Manuscript for *Physiological Reviews*

CD36 as a gatekeeper of cardiac lipid metabolism and therapeutic target for metabolic disease

AUTHORS

Jan F.C. Glatz[1], Lisa C. Heather[2], Joost J.F.P. Luiken[1]

[1]*Department of Genetics & Cell Biology, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, The Netherlands*
[2] *Department of Physiology, Anatomy & Genetics, University of Oxford, Oxford, U.K.*

CORRESPONDENCE

glatz@maastrichtuniversity.nl

KEYWORDS

CD36; SR-B2; fatty acid uptake; lipid metabolism; metabolic disease; heart

---

CONTENTS

Chapter 1: Introduction and scope of review

Chapter 2: CD36 as a multifunctional protein

Chapter 3: CD36 facilitates cellular fatty acid utilization

Chapter 4: CD36 translocation to and from the plasma membrane

Chapter 5: CD36 as a receptor for lipid signaling

Chapter 6: Role of CD36 in the pathogenesis of metabolic diseases

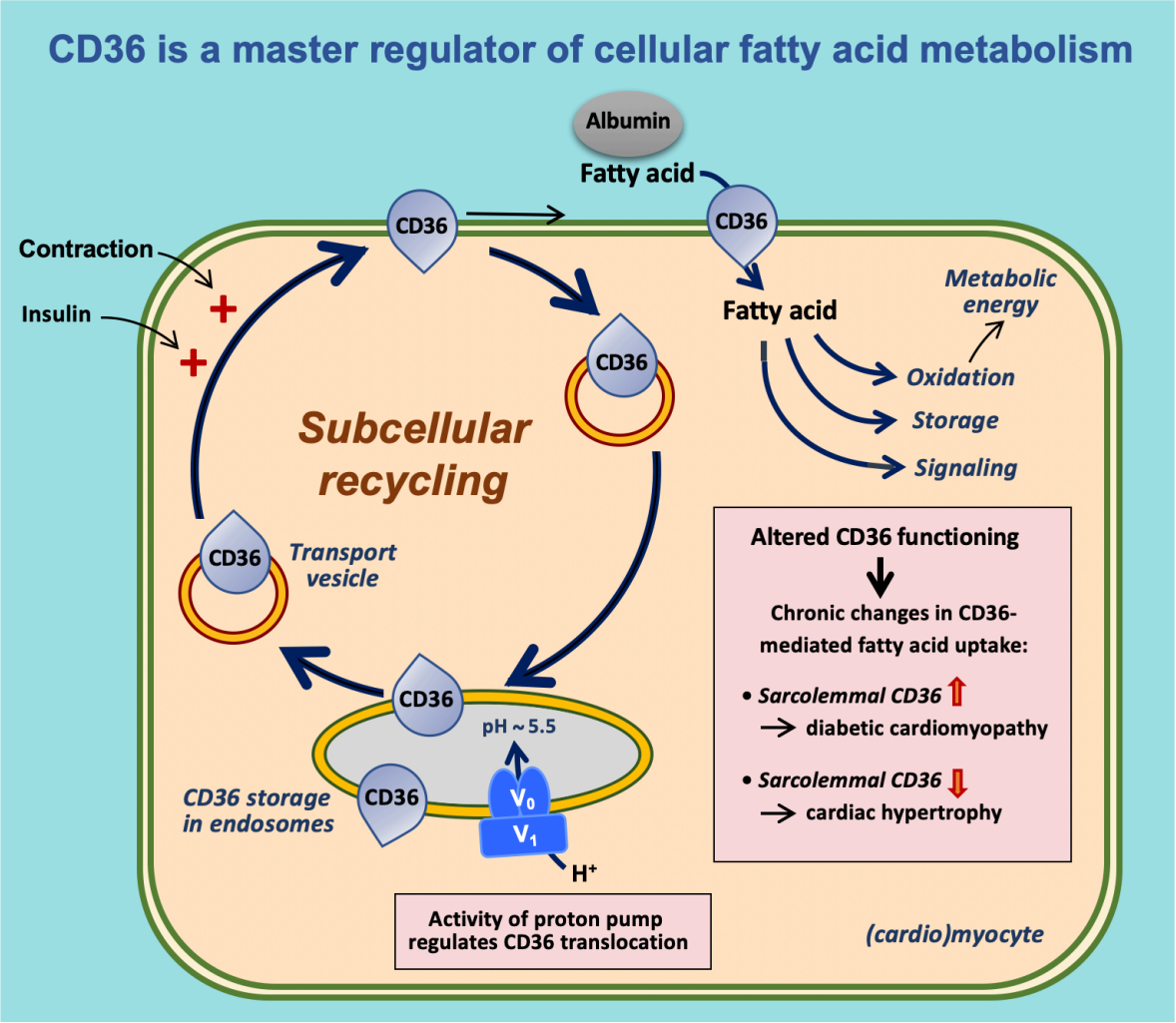
Chapter 7: CD36 as a target for metabolic intervention therapy in heart

Chapter 8: CD36 beyond the heart in metabolic disease

Chapter 9: Concluding remarks and perspective

References

GRAPHICAL ABSTRACT



CLINICAL HIGHLIGHTS

- The transmembrane protein CD36 fulfills multiple functions in cellular lipid metabolism, primarily acting as facilitator of long-chain fatty acid uptake and as receptor for lipid signaling.
- In heart and skeletal myocytes, CD36 reversibly recycles between endosomes (intracellular storage) and the sarcolemma (functional presence) to regulate the rate of fatty acid uptake in order to meet metabolic requirements.
- This dynamic subcellular recycling of CD36 is under control of mechanical, hormonal and nutritional factors, in addition to the transcriptional control of total CD36 content.
- Alterations in CD36 functioning underlie various metabolic diseases, such as diabetic cardiomyopathy, cardiac hypertrophy, non-alcoholic fatty liver disease, and cancer.
- Inhibition of CD36 or modulation of its subcellular localization appear effective strategies for treatment of metabolic diseases associated with abnormal lipid metabolism.

