

Review

# Long-chain fatty acid uptake and FAT/CD36 translocation in heart and skeletal muscle

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## Abstract

Cellular long-chain fatty acid (LCFA) uptake constitutes a process that is not yet fully understood. LCFA uptake likely involves both passive diffusion and protein-mediated transport. Several lines of evidence support the involvement of a number of plasma membrane-associated proteins, including fatty acid translocase (FAT)/CD36, plasma membrane-bound fatty acid binding protein (FABPpm), and fatty acid transport protein (FATP). In heart and skeletal muscle primary attention has been given to unravel the mechanisms by which FAT/CD36 expression and function are regulated. It appears that both insulin and contractions induce the translocation of intracellular stored FAT/CD36 to the plasma membrane to increase cellular LCFA uptake. This review focuses on this novel mechanism of regulation of LCFA uptake in heart and skeletal muscle in health and disease. The distinct signaling pathways underlying insulin-induced and contraction-induced FAT/CD36 translocation will be discussed and a comparison will be made with the well-defined glucose transport system involving the glucose transporter GLUT4. Finally, it is hypothesized that malfunctioning of recycling of these transporters may lead to intracellular triacylglycerol (TAG) accumulation and cellular insulin resistance. Current data indicate a pivotal role for FAT/CD36 in the regulation of LCFA utilization in heart and skeletal muscle under normal conditions as well as during the altered LCFA utilization observed in obesity and insulin resistance. Hence, FAT/CD36 might provide a useful therapeutic target for the prevention or treatment of insulin resistance.

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**Keywords:** Long-chain fatty acid uptake; FAT/CD36; Insulin; PI(3)K; Contraction; GLUT4

## 1. Introduction

In skeletal muscle and heart, the oxidation of long-chain fatty acids (LCFA) provides much of the energy needed for proper function. Since intramuscular storage sites are a limited source, these tissues rely heavily on the continuous supply of exogenous LCFA mainly derived from adipose tissue. Although the single-pass extraction of albumin-bound LCFA from the circulation is very efficient in both heart [1] and muscle [2,3], LCFA uptake into these tissues involves the passage of LCFA across many barriers, as reviewed previously [1,4,5]. The plasma membrane is the final barrier to be crossed before LCFA reach the interior of the muscle cells (i.e., the cytoplasm), where LCFA transfer between intracellular membranes is facilitated by binding to soluble fatty acid-binding proteins (FABP) [6,7]. Specifically, the predominant FABP isoform in muscle tissues, heart-type FABPc (H-FABPc) is

*Abbreviations:* ACC, acetyl-CoA carboxylase; AICAR, 5'-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside; AMPK, adenosine monophosphate (AMP)-activated protein kinase; Akt/PKB, protein kinase B; cAMP, adenosine 3':5'-cyclic monophosphate; DAG, diacylglycerol; FABPc, cytoplasmic fatty acid-binding protein; FABPpm, plasmalemmal fatty acid-binding protein; FAT/CD36, fatty acid translocase; FATP, fatty acid transport protein; IBMX, 3-isobutyl-1-methylxanthine; LCFA, long-chain fatty acid; MAPK, mitogen-activated protein kinase; PDE, phosphodiesterase; PKA, protein kinase A; PKC, protein kinase C; PL, phospholipids; PI(3)K, phosphatidylinositol-3-OH-kinase; TAG, triacylglycerol; TfR, transferrin receptor; VLACS, very long-chain acyl-CoA synthetase

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responsible for delivery of LCFA from the sarcolemma through the cytoplasm to the outer mitochondrial membrane, the site of acyl-CoA synthetase. This enzyme converts LCFA into acyl-CoA to make it available for (i) triacylglycerol synthesis or (ii) mitochondrial  $\beta$ -oxidation. When destined for  $\beta$ -oxidation, acyl-CoA will cross the mitochondrial outer and inner membranes by virtue of the carnitine shuttle, in which the first step, i.e., the conversion of acyl-CoA into acyl-carnitine as mediated by carnitine palmitoyl-transferase I (CPT I), is an important control step in cellular energy metabolism [8,9].

Several factors have been suggested to control the level of LCFA utilization both at rest and during exercise. There is sufficient evidence to suggest that LCFA utilization is regulated at the level of adipose tissue by control of the rate of lipolysis, export of LCFA from adipose tissue, and their subsequent transport by the vascular system to the heart and skeletal muscle [10,11]. In addition, alterations in lipoprotein lipase activity will also contribute to the regulation of LCFA supply to the muscle [12]. On the other hand, there is evidence to indicate that LCFA utilization is regulated at the level of the myocytes themselves. Generally, it is believed that LCFA transport into the mitochondria by the rate-limiting enzyme CPT I is the most regulated step in the muscular control of LCFA utilization. For extended overviews of the potential regulators of CPT I activity and LCFA oxidation, the reader is referred to recent reviews by Lopaschuk [8,13] on heart and Jeukendrup [14] on skeletal muscle.

Another site that more recently has been suggested to play a role in the regulation of LCFA utilization by heart and skeletal muscle is the initial step in cellular uptake of LCFA, namely their trapping and passage across the plasma membrane. It is well documented that the transfer of LCFA through the plasma membrane barrier is facilitated by membrane-associated LCFA binding proteins (reviewed by [15,16]). In addition to their facilitatory role in LCFA transport, a number of recent studies have suggested a regulatory role for these LCFA transport proteins in LCFA metabolism as well [3,17–19]. Although it is not known whether LCFA transport across the plasma membrane may become rate-limiting under specific conditions, it has been demonstrated that regulation of LCFA uptake in response to acute stimuli, such as insulin and muscular contractions, involves the translocation of one of the proteins involved, i.e., fatty acid translocase (FAT)/CD36, from intracellular storage compartments towards to plasma membrane [20,21]. Moreover, long-term regulation of LCFA uptake in trained men has also been observed to involve an upregulation of the muscular expression of this and other putative LCFA membrane transporters [19,22,23]. Therefore, currently much effort is being made to define the signaling pathways involved in the expression and translocation of FAT/CD36 in heart and skeletal muscle in response to insulin and exercise. Full understanding of the significance of LCFA transport proteins to overall LCFA utilization in health and disease will contribute to developing alternative approaches to manipulate substrate utilization by heart and skeletal muscle.

The outline of this review is, therefore, first to give a brief background into the mechanism of cellular LCFA uptake, then

to describe the underlying mechanism and signaling pathways involved in the acute regulation of LCFA uptake by recycling of FAT/CD36 between intracellular storage compartments and the plasma membrane. Finally, the attention will be drawn on the impact of physiological stimuli (chronic exercise, aging, dietary interventions) and selected pathophysiological conditions (diabetes, obesity) on this novel regulatory mechanism of LCFA transport across the plasma membrane.

The data presented in this review are primarily derived from studies performed in rodent cardiac and skeletal muscle. However, where available, the results of human studies will be included.

## 2. Transmembrane transport of LCFA

### 2.1. Putative transport proteins involved in transmembrane transport of LCFA in heart and skeletal muscle

The plasma membrane of most tissue cells contains a sophisticated set of substrate specific transporter proteins, including glucose transporter isoforms (GLUTs), monocarboxylate transporters (MCTs), and multiple transport systems for the cellular exchange of amino acids [24–29]. Likewise, several plasma membrane-associated proteins have been identified as candidate LCFA transport proteins according to their ‘putative’ role in protein-mediated LCFA transport [16,30–32]. Substantial evidence for a prominent role in the transmembrane movement of LCFA in heart and skeletal muscle is now available for fatty acid translocase (FAT)/CD36, plasmalemmal fatty acid binding protein (FABPpm), fatty acid transport protein (FATP) 1 and the heart-specific FATP6 [3,23,33–40]. For an extended review on this topic, see [15].

