Regulation of sarcolemmal glucose and fatty acid transporters in cardiac disease

Robert W. Schwenk1, Joost J.F.P. Luiken1, Arend Bonen2, and Jan F.C. Glatz1*

1Department of Molecular Genetics, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands; and 2Department of Human Health and Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada

Received 1 February 2008; revised 4 April 2008; accepted 6 May 2008; online publish-ahead-of-print 9 May 2008

Time for primary review: 32 days

Circulating long-chain fatty acids (LCFA) and glucose are the main sources for energy production in the heart. In the healthy heart the ratio of glucose and LCFA oxidation is sensitively balanced and chronic alterations in this substrate mix are closely associated with cardiac dysfunction. While it has been accepted for several years that cardiac glucose uptake is mediated by facilitated transport, i.e. by means of the glucose transport proteins GLUT1 and GLUT4, only in the last few years it has become clear that proteins with high-affinity binding sites to LCFA, referred to as LCFA transporters, are responsible for bulk LCFA uptake. Similar to the GLUTs, the LCFA transporters CD36 and FABPpm can be recruited from an intracellular storage compartment to the sarcolemma to increase the rate of substrate uptake. Permanent relocation of LCFA transporters, mainly CD36, from intracellular stores to the sarcolemma is accompanied by accumulation of lipids and lipid metabolites in the heart. As a consequence, insulin signalling and glucose utilization are impaired, leading to decreased contractile activity of the heart. These observations underline the particular role and interplay of substrate carriers for glucose and LCFA in modulating cardiac metabolism, and the development of heart failure. The signalling and trafficking pathways and subcellular machinery regulating translocation of glucose and LCFA transporters are beginning to be unravelled. More knowledge on substrate transporter recycling, especially the similarities and differences between glucose and LCFA transporters, is expected to enable novel therapies aimed at changing the subcellular distribution of glucose and LCFA transporters, thereby manipulating the substrate preference of the diseased heart to help restore cardiac function.

KEYWORDS
Sarcolemmal transport; Cardiomyocytes; GLUT4; CD36; Cardiac hypertrophy; Diabetic cardiomyopathy

1. Introduction

Dependent on substrate availability, the heart may adapt its ratio of glucose and long-chain fatty acid (LCFA) oxidation, an effect referred to as the glucose/fatty acid- or Randle-cycle.1 Thus, when increased amounts of LCFA are circulating, LCFA oxidation is increased while glucose oxidation is decreased.2 In contrast, reducing LCFA metabolism in the heart by either reducing LCFA levels in the blood or directly inhibiting mitochondrial β-oxidation leads to increased rates of pyruvate oxidation.3 As a result, substrate availability is one of the primary factors determining substrate preference (glucose vs. LCFA).

In addition, the heart is capable of altering its substrate utilization under conditions in which the substrate availability is not immediately changed, as for example during the transition from rest to exercise. Under such conditions, the transport of glucose and LCFA across the plasma membrane as well as the transport of their derivatives into the mitochondrial matrix are rate limiting for cardiac substrate utilization. Glucose is transported across the plasma membrane along its concentration gradient by glucose transport proteins. Because of their physicochemical nature, LCFA might cross the lipid bilayer by passive diffusion. However, distinct LCFA binding and transport proteins have been identified and were found to be crucial for proper LCFA uptake. Studies during the last few years indicate that the intracellular distribution of glucose and LCFA transporters is acutely modulated by both insulin treatment and during myocyte contraction. Both stimuli increase the rate of glucose and LCFA uptake in parallel. However, the metabolic fate of LCFA taken up by insulin treatment vs. contraction stimulation is different. While insulin promotes the storage of both substrates, contraction activates oxidative pathways to generate adenosine triphosphate (ATP).

An imbalance of glucose and LCFA oxidation is associated with various pathological states. During obesity an increase in the LCFA release from the abdominal fat depots leads to an elevated level of circulating LCFA and increased uptake by cardiac and skeletal muscle.4 The latter not only leads

* Corresponding author. Tel: +31 43 388 1208/1998; fax: +31 43 388 4574. E-mail address: glatz@gen.unimaas.nl

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to accumulation of LCFA in non-adipose tissues but also inhibits glucose oxidation and reduces insulin sensitivity. The consequences for the heart were found to be a decreased cardiac efficiency and the onset of diabetic cardiomyopathy. Therefore, a deranged glucose/LCFA metabolism is regarded as a strong risk factor contributing to the rapidly increasing number of patients with obesity, type-2 diabetes, and cardiovascular diseases in western countries.