Abstract

The multifunctional membrane glycoprotein CD36 is expressed in different types of cells and plays a key regulatory role in cellular lipid metabolism. CD36 facilitates the cellular uptake of long-chain fatty acids, mediates lipid signaling, and regulates storage and oxidation of lipids in various tissues with active lipid metabolism. CD36 deficiency leads to marked impairments in peripheral lipid metabolism, which consequently impacts on the cellular utilization of multiple different fuels, due to the integrated nature of metabolism. The functional presence of CD36 at the plasma membrane is regulated by its reversible subcellular recycling from and to endosomes, and is under the control of mechanical, hormonal and nutritional factors. Aberrations in this dynamic role of CD36 are causally associated with various metabolic diseases, in particular insulin resistance, diabetic cardiomyopathy, and non-alcoholic fatty liver disease. Recent research in cardiac muscle has disclosed the endosomal proton pump v-ATPase as a key enzyme regulating subcellular CD36 recycling and being the site of interaction among various substrates to determine cellular substrate preference. In addition, evidence is accumulating that interventions targeting CD36 directly or modulating its subcellular recycling are effective for the treatment of metabolic diseases. In conclusion, subcellular CD36 recycling is the major adaptive mechanism of cellular uptake of long-chain fatty acids, and appears a suitable target for metabolic modulation therapy to mend failing organs with active lipid metabolism.

[Abstract: 223 words]

[Main text: 20,132 words]

Chapter 1. Introduction and scope of review

Cluster of Differentiation 36 (CD36), officially designated scavenger receptor B2 (SR-B2) but also known as fatty acid translocase (FAT), was first described in 1977, and implicated in platelet aggregation and the adherence of erythrocytes infected with the malaria parasite (1). Subsequently, it was established that CD36 functions as a scavenger receptor for oxidized low-density lipoproteins (LDL) on macrophages (2). However, further research led to another role being assigned to CD36, as a membrane fatty acid transporter. In 1993, CD36 was shown to facilitate the cellular uptake of long-chain fatty acids (3), the predominant fuel stored within the mammalian body and used by many tissues including the intestine, adipose tissue, liver, and cardiac and skeletal muscles. Thus, CD36 plays a critical role in the transfer of this substrate from the blood to lipid metabolizing tissues, directly regulating fat oxidation to meet energy requirements and lipid storage for future use.

CD36 is a transmembrane protein that consists of a single peptide chain of 472 amino acids with a molecular mass of 88 kDa. CD36 undergoes extensive post-translational modifications, including glycosylation, palmitoylation, ubiquitination, and phosphorylation. In addition, CD36 can reversibly move between intracellular compartments and the plasma membrane in response to specific signals. Taken together, this makes CD36 a highly-regulated dynamic protein with multiple mechanisms controlling its function. Since its first mentioning the number of articles on CD36 has increased steadily to reach >500 per year currently, the total number being over 8,000 with about 2,000 on heart or cardiovascular disease (PubMed search 2023). Yet, despite its identification many decades ago, the protein structure and general membrane topology were only elucidated in 2016.

The present review focuses on CD36 as master regulator of cellular fatty acid homeostasis and lipid metabolism, its consequent pivotal role in the development of metabolic disease, and its emerging appearance as a target for therapy. The emphasis will be on the fatty acid transport function of CD36 within the cardiovascular system, as it has been most extensively studied in this context. We discuss the role of CD36 in other tissues including skeletal muscle, liver, adipose tissue, and immune cells, but also refer the reader to more comprehensive reviews

on CD36 in atherosclerosis (4, 5), in liver and non-alcoholic fatty liver disease (6, 7), and in cancer (8-11) .

Chapter 2. CD36 as a multifunctional protein

2.1 CD36 discovery and structure

CD36 was first reported in 1977 as glycoprotein IV (GP IIIb or GP IV), the fourth major band observed upon SDS-polyacrylamide gel electrophoresis of human platelet membranes (1). Ten years later, the protein was shown to be identical to the antigen recognized by the monoclonal antibody OKM5, a marker for monocytes and macrophages, and designated as the leukocyte differentiation antigen (or cluster-designated antigen) CD36 (12). In that same year CD36 was reported to be the cellular receptor for thrombospondin (13), implicating a role of CD36 in irreversible platelet aggregation, as well as in the adherence of erythrocytes infected with the malaria parasite, *Plasmodium falciparum*, to the endothelium (14). Subsequently, it was discovered that CD36 is a macrophage receptor for oxidized LDL (oxLDL), thereby establishing its role as a scavenger receptor (2). CD36 is currently known to be a member of a superfamily of scavenger receptor proteins (class B), which also includes scavenger receptor (SR)-B1 and lysosomal integral membrane protein-2 (LIMP-2). As a result, CD36 is now officially designated as SR-B2 (15). Pioneering work of Abumrad and Grimaldi first identified a role for CD36 in adipocytes as a membrane fatty acid transporter (3), and subsequent work confirmed its expression in many highly oxidative and fatty acid-dependent tissues (16) (www.proteinatlas.org), demonstrating an important systemic role for CD36.

The structure of CD36 consists of two transmembrane domains, a relatively large extracellular domain, small cytoplasmic tails and both the amino and carboxyl termini located within the cytoplasm (17) (**FIGURE 1**). The extracellular domain contains nine *N*-linked glycosylation sites, which contributes to the stability of the protein (18). The extracellular domain of human CD36 (residues 35–439) has been studied by X-ray crystallography at 2.07 Å resolution (19). This analysis demonstrated the presence of one or two hydrophobic channels within the extracellular domain which putatively function in the transport of fatty acids towards the plasma membrane (19), and perhaps also that of cholesterol (20). The structure of CD36 is not

reminiscent of a typical membrane transporter and, therefore, has caused confusion for many years when trying to elucidate how CD36 facilitates transmembrane fatty acid translocation (see Chapter 3).

2.2 Tissue expression profile of CD36

CD36 is not expressed ubiquitously, yet is present in a variety of mammalian cell types, including hematopoietic cells (platelets, monocytes, macrophages), endothelial cells, specialized epithelial cells in the breast and eye, lung pneumocytes, enterocytes, and insulin-responsive cells, such as adipocytes, and cardiac and skeletal myocytes (www.proteinatlas.org). In liver, the basal expression of CD36 is very low but can be markedly upregulated under hyperlipidemic conditions through activation of nuclear receptors, including PPARγ and liver X receptor (LXR), to contribute to the onset of hepatic steatosis (21-23). The CD36 expression pattern is paralleled by a similar broad number of functions, which are mostly related to the regulation of lipid metabolism and of innate immunity in both health and disease. Specifically, CD36 is involved in inflammatory responses and atherothrombotic diseases, intestinal fat absorption, lipid storage in adipose tissue, and lipid utilization by cardiac and skeletal muscle. CD36 has been linked to metabolic disorders (such as obesity and diabetes), Alzheimer's disease, cancer and multiple sclerosis (reviewed in (24, 25)) Interestingly, CD36 is also expressed in human taste bud cells where it functions as a fatty acid sensor acting in oral fat perception and digestive anticipation (see Chapter 5) (26, 27).

