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REVIEW ARTICLE

Signaling pathways involved in cardiac energy metabolism

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(Received 13 June 2016, revised 9 July 2016, accepted 11 July 2016, available online 23 July 2016)

doi:10.1002/1873-3468.12297

Edited by Wilhelm Just

Various signaling pathways coordinate energy metabolism and contractile function in the heart. Myocardial uptake of long-chain fatty acids largely occurs by facilitated diffusion, involving the membrane-associated protein, CD36. Glucose uptake, the rate-limiting step in glucose utilization, is mediated predominantly by the glucose transporter protein, GLUT4. Insulin and contraction-mediated AMPK signaling each are implicated in tightly regulating these myocardial 'gate-keepers' of energy balance, that is, CD36 and GLUT4. The insulin and AMPK signaling cascades are complex and their cross-talk is only beginning to be understood. Moreover, transcriptional regulation of the CD36 and GLUT4 is significantly understudied. This review focuses on recent advances on the role of these signaling pathways and transcription factors involved in the regulation of CD36 and GLUT4.

Keywords: AMPK; CD36; GLUT4; insulin

The heart has the highest energy requirements of all organs in the body. To function properly it is dependent on a continuous production of intracellular ATP and for this requires a constant and plentiful supply of fuel. Glucose and long-chain fatty acids are the major metabolic substrates with which the heart sustains mechanical performance [1,2]. Current research has initiated a renewed interest in the regulation of cardiac utilization of these substrates, especially in the context of cardio-metabolic diseases.

Glucose catabolism in not only cardiac but also in skeletal muscle begins with the breakdown of glucose, which encompasses glycolysis and glucose oxidation. Glycolysis is the initial sequence of reactions involved in the breakdown of glucose to pyruvate. This process occurs outside of the mitochondria, and can be carried out anaerobically. However, the ATP produced through glycolysis contributes less than 10% of the overall ATP production in the healthy heart [3]. The pyruvate generated from glycolysis is further metabolized within the mitochondria to produce the majority of carbohydrate-derived ATP (glucose oxidation) [4]. Glucose uptake has been established firmly as the rate-limiting step in glucose utilization by cardiac and muscle cells [1–3]. Both cardiac and skeletal muscles oxidize glucose to produce energy or temporarily store glucose as glycogen. The principal glucose transporter protein that mediates myocellular glucose uptake is GLUT4, which is an isoform of a family of sugar transporter proteins containing 12-transmembrane domains (SLC2A4 gene) [5]. The GLUT4 glucose transporter is thus a major mediator of glucose removal from the circulation and, in view of the large total skeletal muscle mass, a key regulator of whole-body glucose homeostasis.

While glucose oxidation is a critical source of myocardial ATP, the healthy adult heart obtains approximately 50–70% of its required ATP from

Abbreviations
AMPK, AMP-activated protein kinase; FAT, fatty acid translocase; FDG, ^{18}F-fluoro-2-deoxyglucose; FoxO, forkhead box O; FTHA, ^{18}F-fluoro-6-thioheptadecanoic acid; GSVs, GLUT4 storage vesicles; IRS, IR substrate; MRI, magnetic resonance imaging; PET, positron emission tomography; PKC, protein kinase C; ZFP, zinc finger protein.

(long-chain) fatty acids [5]. Therefore, mitochondrial oxidation of lipids provides a major source of ATP for the heart, and the cellular processes that regulate lipid uptake and utilization are important contributors to maintaining proper myocardial energetic status. Although numerous proteins are coordinately regulated in order to ensure proper fatty acid utilization in the cardiomyocyte, a key first step in this process is the entry of fatty acids into the cell [5]. An important protein involved in the transport of fatty acids into the cardiomyocyte is the plasma membrane-associated protein designated as fatty acid translocase (FAT; also known as CD36). While multiple proteins are involved in facilitating fatty acid uptake into the heart, CD36 accounts for approximately 70% of the total fatty acid taken up by cardiomyocytes [6]. As such, myocardial metabolism of fatty acids may depend upon proper CD36 function. Consistent with this, changes in CD36 cellular content and/or function have been implicated in the alteration of myocardial metabolism in the pathophysiology of certain cardiovascular diseases [6].