Recent studies have documented that the amount of substrate transporters within the sarcolemma presents a key regulatory step of myocardial glucose and LCFA utilization. Hence, selectively modulating glucose and LCFA transporter distribution so as to favour the uptake of either glucose or LCFA might be an attractive approach to restore cardiac metabolism of the diseased heart. In this review we will summarize the current knowledge about the signalling and trafficking pathways that determine cardiac glucose and LCFA transporter distribution. Additionally, we will focus on the similarities and differences of GLUT4 and CD36 recruitment in response to these signalling pathways as well as the altered distribution of the substrate transporters observed in several heart diseases.

2. Transmembrane transport of glucose and fatty acids in cardiac myocytes

In order to keep the concentration gradient of glucose and LCFA directed inwardly, the intracellular concentration of unbound glucose and LCFA is kept low by enzymatic trapping of the substrates upon entering the cardiac myocyte. Glucose is rapidly converted into glucose-6-phosphate and either stored as glycogen or further oxidized through the glycolytic pathway. LCFA are rapidly converted into acyl-CoA, but in order to need to be bound to the heart-type cytoplasmic fatty acid-binding protein (H-FABP<sub>p</sub>) in order to be transported to the outer mitochondrial membrane, the main site of fatty acyl-CoA synthetase (ACS). Alternatively, LCFA can also be converted to LCFA-CoA directly upon entering the cell by fatty acid transport proteins (FATP). In the working heart, the bulk of LCFA are devoted to mitochondrial β-oxidation, and only a minor portion is stored in lipid droplets.

Because of its hydrophilic nature, glucose is unable to pass the lipid bilayer of the plasma membrane by simple diffusion. In cardiomyocytes two members of the glucose transporter family GLUT are present, GLUT1 and GLUT4. While GLUT1 is regarded as the basal glucose transporter, GLUT4 is responsible for the increase in glucose uptake upon insulin stimulation and elevated contractile activity. The biophysical properties of LCFA do not rule out the possibility of passive diffusion, but surprisingly passive diffusion only contributes to a minor degree to LCFA uptake into the heart. Indeed, LCFA have been found to rapidly partition into the outer layer of the sarcolemmal bilayer, but their rate of flip-flop across the bilayer to the inner side is low (although this is disputed by others). In agreement, kinetic studies and inhibition studies showed that the bulk of LCFA uptake is a protein-mediated process.

Distinct proteins are proposed as putative LCFA transporters, i.e. CD36, the plasma membrane-associated fatty acid-binding protein (FABP<sub>p</sub>) and FATPs. In the heart, CD36 is responsible for the majority of LCFA uptake, which was shown by specific inhibition of the protein with sulfo-N-succimidyl esters of LCFA. CD36 and FABP<sub>p</sub> most likely act in concert to mediate LCFA uptake by cardiomyocytes. So far, the precise mechanism of CD36-facilitated LCFA uptake is incompletely understood and different mechanisms have been proposed. CD36 and FABP<sub>p</sub> might serve as acceptors of albumin-bound extracellular LCFA to increase their local concentration at the cell surface. Besides facilitating the diffusion of LCFA across the sarcolemma via flip-flop or acting as a transporter itself, CD36 might also bring LCFA into the periphery of FATP to accelerate the rate of trans-membrane transport of LCFA (Figure 1). The colocalization of FATP1, FATP6, and CD36 within distinct areas of the plasma membrane, makes an interaction likely. Whether and to which extent an interaction of the CD36/ FABP<sub>p</sub> complex with one or more of the FATP species occurs is currently not known.