Notably, all of the putative LCFA membrane transporters have other established functions, either related (FATP1, FAT/CD36) or unrelated (FABPpm, FAT/CD36) to fatty acid metabolism. FABPpm is identical to mitochondrial aspartate aminotransferase (mAspAT) [41,42]. The reason why FABPpm is a bifunctional protein with two unrelated and entirely different functions is, however, still unknown. FATP1 has been found to exhibit intrinsic acyl-CoA synthetase activity with a broad specificity for both LCFA and very long-chain fatty acids [43–46]. Hence, FATP1 has been proposed to be a bifunctional protein, but it remains to be investigated whether LCFA import is driven by this intrinsic acyl-CoA synthetase activity or whether transport and activation are indeed two distinct functions [46]. FAT/CD36 is the rat homolog of human platelet CD36, also known as glycoprotein (GP) IIIb, GPIV, and PASIV [47,48]. CD36 has been first described as a platelet membrane component and was later shown to act as a receptor for thrombospondin-1 and a class B scavenger receptor B involved in the binding of modified and native lipoproteins and anionic phospholipids [47–49]. Apart from these functions unrelated to fatty acid metabolism, a recent report by Campbell et al. [50] suggested a role for FAT/CD36 in mitochondrial acyl-CoA uptake, revealing a novel role of FAT/CD36 in overall LCFA metabolism.

## 2.2. LCFA uptake into heart and skeletal muscle

Although different steps are involved in membrane transport of LCFA (adsorption, transmembrane movement, desorption) [51] their transmembrane movement through the lipid bilayers is the most controversial part. Due to their hydrophobic nature, LCFA are expected to rapidly diffuse through the lipid bilayer with the transmembrane gradient as driving force [52,53]. With respect to transmembrane movement, most studies have reported extremely fast rates for transport of LCFA across lipid membranes indicating that the lipid bilayer presents a negligible barrier to flip-flop [52–55]. Remarkably, in a recent report by Cupp et al. [56], it was argued that the methods to describe rapid flip-flop in these studies have not been interpreted correctly. In contrast, significantly slower flip-flop rates were found in this study, indicating that the lipid bilayer component of biological membranes might present a significant constraint to transport of LCFA across cell membranes [56], thus favouring a role for protein-mediated transport in this step.

Already since the early 1980s, specific features of protein-mediated transport (e.g. saturation and competitive inhibition) have been observed in cardiac and skeletal muscle [57–60]. The existence of a protein-mediated transport mechanism [34,61] was clearly emphasized by the introduction of giant sarcolemmal vesicles in which LCFA transport across the plasma membrane is divorced from subsequent LCFA metabolism (for review see [62]). In contrast to the traditionally held view that passive diffusion is sufficient to meet cellular LCFA demands [51–53], evidence now suggests the involvement of both diffusion and protein-mediated transport as co-existing components in transmembrane movement of LCFA [7,34,36,61,63].

General transport inhibitors such as trypsin and phloretin have frequently been used to study the quantitative contribution of protein-mediated transport to overall LCFA uptake. Although these inhibitors have demonstrated that in skeletal muscle and heart transporters are involved in the bulk uptake of LCFA (50–80%), they do not discriminate between the individual proteins involved in LCFA transport. The development of a polyclonal antiserum against FABPpm [64] and sulfo-*N*-succinimidyl esters of LCFA [65], which have been shown to bind specifically and inactivate FAT/CD36 [66], have advocated an important role of both proteins in LCFA transport in heart and skeletal muscle. In particular, sulfo-*N*-succinimidyl oleate (SSO) might represent a valuable tool to further enhance our understanding of FAT/CD36 in LCFA transport because this nucleophilic LCFA derivative does neither interact with FABPpm or FATP nor directly interferes with intracellular transport processes (reviewed by [66]). To date, no specific inhibitor has been developed for any of the FATP family members, making it difficult to assess their functional significance in LCFA transport.

It should be stressed, that the protein-mediated LCFA uptake system has been termed a LCFA transport system for matter of convenience, since it displays most of the features of a classic transport system [62]. However, despite their

apparent transport-like function, current insight into the membrane topology of FABPpm, FAT/CD36 and FATP1 does not suggest these membrane-associated proteins to act as typical transporters [32,67]. Thus, instead of functioning as classical pores/channels, FAT/CD36 and FABPpm have been suggested to mediate LCFA uptake by accelerating fatty acid dissociation from albumin (Fig. 1; arrow 2) [41,43,68–70]. In addition, FAT/CD36 has been proposed to promote facilitated diffusion by catalyzing the integration of protonized LCFA into the outer phospholipid bilayer [69]. Consequently, accumulation of LCFA in the outer layer would occur, creating a diffusional gradient across the plasma membrane, thus facilitating LCFA flip-flop to the inner membrane [69].

Another aspect of their functioning is that each of these transporters might not operate independently but interact with other transporters to form a functional transport unit. An interaction between FAT/CD36 and FABPpm in the fatty acid uptake process has previously been proposed [7,15,68]. The view that both proteins might act in conjunction is plausible, since specific inhibition of either FAT/CD36 or FABPpm reduced LCFA uptake into the heart to a similar extent (~80%), while simultaneous inhibition of FAT/CD36 and FABPpm did not lead to an additional decrease in LCFA uptake [61].

In addition, FATP1 and FATP6 are co-expressed with FAT/CD36 in cardiac sarcolemmal regions [40,71], suggesting that LCFA uptake might take place via an interaction of FAT/CD36 with both members of the FATP family as well (Fig. 1; arrow 3), as proposed in earlier reports [15,35,40,70]. Alternatively, FATP1 and FATP6 might directly transfer LCFA across the plasma membrane (Fig. 1; arrow 4) [35,72]. Since VLACS activity has been reported for FATP1, LCFA uptake across lipid bilayers might thus be facilitated via esterification-coupled influx, also referred to as ‘metabolic trapping’ [40,43]. LCFA would subsequently bind to FABPc for transcytoplasmic transport to subcellular targets [6,7,73].

In the past years, animal models have become available that have allowed exploration of the question of the *in vivo* relevance of plasma membrane LCFA transporters [74–77]. Studies in FAT/CD36 transgenic and null mice emphasized a physiological role for FAT/CD36 by illustrating that altered changes in muscle LCFA uptake rates paralleled the changes in FAT/CD36 expression [74–76,78,79]. Yet, given the multifunctional role of this LCFA transport protein and the fact that FAT/CD36 null mice are viable and function normally, it has been argued that these animal models do not necessarily provide a conclusive demonstration of the role of membrane transporters in LCFA transport [49]. Nevertheless, FAT/CD36 null mice show impaired uptake of the LCFA analog 15-(*p*-iodophenyl)-3-(*R*, *S*) methyl pentadecanoic acid (BMIPP) into heart and skeletal muscles *in vivo* [74]. In addition, more recently Kuang et al. [79] studied substrate utilization by the isolated working heart of FAT/CD36 deficient mice to show that despite a 40–60% reduction in the LCFA oxidation rate, a proper cardiac function was maintained because of a compensatory increase in the rate of glucose oxidation. These findings demonstrate both the role

