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## Regulation of cardiac long-chain fatty acid and glucose uptake by translocation of substrate transporters

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**Abstract** Cardiac uptake of long-chain fatty acids (FA) is mediated predominantly by two membrane-associated proteins, the 43-kDa plasma membrane fatty acid-binding protein (FABPpm) and the 88-kDa fatty acid translocase/CD36 (FAT/CD36). While FABPpm is present constitutively in the sarcolemma, FAT/CD36 is recycled between an intracellular membrane compartment and the sarcolemma. Since the amount of sarcolemmal FAT/CD36 is a major determinant of cellular FA uptake, understanding of the regulation of its recycling is likely to provide new insights into altering substrate preference of the heart. FAT/CD36 recycling displays a remarkable similarity with that of the two glucose transporters (GLUT) in the heart, GLUT1 and GLUT4. Translocation of all three transporters is induced by insulin and by contraction, which stimuli activate distinct signalling cascades. The insulin pathway involves phosphatidylinositol-3 kinase (PI3K) whilst the contraction pathway is dependent on AMP-activated protein kinase (AMPK). For the identification of additional protein components involved in the regulation

of FAT/CD36 recycling, valuable lessons can be learned from GLUT1 and GLUT4 recycling. Especially GLUT4 recycling is an intensively studied process in which a number of signalling proteins, both upstream and downstream from PI3 K and AMPK, have been identified, as well as proteins that are part of the translocation machinery involving Rab GTPases and soluble *N*-ethylmaleimide attachment protein receptors (SNAREs). Comparison of the magnitude of the effects of insulin and contraction on substrate uptake and on transporter appearance in the sarcolemma have revealed that FAT/CD36 recycling resembles GLUT1 recycling more closely than that of GLUT4. This pinpoints the recycling compartment and excludes a pre-endosomal storage compartment as the intracellular storage site for FAT/CD36. Further research will probably establish whether FAT/CD36 translocation is (partly) coupled to that of one or both GLUTs or, alternatively, whether it is a distinct process that also can be induced independently of GLUT1 or GLUT4 movement. In the latter case, a unique set of proteins would be dedicated to FAT/CD36 recycling, which would then provide an attractive target for manipulating cardiac substrate preference.

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**Abbreviations and definitions** *AICAR*: 5-aminoimidazole-4-carboxamide-1- $\beta$ -D-ribofuranoside, cell-permeable activator of AMPK · *AMPK*: AMP-activated protein kinase · *amrinone*: specific inhibitor of phosphodiesterase III · *CPT-I*: carnitine palmitoyl transferase I · *dibutyryl cyclic AMP*: cell-permeable analogue of cyclic AMP, mimics cyclic AMP-activated signalling · *DNP*: 2,4-dinitrophenol, mitochondrial uncoupling agent · *ERK*: extracellular signal-regulated kinase · *etomoxir*: specific inhibitor of CPT-I · *FA*: long-chain fatty acid(s) · *FABPpm*: plasma membrane fatty acid-binding protein · *FAT/CD36*: fatty acid translocase/CD36 · *GTP $\gamma$ S*: guanosine 5'-O-(3-thiotriphosphate), non-

hydrolysable GTP analogue, locks Rab proteins in a persistently active state · *IGF-II*: insulin-like growth factor II · *5-iodotubercidin*: specific inhibitor of adenosine kinase, prevents conversion of AICAR into ZMP · *IRAP*: insulin-responsive aminopeptidase · *IRS*: insulin receptor substrate · *isoproterenol*: potent  $\beta$ -agonist · *myristoylated PKC $\zeta$  pseudosubstrate*: cell-permeable, specific inhibitor of atypical PKCs · *oligomycin*: potent inhibitor of mitochondrial  $F_1F_0$ -ATPase · *PD98059*: specific inhibitor of mitogen-activated protein kinase signalling · *PI3K*: phosphatidylinositol-3 kinase · *PKB (PKB/Akt)*: protein kinase B · *PKC*: protein kinase C · *rotenone*: inhibitor of electron transfer in mitochondria · *SCAMP*: secretory carrier membrane protein · *SNAP23*: synaptosomal-associated protein (23 kDa) · *SNARE*: soluble N-ethylmaleimide attachment protein receptor · *SSO*: sulphonyl-succinimidylester, specific inhibitor of transport function of FAT/CD36 · *VAMP2 (-3)*: vesicle-associated membrane protein-2 (-3) · *VAP33*: vesicle-associated protein 33 · *wortmannin*: inhibitor of PI3K · *ZMP*: 5'-monophosphate of AICAR

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## Introduction

Long-chain fatty acids (FA) and glucose are the major substrates with which the heart sustains mechanical performance [122]. Currently, there is renewed interest in the regulation of cardiac utilization of these substrates because novel regulatory sites have been identified. Glucose uptake has been established firmly as the rate-limiting step in glucose utilization by muscle cells [102]. Internalized glucose is phosphorylated rapidly to glucose-6-phosphate, maintaining intracellularly sequestered glucose at a low level even when exogenous glucose concentrations are high. As a consequence, the outside-to-inside glucose gradient across the sarcolemma will remain steep and drives glucose towards cellular uptake. Similarly, FA uptake is governed by the FA gradient across the sarcolemma [122]. Even so, the first step in cellular FA metabolism, the thiolinkage of FA with coenzyme A to form acyl-CoA is a rapid event [86, 122]. Indeed, this rapid trapping of FA following cellular entry ensures that at high exogenous FA concentrations, i.e. at levels beyond which FA uptake is saturated, the intracellular level of free (i.e. non-metabolized) FA does not rise [67]. The fact that for both substrates—glucose and FA—transsarcolemmal transport appears to be the rate-limiting step implies that cardiac substrate preference is determined mainly at the level of the sarcolemma rather than by competition for oxidation, as proposed by Randle et al. [94]. For a better understanding of the interplay between FA uptake and glucose uptake at the sarcolemmal level, unravelling of the molecular mechanisms underlying these processes is of pivotal importance. Newer studies have indicated that specific membrane proteins are involved in the regulation of both cellular FA and glucose uptake.

This article begins with a review of the initial studies that identified the proteins involved in cardiac FA uptake.

One of these putative FA transporters, the fatty acid translocase/CD36 (FAT/CD36), recycles between the sarcolemma and intracellular stores and its translocation to the cell surface appears to be crucial for regulation of cardiac FA uptake. To unmask the components of the cellular FAT/CD36 translocation machinery and to obtain insight into the regulation of cardiac substrate preference, it is helpful to compare this event with what is already known about the translocation of GLUT1 and GLUT4, the major cardiac glucose transporters. Because GLUT4 recycling has been studied in more detail in skeletal muscle and adipose tissue, the mechanisms disclosed in these tissues will be discussed where appropriate. We realize that care must be taken in extrapolating findings from skeletal muscle to a cardiac setting, given the subtle differences in cardiac and skeletal muscle metabolism. But overall, the similarities far outweigh the differences. Moreover, GLUT4 translocation has been best investigated in adipocytes, which share with heart and skeletal muscle the ability to respond metabolically to insulin but, in contrast, lack, of course, exercise metabolism. Finally, an attempt has been made to integrate the various findings into an overall scheme of regulation of cardiac substrate transport by intracellular recycling of glucose and FA transporters.