The cellular content of CD36 increases markedly with ageing, as documented for cardiac muscle (28, 29), and is higher in females than in males (30). This gender difference most likely is explained by specific action of female gonadal hormones (30).

Besides the transmembrane form also a soluble form of CD36 occurs in the circulation and is designated as sCD36 or known as the CD36 receptor ectodomain (31). This form is generated following the abscission of the extracellular domain of the transmembrane form of CD36, most likely through proteolytic shedding by ADAMs (a disentegrin and metalloproteinase) (reviewed in (32)). Plasma sCD36 has been reported to reflect the tissue expression of CD36 and therefore has been proposed as biomarker for various pathological conditions.

2.3 CD36 gene and mutations

The gene encoding CD36 is located on chromosome 7q21.11 and spans about 77 kb. A total of 19 exons were found in the *CD36* gene and at least 20 alternatively spliced transcript variants. A large number of CD36 gene variants have been described, with a notable number leading to either the absence or down-regulation of CD36 protein expression in specific cells, to conformational changes, or to inactivation of the ligand binding region in the extracellular domain. Several of these variants have been associated with the occurrence of thrombocytopenia, malaria, and atherosclerosis and other cardiovascular diseases, insights that have contributed substantially to our understanding of the function and physiological significance of CD36 (reviewed in (33)). Specifically, studies in different populations have disclosed that multiple variants of the *CD36* gene impact on fatty acid processing and metabolism, obesity and metabolic diseases.

A promotor polymorphism in the *CD36* gene was reported that showed a direct association with the presence of type 2 diabetes in Caucasian subjects (34). Similarly, studies on several single nucleotide polymorphisms (SNPs) in the CD36 gene have revealed an association of CD36 genetic variants with interindividual variability in lipid metabolism and the risk of lipid-induced complications (35-42). Individuals with CD36 deficiency due to specific gene mutations (e.g., C478T, i.e., Pro90Ser) were found to have a markedly reduced cardiac fatty acid uptake rate that was associated with mild heart disease (New York Heart Association class I–II) (36, 43, 44). In addition, given the expression of CD36 in taste bud cells of lingual surface (26, 45), gene variants of CD36 might influence oral fat sensing and thereby impact on the individual's preference and intake of fat, which, in turn, influences the development of obesity (40, 46, 47). Some mutations in the CD36 gene have been found to be beneficial to the host, as specific gene variants leading to CD36 deficiency reduce parasite sequestration in red blood cells to protect against the severity of malaria (48).

Initial genetic studies in spontaneously hypertensive rats (SHR) identified CD36 sequence variants to cause CD36 protein deficiency in adipocytes and to underlay insulin resistance in this rat strain (49). However, subsequent more detailed biochemical studies revealed the SHR to express aberrant CD36 mRNAs together with functional CD36 protein, albeit at reduced

levels, in all tissues with active lipid metabolism (50) indicating that the SHR is not a natural CD36 null model. We will discuss the pathology in this SHR model in detail in section 6.

2.4 Transcriptional control of CD36

The expression of CD36 is transcriptionally controlled mainly by agonists of members of the family of nuclear transcription factors called the peroxisome proliferator-activated receptors (PPAR), in particular PPARα in heart and liver, PPARβ in heart and muscle, and PPARγ in adipose tissue and liver (51). PPARs are activated by fatty acids and their metabolites, and regulate the transcription of a large variety of genes implicated in metabolism, inflammation, proliferation, and differentiation in distinct cell types (52, 53). PPARs can directly regulate the transcription of target genes by forming a heterodimer with the retinoid X receptor, and binding to PPAR response element (PPRE) sequences in the promotor or transcribed regions of target genes (52). The CD36 gene in mice (54) and humans (55) possesses one or more functional PPREs close to the transcription start site. The chronic regulation of CD36 by either saturated or unsaturated fatty acids through activation of PPAR results in its expression specifically in tissues with high rates of lipid metabolism (53, 56-59). In addition, most other lipid metabolic genes are transcriptionally controlled by PPARs so that activation of this system leads to the coordinated expression of various proteins and enzymes involved in lipid metabolism. Interestingly, by virtue of its facilitatory function in cellular fatty acid uptake (see Chapter 3) CD36 mediates the delivery of ligands to PPAR eventually resulting in a feed-forward cycle of increased cellular fatty acid uptake.

The CD36 gene has a complicated promotor structure with alternative transcription start sites (60), which has caused confusion on the presence of a PPRE in the upstream promotor region. Initial studies described the absence of PPRE and suggested that transcriptional activation of the gene by PPAR ligands would be indirectly dependent on PPAR (61). In later studies, however, the PPRE sites have been identified and validated in the distal part of the CD36 promotor (62, 63). Furthermore, both the rodent and human CD36 genes are now known to have three independent promoters that are differentially used in various tissues (61, 64). This multiple promoter structure allows the production of mRNA's with diverse 5'-noncoding sequences to further achieve tissue-specific transcriptional regulation as well as differences in translational efficiencies or stabilities of the mRNAs among the PPAR isoforms (64). Besides

PPARs, the translational activators CCAAT/enhancer-binding protein-a and hypoxia-inducible factor-1 (HIF-1) also have been implicated in the regulation of CD36 expression (reviewed in (65)).

More recently, it was reported that in mice and humans micro-RNA-320 (miR-320) acts as a small activating RNA in the nucleus at the level of transcription, with CD36 being the key target gene (66). Upregulation of miR-320 promotes cardiac lipid uptake without affecting fatty acid oxidation, thus inducing lipotoxicity which eventually may lead to diabetic cardiomyopathy (see Section 6.2) (66). Conversely, novel-miR-87 was found to inhibit CD36 gene transcription in primary intramuscular preadipocytes thereby inhibiting their differentiation (67), while both miR-29a (68) and miR-100 (69) suppress CD36 expression in hepatocytes.