Besides their presence at the sarcolemma, most of the substrate transporters have been found to be present also in intracellular compartments. The sarcolemmal pool forms the functional pool of these transporters, whereas the intracellular pools make up the storage depots. Regulation of substrate transport can occur via translocation of transporters from these intracellular depots to the sarcolemma, thereby increasing the functional transporter pool. This transporter translocation is a vesicle-mediated process. A number of physiological stimuli, i.e. contractile activity and insulin, have been found to induce transporter translocation. GLUT4 was the first substrate transporter identified to travel between intracellular compartments and the cell surface. Despite some unresolved issues, the general consensus is that the majority of cardiac GLUT4 is stored in at least two distinct intracellular compartments, i.e. a non-endosomal compartment, referred to as the GLUT4 storage compartment, and an endosomal compartment within the recycling endosomes (Figure 2). The non-endosomal compartment contains the bulk of the intracellularly stored GLUT4. Stimulation with insulin induces a massive translocation of GLUT4 vesicles from the non-endosomal compartment to the recycling endosomes and from there to the plasma membrane. The shift of GLUT4 to the plasma membrane accounts for the multiple-fold increase in glucose uptake observed after insulin stimulation. GLUT4 translocated to the plasma membrane in response to contraction stimulation is recruited from the endosomal compartment. Although the endosomal compartment provides GLUT4 for translocation due to various stimuli, it contributes only marginally to insulin-stimulated GLUT4 translocation. GLUT1 has long been considered to be involved in basal glucose uptake only, but at least in the heart, GLUT1 has been found to be present within recycling endosomes from where it can be translocated to increase glucose uptake in response to insulin and contraction.

The concept that LCFA uptake also can be regulated by transporter translocation is relatively new and was based on the discovery that in skeletal muscle and heart a marked portion of the cellular CD36 was detected in intracellular membrane compartments, presumably endosomes. Importantly, CD36 translocation from these compartments has been found to be responsible for stimulus-induced LCFA uptake. The subcellular localization of CD36 resembles that of GLUT1 rather than GLUT4: a 50–50% distribution
between recycling endosomes and the sarcolemma. There is evidence for intracellular storage of FATP1 in adipocytes, but in the heart it is likely constitutively present at the sarcolemma. FATP4 and -6 have been found to be present at the sarcolemma, but there is no evidence yet for an intracellular localization of both FATP isoforms.

Because the sarcolemma harbours multiple subdomains with entirely distinct functions, a further refinement of subdomain-specific sarcolemmal localization of individual sets of substrate transporters may reveal additional levels of regulation of substrate uptake. For instance, sarcolemmal GLUT4 has been found to be present both within and outside of caveolae, but the role of caveolae in the regulation of glucose uptake is yet unknown. Interestingly, at least in adipocytes sarcolemmal CD36 also has been reported to be present both inside and outside of caveolae, with the caveolar population presenting the functional pool of CD36. An attractive topic for future research is the investigation whether these observations can be extrapolated to the myocardium.

3. Signalling pathways that stimulate glucose and fatty acid uptake

The energetic demands of the heart could be covered for a short period by the breakdown of intracellularly stored glycogen and lipid droplets but longer duration would rely on the uptake of exogenous glucose and LCFA. Circulating insulin and elevated contractile activity are the two major signals responsible for acute increases in cardiac substrate uptake, enabled by inducing transporter translocation from intracellular stores to the sarcolemma as explained above. Both stimuli activate discrete signalling pathways, yet recent data indicate different points where these pathways merge (Figure 2). The latter indicates a conserved mechanism in the initiation of transporter translocation that is shared by both insulin and contraction stimulation.

3.1 Insulin signalling pathway

In response to increased levels of glucose and amino acids in the blood, e.g. after a meal, insulin is secreted from \( \beta \)-cells in the pancreas. After binding to its receptor at the surface of muscle and adipose tissue, insulin activates the tyrosine kinase activity of the insulin receptor. Activation of the insulin receptor kinase results in phosphorylation of a number of specific substrates, e.g. members of the insulin-receptor substrate family (IRS1-4) and the protooncogen Cbl. In skeletal and cardiac muscle the activation of the regulatory subunit \( p_{85} \) of phosphatidyl-inositol-3-kinase (PI3K) by IRS1 is the main signal that leads to insulin-stimulated uptake of glucose and LCFA. Activation of the insulin receptor kinase results in phosphorylation of a number of specific substrates, e.g. members of the insulin-receptor substrate family (IRS1-4) and the protooncogen Cbl. In skeletal and cardiac muscle the activation of the regulatory subunit \( p_{85} \) of phosphatidyl-inositol-3-kinase (PI3K) by IRS1 is the main signal that leads to insulin-stimulated uptake of glucose and LCFA. However, it is still debated whether or not it is functionally operative in cardiac and skeletal muscle. Perhaps dual input of both pathways is necessary for full activation of GLUT4- (and maybe CD36-) translocation also in muscle tissue.