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## Regulation of cardiac FA uptake

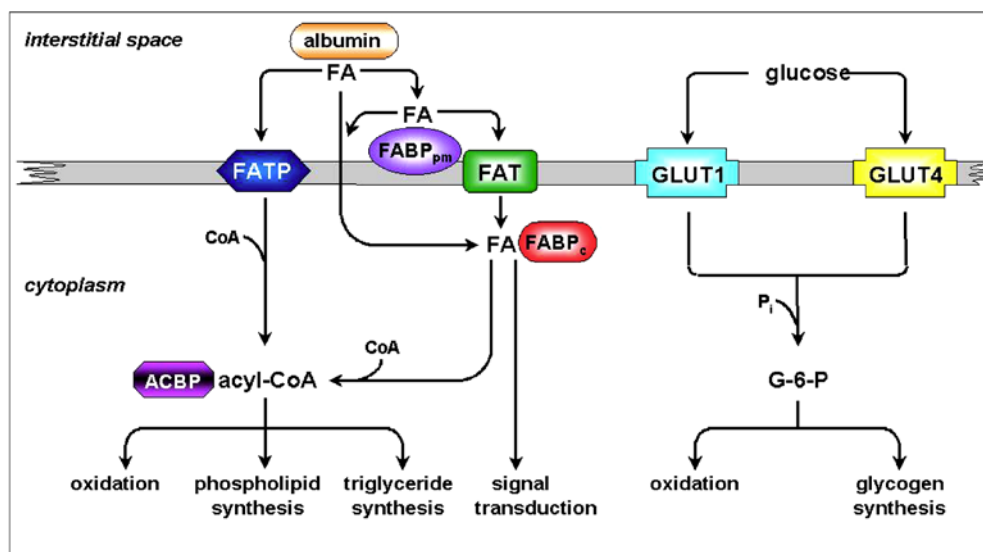
Cellular FA uptake has long been considered to be a passive mechanism, dependent on the metabolic rate and governed by physicochemical properties of these lipophilic substrates [98]. Especially partitioning of FA between albumin and plasma membranes has been postulated to be dependent on  $K_{off}$  rates for albumin and  $K_{on}$  rates for phospholipid bilayers [38]. However, the saturable nature of the FA uptake process, as observed in isolated cardiac myocytes, and its sensitivity to competitive inhibition suggest that a protein component is involved in cellular FA uptake [67, 68, 110]. The use of a variety of transport inhibitors with different modes of action has indicated that the protein component is responsible for the majority of FA uptake. While, in theory, all kinetic evidence could still be explained by saturability of metabolism [39], the observation that transport inhibition and substrate competition also occur in giant vesicles derived from the sarcolemma of cardiac myocytes, in which FA metabolism is entirely absent, adds powerful evidence in favour of the dependence of the heart on sarcolemmal proteins that act as FA transporters or FA transport facilitators [68]. The residual FA uptake, i.e. the non-inhibitable component (20–30% of total FA uptake), then most likely would be due to passive diffusion. While the list of proteins that have been proposed to act as putative transporters for FA is still growing, there is functional evidence for four of them. These are the 40-kDa plasma membrane FA binding protein (FABPpm) [48], the 88-kDa FAT/CD36 [47] and two members of the family of FA transport proteins (FATPs), the 63-kDa FATP1 [100] and the 70-kDa FATP6

[34]. All these putative transporters have been detected in the heart at both the mRNA and protein levels [68, 124]. In addition, all these transporters are localized at the sarcolemma, a prerequisite for their functioning in the cellular uptake of FA. Cellular FA uptake rates and relative abundance correlate positively for FAT/CD36 and FABPpm in white and red skeletal muscle and heart but not for FATP1 [68]. This may argue against a functional role for FATPs in the bulk FA uptake by the heart, but does not exclude a more specific function in the heart, for instance in preferential uptake of FA occurring at lower concentrations or in coupling FA uptake directly to a certain metabolic process. It also does not exclude a significant contribution of FATPs to bulk FA uptake in non-muscle tissues. Interestingly, the FATPs possess acyl-coenzyme A synthetase activity [16], so that these proteins may increase FA uptake by metabolic trapping.

The sulpho-*N*-succinimide esters of FA have been proven a valuable tool for studying the role of FAT/CD36 in cellular FA uptake, because they do not influence other uptake processes nor do they interfere with the action of the other putative FA transporters [17]. Sulpho-*N*-succinimidyloleate (SSO) inhibits cardiac FA uptake by ~80%, which is similar to the inhibitory potential of a polyclonal anti-FABPpm antiserum. Since, however, this antiserum and SSO together do not inhibit FA uptake further [68], i.e. both agents act non-additively, not only at maximally inhibiting concentrations but also at partially inhibiting concentrations, both FABPpm and FAT/CD36

would seem to act as two components of a single FA uptake system [68]. Assessment of the membrane topology of both transporters has revealed that FABPpm is a peripheral membrane protein at the outer leaflet [112], while FAT/CD36 is an integral membrane protein with one or two membrane spanning regions [1, 87]. It is likely that in this putative uptake system FABPpm acts as a receptor for FA, facilitating the diffusion of the FA-albumin complex through the unstirred fluid layer, and that FAT/CD36 mediates the transmembrane passage of FA, possibly by facilitating a flip-flop across the bilayer. Whether FABPpm and FAT/CD36 are, at some stage, in physical contact with each other remains to be established (Fig. 1).

Our studies on the influence of short-term contractile activity on FA uptake into skeletal muscle have provided the first data showing that FA uptake is subject to short-term regulation [6]: the induction of tetanic contraction markedly enhances muscle FA uptake. Because this increase is sensitive to inhibition by SSO, it appears to involve FAT/CD36. Because there was no de novo protein synthesis of FAT/CD36 during this contraction period, the acute increase in FA uptake can only be explained by either an increase in intrinsic activity of FAT/CD36 through a covalent modification or translocation to the sarcolemma from previously unrecognized intracellular membrane stores. This latter mechanism has been shown to occur in contracting muscle, and bears striking resemblance to the up-regulation of glucose uptake by



**Fig. 1** Transporters involved in the uptake of long-chain fatty acids (FA) and glucose across the sarcolemma of cardiac myocytes and their subsequent intracellular transport to their sites of metabolic conversion. For FA the scheme is as follows. Following the dissociation of FA from plasma albumin, FA could either partition directly into the phospholipid bilayer or be bound by plasma membrane FA binding protein (FABP<sub>pm</sub>), FA translocase (FAT/CD36) or FA transport protein (FATP). The subsequent transmembrane migration of FA could take place either without the involvement of membrane proteins (passive diffusion), or with a membrane protein acting solely as an acceptor for FA (FABP<sub>pm</sub>) or as a true translocator (FAT, FATP). At the inner side of the

sarcolemma, FA binds to cytoplasmic (intracellular) FA binding proteins (FABP<sub>c</sub>) before entering metabolic or other pathways after activation to fatty acyl-coenzyme A (acyl-CoA) and subsequent transport by acyl-coenzyme A binding protein (ACBP). FATP is involved in the uptake and simultaneous activation of FA. For glucose the scheme is as follows. Unlike FA, glucose, by virtue of its hydrophilic properties, does not need soluble carrier proteins, but is dependent on the presence of glucose transporters (GLUT)-1 and GLUT4 for transmembrane migration. Its intracellular arrival is rapidly followed by phosphorylation to glucose-6-phosphate (G-6-P) and subsequent entrance into metabolic pathways

the well-described translocation of GLUT4 [6]. These initial findings prompted us to investigate the possibility that cardiac FA uptake is also regulated by FAT/CD36 translocation. As a starting point for the identification of possible cellular components involved in FAT/CD36 translocation in cardiac myocytes, we speculated that valuable lessons could be learned from knowledge of GLUT4 translocation in the heart. We will therefore first review the present status of knowledge on the regulation of cardiac glucose uptake.