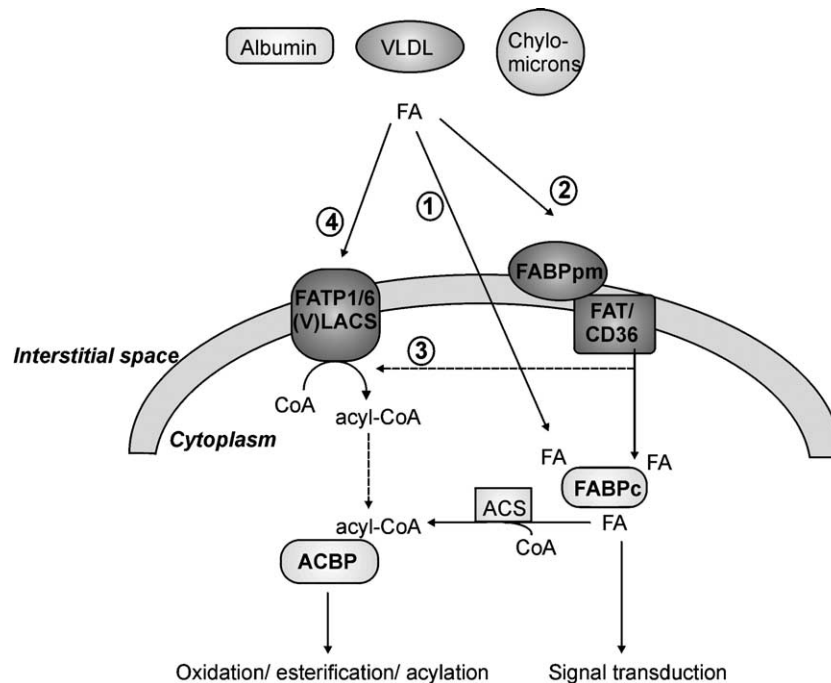


Fig. 1. Schematic representation of LCFA transport in heart and skeletal muscle. The numbers 1–4 illustrate the proposed routes of LCFA uptake. Besides passive diffusion (1), there is evidence to suggest that the interaction of FAT/CD36 and FABPpm in protein-mediated LCFA transport accelerates the dissociation of LCFA from albumin (2). FAT/CD36 has also been proposed to interact with FATP for subsequent uptake of LCFA (3). Alternatively, FATP has been suggested to transfer LCFA directly across the plasma membrane (4). Once in the cytoplasm, LCFA are bound to FABPc, or upon activation to acyl-CoA at the AMP-binding site of FATP the formed acyl-CoA esters will bind to acyl-CoA-binding protein (ACBP) before entering metabolic or other pathways (redrawn from [7,59]).

of FAT/CD36 in cardiac LCFA utilization as well as the metabolic flexibility of the heart.

### 2.3. LCFA uptake in heart and skeletal muscle: a role for lipid rafts and caveolae?

In addressing the possible mechanisms involved in LCFA uptake, the existence of lipid rafts and caveolae is worthwhile mentioning. Lipid rafts are specialized microdomains of the plasma membrane that are enriched in sphingolipids and cholesterol [80]. A specific subset of lipid rafts is formed by the caveolae, dynamic structures that form invaginations of the plasma membrane, and can excise from this membrane to form cytoplasmic vesicles. The coat structure of caveolae is formed by the caveolins, a family of integral membrane proteins [81,82]. Each isoform has a specific tissue-dependent distribution pattern. Caveolin-1 and -2 are predominantly found in adipocytes and endothelial cells, whereas caveolin-3 is expressed exclusively in all muscle cell types [83]. In addition to their roles in cholesterol homeostasis, vesicular transport and signal transduction [84–87], several studies in cell lines and caveolin-1 knockout mice have proposed a role for lipid rafts/caveolae and caveolin-1 in cellular LCFA uptake [80,82,88–92]. In adipocytes where FAT/CD36 is abundantly expressed [93], FAT/CD36 has been found in lipid rafts and caveolae [80,94], suggesting that lipid rafts regulate the expression and function of FAT/CD36 at the level of the plasma membrane [80]. Furthermore, a role of caveolin-1 in LCFA uptake has been suggested in human endothelial cells [91]. A colocalization of FAT/CD36 and caveolin-1 has been observed in

different cell types [94,95] and when cotransfected with caveolin-1, FAT/CD36 was targeted to the plasma membrane in COS-7 cells [95], indicating a facilitative role for caveolin-1 in intracellular trafficking, functioning and regulation of FAT/CD36 [95].

In contrast to their aforementioned role in cellular LCFA uptake in adipocytes [80,92,94], there is yet insufficient data to indicate a role for lipid rafts/caveolae in LCFA uptake and regulation of FAT/CD36 in cardiac and skeletal muscle. In different muscle types, caveolin-3 expression correlates positively with that of LCFA transport and transporters, including FAT/CD36, with each of these parameters being highest in heart [96–98], suggesting that caveolae assist in muscle LCFA transport. Furthermore, their combined presence in sarcolemmal giant vesicles, which are derived from specific plasma membrane regions [96], suggests that both proteins are in each others neighbourhood.

### 3. Acute regulation of LCFA uptake at the plasma membrane level: a role for FAT/CD36

In recent years, evidence has emerged that the plasma membrane is an important site for controlling LCFA utilization in heart and skeletal muscle, in addition to the entry of acyl-CoA into the mitochondria [8,10,99]. Both acute exercise and the hormone insulin have been shown to regulate LCFA uptake by altering the presence of FAT/CD36 at the plasma membrane of muscle cells as outlined in detail below. Translocation of FAT/CD36 has also been described in non-muscle cells, such as platelets [100,101] and type II pneumocytes [102]. Although

‘translocation’ is not an uncommon phenomenon as previously seen in the regulation of glucose uptake by GLUT4, we are only beginning to appreciate this event as a regulatory mechanism in LCFA uptake in both tissues. Identifying the components and intracellular signaling pathways involved in the induction of FAT/CD36 translocation by different stimuli will lead to a better understanding not only of the regulation of cellular LCFA uptake but also of the consequences of metabolic diseases for cardiac substrate handling.

### 3.1. Effect of short-term electrical contractions on LCFA uptake

The first line of evidence to support a regulatory mechanism of LCFA uptake at the plasma membrane was provided by studies in individuals undergoing regular exercise [3] and by applying short-term electrical contractions (<30 min) to rat skeletal muscle in vivo [103,104]. Although direct proof was lacking, it was suggested that LCFA uptake in contracting muscles could be linked to either an activation or translocation of LCFA transporters to the membrane. In subsequent work, several lines of evidence demonstrated that LCFA uptake at the plasma membrane could be regulated by increasing the amount of FAT/CD36 on the cell surface [20]. Since LCFA uptake was increased in giant vesicles prepared from contracting muscle (Fig. 2A), and this increase was inhibited by sulpho-*N*-succinimidyl-LCFA esters, it was concluded that regulation of LCFA uptake by short-term

electrical contractions involved a FAT/CD36-mediated mechanism [20]. Subsequently, fractionation studies in resting muscle confirmed the presence of FAT/CD36 both at the surface and in an intracellular membrane compartment. The onset of contractions caused a marked decrease in the amount of intracellular FAT/CD36, whereas FAT/CD36 at the plasma membrane was simultaneously increased (Fig. 3) [20]. Since there is no de novo synthesis of FAT/CD36 within the short period (30 min) of muscle contractions, acute regulation of LCFA uptake most likely occurs through a translocation of FAT/CD36 from an intracellular membrane compartment to the plasma membrane [20].

More recently, we [105] investigated the effect of contractions on FAT/CD36 and LCFA uptake in adult cardiac myocytes by using electrical field stimulation. Stimulation (4 Hz, 30 min) increased palmitate uptake (Fig. 2B) and oxidation, while esterification into cellular lipid pools was not significantly altered [105]. Thus, during contractions the extra LCFA taken up are efficiently channelled into mitochondrial  $\beta$ -oxidation. The increase in palmitate uptake was blocked in the presence of sulfo-*N*-succinimidyl-LCFA esters, indicating that LCFA uptake in contracting cardiac myocytes as in skeletal muscle is increased due to increased flux through FAT/CD36 [105]. Subcellular fractionation of cardiac myocytes yielded solid evidence for a translocation of FAT/CD36, similar to that in skeletal muscle, as the underlying mechanism to increase LCFA uptake during contraction [106] (Fig. 3).

