

# Cytoplasmic fatty acid binding protein sensing fatty acids for peroxisome proliferator activated receptor activation

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**Abstract.** Translation of nutrient stimuli through intracellular signaling is important for adaptation and regulation of metabolic processes, while deregulation by either genetic or environmental factors predisposes towards the development of metabolic disorders. Besides providing energy, fatty acids act as prominent signaling molecules by altering cell membrane structures, affecting the lipid modification status of proteins, and by modulating ligand-activated nuclear receptor activity. Given their highly hydrophobic nature, fatty acids in the aqueous intracellular compartment are bound to small intracellular lipid binding

proteins which function as intracellular carriers of these hydrophobic components. This review describes recent advances in identifying intracellular pathways for cytosolic fatty acid signaling through ligand-activated receptors by means of small intracellular lipid binding proteins. The mechanism behind intracellular fatty acid transport and subsequent nuclear receptor activation is an emerging concept, and advances in understanding this process provide new potential therapeutic targets towards the treatment of metabolic disorders.

**Keywords.** Fatty acid, PPAR (peroxisome proliferation-activated receptor), fatty acid binding protein, lipid binding protein.

## Introduction

The dramatic increase in obesity and diabetes in westernized countries over the last years has led to a change in dietary awareness, which in turn led to the development of fat-free foods that contain little or no fat. While the reduction of saturated and trans-fatty acids is extremely beneficial for human health from a nutritional point of view, several types of mono- and polyunsaturated fatty acids are an important, even essential part of a balanced diet. Two prominent members of this group of fatty acids are linoleic acid and  $\alpha$ -linolenic acid, which cannot be endogenously synthesized and are important building blocks for a wide variety of complex lipids and hormones [1–3]. These fatty acids as well as their derivatives have therefore been termed essential fatty acids (EFAs). The importance of EFAs was demonstrated in several epidemiological studies, which showed that clinical

symptoms of EFA deficiency include changes in brain and body weight, increased susceptibility to infections and degenerative changes in kidneys, lungs and liver [2–6]. On the other hand epidemiological evidence supports the notion that high intake of EFAs protects against coronary heart disease and diabetes [7, 8]. EFA deficiency has been recognized and described as prevalent in non-westernized countries, and often is associated with protein-energy malnutrition. However, some reports speculate that a high number of people suffer from EFA deficiency in the United States and other westernized countries [9]. The reasons for this deficiency are attributed either to complete absence of fat in the diet or a diet containing solely saturated fats, proteins and carbohydrates. How do EFAs in general and polyunsaturated fatty acids in particular affect cellular metabolism to such an extent that they are implicated in a wide variety of diseases? One reason is that these fatty acids as well as

their derivatives are precursors for complex lipids and endogenous signaling factors. Second, they are also potent regulators of gene transcription through activation of ligand-induced nuclear receptors. Thus, these fatty acids serve as important signal transduction molecules that induce pathways involved in fatty acid trafficking, metabolism and conversion of lipids, thereby regulating whole-body metabolism.

### The family of peroxisome proliferators-activated receptors

Peroxisome proliferators-activated receptors (PPARs) were first identified by Issemann and Green in 1990, when they reported the cloning and characterization of ligand-activated nuclear transcription factors [10]. The name of this family of transcription factors was derived from the fact that PPARs are activated by peroxisome proliferators (PPs), a class of xenobiotics, including hypolipidemic drugs like fibrates, which induce proliferation of peroxisomes in hepatocytes [11–14]. It very quickly became evident that not only are PPARs activated by xenobiotics, but also that fatty acids are potent activators of these transcription factors, thus establishing the role of PPARs as fatty acid sensors [15–19]. To date, three PPAR subtypes have been described, namely PPAR $\alpha$ , PPAR $\beta$  (also designated PPAR $\delta$ , NUC1 or FAAR) and PPAR $\gamma$ . All three factors show a distinct tissue distribution pattern. PPAR $\alpha$  is highly expressed in liver, kidney, testis and skeletal muscle, while PPAR $\gamma$  is mainly expressed in adipose tissue and to a lesser extent in the large intestine and spleen. PPAR $\beta$  in contrast to its family members is expressed ubiquitously [18, 19]. The members of the PPAR family share a domain structure which is commonly found in ligand-activated hormone receptors, constituting of a N-terminal A/B domain containing a ligand-independent transactivation function (AF-1), a C domain functioning as a DNA binding domain (DBD), a hinge D domain connecting the E/F domain, which contains the ligand binding domain (LBD), and a ligand-dependent transactivation function (AF-2) [20, 21]. All three subtypes have in common the ability to heterodimerize with retinoid X receptors (RXRs) to activate gene transcription. The PPAR/RXR heterodimer interacts in target genes with PPAR response elements (PPREs). Consensus PPREs are direct repeats (DRs) of AGGTCA separated by one nucleotide (DR1), with a 5' extension of AACT which allows for additional specificity [22, 23].