Although there appears to be a preferential use of fatty acids for energy production, the heart has the ability to rapidly respond to changes in substrate availability by switching to another substrate for the generation of ATP so as to continuously secure its energy demand. This dynamic balance of substrate utilization was first described by Philip Randle in 1963 [7,8]. In essence, the Randle cycle explains the metabolic process of energy production as one that involves the competition of glucose and fatty acids [8]. In addition, substrate selection occurs based on the availability of the substrate as well as the energy demand in tissues. Since the heart requires a steady supply of energy, the ability of the cardiomyocyte to rapidly switch to different substrates based on their availability is a necessary component of a healthy heart [9]. Evidence is accumulating that the substrate transporters, GLUT4 and CD36, also serve a role in this dynamic balance of substrate utilization.

This article reviews the known molecular and cellular regulatory mechanisms for the substrate transporters GLUT4 and CD36 with a focus on heart and skeletal muscle, their integration with insulin signaling and contraction, and the profound effects that GLUT4 and CD36 exert on whole-body substrate metabolism.

Signaling pathways regulating GLUT4 trafficking

GLUT4 is one of 13 sugar transporter proteins (GLUT1–GLUT12, and H^+/myo-inositol transporter) encoded in the human genome [10,11] that catalyzes hexose transport across cell membranes through an ATP-independent, facilitative diffusion mechanism [12]. The heart expresses mainly GLUT1 and GLUT4, with GLUT4 expression being approximately four times higher than that of GLUT1. GLUT1 is constitutively expressed at the sarcolemma and is involved in basal glucose uptake, whereas GLUT4 displays the unique characteristic of a mostly intracellular disposition in the unstimulated state that is acutely redistributed to the plasma membrane in response to insulin, contraction, and other stimuli [13,14].

Insulin and contraction are the two key stimuli that acutely regulate GLUT4 recruitment to the cell surfaces of heart, skeletal muscle, and adipose cells independent of transcription or translation [15,16]. Nevertheless, these physiological stimuli initiate distinct signaling mechanisms but both lead to enhanced GLUT4 translocation and glucose uptake. The insulin signaling pathway to GLUT4 has been discussed in detail in recent reviews [13,17,18]. Detailed *in vivo* studies using genetically engineered mouse models [19] and, more recently, by siRNA knockdown experiments in cultured cells [20,21] have demonstrated that the canonical insulin signaling pathway is triggered by activation of the IR tyrosine kinase leading to tyrosine phosphorylation of IR substrate (IRS) proteins and their recruitment of PI 3-kinase. The latter kinase catalyzes conversion of phosphatidylinositol (4,5)P2 to phosphatidylinositol (3,4,5)P3 (denoted PIP3). PIP3, in turn, triggers the activation of the protein kinase Akt through the actions of two intermediate protein kinases, PDK1 and Rictor/mTOR [22,23]. Interestingly, Akt2 rather than the Akt1 or Akt3 isoforms appears to control GLUT4 trafficking in adipose and muscle cells as well as mediate insulin signaling to control glucose output in liver [24,25]. Substrates of Akt2 that may mediate the insulin effects on the machinery of GLUT4 trafficking are being actively investigated. The GTPase-activating protein TBC1D4, denoted AS160, is such a substrate [26]. AS160 catalyzes inactivation of critical organizers of intracellular membrane trafficking, Rab proteins 2A, 8A, 10, and 14 *in vitro* [27]. Expression of mutant AS160 lacking Akt-specific phosphorylation sites inhibits insulin-stimulated GLUT4 translocation [28], suggesting that it is a negative regulator that is itself inhibited by insulin through Akt. AS160 plays a role in insulin-stimulated GLUT4 exocytosis but not in the inhibition by insulin of GLUT4 endocytosis [29]. However, AS160 knockdown only partially releases the pool of intracellular GLUT4 mobilized by insulin, and careful analysis suggests that other unknown Akt substrate proteins must make major contributions to overall GLUT4 regulation by

insulin [30,31]. In this context, recent studies have identified TUG (tether containing a UBX domain for GLUT4) protein, encoded by the *Aspscr1* gene, to coordinate with signals through Akt2 to AS160/TBC1D4 and TBC1D1 [32]. The proposed model suggests that TUG traps GLUT4 in intracellular, insulin-responsive vesicles termed GLUT4 storage vesicles (GSVs). Insulin triggers TUG cleavage to release the GSVs; GLUT4 then recycles through endosomes during ongoing insulin exposure. The TUG C-terminus binds a GSV-anchoring site comprising Golgin-160 and possibly other proteins [32]. Adding to the complexity, Sirtuin 2 (SIRT2), a NAD^+-dependent deacetylase, binds TUG and de-acetylates the TUG peptide. SIRT2 overexpression reduced TUG acetylation and redistributed GLUT4 and IRAP to the plasma membrane in 3T3-L1 adipocytes [32] (Fig. 1).