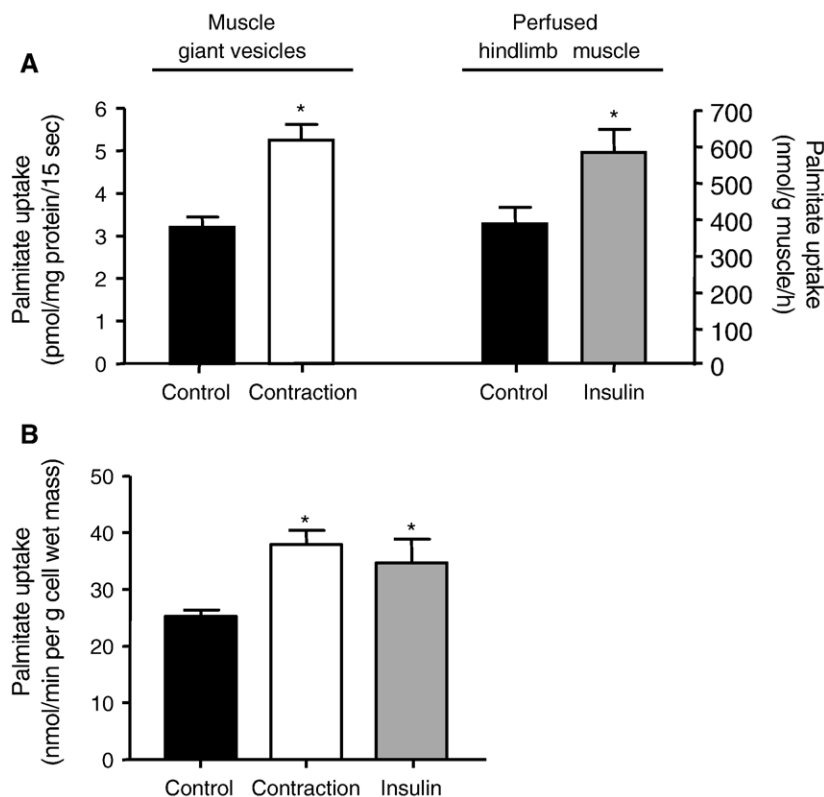


Fig. 2. Effect of insulin and contractions on palmitate uptake in muscle giant vesicles, hindlimb muscle and cardiac myocytes. Palmitate uptake is increased in giant vesicles derived from 30 min stimulated skeletal muscle (A, left panel) and in cardiac myocytes stimulated to contract (B). In addition to contractions, palmitate uptake is enhanced by the hormone insulin in both perfused hindlimb muscle (A, right panel) and cardiac myocytes (B) (redrawn from [20,106,150]).

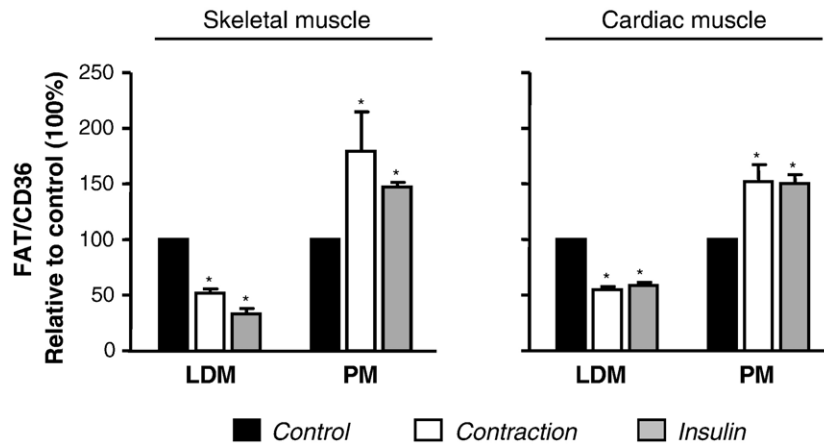


Fig. 3. Effect of insulin and contractions on cellular distribution of FAT/CD36 in heart and skeletal muscle. In the presence of insulin, the FAT/CD36 content in the intracellular fraction (LDM) is reduced, while concomitantly the expression of FAT/CD36 in the plasma membrane (PM) fraction is increased in both muscle (left panel) and heart (right panel). Likewise, contractions induce the translocation of intracellularly stored FAT/CD36 (LDM) to the plasma membrane (PM) in skeletal muscle (left panel) and heart (right panel) (redrawn from [20,106,150,151]).

### 3.2. Contraction-mediated signaling pathway

Exercise is known to stimulate components of several intracellular signaling pathways [107]. The underlying mechanism of contraction-induced translocation of FAT/CD36 thus might be linked to either the activation of the sympathetic nervous system, the actual metabolic status of the muscle during contraction, or the intramyocellular  $\text{Ca}^{2+}$ -concentration (for review, see [108]). Likely candidates for kinases that may regulate LCFA transport in heart and muscle cells are therefore protein kinase A (PKA), adenosine monophosphate (AMP)-activated protein kinase (AMPK), members of the protein kinase C (PKC) family, and mitogen-activated protein kinase (MAPK).

#### 3.2.1. Protein kinase A

It is well documented that myocyte contractility is regulated by intracellular levels of adenosine 3':5'-cyclic monophosphate (cAMP). Effects of cAMP on contraction are mediated through protein-kinase A (PKA)-induced phosphorylation of proteins involved in myocardial  $\text{Ca}^{2+}$  regulation [109]. At least in heart, PKA has been shown to inhibit acetyl-CoA carboxylase (ACC), the rate-limiting enzyme in malonyl-CoA formation which has been shown to control LCFA oxidation [110].

Recently, it was confirmed that in rat cardiac myocytes LCFA oxidation is enhanced and esterification is simultaneously reduced via a cAMP-dependent PKA mechanism [111]. However, in this study there was no change in net LCFA uptake when PKA was either activated by the  $\beta$ -agonist isoproterenol or by the cell permeable analogue dibutyryl cAMP [111]. In contrast, by applying an alternative strategy to increase intracellular cAMP levels, i.e., by inhibition of cAMP breakdown via several phosphodiesterase (PDE) inhibitors, LCFA uptake was differently affected. For instance, amrinone and zaprinast did not enhance LCFA uptake. Interestingly, other PDE inhibitors, such as 3-isobutyl-1-methylxanthine (IBMX), milrinone and dipyridamole (DPY) did stimulate LCFA uptake in isolated cardiac

myocytes. Evidently, the mechanism of action of these latter PDE inhibitors is unrelated to the induced elevation of cAMP [111]. Both IBMX and milrinone were found to enhance the intrinsic activity of FAT/CD36 at the plasma membrane, while DPY induced a translocation of FAT/CD36 from intracellular stores to the membrane to increase LCFA sarcolemmal uptake [111]. With respect to IBMX and milrinone, a common mechanism to increase the intrinsic activity of a given protein includes a Ser/Thr phosphorylation. FAT/CD36 contains several potential phosphorylation sites [112], suggesting that IBMX stimulates cardiac LCFA uptake by FAT/CD36 phosphorylation. It can only be speculated (see Section 3.2.4) which signaling mechanisms are involved. With respect to DPY, another well-described cellular target of DPY is the adenosine transporter which is markedly inhibited in its presence [113]. However, neither varying concentrations of adenosine nor the adenosine uptake inhibitor nitrobenzylthioinosine influenced cardiac LCFA uptake [114], excluding a role of adenosine in DPY-stimulated LCFA uptake. Interestingly, the stimulatory action of DPY on LCFA uptake is non-additive to that of AMPK-activating stimuli (see next section).