Taken together, the expression of the CD36 gene is complex and under control of several effectors enabling its precise regulation in a tissue-specific fashion and in coordination with intracellular metabolism for lipid homeostasis at the whole-body level.

2.5 Co-translational and post-translational modifications of CD36 structure

A particular characteristic of CD36 is its extensive co-translational and post-translational modifications that comprises glycosylation, O-GlcNAcylation, phosphorylation, palmitoylation and ubiquitination, and markedly expands the molecular mass of the protein from approximately 53 kDa to 88 kDa (18). This latter difference is due mainly to extensive glycosylation. Generally, *N*-linked glycosylation at asparagine residues occurs co-translationally within the endoplasmic reticulum and Golgi, and leads to the non-reversible coupling of complex oligosaccharide structures to proteins. Glycosylation is critical to correct folding, stability, transport to the cell surface, and function of membrane proteins (70). The extracellular loop of CD36 contains nine observed *N*-glycosylation sites, and their glycosylation is necessary for proper folding and trafficking to the plasma membrane, although partially glycosylated CD36 mutants also properly distribute to the cell surface (71). Whether, and if so, to what extent, differences in glycosylation would directly affect the physiological function of CD36 is not known.

CD36 has been found to be modified also by O-linked β-*N*-acetylglucosamine (O-GlcNAc), which is a dynamic process that occurs in the cytoplasm and involves a reversible binding of a single O-GlcNAc moiety to serine/threonine residues (72). Increased O-GlcNAcylation of CD36, induced by increasing glucosamine availability and subsequent stimulation of the hexosamine biosynthesis pathway, enhances myocellular fatty acid uptake suggesting a role for O-GlcNAcylation in CD36 function (73, 74).

The extracellular loop of CD36 contains two phosphorylation sites, at Thr92 and Ser237, which are phosphorylated by protein kinase C (PKC) (75) and by protein kinase A (PKA) (76), respectively, and dephosphorylated by specific phosphatases (77). CD36 phosphorylation at Thr92 has been reported to occur intracellularly in the Golgi, to be retained upon trafficking of CD36 to the cell membrane, and to inhibit the binding of thrombospondin-1 in platelets (75). In contrast, CD36 phosphorylation at Ser237 in platelets is under control of an ecto-kinase pathway involving PKA and inhibits fatty acid uptake in a reversible manner (78). However, in heart and muscle CD36 phosphorylation is regarded of minor significance for CD36 function in myocellular fatty acid uptake, likely due to the virtual absence of ecto-protein kinases in the interstitial space of heart and muscle (18).

Palmitoylation (or S-acylation) of CD36 appears of crucial importance for its maturation and proper functioning (reviewed in (79)). Both the NH_2- and COOH-termini of CD36 are each doubly palmitoylated, in human CD36 corresponding to cysteine residues 3, 7, 464, and 468 (80), enabling the protein to anchor into the lipid membrane (**FIGURE 1**). S-palmitoylation refers to the reversible chemical linkage of a palmitate (C16) molecule to cysteine residues and is regulated by specific enzymes, i.e., palmitoyl-acyltransferases (PATs) and palmitoyl-protein thioesterases (PPTs) (also designated acyl-protein thioesterases, APTs) (79). Regulation of the activities of PAT and PPT enzymes allows for short-term (minutes) changes in intracellular CD36 processing, in subcellular localization and trafficking, and in membrane interactions. Palmitoylation of all four intracellular cysteines is required for proper translocation of CD36 to the plasma membrane (81), whereas de-palmitoylation of CD36 appears a requirement for its internalization from the plasma membrane (see Chapter 3.2) (82). Interestingly, both insulin and activation of AMP-activated kinase (AMPK) enhance

palmitoylation of CD36 (see Chapter 4.2) (83). Protein palmitoylation is the topic of a vast developing field (84, 85).

Finally, the small intracellular C-terminal segment of CD36 also contains two ubiquitination sites, which are involved in directing CD36 towards proteasomal degradation (**FIGURE 1**). Specifically, poly-ubiquitination occurs at Lys469 and Lys472 of CD36 (86). The degree of poly-ubiquitination is increased by fatty acids and decreased by insulin within a short time frame (<30 min) (86), suggesting that proteasomal degradation of CD36 is a significant contributor to the short-term regulation of the cellular CD36 protein level. Interestingly, in liver CD36 also is mono-ubiquitinated by Parkin, an E3 ubiquitin ligase, leading to stabilization of CD36 and increased hepatic fatty acid uptake (87). It is unclear, however, whether Parkin and/or mono-ubiquitination is involved in the physiological modulation of CD36 in other tissues such as heart and muscle (18).

In summary, CD36 is subject to various types of co-translational and post-translational modifications in a tissue-, condition- and time-dependent manner. Each of these modifications is either crucial for proper maturation and subcellular trafficking of CD36, or impacts on its cellular content and/or physiological function.