Contraction-induced AS160 phosphorylation is mediated through the AMP-activated protein kinase (AMPK) pathway, providing a potential convergence among insulin, contraction and exercise-mediated signaling to GLUT4 [33,34]. On the other hand, simultaneously disrupting AMPK and Akt failed to completely inhibit contraction-induced AS160 phosphorylation, which observation is consistent with the existence of additional signals leading to GLUT4 translocation. Two cellular consequences of contraction, a transient increase in intracellular calcium concentration ($[Ca^{2+}]_i$) and an increase in [AMP]/[ATP] ratio, are thought to contribute to enhanced GLUT4 translocation to the cell surface [33,34]. The former signal is mediated through activations of the protein kinase CaMKII and perhaps conventional protein kinase C [16,33]. However, the role of AMPK in contraction-induced glucose uptake has been questioned. Muscle-specific overexpression of a dominant-inhibitory catalytic subunit of AMPK (i.e., α2-AMPK, the major catalytic isoform in skeletal muscle) reduced contraction-mediated glucose uptake by only 30–40% [35]. Nevertheless, as has been reported previously, enhanced muscle glucose uptake in response to activation of AMPK by AICAR (an AMP analog) is not an

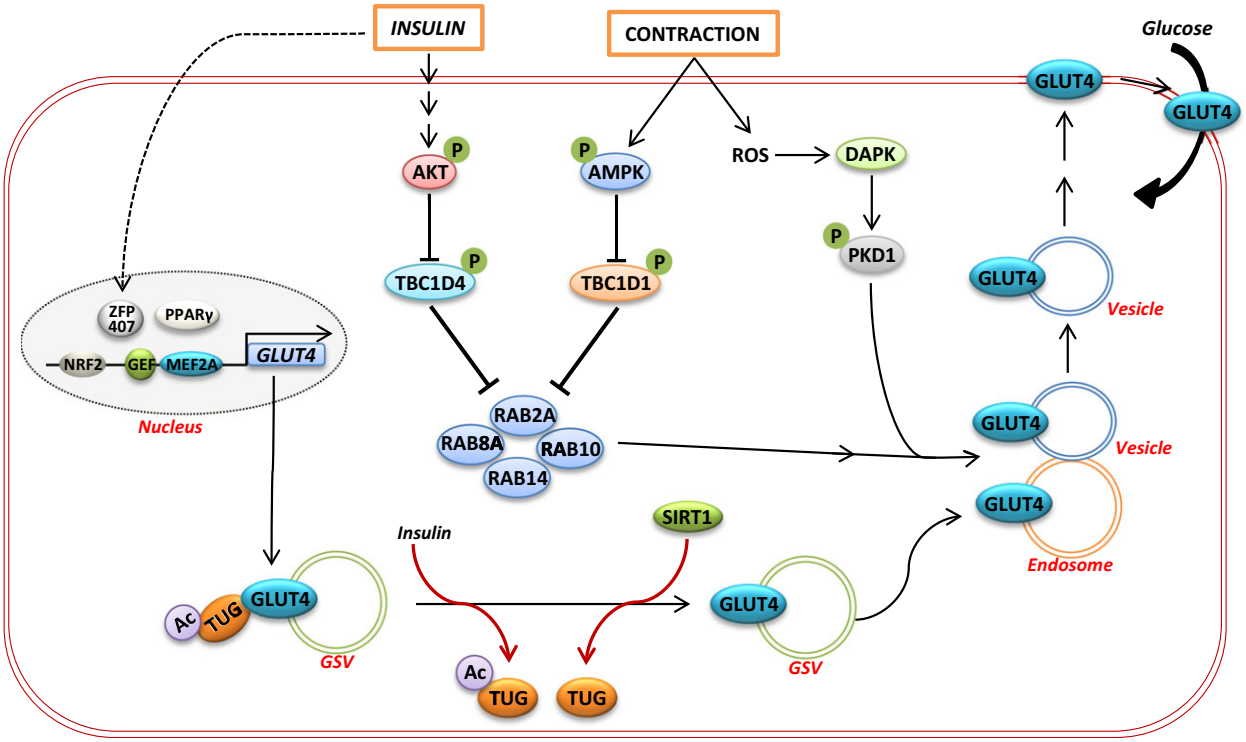

Fig. 1. Convergence of signaling pathways initiated by insulin and contraction leading to GLUT4 regulation. Insulin signaling through the PI3K pathway and muscle contraction through elevated AMP/ATP ratios, elevated ROS, and intracellular $[Ca^{2+}]$ levels lead to activation of downstream protein kinases (AKT, AMPK, PKD) that phosphorylate putative effectors that modulate steps in the GLUT4 trafficking pathways. AS160 is one such substrate that appears to negatively regulate an early step in GLUT4 exocytosis, via inhibition of RAB GTPases. Additionally, acetylated TUG has been identified as a novel interacting partner and negative regulator of GLUT4 translocation. Insulin dissociates TUG from GLUT4, whereas SirT1 deacetylates TUG, both actions ultimately facilitating GLUT4 translocation. At the gene expression level, several transcription factors have been reported to regulate GLUT4 promoter activity. Dashed lines imply pathways yet to be experimentally delineated. See text for further details.

additive to that achieved through muscle contraction, indicating that AMPK is a component of the contraction signaling mechanism [36]. Thus, existing evidence indicates that an AMPK-mediated pathway may be one of several redundant contraction-induced signaling mechanisms leading to GLUT4 translocation to the cell surface. In addition to AMP, other second messengers become elevated during contraction, such as intracellular ROS, Ca^{2+}, and diacylglycerol (DAG) [37]. Both latter second messengers