

Fatty acid transport across the cell membrane: Regulation by fatty acid transporters

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Fatty acid transport across the cell membrane: Regulation by fatty acid transporters

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ABSTRACT

Transport of long-chain fatty acids across the cell membrane has long been thought to occur by passive diffusion. However, in recent years there has been a fundamental shift in understanding, and it is now generally recognized that fatty acids cross the cell membrane via a protein-mediated mechanism. Membrane-associated fatty acid-binding proteins ('fatty acid transporters') not only facilitate but also regulate cellular fatty acid uptake, for instance through their inducible rapid (and reversible) translocation from intracellular storage pools to the cell membrane. A number of fatty acid transporters have been identified, including CD36, plasma membrane-associated fatty acid-binding protein (FABP_{pm}), and a family of fatty acid transport proteins (FATP1–6). Fatty acid transporters are also implicated in metabolic disease, such as insulin resistance and type-2 diabetes. In this report we briefly review current understanding of the mechanism of transmembrane fatty acid transport, and the function of fatty acid transporters in healthy cardiac and skeletal muscle, and in insulin resistance/type-2 diabetes. Fatty acid transporters hold promise as a future target to rectify lipid fluxes in the body and regain metabolic homeostasis.

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1. Introduction

The solubility of long-chain fatty acids in aqueous solutions is extremely low, i.e., in the range of 1–10 nM [1]. The presence of proteins with the ability to bind fatty acids (for convenience used to designate 'long-chain fatty acids') dramatically increases the total amount of fatty acids that can be present in the aqueous phase. For instance, albumin occurs in plasma and interstitium at a concentration of 300–600 μM and can accommodate up to 1–2 mM fatty acids [2]. Likewise, cytoplasmic fatty acid-binding protein (FABP_c) is abundantly present in the soluble cytoplasm of cells with an active fatty acid metabolism and can accommodate up to 150–300 μM fatty acids [3,4]. As a result, albumin and FABP_c act as extracellular and intracellular buffers, respectively, for fatty acids (Fig. 1). Consistent with this, the average concentration of (non-protein bound) fatty acids in plasma from healthy subjects

was found to be 7.5 ± 2.5 nM [5], indicating that of the total amount of fatty acids in plasma only < 1 part in 10⁵ is present in the aqueous phase.

The above data also indicate that under normal physiological conditions, with a total plasma fatty acid concentration of 100–400 μM and a total cytosolic fatty acid concentration < 50 μM, both albumin and FABP_c are in such abundance that fluctuations in their presence will hardly affect their fatty acid buffering function. Thus, studies on fatty acid uptake by hindlimb muscle from mice lacking (heart-type) FABP_c showed that a 65% reduction in FABP_c in heterozygous mice did not affect the rate of muscle fatty acid uptake while the full ablation of FABP_c (homozygous null mice) decreased fatty acid uptake markedly [6]. This latter study illustrates that FABP_c inside the cell functions as a sink for incoming fatty acids, yet plays a merely permissive action in cellular fatty acid uptake.

2. Mechanism of transmembrane fatty acid transport

During the preceding two decades there has been considerable discussion on the mechanism by which fatty acids enter cells, particularly whether fatty acid transport across the plasma membrane occurs by simple ('passive') diffusion, or whether fatty acid uptake is facilitated by (one or more) membrane-associated proteins (for review see [7,8]). While the various experimental

Abbreviations: FABP_c, cytoplasmic fatty acid-binding protein; FABP_{pm}, plasma membrane fatty acid-binding protein; FATP, fatty acid transport protein; GLUT4, glucose transporter-4; ACS, acyl-CoA synthetase; CPT, carnitine palmitoyltransferase; AMPK, AMP-activated kinase

