely mask the biology of FABP4-deficiency, and *Fabp4*^{−/−} mice were protected from obesity-induced insulin resistance and hyperglycaemia.^{75,77} This protection was thought to be the result of changes in the composition and metabolism of lipids and resistance to chronic inflammation in the setting of obesity.⁷⁵

Despite apparently normal glucose homeostasis, lean *Fabp4*^{−/−} mice have reduced lipolysis and increased lipogenesis, as well as a blunted insulin secretion response to β -adrenergic stimulation,^{64,65} which suggests that FABP4 or an FABP4-regulated lipid signal might be involved in modulating insulin secretion. Interestingly, transgenic overexpression of FABP5 increases lipolysis and decreases cellular free fatty acid levels.⁶⁵ To understand the role of FABP4 in the absence of compensatory FABP5 expression, *Fabp4*^{−/−} mice were crossed with FABP5-deficient mice.⁶¹ *Fabp5*^{−/−} mice are themselves moderately protected from diet-induced obesity and insulin resistance, and FABP5 overexpression is sufficient to impair glucose tolerance.⁷⁸ The *Fabp4*–*Fabp5* double mutant (*Fabp4*–*5*^{−/−}) mice were protected from high fat diet-induced weight gain and had a more dramatic improvement in insulin sensitivity and glucose homeostasis than either of the single FABP4 or FABP5 deficiency models.⁶¹ Furthermore, *Fabp4*–*5*^{−/−} mice were protected from high-fat diet-induced hepatic steatosis, with increased muscle AMP kinase activity and reduced hepatic expression of the key fatty acid metabolism enzyme *Scd1* (encoding acyl-CoA desaturase 1), which could in part underlie the reduced lipid accumulation in this model.⁶¹ In the leptin-deficient *ob/ob* model, combined deficiency of FABP4 and FABP5 did not reduce obesity

but did improve glucose tolerance and insulin sensitivity, resulting in reduced hepatic *Scd1* expression, and protection against the development of fatty liver.⁷⁹ In further studies using *Fabp4*^{-5-/-} mice, a robust increase in *de novo* lipogenesis in adipose tissue results in increased levels of circulating palmitoleate (C16:1n7), which suppresses *Scd1* expression in liver and increases insulin sensitivity.⁸⁰ Increased *de novo* lipogenesis has also been seen in FABP4-deficient macrophages, resulting in increased production of palmitoleate, which mediates protection from lipid-induced endoplasmic reticulum stress in these cells.⁸¹ The effect of palmitoleate is in part mediated through free fatty acid receptor 4 (also known as GPR120);⁸² and in contrast to *Fabp4*^{-5-/-} mice, in the setting of GPR120 deficiency palmitoleate levels are reduced, which leads to increased *Scd1* expression, insulin resistance and glucose intolerance.⁸³ The results of these studies demonstrate that in adipose tissue, FABPs are essential for dietary intake to dominate the lipid composition of the tissue. In their absence, adipose tissue is unaffected by the diet, which results in activation of *de novo* lipogenesis. Furthermore, these findings also illustrate the potential importance of adipose tissue *de novo* lipogenesis in controlling systemic metabolism, which under most conditions remains limited.

Surprisingly, although FABP4 is highly upregulated during preadipocyte differentiation, adipogenesis and adipose tissue formation proceed normally or are enhanced in *Fabp4*^{-/-} and *Fabp4*^{-5-/-} cells and mice.^{61,75} This finding demonstrates that FABP function is not required for specification of the adipocyte lineage and correlates with FABP4 having a potential negative feedback regulatory effect on PPAR γ . However, in the context of FABP4 deficiency, the conversion of glucose to fatty acids is elevated twofold,⁶³ which suggests that FABP4 might suppress lipogenesis in adipocytes, thereby directly contributing to the observed improvements in fasting glucose and insulin in obese FABP4-deficient mice.⁶³ The resulting elevation of circulating levels of fatty acids leads to suppression of the 1c isoform of *Srebp1* expression in the liver,⁶¹ which might contribute to the protection from development of fatty liver seen in obese FABP4-deficient mice. In addition, basal and stimulated levels of lipolysis are significantly decreased in adipocytes from *Fabp4*^{-/-} mice, despite the compensatory increase in FABP5 expression.^{62,64}

Decreased efficiency of lipolysis in FABP4-deficient mice probably occurs via modulation of the important lipolytic enzyme hormone-sensitive lipase (HSL), which binds to and is activated by FABP4 in the presence of fatty acids.^{49,84,85} Whether other adipose tissue lipases also interact with FABP4 or FABP5 is unknown. Using a combination of yeast-two hybrid and glutathione *S*-transferase-pull down assays, FABP4 was shown to interact with HSL via a domain contained on the helix-turn-helix motif defined by Asp17, Asp18, Lys21 and Arg30.⁸⁴ Asp17–Arg30 and Asp18–Lys21 in FABP4 form two ion pairs that interact with similarly charged residues on HSL (for example, Asp18 of FABP4 interacts with Lys196 of HSL) to form a complex on the lipid droplet surface.⁸⁵ This interaction facilitates fatty acid transfer and is consistent with the model by which FABP4 facilitates lipolysis. Given this function, it would be interesting to evaluate the role of FABP4 in the adipose tissue of hibernating mammals, which exhibits a dramatic seasonal cycling between lipolysis and lipogenesis.⁸⁶

FABPs in immunity and inflammation

Lipids have a central role in immune cell signalling and, accordingly, FABPs have been implicated in immune cell biology. The major regulator of adipocyte differentiation, PPAR γ , is expressed in monocytes in response to oxidized LDL, and accumulates at high levels in atherosclerotic lesions.⁸⁷ PPAR γ promotes FABP4 expression in macrophages,³⁹ and indeed FABP4 is highly upregulated during foam cell formation in response to lipid loading.⁸⁸ Rapamycin, an immunosuppressant that can induce hyperlipidaemia, also induces FABP4 expression in macrophages.⁸⁹ Furthermore, liver X receptor agonists, which can increase expression of genes involved in cholesterol transport and triglyceride synthesis such as apolipoprotein E and cholesteryl ester transfer protein, also induce FABP4 expression in macrophages, and the FABP4 promoter contains a conserved liver X receptor response element.⁹⁰

The relationship between PPARs and FABP activity is complex and might be context-dependent. Although PPAR γ can initiate FABP4 expression and much evidence has been presented that FABPs can mediate ligand delivery to PPARs,⁶⁷ the metabolic effects of FABP4 action (specifically the activation of lipolysis and suppression of lipogenesis) seem to be in opposition to those of PPAR γ . Indeed, PPAR γ activity is increased in FABP4-deficient macrophages, resulting in accelerated cholesterol efflux and decreased cholesterol content.⁷⁴ Similarly, PPAR γ activity is elevated in FABP5-deficient macrophages.⁹¹ These data support a model in which FABP4 (and possibly FABP5) can regulate PPAR γ activity by controlling ligand availability. Furthermore, FABP4 can increase proteasomal degradation of PPAR γ ,⁹² and consequently the net effect of FABP4 seems to be to suppress PPAR γ activity (Figure 2).

In addition, FABP4 and FABP5 bind and stabilize leukotriene A₄,^{93,94} and FABP4-deficient and FABP5-deficient macrophages have impaired activation of prostaglandin G/H synthase 2 expression and production of prostaglandin E₂,^{74,91} which suggests that these FABPs can help to determine macrophage eicosanoid balance. Eicosanoids mediate adipose tissue inflammation in obesity; for example, leukotriene B₄ binding to its cognate receptor on macrophages induces the expression of C-C motif chemokine 2 (commonly known as monocyte chemoattractant protein 1 [MCP-1]), which leads to potentiated inflammation, and *Blt1*^{-/-} mice (which lack leukotriene B₄ receptor 1) are protected from obesity-linked macrophage recruitment to adipose depots and exhibit attenuated inflammation.⁹⁵

FABPs, and FABP4 in particular, have pronounced effects on cellular and systemic lipid and signalling mediator composition, with important consequences for inflammatory processes. For example, FABP4-deficient macrophages have reduced basal and stimulated expression of proinflammatory cytokines including MCP-1 and TNF,⁹⁶ as well as decreased IKK- β /NF- κ B activity and deficient activation-induced expression of inducible nitric oxide synthase.⁷⁴ One of the intriguing results of FABP-deficiency in adipose tissue is the activation of *de novo* lipogenesis and the resulting production of the related products from the target cells. For example, *Fabp4*-5^{-/-} mice have increased levels of palmitoleate, particularly in adipocytes, macrophages, and the fatty acid fraction of serum, but not liver.⁸⁰ In subsequent studies this lipokine was shown to act on the liver as well as other cells and tissues such as

macrophages to regulate lipid metabolism and inflammatory responses.^{80,97} In macrophages, FABP-deficiency provides strong protection against lipotoxicity. The addition of monounsaturated fatty acids such as palmitoleate to macrophages induces expression of *UCP2*, which encodes mitochondrial uncoupling protein 2.⁹⁸ Moreover, macrophages isolated from *Fabp4*^{-/-} mice have increased palmitoleate levels and increased expression of *UCP2*, and silencing of *UCP2* in FABP4-deficient macrophages attenuates NF-κB signalling and the inflammatory response to lipopolysaccharide.⁹⁸ Correspondingly, increased expression of *UCP2* in macrophages is sufficient to polarize cells towards the M2 phenotype, reduce endoplasmic reticulum stress and diminish oxidative stress.⁹⁸ Furthermore, in protein–protein interaction studies, FABP4 interacts directly with tyrosine-protein kinase JAK2 and might attenuate cytokine signalling within macrophages.⁹⁹ Finally, the lipotoxicity that occurs in the context of atherogenesis induces the unfolded protein response within macrophages; FABP4 seems to be critical for this process and, as a result, FABP4-deficient macrophages are protected from cholesterol or palmitate-induced endoplasmic reticulum stress and cell death *in vitro* and *in vivo*.⁸¹