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## Regulation of cardiac glucose uptake

Cellular uptake of glucose is primarily a protein-mediated process because of the inability of hydrophilic compounds to traverse phospholipid bilayers. This facilitated transport is mediated by members of the GLUT family that belong to a much larger superfamily of 12-transmembrane segment transporters. Twelve GLUT isoforms have been described to date [51, 111]. The major isoforms present in heart and skeletal muscle are GLUT1 and GLUT4 (Fig. 1). A key mechanism in short-term regulation of glucose uptake is the translocation of both glucose transporters from intracellular stores to the sarcolemma through vesicular trafficking. Insulin and contraction are the two major physiological stimuli of cardiac glucose transport. Of these transporters, the translocation of GLUT4 is by far the most intensively investigated, especially in response to insulin. We will thus first review the signalling pathways and vesicular trafficking components involved in the translocation of GLUT4 in response by either of both stimuli. Subsequently, we will summarize the major findings concerning the regulation of cardiac glucose uptake by GLUT1, and finally compare the subcellular distribution of GLUT1 in cardiac myocytes with that of GLUT4.

### GLUT4 and insulin

Under basal, non-stimulated conditions, the majority of GLUT4 is stored intracellularly [103, 135]. Insulin is able to translocate GLUT4 to the cell surface primarily by stimulation of exocytosis [108]. Insulin also decreases the rate of GLUT4 endocytosis approximately two- to threefold in adipose cells [44, 54]. The magnitude of the stimulatory effect of insulin on glucose uptake by cardiac myocytes is reportedly between 2- and 14-fold, and is attributable mainly to GLUT4 translocation [20, 27, 28, 29, 67, 93]. The sequence of intracellular events has been a topic of intensive research and is known to involve activation of the insulin receptor, tyrosine phosphorylation of insulin receptor substrates (IRSs) followed by their binding to and activation of phosphatidylinositol (PI)-3 kinase (PI3K) and subsequent production of PI-3,4,5-(PO<sub>4</sub>)<sub>3</sub>. Downstream from PI3K, transduction of the signal becomes more obscure, but it is recognized that protein kinase B (PKB/Akt) and atypical protein kinase C (PKC)

isoforms are involved in adipose and skeletal muscle cells [25, 125]. Interestingly, insulin treatment of cardiac myocytes caused the association of PKB/Akt to GLUT4-containing vesicles [58], as has been also shown for adipocytes [11, 43]. This suggests that PKB/Akt phosphorylates component proteins of GLUT4-containing vesicles in response to insulin [62]. More recently, a novel, PI3K-independent signalling pathway with significance in insulin-induced glucose transport has been identified in adipose cells; this pathway originates in caveolae or in lipid rafts, involves tyrosine phosphorylation of the proto-oncogene *c-Cbl* and results in the activation of a small GTP-binding protein, TC10, a member of the Rho family [59].

The translocation machinery involved in vesicle-mediated shuttling of GLUT4 exhibits many of the features of transport of synaptic vesicles in the presynaptic nerve terminal. The specificity of the transport of these vesicles is safeguarded by soluble *N*-ethylmaleimide attachment protein receptor (SNARE) proteins according to the principles of the so-called SNARE hypothesis [9, 13]. The hypothesis postulates that, in vesicle trafficking events, a unique vesicle-associated protein (v-SNARE) specifically recognizes and interacts with a cognate target (t-SNARE) protein localized at the target membrane. This hypothesis also provides an attractive framework to ensure that intracellular GLUT4 vesicles, which are mobilized by insulin, are properly targeted to the plasma membrane and do not fuse randomly with other membranes. Indeed, homologues of v-SNAREs—vesicle-associated membrane protein-2 (VAMP2) and VAMP3 and the t-SNAREs syntaxin-4 and 23-kDa synaptosomal-associated protein (SNAP23)—have been identified in adipocytes and skeletal myocytes [101, 107]. Specifically VAMP2 is localized in intracellular GLUT4 containing vesicles and syntaxin-4 and SNAP23 exist almost exclusively in the plasma membrane. Disruption of the function of the syntaxin-4-SNAP23-VAMP2 SNARE complex selectively inhibits the insulin-induced translocation of GLUT4 to the cell surface but not the recruitment of GLUT1 in adipose cells [125]. VAMP3 or cellubrevin is associated with intracellular GLUT4 from endosomal nature in skeletal and cardiac muscles [101] and probably participates in the trafficking of GLUT4 within endosomes or from endosomes to the GLUT4 storage compartment. Several proteins are implicated in regulating the formation of syntaxin-4, SNAP23 and VAMP2 complex in response to insulin. Synip binds to syntaxin-4 in an insulin-dependent manner and prevents VAMP2 binding [80]. Pantophysin and vesicle-associated protein-33 (VAP33) bind to VAMP2 and prevent the association of the v-SNARE into the complex [8, 30].

Other proteins involved in the insulin-induced translocation of GLUT4 are members of the Ras-related Rab GTP-binding proteins. All Rabs bind and hydrolyse GTP, and in their cycling between GTP-bound (active) and GDP-bound (inactive) conformations these proteins act as molecular switches in vesicular trafficking [132]. Although Rabs have not been detected in the SNARE

complex, they are probably required for the assembly of this complex, as has been shown in yeast [104]. Members of the Rab family are associated with distinct intracellular compartments involved in endocytic and exocytic processes, indicating that the individual Rab proteins have specialized transport functions. The non-hydrolysable GTP analogue GTP $\gamma$ S, which keeps GTP-binding proteins in a permanently active state, has been instrumental in establishing a role for Rab proteins in the translocation of GLUT4. In adipocytes, which had been permeabilized to allow the cellular entry of non-permeant nucleoside phosphates, the increase in Rab activity upon GTP $\gamma$ S addition induces the translocation of GLUT4 to the plasma membrane in an insulin-like manner [3]. These findings have been confirmed in permeabilized cardiac myocytes [5].

The few studies to date addressing the presence of rab proteins in the heart have revealed the presence of rab1, -3, -4, -5, -6 and -11 [119, 120, 128]. While rab1 and -6 are involved in antegrade and retrograde vesicular trafficking between the endoplasmic reticulum and Golgi stacks [78, 89], rab3, -4, -5 and -11 are candidates for regulating GLUT4 recycling. rab3 and -4 translocate to the plasma membrane of cardiac myocytes in response to insulin [120]. rab4, but not rab3, has been detected in immunopurified GLUT4-containing intracellular vesicles obtained from rat heart [120]. Subsequently, a functional involvement of Rab4 has been demonstrated through its over-expression in H9c2 cardiac myoblasts. Basal glucose uptake is reduced moderately in these cells but insulin-stimulated glucose uptake and surface GLUT4 content increase fourfold, indicating that Rab4 is a key player in the insulin responsiveness of GLUT4 translocation in the heart [21]. More recently, Rab11 has been found to participate in insulin-inducible GLUT4 translocation in cardiac myocytes [57]. Rab11 associates specifically with recycling endosomes in mammalian cell lines [106, 117]. Its association with GLUT4-containing vesicles suggests a role for recycling endosomes in trafficking of GLUT4. Rab5 has been localized to the plasma membrane, clathrin coated vesicles and early endosomes and is postulated to be functioning in endocytic processes, as opposed to both Rab4 and Rab11 that are more likely to be involved in exocytosis [10, 12, 115]. Interestingly, in adipocytes, microinjection of anti-Rab5 increases basal levels of GLUT4 and inhibits GLUT4 internalization upon insulin withdrawal [45]. This suggests a role for Rab5, which associates with the motor protein dynein, in the regulation of GLUT4 distribution in adipose cells.