PI3K consists of a catalytic \( p_{110} \) subunit and a regulatory \( p_{85} \) subunit and catalyses the phosphorylation of phosphoinositides to phosphatidyl-inositol-3-phosphates, most importantly phosphatidylinositol-3,4,5-tri-phosphate (PIP3). In turn, PIP3 activates different kinases, among others the phosphoinositide-dependent kinase (PDK), the serine/threonine protein kinase B (PKB/Akt) and the atypical protein kinase C PKC\( \zeta \). Subsequently, PDK phosphorylates PKB/Akt and the two atypical protein kinase C isoforms, PKC\( \lambda \), and PKC\( \zeta \). Taken together, Akt and the atypical PKCs contribute in parallel to insulin-induced GLUT4 translocation.
The functional consequence of Akt activation is the subsequent phosphorylation and inhibition of the Akt substrate of 160 kDa (AS160). As a negative regulator of GLUT4 translocation AS160 restrains GLUT4 in its storage compartment in the basal state. The inhibition of AS160 therefore is a crucial step to release GLUT4 for translocation to the plasma membrane in response to insulin. AS160 is a negative regulator of Rab GTPases, promoting their inactive, guanosine diphosphate (GDP)-bound form. For its substrates Rab8a, Rab10, and Rab14 an involvement in insulin-stimulated GLUT4 translocation was confirmed. Other GTPases such as Rab4a and Rab11a, which are not substrates of AS160, have also been shown to be involved in insulin-independent sequestration of myocardin-dependent GLUT4. Other Rab proteins in insulin-induced CD36 translocation is currently not known.

Myocardial GLUT4, GLUT1, and CD36 are simultaneously translocated to the plasma membrane in response to insulin. However, the majority of intracellular GLUT4 and CD36 appear to be sequestrated in separate compartments.

As mentioned in the previous section, in non-stimulated myocytes GLUT4 is stored in the GLUT4 storage compartment and in recycling endosomes, whereas CD36 and GLUT1 are only stored in the recycling endosomes. The storage compartment harbours the largest pool of insulin-responsive GLUT4, which is transported to the plasma membrane through budding of small membrane vesicles. The GLUT4-containing transport vesicles mobilized from the non-endosomal storage compartment do not migrate directly to the cell surface, but first fuse with the recycling endosomes. Simultaneously, transport vesicles will be assembled from insulin-sensitive subcompartments within the recycling endosomes for further transport of GLUT4 to the sarcolemma. Possibly, endosomal CD36 and GLUT1 translocate independently on separate transport vesicles to the sarcolemma.

In rat cardiomyocytes the translocation of these substrate transporters is connected with an equivalent increase of glucose and LCFA uptake rates. In contrast to the...
GLUTs and CD36, the amount of sarcolemmal FABPpm is virtually not or only slightly increased after insulin-stimulation while the cell surface abundance of FATP1 and FATP6 is not altered. Overall, CD36 and not the other LCFA transporters are implicated in the dynamics of insulin-mediated LCFA uptake into the heart.

3.2 Contraction pathway
An increase in contractile activity results in a rapid rise in the concentration of a number of second messengers in isolated cardiac myocytes, such as adenosine monophosphate (AMP), cyclic AMP, Ca\(^{2+}\), and reactive oxygen species. Together these second messengers activate a complex network of signalling events. Among all the protein kinases activated by contraction [e.g. protein kinase A (PKA), protein kinases C (PKC) \(\delta\) and \(\epsilon\), extracellularly-regulated protein kinases (ERK) 1 and 2, mitogen-activated protein kinase (MAPK) and Ca\(^{2+}\)-calmodulin-dependent protein kinases (CaMK)], especially activation of AMP-activated protein kinase (AMPK) is known to have metabolic implications in contraction-induced substrate uptake.

Multiple genes encode different isoforms for each of the three subunits (\(\alpha\), \(\beta\), and \(\gamma\)) of AMPK, leading to up to 12 possible combinations. The \(\alpha\)-subunit contains the catalytic domain and is connected to the regulatory \(\beta\)- and \(\gamma\)-subunits at its C-terminus. Both isoforms of the \(\alpha\)-subunit (\(\alpha_1\) and \(\alpha_2\)) contribute to the increase in glucose uptake during exercise and are upregulated to compensate the loss of function of the other isoform as shown in skeletal muscle of AMPK\(\alpha_1\) and AMPK\(\alpha_2\) knockout mice. However, AMPK\(\alpha_1\) upregulation is not sufficient to prevent a severe drop in ATP and phosphocreatine during contraction in AMPK\(\alpha_2\) knockout mice. AMPK is activated by the binding of AMP to its regulatory \(\gamma\)-subunit. Furthermore it is phosphorylated at Thr172 of the \(\alpha\)-subunit by tumour suppressor LKB1. The phosphorylation by LKB1 is due to a conformational change of AMPK after AMP-binding and leads to a further increase of the activity of the enzyme. Another AMPK kinase identified in LKB1-deficient cells is CaMKK\(\beta\)2. Thus, the presence of CaMKK\(\beta\) in the heart makes a regulation of cardiac AMPK activity by CaMKK\(\beta\) likely.