Given the expression of FABP4 in both macrophages and adipocytes, and the close association between insulin resistance, inflammation and atherogenesis,¹⁰⁰ a role for FABP4 in the pathogenesis of atherosclerosis is perhaps unsurprising. FABP4 is expressed in atherosclerotic lesions and foam cells and, in the atherogenic *ApoE*^{-/-} mouse model, FABP4 deficiency results in reduced macrophage accumulation in plaques and reduced plaque area in early and late stages of disease development.^{101–103} In further support for this crucial role, a small molecule FABP4 inhibitor, BMS309403, potently reduced lesion area in a mouse model of atherosclerosis.¹⁰⁴ Indeed, both in genetic and chemical models of FABP4 inhibition, the anti-atherogenic phenotype seems to be the most robust.^{104,105} In bone marrow transplant experiments, the protection from atherosclerosis occurs largely via FABP actions in haematopoietic cells,^{96,102} and the findings from these studies suggest that FABP5 has a similar but smaller role in this process.⁹¹ Although FABP5 does not seem to be upregulated in *FABP4*^{-/-} macrophages,^{96,102} combined FABP4–FABP5 deficiency provides moderate additional benefit in the *ApoE*^{-/-} background by improving glucose homeostasis, resulting in reduced plaque formation and prolonged survival.¹⁰⁵

In addition to macrophages, FABP4 is also expressed in myeloid dendritic cells, and is upregulated during their differentiation.¹⁰⁶ Dendritic cells that are FABP4-deficient produce lower levels of cytokines including IL-12 and TNF, and have reduced capacity to activate T cells.¹⁰⁶ These data, and the finding that FABP-deficient mice are protected against experimental autoimmune encephalomyelitis,¹⁰⁷ suggest that in addition to its immunometabolic roles, FABP4 might have wide-ranging effects on adaptive immunity, autoimmunity and susceptibility to infection. This is probably the result of the FABP4 effect on metabolic programming of its target cells, and needs to be further explored in mechanistic and functional studies.

Other intracellular FABP functions

Although the first expression profiles of FABP4 suggested this protein had a very limited tissue distribution, detailed analysis has revealed additional sites of expression and function

in specific conditions. For example, both FABP4 and FABP5 are expressed in bronchial epithelial cells upon exposure to IL-4 and IL-13, which suggests a potential role for these FABPs in the pathogenesis of allergic asthma.⁴⁰ Indeed, FABP4 expression is induced in the airway in the ovalbumin challenge model of allergic inflammation, and FABP4-deficient mice are protected from inflammatory cell infiltration in this model.⁴⁰ Other investigators have described potential roles for FABP4 in endothelial cell growth and blood vessel branching,^{108–113} and FABP4 has also been implicated in cancer cell growth and metastasis.^{114–116} The mechanisms that underly these effects are not fully understood and indeed might be pleiotropic; for example, whether the protection from asthma in *Fabp4*^{−/−} mice⁴⁰ is due to changes in immune responses, alterations in bronchoepithelial cells, other stromal cells or both, is unclear. Finally, conditions of ectopic lipid accumulation in tissues, especially in the presence of inflammatory and stress responses, might produce a permissive milieu that activates aberrant FABP expression in cells such as hepatocytes, or trigger expression from resident immune cells such as Kupffer cells. Although our understanding of FABP function in different cell types has increased, the development of tissue specific gain-of-function and loss-of-function models might help to fully define the targets and related functions under different conditions.

Extracellular FABPs

FABPs have been primarily studied as intracellular proteins. However, after the development of highly sensitive detection systems and serum proteomics, multiple FABP isoforms have been detected in the circulation.¹¹⁷ In general, these circulating FABPs are seen as markers of cell injury or death. For example, extracellular FABP3 was first detected in mice during experimental ischaemia–reperfusion injury owing to leakage of the protein through the plasma membrane, which might impair the capacity of cardiac myocytes to handle their lipid load.¹¹⁸ FABP3 can also be detected in the plasma and urine of patients following muscle injury or acute myocardial infarction.^{119,120} In healthy individuals, the plasma level of FABP2 is near or below the lower limit of detection, but is increased by approximately fourfold in patients with ischaemic bowel disease.¹²¹ Furthermore, in a human surgical model of intestinal ischaemia, FABP2 levels in venous blood correlate with the level of tissue damage.¹²² Similarly, elevated plasma levels of FABP1 have been associated with liver transplant rejection¹¹⁷ and are a biomarker for chronic hepatitis C infection.¹²³

The lack of signal peptide sequence has led to the assumption that all FABPs detected extracellularly might result from cell leakage or death. However, evidence has been presented supporting regulated secretion of some of these proteins. For example, FABP1 is detectable in bile in the absence of cellular injury,¹²⁴ and mammary-derived growth inhibitor, which is thought to represent a combination of FABP4 and FABP3,¹²⁵ is present in milk.^{126,127} Consequently, at least some FABPs might undergo regulated release and, in addition to their usefulness as biomarkers, might have active, physiologically relevant extracellular activities. Studies from our laboratory have shown that FABP4 is secreted from cultured adipocytes and adipose tissue explants in a regulated manner in the absence of cell death, and in mice plasma FABP4 levels are elevated in response to fasting-related and lipolytic signals.¹²⁸ In addition, FABP4 plasma levels are elevated in experimental models

of obesity, as well as in patients with obesity, and correlate with aspects of metabolic syndrome such as insulin resistance.¹²⁹ Interestingly, regulated secretion of FABP4 has also been demonstrated in cultured macrophages,¹³⁰ although bone marrow transplant experiments in mice suggest that the majority of FABP4 in the circulation is generated from the stromal compartment rather than bone marrow-derived cells.¹²⁸

Circulating FABP4 and metabolic pathology

Nearly 100 articles have been published regarding links between plasma levels of FABP4 and different pathologies in multiple populations, and almost half of these papers identify a positive correlation between levels of plasma FABP4, obesity and type 2 diabetes mellitus. One of the largest of these studies was a cross-sectional analysis of the 3,658 individuals within the Framingham Heart Study, in which increased plasma FABP4 levels correlated with increased BMI and insulin resistance, as well as dyslipidaemia.¹³¹ Examination of the 1,847 participants in the Hong Kong Cardiovascular Risk Factor Prevalence Study revealed that elevated FABP4 correlated with increased BMI, insulin resistance, elevated LDL cholesterol, and even reduced survival from cardiovascular disease.¹³² In a follow-up study of >1,000 patients with cardiovascular disease in Germany, those patients who experienced secondary cardiovascular disease events had significantly higher plasma FABP4, and FABP4 levels were also higher in patients with type 2 diabetes mellitus, hypertension and elevated triglyceride levels than in patients without those complications.¹³³ In other small studies, plasma FABP4 can predict the development of type 2 diabetes mellitus independent of the presence of obesity,¹³⁴ is an independent biomarker of insulin resistance,¹³⁵ predicts early death in patients with stroke,¹³⁶ and is elevated in patients with severe obstructive sleep apnoea syndrome.¹³⁷ Independently of obesity, circulating FABP4 is also higher in patients with breast cancer than in healthy individuals,¹³⁸ is associated with vascular inflammation,¹³⁹ and is predictive of the development of insulin resistance in recipients of liver transplants.¹⁴⁰ Consequently, a large and growing body of evidence supports a strong link between circulating levels of FABP4, obesity and metabolic disease (Figure 3, Supplementary file 1).

Although in many studies a link between weight, adiposity and circulating levels of FABP4 has been observed, in other analyses the relationship seems to be more complicated. This is particularly true in diseases with severe or rapid loss of adiposity such as lipodystrophies and anorexia nervosa, or uncontrolled lipolysis and lack of insulin such as in type 1 diabetes mellitus,^{141–143} which might introduce massive lipid breakdown or adipose tissue injury. This effect could result in increased levels of circulating FABP4 despite reducing the rate of secretion from adipocytes and is likely to introduce complex patterns of regulation and disease correlations.

The correlation between metabolic disease and circulating levels of FABP4 is also supported by a number of genetic studies in humans. A polymorphism in the promoter of *FABP4* (–87T>C; rs77878271) that results in reduced expression of this gene is associated with reduced risk of coronary heart disease and with reduced risk of developing type 2 diabetes mellitus among patients with obesity.¹⁴⁴ In an independent study, the same variant in the *FABP4* promoter resulted in reduced *FABP4* expression and was associated with decreased

total cholesterol levels and reduced risk of myocardial infarction in patients with advanced atherosclerosis.¹⁴⁵ Moreover, in other small studies an association between FABP4 polymorphisms, plasma levels of FABP4 and metabolic outcome in polycystic ovary syndrome,¹⁴⁶ and obstructive sleep apnoea¹⁴⁷ have been found. Consequently, overwhelming biochemical and genetic evidence demonstrates an important correlation between circulating levels of FABP4 and metabolic disease, which raises the possibility that targeting this form of the molecule might be a useful therapeutic intervention.