It is now generally accepted that, in insulin-sensitive tissues, GLUT4 is found at least in two distinct intracellular compartments [2, 84, 101, 107]. In rat skeletal muscle, the insulin-responsive intracellular pool with the lowest buoyant density coincides with endosomal proteins such as the transferrin receptor, annexin II, secretory carrier membrane proteins (SCAMPs) or cellubrevin, while the heavier fraction containing the majority of intracellular GLUT4 is depleted of the transferrin receptor and is enriched in an 160-kDa insulin-responsive amino-

peptidase (IRAP) and in VAMP2 [2, 84, 101]. In rat cardiac myocytes, GLUT4 is distributed in an endosomal pool that contains SCAMPs, GLUT1, insulin-like growth factor (IGF)-II receptors and into a unique non-endosomal intracellular membrane pool [4, 29]. It is likely that rab11 associates with the endosomal GLUT4 pool in rat heart [57]. Based on these observations in skeletal muscle and in cardiac myocytes, we propose that GLUT4 is distributed in two major pools: a GLUT4 storage compartment that contains most of GLUT4 and is translocated to the sarcolemma in response to insulin, and an endosomal GLUT4 compartment that may respond to other signals. The GLUT4 storage compartment is enriched in IRAP and in VAMP2 which is in keeping with the observations performed in adipose cells. [18, 53]. The mechanisms that control the generation of the GLUT4 trafficking pathway remain unknown. In this regard, muscle denervation causes a marked repression of GLUT4, but does not affect the level of expression of component proteins of intracellular vesicles such as IRAP, VAMP2, transferrin receptors, IGF-II receptors or SCAMPs [134]. In addition, in muscle from GLUT4<sup>-/-</sup> mice, trafficking of the transferrin receptor is not different from that in the wild-type, while IRAP displays an abnormal subcellular distribution and an impaired recruitment to the sarcolemma in response to insulin [49]. These findings suggest that despite the fact that GLUT4 is not involved in the expression of other component proteins, this glucose transporter has a pivotal role in defining the exocytic storage compartment. The storage compartment and its component proteins are considered to be uniquely dedicated to trafficking of GLUT4 in response to insulin. In contrast, the recycling endosomes fulfil a variety of other functions besides GLUT4 recruitment [12, 106, 115, 117].

#### GLUT4 and contraction

The ability to induce GLUT4 translocation is not restricted to insulin, but can also be achieved by a drop in oxygen supply (anoxia/ischaemia) and by mitochondrial inhibitors [127] and, physiologically most relevant, by an increase in mechanical activity of the heart [41]. All these factors induce GLUT4 translocation independently of insulin signalling, because inhibition of PI3K does not interfere with their ability to induce GLUT4 translocation [75, 116, 129]. The notion that these factors each induce metabolic stress suggests that they share a common signalling pathway. Recently, this pathway has been shown to include AMP-activated protein kinase (AMPK), which is activated by a stress-induced rise in intracellular AMP and a concomitant drop in ATP [40, 91]. Other evidence for an involvement of AMPK comes from studies using the cell-permeable agent 5-aminoimidazole-4-carboxamide-1- $\beta$ -D-ribofuranoside (AICAR). This adenosine analogue is taken up readily by cells and subsequently 5'-phosphorylated to ZMP, which in turn activates AMPK selectively and increases glucose uptake in a manner that is not

reversible by addition of the PI3K-specific inhibitor wortmannin [42, 79]. However, the involvement of AMPK is not undisputed. For instance, AMPK activation in skeletal muscle is prevented by preloading with glycogen prior to exercise, but nonetheless contraction-inducible glucose uptake is preserved [19]. On the other hand, expression in mouse muscle of a dominant inhibitory mutant of AMPK completely blocks the stimulation of glucose uptake by AICAR or hypoxia [81]. The difficulty in establishing a causal link between AMPK activation and glucose uptake resides in the fact that a specific inhibitor of AMPK is not yet available. The downstream targets of AMPK that are involved in GLUT4 translocation await their identification. The ability of specific protein kinase C (PKC) inhibitors to inhibit contraction-inducible glucose uptake into skeletal muscle suggests an involvement of one or more PKC isoforms. Furthermore, PKC $\zeta$  and components of the extracellular signal-regulated kinase (ERK) pathway have been found to be downstream from AMPK, based on the ability of their specific inhibitors myristoylated PKC $\zeta$  pseudosubstrate and PD98059, respectively, to inhibit AICAR-induced GLUT4 translocation in mouse skeletal muscle [14].

## GLUT1

GLUT1 has a much more ubiquitous tissue distribution than GLUT4, which is restricted mainly to insulin-sensitive, extrahepatic tissues. Compared with other tissues, the heart expresses a relatively large amount of GLUT1 [29]. In contrast to GLUT4, which is stored predominantly intracellularly, GLUT1 has a more pronounced sarcolemmal localization and has been regarded generally as mediating basal glucose uptake. However, for the heart evidence is accumulating that intracellularly stored GLUT1 can also be recruited by insulin [4, 29] and by contraction or manipulations acting on contraction signalling in perfused hearts or in isolated cardiac myocytes [4, 127]. Quantitatively, the effect of insulin on the surface appearance of GLUT1 (1.5- to 2.7-fold increase) is moderate compared with that of GLUT4 (2.6- to 5.6-fold increase; [23, 29, 95]). It is likely that some of the variability observed in the different studies stems from the utilization of perfused rat heart preparations on the one hand and isolated rat cardiomyocytes on the other. The latter model is characterized by a lower basal glucose transport rate due to a lower abundance of GLUT1 in the plasma membrane under basal conditions. In contrast to the effect of insulin, the effect of mitochondrial inhibitor rotenone (which activates contraction signalling) on the extent of translocation of GLUT1 is similar to that of GLUT4 [127]. The signalling mechanisms involved in the translocation of GLUT1 are remarkably similar to that of GLUT4 in that PI3K is involved in insulin-induced GLUT1 translocation, but not in ischemia (contraction-signalling)-induced GLUT1 translocation [23]. The effect of ischaemia [23, 131] or rotenone [4, 127] on surface

GLUT1 is of a similar magnitude to that of insulin, i.e. an ~1.5-fold increase. The comparison of the effects of insulin and contraction signaling on translocation of GLUT1 and GLUT4 will be used below (see section Comparison of glucose and FA uptake) to shed light on possible similarities with recycling of FA transporters.

## Intracellular storage of GLUT1 and GLUT4

Comparison of intracellular storage of GLUT4 with that of GLUT1 in non-stimulated cardiac myocytes, using immunotitration with an anti-GLUT4 antibody, has identified two distinct intracellular GLUT4 storage sites [4, 29]. One site of intracellular GLUT4 is shared with the complete intracellular population of GLUT1, and contains other endosomal proteins such as the transferrin receptor and Rab11, identifying this site as the recycling endosomes [57]. The other site is entirely devoid of GLUT1 [4] and most probably represents the GLUT4 storage compartment. Interestingly, rotenone induces GLUT1 and GLUT4 translocation mainly from the endosomal compartment, while insulin recruits GLUT4 from the storage compartment and also GLUT1 from the endosomal compartment [4]. The separation between intracellular GLUT1 and the GLUT4-specific storage compartment is further substantiated by over-expression of Rab4 in cardiac myocytes. Rab4 over-expression decreases the cell surface abundance of GLUT4, while not affecting GLUT1 distribution [21]. Moreover, a synthetic peptide corresponding to the N-terminus of VAMP2 inhibits insulin-inducible GLUT4 translocation, but not insulin-inducible GLUT1 translocation in adipocytes [77].