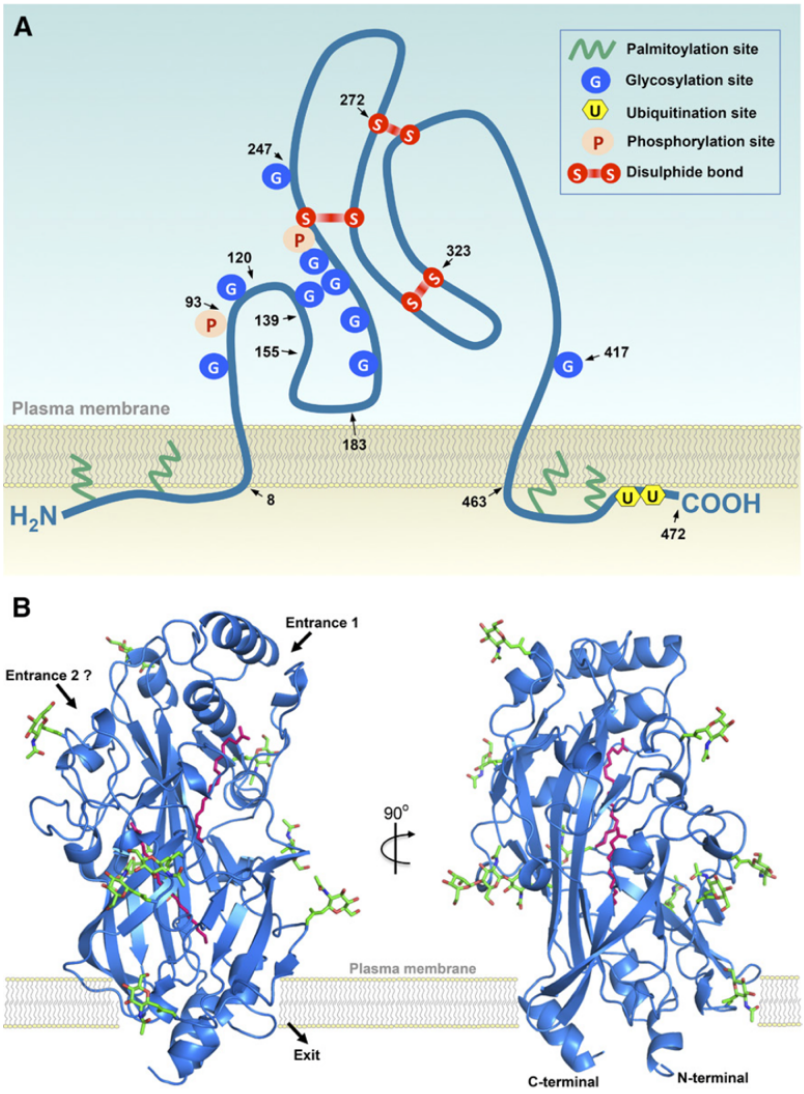


FIGURE 1. The two- and three-dimensional structure of CD36 and its binding of fatty acids.
A. Two-dimensional structure showing the membrane orientation of CD36, the sites of co-translational and post-translational modifications, and the three sulfide bridges. CD36 consists of five regions: *N*-terminal intracellular domain (residues 1–7), *N*-terminal transmembrane domain (residues 8–29), extracellular domain (residues 30–439), C-terminal transmembrane domain (residues 440-461), and C-terminal cytosolic domain (residues 462-472).
B. Two views of the three-dimensional structure of CD36. The ribbon diagram shows the structure in blue with the nine *N*-linked glycosylation sites and associated sugars in green. Two palmitic acid molecules, bound in the central cavity of the protein, are shown as pink sticks. The two putative entrances for fatty acids to reach this central cavity, and the putative exit are indicated by black arrows. Entrance 1 is regarded more likely than entrance 2. Note that the positioning of CD36 with respect to the membrane and its assumed interactions with phospholipids and other membrane constituents have not yet been delineated in detail and, therefore, are only vaguely indicated. Reproduced from (88) with permission.

Chapter 3 . CD36 facilitates cellular fatty acid utilization

3.1 The need for membrane proteins to facilitate cellular fatty acid uptake

Metabolism of substrates provides both the energy and biosynthetic intermediates essential to fuel cell function. Therefore, in a healthy cell it is a prerequisite that the rate of uptake of substrates, including that of long-chain fatty acids, is constantly in tune with their rate of cellular utilization. Too low uptake rates could elicit a substrate deficit, e.g., a shortfall of energy-providing substrates, while too high uptake rates impose the risk for substrate accumulation and putative toxicity. In the case of long-chain fatty acids, proper regulation of their transport across the plasma membrane is of fundamental importance given that fatty acids fuel energy provision, are components of membranes, and are distinct signaling molecules. In addition, fatty acids must be handled with care by the cell because they may act as detergents and thus non-selectively impair various cellular processes. Given this delicate balance, in the past decades much effort has been made to unravel both the molecular mechanism and regulation of cellular fatty acid uptake.

For many years, there was great debate in the field regarding the general concept of fatty acid uptake. Two lines of arguments existed; one view was that due to their lipophilic nature fatty acids would be able to pass biological membranes without the involvement of membrane proteins (89, 90). The opposing argument was that membrane-associated proteins would be involved to provide a mechanism that allowed cellular regulation of fatty acid uptake (91-93). The consensus view recently reached is that specific membrane-associated proteins, in particular CD36, facilitate both the entry of fatty acids into the membrane and their desorption from the membrane, while the actual transmembrane translocation of fatty acids occurs by their spontaneous ‘flip-flop’ between the two leaflets of the phospholipid membrane (94). Desorption from the membrane is the rate-limiting step of transmembrane transport of fatty acids (90, 95). As a result, the molecular mechanism of cellular fatty acid uptake comprises an integration of simple diffusion (within the lipid bilayer of the membrane) and facilitated diffusion (capture of fatty acids into the membrane and their release from the membrane). Membrane-associated proteins thereby provide a hydrophobic pore for the fatty acid to overcome the unstirred water layer and obtain access to the lipid membrane. Regulation of the rate of fatty acid uptake (or release) occurs by adjusting the amount and/or

activity of these membrane-associated proteins in the cell membrane while the actual rate and direction of fatty acid movement are determined by the transmembrane gradient of fatty acids (94).

With respect to terminology, it is of note that CD36 facilitates transmembrane transport of (long-chain) fatty acids, but does not act as a classical transporter or translocase. Therefore, in the context of CD36 the older terms 'fatty acid translocase (FAT)' (3) and 'fatty acid transporter' are misleading and should be avoided, although they may be used for convenience.

3.2 CD36 as primary facilitator, working in sequence with other fatty acid transporters

In blood plasma and interstitium, long-chain fatty acids are transported bound to albumin. The aqueous solubility of common fatty acid species is extremely low (for instance, for palmitate in the nanomolar range) (96) so that under physiological conditions >99,99% of fatty acids is complexed to albumin. Likewise, transport of fatty acids within the cellular cytoplasm is facilitated by cytoplasmic fatty acid-binding protein ($FABP_c$), relatively small proteins (12–15 kDa) of which nine distinct types exist (97, 98). As a result, cellular fatty acid uptake involves the transfer of the highly hydrophobic fatty acid from its extracellular carrier albumin through the lipid membrane towards its intracellular carrier $FABP_c$.