A major substrate of AMPK in the heart is acetyl-CoA carboxylase (ACC), which is phosphorylated at Ser219. Ser219 phosphorylation is inhibitory to ACC activity and, therefore, decreases the conversion of acetyl-CoA into malonyl-CoA. Because malonyl-CoA is a negative regulator of CPT1, the rate-limiting enzyme in mitochondrial \(\beta\)-oxidation, ACC-phosphorylation leads to an increase in LCFA oxidation.

The contraction-induced rapid induction of the translocation of GLUT4, CD36, and FABPpm from intracellular stores to the sarcolemma is also mediated by AMPK. The sequence of events downstream of AMPK leading to translocation of these transporters is largely unknown, but might also involve AS160. The intracellular source of GLUT4 and CD36 mobilized upon AMPK activation is likely formed by contraction-sensitive subcompartments within the recycling endosomes, whereas the intracellular source of recruitable FABPpm is not known. It is also not known whether GLUT4 and CD36 travel together or, alternatively, on separate vesicles to the sarcolemma upon AMPK activation.

A recently identified contraction-activated protein kinase is protein kinase D-1 (PKD1). A pharmacological blockade of PKD1 is associated with inhibition of contraction-induced GLUT4 translocation and glucose uptake, suggesting that, independent of AMPK, PKD1 could also be involved in stimulation of cardiac substrate uptake upon an increase in contractile activity. Interestingly, contraction-induced PKD1 activation in cardiomyocytes was observed to be fully independent of AMP levels and AMPK signalling, as it was preserved in AMPK\(\alpha_2\) knockout mice. This suggests that the contraction-responsive endosomes need the combined input from (at least) two separate pathways to initiate budding of vesicles for carrying substrate transporters to the sarcolemma. Because also insulin-induced GLUT4 translocation is dependent on activation of two independent signalling pathways, PI3K and Cbl, the dual input might be a novel concept in stimulus-induced transporter translocation in order to safeguard the specificity of the translocation events. It is currently unknown, whether PKD is also involved in contraction-induced CD36 translocation and LCFA uptake.

3.3 Levels where insulin- and contraction-dependent signalling merge
Both insulin treatment and contraction stimulation increase glucose and LCFA uptake by inducing the translocation of substrate transporters from intracellular compartments to the plasma membrane. The effects of insulin and contraction on transporter translocation and substrate uptake are additive, indicating that separate mechanisms are involved in insulin- vs. contraction-induced substrate uptake. Indeed, PI3K is involved in insulin-induced GLUT4- and CD36 translocation, but not in contraction-induced GLUT4- and CD36 translocation, whereas AMPK (and PKD) are involved in contraction-induced GLUT4- and CD36 translocation but not in insulin-induced GLUT4- and CD36 translocation. However, several junctions downstream of insulin and contraction signalling have been revealed.

AS160, which is involved in insulin-stimulated GLUT4 translocation, also has been shown to be a substrate of AMPK upon contraction stimulation, and necessary for contraction-induced glucose uptake. Moreover, insulin-induced AS160 phosphorylation is additive to contraction-induced AS160 phosphorylation. Together, these observations indicate that the insulin signalling and contraction pathways meet at the level of AS160. Its inhibition is necessary to induce the translocation of GLUT4 and potentially also the translocation of CD36.