Extracellular function of FABP4

Given the wide range of pathophysiological associations of high levels of circulating FABP4, the functions of this form of FABP4 are likely to be pleiotropic (Figure 4). The findings in *Fabp4*^{-/-} mice, which have preserved glucose homeostasis during obesity,⁷⁵ and the correlation between plasma FABP4 levels and type 2 diabetes mellitus in humans (Figure 3, Supplementary file 1) suggest that circulating FABP4 might function as an adipokine in metabolism. Indeed, *in vitro*, addition of exogenous FABP4 to primary hepatocytes can induce a moderate increase in expression of gluconeogenic genes such as glucose-6-phosphatase, and glucose production, and infusion of FABP4 into *Fabp4*^{-/-} mice in the clamp setting increases gluconeogenic enzyme activity and hepatic glucose production.¹²⁸ As dysregulated hepatic glucose production is a major contributor to the diabetic phenotype,¹⁴⁸ this finding suggests that FABP4 is a critical factor connecting adipose tissue status to liver function and systemic metabolism. In addition, neutralization of the FABP4 protein with a polyclonal antibody significantly improved glucose tolerance and insulin sensitivity in obese mice, providing strong support for the concept that circulating FABP4 is a major contributor to metabolic decline in obesity.¹²⁸ In one study, FABP4 modestly increased insulin secretion from β cells *in vitro* when delivered with the fatty acid linoleate.¹⁴⁹ However, whether this action is due to the lipid cargo alone or related to FABP4 is unclear. Furthermore, reduced insulin secretion in the setting of adrenergic stimulation has been previously described in FABP4-deficient mice, which was attributed to alteration of free fatty acid profile.⁶⁴ Indeed, delivery of exogenous FABP4 can induce insulin resistance without altering β cell responsiveness,¹⁵⁰ consequently FABP4 might initiate hyperinsulinaemia downstream of insulin resistance.

The established link between circulating levels of FABP4, atherosclerosis and cardiovascular disease (Figure 3, Supplementary file 1) has led some researchers to investigate the potential function of circulating FABP4 in endothelial cell biology and cardiac function. Exogenous FABP4 can induce replication and migration of human coronary artery smooth muscle cells¹⁵¹ and inhibit expression of *NOS3* in human umbilical vein endothelial cells.¹⁵² Exogenous FABP4 also suppresses contraction in cultured cardiomyocytes *in vitro*, although whether this effect is a specific FABP4 function is unclear.¹⁵³ This activity of FABP4 on cardiomyocyte function is potentiated in the presence of epoxyeicosatrienoic acids, which supports the concept that fatty acid ligands can modulate the function of FABP4.¹⁵⁴ In addition, FABPs themselves regulate the production of lipids that have effects on cardiomyocytes, therefore FABPs are likely to exert both direct and indirect effects on cardiac function. For example, palmitoleate, a lipid produced at high levels in adipocytes lacking FABP4, is required to sustain cardiac function in the python.¹⁵⁵

In mice, the most dramatic effect of genetic deficiency in *FABP4* has been observed in models of atherosclerosis,^{101–103} but the contribution of circulating levels of FABP4 to inflammatory and cardiovascular function has not yet been defined. In addition, determining the extracellular roles of other FABP family members, and the degree to which each can functionally compensate for the others, will be a crucial question for future research. Continuing investigation of the relative affinities of FABP isoforms for different ligands will also help to determine their roles in delivering and modulating extracellular lipid.

Regulation of FABP4 secretion

Given the lack of a signal peptide, one can reason that regulated FABP4 secretion might occur via a non-classical pathway. Indeed, inhibitors of classical secretion do not alter FABP4 release from cultured adipocytes.¹²⁸ As described previously in the text, circulating FABP4 functions by enhancing hepatic glucose production; plasma FABP4 levels are elevated during fasting, and FABP4 secretion is suppressed by insulin.¹²⁸ In addition, in clamp studies, administration of exogenous FABP4 impairs insulin sensitivity while FABP4 neutralization improves glucose disposal.¹²⁸ Taken together, these findings indicate that FABP4 is an adipose-derived factor that integrates with components of the fasting response to maintain glucose homeostasis. Secretion of FABP4 is responsive to signals that induce lipolysis, including β -adrenergic receptor agonists, forskolin and isobutyl-methylxanthine,¹²⁸ and chemical inhibition or genetic deficiency of HSL and patatin-like phospholipase domain-containing protein 2 (also known as adipose triglyceride lipase) abrogate β -adrenergic-induced FABP4 secretion.¹⁵⁶ Interestingly, this effect might be linked to the lipolysis-induced increase in free fatty acids, and indeed lipid infusion *in vivo* and lipid treatment of adipocytes *in vitro* is sufficient to increase FABP4 secretion.¹⁵⁶ Increased FABP4 release has also been seen in adipocytes exposed to hypoxia,¹⁴⁹ which might in part explain the increased level of FABP4 in circulation observed in obese rodent models and humans with obesity, in whom adipose tissue is poorly oxygenated.¹⁵⁷ In adipocytes, FABP4 localizes to multivesicular bodies and its presence in these structures is enhanced upon stimulation of lipolysis.¹⁵⁶ Fractionation of the media of cultured adipocytes suggests that FABP4 is present in caveolin-1 positive microvesicles,¹⁵⁸ and in electron microscopy and cell fractionation experiments FABP4 is localized to vesicles that are ~100 nm in diameter and positive for the exosome markers CD36 and programmed cell death 6-interacting protein (also known as ALIX).¹⁵⁶ Remarkably, FABP4 localization to these vesicles also increases in response to lipolytic signals.¹⁵⁶ In addition, increasing the concentration of intracellular calcium increases FABP4 secretion from both macrophages and adipocytes (Figure 5).^{130,158} Interestingly, FABP4 secretion via an exosome-like vesicular pathway in adipocytes is not entirely dependent on its lipid-binding activity, as mutants in the lipid binding domain of FABP4 can still be secreted, albeit at a lower efficiency than the wild-type protein.¹⁵⁶ Although this vesicular secretory pathway has been clearly demonstrated in experimental studies, the vast majority of FABP4 present in the serum is in a free form.¹⁵⁶ Whether multiple routes of FABP4 secretion exist that perhaps support different forms of the molecule is unclear. Improved understanding of the molecular mechanisms that drive FABP4 secretion has the potential to lead to novel therapeutic interventions that modulate level of this hormone and its subsequent metabolic effects.

Therapeutic implications of FABPs

Much of the therapeutic research surrounding FABP activity has been focused on FABP4. These studies have been based on animal models of obesity, diabetes mellitus and cardiovascular disease, as well as genetic association studies, and population and longitudinal studies in humans in which plasma FABP4 levels are associated with disease risk and adverse prognosis. The similarities in cellular, molecular and biochemical studies between humans and model systems all suggest that targeting FABP4 in these chronic immunometabolic diseases might be a useful therapeutic approach. For example, treating genetically obese mice with BMS309403 improves glucose homeostasis and insulin sensitivity, reduces adipose tissue inflammation, and reduces lipid accumulation in the liver.¹⁰⁴ This synthetic small molecule FABP4 inhibitor also significantly limited atherosclerotic lesion formation and improved endothelial function in *ApoE*^{-/-} mice,^{104,159} and reduced acute liver injury and high fat high cholesterol diet-induced non-alcoholic fatty liver disease.¹⁶⁰ At the level of the macrophage, another small molecule FABP4 inhibitor, HTS01037, induces *UCP2* expression and attenuates proinflammatory NF- κ B signalling.⁹⁸ Other FABP4 inhibitors have been developed,^{161–165} although their *in vivo* efficacy remains incompletely characterized. However, some have been shown to improve glucose homeostasis and hepatic lipid accumulation in mice with diet-induced obesity.¹⁶⁵

In addition to novel synthetic small molecule FABP4 inhibitors, a screen of FDA-approved drugs resulted in the discovery of several FABP4-binding molecules.¹⁶⁶ Among these, a broad-spectrum antibiotic, levofloxacin, was identified as a high-affinity inhibitor of FABP4.¹⁶⁶ In addition, benzbromarone, a drug with uricosuric properties, was also identified as an inhibitor of FABP4 and can significantly reduce blood glucose in the *db/db* mouse model of obesity and type 2 diabetes mellitus.¹⁶⁷ The metabolic phenotypes generated by these molecules or their derivatives will be interesting to study, although in general the small molecule approach might be challenging due to issues of nonspecificity and off-target effects inherent with this therapeutic strategy. In addition, whether small molecule-mediated inhibition of both intracellular and extracellular FABP4 actions will be desirable therapeutically is unknown, as some intracellular actions might be important for normal adipocyte function.

Major advances have been made in targeting molecules in specific cell types using modified carriers for small interfering RNAs. For example, targeting TNF or osteopontin using β 1,3-D-glucan-encapsulated siRNAs in adipose tissue or macrophages of obese animals improved insulin sensitivity and glucose tolerance.^{168,169} Another elegant strategy to target FABP4 in adipose tissue has been developed using short-hairpin RNAs (shRNAs) coupled to a peptide sequence that facilitates specific delivery to adipose tissue *in vivo*, which resulted in a marked suppression of FABP4 expression.¹⁷⁰ Targeting FABP4 in adipose tissue using this method increased insulin sensitivity and glucose tolerance and reduced blood glucose levels, which recapitulates the effects of *FABP4* genetic deficiency and demonstrates the feasibility of therapeutically targeting FABP4 *in vivo* using interfering RNA.¹⁷⁰

Owing to the emerging insights related to the biological function of secreted FABP4, especially in the context of metabolic diseases, it was interesting to consider the possibility

of targeting FABP4 using antibody-mediated neutralization. Surprisingly, treatment of obese diabetic mice with a polyclonal antibody targeting FABP4 significantly improved insulin sensitivity and glucose homeostasis.¹²⁸ Translation of this strategy into clinical efficacy will require the development of potent monoclonal antibodies, and to date only one group of investigators has reported the application of an FABP4 monoclonal antibody to obese diabetic mice, which modestly improved glucose tolerance with no effect on insulin sensitivity.¹⁷¹ In addition, the extent to which FABP4 biology in metabolic disease is dictated by the secreted versus the cellular forms must be defined, and how other FABP proteins might be capable of functional compensation also needs to be understood.

Interesting possibilities also exist for the use of FABP4 as a target in cancer, including ovarian, breast and prostate malignancies. Although the data are less prevalent for this use, the role for FABP4 in stroma–tumour interactions and the energy extraction of cancer cells from adipose depots or sites of metastasis (for example, in ovarian tumours¹¹⁴ and prostate cancer¹⁷²) justify further study and testing of the advanced therapeutic molecules targeting FABP4.

