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Cardiac substrate uptake and metabolism in obesity and type-2 diabetes: Role of sarcolemmal substrate transporters

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Abstract

Cardiovascular disease is the primary cause of death in obesity and type-2 diabetes mellitus (T2DM). Alterations in substrate metabolism are believed to be involved in the development of both cardiac dysfunction and insulin resistance in these conditions. Under physiological circumstances the heart utilizes predominantly long-chain fatty acids (LCFAs) (60–70%), with the remainder covered by carbohydrates, i.e., glucose (20%) and lactate (10%). The cellular uptake of both LCFA and glucose is regulated by the sarcolemmal amount of specific transport proteins, i.e., fatty acid translocase (FAT)/CD36 and GLUT4, respectively. These transport proteins are not only present at the sarcolemma, but also in intracellular storage compartments. Both an increased workload and the hormone insulin induce translocation of FAT/CD36 and GLUT4 to the sarcolemma. In this review, recent findings on the insulin and contraction signalling pathways involved in substrate uptake and utilization by cardiac myocytes under physiological conditions are discussed. New insights in alterations in substrate uptake and utilization during insulin resistance and its progression towards T2DM suggest a pivotal role for substrate transporters. During the development of obesity towards T2DM alterations in cardiac lipid homeostasis were found to precede alterations in glucose homeostasis. In the early stages of T2DM, relocation of FAT/CD36 to the sarcolemma is associated with the myocardial accumulation of triacylglycerols (TAGs) eventually leading to an impaired insulin-stimulated GLUT4-translocation. These novel insights may result in new strategies for the prevention of development of cardiac dysfunction and insulin resistance in obesity and T2DM. (Mol Cell Biochem **299:** 5–18, 2007)

Key words: CD36, fatty acid uptake, GLUT4, type-2 diabetes

Introduction

In both Europe and the United States of America the incidence of obesity and type-2 diabetes mellitus (T2DM) is drastically increasing [1–5]. Obesity has been defined as having too much fat body mass compared to lean body mass. A western lifestyle and genetic factors play major roles in the development of obesity [6, 7]. It is well known that obesity

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influences the total body metabolism of both lipids and carbohydrates, the regulation of blood pressure, and thrombotic and inflammatory reactions [8, 9]. Most obese patients develop insulin resistance, which is characterized by impairment in the insulin-mediated glucose uptake by muscle and adipose tissue. Insulin resistance is also one of the key features of patients with T2DM. In T2DM insulin resistance occurs before the onset of hyperglycemia.

Although the heart plays a minor role in the development of whole-body insulin resistance, cardiovascular complications are one of the main causes of morbidity and death in insulin resistant obese and T2DM patients [10, 11]. Obesity and T2DM are both independent risk factors for the development of cardiac dysfunctioning [12–14]. In T2DM patients who have no clinically manifested cardiovascular diseases, the left ventricle mass is increased combined with a diastolic dysfunctioning, whereas systolic functioning is normal [15–17]. These structural and functional alterations in the hearts of T2DM patients are referred to as diabetic cardiomyopathy, which has a unique entity [13, 18]. During the development of T2DM this diabetic cardiomyopathy can progress towards heart failure. Although diabetic cardiomyopathy is an outcome of a multifactorial process, a disturbance in substrate metabolism of the heart is thought to be crucial in the development of cardiac dysfunctioning.

In this review we will address recent findings on the role of sarcolemma-associated substrate transporters and their regulation in myocardial substrate utilization in obesity and T2DM. These findings lead us to hypothesize that disturbances in the regulation of cardiac long-chain fatty acid (LCFA) utilization play a prominent role in the development of cardiac insulin resistance and cardiac dysfunctioning. First, we discuss novel aspects of cardiac substrate metabolism under physiological conditions, especially its regulation by an increased workload and by the hormone insulin. Second, alterations of cardiac substrate metabolism in obesity and T2DM will be discussed. Finally, possible therapeutic strategies aiming at correcting cardiac substrate metabolism in disease are addressed.

Mechanism of glucose and long-chain fatty acid uptake and metabolism in the heart

The heart predominantly consists of specialized muscle cells, cardiac myocytes, which perform a constant contraction and relaxation cycle in a coordinated fashion. To be able to contract cardiac myocytes need to generate energy, which is derived from ATP hydrolysis [19]. ATP itself is generated during the oxidation of substrates. Cardiac myocytes are omnivores and can utilize fatty acids, carbohydrates, and ketone bodies [20]. Under physiological conditions these cells oxidize predominantly long-chain fatty acids (LCFA) (60–70%), with the remainder covered by carbohydrates, i.e., glucose (20%) and lactate (10%) [21, 22]. Before intracellular oxidation, these substrates have to enter the cardiac myocytes by crossing the sarcolemma.

The uptake of extracellular glucose by cardiac myocytes is regulated by the amount and activity of glucose transporters at the sacrolemma [23]. In the heart two glucose transport proteins, i.e., GLUT1 and GLUT4 are present, of which GLUT4 is most abundant [24] (Fig. 1). Both GLUT1 and GLUT4 are not only located at the sarcolemma, but also in intracellular storage compartments [25]. Upon entering cardiac myocytes, glucose is rapidly phosphorylated into glucose-6-phosphate by hexokinase, resulting in intracellular trapping. Thereafter, glucose-6-phosphate can either be stored as glycogen or enter the glycolytic pathway. The end product of glycolysis, pyruvate, crosses the mitochondrial membranes and is converted into acetyl-CoA by the enzymatic action of pyruvate dehydrogenase (PDH). Acetyl-CoA undergoes further mitochondrial metabolism resulting in the synthesis of ATP by oxidative phosphorylation.

LCFAs destined for cardiac utilization are present in the circulation in two forms, (i) in complex with albumin and (ii) esterified in the lipid core of very-low density lipoproteins (VLDLs) and chylomicrons [26, 27]. While albumin-bound LCFAs dissociated easily from albumin, the esterified LCFAs have to be hydrolyzed by the action of lipoprotein lipase at the luminal surface of the endothelium before they become available. Thereafter, LCFAs are transported across the endothelium, via a mechanism that is yet unclear. Once present in the interstitial space LCFAs are complexed with albumin, their transport vehicle through this aqueous compartment (Fig. 1). Prior to their transport across the sarcolemma, LCFAs dissociate again from albumin. Although it was originally believed that the transport of LCFAs across the sarcolemma into cardiac myocytes occurs solely by passive diffusion [28, 29], now it is generally accepted that the bulk of LCFAs are taken up via a protein-mediated (facilitated) transport system [30, 31].