It is firmly established that, at least in skeletal muscle, PI3K-mediated PKC-\(\zeta\) activation is necessary for insulin-induced GLUT4 translocation. Treadmill exercise also activates PKC\(\zeta\), and insulin even increases this effect. In the same study, the stimulatory effects of the pharmacological AMPK activator 5-aminimidazole-4-carboxamide-1-\(\beta\)-D-ribofuranoside (AICAR) on GLUT4 translocation and glucose uptake were blocked by pre-treatment of muscle cells with myristoylated PKC\(\zeta\) pseudosubstrate, indicating the involvement of PKC-\(\zeta\) in AMPK-mediated substrate utilization. As already mentioned, activation of PKC-\(\zeta\) by insulin involves combined phosphorylation by PDK1 and binding to PIP\(\gamma\) produced by PI3K. Activation of PKC-\(\zeta\) downstream of AMPK requires sequential activation of proline-rich tyrosine
kinase-2, ERK pathway components, and phospholipase D.\textsuperscript{61} It remains to be established whether these PKC-\(\zeta\) observations made in skeletal muscle can be extrapolated to the heart, and also whether PKC-\(\zeta\) is involved in insulin- as well as contraction-induced CD36 translocation.

Taken together, the proteins involved in the insulin-induced transporter translocation machinery and contraction-induced transporter translocation machinery can be divided into two classes: (i) proteins that are specific to either machinery so as to ensure that insulin-responsive compartments are activated to mobilize transporter-containing vesicles in response to insulin (e.g., Akt/PKB) and contraction-responsive compartments in response to contraction (e.g., AMPK) and (ii) proteins that are common to both machineries, likely because of their general involvement in vesicle budding and translocation. In this respect, inhibition of AS160 appears necessary for activation of GLUT4 vesicles to translocate to the plasma membrane, and the function of PKC\(\zeta\) appears to be the remodelling of the actin cytoskeleton, which is the transport route for the GLUT4-containing vesicles.\textsuperscript{63} Both, the activation of GLUT4 vesicles and their transport along the actin cytoskeleton are general preconditions to increase sarcolemmal GLUT4 and glucose uptake in response to insulin or increased workload. Therefore, both signalling pathways might converge at the level of GLUT4 translocation and interact with the same regulatory elements. A similar mechanism may operate in case of CD36 translocation.

4. Transporter regulation in cardiac disease

In a number of cardiac diseases the contribution of the main substrates glucose and LCFA to cardiac energy production is chronically altered. For instance, the development of pathologic cardiac hypertrophy and cardiac failure is characterized by a gradual decrease in LCFA utilization, partly compensated for by increased glucose utilization.\textsuperscript{2,64} In contrast, in case of diabetes the heart suffers from impaired glucose uptake and relies almost completely on LCFA. As a result, the balance between the utilization of glucose and LCFA, which under normal conditions is distinctive and finely tuned, is upset in cardiac disease to the marked preference for a single substrate class. Interestingly, a genetic limitation of the heart in the use of either glucose or LCFA, which forces a shift in this substrate balance, elicits cardiac malfunctioning.\textsuperscript{65} These observations led to the notion that a disruption of the cardiac substrate balance and cardiac pathology appear invariably linked (Figure 3).\textsuperscript{8}

Several studies, mostly in rodents but some also in humans, have shown that a shift in substrate balance as occurs in different cardiac pathologies can be linked to altered regulation of sarcolemmal substrate transporters as will be discussed later.

4.1 Cardiac hypertrophy and heart failure: lessons from animal models

During the development of pathologic cardiac hypertrophy the shift in substrate preference towards increased glucose utilization is accompanied by impaired cardiac functioning, and further worsens when hypertrophy develops towards cardiac failure.\textsuperscript{14,64} For instance, in two frequently used rat models of cardiac hypertrophy, i.e. the aortic-constricted rat and the myocardial-infarcted rat,\textsuperscript{66} there is a shift from LCFA utilization to glucose utilization with a concomitant down-regulation of lipid metabolic enzymes and LCFA transporters.\textsuperscript{67–70} Conversely, inhibition of cardiac LCFA utilization by blocking mitochondrial \(\beta\)-oxidation leads to elevated glucose utilization, and ultimately elicits the development of cardiac hypertrophy.\textsuperscript{71,72} Also inborn errors of \(\beta\)-oxidation are a cause of hypertrophic cardiomyopathies in man.\textsuperscript{73}

It has been reported that rats chronically treated with sulfo-N-succimidyl-palmitate, a specific inhibitor of CD36, develop cardiac hypertrophy.\textsuperscript{74} The same was observed in mice with a null mutation in the genes for CD36,\textsuperscript{75} FABPc\textsuperscript{76} as well as the spontaneously hypertensive rat lacking CD36 expression.\textsuperscript{77} As a consequence of the CD36...
deficiency, the contraction-stimulated increase in LCFA uptake is missing, showing the impact of CD36 for acute regulation of LCFA uptake into cardiomyocytes. In CD36 null mice, glucose clearance after a high carbohydrate/low fat diet occurs faster than in wild-type mice, concomitant with the switch in substrate utilization from LCFA to glucose. The observations in CD36 null mice match to a study in a Japanese population in which inherited hypertrophic cardiomyopathy showed a high prevalence of a mutation or deficiency in CD36. Studies in aortic-constricted rats also revealed that the impaired LCFA utilization was not due to limitations in mitochondrial oxidation but rather may reside at the level of LCFA transport across the sarcolemma.