Interestingly, PPAR subtypes vary not only with respect to tissue expression but also in their ability to bind and be activated by ligands. The LBDs of the

three PPAR subtypes have a divergent amino acid sequence, thus allowing a broad specificity of ligands [24]. As mentioned above, in addition to xenobiotics PPARs can bind natural occurring fatty acids and fatty acid derivatives. Natural ligands of PPAR $\alpha$ ,  $\beta$  and  $\gamma$  are a variety of long-chain fatty acids and, in particular, polyunsaturated fatty acids, such as docosahexaenoic acid, EPA, linoleic acid, linolenic acid and arachidonic acid, with varying degrees of affinity depending on the method of analysis (Table 1). Fatty acid metabolites that have been shown to activate PPARs are the oxidized metabolites of linoleic acid like 9-hydroxy-octadecadienoic acid (9-HODE) and 13-HODE, which bind and activate both PPAR $\alpha$  and PPAR $\gamma$  [25, 26]. Furthermore, the prostaglandin 15-deoxy- $\Delta$ 12,14 PGJ2 was identified as a specific ligand of PPAR $\gamma$  [27, 28].

PPARs have been shown to be important regulators of whole-body glucose to lipid metabolism. While the complete description of target genes and physiological functions is beyond the scope of this review, mouse models with targeted deletions of the three PPAR subtypes should be mentioned here, as they can be used to describe the major physiological functions of PPARs. The generation of PPAR $\alpha$ -deficient mice confirmed the role of PPAR $\alpha$  in peroxisome biogenesis and fatty acid  $\beta$ -oxidation in the liver [29–32]. PPAR $\alpha$ -deficient mice fail to exhibit peroxisome proliferation or activation of fatty acid oxidation target genes when exposed to PPAR $\alpha$  agonists. Furthermore, these mice accumulate increased hepatic triglycerides in response to feeding. During fasting these mice develop severe hypoglycemia due to the fact that they cannot derive energy from fatty acids due to impaired  $\beta$ -oxidation [29, 30, 32].

In contrast to PPAR $\alpha$ , PPAR $\gamma$ -deficient mice die during intra-uterine development owing to defects in the placenta, thus limiting research to the study of heterozygous PPAR $\gamma$  mice and tissue-specific deletions of this receptor. Interestingly, even though PPAR $\gamma$  was shown in cell culture to be a key regulator of adipogenesis, heterozygous mice on a normal commercial diet showed no effect on adipose tissue accretion. Rather, PPAR $\gamma$  heterozygous mice displayed a higher insulin-stimulated glucose disposal rate as well as reduced levels of plasma insulin during glucose tolerance tests and enhanced insulin-mediated suppression of hepatic glucose production in comparison to wild-type mice [33]. When challenged by a high fat diet, PPAR $\gamma$  heterozygous mice exhibit less adipose tissue generation, concomitant with increased insulin sensitivity in comparison to their wild-type counterparts [33]. Targeted deletion of PPAR $\gamma$  in adipose tissue resulted in marked adipocyte hypocellularity and hypertrophy, elevated levels of

**Table 1.** Ligand binding of natural and synthetic compounds to different PPAR subtypes. The table is a compilation of binding data [27, 28, 47, 79, 83–91].

n.b., no binding; n.d. not measured; + - +++ indicates increasing binding affinity.

Ligand	PPAR $\alpha$	PPAR $\beta$	PPAR $\gamma$
Palmitic acid (16:0)	n.b. - +	+	+
Stearic acid (18:0)	n.b - +	+	+
Palmitoleic acid (16:1)	+	n.d.	n.d.
Oleic acid (18:1)	++ - +++	+	+
Linoleic acid (18:2)	+++	+ - ++	++
Linolenic acid (18:3)	+++	+ - ++	++
AHA (20:4)	+ - ++	+	+
DHA (20:4)	+ - ++	n.b - +	++
Phytanic acid	+++	n.b.	n.b.
15d- $\Delta^{12,14}$ -PGJ <sub>2</sub>	n.b.	n.b.	+++
Wy14,643	+++	n.b.	n.b. - +
ETYA	+++	+++	+
Bezafibrate	+ - ++	++ - +++	n.b.
LtB4	++	n.b.	n.b.
Troglitazon	n.b.	+	++ - +++

plasma free fatty acids and triglyceride, and decreased levels of plasma leptin [34]. In addition, these mice have increased hepatic gluconeogenesis and insulin resistance. Despite these defects, blood glucose, glucose and insulin tolerance, and insulin-stimulated skeletal muscle glucose uptake were all comparable to those of control mice. However, targeted mice were significantly more susceptible to high-fat diet-induced steatosis, hyperinsulinemia and insulin resistance [34]. In contrast to the well-established roles of PPAR $\gamma$  and PPAR $\alpha$  in lipid metabolism, less is known of PPAR $\beta$  in regulation of lipid and glucose metabolism. It was recently demonstrated that targeted activation of PPAR $\beta$  in adipose tissue induces expression of genes required for fatty acid oxidation and energy dissipation, which in turn leads to improved lipid profiles and reduced adiposity. Mice overexpressing PPAR $\beta$  in adipose tissue are completely resistant to both high-fat diet-induced and genetically predisposed obesity. In accordance, PPAR $\beta$ -deficient mice challenged with a high-fat diet show reduced energy uncoupling and are prone to obesity [35]. Activation of PPAR $\beta$  in adipocytes and skeletal muscle cells promotes fatty acid oxidation and utilization, suggesting that PPAR $\beta$  has a role more similar to PPAR $\alpha$  by regulating lipid catabolism rather than PPAR $\gamma$ , which affects adipocyte differentiation and lipid storage [36, 37].