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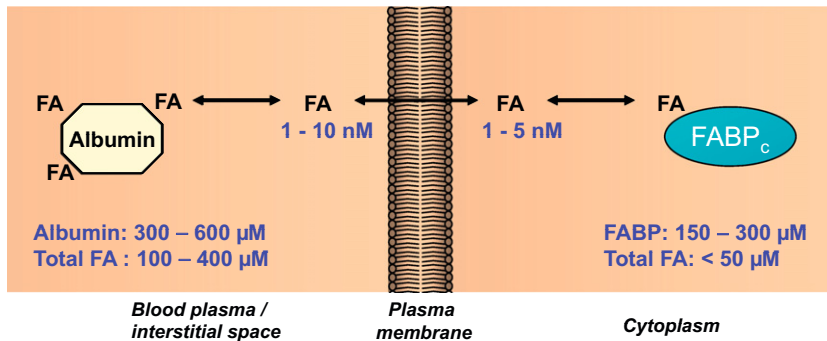


Fig. 1. Quantitative comparison of the presence of albumin in the extracellular space, cytoplasmic fatty acid-binding protein (FABP_c) inside cells, and fatty acid concentrations under normal physiological conditions. Albumin and FABP_c provide a buffer for the extremely low aqueous concentration of (long-chain) fatty acids. Albumin has 3–6 binding sites for fatty acids. FABP_c occurs in 9 distinct types of which liver-type FABP_c has two ligand binding sites while all other types have only a single ligand binding site [58,59].

studies provide support for both of these possibilities, evidence in support of a protein-mediated fatty acid uptake system is now believed to be the dominant means by which fatty acids are taken up by metabolically important tissues.

The amphipathic nature of the fatty acid molecule, with a nonpolar chain and a polar head group, provides it with the biophysical properties for entry into the phospholipid bilayer of the cell membrane. Subsequent transfer of the fatty acid from the outer leaflet of the bilayer to the inner leaflet ('flip-flop') is hampered by the charge of the polar head group. However, in the vicinity of the membrane the apparent pK_a of the fatty acid shifts from about 4.5 in aqueous solutions to about 7.6 (independent of fatty acid type), as a result of which about half of the fatty acids are present in the un-ionized, i.e., protonated, form [9]. This uncharged species can then easily flip-flop to the inner leaflet of the membrane, whereafter a proton is donated to the interior solution and the fatty acid is available for desorption.

This mechanism suggests that biological membranes do not form a barrier for fatty acids. Indeed, Hamilton and co-workers have compared the permeabilities of various molecules and (long-chain) fatty acids in a phospholipid bilayer to conclude that the permeability of fatty acids is several orders of magnitude larger than that of water, glucose, and other small non-electrolytes [10]. In addition, measured values of desorption kinetics revealed half-times in the milliseconds to seconds time range for fatty acids, which is fast enough to support intracellular metabolism. Together, these data suggest that (i) fatty acids can rapidly pass phospholipid bilayers without the help of membrane proteins and (ii) membrane proteins are not needed for the purpose of releasing fatty acids into the cytosol [11].

If fatty acids could freely diffuse across biological membranes, the direction and rate of fatty acid movement would depend on fatty acid delivery to the tissue and on the transmembrane gradient of fatty acids. Such a mechanism would be difficult to control and, moreover, may not meet (changes in) metabolic demands in tissues, e.g., muscle upon the initiation of contraction. Thus, from a physiological perspective it would be highly desirable to regulate fatty acid entry into the cell, especially (i) to ensure fatty acid uptake when its extracellular concentration is low, (ii) to limit uptake when the extracellular concentration is high, (iii) to potentially select for specific fatty acid types, and (iv) to allow rapid adjustments in fatty acid provision to meet fluctuations in metabolic demands [12].

Since the early 1980s several investigators have searched for membrane-associated proteins able to bind fatty acids and that may function to facilitate and/or regulate transmembrane fatty acid transport. To date, various membrane proteins have been identified that facilitate the cellular uptake of fatty acids (Fig. 2).