Because cardiac hypertrophy is linked to a shift of cardiac metabolism towards glucose utilization, it was surprising to see that the homozygous knockout of GLUT4 in mice resulted not in the development of diabetes but rather in the development of cardiac hypertrophy. The loss of GLUT4 obviously induced a compensatory upregulation of GLUT1. Enhanced expression of GLUT1 has already been associated with failure of postinfarcted rat hearts; however, the study gives no information about the cellular distribution of glucose transporters in the analysed hearts. Nevertheless, overexpression of GLUT1 acts cardioprotective in the pressure-overloaded heart, because of the higher efficiency of glucose compared with LCFA utilization with respect to oxygen consumption. Apparently, cardiac function is highly dependent on GLUT4-mediated glucose transport, a complete loss of GLUT4 leads to compensatory GLUT1 activity and downregulation of proteins required in LCFA utilization. This mechanism prevents the onset of diabetic cardiomyopathy, but still is unable to maintain or restore cardiac function. It might be speculated that parts of the GLUT4 trafficking machinery are essential for correct adjustment of balanced glucose and LCFA utilization.

4.2 Diabetic cardiomyopathy: lessons from animal models

Sarcolemmal substrate transporters have been found also to be involved in the aberrations in substrate utilization found in the diabetic heart. In animal models of type-2 diabetes (obese Zucker rats, high-fat diet-fed rats, rats fed sucrose during weanling), LCFA uptake into heart as well as skeletal muscle was chronically increased due to increased abundance of CD36, but not FABPpm, at the sarcolemma. In hearts of db/db mice, the elevated LCFA influx is accompanied by a relocation of both CD36 and FABPpm to the sarcolemma. The increase in the CD36 functional pool is not due to increased total tissue expression, but rather to a permanent relocation of this transporter from intracellular stores (recycling endosomes) to the sarcolemma. At least, obese Zucker rats and high-fat diet-fed rats are known to develop diabetic cardiomyopathy. Evidence is accumulating that this permanent CD36 relocation is an early key event in the etiology of diabetic cardiomyopathy, and precedes changes in insulin-induced GLUT4 translocation and a decrease in cardiac functioning.

The increased LCFA influx into the diabetic heart results in an increased oxidation of LCFA, so that LCFA oxidation becomes responsible for ~90% of total cardiac energy production. Nonetheless, the LCFA influx exceeds the mitochondrial oxidative capacity of the heart, giving rise to the accumulation of non-oxidative lipid metabolites and expansion of lipid droplets. Especially, this lipid accumulation in the diabetic heart has been considered as a hallmark in the etiology of diabetic cardiomyopathy. Notably, elevated levels of diacylglycerols and ceramides have been postulated to interfere with insulin signalling (and, hence, insulin-induced GLUT4 translocation) through activation of Ser/Thr kinase cascades (involving members of the PKC family) that result in increased Ser-phosphorylation of the insulin receptor, which inhibits its intrinsic tyrosine kinase activity and the subsequent activation of PI3K and Akt/PKB. The ability of the cell-impermeable CD36 inhibitor sulfo-N-succimidyl-oleate (SSO) to reduce the rate of esterification of LCFA into triacylglycerides in cardiac myocytes from obese Zucker rats to levels observed in nondiabetic rats provides direct evidence that sarcolemmal CD36 is causal to the accumulation of LCFA metabolites in the type-2 diabetic heart. Hence, CD36 would be an attractive target to design clinical therapies against lipid-induced diabetic cardiomyopathy.

A molecular event causal to the permanent CD36 relocation in the diabetic heart might be the elevated basal activity of Akt/PKB. This increase in basal Akt/PKB phosphorylation could be due to chronically increased circulating insulin levels as observed in obese Zucker rats or due to PI3K-independent signalling cascades in hearts of high-fat fed rats that do not display hyperinsulinemia. Elucidation of the signalling kinases responsible for chronically increased basal phosphorylation of Akt/PKB might lead to novel therapies using agents that directly inhibit these kinases, and hence lower basal Akt/PKB phosphorylation, so as to prevent permanent CD36 relocation to the sarcolemma and the resulting loss in cardiac function.