Taken together, these findings are compatible with the existence of a minimum of two and a maximum of three distinct glucose transporter pools in the heart. The first pool is the GLUT4 storage compartment, which contains GLUT4 but not GLUT1 and permits the translocation of GLUT4 to the plasma membrane in response to insulin. The second pool is formed by the endosomal compartment that contains both GLUT4 and GLUT1 and responds to rotenone and to insulin; rotenone recruits GLUT4 and GLUT1, whereas insulin only recruits GLUT1 from the endosomal pool. This pool may in theory permit the response to contraction. A third pool, for which there is not yet experimental support in cardiac myocytes, may be proposed: the contraction-responsive GLUT4 compartment. We propose this third compartment on the basis of observations in skeletal muscle that indicate the existence of a postendocytic, contraction-responsive GLUT4 compartment. However, we stress that this compartment may not exist in cardiac myocytes. Currently available estimates in skeletal muscle indicate that the storage pool contains 40–60% of the intracellular GLUT4.

## Comparison of glucose and FA uptake

The similarities between the regulation of cardiac FA and glucose uptake are striking. Notably, both processes are up-regulated by the same physiological stimuli and, moreover, both use the same basic mechanism of regulation, i.e. recycling of substrate transporters between intracellular membrane compartments and the sarcolemma. However, subtle differences exist. These similarities and differences are being discussed alongside the following issues.

### Involvement of passive diffusion

The uptake of glucose is dependent on the involvement of transporters, as the hydrophilic substrate is unable to cross the phospholipid bilayer. However, FA readily partitions in biological membranes [38, 39] and its uptake is inhibited incompletely by transport inhibitors or protein-modifying agents [68]. Since the rate of passive diffusion is governed by physicochemical properties of the phospholipid bilayer and obeys the laws of thermodynamics, the process is unlikely to be subject to acute regulation. We can assume safely that passive diffusion of FA is not affected directly by contraction or insulin, since the absolute rate of non-inhibitable FA uptake is not altered in the presence of these stimuli [70, 72]. This implies that the passive diffusion component in the FA uptake process has a damping effect on the stimulation of FA uptake by insulin or contraction.

### Storage of FA and glucose transporters in non-stimulated cardiac myocytes

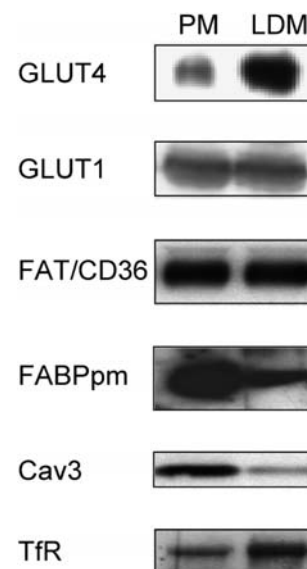
There is general agreement that the majority of GLUT4 is stored intracellularly under basal conditions (see above, GLUT4 and insulin). In concordance, our fractionation studies (Fig. 2) demonstrate a markedly (two- to threefold) greater abundance of GLUT4 in a low-density microsomal fraction (LDM; intracellular membrane fraction, presumably containing endosomes) than in a plasma membrane fraction (PM). In addition, we have confirmed that, under basal conditions, GLUT1 has a more pronounced sarcolemmal localization than GLUT4, because the amount of GLUT1 in the PM is similar to that in the LDM. Examining the presence of FAT/CD36 within these fractions, it is obvious that its equal distribution between PM and LDM more closely resembles that of GLUT1 than that of GLUT4 (Fig. 2). In addition, FAT/CD36 is present equally in both sarcolemmal and microsomal membranes in non-stimulated cardiac myocytes [83]. Moreover, Western blot analysis of immunoabsorbed GLUT4 vesicles reveals that these vesicles lack FAT/CD36, providing convincing evidence for an intracellular storage of FAT/CD36 that is different from that of GLUT4. FAT/CD36 also colocalizes with Rab11. Combining these pieces of evidence carefully pinpoints the endosomes, harbouring the complete population of intracellular GLUT1, as

suitable candidates for intracellular storage of FAT/CD36. It remains to be established whether the intracellular localization of the storage compartment marker IRAP is distinct from that of FAT/CD36.

In contrast to FAT/CD36, FABPpm is markedly more abundantly expressed in the PM than in the LDM under basal (non-stimulated) conditions, suggesting that its storage within an endosomal compartment is relatively small compared with that of other transporters. Translocation processes will thus make only a minor contribution to the regulation of the sarcolemmal content of FABPpm. As a verification of the fractionation procedure, the sarcolemmal marker caveolin-3 resides mainly in the PM fraction, and the transferrin receptor mainly in the LDM.

### Regulation by insulin

While it is appreciated that insulin favours esterification of FA into cellular triacylglycerol stores in skeletal muscle [22, 71, 85] and heart [72], the ability of insulin to stimulate FA utilization at the sarcolemmal level has only been explored recently. In cardiac myocytes insulin stimulates FA uptake 1.5-fold [72]. This stimulation can be prevented by SSO and by wortmannin indicating that (i) FAT/CD36 is responsible for the increase in FA uptake and (ii) activation of PI3K is required. Furthermore, insulin decreases the amount of FAT/CD36 in the LDM fraction by 47% and simultaneously increases the amount



**Fig. 2** Comparison of cellular distribution of GLUT4, GLUT1 and FAT/CD36, caveolin-3 (*Cav3*) and the transferrin receptor (*TfR*) in the plasma membrane (PM) or low-density microsomal (LDM) fractions under basal (non-stimulated) conditions. GLUT1 and GLUT4 were both detected at 45 kDa, FAT/CD36 at 88 kDa, caveolin-3 at 24 kDa and the transferrin receptor at 95 kDa. It must be mentioned that, despite the assumption that the GLUT4 specific storage pool is missing in the LDM fraction, the minute presence of GLUT4 in the sarcolemma of non-stimulated cardiac myocytes, compared with that of GLUT1, still allows the conclusion that basal GLUT4 localization is more intracellular than that of GLUT1

of FAT/CD36 in the PM fraction 1.5-fold [72, 74]. It is therefore likely that insulin induces FA uptake through translocation of FAT/CD36 from intracellular stores to the sarcolemma. Apparently, insulin mobilizes FAT/CD36 to the sarcolemma, just as it mobilizes GLUT1 and GLUT4 from intracellular stores. However, the magnitude of the effect of insulin on FAT/CD36 translocation is more similar to that of GLUT1 (the surface appearance of which is also enhanced 1.5-fold) than that of GLUT4 (the surface appearance of which more than doubles). Combined with the similarity of FAT/CD36 subcellular distribution to that of GLUT1 rather than GLUT4 under basal, non-stimulated conditions (see previous section), this adds further evidence in favour of the hypothesis that storage of intracellular FAT/CD36, just as that of GLUT1, is confined to the endosomal compartment.

The involvement of insulin in the regulation of FA uptake is an entirely novel role for this hormone, and could have consequences for altered FA fluxes in syndromes of insulin deficiency or resistance. The relatively modest stimulation of FA uptake by insulin, compared with that of glucose, does not mean that this effect of insulin is functionally less significant. In this respect, intracellular FA are known to be powerful mediators of signal transduction as they are the main physiological ligands of the peroxisome proliferator-activated receptors [33]. Furthermore, intracellular FA and metabolites are more toxic to cells than glucose and its metabolites. For instance, high levels of intracellular “free” FA exert detergent-like effects on biological membranes and inhibit the catalytic activity of many enzymes [35] and accumulation of certain FA metabolites, such as acyl-CoA and acyl-carnitine, cause arrhythmias and can impair cardiac functioning severely [63]. This implies that cellular systems cannot allow the intracellular FA concentration to fluctuate to the same extent as with glucose.