Conclusions

Studies conducted in the past 20 years have clearly demonstrated the exciting biological functions carried out by the simple proteins that bind to fatty acids. In this Review we discussed the biology of FABPs, primarily FABP4 (also known as aP2), which is the best characterized member of this family with important roles in metabolic homeostasis and immunometabolic diseases. However, ongoing studies are identifying interesting biological functions of the other isoforms, and in the coming years, we should have further exciting insights into their functions in physiology and pathophysiology.

Two developments have marked critical paradigm shifts in our understanding of FABPs. The first relates to their mechanistic complexity and vast biological diversity, which illustrates that these proteins are far more than simple reservoirs or buffers for excess fatty acids. The second is the finding that these proteins do not act only as intracellular mediators but also have extracellular functions. The discovery that some FABPs undergo regulated secretion and exert specific biological functions in tissues other than their origin reveals that this family of proteins are perhaps the most versatile endocrine hormones and potent integrators of tissue status, especially lipid homeostasis, with systemic metabolic homeostasis.

However, with progress comes complexity and, as the fields of FABP and lipid biology research have advanced, the puzzle of how these molecules interact has grown exponentially. In our opinion, one of the most important areas of future investigation involves resolution of how different FABPs interact with various ligands, and the biological implications of these interactions, as well as an understanding of the physiological, dietary and pathophysiological conditions under which FABP biology can be altered by the lipid milieu and composition. New research has revealed the presence of metabolically-active lipids produced in state-specific settings.^{80,155,173,174} In combination with the numerous lipid binding proteins that also show hormonal activity, there seem to be infinite

permutations by which these lipids might integrate metabolic and inflammatory status and transmit signals. Unraveling these details will require a concerted effort, but also provide an exciting opportunity for the development of novel therapeutic approaches.

The second area of future investigation relates to the mechanisms of FABP4 action, especially the most proximal steps that initiate the signalling cascades that are characterized as downstream of FABP action. Specifically, the determination of the molecular organization of FABP4 within an extracellular vesicle, and what determinants on FABP4 define packaging and release from these vesicular compartments is an important consideration. Finally, do cell surface molecules exist that interact with FABPs to propagate their signal, and what protein complexes and lipid ligands, inside or outside of the cell, assemble as signalling nodes for these molecules? Resolution of these critical questions will greatly enhance our current understanding of not only FABPs but also the diverse functions of lipids as signalling intermediates and endocrine hormones.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank members of the Hotamisligil and Bernlohr laboratories for helpful discussions. We thank A. P. Arruda for assistance in generating the initial figures, and K. Claiborn for critical reading and editing of the manuscript. The Hotamisligil laboratory is supported in this area by research funding from the NIH (grant number DK064360) and a sponsored research agreement with Union Chimique Belge. The Bernlohr laboratory is supported by the NIH (grant number DK053189).

References

1. Poveda JA, et al. Lipid modulation of ion channels through specific binding sites. *Biochim Biophys Acta*. 2014; 1838:1560–1567. [PubMed: 24211605]
2. Wahli W, Michalik L. PPARs at the crossroads of lipid signaling and inflammation. *Trends Endocrinol Metab*. 2012; 23:351–363. [PubMed: 22704720]
3. Shimizu T. Lipid mediators in health and disease: enzymes and receptors as therapeutic targets for the regulation of immunity and inflammation. *Annu Rev Pharmacol Toxicol*. 2009; 49:123–150. [PubMed: 18834304]
4. Dresner A, et al. Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. *J Clin Invest*. 1999; 103:253–259. [PubMed: 9916137]
5. Cho H, et al. Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB β). *Science*. 2001; 292:1728–1731. [PubMed: 11387480]
6. Kim JK, et al. Prevention of fat-induced insulin resistance by salicylate. *J Clin Invest*. 2001; 108:437–446. [PubMed: 11489937]
7. Solinas G, Naugler W, Galimi F, Lee MS, Karin M. Saturated fatty acids inhibit induction of insulin gene transcription by JNK-mediated phosphorylation of insulin-receptor substrates. *Proc Natl Acad Sci USA*. 2006; 103:16454–16459. [PubMed: 17050683]
8. Nguyen MT, et al. JNK and tumor necrosis factor- α mediate free fatty acid-induced insulin resistance in 3T3-L1 adipocytes. *J Biol Chem*. 2005; 280:35361–35371. [PubMed: 16085647]
9. Shi H, et al. TLR4 links innate immunity and fatty acid-induced insulin resistance. *J Clin Invest*. 2006; 116:3015–3025. [PubMed: 17053832]
10. Severeid L, Connor WE, Long JP. The depressant effect of fatty acids on the isolated rabbit heart. *Proc Soc Exp Biol Med*. 1969; 131:1239–1243. [PubMed: 5811981]

11. Gordon GB. Saturated free fatty acid toxicity. II. Lipid accumulation, ultrastructural alterations, and toxicity in mammalian cells in culture. *Exp Mol Pathol.* 1977; 27:262–276. [PubMed: 561704]
12. Ockner RK, Manning JA, Poppenhausen RB, Ho WK. A binding protein for fatty acids in cytosol of intestinal mucosa, liver, myocardium, and other tissues. *Science.* 1972; 177:56–58. [PubMed: 5041774]
13. Ockner RK, Manning JA. Fatty acid-binding protein in small intestine. Identification, isolation, and evidence for its role in cellular fatty acid transport. *J Clin Invest.* 1974; 54:326–338. [PubMed: 4211161]
14. Furuhashi M, Hotamisligil GS. Fatty acid-binding proteins: role in metabolic diseases and potential as drug targets. *Nat Rev Drug Discov.* 2008; 7:489–503. [PubMed: 18511927]
15. Ockner RK, Manning JA. Fatty acid binding protein. Role in esterification of absorbed long chain fatty acid in rat intestine. *J Clin Invest.* 1976; 58:632–641. [PubMed: 986401]
16. Li LO, Klett EL, Coleman RA. Acyl-CoA synthesis, lipid metabolism and lipotoxicity. *Biochim Biophys Acta.* 2010; 1801:246–251. [PubMed: 19818872]
17. Ockner RK, Manning JA, Kane JP. Fatty acid binding protein. Isolation from rat liver, characterization, and immunochemical quantification. *J Biol Chem.* 1982; 257:7872–7878. [PubMed: 6806286]
18. Gordon JI, Alpers DH, Ockner RK, Strauss AW. The nucleotide sequence of rat liver fatty acid binding protein mRNA. *J Biol Chem.* 1983; 258:3356–3363. [PubMed: 6298233]
19. Bass NM, Raghupathy E, Rhoads DE, Manning JA, Ockner RK. Partial purification of molecular weight 12,000 fatty acid binding proteins from rat brain and their effect on synaptosomal Na⁺-dependent amino acid uptake. *Biochemistry.* 1984; 23:6539–6544. [PubMed: 6529568]
20. Haq RU, Shrago E, Christodoulides L, Ketterer B. Purification and characterization of fatty acid binding protein in mammalian lung. *Exp Lung Res.* 1985; 9:43–55. [PubMed: 3933967]
21. Sweetser DA, Lowe JB, Gordon JI. The nucleotide sequence of the rat liver fatty acid-binding protein gene. Evidence that exon 1 encodes an oligopeptide domain shared by a family of proteins which bind hydrophobic ligands. *J Biol Chem.* 1986; 261:5553–5561. [PubMed: 3007511]
22. Sacchettini JC, Said B, Schulz H, Gordon JI. Rat heart fatty acid-binding protein is highly homologous to the murine adipocyte 422 protein and the P2 protein of peripheral nerve myelin. *J Biol Chem.* 1986; 261:8218–8223. [PubMed: 2424895]
23. Madsen P, Rasmussen HH, Leffers H, Honore B, Celis JE. Molecular cloning and expression of a novel keratinocyte protein (psoriasis-associated fatty acid-binding protein [PA-FABP]) that is highly up-regulated in psoriatic skin and that shares similarity to fatty acid-binding proteins. *J Invest Dermatol.* 1992; 99:299–305. [PubMed: 1512466]
24. Kurtz A, et al. The expression pattern of a novel gene encoding brain-fatty acid binding protein correlates with neuronal and glial cell development. *Development.* 1994; 120:2637–2649. [PubMed: 7956838]
25. Watanabe R, et al. Molecular cloning of a cDNA encoding a novel fatty acid-binding protein from rat skin. *Biochem Biophys Res Commun.* 1994; 200:253–259. [PubMed: 8166694]
26. Spiegelman BM, Green H. Control of specific protein biosynthesis during the adipose conversion of 3T3 cells. *J Biol Chem.* 1980; 255:8811–8818. [PubMed: 6773950]
27. Bernlohr DA, Angus CW, Lane MD, Bolanowski MA, Kelly TJ Jr. Expression of specific mRNAs during adipose differentiation: identification of an mRNA encoding a homologue of myelin P2 protein. *Proc Natl Acad Sci USA.* 1984; 81:5468–5472. [PubMed: 6206497]
28. Haq RU, Christodoulides L, Ketterer B, Shrago E. Characterization and purification of fatty acid-binding protein in rat and human adipose tissue. *Biochim Biophys Acta.* 1982; 713:193–198. [PubMed: 6817805]
29. LaLonde JM, Bernlohr DA, Banaszak LJ. The up-and-down β -barrel proteins. *FASEB J.* 1994; 8:1240–1247. [PubMed: 8001736]
30. Storch J, Thumser AE. Tissue-specific functions in the fatty acid-binding protein family. *J Biol Chem.* 2010; 285:32679–32683. [PubMed: 20716527]
31. Zezulak KM, Green H. Specificity of gene expression in adipocytes. *Mol Cell Biol.* 1985; 5:419–421. [PubMed: 3974575]