Several putative LCFA transport proteins have been identified and are thought to act in concert to facilitate cardiac LCFA uptake. The molecular mechanism however is incompletely understood [32]. Evidence is accumulating that the 88 kDa fatty acid translocase (FAT), a rat homologue of human CD36, is the main putative LCFA transport protein (reviewed by Brinkmann *et al.* [33). FAT/CD36, like GLUT4, is not only present at the sarcolemma, but also in intracellular storage compartments [34]. A strong positive correlation between the amount of FAT/CD36 present at the sarcolemma and the uptake of LCFA has been observed in cardiac myocytes [35]. Moreover, by blocking FAT/CD36 with sulfo-*N*-succinimidyl oleate (SSO), which specifically binds to FAT/CD36, myocardial LCFA uptake is markedly inhibited [36]. In addition

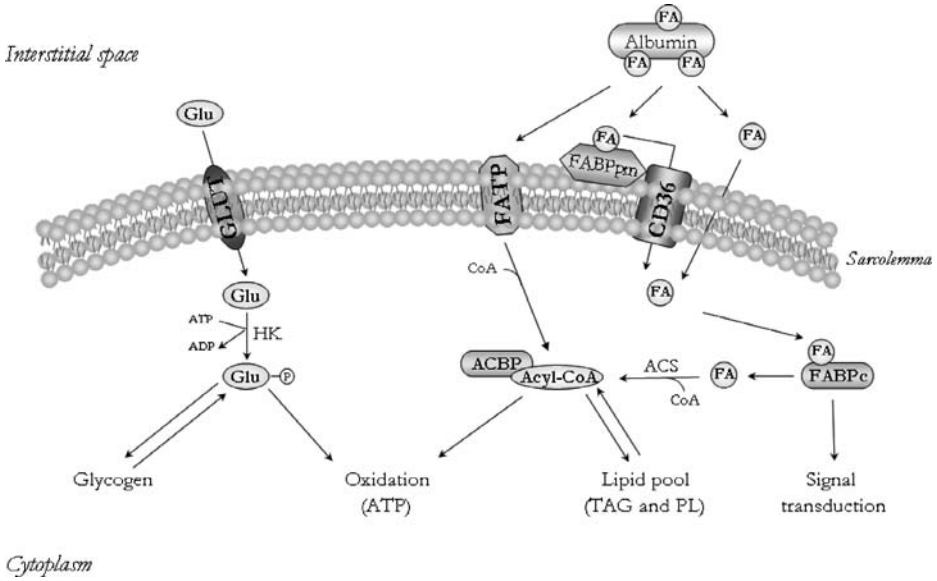

Fig. 1. Schemtaic presentation of glucose and long-chain fatty acid uptake and metabolism in cardiac myocytes under physiological conditions. Abbreviations: Glu, glucose; FA, long-chain fatty acid; CD36, fatty acid translocase (FAT/CD36); FABPpm, plasmalemmal fatty acid-binding protein; FABPc, cytoplasmic fatty acid-binding protein; FATP, fatty acid transport protein; ACBP, acyl-CoA binding protein; GLUT1 or -4, glucose transport protein either 1 or 4; HK, hexokinase; ACS, acyl-CoA synthetase; TAG, triacylglycerol, PL, phospholipids.

to FAT/CD36, other proteins play a role in the sarcolemmal transport system. First, at the outer leaflet of the sarcolemma a 43 kDa fatty acid binding protein (FABPpm) is present [37]. By specifically inhibiting FABPpm with an anti-FABPpm antibody LCFA transport across the sarcolemmal membrane was decreased [31]. Interestingly, the inhibitory actions of SSO and anti-FABPpm were non-additive, suggesting the FAT/CD36 and FABPpm act together in the LCFA uptake process [31]. Second, two isoforms of the fatty acid transport protein (FATP) family, i.e., FATP1 and FATP6, are present in cardiac myocytes and each exhibit acyl-CoA synthetase activity (reviewed by Stahl *et al*. [38]). FATP6 is expressed exclusively in the heart, and also more abundant than FATP1 [39, 40]. Since FATP is associated with the sarcolemma and colocalizes with FAT/CD36 it is suggested that these two LCFA transport proteins act in concert [40].

Once inside the cardiac myocyte, LCFAs bind to 15 kDa cytoplasmic heart-type fatty acid binding protein (H-FABPc) (Fig. 1). Using this protein as a vehicle non-esterified LCFAs are transported inside the cardiac myocytes towards their site of metabolic conversion or action [41, 42]. Subsequently, they are activated into acyl-CoAs by the enzyme acyl-CoA synthetase (ACS) [43]. These acyl-CoAs can bind to the cytoplasmic acyl-CoA binding protein (ACBP) [44, 45]. Acyl-CoAs either enter the mitochondria or are incorporated into the intracellular lipid pools. Transport of acyl-CoAs into the mitochondria is mediated by the concerted action of three carnitine-dependent enzymes [46]. At the outer mitochondrial membrane carnitine palmitoyl transferase I (CPT-I) is present, catalyzing the formation of acylcarnitine. Thereafter, carnitine/acylcarnitine transferase (CACT) transports acylcarnitine into the mitochondria and at the inner mitochondrial membrane, CPT-II generates acyl-CoA. In the mitochondrial LCFA uptake process CPT-I is the key regulatory enzyme. Once inside the mitochondria acyl-CoAs are oxidized via β-oxidation and the endproduct, acetyl-CoA, is gradually degraded in the citric acid cycle [47, 48]. At this point the pathways for glucose and LCFA oxidation merge.

Interestingly, whereas oxidation and esterification of LCFAs occur within minutes, within days intracellular LCFAs also exert effects on their own utilization. These LCFAs induce the expression of genes involved in LCFA metabolism by activating a family of ligand-activated nuclear receptor, the peroxisome proliferator activated receptors (PPARs) that consists of three isoforms, i.e., α, β/δ and γ [49, 50]. In cardiac myocytes, PPARα and β/δ are the predominant isoforms and the abundance of PPARγ is very low [51]. Gilde *et al*. [51] demonstrated that in neonatal cardiac myocytes activation of PPARα and β/δ, but not that of PPARγ, results in an increased expression of genes encoding for proteins involved in cardiac LCFA utilization, including FAT/CD36 and CPT1.