are known activators of members of the conventional and novel protein kinase C (PKC) subfamilies, as well as the closely related PKD family, with its founding member PKD1. Moreover, the classical PKC activators phorbol esters (cell-permeable DAG analogs) additionally activate PKD1. Several lines of evidence suggest a crucial role for PKD1 in contraction-induced glucose uptake. Pharmacological PKC inhibitors (which also inhibit PKD1) and RNAi-mediated silencing of PKD1 each completely abolish contraction-induced GLUT4 translocation and glucose uptake in cardiomyocytes [38,39]. Similarly, in cardiomyocytes from cardio-specific PKD1-KO mice, contraction-induced glucose uptake was entirely lost [38]. Furthermore, adenoviral overexpression of PKD1 in cultured cardiomyocytes [40] and transgenic mice overexpressing constitutively active cardiac PKD1 [41] each were shown to elevate both basal and contraction-induced glucose uptake. Substantial evidence also indicates that atypical PKC-λ/ζ acts downstream of PI 3-kinase to relay insulin and contraction signals to GLUT4 translocation [42,43]. However, there are conflicting results using RNAi regarding the importance of PKC-λ/ζ on insulin-stimulated glucose uptake in adipocytes [19,41]. Thus, the precise roles of atypical PKC-λ/ζ in GLUT4 regulation need further clarification, perhaps from tissue-specific knockout mice [44].

Endogenous regulation of GLUT4 expression

The profound effects on whole-body glucose homeostasis observed in mouse models of GLUT4 deficiency or overexpression heighten the potential physiological importance of changes in endogenous GLUT4 expression in different states. For these reasons the mechanisms that regulate GLUT4 expression are important to clarify, and there is much fertile territory for exploration in this field. Tissue-specific expression of GLUT4 in adipose tissue, skeletal muscle, and cardiac muscle, as well as its regulation by fasting and refeeding, is conferred within a 2.4-kb DNA segment at the 5′ region of the GLUT4 gene [45]. For skeletal muscle-specific expression, a region between −522 and +420 bp has been inferred in transgenic mice to be important [46]. This region contains an apparent myocyte enhancer factor (MEF)2-binding domain at −466 to −457 bp that is critical for specifying tissue expression [47] and increased GLUT4 expression during muscle regeneration [48]. Near this same region it has been proposed that thyroid hormone receptor and myoD form a complex with MEF2 to regulate GLUT4 expression [49]. Another domain that has been implicated in the tissue-specific expression of GLUT4 is termed Domain I and includes the region −742 to −712 bp relative to the initiation site for transcription [50]. Moreover, a factor termed GEF appears to operate in this region in association with MEF2A [51].