32. Bernlohr DA, Doering TL, Kelly TJ Jr, Lane MD. Tissue specific expression of p422 protein, a putative lipid carrier, in mouse adipocytes. *Biochem Biophys Res Commun*. 1985; 132:850–855. [PubMed: 2415129]
33. Matarese V, Bernlohr DA. Purification of murine adipocyte lipid-binding protein. Characterization as a fatty acid- and retinoic acid-binding protein. *J Biol Chem*. 1988; 263:14544–14551. [PubMed: 2844775]
34. Blake WL, Clarke SD. Induction of adipose fatty acid binding protein (a-FABP) by insulin-like growth factor-1 (IGF-1) in 3T3-L1 preadipocytes. *Biochem Biophys Res Commun*. 1990; 173:87–91. [PubMed: 1701639]
35. Amri EZ, Ailhaud G, Grimaldi P. Regulation of adipose cell differentiation. II. Kinetics of induction of the aP2 gene by fatty acids and modulation by dexamethasone. *J Lipid Res*. 1991; 32:1457–1463. [PubMed: 1753216]
36. Amri EZ, Bertrand B, Ailhaud G, Grimaldi P. Regulation of adipose cell differentiation. I. Fatty acids are inducers of the aP2 gene expression. *J Lipid Res*. 1991; 32:1449–1456. [PubMed: 1753215]
37. Distel RJ, Robinson GS, Spiegelman BM. Fatty acid regulation of gene expression. Transcriptional and post-transcriptional mechanisms. *J Biol Chem*. 1992; 267:5937–5941. [PubMed: 1372897]
38. Tontonoz P, Hu E, Spiegelman BM. Stimulation of adipogenesis in fibroblasts by PPAR γ 2, a lipid-activated transcription factor. *Cell*. 1994; 79:1147–1156. [PubMed: 8001151]
39. Pelton PD, Zhou L, Demarest KT, Burris TP. PPAR γ activation induces the expression of the adipocyte fatty acid binding protein gene in human monocytes. *Biochem Biophys Res Commun*. 1999; 261:456–458. [PubMed: 10425206]
40. Shum BO, et al. The adipocyte fatty acid-binding protein aP2 is required in allergic airway inflammation. *J Clin Invest*. 2006; 116:2183–2192. [PubMed: 16841093]
41. Wootan MG, Bass NM, Bernlohr DA, Storch J. Fatty acid binding sites of rodent adipocyte and heart fatty acid binding proteins: characterization using fluorescent fatty acids. *Biochemistry*. 1990; 29:9305–9311. [PubMed: 2248947]
42. Xu Z, Bernlohr DA, Banaszak LJ. The adipocyte lipid-binding protein at 1.6-Å resolution. Crystal structures of the apoprotein and with bound saturated and unsaturated fatty acids. *J Biol Chem*. 1993; 268:7874–7884. [PubMed: 8463311]
43. Richieri GV, Ogata RT, Kleinfeld AM. Equilibrium constants for the binding of fatty acids with fatty acid-binding proteins from adipocyte, intestine, heart, and liver measured with the fluorescent probe ADIFAB. *J Biol Chem*. 1994; 269:23918–23930. [PubMed: 7929039]
44. Wootan MG, Bernlohr DA, Storch J. Mechanism of fluorescent fatty acid transfer from adipocyte fatty acid binding protein to membranes. *Biochemistry*. 1993; 32:8622–8627. [PubMed: 8357805]
45. Gericke A, Smith ER, Moore DJ, Mendelsohn R, Storch J. Adipocyte fatty acid-binding protein: interaction with phospholipid membranes and thermal stability studied by FTIR spectroscopy. *Biochemistry*. 1997; 36:8311–8317. [PubMed: 9204877]
46. Herr FM, Matarese V, Bernlohr DA, Storch J. Surface lysine residues modulate the collisional transfer of fatty acid from adipocyte fatty acid binding protein to membranes. *Biochemistry*. 1995; 34:11840–11845. [PubMed: 7547918]
47. LiCata VJ, Bernlohr DA. Surface properties of adipocyte lipid-binding protein: response to lipid binding, and comparison with homologous proteins. *Proteins*. 1998; 33:577–589. [PubMed: 9849941]
48. Banaszak L, et al. Lipid-binding proteins: a family of fatty acid and retinoid transport proteins. *Adv Protein Chem*. 1994; 45:89–151. [PubMed: 8154375]
49. Jenkins-Kruchten AE, et al. Fatty acid-binding protein-hormone-sensitive lipase interaction. Fatty acid dependence on binding. *J Biol Chem*. 2003; 278:47636–47643. [PubMed: 13129924]
50. Hellberg K, et al. X-ray crystallographic analysis of adipocyte fatty acid binding protein (aP2) modified with 4-hydroxy-2-nonenal. *Protein Sci*. 2010; 19:1480–1489. [PubMed: 20509169]
51. Borchers T, Spener F. Involvement of arginine in the binding of heme and fatty acids to fatty acid-binding protein from bovine liver. *Mol Cell Biochem*. 1993; 123:23–27. [PubMed: 8232264]

52. Jenkins AE, Hockenberry JA, Nguyen T, Bernlohr DA. Testing of the portal hypothesis: analysis of a V32G, F57G, K58G mutant of the fatty acid binding protein of the murine adipocyte. *Biochemistry*. 2002; 41:2022–2027. [PubMed: 11827549]
53. Sha RS, Kane CD, Xu Z, Banaszak LJ, Bernlohr DA. Modulation of ligand binding affinity of the adipocyte lipid-binding protein by selective mutation. Analysis *in vitro* and *in situ*. *J Biol Chem*. 1993; 268:7885–7892. [PubMed: 8463312]
54. Grimsrud PA, Picklo MJ Sr, Griffin TJ, Bernlohr DA. Carbonylation of adipose proteins in obesity and insulin resistance: identification of adipocyte fatty acid-binding protein as a cellular target of 4-hydroxynonenal. *Mol Cell Proteomics*. 2007; 6:624–637. [PubMed: 17205980]
55. Chabowski A, Gorski J, Luiken JJ, Glatz JF, Bonen A. Evidence for concerted action of FAT/CD36 and FABPpm to increase fatty acid transport across the plasma membrane. *Prostaglandins Leukot Essent Fatty Acids*. 2007; 77:345–353. [PubMed: 18240411]
56. Glatz JF, Luiken JJ, Bonen A. Membrane fatty acid transporters as regulators of lipid metabolism: implications for metabolic disease. *Physiol Rev*. 2010; 90:367–417. [PubMed: 20086080]
57. Spitsberg VL, Matitashvili E, Gorewit RC. Association and coexpression of fatty-acid-binding protein and glycoprotein CD36 in the bovine mammary gland. *Eur J Biochem*. 1995; 230:872–878. [PubMed: 7541353]
58. Woodford JK, Jefferson JR, Wood WG, Hubbell T, Schroeder F. Expression of liver fatty acid binding protein alters plasma membrane lipid composition and structure in transfected L-cell fibroblasts. *Biochim Biophys Acta*. 1993; 1145:257–265. [PubMed: 8431458]
59. Iso T, et al. Capillary endothelial fatty acid binding proteins 4 and 5 play a critical role in fatty acid uptake in heart and skeletal muscle. *Arterioscler Thromb Vasc Biol*. 2013; 33:2549–2557. [PubMed: 23968980]
60. Murphy EJ, Prows DR, Stiles T, Schroeder F. Liver and intestinal fatty acid-binding protein expression increases phospholipid content and alters phospholipid fatty acid composition in L-cell fibroblasts. *Lipids*. 2000; 35:729–738. [PubMed: 10941873]
61. Maeda K, et al. Adipocyte/macrophage fatty acid binding proteins control integrated metabolic responses in obesity and diabetes. *Cell Metab*. 2005; 1:107–119. [PubMed: 16054052]
62. Coe NR, Simpson MA, Bernlohr DA. Targeted disruption of the adipocyte lipid-binding protein (aP2 protein) gene impairs fat cell lipolysis and increases cellular fatty acid levels. *J Lipid Res*. 1999; 40:967–972. [PubMed: 10224167]
63. Shaughnessy S, Smith ER, Kodukula S, Storch J, Fried SK. Adipocyte metabolism in adipocyte fatty acid binding protein knockout mice (aP2^{-/-}) after short-term high-fat feeding: functional compensation by the keratinocyte [correction of keratinocyte] fatty acid binding protein. *Diabetes*. 2000; 49:904–911. [PubMed: 10866041]
64. Scheja L, et al. Altered insulin secretion associated with reduced lipolytic efficiency in aP2^{-/-} mice. *Diabetes*. 1999; 48:1987–1994. [PubMed: 10512363]
65. Hertzel AV, et al. Lipid metabolism and adipokine levels in fatty acid-binding protein null and transgenic mice. *Am J Physiol Endocrinol Metab*. 2006; 290:E814–E823. [PubMed: 16303844]
66. Storch J, Thumser AE. The fatty acid transport function of fatty acid-binding proteins. *Biochim Biophys Acta*. 2000; 1486:28–44. [PubMed: 10856711]
67. Schroeder F, et al. Role of fatty acid binding proteins and long chain fatty acids in modulating nuclear receptors and gene transcription. *Lipids*. 2008; 43:1–17. [PubMed: 17882463]
68. Huang H, Starodub O, McIntosh A, Kier AB, Schroeder F. Liver fatty acid-binding protein targets fatty acids to the nucleus. Real time confocal and multiphoton fluorescence imaging in living cells. *J Biol Chem*. 2002; 277:29139–29151. [PubMed: 12023965]
69. Yu S, Levi L, Siegel R, Noy N. Retinoic acid induces neurogenesis by activating both retinoic acid receptors (RARs) and peroxisome proliferator-activated receptor β/δ (PPAR β/δ). *J Biol Chem*. 2012; 287:42195–42205. [PubMed: 23105114]
70. Yu S, Levi L, Casadesus G, Kunos G, Noy N. Fatty acid-binding protein 5 (FABP5) regulates cognitive function both by decreasing anandamide levels and by activating the nuclear receptor peroxisome proliferator-activated receptor β/δ (PPAR β/δ) in the brain. *J Biol Chem*. 2014; 289:12748–12758. [PubMed: 24644281]