### Regulation by contraction

While the consequences of an increase in workload on cardiac energy metabolism have been investigated extensively [66, 113], the impact of an increase in contractile activity on FA uptake by cardiac myocytes has been explored only poorly. When cardiac myocytes are stimulated electrically at varying stimulation frequencies, both FA uptake and glucose uptake rise with stimulation frequency. At 4 Hz, this increase is 1.5-fold for both substrates [70]. The contraction-induced increase in FA uptake is blocked completely by SSO. The increase in FA uptake in the presence of contraction is thus linked causally to increased involvement of FAT/CD36. In the search for a signal transduction pathway, we can exclude a role for PI3K since wortmannin does not inhibit contraction-inducible FA uptake [72], indicating that different mechanisms are involved in contraction-inducible and insulin-inducible FA uptake. We can also exclude a role for cyclic AMP-dependent protein kinase A, because

manipulations increasing intracellular cyclic AMP, e.g. the membrane-permeant cyclic AMP analogue dibutyryl cyclic AMP, the  $\beta$ -agonist isoproterenol and the phosphodiesterase III inhibitor amrinone, all fail to alter FA uptake into cardiac myocytes [73].

Another attractive candidate signalling enzyme for the mediation of contraction-inducible FA uptake is AMPK, because it is already known to be involved in another aspect of cardiac FA utilization. An important target for AMPK is namely acetyl-CoA carboxylase, which AMPK inhibits by phosphorylation, resulting in a drop in intracellular malonyl-CoA levels and a de-inhibition of carnitine palmitoyltransferase-I (CPT-I) [65]. The combined induction of FA uptake and of CPT-I activity through AMPK activation is then likely to be metabolically efficient, as this allows the extra incoming FA to be channelled preferentially into mitochondrial  $\beta$ -oxidation. In addition, as mentioned earlier, AMPK is known to be involved in stimulation of glucose uptake by translocation of GLUT4 to the sarcolemma (see above: GLUT4 and contraction). Indeed, electrical stimulation of cardiac myocytes elevates the intracellular AMP/ATP ratio 1.9-fold and stimulates AMPK activity 3.3-fold [74]. Pharmacological evidence for the involvement of AMPK in contraction-inducible FA uptake has been provided by studying the effects on FA uptake of the mitochondrial inhibitor oligomycin and the cell-permeable adenosine analogue AICAR, both of which stimulate glucose uptake and GLUT4 translocation (see above: GLUT4 and contraction). Both oligomycin and AICAR stimulate AMPK activity markedly (more than fivefold) and FA uptake up to twofold [74]. Moreover, 4-Hz stimulation loses its enhancing effect on FA uptake into cardiac myocytes when the latter are treated simultaneously with either oligomycin or AICAR, indicating a common mechanism. Additional evidence for a common mechanism in the stimulating effects of contractions, oligomycin and AICAR on FA uptake is the observation that the PI3K inhibitor oligomycin is unable to block either action. The mechanism has been shown to involve a translocation of FAT/CD36 from intracellular stores to the sarcolemma. In this respect, subcellular fractionation experiments indicate that activation of AMP kinase by oligomycin results in a 49% depletion of FAT/CD36 from an intracellular membrane compartment and a concomitant 1.5-fold increase at the sarcolemma [74]. Remarkably, the relative extent of up-regulation of FAT/CD36 at the cell surface by AMPK activation is very similar to that of GLUT1 and GLUT4. Since both contraction-inducible GLUT1 and GLUT4 are stored in the recycling endosomes, it may be speculated that they share this intracellular storage site with contraction-inducible FAT/CD36. Thus, these recycling endosomes harbour FAT/CD36 that is mobilized by contraction-induced activation of AMPK as well as by insulin-induced activation of PI3K (see previous section). Because insulin and contraction are likely to operate independently of each other, this implies that there must be distinct sorting mechanisms, to respond to either stimulus, present within the recycling endosomes. However, because a



specific inhibitor of AMPK is not yet available, a causal relationship between AMPK activation and FAT/CD36 translocation, just as with GLUT4 translocation, is difficult to establish. To date, the strongest evidence for such a relationship comes from the ability of 5-iodotubercidin, an adenosine kinase inhibitor that prevents the conversion of AICAR to ZMP, to block the AICAR-induced increase in FA uptake completely [74].

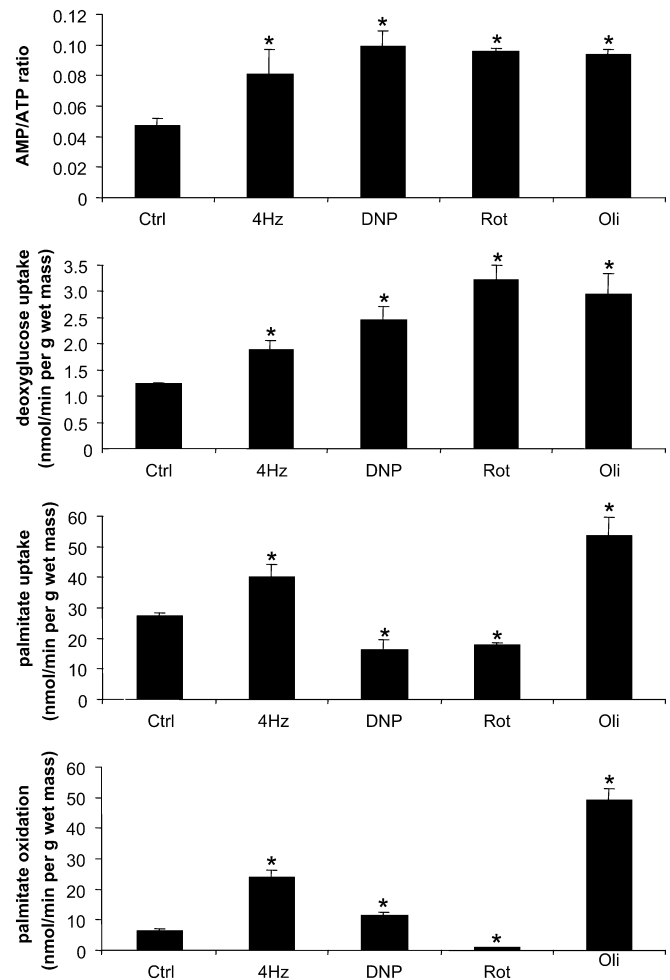
#### Differential effects of various mitochondrial inhibitors on uptake of FA and glucose

Mitochondrial inhibitors have been used frequently to mimic pharmacologically the effect of contraction on ATP utilization. Accordingly, rotenone, 2,4-dinitrophenol (DNP) and oligomycin all similarly enhance the AMP level twofold (Fig. 3), leading to activation of AMPK. In addition all of them have proven successful in stimulating glucose uptake and GLUT4 translocation (Fig. 3, see also [5, 31, 127]). However, only oligomycin enhances FA uptake, whereas DNP and rotenone inhibit this process rather substantially (Fig. 3). Remarkably, the underlying mechanisms between DNP and rotenone must be different, since these inhibitors exert opposite effects on FA oxidation (Fig. 3).

With respect to rotenone, its blockade of the electron transport chain arrests the Krebs' cycle and thus reduces FA oxidation. Our earlier work suggested that palmitate uptake and oxidation are tightly coupled. For example, treatment of cardiac myocytes with the CPT-I inhibitor etomoxir completely arrests FA oxidation and inhibits FA uptake by 50% [67]. In addition, etomoxir has no direct effect on FA uptake, i.e. at the level of the sarcolemma [36], indicating that the etomoxir-imposed inhibition of FA uptake is secondary to the fall in FA oxidation. Likewise, rotenone inhibits FA uptake through a blockade of FA oxidation.