Additionally, it has been reported that the synthetic thiazolidinedione ligands of peroxisome proliferator-activated receptor (PPAR)γ improve insulin sensitivity in type 2 diabetes and induce GLUT4 mRNA expression in adipose tissue and muscle. In primary rat adipocytes and CHO-K1 cells, PPARγ1 and PPARγ2 repressed GLUT4 promoter activity, whereas this repression was completely alleviated by rosiglitazone [52]. The –66 to +163 bp GLUT4 promoter region was sufficient to mediate PPARγ inhibitory effects [52]. In conjunction to this finding, a recent study has reported that zinc finger protein (ZFP) 407 regulates insulin-stimulated glucose uptake in adipocytes [53]. ZFP407 deficiency was attributed to a reduction in GLUT4 mRNA and protein levels. The decrease in GLUT4 was due to both decreased transcription of *Glut4* mRNA and decreased efficiency of *Glut4* pre-mRNA splicing. Interestingly, ZFP407 coordinately regulated this decrease in transcription with an increase in the stability of *Glut4* mRNA, resulting in opposing effects on steady-state *Glut4* mRNA levels [53]. Additionally, cytochrome P450 isoform 2E1 (CYP2E1) has also been identified as a novel negative regulator of GLUT4 gene expression in insulin-sensitive cells [54]. This inhibitor effect of CYP2E1 on GLUT4 gene expression was shown to be mediated by the binding of transcription factor NF-E2-related factor 2 (NRF2) to the distal promoter region of GLUT4 [54]. These and other data suggest a complex mode of GLUT4 regulation at the transcriptional level that is incompletely understood at present and warrants extensive research.

Signaling pathways regulating CD36 trafficking

CD36 is a multifunctional immuno-metabolic receptor with various ligands. One of its physiological functions in the heart is facilitating the high-affinity uptake of

fatty acids from albumin and from triacylglycerol-rich lipoproteins. The protein is expressed in endothelial cells and cardiomyocytes and at both sites is likely to contribute to fatty acid uptake by the myocardium. CD36 also transduces intracellular signaling events that influence how the fatty acid is utilized and that mediate metabolic effects of fatty acid in the heart [55]. CD36 was identified as a cellular fatty acid 'transporter' (for convenience this term is used although it was later found that CD36 does not act as a true membrane transporter for fatty acids but rather facilitates the uptake process) in 1993 based on work with isolated adipocytes [56]. Physiological relevance of this function of CD36, especially as related to heart metabolism, has been extensively reviewed [2,55]. CD36 was shown to traffic between the cell surface and intracellular compartments (specifically endosomes) and is recruited to the sarcolemma by either insulin and/or AMPK in a vesicle-mediated process [57,58] that is controlled by the Rab GTPase-activating protein AS160 and its target GTPase, Rab8a [58].

In humans, CD36 is composed of 472 amino acids that make up a hairpin-like structure with a large extracellular loop on the extracellular surface of the plasma membrane. The extracellular domain of the transmembrane protein is heavily glycosylated resulting in an increase in the apparent mass from 53 kDa (estimated) to 88 kDa (observed) [58]. The protein also has two phosphorylation sites, three external disulfide bridges, and contains four palmitoylation sites, two each at the extreme NH_2 and COOH termini [58]. Finally, the COOH terminal domain of CD36 contains two ubiquitination sites, indicating that CD36 is a potential target for various signaling modulators in the context of metabolic homeostasis. Currently, two major signaling pathways, insulin-mediated signaling and contraction stimuli, for CD36 trafficking have been explored in detail. Each relies on specific signaling cascades, which are tightly regulated by an intricate network of kinases (Fig. 2).

In addition to the major role of insulin signaling in controlling GLUT4 translocation, a number of kinases in this pathway are involved also in the regulation and intracellular translocation of CD36 [59]. Interestingly, and in contrast to the Randle cycle phenomenon, which suggests competition between glucose and fatty