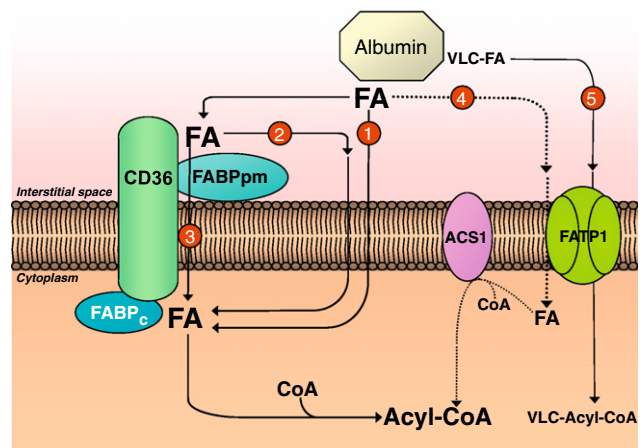


Fig. 2. Schematic representation of the current view of fatty acid transport across the cell membrane. Because the exact mechanism of transmembrane translocation of fatty acids is still unknown, different models have been suggested. (1) In view of their hydrophobic nature, fatty acids could cross the membrane by simple diffusion. (2) Alternatively, CD36 (88 kDa; also referred to as 'fatty acid translocase'), alone or together with the peripheral membrane protein FABP_{pm} (plasma membrane-associated fatty acid-binding protein; 43 kDa) accepts fatty acids at the cell surface to increase their local concentration and thus increase the number of fatty acid diffusion events. (3) It is also possible that CD36 itself actively transports fatty acids across the membrane. Once at the inner side of the membrane fatty acids are bound by cytoplasmic FABP (FABP_c) before entering metabolic or signalling pathways. (4) Additionally, a minority of fatty acids are thought to be transported by fatty acid transport proteins and rapidly activated by plasma membrane acyl-CoA synthetase (ACS1) to form acyl-CoA esters. (5) Very-long-chain fatty acids (> C22) are preferentially transported by FATPs and by action of their synthetase activity directly converted into very-long-chain acyl-CoA esters (uptake by vectorial acylation). Adapted from [27].

For convenience, these proteins are generally referred to as 'fatty acid transporters'. The prevalent view is that these fatty acid transporters act as acceptors for fatty acids whereafter the fatty acids make their way through the cell membrane by simple diffusion (route 2 in Fig. 2). At the inner side of the membrane, the (transmembrane) proteins may provide a docking site for FABP_c or for enzymes that act on fatty acids (such as acyl-CoA synthetase) (Fig. 2). Thus, these proteins may function to sequester fatty acids in the membrane, and help organize them within specific membrane domains so as to make the fatty acids readily available for subsequent aqueous transport and/or enzymatic conversion.

The membrane-associated (putative) fatty acid transporters CD36, FABP_{pm} and FATP_s (Fig. 2) differ in molecular mass

and degree of post-translational modification; some show a characteristic pattern of tissue distribution, while others are ubiquitously expressed. Interestingly, the FATPs form a family of 6 proteins. For most of these proteins there is evidence from studies in cell lines and/or in genetically altered animals that they facilitate cellular long-chain fatty acid uptake [13].

3. Function of fatty acid transporters in muscle

In skeletal muscle CD36, FABP_{pm}, FATP1, and FATP4 are co-expressed. This makes this tissue suitable especially to compare the significance of the various transporters for fatty acid uptake and utilization, that is, to examine whether they all function as membrane-bound transporters and whether they channel fatty acids to specific intracellular targets, particularly oxidation and/or esterification. A recent study compared the transport efficiencies of these four proteins and their possible differential effects on fatty acid utilization in rat skeletal muscle [14]. Because knock-out animals show compensatory responses in the expression of the other transporters, these animals could not be used for such studies. Instead, the genes for each of these transporters were upregulated independently in skeletal muscle only, under controlled conditions and within a physiologically meaningful range. For this, respective cDNAs cloned into plasmid DNAs were electrotransfected in the intact tibialis anterior muscle of one leg *in vivo*, while electrotransfection of the empty plasmid in the contralateral muscle served as control [13]. After 2 weeks, the respective fatty acid transporters were overexpressed by 1.5–2.2-fold while in each case the muscle contents of the other transporters remained unchanged. All transporters increased fatty acid transport, but CD36 and FATP4 were 2.3- and 1.7-fold more effective than FABP_{pm} and FATP1, respectively (Fig. 3, panel A) [14]. Overexpression of the transporters failed to alter the rates of fatty acid esterification into triacylglyceroles, but increased the rates of fatty acid oxidation (Fig. 3, panel B). Interestingly, the effects of CD36 and FABP_{pm} were 3-fold greater than for FATP1 and FATP4 (Fig. 3, panel B).