This novel role of CD36 in the development of insulin resistance can be extrapolated to the human setting: in skeletal muscle biopsies from obese and type-2 diabetic subjects CD36 was found to be relocated to the sarcolemma, and this upregulation appeared closely correlated with muscular triacylglycerol accumulation. Based on the great similarity in the regulation of intermediary metabolism between heart and skeletal muscle, the notion arises that besides in rodents, CD36 most likely is a critical factor in the etiology of human diabetic cardiomyopathy as well.

In comparison with CD36 and FABPpm, fewer studies have been performed on the role of FATPs in the development of diabetic cardiomyopathy. The generation of transgenic mice with cardiac-specific overexpression of FATP1 provided additional evidence to the concept that upregulation of cardiac LCFA uptake eventually results in a loss of cardiac function. Conversely, knocking out FATP1 gene expression is protective against lipid-induced insulin resistance, at least in mouse skeletal muscle. Nonetheless there is little evidence for a direct role of FATP1 in the etiology of diabetic cardiomyopathy in obese Zucker rats and high-fat diet-fed rats, because neither cardiac expression nor subcellular localization of this transporter is altered in these rodent models.

Concluding remarks

Myocardial utilization of the main metabolic substrates glucose and LCFA is regulated not only by substrate availability and competition at the level of mitochondria (Randle-cycle) but also at the site of cellular entry. The
abundance of substrate transporters at the sarcolemma governs their rate of uptake, independent of the intracellular channelling of glucose and LCFA. The sarcolemmal amount of selected transporters, in particular GLUT4 and CD36, is acutely regulated by endogenous stimuli such as insulin or during elevated muscular work. Although these stimuli activate two largely distinct signalling pathways, they both result in simultaneous translocation of GLUT4 and CD36 from intracellular stores to the plasma membrane, leading to a simultaneous increase in myocardial glucose and LCFA uptake.

The heart appears sensitive to a chronic change in the balance of glucose and LCFA utilization, as both a shift towards an increased glucose contribution or to an increased LCFA contribution to total ATP-production is invariably linked to cardiac disease. These chronic changes in substrate utilization are paralleled by concomitant changes in sarcolemmal presence of substrate transporters. Therefore, it has been suggested that modulating the cellular distribution of, in particular, the substrate transporters GLUT4 and CD36 in the diseased heart may normalize cardiac metabolism and restore cardiac function. While insulin and contraction lead to the simultaneous recruitment of GLUT4 and CD36, preliminary studies in rat cardiomyocytes indicate that arsenite98 and dipyridamole 99 specifically recruit these transporters leading to selectively increased glucose and LCFA utilization, respectively. The intracellular targets of these pharmacological compounds are not known, but the possibility to translocate selectively either one transporter suggests that GLUT4 and CD36 may be stored in specific endosomal subcompartments and are recruited by distinct sorting mechanisms. Specificity at the level of these compartments might be mediated by distinct Rab-GTPases, of which some are substrates of AS160. Numerous members of this protein family have already been implicated in GLUT4 vesicle transport and themselves are strongly regulated by specific effector proteins. Recently, we found that Rab11a-mediated GLUT4 and CD36 trafficking is dependent on different subsets of Rab11a effector proteins.100 These findings elude to the general concept that selective transporter translocation can be achieved by activating or inhibiting specific trafficking proteins. Accordingly, future studies should focus on a detailed identification of the trafficking machineries dedicated to GLUT4 and CD36 translocation. Especially, trafficking proteins not shared by both machineries are candidate targets for future therapies to regulate subcellular GLUT4 and CD36 distribution separately aimed at restoring cardiac metabolism and cardiac function.

Conflict of interest: none of the authors reports a conflict of interest.

Funding
Our studies were supported by the Netherlands Organization for Health Research and Development (ZonMW grant 912-04-075), the European Commission (Integrated Project LSHM-CT-2004-005272, Exogenesis), and the Heart and Stroke Foundation of Ontario. J.J.F.P.L. is the recipient of a VIDI-Innovational Research Grant from ZonMW (grant 016.036.305), A.B. is the Canada Research Chair in Metabolism and Health and J.F.C.G. is Netherlands Heart Foundation Professor of Cardiac Metabolism.

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