The fall in palmitate uptake by cardiac myocytes treated with the uncoupling agent DNP, however, is not due to an arrest of aerobic metabolism. Accordingly, despite the decrease in FA uptake FA oxidation in the presence of DNP is increased. This indicates that the reduction in FA uptake is compensated for by more efficient channelling of FA into oxidative pathways, probably at the expense of esterification into cellular lipid pools. This channelling is then made possible by the AMPK-induced phosphorylation and inhibition of acetyl-CoA carboxylase and a concomitant increase in mitochondrial  $\beta$ -oxidation [65]. As an explanation for its inhibitory action on FA uptake, we speculate that this mitochondrial uncoupler has non-specific side effects on proton gradients across other membranes at the cellular level, and consequently destroys the membrane potential at the level of the sarcolemma. Since FA uptake is reportedly dependent on the membrane potential [24, 126], DNP's inhibitory effect on FA uptake is then caused through this mechanism.

Of all tested mitochondrial inhibitors, oligomycin mimics most closely the effects of contraction on substrate



**Fig. 3** Effects of mitochondrial inhibitors on cardiomyocyte AMP levels, glucose uptake and FA uptake and oxidation by cardiac myocytes: comparison with electrical stimulation. Cell suspensions were incubated for 15 min in the absence of additions (control, *Ctrl*), in the presence of electrical field stimulation at 200 V and 4 Hz (4 Hz), or with 10  $\mu$ M 2,4-dinitrophenol (*DNP*), 3  $\mu$ M rotenone (*ROT*), or 30  $\mu$ M oligomycin (*Oli*) prior to the metabolic measurements. Cardiomyocyte AMP levels are normalized to those of ATP, which does not change significantly in the presence of the different manipulations [74]. The cellular content of adenosine phosphates was determined by HPLC according to [121]. Determination of deoxyglucose uptake (3 min), palmitate uptake (3 min) and oxidation (30 min) was carried out as described earlier [67]. Means $\pm$ SD,  $n=4$  experiments on different cardiomyocyte preparations. \* $P<0.05$  vs. control

utilization by myocytes in that it enhances both FA and glucose uptake. Oligomycin is a potent inhibitor of the mitochondrial  $F_1F_0$ -ATPase and, as such, has long been used to inhibit state-3 respiration in isolated mitochondria [37]. Because of the tight coupling between FA oxidation and FA uptake, oligomycin would be expected to reduce FA uptake under these anaerobic conditions rather than to stimulate it. However, a recent study has demonstrated that the concentration of oligomycin required for 50% inhibition ( $IC_{50}$ ) of the mitochondrial  $F_1F_0$ -ATPase is fivefold lower than its  $IC_{50}$  for inhibition of electron flux through the respiratory chain [130]. This discrepancy opens a window of oligomycin concentrations at which the

intracellular AMP is elevated while, simultaneously, oxygen consumption is not inhibited. Accordingly, at concentrations of 5–100  $\mu\text{M}$ , oligomycin elevates intracellular AMP and simultaneously enhances FA uptake and oxidation [74].

In conclusion, DNP and oligomycin share with contractile activity the ability to efficiently channel FA upon its cellular entry towards oxidative metabolism. It remains to be established whether the AMP-enhancing action of rotenone, despite its mitigating action on FA oxidation, is still sufficient to trigger translocation of FAT/CD36 to the cell surface. If so, the coupling between translocation of the GLUTs and translocation of FAT/CD36 would be retained, despite the opposite effects of rotenone on FA and glucose uptake (see above, GLUT1).

#### Mutual dependence of FA transporters: comparison with glucose transporters

As mentioned above (Regulation of cardiac FA uptake), FAT/CD36 and FABPpm together are responsible for 70–80% of the FA influx into the heart. However, FAT/CD36- and FABPpm-mediated FA uptakes are not additive. In contrast, glucose uptake into adipose and muscle tissues is regarded as the sum of the transport actions of GLUT1 and GLUT4 [82]. Hence, the two cardiac FA transporters have a different mutual relationship than the two cardiac glucose transporters. The two cardiac glucose transporters GLUT1 and GLUT4, although translocated to the sarcolemma by the same stimuli, operate independently of each other and, therefore in parallel. In contrast, the two FA transporters FAT/CD36 and FABPpm most probably act as two components of a single transport system, and thus are positioned in series. As a putative receptor for FA, FABPpm is present constitutively at the cell surface, while FAT/CD36 recycles between endosomes and the sarcolemma. Of these two transporters, FAT/CD36 is the major site of flux control. This notion is based on the following lines of evidence. First, in heart and other tissues FABPpm is an abundantly expressed protein [133] so that its availability is unlikely to be rate limiting. Second, there is a better relationship between the amount of sarcolemmal FAT/CD36 and FA uptake rates into giant vesicles derived from different mammalian tissues than between sarcolemmal FABPpm and giant vesicle FA uptake rates, both under basal [61, 68] and pathological conditions [7]. Third, the relative increase in FA uptake into cardiac myocytes elicited by insulin or contraction is similar to the increases in the sarcolemmal content of FAT/CD36 due to translocation from endosomal sources [72, 74]. Hence, insulin or the onset of contraction activates signalling events resulting in translocation of FAT/CD36 to the sarcolemma, after which, supposedly, an interaction will be established with FABPpm. Whether specific sarcolemmal microdomains exist that may act as docking sites for endosomally delivered FAT/CD36 and simultaneously harbour significant populations of FABPpm is an intriguing possibility that remains to be established. An

interesting candidate domain could be presented by the caveolae that are known to accommodate a number of receptors and transporters. These 100-nm, flask-shaped invaginations of the sarcolemma are abundant in cardiac myocytes [26, 64]. In addition, cardiac myocytes express relative large quantities of caveolin-3, the major constituent of caveolae [105]. Interestingly, it has been established that cholesterol-complexing agents that disrupt the structural integrity of caveolae markedly reduce FA uptake into hepatoma cells [90]. These findings suggest an important role for caveolae in cellular FA uptake. With respect to glucose uptake and GLUT4 recycling, there are also indications for an involvement of caveolae, although this still is a controversial issue. For instance, caveolin-3 does not colocalize with GLUT4 at the cell surface of skeletal muscle fibres [92]. In adipocytes, however, insulin induces the translocation of GLUT4 to caveolae [55, 76]. Furthermore, adipocytic caveolae are known to play a crucial role in internalization of GLUT4 upon insulin withdrawal [97]. Whether caveolae play comparable roles in GLUT4 and FAT/CD36 recycling is another intriguing research question.

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#### Integrated model for recycling of FA and glucose transporters in cardiac myocytes

The available evidence suggests that intracellular FAT/CD36 is stored in a single endosomal pool. We propose that this intracellular pool is identical to the endosomal compartment that contains both GLUT4 and GLUT1, and that this compartment responds to AMPK activation and to insulin signalling. As a consequence, the FAT/CD36-containing pool represents an endosomal population and also contains both GLUT1 and GLUT4. The additivity of AMPK activation and insulin signalling with respect to FA and glucose uptake [72, 74, 75, 116, 129] indicates that within this endosomal compartment there must be (at least) two independently operating sorting mechanisms for recruitment of FAT/CD36 and GLUTs, most probably involving different SNARE proteins and Rabs. A candidate Rab protein in insulin-inducible transporter translocation from this compartment could be Rab11, because it is associated with both GLUT4 and FAT/CD36 and redistributes to the sarcolemma upon insulin stimulation [57, 83, 106, 117]. Its counterpart involved in AMPK-inducible transporter translocation is still hypothetical.