In search of proteins participating in the transmembrane step of cellular fatty acid uptake, several research groups identified both peripheral and integral membrane proteins. These include plasma membrane fatty acid-binding protein ($FABP_{pm}$; 40–43 kDa) (99), fatty acid-transport protein (FATP; 63 kDa) isoforms 1–6 (100), fatty acid translocase/CD36 (88 kDa) (3), and caveolin-1 (21–24 kDa) (101) (reviewed in (102-104)). $FABP_{pm}$ exists on the extracellular surface of the cell, CD36 is a transmembrane protein (Section 2.1), whereas FATP occupies a more cytosolic location in relation to the cell membrane. The current working model is that the various proteins operate in sequence with CD36, facilitating in a coordinated fashion the movement of the fatty acid away from albumin, into the lipid membrane and subsequent dissociation into the binding cavity of $FABP_c$ (**FIGURE 2**). $FABP_{pm}$ displays protein–protein interaction with CD36 (105) and as functional complex may assist in sequestering and presenting the fatty acid to the membrane (106). Caveolin is potentially involved in defining

specific membrane microdomains (such as flask-shaped caveolae, or lipid rafts) to cluster the various proteins and enable an efficient uptake process (see also section 4.3) (107, 108). Initially, FATPs were identified as transporters, but have now been reassigned based on their inherent enzyme activity. The FATP isoforms were found to have acyl-CoA synthetase activity, converting the fatty acid into fatty acyl-CoA straight after it is associated with the membrane, resulting in membrane-mediated metabolic trapping of fatty acids (also designated vectorial acylation) (103).

An *in vivo* approach to investigate the function of the various fatty acid transporters is by electro-transfection of plasmid DNA containing the transporter of interest to increase the expression levels specifically in a tissue of interest. Electro-transfection of $FABP_{pm}$, CD36, FATP1 and FATP4 into muscle was associated with increased fatty acid uptake and oxidation, but no change in esterification two weeks after transfection (109). This approach has also been utilized to try to understand how these transporters work in sequence, and interestingly suggested a different mechanism of action to that predicted from pharmacological transporter manipulation. Electro-transfection of CD36 or of $FABP_{pm}$ had different effects on the magnitude of increase in fatty acid uptake, with the greater contribution from CD36. When both transporters were simultaneously transfected the effects were additive, suggesting an independent rather than collaborative mechanism of action for each transporter (110).

CD36 also has been implicated in an endocytotic pathway to take up fatty acids into adipose tissue (82). In this process, binding of fatty acids to CD36 triggers a signaling cascade that de-palmitoylates CD36 and stimulates caveolae-dependent CD36 internalization as the mechanism to deliver fatty acids into the cell (82). It is not yet clear whether such system also operates in other tissues and to what extent it would contribute to the overall rate of cellular fatty acid uptake. Finally, circulating exosomes have been reported to express functional CD36 that may be involved in the delivery of fatty acids to the heart, especially in the postprandial state where circulating exosomes show higher levels of CD36 (111).

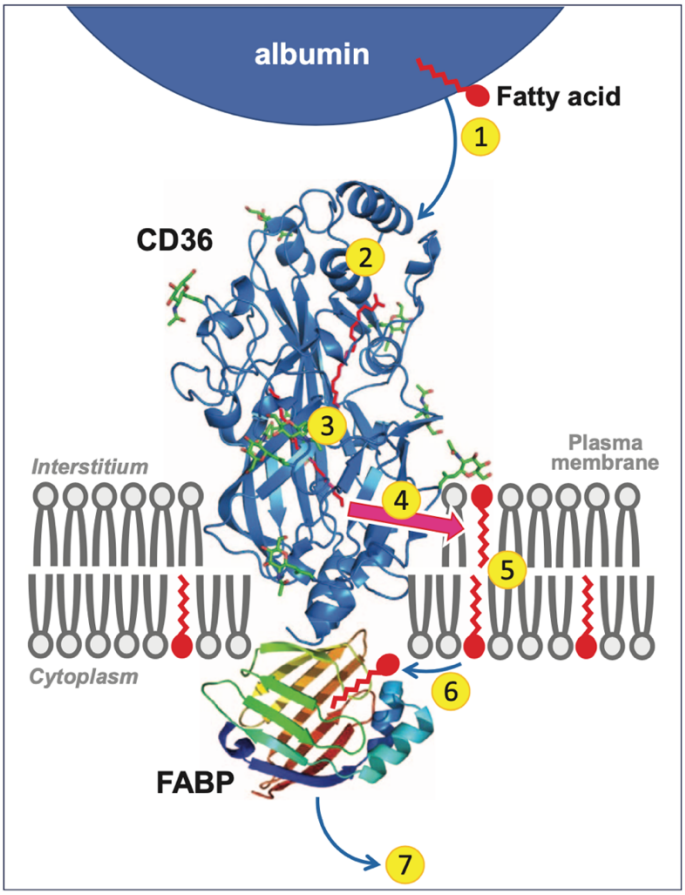


FIGURE 2. Schematic illustration of the sequential steps involved in the uptake of long-chain fatty acids by cells. **1**. Release of fatty acids from (interstitial) albumin; although at any given time the aqueous concentration of fatty acids is extremely low, once a fatty acid is bound by CD36 it is immediately replenished from the large pool of albumin-bound fatty acids. **2.** Binding in the hydrophobic cavity of CD36 which can accommodate up to two fatty acids at a time. **3**. Guidance of the fatty acid through the CD36 ectodomain interior to pass the unstirred water layer and be exposed to the plasma membrane surface. **4**. Exit of the fatty acid from CD36 to the outer leaflet of the phospholipid bilayer (membrane adsorption step). **5**. Transmembrane translocation ('flip-flop') of single fatty acids (membrane translocation step). **6**. Desorption of fatty acids from the inner leaflet of the phospholipid bilayer and binding to the interior of $FABP_c$ that is anchored by binding to the intracellular part of CD36 (membrane desorption step). **7**. Diffusion into the soluble cytoplasm of the fatty acid–$FABP_c$ complex towards sites of intracellular fatty acid metabolism. Note that proteins and membranes, and their putative mutual interactions are not drawn to scale, while the putative interactions of CD36 with $FABP_{pm}$ and possibly with other proteins are not shown. Reproduced with permission from (94).