Binding and activation of PPARs through hydrophilic xenobiotic compounds is easily explained and understood; however, binding and activation of PPARs through naturally occurring fatty acids poses a problem, as fatty acids, which are by nature hydrophobic

molecules, are not readily available in their free form because of their insolubility in aqueous cellular compartments. It became evident in recent years that a transport system for these insoluble compounds exists to provide receptors with ligands for target gene activation. This mechanism is represented by a class of small lipid binding proteins (iLBPs) that are found intracellularly in a wide variety of tissues and have been shown to bind and transport fatty acids.

### Fatty acid binding proteins

Members of this family are the soluble 14–15-kDa iLBPs, which bind type-specifically retinoids, fatty acids and bile acids in a non-covalent manner. To date, 14 different cytoplasmic subtypes have been identified. The group of iLBPs contains the family of fatty acid binding proteins (FABPs), which so far encompasses 8 members [38–40]. Expression of FABPs occurs in a regulated and tissue-specific manner, and their putative functions include shuttling of fatty acids to specific enzymes and cellular compartments, modulation of intracellular lipid metabolism and regulation of gene expression [41–43]. A growing body of experimental evidence has defined the roles of these proteins in mammalian physiology.

Liver FABP (L-FABP) is abundantly found in hepatocytes but is also expressed in other tissues, including the stomach, pancreas, intestine and kidney [44]. The L-FABP gene promoter contains a PPRE, and it was shown that L-FABP is a direct target of PPAR $\alpha$

**Table 2.** Ligand binding of natural and synthetic compounds to different FABP subtypes. The table is a compilation of binding data [47, 52, 53, 84, 92–97].

n.b., no binding; n.d. not measured; + – +++ indicates increasing binding affinity.

Ligand	L-FABP	L-FABP	A-FABP	E-FABP	H-FABP
Palmitic acid (16:0)	+++	+	++	++	+++
Stearic acid (18:0)	+ – +++	+	++	++	+++
Palmitoleic acid (16:1)	++	+	++	++	+++
Oleic acid (18:1)	++ – +++	+	++	++	+++
Linoleic acid (18:2)	++	+	++	++	++
Linolenic acid (18:3)	+ – ++	+	+	++	++
AHA (20:4)	++	+	+ – ++	++	++
DHA (20:4)	++ – +++	+	++	++	+
Phytanic acid	+++	+	n.d.	n.d.	n.d.
15d- $\Delta^{12,14}$ -PGJ <sub>2</sub>	+	n.d.	n.b.	n.d.	n.d.
Wy14,643	++	n.b. – +	n.b.	n.d.	n.d.
ETYA	++	++	n.d.	n.d.	n.d.
Bezafibrate	++	+	n.d.	n.d.	n.d.
LtB4	n.b. – +	n.d.	n.d.	n.d.	n.d.
Troglitazon	+	n.b. – +	++	+	n.d.

[45–49]. Furthermore, messenger RNA levels are increased by fatty acids, dicarboxylic acids and retinoic acid as well [50]. Unlike the other members of the FABP family, L-FABP is able to bind two ligands simultaneously [51–53]. Peroxisome proliferators in general bind L-FABP with low affinity, whereas the fatty acids are bound with varying affinity, depending on length and degree of saturation (Table 2). The main known molecular function of L-FABP is the reversible binding of hydrophobic ligands, which correlates with fatty acid metabolism *in vivo*. In line with this, several changes in fatty acid metabolism in correlation with intracellular L-FABP concentration have been reported *in vitro*, including regulation of fatty acid uptake and  $\beta$ -oxidation [54–57].

The adipocyte FABP (A-FABP, also known as ap2 and ALBP) was first identified in adipose tissue, where it is an abundant cytoplasmic protein [58, 59]. A-FABP has been shown to bind both saturated and unsaturated fatty acids with similar affinities. In addition to fatty acids, it was shown to bind troglitazone, a potent antidiabetic PPAR $\gamma$  activator. No binding was observed for other PPAR $\gamma$  ligands such as prostaglandins (Table 2). Expression of A-FABP is highly regulated during differentiation of adipocytes, and its messenger RNA is transcriptionally controlled by fatty acids [60]. Recent studies in A-FABP-deficient mice have shown that A-FABP impacts several aspects of the metabolic syndrome. First, targeted disruption of A-FABP in mice reduces the hyperinsulinemia and insulin resistance associated with dietary or genetic obesity [61, 62]. Second, A-FABP contributes to improved sys-

temic glucose and lipid metabolism in the setting of dietary or genetic obesity and influences the rate of adipocyte lipolysis [61, 62]. Recent studies have demonstrated that A-FABP plays an important role in macrophage metabolism and that it protects macrophages from the excess uptake of oxidized LDL thereby inhibiting foam cell formation [63, 64].