Contraction pathway in cardiac myocytes

Cardiac myocytes possess a specialized contractile machinery that enables them to contract and relax in a regular fashion

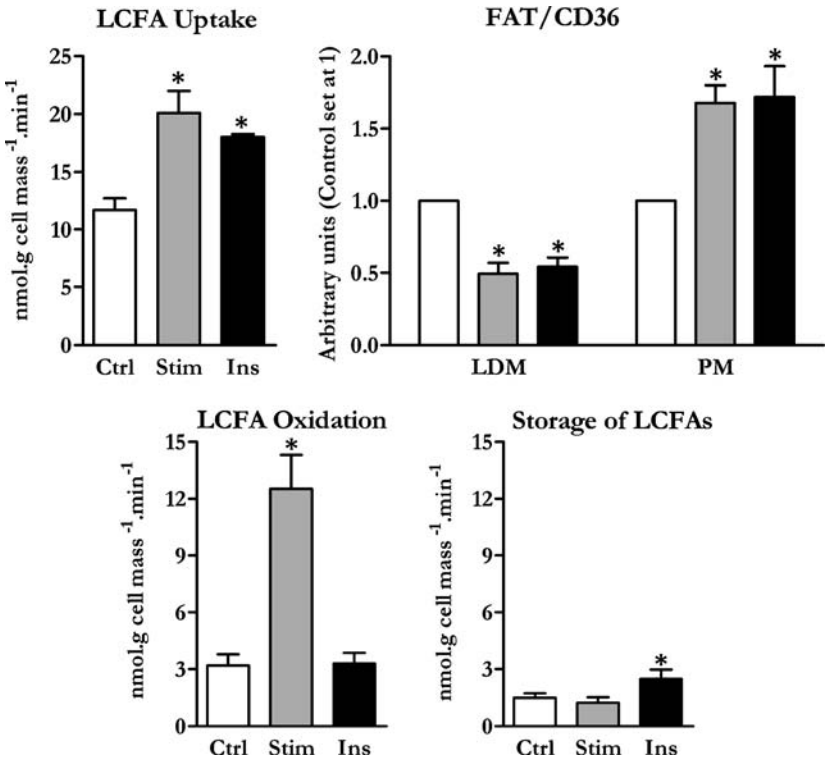

Fig. 2. Effects of cellular contractions and insulin on LCFA utilization and CD36 subcellular localization in cardiac myocytes. Isolated rat cardiac myocytes from lean Zucker rats were incubated for 15 min at 37 °C under continuous shaking either non-treated (Ctrl), electrically stimulated (Stim) or treated with 10 nmol/l insulin (Ins). The ^{14}C-palmitate uptake rate was measured in 3 min and the rates of oxidation and storage of ^{14}C-palmitate was measured in 20 min. Moreover, the treated cardiac myocytes were subcellular fractionated into low-density microsomes (LDM) and plasma membrane (PM) fractions. By Western blotting using MO25, an antibody directed against FAT/CD36, the protein content of FAT/CD36 was measured in these two fractions. Data on ^{14}C-palmitate utilization are expressed as means ± S.E.M. and presented as nmol g cell mass^{-1} min^{-1}. The protein content of FAT/CD36 was expressed as percentage of control values (control set at 1). *Significantly different form control values, $P < 0.05$.

[52]. The contraction process is an energy requiring process, which is accounted for by increased utilization of substrates by cardiac myocytes. It is demonstrated that in contracting isolated rat cardiac myocytes translocation of GLUT4 [53, 54] and FAT/CD36 [55] from intracellular storage compartments towards the sarcolemma occurs, resulting in an increased glucose and LCFA uptake, respectively. Moreover, cellular contractions do not only induce FAT/CD36-mediated LCFA uptake, but also the LCFA oxidation rate, whereas the LCFA storage rate into TAGs remains unaltered (Fig. 2). Several proteins, i.e., protein kinase A, AMP-activated kinase and protein kinases C, are potential candidates to be involved in the signalling pathway activated upon cellular contractions. These proteins and their role in the regulation of cardiac substrate utilization and substrate transport proteins localization are subject of intensive studies, the outcome of which will be discussed in detail below.

Protein kinase A

Protein kinase A (PKA) is a serine/threonine kinase and activated by cyclic AMP (cAMP) [56, 57]. It is inactive enzyme consisting of two catalytic subunits and a regulatory dimer. Binding of cAMP to the regulatory dimer results in the dissociation of the dimer from the catalytic PKA subunits, resulting in the activation of PKA. Activated PKA phosphorylates other proteins containing specific serine/threonine residues (see for review [56, 57]). Intramyocellular cAMP levels increase during acute exercise. Upon ß-adrenergic receptor stimulation a sarcolemmal heterotrimeric GTP-binding protein is activated, which stimulates adenylyl cyclase to produce cAMP. Activation of PKA by cAMP results in the phosphorylation of several proteins involved in chronotropic and inotropic mechanisms in the cardiac myocytes, i.e., L-type-Ca^{2+} channels, and myosin binding protein [58, 59]. Moreover, activation of PKA results in the phosphorylation of acetyl-CoA carboxylase (ACC) at serine-79 (Ser79) and Ser1200 [60, 61]. In the heart two isoforms of ACC with subunit sizes of 265 (α) and 280 (β) kDa have been identified. ACCβ is the major cardiac isoform and is involved in the regulation of LCFA metabolism [62]. Phosphorylation of ACC at critical sites, i.e., Ser1200 [63], inhibits its activity, resulting in a fall of intramyocellular malonyl-CoA thereby deinhibiting the catalytic activity of CPT-I and accelerating

β-oxidation [46] (Fig. 3). In contrast to the stimulatory effect of cAMP on LCFA oxidation, cardiac LCFA uptake is not regulated by intramyocellular cAMP levels and thus the activation of PKA [64]. In cardiac myocytes, in which cAMP levels are pharmacologically increased, LCFA uptake [65] and subcellular distribution of FAT/CD36 [64] were unaltered. Collectively, these findings indicate that PKA directs intracellular LCFAs towards mitochondrial β-oxidation, whereas the subcellular localization of FAT/CD36 translocation and hence cellular LCFA uptake remain unaltered.