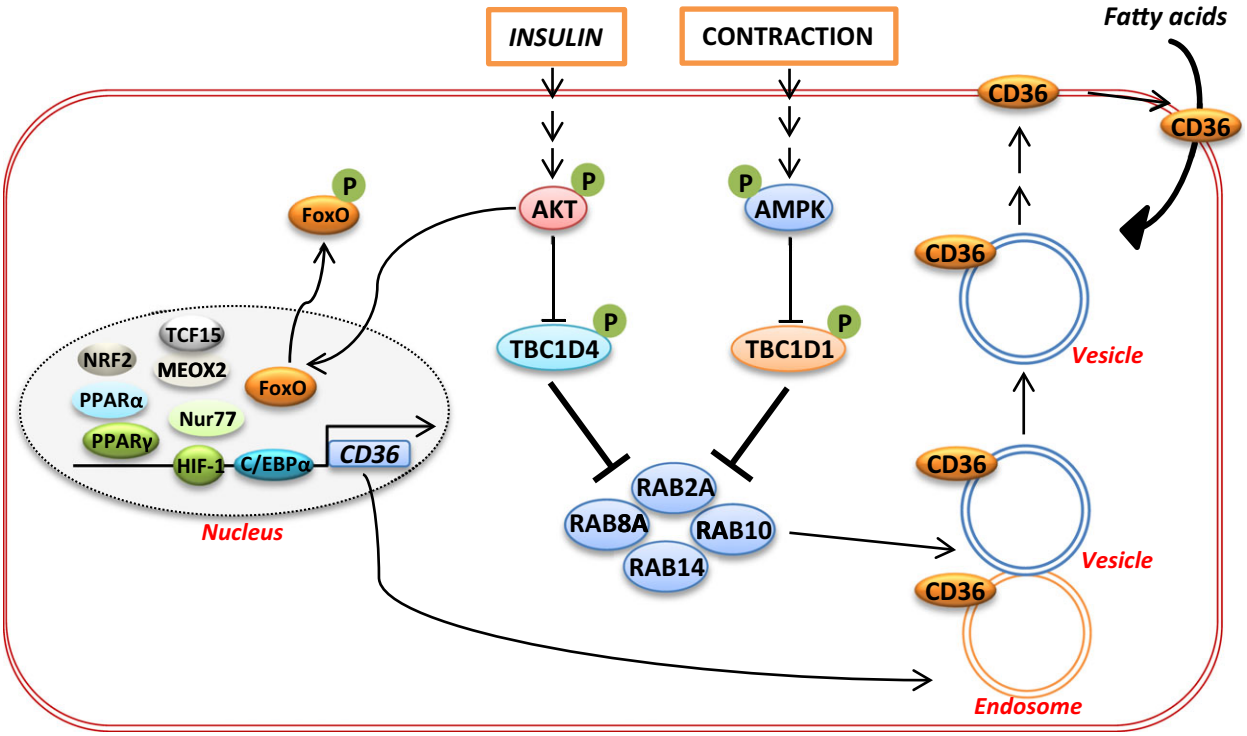

Fig. 2. Convergence of signaling pathways initiated by insulin and contraction leading to CD36 regulation. Insulin signaling through the PI3K pathway and muscle contraction through elevated AMP/ATP ratios lead to activation of downstream protein kinases (AKT, AMPK) that phosphorylate putative effectors that modulate steps in the CD36 trafficking pathways. AS160 is one such substrate that appears to negatively regulate an early step in CD36 exocytosis, via inhibition of RAB GTPases. At the gene expression level, several transcription factors have been reported to regulate CD36 promoter activity. See text for further details.

acid oxidation, insulin signaling is responsible for the translocation of both GLUT4 and CD36 to the sarcolemma [59]. For example, phosphatidylinositol-3-kinase (PI3K), a key component of the insulin signaling pathway, is thought to be a key player in CD36 translocation as PI3K-specific inhibitors, such as wortmannin and LY-294002, have been shown to negate these insulin-mediated changes in lipid metabolism [60]. Additionally, PI-2, 4, 5-triphosphate (PIP3), a product of PI3K, is involved in the activation of Akt2 [61], while PKC-λ/ζ [62] plays a role in CD36 translocation as well. Furthermore, the PI3K–Akt signaling pathway is also involved in the phosphorylation of the transcription factor forkhead box O (FoxO), which results in its nuclear export and inactivation (Fig. 2). The transcriptional activity of FoxO has been implicated in promoters of metabolic genes, which includes CD36 [63]. Thus, insulin signaling has an added effect of increasing CD36 expression in addition to increasing CD36 availability at the sarcolemma [63]. Notably, FoxOs can also be inhibited by the deacetylase SirT1. Thus, the role of Sirtuins, in general, and SirT1, in particular, can be of potential interest in the context of CD36 gene regulation.

Taken together, insulin is involved in not only the translocation of both GLUT4 and CD36 but also in upregulating their expression [59]. In the context of our understanding of the Randle cycle, how or why insulin simultaneously promotes the potential for both fatty acid entry and glucose entry has still not been addressed. In this regard, experiments designed to titrate the insulin concentration and subsequently determine the time responsiveness of GLUT4 and of CD36 translocation are of potential interest. On the other hand, the observations illustrate that the heart is an omnivorous organ in that it readily extracts all available substrates for storage purposes (Fig. 2). Namely, both glucose and fatty acids will be directed by further insulin actions (e.g., insulin-mediated dephosphorylation of glycogen synthase and phosphorylation of GPAT, respectively) toward synthetic pathways (to produce glycogen and lipid droplets, respectively).