Heart FABP (H-FABP, also known as M-FABP) has been isolated from a wide range of tissues, including cardiac muscle, skeletal muscle, renal cortex, testis and brain [65]. Levels of H-FABP are influenced by testosterone, exercise and circadian rhythms. The important role of heart FABP in regulation of  $\beta$ -oxidation in cardiac muscle was discovered with the creation of mice deficient in heart FABP, which exhibits a severe defect in cardiac myocyte long-chain fatty acid cellular transport and  $\beta$ -oxidation [66]. H-FABP has been shown to bind various fatty acids, although some reports indicate a preference for unsaturated fatty acids. No other ligands have been identified for H-FABP (Table 2).

Epidermal type FABP (E-FABP, also known as keratinocyte FABP) is expressed ubiquitously in various tissues [40]. Similarly to A-FABP, E-FABP binds a variety of fatty acids and synthetic compounds with similar affinities (Table 2). The generation of E-FABP-deficient mice demonstrated that E-FABP is responsible for the water permeability barrier of the skin [67]. This mild phenotype, restricted to the skin, could be explained by the fact that E-FABP is seldom the only FABP subtype expressed in a certain tissue. Also, in the liver of E-FABP-deficient mice H-FABP

is upregulated, which might compensate for the loss of E-FABP. Interestingly, E-FABP itself is dramatically increased in A-FABP-deficient adipocytes [61, 62], indicating that these proteins are part of a highly redundant compensatory mechanism.

### **FABPs and PPARs: a signaling pathway for fatty acids**

Since fatty acids have been shown to be regulators of nuclear receptor activity, it was hypothesized that due to the lipophilic nature of these compounds an intracellular transport system would need to exist to shuttle fatty acids from the cytoplasmic membrane to the nucleus for targeted receptor activation. Dong et al. described such a mechanism by providing experimental evidence that Crebp facilitates the transport of 9-cis retinoic acid to the transcription factor RXR for activation of the latter receptor [68]. These findings have been extended in recent years to encompass regulation of PPAR activity by fatty acids through cytosolic FABPs, which show ligand binding affinity similar to their receptors.

The first study demonstrating a direct link between FABP expression and PPAR activation was performed by Hertzler and Bernlohr [60]. By coexpressing PPAR $\gamma$  and A-FABP in CV-1 cells, they demonstrated enhanced activation of PPAR $\gamma$ , as measured in a reporter gene assay. This increase in PPAR $\gamma$  activation showed a significant positive correlation to A-FABP expression. From these findings the authors suggested that A-FABP functions as a positive factor in fatty acid signaling by directly targeting and delivering fatty acids and/or metabolites to PPAR to activate the lipid signal transduction pathway. Several further studies on the regulation of PPAR activity through FABPs were published in the following years analyzing different pairs of FABPs/PPARs based on their relative tissue distribution.

The L-FABP type family member was reported to regulate activity of different PPAR subtypes in hepatocytes. It was shown that L-FABP and PPAR $\alpha$  as well as PPAR $\gamma$  colocalize to the nucleus of hepatocytes and that both proteins interact as demonstrated by direct immunoprecipitation and mammalian two-hybrid assays [69]. These studies, linking activation of the nuclear receptor and FABP concentration, were performed in a series of HepG2 cell clones, which expressed L-FABP at various levels, generated by stable antisense L-FABP transfection [57]. PPAR activation was quantified using a reporter gene approach, and the relationship between intracellular FABP concentration and PPAR $\alpha$  and PPAR $\gamma$  activation, respectively, was determined in the pres-

ence of various fatty acids and hypolipidemic drugs which had been shown to be activators of either receptor. The correlation of transactivation versus L-FABP concentration revealed a positive linear correlation for all fatty acids and hypolipidemic drugs tested. Similar to PPAR $\alpha$  a linear correlation was found between intracellular L-FABP concentration and PPAR $\gamma$  activation. The observed protein-protein interaction of L-FABP and PPAR $\alpha/\gamma$  was analyzed for its dependency on the ligand status of L-FABP, and it was shown that the addition of either linoleic acid or Wy14,643 did not affect the interaction of L-FABP and PPAR $\alpha$ . Thus it was suggested that L-FABP in the hepatocyte functions as a transporter of fatty acids and xenobiotics but that interaction of the two proteins is independent of a bound ligand.