AMP kinase

AMP kinase (AMPK) is a heterotrimeric protein consisting of one catalytic subunit, and two regulatory subunits. Activation of AMP kinase occurs through phosphorylation of threonine-172 (Thr172) in the catalytic domain by one or more upstream AMPK kinases (AMPKKs), such as the recently discovered LKB1 [66], and allosterically by binding of AMP to one of the regulatory subunits [67, 68]. Coven and coworkers [69] demonstrated that AMPK is activated in the rat heart during exercise, emphasizing the importance of AMPK in this organ. In muscle cells AMPK can be pharmacologically activated by 5-aminoimidazole-4-carboxamide-1-ß-D-ribofuranoside (AICAR), which is intracellularly phosphorylated into AICAR monophosphate (ZMP) that closely resembles the chemical structure of AMP [70, 71]. It has been demonstrated that in both electrically stimulated and AICAR-treated cardiac myocytes GLUT4 translocates to the sarcolemma thereby increasing glucose uptake [53, 54]. Since not only GLUT4-mediated glucose uptake but also FAT/CD36-mediated LCFA uptake was induced in cardiac myocytes during cellular contractions (Fig. 2), we were interested in whether AMPK is involved in FAT/CD36 translocation. Subsequently, we were the first to demonstrate that in cardiac myocytes an increased AMPK activity is involved in translocation of FAT/CD36 from intracellular storage compartments to the sarcolemma [72] (Fig. 3). Besides its role in the regulation of substrate transporter localization, AMPK activity directly influences cardiac LCFA oxidation. Activation of AMPK causes phosphorylation of ACC at Ser79, Ser1200 and Ser1215 [73, 74]. Phosphorylation of ACC at Ser79 by AMPK results in the inhibition of ACC activity thereby lowering intracellular malonyl-CoA which eventually leads to an increased LCFA oxidation [63]. Collectively, these findings demonstrate that activation of AMPK is involved in inducing both FAT/CD36-mediated LCFA uptake and LCFA oxidation.

Protein kinase C

Protein kinases C (PKCs) are a family of serine/threonine kinases consisting of 12 members which are divided into three subfamilies based on their structure and ligand-binding domains, i.e., conventional (c), novel (n) and atypical (a) PKCs (see for review Newton *et al.* [75]). cPKCs are activated by Ca^{2+} and diacylglycerols (DAGs), nPKCs are insensitive to Ca^{2+} but can be activated by DAGs and aPKCs are insensitive to both Ca^{2+} and DAGs [76, 77]. In the heart one cPKC, PKCα, two nPKCs, PKCδ and ε and one aPKC, PKCζ have been detected [78]. It has been demonstrated that PKCs are involved in regulating contractile function by phosphorylating components of the cardiac contractile machinery [79, 80]. Moreover, during cellular contractions intramyocellular Ca^{2+} levels increase through an increased uptake of Ca^{2+} via sarcolemmal L-type Ca^{2+} channels by the cardiac myocyte, which then triggers the Ca^{2+} release from the sarcoplasmatic reticulum [81]. This switches on the contractile machinery and relaxation is initiated once Ca^{2+} is transported out of the cytoplasm [52, 82]. Evidence is accumulating that activation of conventional and novel PKCs are involved in the contraction-induced substrate uptake by cardiac myocytes. Recently, we demonstrated that in cardiac myocytes treated with phorbol 12-myristate 13-acetate (PMA), a cell-permeable analog of DAG that induces PKC activity, both glucose and LCFA uptake are enhanced [83]. Moreover, in PMA-treated cardiac myocytes LCFA uptake can be inhibited by SSO to the same residual level as in non-treated cardiac myocytes, demonstrating the involvement of FAT/CD36 in the increase of LCFA uptake by PMA. Besides conventional and novel PKC, several studies in both rodent and human skeletal muscle demonstrated that upon exercise and electrical stimulation aPKCs translocate to the membrane and hence are activated [84–86]. These findings indicate a possible role for aPKCs in the effect of cellular contractions in cardiac myocytes. Taken together, activation of the PKC family is believed to be involved in contraction-induced substrate uptake by cardiac myocytes, although the specific PKC isoform and the molecular mechanism remains unknown.

Insulin signalling pathway in cardiac myocytes

Similar to the effects of cellular mechanical activity, the hormone insulin regulates substrate utilization and localization of substrate transport proteins in muscle tissues [88, 89]. Insulin is involved in the maintenance of normal plasma concentrations of glucose and lipids [90]. Upon binding of insulin to its $\alpha 2/\beta 2$ tetrameric receptor at the sarcolemma of the cardiac myocyte the intracellular tyrosine kinase activity of this receptor is activated. This activation catalyzes phosphorylation of tyrosine residues at the insulin receptor interacting proteins, i.e., insulin receptor substrate 1 (IRS-1). The two major signalling pathways in cardiac myocytes subsequently