#### 3.2.2. AMP-activated protein kinase

AMPK, which acts as a metabolic sensor, can be activated by a change in energy state of the cell, as reflected by increases in the AMP/ATP ratio [115] and the creatine/phosphocreatine ratio [116]. Exercise is an important physiological stimulator of the AMP/ATP ratio, but this ratio can also be increased pharmacologically by the mitochondrial inhibitor oligomycin [106] (Fig. 4). The cell-permeable adenosine analogue 5'-aminoimidazole-4-carboxamide-1- $\beta$ -ribofuranoside (AICAR), through formation of AICAR monophosphate (ZMP), can also activate AMPK [117]. In addition, AMPK can be allosterically activated through phosphorylation by an upstream AMPK-kinase (AMPKK) [118].

AMPK activation has been shown to promote LCFA oxidation in heart [119,120] and in skeletal muscle [117,121]

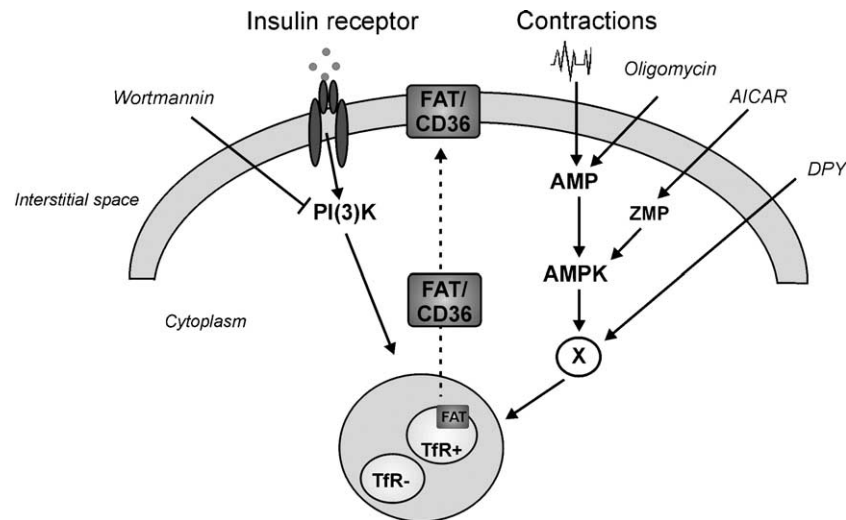


Fig. 4. Schematic representation of the main signaling components involved in the mobilization of FAT/CD36 by insulin and contractions in heart and skeletal muscle. Insulin recruits intracellular FAT/CD36 via a phosphatidylinositol-3-OH-kinase (PI(3)K)-dependent mechanism, since the PI(3)K-inhibitor wortmannin reduces LCFA uptake [151]. Cellular contractions elevate intracellular AMP, activate AMP-activated protein kinase (AMPK) and hence induce FAT/CD36 translocation [106]. LCFA uptake can be enhanced by pharmacological activation of contraction-induced signaling by the mitochondrial  $F_1F_0$ -ATPase inhibitor oligomycin and the cell permeable adenosine analog 5-aminoimidazole-4-carboxamide-1- $\beta$ -D-ribofuranoside (AICAR) [106]. The PDE inhibitor dipyridamole (DPY) provokes FAT/CD36 translocation via stimulation of an unknown target (X) downstream of AMPK [114].

through the phosphorylation and inhibition of acetyl-CoA carboxylase (ACC). ACC produces malonyl-CoA, which inhibits carnitine palmitoyltransferase I (CPT1), the enzyme controlling the transfer of LCFAs into the mitochondria [117]. Thus, in these tissues activation of AMPK results successively in a reduction of ACC activity, a decrease in malonyl-CoA levels, and a stimulation of LCFA oxidation. For an extended overview of the putative roles of AMPK on metabolism and exercise, the reader is referred to recent reviews by Hardie [122] and Aschenbach et al. [123].

The ability of AICAR and oligomycin to stimulate LCFA uptake in a non-additive manner to electrical stimulation provided the first evidence that AMPK activation is involved in contraction-induced FAT/CD36 translocation in cardiac myocytes [106]. Previously, non-specific effects of AICAR, unrelated to AMPK activation, have been reported on intermediary metabolism [124,125]. In the presence of the adenosine kinase inhibitor 5-iodotubercidin, the effect of AICAR on LCFA uptake was, however, abolished, indicating that AMPK activation by ZMP formation is necessary for AICAR to stimulate LCFA uptake [106]. As a result, the activation of AMPK has a wider impact on substrate metabolism than had previously been presumed. Also, DPY stimulates LCFA uptake in a non-additive manner to AMPK-activating stimuli [114]. Nonetheless, DPY does not activate AMPK suggesting that DPY stimulates a signaling enzyme downstream of AMPK leading to FAT/CD36 translocation, as illustrated in Fig. 4.

Activation of AMPK not only promotes LCFA oxidation [117,119–121,126,127] and glucose uptake through translocation of the glucose transporter GLUT4 [117,128,129] but also has a potent effect on LCFA uptake through induction of FAT/CD36 translocation [106]. This simultaneous regulation of LCFA uptake and oxidation allows an efficient channelling of

the incoming LCFA into mitochondrial  $\beta$ -oxidation during increased energy demands.

### 3.2.3. Protein kinase C

Other candidate kinases mediating contraction-induced FAT/CD36 translocation include the PKC family consisting of at least twelve isoforms. There are three classes of PKC isoforms [130], depending on their ability to be activated by  $Ca^{2+}$  or by the lipid metabolite diacylglycerol (DAG): (i) the class of conventional PKC isoforms, activated by both  $Ca^{2+}$  and DAG; (ii) novel PKC isoforms, activated by DAG only; (iii) atypical PKC isoforms, activated by neither  $Ca^{2+}$  nor DAG but by other lipids such as phosphatidylinositol phosphates and phosphatidic acid. Muscle contraction has been shown to elevate both DAG and phosphatidic acid [122], and hence activate PKC signaling [131,132], suggesting a role for this protein kinase family in contraction-induced changes in muscle metabolism [121]. More recently, it was observed that contractions induce isoform-specific PKC activation in electrically stimulated cardiac myocytes [133,134]. The phorbol ester, phorbol 12-myristate 13-acetate (PMA), is a cell-permeable DAG analogue frequently used to pharmacologically activate the conventional and novel PKC isoforms. PMA has been shown to induce the translocation of GLUT4 in adipocytes, leading to enhanced glucose uptake [135,136]. Recently, it was shown that PMA stimulates both glucose and LCFA uptake in rat cardiac myocytes, but PMA was without effect on AMPK activation [137]. Nonetheless, in the presence of specific PKC inhibitors, AMPK activation does not result in substrate transporter translocation [137]. Conventional and/or novel PKC isoforms might, therefore, be involved in contraction-induced translocation of both GLUT4 and FAT/CD36 in cardiac myocytes as a potential downstream target of AMPK. Interestingly,  $Ca^{2+}$ -modulating agents do not stimulate sub-

strate uptake by cardiac myocytes, hence  $\text{Ca}^{2+}$ -signaling is probably not necessary for the induction of substrate transporter translocation [137]. These observations suggest that downstream of AMPK, but upstream of PKC, a lipid metabolizing enzyme must be activated to generate DAG necessary for subsequent PKC activation and translocation of GLUT4 and FAT/CD36.