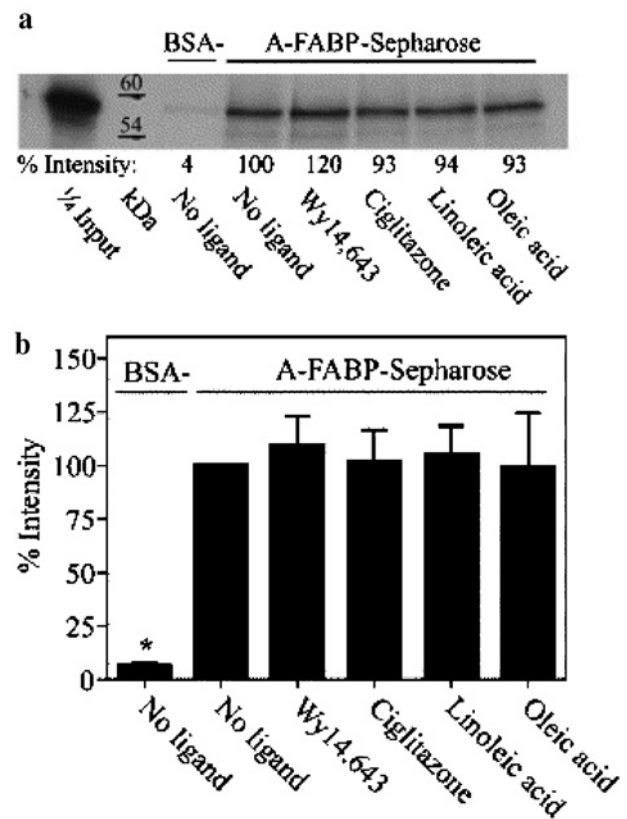
In addition to the positive correlation between PPAR activation and FABPs, in 2000 a study was published that put forth the hypothesis that PPAR activation and FABPs might be inversely correlated under certain condition [70]. The authors studied tetradecylthioacetic acid (TTA)-stimulated activation of all three PPAR subtypes in response to A-FABP and E-FABP overexpression in CV-1 cells. Under all conditions the authors found an inverse relationship between PPAR activation (all three subtypes) and intracellular FABP concentration, which contrasted with the published data so far. Interestingly, when BRL49653 was used as an activator of PPAR $\gamma$ , A-FABP had no significant effect on ligand-dependent activation, whereas co-transfection with E-FABP resulted in a decrease in transactivation potential similar to the decrease observed for TTA-induced transactivation. In accordance with other studies they found nuclear expression of both A-FABP and E-FABP in their experiment. The divergent findings between this and other studies are interesting because of the possibility that different FABPs regulate PPAR activity in a different manner. Since all studies that have been published so far showing a direct correlation between FABP and PPAR activity have been performed in cell culture systems, it is possible that in some tissues FABPs might function as negative regulators of PPAR activity. One caveat when comparing these studies to others, however, is the ligand that was used in these experiments. Although TTA is a true ligand of all the PPAR subtypes, TTA is unable to undergo  $\beta$ -oxidation thus leading to a unphysiological buildup of the free TTA as well as its CoA (coenzyme A) ester over the course of the 2-day incubation. This in turn might lead to nonspecific effects, such as the repression of nuclear receptor activity, which could explain the divergent findings.

A more recent study on FABP-PPAR interaction was published by Tan et al. in 2002. The authors showed

that A-, H- and E-FABP selectively enhance the activities of different PPAR subtypes [71]. Similar to the above-mentioned experiments, the activity of PPAR was measured by cotransfecting a PPAR, responsive reporter gene together with an expression vector for either FABP or PPAR subtype before treating the cells with various ligands. H-FABP augmented the transcriptional activity of PPAR $\alpha$  under all conditions. A-FABP markedly enhanced the transcriptional activity of PPAR $\gamma$  both in the absence and in the presence of an exogenous ligand, while the effect of E-FABP on PPAR $\gamma$  expression was not significant. On the other hand, E-FABP markedly activated PPAR $\beta$  in a dose-dependent manner, while A-FABP had no effect on the activity of this receptor. Furthermore, the authors showed that E-FABP or A-FABP fused with GFP upon addition of the PPAR $\gamma$  ligand rosiglitazone resulted in a dramatic redistribution of A-FABP into the nucleus, while E-FABP relocated to the nucleus upon addition of the synthetic PPAR $\beta$  ligand L165041. In coprecipitation FABP experiments the authors could show a weak association between PPAR $\gamma$  and GST-tagged or histidine-tagged A-FABP in the absence of ligand, but the interaction was significantly stabilized in the presence of the PPAR $\gamma$  ligand troglitazone, while A-FABP did not form an observable complex with PPAR $\beta$  either in the presence or absence of ligand. Conversely, E-FABP associated with PPAR $\beta$  in the presence of the PPAR $\beta$  ligand L165041, while it did not associate with PPAR $\gamma$  in the absence of ligand or in the presence of either troglitazone.