71. Tan NS, et al. Selective cooperation between fatty acid binding proteins and peroxisome proliferator-activated receptors in regulating transcription. *Mol Cell Biol.* 2002; 22:5114–5127. [PubMed: 12077340]
72. Adida A, Spener F. Adipocyte-type fatty acid-binding protein as inter-compartmental shuttle for peroxisome proliferator activated receptor γ agonists in cultured cell. *Biochim Biophys Acta.* 2006; 1761:172–181. [PubMed: 16574478]
73. Ayers SD, Nedrow KL, Gillilan RE, Noy N. Continuous nucleocytoplasmic shuttling underlies transcriptional activation of PPAR γ by FABP4. *Biochemistry.* 2007; 46:6744–6752. [PubMed: 17516629]
74. Makowski L, Brittingham KC, Reynolds JM, Suttles J, Hotamisligil GS. The fatty acid-binding protein, aP2, coordinates macrophage cholesterol trafficking and inflammatory activity. Macrophage expression of aP2 impacts peroxisome proliferator-activated receptor γ and I κ B kinase activities. *J Biol Chem.* 2005; 280:12888–12895. [PubMed: 15684432]
75. Hotamisligil GS, et al. Uncoupling of obesity from insulin resistance through a targeted mutation in aP2, the adipocyte fatty acid binding protein. *Science.* 1996; 274:1377–1379. [PubMed: 8910278]
76. Bernlohr DA, Coe NR, Simpson MA, Hertzler AV. Regulation of gene expression in adipose cells by polyunsaturated fatty acids. *Adv Exp Med Biol.* 1997; 422:145–156. [PubMed: 9361822]
77. Uysal KT, Scheja L, Wiesbrock SM, Bonner-Weir S, Hotamisligil GS. Improved glucose and lipid metabolism in genetically obese mice lacking aP2. *Endocrinology.* 2000; 141:3388–3396. [PubMed: 10965911]
78. Maeda K, et al. Role of the fatty acid binding protein mal1 in obesity and insulin resistance. *Diabetes.* 2003; 52:300–307. [PubMed: 12540600]
79. Cao H, et al. Regulation of metabolic responses by adipocyte/macrophage fatty acid-binding proteins in leptin-deficient mice. *Diabetes.* 2006; 55:1915–1922. [PubMed: 16804058]
80. Cao H, et al. Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism. *Cell.* 2008; 134:933–944. [PubMed: 18805087]
81. Erbay E, et al. Reducing endoplasmic reticulum stress through a macrophage lipid chaperone alleviates atherosclerosis. *Nat Med.* 2009; 15:1383–1391. [PubMed: 19966778]
82. Oh DY, et al. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell.* 2010; 142:687–698. [PubMed: 20813258]
83. Ichimura A, et al. Dysfunction of lipid sensor GPR120 leads to obesity in both mouse and human. *Nature.* 2012; 483:350–354. [PubMed: 22343897]
84. Shen WJ, Sridhar K, Bernlohr DA, Kraemer FB. Interaction of rat hormone-sensitive lipase with adipocyte lipid-binding protein. *Proc Natl Acad Sci USA.* 1999; 96:5528–5532. [PubMed: 10318917]
85. Smith AJ, et al. Physical association between the adipocyte fatty acid-binding protein and hormone-sensitive lipase: a fluorescence resonance energy transfer analysis. *J Biol Chem.* 2004; 279:52399–52405. [PubMed: 15456755]
86. Hampton M, et al. Deep sequencing the transcriptome reveals seasonal adaptive mechanisms in a hibernating mammal. *PLoS ONE.* 2011; 6:e27021. [PubMed: 22046435]
87. Tontonoz P, Nagy L, Alvarez JG, Thomazy VA, Evans RM. PPAR γ promotes monocyte/macrophage differentiation and uptake of oxidized LDL. *Cell.* 1998; 93:241–252. [PubMed: 9568716]
88. Fu Y, Luo N, Lopes-Virella MF, Garvey WT. The adipocyte lipid binding protein (ALBP/aP2) gene facilitates foam cell formation in human THP-1 macrophages. *Atherosclerosis.* 2002; 165:259–269. [PubMed: 12417276]
89. Liu QY, Nambi P. Sirolimus upregulates aP2 expression in human monocytes and macrophages. *Transplant Proc.* 2004; 36:3229–3231. [PubMed: 15686734]
90. Liu QY, Quinet E, Nambi P. Adipocyte fatty acid-binding protein (aP2), a newly identified LXR target gene, is induced by LXR agonists in human THP-1 cells. *Mol Cell Biochem.* 2007; 302:203–213. [PubMed: 17396233]

91. Babaev VR, et al. Macrophage Mal1 deficiency suppresses atherosclerosis in low-density lipoprotein receptor-null mice by activating peroxisome proliferator-activated receptor- γ -regulated genes. *Arterioscler Thromb Vasc Biol.* 2011; 31:1283–1290. [PubMed: 21474828]
92. Garin-Shkolnik T, Rudich A, Hotamisligil GS, Rubinstein M. FABP4 attenuates PPAR γ and adipogenesis and is inversely correlated with PPAR γ in adipose tissues. *Diabetes.* 2014; 63:900–911. [PubMed: 24319114]
93. Zimmer JS, Dyckes DF, Bernlohr DA, Murphy RC. Fatty acid binding proteins stabilize leukotriene A4: competition with arachidonic acid but not other lipoxygenase products. *J Lipid Res.* 2004; 45:2138–2144. [PubMed: 15342681]
94. Dickinson Zimmer JS, Voelker DR, Bernlohr DA, Murphy RC. Stabilization of leukotriene A4 by epithelial fatty acid-binding protein in the rat basophilic leukemia cell. *J Biol Chem.* 2004; 279:7420–7426. [PubMed: 14676186]
95. Spite M, et al. Deficiency of the leukotriene B4 receptor, BLT-1, protects against systemic insulin resistance in diet-induced obesity. *J Immunol.* 2011; 187:1942–1949. [PubMed: 21742977]
96. Layne MD, et al. Role of macrophage-expressed adipocyte fatty acid binding protein in the development of accelerated atherosclerosis in hypercholesterolemic mice. *FASEB J.* 2001; 15:2733–2735. [PubMed: 11606480]
97. Chan, KL., et al. Palmitoleate reverses high fat-induced pro-inflammatory macrophage polarization via AMPK. *J Biol Chem.* <http://dx.doi.org/10.1074/jbc.M115.646992>
98. Xu H, et al. Uncoupling lipid metabolism from inflammation through fatty acid binding protein-dependent expression of UCP2. *Mol Cell Biol.* 2015; 35:1055–1065. [PubMed: 25582199]
99. Thompson BR, Mazurkiewicz-Munoz AM, Suttles J, Carter-Su C, Bernlohr DA. Interaction of adipocyte fatty acid-binding protein (AFABP) and JAK2: AFABP/aP2 as a regulator of JAK2 signaling. *J Biol Chem.* 2009; 284:13473–13480. [PubMed: 19318353]
100. Semenkovich CF. Insulin resistance and atherosclerosis. *J Clin Invest.* 2006; 116:1813–1822. [PubMed: 16823479]
101. Perrella MA, et al. Absence of adipocyte fatty acid binding protein prevents the development of accelerated atherosclerosis in hypercholesterolemic mice. *FASEB J.* 2001; 15:1774–1776. [PubMed: 11481226]
102. Makowski L, et al. Lack of macrophage fatty-acid-binding protein aP2 protects mice deficient in apolipoprotein E against atherosclerosis. *Nat Med.* 2001; 7:699–705. [PubMed: 11385507]
103. Boord JB, et al. Adipocyte fatty acid-binding protein, aP2, alters late atherosclerotic lesion formation in severe hypercholesterolemia. *Arterioscler Thromb Vasc Biol.* 2002; 22:1686–1691. [PubMed: 12377750]
104. Furuhashi M, et al. Treatment of diabetes and atherosclerosis by inhibiting fatty-acid-binding protein aP2. *Nature.* 2007; 447:959–965. [PubMed: 17554340]
105. Boord JB, et al. Combined adipocyte-macrophage fatty acid-binding protein deficiency improves metabolism, atherosclerosis, and survival in apolipoprotein E-deficient mice. *Circulation.* 2004; 110:1492–1498. [PubMed: 15353487]
106. Rolph MS, et al. Regulation of dendritic cell function and T cell priming by the fatty acid-binding protein AP2. *J Immunol.* 2006; 177:7794–7801. [PubMed: 17114450]
107. Reynolds JM, et al. Deficiency of fatty acid-binding proteins in mice confers protection from development of experimental autoimmune encephalomyelitis. *J Immunol.* 2007; 179:313–321. [PubMed: 17579051]
108. Elmasri H, et al. Fatty acid binding protein 4 is a target of VEGF and a regulator of cell proliferation in endothelial cells. *FASEB J.* 2009; 23:3865–3873. [PubMed: 19625659]
109. Harjes U, Bridges E, McIntyre A, Fielding BA, Harris AL. Fatty acid-binding protein 4, a point of convergence for angiogenic and metabolic signaling pathways in endothelial cells. *J Biol Chem.* 2014; 289:23168–23176. [PubMed: 24939870]
110. Daly C, et al. Angiopoietin-1 modulates endothelial cell function and gene expression via the transcription factor FKHR (FOXO1). *Genes Dev.* 2004; 18:1060–1071. [PubMed: 15132996]
111. Elmasri H, et al. Endothelial cell-fatty acid binding protein 4 promotes angiogenesis: role of stem cell factor/c-kit pathway. *Angiogenesis.* 2012; 15:457–468. [PubMed: 22562362]