### 3.2.4. MAPK signaling

Mitogen-activated protein kinases (MAPKs) are ubiquitous signaling proteins involved in control of cell growth, differentiation and adaptation. Four parallel MAPK signaling cascades have been identified and the MAPK family members include the classic extracellular signal-regulated kinase (ERK) 1 (p44 MAPK) and 2 (p42 MAPK), the stress-activated protein kinase (SAPK)/Jun N-terminal kinases (JNK), p38 MAPK, and ERK3, -4 and -5 [138]. Phosphorylation is required for the activation of MAPK, and for the subsequent phosphorylation of different cytoplasmic targets or translocation to the nucleus to affect transcription [139]. Cardiac and skeletal muscle cells have been shown to express components of MAPK signaling pathways [140,141], and MAPK signaling has been suggested to mediate substrate uptake and metabolism in muscle in response to exercise [107,140,142,143]. Physical exercise is a potent stimulator of p42/44 MAPK and p38 MAPK signaling in human and skeletal muscle [107,141,144]. Activation of upstream kinases and downstream targets of p42/44 and p38 MAPK signaling have been seen in response to exercise and muscle contraction evoked by electrical stimulation [141,143,145,146]. Substantial evidence has ruled out a role for contraction-activated p42/44 MAPK signaling in the acute regulation of glucose transport and glycogen synthesis in skeletal muscle [140,142]. In contrast, activation of p38 MAPK is likely to participate in the stimulation of glucose uptake in contracting skeletal muscles [143]. Partial reductions in contraction-induced glucose uptake have been observed in rat skeletal muscle by SB203580, a specific inhibitor of the p38 MAPK $\alpha$  and - $\beta$  isoforms [143]. Since p38 MAPK is not likely to interfere with GLUT4 translocation from intracellular stores to the cell surface [147], p38 MAPK has been proposed to function as an integral component of the signaling pathway regulating GLUT4 activity.

With respect to LCFA uptake, an increase in intrinsic activity of FAT/CD36 has been seen in cardiac myocytes upon incubation with the PDE inhibitors milrinone and IBMX [111] (see also Section 3.2.1) (Fig. 4). However, it is highly speculative to suggest a role for MAPK herein. To date, it remains to be investigated whether MAPK pathways might be involved in the acute regulation of contraction-induced LCFA uptake by FAT/CD36.

### 3.3. Effect of insulin on regulation of LCFA transport

Insulin is the most potent anabolic hormone in mammals and has a profound impact on cellular metabolism in general (for review see [148]). Insulin promotes the synthesis and storage of carbohydrates, lipids and proteins, inhibits their

degradation and release into the circulation, and stimulates glucose and amino acid uptake into cells.

Recently, evidence was provided for a stimulatory role of insulin on LCFA uptake in both cardiac myocytes and skeletal muscles [149–151] (Fig. 2). This acute effect of insulin on LCFA uptake was attributable to a translocation of intracellular stored FAT/CD36 to the plasma membrane, thereby promoting sarcolemmal LCFA uptake, both in vitro and in vivo [150,151] (Fig. 3). Notably, in the presence of insulin, the extra palmitate taken up is exclusively directed toward lipid esterification into triacylglycerols (TAG) [149,150]. Furthermore, in skeletal muscle, insulin has been shown to increase LCFA esterification while simultaneously reducing LCFA oxidation [149]. This channelling of LCFA into TAG might be due to simultaneous activation of glycerol-3-phosphate acyltransferase (GPAT), the enzyme that catalyses the initial and committed step in TAG synthesis [121]. In addition, negative regulation of AMPK signaling by insulin resulting in a reduced CPT1 activity could further add to redirecting LCFA from oxidative pathways into storage [152–155]. Finally, insulin is believed to be the most important anti-lipolytic hormone and might contribute, via lowered cAMP levels, to the increase in LCFA esterification by inhibiting hormone sensitive lipase, a key enzyme in the mobilization of LCFA from stored TAG [156].

### 3.4. Signaling events involved in insulin-induced FAT/CD36 translocation

#### 3.4.1. PI(3)K-dependent insulin signaling pathway

In unraveling the signaling pathway involved in the stimulation of LCFA uptake by insulin-induced FAT/CD36 translocation, it was observed that blocking phosphatidylinositol-3-OH kinase (PI(3)K) by structurally unrelated PI(3)K inhibitors (wortmannin, LY-294002) prevented the insulin-induced translocation of FAT/CD36 in cardiac and skeletal muscles [150,151]. This clearly shows that a PI(3)K-dependent mechanism is involved in the signal transduction pathway utilized by insulin to stimulate LCFA uptake in these tissues. Since activation of PI(3)K is known to promote glucose uptake through translocation of GLUT4 [157], LCFA uptake presumably requires a similar mechanism, including PI(3)K activation. Therefore, insulin stimulation of PI(3)K in LCFA uptake most likely also includes autophosphorylation of the insulin receptor, activation of intrinsic receptor kinase activity, and subsequent phosphorylation of insulin receptor substrates (IRS-1/-2) [158]. Two downstream effectors of PI(3)K have been proposed in insulin-stimulated GLUT4 translocation, i.e., the serine/threonine Akt also referred to as protein kinase B (Akt/PKB) [159], and atypical (and phorbol ester insensitive) isoforms of protein kinase C, i.e., PKC  $\zeta$ , and PKC  $\lambda$  [160]. Whether similar downstream targets of PI(3)K are required for FAT/CD36 translocation is currently unknown.

#### 3.4.2. MAPK signaling

Besides the classical IRS/PI(3)K-PKB axis, other signaling pathways are activated by insulin and contribute to changes in muscle metabolism, among which the mitogen-activated

protein kinases (MAPK) superfamily. While contractions have been shown to activate MAPK signaling (section 3.2.4), insulin has also been shown to activate MAPK signaling in skeletal muscle including both p38 MAPK and p42/44 MAPK [107,142,143,161]. The classic ERK1 and ERK2 MAPK (p42/44 MAPK), however, do not play a major role in mediating insulin's metabolic responses. Namely, the activation of glucose and amino acid transport and of glycogen synthase in response to insulin have been shown to be independent of p42/44 MAPK signaling [142]. However, the role of insulin induction of p42/44 MAPK in enhancing LCFA transport is completely unknown at present. Furthermore, insulin activates p38 MAPK $\alpha$  and p38 MAPK $\beta$  [143,161,162], and inhibition of p38 MAPK has been shown to reduce the insulin-stimulated glucose uptake in skeletal muscle. Because p38 MAPK is not involved in the regulation of GLUT4 translocation to the plasma membrane (see also Section 3.2.4), p38 MAPK has been proposed to regulate glucose transport by increasing the intrinsic activity of GLUT4 on the membrane [143,161,163]. A possible analogous role of p38 MAPK in enhancing the intrinsic activity of FAT/CD36 by IBMX/milrinone (see Section 3.2.1) remains speculative.

### 3.5. FAT/CD36 intracellular storage pools

From the novel observations outlined above, it can be pointed out that there is a remarkable parallel between the acute regulation of FAT/CD36-mediated LCFA uptake and the regulation of glucose uptake by GLUT4 in heart and skeletal muscles. In line with glucose uptake, insulin and contractions exhibit an additive effect on LCFA uptake in heart and skeletal muscle [106,149,151]. Notably, PI(3)K-signaling is not involved in the regulation of LCFA uptake in electrostimulated and AICAR-/oligomycin-treated cardiomyocytes [106,151], and vice versa AMPK activation is not part of the insulin-signaling pathway involved in the recruitment of FAT/CD36 [106]. Therefore, it might be emphasized that at least two separate signaling pathways exist by which FAT/CD36 can be recruited to the cell surface. Since identical signaling pathways are involved in the intracellular recruitment of GLUT4 [129,164,165], it might be hypothesized that both FAT/CD36 and GLUT4 reside in similar intracellular storage compartments. Identification of GLUT4 containing intracellular pools revealed the existence of both large and small depots, representing the GLUT4 specific pre-endosomal storage compartment and the recycling endosomal compartment, respectively [166–168]. These small depots, i.e., recycling endosomes, can be subdivided into transferrin receptor (TfR)-positive depots primarily recruited by contractions, and TfR-negative depots mainly recruited by insulin [167,169].