Just as insulin, contraction simultaneously stimulates the translocation of GLUT4 and CD36. Contraction-induced CD36 translocation, similarly to that of GLUT4, is AMPK dependent [64]. Furthermore, similar to the PI3K–Akt signaling pathway, FoxO is downstream of the AMPK pathway, and thus may lead to enhanced CD36 availability for translocation to the membrane [65]. Although AMPK activation induces sarcolemmal recruitment of CD36 [64], on the flip side CD36 has also been recently reported to regulate AMPK activation [66]. CD36 was shown to be important for coordinating the dynamic protein interactions within a molecular complex consisting of the CD36 partner tyrosine kinase Fyn, the AMPK kinase LKB1, and AMPK. CD36 expression maintains AMPK quiescent by allowing Fyn to access and phosphorylate LKB1, promoting its nuclear sequestration away from AMPK [66]. Palmitate binding to CD36 within minutes activates AMPK via its ability to dissociate Fyn from the complex as CD36 is internalized into LKB1-rich vesicles. Thus, while CD36 keeps AMPK inhibited, palmitate binding acts to reverse this inhibition by activating AMPK [66]. This feedback signaling loop would serve to adjust the capacity for fatty acid oxidation to match fatty acid availability, and would explain earlier observations made in cardiomyocytes, where both sarcolemmal CD36 recruitment and AMPK activation were found to be important for fatty acid oxidation [67,68]. As a result, contraction-mediated signaling and CD36 translocation to the plasma membrane may provide an important mechanism for the heart to increase fatty acid supply in response to long-term changes in metabolic demand.

Endogenous regulation of CD36 expression

In humans, earlier work has shown that approximately 40% of patients with hypertrophic cardiomyopathy had decreased or abnormal CD36 expression associated with impaired myocardial fatty acid uptake [55]. Animal models of altered CD36 expression have shown detrimental cardiovascular outcomes related to both decreased and increased CD36 expression [extensively reviewed in 6,55]. Some studies have suggested cardiac abnormalities in mice lacking CD36 [69,70], suggesting that the loss of CD36 is detrimental to the heart. In contrast to the loss of CD36 activity, abnormal accumulation of CD36 at the sarcolemma may also cause cardiac dysfunction [59]. For example, recent work has shown that during the development of insulin resistance, CD36 becomes permanently localized at the sarcolemma at the cost of intracellular storage [60]. The resulting increase in fatty acid entry into cardiomyocytes has been suggested to be an important contributor to increased intracellular stores of triacylglycerol observed in hearts from diabetics and in animal models of type 2 diabetes [60]. Moreover, this increased storage may contribute to diabetic cardiomyopathy [60]. Thus, normal cardiac function is dependent on the proper amount and intracellular localization of CD36 while deviations from its optimal localization and expression likely play a role in many

cardiovascular diseases that involve abnormal fatty acid utilization.

Cellular CD36 content is regulated at different levels, including gene expression, mRNA stability, and protein expression in a cell- and tissue-specific manner [71]. Different physiological conditions, where the nutritional and/or hormonal status of the individual is affected, have been shown to impact on CD36 levels present in the plasma membrane. Regulation at the level of mRNA expression in skeletal muscle has been reported to include starvation, refeeding, and exercise [72], but only few studies have reported the molecular mechanisms underlying these effects. A previous study has reported that CD36 expression was activated during 3T3-L1 adipocyte differentiation, and CD36 protein levels were positively correlated with CCAAT/enhancer-binding protein α (C/EBPα) and PPARγ [73]. Overexpression of C/EBPα or C/EBPβ increased CD36 mRNA and protein levels in several types of cells. Restoration of C/EBPα or C/EBPβ expression in C/EBPα- or C/EBPβ-deficient mouse embryonic fibroblasts increased CD36 expression. A C/EBP-responsive element was identified in the CD36 promoter using 5′ and specific site mutations, thereby identifying as a C/EBPα as a transcriptional regulator of CD36 gene expression [73]. In another study, sequence analysis of the human CD36 promoter region revealed a functional hypoxia-inducible factor (HIF)-1-, the major hypoxia effector, binding site [74]. These responses were reliant upon ROS production. Interestingly, this study also demonstrated that inhibition of the PI3K pathway blocked the HIF-1-dependent induction of CD36 expression and promoter activity, thus indicating a novel mechanism interlinking hypoxia, ROS, and PI3K signaling pathways in the context of CD36 gene regulation [74]. Additionally, although PPARα, PPARγ [75,76], NR4A (Nur77) [77], Nrf2 [78], and Meox2/Tcf15 heterodimer [79] have been shown each to affect CD36 expression, no direct binding of these transcription factors to the *Cd36* promoter have been described. Furthermore, recent analyses of the *Cd36* gene have revealed a complicated promoter structure with alternative transcription start sites [80]. Alternative promoter usage has been shown to contribute to tissue-specific regulation of CD36 expression in both mice and humans [80]. Although the alternative promoters are mapped in the human and murine *Cd36* genes, less information is available for the rat gene. This intricate fine tuning of *Cd36* gene regulation would be consistent with the concept that the metabolic effects of CD36 involve its ability to function under the control of different molecular complexes of several functional proteins.