Thus, at least in skeletal muscle fatty acid transporters exhibit different capacities for fatty acid transport and metabolism: *in vivo* CD36 and FATP4 are the most effective plasmalemmal fatty acid transporters, whereas CD36 and FABP_{pm} are key players for promoting fatty acid oxidation.

4. Acute regulation of muscle fatty acid transport

The presence of CD36 not only on the cell membrane but also in intracellular compartments, notably endosomes, triggered studies to identify whether regulation of fatty acid transport could occur by translocation of CD36 from endosomes to the cell membrane to increase fatty acid uptake. Such a mechanism would be analogous to the well-known regulation of glucose uptake which involves the translocation of the glucose transporter GLUT4 from an intracellular storage depot to the cell membrane. A series of studies showed that in heart and skeletal muscles both muscle contraction and insulin stimulate, within minutes, the translocation of CD36 from an endosomal compartment to the cell membrane to markedly (up to 2-fold) increase fatty acid uptake [15–17]. The contraction-induced translocation is mediated by AMP-kinase and occurs independently of the insulin-induced translocation which is mediated by PI3-kinase. In each case, both CD36 and GLUT4 are recruited in the same time frame resulting in increased uptake rates for both fatty acids and glucose [15,18,19]. Similar to what is known for GLUT4, the translocation of CD36 is rapid and reversible, with CD36 being internalized on the same time scale (minutes) as its recruitment [15,20]. Taken together, the regulation of fatty acid uptake by heart and muscle appears to display a striking similarity with the regulation of glucose uptake, in both cases involving the recycling of substrate transporters that is under the control of (at least a number of) the same triggers (Fig. 4).

Others have confirmed that contractile activity increases fatty acid transport via the translocation of CD36 in muscle [21]. Muscle contraction also increases the translocation to the cell membrane of FABP_{pm}, FATP1, and FATP4 [22]. Nevertheless, studies in heart and muscle obtained from CD36 knock-out animals have indicated that CD36 is fundamental to enabling the contraction-induced increase in fatty acid uptake, as its ablation almost completely blunted this increase in rate of uptake [23,24].

Considerable effort is being made to unravel both the signalling and trafficking pathways involved in the recycling of CD36 in heart and muscle, in particular in relation to that of GLUT4. Knowledge on the pathways involved is relevant (i) to understand what triggers influence cellular substrate uptake through substrate transporter recycling and (ii) to learn to what extent CD36 and GLUT4 recycling share the same triggers and trafficking machinery and what signals would selectively influence either CD36-mediated fatty acid uptake or GLUT4-mediated