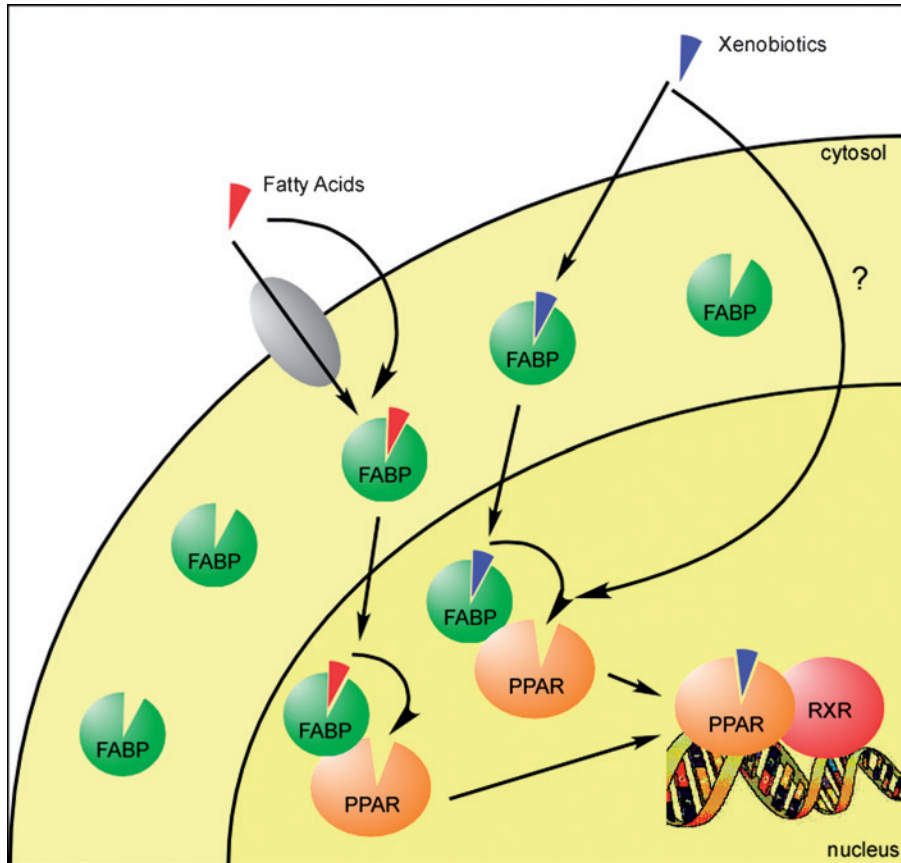
The most recent study was published in 2006 by Adida and Spener, in which the authors analyzed the regulation of PPAR $\gamma$  activity by A-FABP with special emphasis on the contribution of ligand binding to the FABP-PPAR interaction [72]. The authors demonstrated that A-FABP interacted with both the PPAR $\gamma^1$  and  $\gamma^2$  isoform but not with either PPAR $\alpha$  or PPAR $\beta$ . No difference was observed for the binding of A-FABP to either PPAR $\gamma^1$  or PPAR $\gamma^2$ . In addition to the interaction of A-FABP they demonstrated that B-FABP interacted with PPAR $\gamma^1$  and PPAR $\gamma^2$ , respectively, which is the first report for the interaction of this FABP subtype with any nuclear receptor. As shown previously by Helledie et al. [70] and Tan et al. [71], the authors demonstrated that upon ligand binding A-FABP translocates to the nucleus after a 4-h treatment with linoleic acid. Furthermore, the authors demonstrated that this nuclear accumulation is independent of putative A-FABP phosphorylation at position Tyr19 but is directly due to ligand binding of A-FABP. This was shown by employing a fatty binding-deficient mutant of A-FABP (Arg126) [73]. The mutant fatty acid binding-deficient A-FABP

shows localization similar to wildtype A-FABP in the absence of exogenous fatty acids but fails to translocate to the nucleus in the presence of exogenous fatty acids, thus demonstrating that ligand binding of A-FABP is a requirement for nuclear redistribution of A-FABP. In contrast to the results reported by Tan et al. [71], the authors of this study did not find a ligand dependency of FABP-PPAR interaction. This was shown by using delipidated A-FABP bound to sepharose, which interacted with PPAR $\gamma$  in the same intensity independent of the presence of exogenous ligand (Fig. 1a and b).



**Figure 1.** Effects of ligands on A-FABP/PPAR $\gamma$ -DEF interaction. (a) [ $^{35}\text{S}$ ]-PPAR (Input) was precipitated with A-FABP. Bound PPAR was eluted with  $2 \times$  SDS loading buffer after 3 washes with HEDK-100 buffer. After separation of proteins by SDS-polyacrylamide gel electrophoresis (13.5%), radioactively labeled proteins were visualized by autoradiography. Representative example from 2 (PPAR $\alpha,\beta$ ) to 3 independent experiments (PPAR $\gamma_{1,2}$ ); (b) ensitometric analysis of 3 independent experiments. The intensity of the band corresponding to A-FABP-Sepharose with no ligand was set to 100%. \*Significant difference in comparison to A-FABP-Sepharose in the absence of ligand by paired *t*-test ( $P < 0.05$ ). Figure taken from Adida et al. [72].