112. Ghelfi E, et al. Fatty acid binding protein 4 regulates VEGF-induced airway angiogenesis and inflammation in a transgenic mouse model: implications for asthma. *Am J Pathol.* 2013; 182:1425–1433. [PubMed: 23391391]
113. Saint-Geniez M, et al. Fatty acid binding protein 4 deficiency protects against oxygen-induced retinopathy in mice. *PLoS ONE.* 2014; 9:e96253. [PubMed: 24802082]
114. Nieman KM, et al. Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth. *Nat Med.* 2011; 17:1498–1503. [PubMed: 22037646]
115. Cataltepe O, et al. Fatty acid binding protein 4 is expressed in distinct endothelial and non-endothelial cell populations in glioblastoma. *Neuropathol Appl Neurobiol.* 2012; 38:400–410. [PubMed: 22093167]
116. Lee D, et al. Expression of fatty acid binding protein 4 is involved in the cell growth of oral squamous cell carcinoma. *Oncol Rep.* 2014; 31:1116–1120. [PubMed: 24425381]
117. Pelsers MM, et al. Liver fatty acid-binding protein as a sensitive serum marker of acute hepatocellular damage in liver transplant recipients. *Clin Chem.* 2002; 48:2055–2057. [PubMed: 12406996]
118. Knowlton AA, Apstein CS, Saouf R, Brecher P. Leakage of heart fatty acid binding protein with ischemia and reperfusion in the rat. *J Mol Cell Cardiol.* 1989; 21:577–583. [PubMed: 2778809]
119. Tanaka T, Hirota Y, Sohmiya K, Nishimura S, Kawamura K. Serum and urinary human heart fatty acid-binding protein in acute myocardial infarction. *Clin Biochem.* 1991; 24:195–201. [PubMed: 2040092]
120. Kleine AH, Glatz JF, Van Nieuwenhoven FA, Van der Vusse GJ. Release of heart fatty acid-binding protein into plasma after acute myocardial infarction in man. *Mol Cell Biochem.* 1992; 116:155–162. [PubMed: 1480144]
121. Kanda T, et al. Intestinal fatty acid-binding protein is a useful diagnostic marker for mesenteric infarction in humans. *Gastroenterology.* 1996; 110:339–343. [PubMed: 8566578]
122. Schellekens DH, et al. Plasma intestinal fatty acid-binding protein levels correlate with morphologic epithelial intestinal damage in a human translational ischemia-reperfusion model. *J Clin Gastroenterol.* 2014; 48:253–260. [PubMed: 24100750]
123. Akbal E, et al. Liver fatty acid-binding protein is a diagnostic marker to detect liver injury due to chronic hepatitis C infection. *Arch Med Res.* 2013; 44:34–38. [PubMed: 23291381]
124. Foucaud L, et al. Output of liver fatty acid-binding protein (L-FABP) in bile. *Biochim Biophys Acta.* 1999; 1436:593–599. [PubMed: 9989289]
125. Specht B, et al. Mammary derived growth inhibitor is not a distinct protein but a mix of heart-type and adipocyte-type fatty acid-binding protein. *J Biol Chem.* 1996; 271:19943–19949. [PubMed: 8702709]
126. Brandt R, et al. A 13-kilodalton protein purified from milk fat globule membranes is closely related to a mammary-derived growth inhibitor. *Biochemistry.* 1988; 27:1420–1425. [PubMed: 3365397]
127. Bronsky J, et al. Adiponectin, adipocyte fatty acid binding protein, and epidermal fatty acid binding protein: proteins newly identified in human breast milk. *Clin Chem.* 2006; 52:1763–1770. [PubMed: 16873294]
128. Cao H, et al. Adipocyte lipid chaperone AP2 is a secreted adipokine regulating hepatic glucose production. *Cell Metab.* 2013; 17:768–778. [PubMed: 23663740]
129. Xu A, et al. Adipocyte fatty acid-binding protein is a plasma biomarker closely associated with obesity and metabolic syndrome. *Clin Chem.* 2006; 52:405–413. [PubMed: 16423904]
130. Schlottmann I, Ehrhart-Bornstein M, Wabitsch M, Bornstein SR, Lamounier-Zepter V. Calcium-dependent release of adipocyte fatty acid binding protein from human adipocytes. *Int J Obes (Lond).* 2014; 38:1221–1227. [PubMed: 24352293]
131. Kaess BM, et al. Cardiometabolic correlates and heritability of fetuin-A, retinol-binding protein 4, and fatty-acid binding protein 4 in the Framingham Heart Study. *J Clin Endocrinol Metab.* 2012; 97:E1943–E1947. [PubMed: 22855337]
132. Chow WS, et al. Elevated circulating adipocyte-fatty acid binding protein levels predict incident cardiovascular events in a community-based cohort: a 12-year prospective study. *J Am Heart Assoc.* 2013; 2:e004176. [PubMed: 23525430]

133. von Eynatten M, et al. Circulating adipocyte-fatty acid-binding protein levels and cardiovascular morbidity and mortality in patients with coronary heart disease: a 10-year prospective study. *Arterioscler Thromb Vasc Biol.* 2012; 32:2327–2335. [PubMed: 22679309]
134. Tso AW, et al. Serum adipocyte fatty acid binding protein as a new biomarker predicting the development of type 2 diabetes: a 10-year prospective study in a Chinese cohort. *Diabetes Care.* 2007; 30:2667–2672. [PubMed: 17620449]
135. Ishimura S, et al. Circulating levels of fatty acid-binding protein family and metabolic phenotype in the general population. *PLoS ONE.* 2013; 8:e81318. [PubMed: 24278421]
136. Tso AW, et al. Serum adipocyte fatty acid-binding protein associated with ischemic stroke and early death. *Neurology.* 2011; 76:1968–1975. [PubMed: 21562251]
137. Balci MM, et al. Serum levels of adipocyte fatty acid-binding protein are independently associated with left ventricular mass and myocardial performance index in obstructive sleep apnea syndrome. *J Investig Med.* 2012; 60:1020–1026.
138. Hancke K, Grubeck D, Hauser N, Kreienberg R, Weiss JM. Adipocyte fatty acid-binding protein as a novel prognostic factor in obese breast cancer patients. *Breast Cancer Res Treat.* 2010; 119:367–367. [PubMed: 19842034]
139. Yoo HJ, et al. Serum adipocyte fatty acid-binding protein is associated independently with vascular inflammation: analysis with ¹⁸F-fluorodeoxyglucose positron emission tomography. *J Clin Endocrinol Metab.* 2011; 96:E488–E492. [PubMed: 21159847]
140. Schmilovitz-Weiss H, et al. Serum adipocyte fatty acid binding protein in liver transplant recipients and the metabolic syndrome. *Ann Hepatol.* 2012; 11:343–349. [PubMed: 22481453]
141. Haluzikova D, et al. Serum concentrations of adipocyte fatty acid binding protein in patients with anorexia nervosa. *Physiol Res.* 2009; 58:577–581. [PubMed: 18657008]
142. Comerford KB, Buchan W, Karakas SE. The effects of weight loss on FABP4 and RBP4 in obese women with metabolic syndrome. *Horm Metab Res.* 2014; 46:224–231. [PubMed: 23979787]
143. Stejskal D, Karpisek M, Bronsky J. Serum adipocyte-fatty acid binding protein discriminates patients with permanent and temporary body weight loss. *J Clin Lab Anal.* 2008; 22:380–382. [PubMed: 18803274]
144. Tuncman G, et al. A genetic variant at the fatty acid-binding protein aP2 locus reduces the risk for hypertriglyceridemia, type 2 diabetes, and cardiovascular disease. *Proc Natl Acad Sci USA.* 2006; 103:6970–6975. [PubMed: 16641093]
145. Saksi J, et al. Low-expression variant of fatty acid-binding protein 4 favors reduced manifestations of atherosclerotic disease and increased plaque stability. *Circ Cardiovasc Genet.* 2014; 7:588–598. [PubMed: 25122052]
146. Wang J, et al. FABP4: a novel candidate gene for polycystic ovary syndrome. *Endocrine.* 2009; 36:392–396. [PubMed: 19844814]
147. Bhushan B, et al. Fatty-acid binding protein 4 gene polymorphisms and plasma levels in children with obstructive sleep apnea. *Sleep Med.* 2011; 12:666–671. [PubMed: 21664182]
148. Unger RH, Cherrington AD. Glucagonocentric restructuring of diabetes: a pathophysiologic and therapeutic makeover. *J Clin Invest.* 2012; 122:4–12. [PubMed: 22214853]
149. Wu LE, et al. Identification of fatty acid binding protein 4 as an adipokine that regulates insulin secretion during obesity. *Mol Metab.* 2014; 3:465–473. [PubMed: 24944906]
150. Kralisch S, et al. Circulating adipocyte fatty acid-binding protein induces insulin resistance in mice *in vivo*. *Obesity (Silver Spring).* 2015; 23:1007–1013. [PubMed: 25865078]
151. Girona J, et al. FABP4 induces vascular smooth muscle cell proliferation and migration through a MAPK-dependent pathway. *PLoS ONE.* 2013; 8:e81914. [PubMed: 24312381]
152. Aragones G, et al. Fatty acid-binding protein 4 impairs the insulin-dependent nitric oxide pathway in vascular endothelial cells. *Cardiovasc Diabetol.* 2012; 11:72. [PubMed: 22709426]
153. Lamounier-Zepter V, et al. Adipocyte fatty acid-binding protein suppresses cardiomyocyte contraction: a new link between obesity and heart disease. *Circ Res.* 2009; 105:326–334. [PubMed: 19608978]
154. Lamounier-Zepter V, et al. Interaction of epoxyeicosatrienoic acids and adipocyte fatty acid-binding protein in the modulation of cardiomyocyte contractility. *Int J Obes (Lond).* 2015; 39:755–761. [PubMed: 25370574]