Genetic and pharmacological studies have indicated a predominant role of CD36 in determining and regulating cellular fatty acid uptake. There is ample evidence that CD36 is the foremost membrane protein facilitating fatty acid transport into adipocytes, enterocytes,

endothelial cells, cardiac myocytes, and skeletal myocytes (102, 112). For instance, in patients with various single nucleotide polymorphisms in the CD36 gene (Chapter 2.2), *in vivo* myocardial fatty acid uptake was virtually absent (36) or markedly reduced (44). In addition, mice with a targeted deletion of CD36 showed markedly reduced (–50% to –80%) fatty acid uptake both by contracting cardiomyocytes *in vitro* (113), by the heart *in vivo* (114, 115), and by *in vivo* adipose tissue (114, 115). For contracting isolated cardiomyocytes it has been calculated that CD36 contributes about 70% to the rate of fatty acid uptake (116). One study reported that merely the presence of CD36 in the sarcolemma is not sufficient and that CD36 requires some form of activation such as an interaction with one or more protein partners (117).

In tissues with a non-fenestrated endothelium such as cardiac muscle, skeletal muscle, and adipose tissue, the capillary endothelium represents a barrier for the transport of fatty acids to parenchymal cells. In cardiac muscle it has been documented that the vast majority of fatty acids, either enzymatically liberated from lipoproteins or released from albumin, traverse the endothelial cells through their cytoplasm, because diffusion of albumin–fatty acid complexes around endothelial cells is too low to explain observed myocardial uptake rates. The trans-endothelial step is facilitated by the abundant expression of both CD36 and (endothelial type) $FABP_c$ in these cells (118, 119). CD36 deletion specifically in endothelial cells markedly reduces the rate of myocardial fatty acid uptake (118). However, given the abundant presence of CD36 in the healthy endothelium (120), trans-endothelial transit of fatty acids most likely is not rate-limiting for delivery of fatty acids to cardiac myocytes (see Chapter 4).

3.3 CD36 and onwards lipid metabolism

Transmembrane fatty acid transport alone will result in equilibration of fatty acids at both sides of the cellular membrane, and culminate in net uptake only when the incoming fatty acids are metabolized. Following uptake, the first metabolic step is the esterification of fatty acids with co-enzyme A to yield fatty acyl-CoA, which is predominantly mediated by specific long chain acyl-CoA synthetases (ACSL) (and to a lesser extent by the FATPs) (121). Acyl-CoA esters cannot leave the cell so this serves to trap the substrate within the cell to funnel it into the downstream metabolic pathways, in particular esterification into triacylglycerol (TAG) for

temporary storage, and subsequent β-oxidation in mitochondria to yield metabolic energy (ATP) (122).

Cellular fatty acid utilization is controlled at three major levels: (*i*) transmembrane uptake of fatty acids by CD36 and other proteins (see above), (*ii*) the activation of fatty acids into acyl-CoA by ACSL, and (*iii*) the delivery of acyl moieties into mitochondria for β-oxidation. The latter is dependent on the activity of carnitine palmitoyltransferase-1 (CPT-1) present at the outer mitochondrial membrane (123). From a cell perspective it would make logical sense that the plasma membrane is the preferred site of control for overall fatty acid uptake and utilization, so as to prevent excess intracellular presence of fatty acids that would result in lipotoxicity. Indeed, the evidence points towards the plasma membrane translocation of fatty acids being the rate governing step (88, 124). Specifically, partial inhibition of carnitine palmitoyltransferase-1 (CPT-1) activity in rats *in vivo* through administration of etomoxir resulted in a 44% reduced cardiac CPT-1 activity but failed to alter the rates of fatty acid uptake (125). While ACSL is required for trapping of incoming fatty acids, its cellular content or activity does not appear limiting to sustain intracellular fatty acid metabolism, as only excess ACSL expression has been linked to enhanced fatty acid uptake and lipotoxicity (121). Thus, trans-sarcolemmal transport might be the primary site for regulation of fatty acid flux while CPT-1 acts as a regulatory site for mitochondrial β-oxidation (122, 126). As will be discussed below (Chapter 4), CD36-facilitated fatty acid uptake is regulated primarily by modulating the presence of CD36 in the plasma membrane. Finally, it should be mentioned that because of its abundant presence in the soluble cytoplasm, $FABP_c$ serves an important yet merely permissive action in the overall fatty acid uptake process (127).

Studies in both rat and human muscle have shown that CD36 is also present at the mitochondrion (128, 129). Mitochondrial CD36 putatively contributes, in combination with ACSL and CPT-1, to the regulation of mitochondrial fatty acid oxidation, for instance by facilitating the unloading of fatty acids from $FABP_c$ for subsequent activation by ACSL at the mitochondrial membrane and entry via CPT-1. The finding that in humans aerobic exercise-induced differences in mitochondrial fatty acid oxidation correlate with both the muscular contents of mitochondrial CPT-1 and CD36 support this concept (130, 131). Other investigators confirmed the presence of CD36 in mouse heart and skeletal muscle

mitochondria but could not observe differences in mitochondrial uptake of fatty acid moieties or export of fatty acid anions between wild-type and CD36 null animals suggesting that CD36 does not have an essential role in mitochondria (132). Perhaps the function of CD36 in mitochondria is limited to specific conditions.

In summary, the cellular uptake of long-chain fatty acids appears to be governed to a large extent by the presence of CD36 in the cell membrane. The corollary is that adjusting the amount of CD36 present in the membrane will regulate the rate of cellular fatty acid uptake.

Chapter 4. CD36 translocation to and from the plasma membrane

Early studies focused on the total amount of CD36 within the cell, and related changes in total CD36 protein to changes in fatty acid uptake rate. However, later studies soon demonstrated that the relationship between CD36 content and uptake was more complicated, due to the location of CD36 in multiple cellular compartments. CD36 is present not only at the plasma membrane, where it is involved in transporting fatty acids into the cell, but also within intracellular compartments where it is inactive (133). Translocation of CD36 from these intracellular compartments to the plasma membrane provides a mechanism to acutely upregulate fatty acid uptake over a short period of time (minutes). Given that CD36-mediated fatty acid uptake presents the rate-governing step in fatty acid utilization (Chapter 3.3), regulation of this step would have the greatest impact on overall tissue fatty acid metabolism. Therefore, intensive research has focused on understanding the intracellular compartments in which CD36 resides and is ready to be recruited, the machinery involved in its subcellular translocation, and the signals that drive this movement of CD36 to and from the plasma membrane.