Future perspectives

As encountered with every biological process, the regulations of GLUT4 and CD36 gene expression and translocation are complex processes and have important further implications for modulation of glucose and fatty acid metabolism, calcium homeostasis, and cellular inflammation. Our tools of using non- and/or supraphysiological *in vitro* systems and gross genetic modifications of animal models may fail to identify all the critical contributors involved in physiological processes that are often redundant or, in most cases, are highly context dependent. Recent insights have shed light into the roles of GLUT4 and CD36 in the heart both under homeostatic and patho-physiological conditions but many questions remain unanswered.

The role of GLUT4 as a dominant regulator of whole-body glucose homeostasis is now well established based on several genetically engineered mouse models overexpressing or deficient of GLUT4. These data validate the notion that either acute or long-term changes in the abundance of GLUT4 on the cell surface of heart, adipose, or muscle cells could provoke systemic changes in glucose disposal *in vivo*. Such changes include decreased GLUT4 expression in adipocytes in obesity and increased GLUT4 expression in cardiac and muscle cells in response to contraction. However, we are still at an early stage of understanding the regulatory molecular mechanisms that underlie GLUT4 expression in these tissues. Few transcription factors involved in GLUT4 gene regulation have been identified, but their exact roles within the various physiological conditions that alter GLUT4 expression need to be further clarified. Other transcriptional regulators also likely play important roles and remain to be discovered.

On the other hand, what role does CD36 play in myocardial fatty acid uptake and how is its function altered in disease? What are the post-translational modifications responsible for the regulation of CD36 metabolic actions as well as dysfunctional CD36 persistence at the sarcolemma under conditions of hyperinsulinemia? Is it possible to prevent or reverse these modifications, and thus ameliorate lipid abnormalities associated with metabolic syndromes and cardiovascular diseases? Would enhancing CD36 expression in the myocardium be beneficial in helping resolve cardiac injury and how will this impact on metabolic reprogramming? In addition to these questions there are several emerging research avenues that need to be addressed. Identifying the molecular partners and interactions that define these regulatory effects would provide great insight into potential therapeutic targets.

It is also highly likely that the ability of CD36 to regulate cellular metabolism is not limited to lipid uptake and utilization. The generation of transgenic animals with inducible and tissue-specific overexpression or deletion of substrate transporters (GLUT4, CD36) is currently underway and will provide specific insight into the role of the substrate transporters in selected tissues as well as their role in the cross-talk among tissues and in whole body homeostasis.

Finally, much progress is needed in translating these research approaches to humans, where common variants in the *Cd36* gene impact its expression level often in a tissue-specific manner [81]. The human genome is complex owing to multiple promoters and transcripts with tissue- or cell-specific distribution [80] and understanding how these transcripts are regulated is likely to have relevance to myocardial metabolism. The emerging availability of noninvasive imaging techniques, such as magnetic resonance imaging (MRI) and positron emission tomography (PET), will allow detailed functional (MRI) and metabolic [e.g., cardiac ^{18}F-fluoro-2-deoxyglucose (FDG) and ^{18}F-fluoro-6-thioheptadecanoic acid (FTHA) PET imaging] characterization of individuals. Subsequently, putative associations between variants in the *Glut4* and/or *Cd36* genes with these phenotypic characteristics then can be explored. Furthermore, potential epigenetic influences of nutrients and of other environmental factors on GLUT4 and CD36 transcription also might provide insight into the etiology of metabolic dysfunction and the associated cardiovascular complications.

Acknowledgements

The authors thank former and current laboratory members for insights, discussion, and data that have contributed to the understanding of this topic.

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