In summary, from the above-mentioned studies that directly address the functional relationship between FABPs and PPARs, it is evident that FABPs affect the



**Figure 2.** FABPs function as cytoplasmic shuttle proteins for ligand activation of PPARs.

activity of PPARs by regulating ligand availability (Fig. 2). Discrepancies between the observed positive correlation of FABP concentration and PPAR activity in three studies and the negative regulation of PPAR activity by FABPs in one study could either be due to tissue-specific or ligand-specific differences for FABP-regulated PPAR activity. An important question that remains unanswered at the moment is the ligand dependency of the interaction between PPARs and FABPs. It was shown that the interaction between L-FABP and PPAR $\alpha$  is ligand-independent; however, two conflicting studies exist with respect to A-FABP and its interaction with PPAR $\gamma$  [71, 72]. By using a fatty acid binding-deficient mutant of A-FABP, Adida and Spener demonstrated that ligand binding of A-FABP is in part responsible for translocation of the protein; however, ligand-independent mechanisms for nuclear translocation exist as well. In contrast to the report published by Tan et al. [71] the authors could not observe any ligand dependency of interaction between A-FABP and PPAR $\gamma$  in the presence or absence of ligand. These differences might be due to the fact that in the study by Tan et al. coprecipitation experiments were carried out with GST-tagged proteins, while the studies by Adida et al. were carried out by unmodified A-FABP. The use of the binding-

deficient control in the latter experiment strongly suggests that interaction of PPAR $\gamma$  and A-FABP is ligand-independent. Thus at present the level of controlling fatty acid flux to the nuclear receptor is achieved by regulating the nuclear import of FABP by so far unknown mechanisms.

In addition to the above-mentioned studies that directly show a dependency of PPAR activation on FABP, further insights into the regulation of PPAR activity through ligand transport by fatty acid binding proteins can be gained by comparison of genetic models of PPAR and FABP ablation, respectively. In the case of L-FABP it has been shown that the single knockout of L-FABP does not affect PPAR $\alpha$  activity in the hepatocyte [74, 75], thus contrasting with the observed data in cell culture. As mentioned above, however, FABPs are part of a redundant system, and E-FABP, which is also expressed in the hepatocyte, might compensate for the loss of L-FABP as E-FABP has been shown to regulate PPAR activity [71]. Furthermore, it will be necessary to study the effect of ligand induced stimulation of PPAR $\alpha$  above the steady-state level as mice chow in general contains low amounts of fatty acids. It is possible that challenging mice with potent PPAR $\alpha$  activators like polyunsaturated fatty acids or hypolipidemic xenobiotics might

result in differences in the levels of PPAR $\alpha$  activation. With respect to interaction of A-FABP/PPAR $\gamma$  and E-FABP/PPAR $\beta$ , a clear-cut relationship seems to exist, in the sense that either FABP can only activate one receptor subtype. Interestingly, PPAR $\gamma$  heterozygous animals are resistant to obesity when challenged by a high-fat diet [33], while A-FABP knockout animals develop dietary obesity. But, unlike control mice, they do not develop insulin resistance or diabetes [61, 62]. However, a caveat exists as E-FABP is upregulated about 20-fold in these animals [62]. Furthermore, adipocytes isolated from E-FABP-deficient mice exhibit enhanced insulin-stimulated glucose transport capacity [76], while mice expressing high levels of E-FABP in adipose tissue display reduced systemic insulin sensitivity [76, 77]. These findings are highly suggestive of the fact that E-FABP plays an important role in controlling adipocyte metabolism and might adequately compensate for the loss of A-FABP. Mice lacking both E-FABP and A-FABP exhibited a striking phenotype with strong protection from diet-induced obesity, insulin resistance, type 2 diabetes and fatty liver disease [78]. These mice have altered cellular and systemic lipid transport and composition, leading to enhanced insulin receptor signaling, enhanced muscle AMP-activated kinase activity and dramatically reduced liver stearoyl-CoA desaturase-1 activity underlying their phenotype [78]. This suggests that E-FABP at a higher rate of expression may compensate for A-FABP-dependant activation of PPAR $\gamma$ . On the other hand, it might be possible that induction of E-FABP expression in accordance with the pair specificity reported by Tan et al. [71] leads to the induction of PPAR $\beta$ , resulting in the observed phenotype. This hypothesis is in contrast to recent findings that suggest that activation of PPAR $\beta$  rather than its deletion leads to expression of genes required for fatty acid oxidation and energy dissipation, which in turn leads to improved lipid profiles and reduced adiposity [35, 37].

Interestingly, a recent study using A-FABP-deficient macrophages demonstrated that lack of A-FABP coincided with defects in cholesterol accumulation and alterations in pro-inflammatory responsiveness [63]. Furthermore, these macrophages exhibited altered lipid composition and enhanced PPAR $\gamma$  activity. The authors conclude that in normal macrophages A-FABP would retain PPAR $\gamma$ -activating fatty acids in the cytosol, thereby repressing its activity. This is in contrast to the reported *in vitro* data, which could be explained either by the fact that the mechanism for the regulation of PPAR activity is tissue-specific. On the other hand it could be possible that similar to what is observed in adipocytes another FABP subtype, such as E-FABP, is upregulated in these macrophages,

leading to the observed phenotype. A study by Boord et al. seems to support this notion, as they demonstrated that ApoE-deficient mice with genetic ablation for both A-FABP and E-FABP were protected from atherosclerosis development [78]. Thus, further studies in macrophages using combined A-FABP/E-FABP-deficient animals will be necessary to assess these questions. In conclusion it must be noted that comparison of the genetic models might not provide good evidence for the activation of PPARs through FABP because several pathways might be regulated in a compensatory manner, and thus the effect of this one pathway on the whole body phenotype might be difficult to dissect.