FAT/CD36 storage resembles that of GLUT1 more closely than that of GLUT4, since GLUT4 is also stored in a unique insulin-sensitive compartment. GLUT1, and probably also FAT/CD36, lack this kind of intracellular storage. This makes the insulin-inducible recruitment of GLUT4 a magnitude greater than its contraction-inducible recruitment, or recruitment of FAT/CD36 or GLUT1 by insulin or contraction. We do not know yet whether GLUT4, GLUT1 and FAT/CD36 travel together within the same recycling endosomal vesicles or whether they are stored in independent subcompartments, but still use the

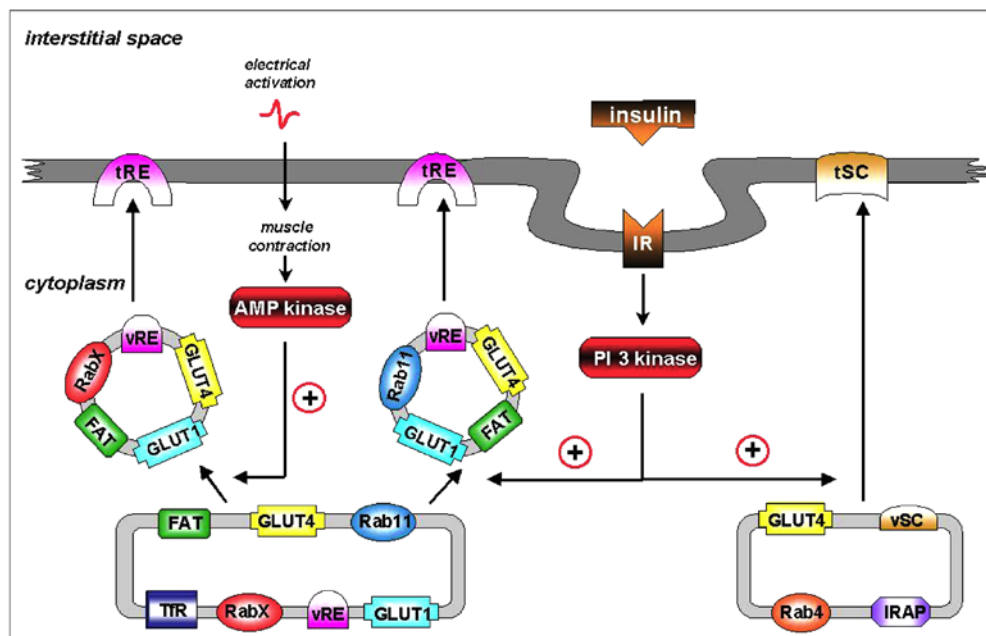
same signalling processes for their recruitment. A hypothetical model for FAT/CD36 recycling in relation to that of GLUT1 and GLUT4 is depicted in Fig. 4.

### Future perspectives

It is becoming increasingly evident that translocation events play a more common role in the regulation of substrate utilization than previously realized after the initial discovery of GLUT4 recycling in adipocytes [56, 60]. This was followed by the observation that another glucose transporter, GLUT1, also shuttles between intracellular stores and the cell surface [99]. At present, we should take into consideration that transporter recycling is also involved in the regulation of FA uptake. While we have established recently that contraction and insulin induce the translocation of FAT/CD36 in skeletal muscle and heart [6, 71, 72, 74], other FA transporters, e.g. FATP-1 and -4, are translocated to the cell surface in adipocytes in response to insulin [109]. Interestingly, in skeletal muscle insulin is able to induce the sodium-dependent uptake of neutral amino acids by translocation of the

amino acid transport system A to the sarcolemma [46]. It is indeed possible that recycling of transporters is the standard, while their constitutive presence at the sarcolemma, in case of FABPpm (see above: Storage of FA and glucose transporters in non-stimulated cardiac myocytes) and also in case of the cardiac monocarboxylate transporter MCT1 [50], is the exception to the rule.

Cardiac disease has often been linked to a change in substrate preference. For example, cardiac hypertrophy is associated with a shift in the utilization from FA to glucose [52, 123], while on the other hand the diabetic heart prefers FA at the expense of glucose [96, 102, 118]. In obese Zucker rats, a rodent model for insulin resistance and obesity, FA uptake into the heart is increased twofold [69]. This increase is not accompanied by a change in protein expression of FAT/CD36 or FABPpm, but is paralleled by an increased abundance of FAT/CD36 at the sarcolemma at the expense of intracellular storage. At present, we do not know whether this permanent relocation of FAT/CD36 to the sarcolemma is a normal physiological response to elevated circulating insulin levels, as observed in obese Zucker rats, or otherwise is due to chronic alterations in the FAT/CD36 translocation



**Fig. 4** Hypothetical model of FAT/CD36 translocation in relation to that of GLUT1 and GLUT4. FAT/CD36, just as the transferrin receptor, GLUT1 and a minor portion of GLUT4, migrates upon activation of AMP-activated protein kinase (AMPK, through muscle contraction) or phosphatidylinositol-3 kinase [PI3K, initiated by insulin binding to the insulin receptor (IR)] from an endosomal compartment (recycling endosomes) to the sarcolemma. AMPK-dependent mobilization is assisted by RabX, a hypothetical member of the Rab family involved in contraction-dependent recycling, whereas PI3K-dependent mobilization is presumably mediated by Rab11. In parallel, the majority of GLUT4 present in the exocytotic storage compartment together with Rab4 and an insulin-responsive aminopeptidase (IRAP) is moved in response to insulin to the sarcolemma. We speculate that the segregation of intracellular FAT/CD36 from the majority of intracellular GLUT4 present in the storage compartment is extended to the level of the sarcolemma. In

agreement with this view, surface GLUT4 derived from the storage compartment, is confined to a specific sarcolemmal region that is distinct from the surface docking site for recycling proteins. Both docking sites possess unique target soluble *N*-ethylmaleimide attachment protein receptors (t-SNAREs) (*tRE* t-SNARE involved in docking of recycling endosomes, *tSC* t-SNARE involved in docking of vesicles from the storage compartment) for binding to their cognate vesicle-SNAREs (v-SNAREs) at the recycling endosomes (*vRE*) or the storage compartment (*vSC*), respectively. Based on this, we envisage two systems, the first being the continuum between the recycling endosomes and their surface docking sites, and the second being vesicular trafficking between the storage compartment and its surface docking site. These systems may operate independently of each other, which precludes a mixing between their putative intracellular and sarcolemmal pools of transporters

machinery. It is therefore of pivotal importance to unravel the molecular mechanisms of FAT/CD36 translocation, notably the rate-limiting step in FA utilization. Especially the differences with the regulation of translocation of glucose transporters can help to identify therapeutic strategies to allow selective manipulation of substrate transporter recruitment and/or internalization, and therefore restore the cardiac substrate balance. It is expected that normalization of cardiac substrate utilization will lead to improvement of cardiac functioning.

An attractive therapeutic target is possibly represented by the endosomal compartment that is involved in storage of FAT/CD36 and GLUTs. These endosomes constitute a highly organized subcellular structure in which a multitude of trafficking processes involved in sorting of proteins or other macromolecules with different destinations are carried out with a remarkable degree of specificity. Alongside the unmasking of the components involved in vesicular trafficking of GLUT4 and the transferrin receptor, it is becoming evident that this sorting is safeguarded by unique molecular mechanisms that we now are just beginning to understand. It will be the challenge in future research to find the specific v-SNAREs and t-SNAREs, Rabs and other proteins involved in vesicle fission and fusion, that are dedicated to trafficking of FAT/CD36 between the cell surface and its intracellular compartment.

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