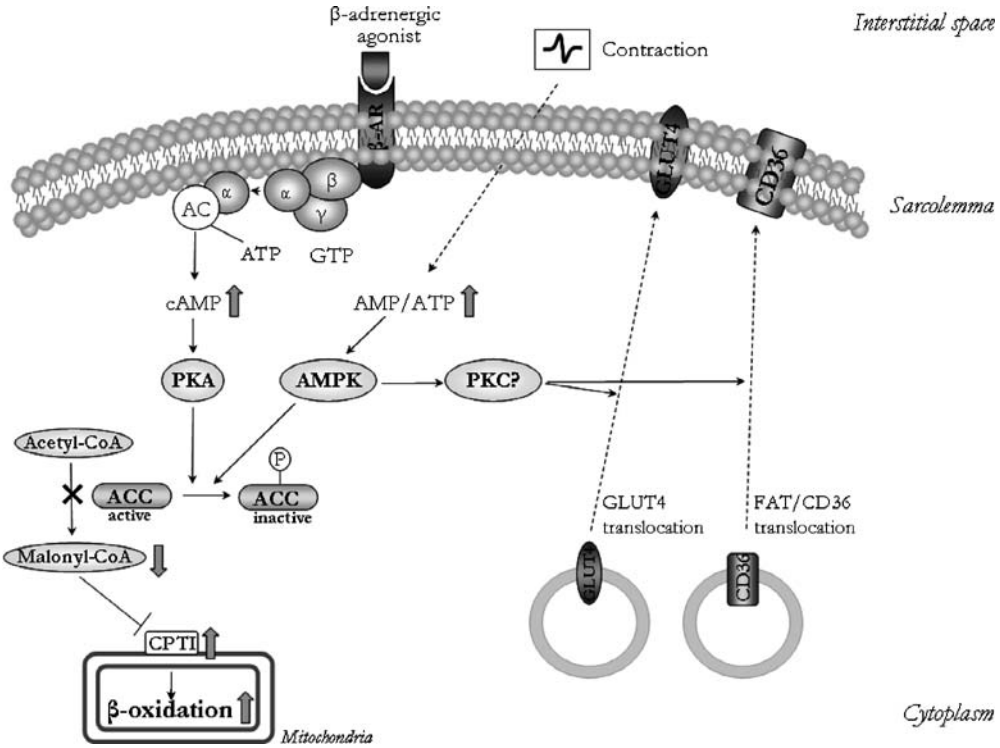

Fig. 3. Components of the contraction pathway involved in substrate utilization in cardiac myocytes. Abbreviations: β-AR, β-adrenergic receptor; AC, adenylyl cyclase; ACC, acetyl-CoA carboxylase; FAT/CD36, fatty acid translocase/CD36; cAMP, cyclic AMP; PKA, protein kinase A; AMPK, AMP kinase; PKC, protein kinase C; CPT-I, carnitine palmitoyl transferase I.

activated by insulin are, (i) the phosphatidylinositol 3 kinase (PI_3K) pathway and (ii) the mitogen activated protein kinase (MAPK) pathway [91]. Whereas the MAPK pathway plays a prominent role in the transcriptional and mitogenic processes, such as alterations in growth, differentiation and regulation of gene expression, the PI_3K pathway is involved in metabolic signalling. Therefore, we will focus on the PI_3K pathway (Fig. 4).

PI_3K consists of two subunits, a 110 kDa catalytic subunit and an 85 kDa regulatory subunit. Once activated, PI_3K translocates to the sarcolemma where it phosphorylates phosphatidylinositol phosphates (PIPs) at the 3 position. Especially the conversion of PI-4,5P_2 to PI-3,4,5P_3 is important for insulin signalling. PIP_3 activates the protein kinase 1, which phosphorylates and thereby activates the serine/threonine kinase, protein kinase B (PKB) [92] and the atypical protein kinase C (aPKC) isoforms λ and ζ [93].

It has been well documented that activation of the PI_3K pathway mediates translocation of the glucose transporter GLUT4 from intracellular storage compartments towards the sarcolemma, thereby increasing the myocardial glucose uptake rate [25, 94]. The metabolic fate of glucose taken up into muscles exposed to elevated levels of insulin includes glycogen synthesis, glucose oxidation and lactate production. Recently is has been shown that insulin does not only induce glucose uptake, but also LCFA uptake in cardiac myocytes [95]. Since FAT/CD36 is just like GLUT4 present at the sarcolemma and in intracellular storage compartments, it was investigated whether insulin induces translocation of FAT/CD36. In adult rat cardiac myocytes, insulin indeed promotes FAT/CD36 translocation from intracellular sites to the sarcolemma depending on activation of PI_3K [95] (Figs. 2 and 4). Moreover, the amount of FAT/CD36 present at the sarcolemma correlates with the myocardial LCFA uptake. Insulin induces not only the FAT/CD36-mediated LCFA uptake but influences also the intramyocellular channeling of LCFAs (Fig. 2). The additional amount of LCFAs entering the cardiac myocytes under the influence of insulin is predominantly stored into the intracellular lipid pool, whereas the LCFA oxidation rate remains unaltered [96].

Cardiac substrate utilization in obesity and type-2 diabetes mellitus

Obesity and T2DM each are strongly associated with insulin resistance [97]. In the development towards full-blown T2DM compensatory hyperinsulinemia maintains normal

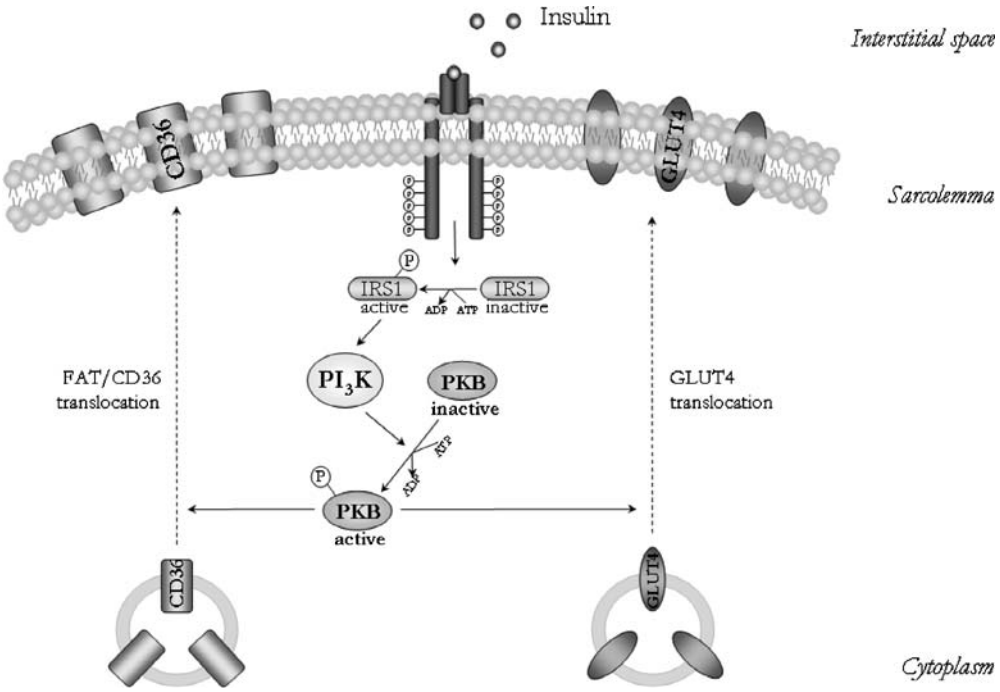

Fig. 4. The insulin signalling pathway involved in substrate uptake by cardiac myocytes. Abbreviations: IRS1, insulin receptor substrate 1; PI_3K, phosphatidylinositol 3 kinase; PKB, protein kinase B.

plasma glucose levels. Eventually hypersecretion of insulin by the pancreatic beta cells is no longer sufficient and hyperglycemia will develop. Besides insulin resistance, T2DM is also strongly associated with the development of diabetic cardiomyopathy. Although, the mechanisms causing cardiac insulin resistance and diabetic cardiomyopathy are incompletely understood, metabolic disturbances in the heart are thought to play an essential role. Because in the heart the expression, subcellular localization and functional regulation of GLUT4 and FAT/CD36 are important parameters determining the rates of substrate utilization, it was of interest to study possible changes in the amount of substrate transport proteins in the insulin-resistant heart.