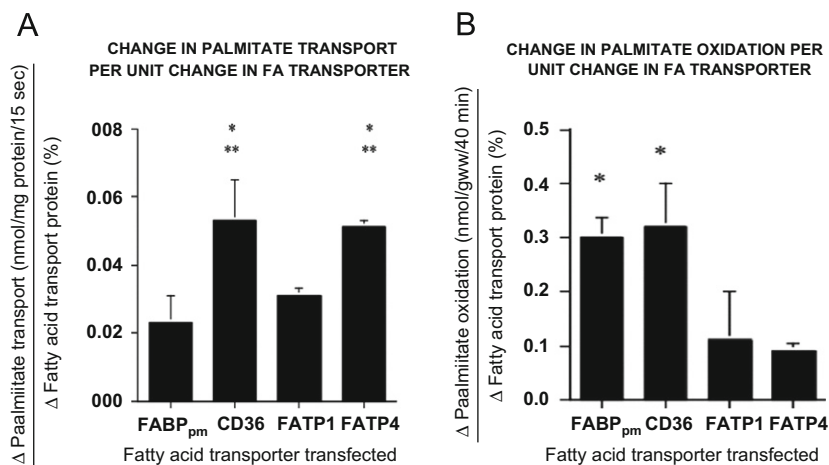


Fig. 3. Effects of fatty acid transporter overexpression *in vivo* in rat hindlimb muscle on fatty acid uptake and utilization. *Panel A:* Change in palmitate transport per unit change in fatty acid transporter *Panel B:* Change in palmitate oxidation per unit change in fatty acid transporter. Data reproduced, with permission, from Nickerson et al. [14].

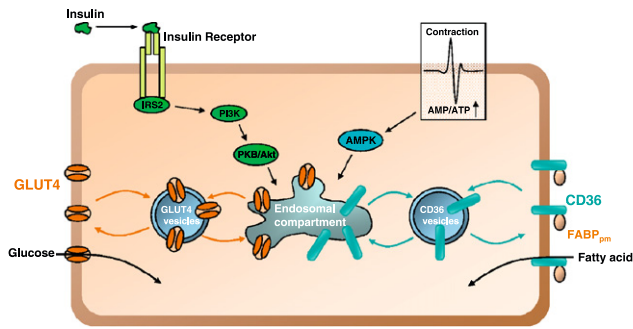


Fig. 4. Schematic representation illustrating the similarity between the regulation of cellular fatty acid uptake by CD36 translocation and that of glucose uptake by GLUT4 translocation. Both muscle contraction and insulin treatment stimulate the simultaneous recruitment of CD36 and GLUT4 from recycling endosomal storage compartments to the cell membrane to increase fatty acid and glucose uptake, respectively. AMPK, AMP-activated kinase; PI3K, PI3-kinase.

glucose uptake (which would alter substrate preference; see below). With respect to the latter, it should be noted that selective recruitment and/or internalization indeed can occur as in studies with cardiac myocytes it was observed that the phosphodiesterase inhibitor dipyridamole mobilizes CD36 from contraction-inducible storage depots while not affecting GLUT4 mobilization [25], while conversely, the protein-sulfhydryl-modifying compound arsenite stimulates GLUT4-mediated glucose uptake but does not affect CD36-mediated fatty acid uptake [26]. Although the intracellular targets of these pharmacological compounds are not yet known, these data are proof of concept that selective substrate transporter recycling is possible.

So far, the signalling pathways involved in CD36 and GLUT4 recycling appear to be very much alike (reviewed in [27]). In contrast, preliminary evidence suggests that the trafficking pathways used for CD36 and for GLUT4 translocation show clear differences with respect to vesicle-associated proteins involved.

Another interesting finding is that CD36 [24,28–30] and FATP1 [30] are also present at the mitochondrial membrane, suggesting their possible involvement in mitochondrial fatty acid oxidation. A number of recent studies have shown strong support for this concept: (i) mitochondria isolated from human muscle following 2 h of aerobic exercise (bicycle ergometry) showed an increase in mitochondrial CD36 content which correlated with an increased rate of mitochondrial fatty acid oxidation [31], (ii) overexpression of CD36 increased mitochondrial fatty acid oxidation [30], and (iii) in CD36 knock-out animals the exercise-induced increase in fatty acid oxidation is ablated [24]. These findings suggest that CD36 translocation (from an as yet unidentified storage pool) to the mitochondria is essential to increase fatty acid oxidation during exercise. The precise mechanism by which CD36 and FATP1 facilitate mitochondrial fatty acid oxidation remains to be elucidated.