Co-localization of FAT/CD36 and the majority of intracellularly stored GLUT4 was, however, not observed, excluding the GLUT4-specific pre-endosomal storage compartment as an intracellular depot of FAT/CD36 [170]. The same authors did find a co-localization between FAT/CD36 and a member of the Rab family, rab11, which predominantly resides within the

recycling endosomes [170]. This observation emphasizes the possible involvement of recycling endosomes in the storage of intracellular FAT/CD36. Further biochemical and/or immunohistochemical studies are needed to examine whether FAT/CD36 is stored intracellularly into similar contraction- and insulin-sensitive depots within the recycling endosomes [171]. In this context, it should be mentioned that the intracellular presence of FAT/CD36 in human skeletal muscle was confirmed by immunohistochemical studies performed by one group [97], but not by another group of researchers [98]. The discrepancy between these observations presently is not clear.

## 4. LCFA uptake in health and disease

### 4.1. Physiological significance of LCFA transporter expression in LCFA uptake

Over the last few years, the physiological role of the putative fatty acid transporters FAT/CD36 and FABPpm in LCFA uptake have become more evident. Regulation of LCFA uptake in response to several stimuli has been observed in cardiac and skeletal muscle. Cardiac gene expression of FAT/CD36 is increased in a rat model of short bowel syndrome to compensate for the decreased supply of LCFA to the heart due to malabsorption of dietary fat intake [172]. Treatment of these rats with a high-fat diet reduced FAT/CD36 expression in the rat myocardium [172].

In muscle, the LCFA uptake system is capable of adapting to changes in muscle activity, diet and aging by changing the expression levels of the putative LCFA transport proteins FAT/CD36 and/or FABPpm. The cellular content of FABPpm has been shown to increase after long-term exercise, such as endurance training [23,173]. In contrast, FABPpm gene and protein expression were not increased after short-term exercise training for 9 days, while LCFA oxidation was significantly increased, and adaptations were seen in FAT/CD36 protein contents [37]. On the other hand, chronic low frequency muscle stimulation has been shown to enhance LCFA uptake, while FAT/CD36 and FABPpm protein expression and sarcolemmal presence were concomitantly increased [19,22]. An increased abundance of skeletal muscle FABPpm has been observed in short-term fasting, a nutritional intervention resulting in an increased reliance on LCFAs as oxidizable fuel [174]. Rapid increases in FAT/CD36 gene expression have been shown after lipid infusion in human adipocytes [175] and in rat skeletal muscle [176]. Similarly, increases in FAT/CD36 protein in skeletal muscle have been reported after high-fat feeding [177]. The increase in LCFA uptake seen in skeletal muscle of aged rats (24-months) has recently been reported to coincide with increased FAT/CD36 and FABPpm protein contents [178].

### 4.2. LCFA transporter relocation

In line with all these observations, a change in the level of gene and protein expression has been commonly seen as

underlying mechanism in this adaptation process. Gene and/or protein expression is, however, not always altered, as observed in chronic leptin administration, in obesity, and after sciatic nerve denervation in which LCFA uptake is significantly changed (Table 1). Palmitate transport rates into giant sarcolemmal vesicles, as well as FAT/CD36 mRNA and protein expression were reduced following 2 weeks of leptin treatment [179]. However, FABPpm mRNA and total protein expression were not altered, whereas less FABPpm was present on the plasma membrane [179]. Recently, additional evidence was provided for an altered subcellular distribution pattern, i.e., relocation, of both FAT/CD36 and FABPpm. We observed that 7 days of muscle inactivity (sciatic nerve denervation) concomitantly reduced LCFA uptake and sarcolemmal expression of FAT/CD36 and FABPpm, whereas transporter protein expression in the denervated hindlimb was unaltered [19]. Reciprocally, increases in plasma membrane expression of FAT/CD36 and FABPpm and in LCFA uptake were observed in heart and skeletal muscle of obese Zucker rats, while protein expression was not altered [180]. Collectively, these data suggest that cycling of these transporters between the plasma membrane and the intracellular depot has been altered chronically. Most likely, this impaired transporter cycling might be due to an increased exocytosis (release) of stored LCFA transporters, a decrease in endocytosis (internalization) of plasma membrane-bound LCFA transporters, or a combination of both.

#### 4.3. LCFA uptake and insulin resistance

Many insulin-resistant states such as obesity and type 2 diabetes have been associated with an increase in plasma LCFA and an accumulation of intramuscular triacylglycerols (TAG) [181]. This intracellular rise in TAG by itself is not harmful, but is an indicator of elevated levels of other lipid intermediates such as DAG, ceramides and long-chain acyl-CoAs [181–183]. These lipid intermediates have been proposed to interfere in the insulin-signaling cascade by activating several isoforms of PKC and by inhibiting Akt/PKB activation. For instance,

DAG might lead to activation of PKC  $\delta$  and  $\epsilon$  [183–185]. These PKC isoforms can phosphorylate and inhibit tyrosine kinase activity of the insulin receptor and IRS-1, thereby preventing insulin to properly stimulate glucose transport into muscle and causing peripheral insulin resistance [183,186]. Ceramides might interfere with insulin signaling at a more downstream level as these metabolites are regarded as specific inhibitors of Akt/PKB activation [186]. Finally, acyl-CoAs might directly affect glucose utilization by altering the activity of glycolytic enzymes [187].

Skeletal muscle accounts for the majority of insulin-stimulated glucose utilization, and is, thus, regarded as the major site of insulin resistance in obesity and type 2 diabetes [188]. However, dramatic increases in myocardial TAG content are positively correlated with the development of insulin resistance as well [189–191]. To date, the mechanism(s) responsible for accumulation of TAG and lipid derivatives in both heart and skeletal muscle are incompletely understood. Several mechanisms have been postulated, and it remains to be investigated whether LCFA-mediated insulin resistance is the result of (i) a decrease in the LCFA oxidation rate, or an increase in LCFA uptake, either by (ii) changes in delivery/supply or, alternatively, (iii) changes in the protein-mediated LCFA uptake process itself.

##### 4.3.1. Ad (i) Oxidation

A defect in LCFA oxidation, i.e., reduced activity of oxidative enzymes in skeletal muscle has been found in obesity and in relation to insulin resistance [192–195]. It is questionable, however, whether an altered oxidation rate might be the primary factor in developing lipid-mediated insulin resistance. Although LCFA oxidation is decreased in skeletal muscle of extremely obese individuals, no reduction in oxidative rate has been observed in skeletal muscle of moderately obese/overweight humans [196–198]. Moreover, accumulation of intramyocellular long-chain fatty acyl-CoAs is not solely a result of reduced LCFA oxidation, since elevations in long-chain fatty acyl-CoAs content have been observed in skeletal muscles of both extremely and moderately obese

Table 1  
Effects of physiological and pathophysiological stimuli on LCFA uptake and expression of the putative LCFA transporters FAT/CD36 and FABPpm in heart and skeletal muscle

Condition	LCFA uptake	Total content		Sarcolemmal content	
		FAT/CD36	FABPpm	FAT/CD36	FABPpm
<i>Interventions (muscle tissue)</i>					
Acute stimulation (20, 105, 111)	↑	=	=	↑	↑
Chronic stimulation (19, 22)	↑	↑	↑	↑	↑
Denervation (19)	↓	=	=	↓	↓
Leptin treatment (179)	↓	↓	=	↓	↓
Aging (178)	↑	↑	↑	nd	nd
<i>Disease-related (cardiac tissue)</i>					
STZ-rats (214)	↑	↑	↑	↑	↑
Obesity (180)	↑	=	=	↑	↑

An increase in total cellular expression of FAT/CD36 and FABPpm is one mechanism by which LCFA uptake is regulated, as observed in skeletal muscle upon chronic stimulation [19,22], during aging [178] and in streptozotocin-treated rats [214]. Regulation of LCFA uptake does not necessarily involve alterations in total protein expression. Instead, FAT/CD36 and FABPpm might be relocated towards the cell surface, as seen in acute stimulation [20,106,215] and in obesity [180], or relocated towards the storage compartment as observed in 7-day denervated hindlimb muscle [19] and upon leptin treatment [179].

humans with a reduction in oxidation rate only occurring in the former [197].