In rodent models of insulin-resistance [98, 99] or early-onset T2DM [100, 101], basal cardiac glucose uptake has not been changed compared to controls. However, basal glucose uptake decreases in hearts of rodent models with full-blown T2DM, concomitant with a reduced total protein content of GLUT4 [100]. In T2DM db/db mice also cardiac glycolysis and glucose oxidation are decreased. Belke and coworkers [100] demonstrated that when overexpressing GLUT4 specifically in the heart of these rodents, changes in glucose utilization were completely normalized. These findings demonstrate that the reduced cardiac glycolytic state in T2DM rodents will likely be due to a reduced sarcolemmal GLUT4 content. Not only the total amount of GLUT4 but also the induction of GLUT4 translocation by insulin and therefore the subcellular localization of GLUT4 is altered in T2DM. Since recruitment of GLUT4 to the sarcolemma upon insulin requires an intact PI_3K pathway, it is believed that alterations in this signalling pathway are responsible for an impaired GLUT4 translocation. Indeed, several studies demonstrate that in the T2DM heart, insulin signalling in cardiac mycoytes is impaired. Insulin stimulated activity of the IRS/PI_3K/PKB pathway was reduced in (i) isolated cardiac myocytes from rats that were exposed to a high fat diet and developed a T2DM phenotype [102], (ii) isolated cardiac myocytes from insulin resistant obese Zucker rats [98, 103] and (iii) hearts from T2DM Goto-Kakizaki rats [101]. At physiological insulin concentrations, insulin-stimulated GLUT4 translocation and glucose uptake are reduced in insulin resistant obese rats [103, 104]. If cardiac myocytes from obese Zucker rats are stimulated with maximal insulin concentrations, GLUT4 translocation and glucose uptake are induced to the same extent as in control rats [99, 103]. However, in hearts of T2DM rodents even at maximal insulin concentrations GLUT4 translocation is impaired. These findings suggests that insulin-induced GLUT4-mediated glucose uptake becomes impaired when progressing from obesity towards T2DM.

While the impairment in myocardial glucose uptake occurs after the onset of T2DM, LCFA uptake in rodent hearts was altered already in the prediabetic state. It was demonstrated that in insulin resistant, obese Zucker rats, myocardial LCFA uptake increases [87, 105]. The increase in

myocardial LCFA uptake coincided with an increased amount of FAT/CD36 at the sarcolemma, whereas the total cellular FAT/CD36 protein content was unaltered. Moreover, in these obese rats, the rate of cardiac LCFA oxidation was unaltered, whereas the cardiac TAG content and the incorporation rate of LCFA into TAG were increased. In T2DM rodents it has been reported that cardiac LCFA oxidation and the activity of 3-hydroxyl-acyl-CoA dehydrogenase, a component of mitochondrial ß-oxidation pathway, increases [100, 106]. However, data obtained by human studies are contradictory to rodent models and do not give a clear view on the cardiac LCFA oxidation during impaired glucose tolerance. In patients with an impaired glucose tolerance no effect on myocardial ^{11}C-palmitate uptake and oxidation was observed [107], whereas in the same patient group the ^{123}I-heptadecanoic acid (HAD) oxidation by the heart was reduced [108]. This discrepancy could be due to the difference in the fatty acid analog used to study oxidation. ^{123}I-HAD is, compared to ^{11}C-palmitate, limited for using it to quantitate myocardial LCFA oxidation [109].

In obese and T2DM subjects and in insulin-resistant, obese and T2DM rodent models [110–112] it has been well established that lipids accumulate in the heart. Young and coworkers [113] demonstrated that in obese rats a mismatch between LCFA uptake and oxidation leads to a deposition of the excess LCFA taken up by the heart in the intracellular lipid pool. Recently, we showed that increased amounts of FAT/CD36 are present at the sarcolemma in cardiac myocytes from insulin-resistant rats, resulting in increased rates of LCFA deposition in TAG and an increased intracellular LCFA content [87, 99]. Moreover, Zhou and coworker [110] demonstrated that the increased cardiac TAG content coincided with increased mRNA levels of glycerol-3-phosphate acyltransferase (GPAT), an enzyme know to direct exogenous LCFA into TAGs [114]. Hence combined elevation of FAT/CD36 and GPAT provides a mechanism for increased LCFA channeling towards intracellular storage. The relation between FAT/CD36 and TAG in the heart of obese Zucker were confirmed in human studies in skeletal muscle. Also in skeletal muscle of humans alterations in FAT/CD36 functioning are causally related to intramyocellular lipid accumulation. The plasmalemmal FAT/CD36 protein content, rates of palmitate transport and the tissue TAG content are increased in skeletal muscle from obese and T2DM subjects, compared to lean controls [115].

Development of lipid-induced cardiac insulin resistance

In obesity and T2DM, the excess myocardial lipid accumulation is related to both myocardial dysfunction and the development of cardiac insulin resistance [116]. Initially it was believed that elevated intracellular levels of LCFA result in Randle cycle effects on metabolism, leading to an in impaired glucose metabolism. However, recent evidence demonstrated that rather than the Randle cycle, intracellular lipid metabolites result in an impaired insulin-stimulated GLUT4 translocation.