5. Trafficking machinery involved in membrane transporter translocation

At present little is known about the mechanisms by which fatty acid transporters travel between different intracellular compartments. However, much effort has been made to unravel proteins involved in the trafficking events responsible for the migration of the glucose transporter GLUT4 from intracellular storage compartments to the cell membrane and vice versa. During recent years evidence has accumulated that the trafficking machinery of CD36 might be closely related to that of GLUT4. For example, morphological studies in Chinese Hamster ovary cells revealed that GLUT4 and CD36 are shifted to the same

microdomains in the plasma membrane upon insulin stimulation [20]. Additionally, the recycling of both GLUT4 and CD36 back into their distinct intracellular storage compartments is dependent on the action of the Rab-GTPase Rab11a [32]. Therefore, our current knowledge about GLUT4 trafficking could give links to gain further insight into the trafficking machinery of fatty acid transport proteins like CD36.

One crucial step in insulin-mediated GLUT4 translocation is the inhibition of AS160 by Akt. AS160 acts as a Rab-GTPase activating protein (Rab-GAP) and restrains GLUT4 in its storage compartment in the basal state [33]. It does so by promoting the inactive form of different Rab-GTPases known to be involved in insulin stimulated GLUT4 translocation [34,35]. Whether AS160 also functions in insulin-induced translocation of fatty acid transporters is currently not known. Interestingly, AS160 has been shown to also be a substrate of AMPK. The inhibition of AS160 by AMPK is important to stimulate glucose uptake during increased contractions, and occurs additive to the inhibition by insulin [36].

Another family of trafficking proteins that are currently under study are soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNARES). These proteins are present at the transport vesicle (v-SNARES) and at the target membrane (t-SNARES), and are mediators of membrane fusion (reviewed in [37]). This process is highly selective as a v-SNARE only interacts with a specific subset of t-SNARES to form a SNARE complex that initiates fusion. For example, the insulin-responsive GLUT4 pool is enriched with the v-SNARE VAMP2 [38]. At the plasma membrane, VAMP2 interacts with the t-SNARES syntaxin4 and SNAP23 to initiate membrane fusion [39]. A negative regulator of this complex formation is Munc18c, which masks the SNAP23 interaction site on syntaxin4 and is moved aside upon insulin stimulation [40]. In contrast to Munc18c, binding of DOC2B to syntaxin4 promotes SNARE complex formation and fusion of GLUT4 vesicles with the plasma membrane [41].

It is likely that similar mechanisms are involved in the translocation of CD36 upon insulin stimulation or the translocation of CD36, FABP_{pm}, FATP1, and FATP4 upon increased muscle contraction.

6. Fatty acid transporters in pathological states

Whole-body lipid homeostasis requires a fine tuning of fatty acid transport and utilization by metabolically active tissues. Because of their facilitatory and regulatory roles in cellular fatty acid uptake and utilization, membrane fatty acid transporters form an integral part of this homeostatic system. As a result, aberrations in lipid metabolism likely will influence the functioning of fatty acid transporters, while changes in fatty acid transporter content or functioning – for instance as induced by dietary or pharmacological interventions – may have an impact on whole-body lipid metabolism and potentially elicit a pathological state.

In the last few years most attention has been given towards examining a possible role for fatty acid transporters in insulin resistance and type-2 diabetes because these are associated with marked changes in lipid metabolism, including increased plasma fatty acid concentrations [42] and increased intramuscular triacylglycerol depots [43]. Excessive accumulation of intramyocellular fatty acids and their metabolites is commonly referred to as lipotoxicity, and is a main contributor to the pathophysiology of insulin resistance and dysfunctioning of heart and skeletal muscle [44,45].