155. Riquelme CA, et al. Fatty acids identified in the Burmese python promote beneficial cardiac growth. *Science*. 2011; 334:528–531. [PubMed: 22034436]
156. Ertunc ME, et al. Secretion of fatty acid binding protein aP2 from adipocytes through a nonclassical pathway in response to adipocyte lipase activity. *J Lipid Res*. 2015; 56:423–434. [PubMed: 25535287]
157. Sun K, Kusminski CM, Scherer PE. Adipose tissue remodeling and obesity. *J Clin Invest*. 2011; 121:2094–2101. [PubMed: 21633177]
158. Kralisch S, et al. Adipocyte fatty acid-binding protein is released from adipocytes by a non-conventional mechanism. *Int J Obes (Lond)*. 2014; 38:1251–1254. [PubMed: 24445660]
159. Lee MY, et al. Chronic administration of BMS309403 improves endothelial function in apolipoprotein E-deficient mice and in cultured human endothelial cells. *Br J Pharmacol*. 2011; 162:1564–1576. [PubMed: 21175571]
160. Hoo RL, et al. Pharmacological inhibition of adipocyte fatty acid binding protein alleviates both acute liver injury and non-alcoholic steatohepatitis in mice. *J Hepatol*. 2013; 58:358–364. [PubMed: 23108115]
161. Lehmann F, et al. Discovery of inhibitors of human adipocyte fatty acid-binding protein, a potential type 2 diabetes target. *Bioorg Med Chem Lett*. 2004; 14:4445–4448. [PubMed: 15357969]
162. Barf T, et al. N-Benzyl-indolo carboxylic acids: design and synthesis of potent and selective adipocyte fatty-acid binding protein (A-FABP) inhibitors. *Bioorg Med Chem Lett*. 2009; 19:1745–1748. [PubMed: 19217286]
163. Liu X, et al. New aromatic substituted pyrazoles as selective inhibitors of human adipocyte fatty acid-binding protein. *Bioorg Med Chem Lett*. 2011; 21:2949–2952. [PubMed: 21481589]
164. Chen J, Wang J, Zhu W. Binding modes of three inhibitors 8CA, F8A and I4A to A-FABP studied based on molecular dynamics simulation. *PLoS ONE*. 2014; 9:e99862. [PubMed: 24918907]
165. Xu Q, et al. Design, synthesis and biological evaluation of thiazole- and indole-based derivatives for the treatment of type II diabetes. *Eur J Med Chem*. 2012; 52:70–81. [PubMed: 22483089]
166. Wang Y, et al. Discovery of FDA-approved drugs as inhibitors of fatty acid binding protein 4 using molecular docking screening. *J Chem Inf Model*. 2014; 54:3046–3050. [PubMed: 25360897]
167. Cai HY, et al. Benzbromarone, an old uricosuric drug, inhibits human fatty acid binding protein 4 *in vitro* and lowers the blood glucose level in *db/db* mice. *Acta Pharmacol Sin*. 2013; 34:1397–1402. [PubMed: 24077632]
168. Aouadi M, et al. Gene silencing in adipose tissue macrophages regulates whole-body metabolism in obese mice. *Proc Natl Acad Sci USA*. 2013; 110:8278–8283. [PubMed: 23630254]
169. Aouadi M, et al. Orally delivered siRNA targeting macrophage Map4k4 suppresses systemic inflammation. *Nature*. 2009; 458:1180–1184. [PubMed: 19407801]
170. Won YW, et al. Oligopeptide complex for targeted non-viral gene delivery to adipocytes. *Nat Mater*. 2014; 13:1157–1164. [PubMed: 25282508]
171. Miao X, et al. The mAb against adipocyte fatty acid-binding protein 2E4 attenuates the inflammation in the mouse model of high-fat diet-induced obesity via toll-like receptor 4 pathway. *Mol Cell Endocrinol*. 2015; 403:1–9. [PubMed: 25596549]
172. Herroon MK, et al. Bone marrow adipocytes promote tumor growth in bone via FABP4-dependent mechanisms. *Oncotarget*. 2013; 4:2108–2123. [PubMed: 24240026]
173. Yore MM, et al. Discovery of a class of endogenous mammalian lipids with anti-diabetic and anti-inflammatory effects. *Cell*. 2014; 159:318–332. [PubMed: 25303528]
174. Maceyka M, Spiegel S. Sphingolipid metabolites in inflammatory disease. *Nature*. 2014; 510:58–67. [PubMed: 24899305]
175. NCBI. Fabp4 [Mus musculus] GenBank: CAJ18597.1. 2005. [online], <http://www.ncbi.nlm.nih.gov/protein/71060107>

Key points

- Fatty acid-binding proteins (FABPs) are versatile proteins that can modulate lipid fluxes, trafficking, signalling and metabolism
- Fatty acid-binding protein, adipocyte (FABP4) regulates metabolic and inflammatory pathways, and in mouse models its inhibition can improve type 2 diabetes mellitus and atherosclerosis
- FABP4 is actively secreted by adipocytes and its levels are increased in obesity; in humans, elevated circulating FABP4 levels are associated with obesity, metabolic disease and cardiac dysfunction
- Circulating FABP4 is secreted through a vesicular pathway and has pleiotropic roles that include the stimulation of hepatic glucose production
- Targeting FABP4 offers a novel therapeutic approach for the treatment of many metabolic diseases
- The signalling components of hormonal FABP4 and determinants of FABP-mediated functions in the context of specific lipid or other cargo are issues that must be addressed in future research

Figure 1.

Ribbon and domain structure of FABP4. The structure of FABP4 is depicted as a ribbon with bound oleic acid in space-filling spheres (carboxylate oxygen atoms in red). Also shown are four domains of FABP4: portal region for ligand entry and exit; charge quartet used for protein–protein interactions (Asp17, Asp18, Lys21 and Arg30); the salt bridge where the fatty acid carboxylate forms ion pairs with basic residues within the cavity (Arg106, Arg126 and Tyr128); the hinge where the helix–turn–helix region rotates to enable access of ligands to the cavity (Glu14, Asn15 and Phe16). Amino acid numbering system is based on the mouse Fabp4 protein (Genbank: CAJ18597.1).¹⁷⁵

Figure 2.

Summary of FABP4 intracellular functions. In studies using cultured cells and mouse models, FABP4 carries out a number of intracellular roles. FABP4 suppresses adipose tissue lipogenesis and promotes lipolysis, with direct effects on the composition of the local and circulating free fatty acid pool. FABP4 is also implicated in modulating eicosanoid balance by affecting both COX2 activity and LTA₄ stability, and upregulates UCP2; all these processes influence macrophage function and adipose tissue inflammation. FABPs might mediate PPAR activity by regulating ligand availability, and genetic models suggest that FABP4 opposes PPAR γ action. FABP4 can also interact with HSL, JAK2 and CD36. Consequently, FABP4 acts on multiple integrated pathways to regulate lipid metabolism and inflammation, impair insulin action, promote glucose production, and contribute to the pathogenesis of immunometabolic diseases such as diabetes mellitus and atherosclerosis. Abbreviations: COX2, cyclooxygenase 2; ER, endoplasmic reticulum; FABP4, fatty acid binding protein 4; HSL, hormone-sensitive lipase; JAK2, janus kinase 2; JNK, c-jun N-terminal kinase; LTA₄, leukotriene A₄; LXR, liver X receptor; PGE₂, prostaglandin E₂; PPAR, peroxisome proliferator-activated receptor; STAT3, signal transducer and activator of transcription 3; UCP2, uncoupling protein 2.

Figure 3.

The association of circulating levels of FABP4 with different human diseases. A summary of studies in humans showing the association of different immunometabolic diseases with circulating levels of FABP4 organized by number of patients. Numbers in the outer circle represent the number of studies in each category. For full list of references included in this figure please see reference list in Supplementary file 1 online. Abbreviations: FABP4, fatty acid binding protein 4; NAFLD, nonalcoholic fatty-liver disease.

Figure 4.

Pleiotropic functions of circulating FABP4. FABP4 secretion from adipose tissue is enhanced in the setting of obesity and insulin resistance. The circulating form of FABP4 can function as a hormone and regulate hepatic glucose production. This form of the protein, and lipids regulated by FABP4 such as palmitoleate, also affect insulin action, hepatic lipid metabolism, and cardiac function. Other potential roles of circulating FABP4 include regulation of tumour growth, immune cell functions, endothelial migration, cardiomyocyte contraction and insulin secretion. Abbreviations: FABP4, fatty acid binding protein 4; FFA, free fatty acid. Modified with permission from Elsevier © Cao, H. *et al.* Adipocyte lipid chaperone aP2 is a secreted adipokine regulating hepatic glucose production. *Cell Metab.* **17**, 768–778 (2013).

Figure 5.

Regulated vesicular secretion of FABP4. The secretion of FABP4 from adipocytes has been demonstrated *in vivo* in response to fasting and signals that induce lipolysis such as β -AR stimulation. Furthermore, the actions of lipolytic enzymes ATGL and HSL (but not MAGL) are required for the induction of FABP4 secretion, and an influx in FFAs is also sufficient for increased secretion. Following lipolytic stimulation, FABP4 is recruited to multivesicular bodies, and secreted in vesicles that have exosome markers including CD36, calveolin, lactadherin (also known as milk fat globule factor 8 protein), TSG101, and ALIX, as shown on the right. The secretion of FABP4 has also been shown to be responsive to Ca^{2+} signalling, and to be inhibited by insulin. Whether alternative paths exist that support FABP4 secretion is currently unknown. Visualization of multivesicular bodies containing labelled FABP4 (arrows) before and after β -AR stimulation are shown in insets.

Abbreviations: β -AR, β -adrenergic receptor; ALIX, programmed cell death 6 interacting protein; ATGL, adipose triglyceride lipase; DAG, diacyl glycerol; FFA, free fatty acid; HSL, hormone-sensitive lipase; MAG, monoacylglycerol; MAGL, monoacylglycerol lipase; MVB, multivesicular body; PKA, protein kinase A; TSG101, tumour susceptibility gene 101 protein.