Elevated plasma LCFA levels; The Randle cycle

Elevated plasma LCFAs levels, one of the main features in obesity and resulting in an increased cellular LCFA uptake, could play a role in the development of insulin resistance. In view to the Randle cycle, it is believed that an increased LCFA oxidation, due to increased availability of intracellular LCFAs, contributes to the development of a decreased glucose uptake in cardiac myocytes [117]. By increasing LCFA oxidation the glycolytic pathway is inhibited due to an accumulation of intracellular acetyl-CoA and citrate leading to increased glucose-6-phosphate concentrations and decreased hexokinase activity [117, 118]. Eventually cardiac glucose uptake and oxidation are reduced. An inverse relation between cardiac glucose uptake and plasma LCFAs in man has been demonstrated [119]. However, the mechanism proposed by Randle occurs under physiological conditions and it is unclear whether this mechanism can be extrapolated to pathophysiological conditions in which plasma LCFA concentrations are chronically elevated [120].

Intracellular lipid metabolites and insulin resistance

A strong positive correlation between the accumulation of intramyocellular TAGs and cardiac insulin resistance has been demonstrated [110, 113]. Lipid metabolites of TAG, i.e., acyl-CoAs, ceramides and DAGs are believed to be involved in the development of insulin resistance. Unfortunately, most studies addressing the effect of these lipid metabolites on insulin resistance are performed in skeletal muscle and not in heart muscle. However, findings in skeletal muscle usually can be extrapolated to the heart muscle due to the similarity in regulation of substrate utilization between skeletal muscle and heart.

Intramyocellular TAGs are believed to directly affect cellular contractions by spatial hindrance of the contractile machinery making it more difficult to sustain contractions with appropriate amplitude in cardiac myocytes [121]. However, the precise mechanism has not been clarified yet. In addition, lipid metabolites such as acyl-CoAs, ceramides and DAGs originating from or in equilibrium with the intracellular TAG pool are believed to influence insulin-induced glucose uptake and GLUT4 translocation.

Acyl-CoAs are precursors for ceramides which are generated via *de novo* synthesis or via degradation of

sphingomyelin [122]. In skeletal muscle from human and rodent models of insulin resistance intracellular ceramide content was increased up to 2-fold [123–126]. *In vitro* treatment of skeletal muscle cell lines, i.e., L6 myotubes and C2C12 myotubes with either a cell-permeable ceramide or palmitate increased the intracellular ceramide content [123, 127–129]. In these ceramide-loaded skeletal muscle cells insulin-stimulated serine phosphorylation of PKB and its activation was reduced, whereas tyrosine phosporylation of the insulin receptor and IRS1 remained unaltered, suggesting that ceramides do not affect insulin signalling upstream of PKB [127, 129]. Moreover, ceramide decreased insulin-stimulated glucose uptake and glycogen synthesis in skeletal muscle cells [123, 130]. Whether ceramides affect GLUT4 translocation in skeletal muscle is unknown, however studies in 3T3 adipocytes showed that ceramides inhibit GLUT4 translocation leading to a decreased glucose uptake [131].

Intracellular DAGs have been found to be markedly increased in skeletal muscle from insulin resistant rodents and humans [132–136]. In skeletal muscle, the activity of conventional and novel PKCs is induced by DAG. It has been demonstrated that activation of these PKCs leads to tyrosine phosphorylation of the insulin receptor and IRS1, thereby inhibiting their activity, which results in a decreases PI_3K stimulation [137]. Itani *et al*. [138, 139] showed that the activation of PKCtheta is significantly increased in skeletal muscle from diabetic patients compared to muscle from non-diabetic controls. Whether DAGs are directly involved in the development of cardiac insulin resistance remains to be elucidated.

FAT/CD36 as a key player in the development of cardiac lipotoxicity; hypothetical model

Available data indicates that FAT/CD36 may have an important role in the etiology of diabetic cardiomyopathy. Abnormalities in the regulation of the sarcolemmal amount of FAT/CD36 could contribute to the development of cardiac lipotoxicity resulting in both cardiac dysfunctioning and a decreased insulin sensitivity of the heart. Based on animal studies, a hypothetical model is presented for the development of impaired insulin-stimulated GLUT4 translocation (Fig. 5).

Already under prediabetic conditions is LCFA uptake by cardiac myocytes markedly increased, due to a permanent relocation of FAT/CD36 to the sarcolemma. Moreover, it has been demonstrated that the total intracellular TAG and FA contents and the incorporation rate of LCFA into intracellular TAG were increased in isolated cardiac myocytes from insulin resistant, obese rats. Interestingly, when sarcolemmal FAT/CD36 activity was inhibited by SSO in these cardiac myocytes both intracellular FA and the incorporation rate of LCFA into intracellular TAG were normalized. The accumulation of TAG, and its metabolites, i.e., acyl-CoAs, ceramides and DAGs are one of the first events in the development of cardiac insulin resistance. Both ceramides and DAG-activated PKCs inhibit the insulin signalling pathway involved in translocation of GLUT4 from intracellular storage compartments to the sarcolemma, leading to a reduced GLUT4-mediated glucose uptake by cardiac myocytes. Interestingly, Bonen *et al*. [115] demonstrated in human skeletal muscle a strong positive correlation between the amount of plasmalemmal FAT/CD36 and the amount of TAG. Moreover both plasmalemmal FAT/CD36 and TAG content increased with the severity of the insulin reistance Collectively, these data suggest a causal link between the activity of FAT/CD36 at the sarcolemma and the development of cardiac lipotoxicity, which eventually leads to cardiac insulin resistance. This model is based on studies performed in rat cardiac myocytes and should be verified in cardiac myocytes from humans.

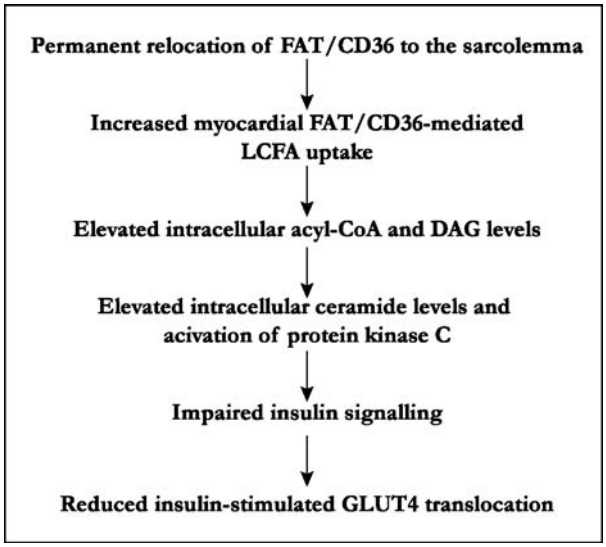

Fig. 5. Hypothetical model for the development of an impaired insulin-stimulated GLUT4-translocation in cardiac myocytes. Abbreviations: LCFA, long-chain fatty acid; FAT/CD36, fatty acid translocase/CD366; DAG, diacylglycerol.