FABPs, as have been shown by independent studies, seem to act as regulators of PPAR activities by providing ligands for nuclear receptor activation. Besides physical interaction of different pairs of FABPs/PPARs the complexity of ligand binding and transactivation of both PPARs and FABPs, is an important regulation point for this signal transduction pathway. First, all binding studies analyzing FABP-ligand interaction have demonstrated that the binding of fatty acids by FABPs is quite strong, and it remains to be elucidated whether it would be possible for PPARs to displace fatty acids bound to FABPs. L-FABP is an exception to this problem, as it can bind two ligands at the same time, with the second ligand being bound with much lower affinity, which would allow its displacement by PPARs. Other exceptions are xenobiotic compounds, known potent activators of PPAR $\alpha$ , which have been shown to bind L-FABP with an approximately 10-fold weaker affinity than fatty acids. This reflects a situation in which, under physiologic conditions, ligands might not be retained by L-FABP and therefore would be more available to bind PPARs. The high-affinity binding of different fatty acids to liver FABP suggests that the ligand-binding protein complex may represent the form in which fatty acids are transported into the nucleus for possible interaction with and delivery to the PPARs. In this scenario, when liver FABP enters the nucleus carrying a fatty acid and a peroxisome proliferator, the PPAR will be able to extract the peroxisome proliferator, whereas the fatty acid could be removed only if bound to the low-affinity binding site. This could explain why xenobiotics act as potent PPAR $\alpha$  activators, despite the presence of large amounts of fatty acids in the cytoplasm and the nucleus. There has been some recent evidence that PPARs can bind to and are activated by fatty acid-CoA esters [79, 80]. Conversely, it has been shown that L-FABP also binds different fatty acid-CoA esters, albeit with lower affinity than fatty acids [53, 81, 82]. Furthermore, it was shown that overexpression of L-FABP in fibroblasts led to



increased targeting of a fluorescently labeled fatty acid-CoA ester or free fatty acids into the nuclear membrane and nucleoplasm [82], where it colocalized with PPAR $\alpha$ . While this study provides only indirect evidence for the transfer of fatty acid-CoA esters to PPAR by L-FABP, it is of importance since fatty acid-CoA esters are bound with lower affinities by L-FABP and might therefore be more readily displaced by PPAR than the tightly bound free fatty acids. A more comprehensive study of binding affinities of fatty acid-CoA esters to PPARs and FABPs will be important to assess the importance of this pathway.

The discrimination of fatty acid leads back to the question of different fatty acid subtypes. It has been shown in several studies that polyunsaturated long and very long chain fatty acids are the strongest activators of PPARs, which also correlates with the binding of these compounds. Interestingly, these differences are not exactly mirrored by FABPs. Even though polyunsaturated fatty acids are usually the preferred ligands of FABP (exception being H-FABP), FABPs still bind the fatty acids with high affinity. Thus it can be envisaged that the activation of PPARs through EFAs and other important polyunsaturated fatty acids which have been shown to be beneficial for health with respect to diabetes and obesity is due to the fact that FABPs discriminate between these fatty acids. It is tempting to speculate that polyunsaturated fatty acids would be more readily transferred to PPARs for target gene activation, while medium and short chain saturated fatty acids were retained by FABPs. In this simplistic view, activation of PPAR through unsaturated fatty acids would lead to increased lipid metabolism, while saturated fatty acids would be retained by FABPs and stored, leading to the development of obesity and insulin resistance.

In conclusion it has to be noted that *in vitro* evidence for a pathway linking FABPs and PPARs with respect to signal transduction has been proven by a number of independent studies (Fig. 2). The exact mechanism for how FABPs are translocated to the nucleus upon ligand binding and how a ligand is transferred between FABP and PPAR remains unclear. Furthermore, a direct link between *in vivo* data gained from studying the genetic knockout models of either PPARs or FABPs remain inconclusive, most likely due to the fact that several compensatory mechanisms exist which may influence and deregulate a severe phenotype like complete ablation of either FABP or PPAR expression. Further studies of these pathways will be needed, as it is evident that PPARs control several major aspects of glucose and lipid metabolism as evidenced by the fact that several important drugs to counter obesity and diabetes are targeted towards those

receptors. It will be of utmost importance to understand the exact mechanism of delivery of these drugs, as the transfer proteins pose one discriminatory step in translocation of those compounds from the extracellular compartment to the nucleus for receptor activation and thus play an important role in modulating receptor activity.

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