In animal models of insulin resistance [46–49] and in humans with insulin resistance and type-2 diabetes [50] it was found that the intramuscular triacylglycerol accumulation is associated with an

increased presence of CD36 at the sarcolemma. The latter is not due to an increase in total tissue CD36 content, but rather to a permanent relocalization of this transporter to the cell membrane. Further studies in rat heart [48] and skeletal muscle (A. Bonen et al., unpublished data) have elucidated that this permanent relocalization is an early event in the development of insulin resistance and its progression towards diabetic cardiomyopathy, and, importantly, appears to precede a change in glucose uptake. These combined observations suggest that early in the development of insulin resistance alterations occur in the signalling and/or trafficking proteins specifically dedicated to CD36 recycling, which would result in a selective and permanent relocation of CD36 to the sarcolemma, without a concurrent change in the subcellular distribution of GLUT4. The increased sarcolemmal CD36 abundance, together with an increased plasma fatty acid concentration, would elicit an increased rate of fatty acid uptake to an excessive level, in turn leading to an increased rate of fatty acid esterification into triacylglycerols and increased concentrations of fatty acid metabolites such as diacylglycerols and ceramides, as has been shown recently [49]. The latter compounds then will interfere with insulin-induced GLUT4 translocation to the sarcolemma so that GLUT4 is retained intracellularly and the rate of glucose uptake is lowered (i.e., insulin resistance). Thus, in this scenario, based on our studies in heart and skeletal muscle, it appears that CD36 can be regarded as a key factor in the development of insulin resistance in cardiac and skeletal muscle [51].

7. Conclusions and remaining issues

Although long-chain fatty acids can move across the cell membrane by simple ('passive') diffusion, consensus is beginning to appear that fatty acid-binding membrane proteins fulfil both a facilitatory and a regulatory role in the process of cellular fatty acid uptake. In general, these membrane-associated proteins function as acceptors for fatty acids, assist in organizing fatty acids within membrane microdomains, and provide a site for desorption of fatty acids for subsequent binding to carrier proteins in the aqueous solution. Fatty acid transport across the cell membrane thus appears to be a highly regulated process, involving various membrane proteins (many of which are co-expressed in the same tissue) which operate through several levels of regulation such as the above-mentioned translocation from intracellular storage sites to the cell membrane, but also by post-translational modification (phosphorylation, palmitoylation, glycosylation) and possibly protein–protein interactions (reviewed in [12,52–54]). The membrane fatty acid transporters are now also implicated in metabolic disease, particularly insulin resistance and its progression to type-2 diabetes. As a corollary, fatty acid transporters are being regarded as a promising therapeutic target to re-direct lipid fluxes in the body in an organ-specific fashion. The potential of such an approach is illustrated by the observation that myocardial lipotoxicity and myocardial dysfunction induced experimentally by PPAR α overexpression could be rescued by ablation of CD36 [55]. However, many aspects of the mechanisms by which these fatty acid transporters function still need to be uncovered in order to design therapies to selectively manipulate their actions. These remaining issues include:

- further disclosing the signalling and trafficking pathways involved in recycling of fatty acid transporters, especially in relation to that of the glucose transporter GLUT4;
- establishing whether protein–protein interaction and post-translational modifications among fatty acid transporters have functional significance, and whether these aspects are changed in the pathological state;

- unraveling the role of membrane microdomains (caveolae, rafts) in the functioning of fatty acid transporters;
- examining whether, and if so, in what manner, fatty acid transporters select for specific fatty acid types and/or target fatty acids towards specific metabolic pathways (oxidation, esterification) or signalling routes (activation of fatty acid responsive genes).

Fatty acid uptake has been studied mostly in heart, muscle, and adipose tissue. Uptake of fatty acids by the brain is less well addressed, despite the fact that brain expresses CD36, FATP1, FATP4 and several FABP isoforms [56,57]. It would be of much interest to study whether these latter proteins are involved in the special fatty acid handling that occurs in brain, for instance, whether these proteins are involved in the preferential and very high rates of uptake of the ω -3 fatty acids (eicosapentaenoic acid and docosahexaenoic acid) during brain development.

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