Therapeutic approaches

During recent years several strategies were followed to normalize altered substrate preference to improve cardiac function and insulin sensitivity in T2DM. Since in full-blown T2DM cardiac glucose oxidation decreases it was thought that by inhibiting LCFA oxidation, glucose uptake and oxidation would be compensatory upregulated and thus normalized, resulting in an improved cardiac function. A manner to inhibit cardiac LCFA oxidation is by blocking the entry of acyl-CoA's into the mitochondria. Etomoxyril-CoA, the biologically active intracellular metabolite of etomoxir, inhibits the enzyme activity of CPT-I in the same manner as its naturally occurring inhibitor, malonyl-CoA [140]. Studies

in which T2DM patients were treated for three days with a high dose of etomoxir (100 mg/day) demonstrated that indeed overall insulin-stimulated glucose uptake increases, basal glucose oxidation increases and basal LCFA oxidation decreases [141, 142]. However, insulin stimulated glucose oxidation was not altered [142]. One of the negative side effects of etomoxir-treatment is that lipids accumulate in non-adipose tissue such as the heart [143]. Etomoxir has no effect on FAT/CD36-mediated LCFA uptake, thus LCFA taken up by cardiac myocytes can only be intracellularly stored [34]. On the long run one would expect severe negative side effects when using etomoxir. Moreover, it has been demonstrated that cardiac hypertrophy occurs after etomoxir administration [144].

Second, manipulations of the AMPK signalling pathway could be an attractive approach to increase glucose uptake in heart and subsequently improve the functioning of the heart. Activating AMPK with exercise results in an increased LCFA oxidation which could eventually lead to a reduction in the accumulation of intracellular TAGs [69]. Since lipid intermediates of TAGs are believed to play a role in the impaired GLUT4 translocation in T2DM, inducing AMPK activity could be beneficial [145]. However, as mentioned before, AMPK activation results not only in an enhanced LCFA oxidation but also LCFA uptake increases in cardiac myocytes [72]. Since we demonstrated in cardiac myocytes from insulin resistant rats that LCFA uptake is still inducible when pharmacologically increasing the AMP/ATP ratio [87], activation of AMPK may not even reduce the amount of intramyocellular TAGs when stimulated.

Third, increasing the activity of protein kinase A is another possibility to improve cardiac insulin sensitivity. Like AMPK, PKA is able to increase LCFA oxidation in cardiac myocytes [60–62]. On the other hand, PKA has no effect on LCFA uptake, whereas AMPK enhances LCFA uptake [64]. Thus, by activating PKA the excess intracellular LCFAs are redistributed from esterification towards β-oxidation, resulting in a decline in intracellular TAG content. Already a 3-fold elevation in intracellular cAMP was sufficient to maximally induce this redistribution [64]. However, a potential drawback of inducing the PKA activity is that this also elicits positive ionotropic effects, which could lead to cardiac hypertrophy [146]. This drawback could be partially circumvented by PKA stimulating agents that have relative small effects on cardiac hyperthrophy, but will sustain metabolic action.

Fourth, modulation of the expression of genes involved in substrate utilization by activating members of the PPAR family is one of the strategies recently followed to increase insulin sensitivity in T2DM. A new class of insulin-sensitizing agents that exert their action through activating PPARγ, are the thiazolidinediones, such as rosiglitazone and troglitazone [147, 148]. Rosiglitazone treatment of (i) insulin resistant obese Zucker rats (12 months old) for 14 days and of (ii) high-fat fed rats treated for 4 days, resulted in normalized insulin-stimulated glucose uptake and GLUT4 transporter expression in isolated cardiac myocytes [98, 149]. Subsequently, TZD-treatment results in improved cardiac function due to a reduction in TAG and ceramide levels in hearts of insulin resistant obese rats [150]. The effects of TZDs seen in hearts of insulin resistant rodents are believed to be indirect, namely through shuttling of LCFAs towards adipose tissue.

Fifth, it is believed that by inhibiting the accumulation of intracellular TAG, cardiac insulin-resistance will decline and cardiac function will improve. The strong positive correlation between the intramyocellular amount of TAG and the sarcolemmal FAT/CD36 content muscle of obese and T2DM patients, suggests that an inhibition of FAT/CD36 could result in an decreased TAG accumulation [87, 115]. *In vitro* it has been demonstrated that FAT/CD36-mediated cellular LCFA uptake can be inhibited by sulfo-*N*-succinimidyl oleate (SSO), which covalently binds to FAT/CD36. Therefore, SSO seems suitable to inhibit the accumulation of TAG in the heart at an early stage of development of T2DM. However, FAT/CD36 is not only expressed in high amount in muscle tissue, but also in white adipose tissue [35]. It is expected that when SSO is administrated *in vivo*, due to a decreased LCFA uptake by muscle tissues, plasma LCFA will rise. The increased plasma LCFA levels could be reduced if more LCFA are taken up by adipose tissue, however, this is impossible since SSO will also block FAT/CD36 in adipose tissue. Ideally an agent should be developed that specifically inhibits FAT/CD36 in muscle tissue and that reaches the circulation after oral intake.

Concluding remarks

Chronically increased protein levels of FAT/CD36 at the sarcolemma play a pivotal role in the development of T2DM, leading to enhanced LCFA uptake, accumulation of intracellular TAGs and impaired insulin-stimulated GLUT4 translocation. The increased sarcolemmal abundance of FAT/CD36 is due an impairment of FAT/CD36 recycling between intracellular storage compartments and the sarcolemma. Pharmacological manipulations aimed at specific signalling processes, i.e., the contraction and insulin signalling pathways involved in substrate utilization, or trafficking steps that will result in selective recruitment of GLUT4 and/or internalization of FAT/CD36 are expected to inhibit the accumulation of TAG and to reverse cardiac insulin resistance and prevent the development of cardiac dysfunction.

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