In hearts of pre-diabetic insulin-resistant obese Zucker rats, LCFA oxidation is only impaired upon fasting [190,199], whereas fasting did not affect LCFA oxidation in the isolated working heart of insulin-resistant JCR:LA-cp rats [191]. Hence, additional factors, i.e., supply or uptake, must be involved to explain the initial accumulation of TAG in insulin resistant heart and skeletal muscle.

#### 4.3.2. *Ad (ii) Supply*

Plasma LCFAs are commonly elevated in obesity and have previously been suggested to represent the link between obesity and insulin resistance [200]. An increase in LCFA delivery into the cell can trigger insulin resistance, as was shown in transgenic mice with muscle overexpression of lipoprotein lipase, the rate controlling enzyme involved in TAG hydrolysis [201]. Generally, there is good evidence to suggest that an increase in circulating LCFAs can induce insulin resistance by interfering with insulin signaling [181,200,202,203]. Acute elevations of plasma LCFA levels produce insulin resistance in healthy and diabetic subjects, while lowering chronically elevated plasma LCFA levels overnight results in significantly improved insulin sensitivity in obese diabetic patients [204–206]. However, plasma LCFAs and TAG are not increased in every model of insulin-resistance [180,201,207]. Notably, an increase in plasma LCFAs and TAG per se does not necessarily result in peripheral insulin resistance, but might be related to improvements in insulin sensitivity instead [208–210]. In mice overexpressing human apoC1, strongly elevated plasma LCFA levels were found in combination with increased insulin sensitivity [208]. Moreover, insulin sensitivity is improved in mice deficient for CD36, in which plasma LCFA and TAG levels are increased, due to the reduced peripheral LCFA uptake [209,210]. In addition, a recent report on FATP1 knockout mice indicated that, despite elevations in plasma LCFA levels, the homozygous null mice were protected from lipid-induced increases in intramuscular fatty acyl-CoA and insulin resistance [77]. Collectively, these studies indicate that insulin resistance is not increased by simply elevating the circulating plasma concentrations of LCFAs. Only when the excess of LCFAs have access to the cellular interior by means of the facilitative action of LCFA transporters, insulin sensitivity is impaired. Thus, altering the rate of LCFA transport into muscle cells may have a key role in insulin resistance.

#### 4.3.3. *Ad (iii) Uptake*

In both cardiac and skeletal muscle giant vesicles and cardiac myocytes obtained from obese, pre-diabetic Zucker rats, the rate of LCFA uptake is markedly upregulated [180,211,212]. The increase in LCFA transport is not associated with an increase in the total expression of FAT/CD36, but is rather associated with an increase in FAT/CD36 on the cell surface [180,211]. Although the observed increase in the cardiac TAG esterification rate was associated to a 2-fold increase in FAT/CD36 abundance at the plasma membrane

[199], more definite evidence for a direct link between increased LCFA flux and sarcolemmal FAT/CD36 levels was provided with the specific FAT/CD36 inhibitor, SSO [66]. FAT/CD36-mediated LCFA uptake, esterification into TAG, and the level of unesterified LCFAs in cardiac myocytes from obese Zucker rats were reduced by treatment of the myocytes with SSO to basal levels also seen in lean littermates [199]. Hence, sarcolemmal FAT/CD36 appears causally related to lipid accumulation in cardiac muscle cells.

Likewise, an elevated LCFA uptake rate has been observed in skeletal muscle of obese and type 2 diabetic patients [198]. Notably, the excess accumulation of intramuscular TAG was strongly associated with an upregulation of the LCFA transport rate across the sarcolemma due to an increased amount of FAT/CD36 on the cell surface [198].

Taken together, it is likely that impaired FAT/CD36 cycling is responsible for elevated LCFA uptake and accumulation of TAG in obese heart and skeletal muscle in rodents and humans. In addition, it is well known that GLUT4 cycling between the cell surface and intracellular depots is impaired in this condition, and that GLUT4 is retained in intracellular depots despite similar levels of total available GLUT4 [213]. Thus, impairment of GLUT4 and FAT/CD36 recycling in obesity and type 2 diabetes results in opposite effects on their subcellular redistribution, i.e., GLUT4 is retained within intracellular depots, whereas FAT/CD36 is retained at the cell surface [180].

Interestingly, in cardiac myocytes of 11-week-old pre-diabetic insulin-resistant obese Zucker rats displaying 7-fold elevated plasma insulin and normal glucose levels, basal and insulin-stimulated glucose uptake were not significantly different from lean littermates [211]. However, basal LCFA uptake was upregulated in cardiac myocytes of obese Zucker rats, and insulin lost the ability to further enhance LCFA uptake in these rats [211]. This suggests that, at least in the heart, alterations in FAT/CD36 regulation might be an early step during the development of insulin resistance.

The above evaluation might raise the paradoxical impression that by channelling LCFA from increased uptake to TAG stores insulin would evoke insulin resistance. However, one has to bear in mind that in the healthy condition insulin is only elevated postprandially and that the effect of insulin on FAT/CD36 translocation is reversible whereby FAT/CD36 is internalized upon normalisation of plasma insulin. In contrast, in early stage of insulin resistance plasma insulin is chronically elevated so that FAT/CD36 translocation is permanently stimulated resulting in its net relocation to the sarcolemma and an increased LCFA uptake rate.

## 5. Future perspectives

Since FAT/CD36 is likely to contribute to the etiology of insulin resistance, FAT/CD36 might provide a useful therapeutic target for the prevention or treatment of insulin resistance. Hence, disclosing the molecular mechanisms of FAT/CD36 translocation is an important area of research. Specifically, insight into the differences between the regulation of the translocation of FAT/CD36 and that of GLUT4 will help to

identify therapeutic strategies to allow selective manipulation of substrate transporter recruitment thereby restoring normal substrate balance. In this respect, it was recently found that the PDE inhibitor dipyridamole (DPY) specifically enhanced cardiac LCFA uptake by recruiting FAT/CD36 to the cell surface [111], while DPY did not influence glucose uptake nor did it induce GLUT4 translocation [114]. The stimulatory action of DPY on LCFA uptake was related to a PI(3)K-independent signaling mechanism and involves an as yet unknown target downstream of AMPK (see Section 3.2.2). It might be speculated that downstream of AMPK the contraction-induced signaling cascade branches off into two separate pathways, each leading to specific mobilization of intracellularly stored GLUT4 and FAT/CD36. Whether in analogy to the contraction-inducible recruitment of FAT/CD36 and GLUT4 from distinct contraction-responsive storage compartments, insulin also induces the recruitment of FAT/CD36 and GLUT4 from distinct insulin-responsive storage compartments, is presently unknown. In order to modify cardiac and skeletal muscle substrate preference, more research is needed to unravel the signaling kinases that are positioned downstream of either PI3K or AMPK, and that are dedicated to the specific recruitment of either FAT/CD36 or GLUT4.

Although FAT/CD36 has been recognised as responsible for bulk LCFA uptake in heart and skeletal muscles, it should be emphasized that still little is known about FABPpm, FATP1 and the heart-specific FATP6. Hormonal regulation of FATP1 by insulin has been observed in adipocytes [17], but to date no information is available whether FATP1 or FATP6 can be regulated in skeletal muscle and heart. Recent data from FATP1 knockout mice indicate an important role for FATP1 in the pathogenesis of type 2 diabetes [77]. Hence, the putative LCFA transporters FAT/CD36 and FATP1 are both likely to represent an attractive therapeutic target for the prevention or treatment of insulin resistance.

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