4.1 CD36 recycles between endosomes and the plasma membrane

A pivotal discovery in the regulation of subcellular CD36 trafficking was the detection of CD36 not only at the cell surface but also intracellularly. The first study reporting on this intracellular localization of CD36 used a subcellular fractionation method on rat hindlimb muscle, thereby revealing two subcellular fractions of CD36, one being enriched in plasmalemmal marker proteins, and one enriched in endosomal compartment marker proteins (133). In subsequent years, a set of intracellular proteins involved in vesicular trafficking and subcellular compartmentalization were found to co-localize with intracellular CD36. This list contains the Rab protein (family of peripheral membrane proteins) Rab 11 (134), the vesicle-SNARE proteins VAMP2 and VAMP3 (135), and the endosomal proton pump vacuolar-type H^+-ATPase (v-ATPase) (136, 137). Hence, this evidence pinpointed the intracellular storage pool of CD36 to the endosomes. Subsequently, microscopical studies of CD36 localization in heart and muscle using immunofluorescence staining revealed, besides a sarcolemmal staining, a strong punctuate staining pattern inside the cytoplasm, which was visible as a subsarcolemmal region of most of the vesicles with fewer vesicles more or less homogeneously present in the

remainder of the cytoplasm (120, 138). It has been estimated that the membrane and endosomal pools of CD36 are equal in size, providing a large inactive pool of CD36 within the cell that can be recruited rapidly in response to stimuli to acutely upregulate fatty acid uptake (134).

Endosomes are an intracellular pool of tubulovesicular structures that recycle between the lysosomes, the trans-Golgi network and the plasma membrane. A main characteristic of the endosomes is that their lumen is acidic with a pH below 6.0 (139, 140), this pH being maintained by v-ATPases. The endosomes have been morphologically and functionally divided into sub-compartments, such as early (or sorting) endosomes, recycling endosomes, late endosomes and endolysosomes. Integrating this sub-classification of the endosomes with the process of CD36 recycling, may lead to the suggestion that CD36 becomes internalized via the early endosomes, then is redistributed to the recycling endosomes to enable its subsequent return to the cell surface.

4.2 Stimuli driving CD36 translocation

The process of moving vesicles from the endosomal compartment to fuse with the plasma membrane is named exocytosis. The reverse process of internalizing membrane proteins inside the cell to form vesicles is termed endocytosis. In heart and muscle it is predicted that CD36 constantly recycles between the endosomes and the sarcolemma, and a net change in the location of CD36 within this system is due to a relative imbalance between these exocytotic and endocytotic pathways. An increased translocation of CD36 to the sarcolemma indicates a net upregulation of exocytosis relative to endocytosis.

4.2.1 Contraction induces CD36 translocation

The first stimulus identified to induce CD36 movement to the membrane was muscle contraction. Short-term tetanic stimulation of the sciatic nerve in rats led to forced contractions of the hindlimb, which was accompanied by increased plasma membrane content and a decrease in the endosomal content of CD36 (133). This translocation occurs within minutes after the onset of the induced exercise, and is reversible upon termination of the exercise. This contraction-induced translocation of CD36 was also found in electrically-stimulated cardiomyocytes from rats (116, 141). The signaling mechanisms have been found

to include AMP-activated kinase (AMPK) as a major regulatory kinase, which is phosphorylated by the upstream kinase liver kinase B1 (LKB1) upon binding of exercise-produced AMP (**FIGURE 3**). Activated AMPK then phosphorylates members of the family of Rab-GTPase activating proteins (RabGAPs; most notably TBC1D1 and TBC1D4/AS160). The subsequent de-inhibition of small Rab-GTPases then allows the actual initiation of the CD36 trafficking process (further explained in section 4.3). The increase in CD36 translocation leads to increased fatty acid uptake, whereby the extra incoming fatty acids are channeled towards increased mitochondrial fatty acid oxidation (141). Thus, the contraction-induced CD36 translocation provides a mechanism to increase fatty acid uptake to support the increased demand for ATP required by the contracting muscle.

4.2.2 Insulin induces CD36 translocation

Hormonal stimulation by insulin has been shown to induce CD36 translocation to the plasma membrane in both heart and muscle (142, 143). This upregulation increases fatty acid uptake in the fed state, and is integrated with the subsequent intracellular changes in fatty acid metabolism. In contrast to contraction-induced CD36 translocation, insulin-induced translocation is associated with redirection of the intracellular fatty acids towards storage into triacylglycerol (TAG) stores, in line with the general anabolic effects of insulin (144). The signaling mechanism involved in insulin-induced CD36 translocation includes the canonical insulin signaling pathway, comprising activation of the insulin receptor followed by subsequential activation of insulin receptor substrate (IRS), phosphatidylinositol-3 kinase, and Akt, leading to phosphorylation of AS160, which is then at the start of initiation of CD36 trafficking to the cell surface (reviewed in (145)) (**FIGURE 3**). Phosphatidylinositol-3 kinase (PI3K) was the first kinase identified in CD36 translocation, based on inhibition of this process by the inhibitors LY294002 and wortmannin (142, 143). Likely, this involvement includes class-I PI3Ks, which are heterodimers consisting of a 110 kDa catalytic subunit and an 85 kDa regulatory subunit producing mainly the lipid species phosphatidylinositol-(3,4,5)-trisphosphate (PIP3), which is essential for activation of Akt. Studies with skeletal muscle from Akt2 knockout mouse indicated that this is the main Akt isoform involved in CD36 translocation (146). With respect to the involved isoform of IRS, no research has been undertaken yet. Atypical PKCs are additionally involved in canonical insulin signaling as they support activation of Akt (145). Indeed, the atypical PKC members λ and ζ are necessary for

CD36 translocation (147), but independently of insulin stimulation. Hence, their basal activity appears sufficient, and likely their stimulation of actin remodeling is important herein (see section 4.3). Taken together, although details on the isoforms of members of the canonical insulin signaling pathway involved in CD36 translocation still need to be filled in, it is evident that insulin-induced CD36 translocation closely resembles the intensively investigated pathway of insulin-stimulated glucose transporter GLUT4 translocation (102, 145).

When both insulin and electrostimulation are applied simultaneously, there is an additive effect on CD36 translocation (141, 148). This provides kinetic evidence that there are (at least) two different sub-compartments within the population of CD36-containing endosomes: one pool specifically responsive to insulin stimulation and the other to contraction-stimulation. Other hormones have also been shown to be implicated in CD36 translocation, as listed in **TABLE 1** (*see page 66 of manuscript*). In most instances, their effects may converge at one of the pathways described above, such as the AMPK pathway.

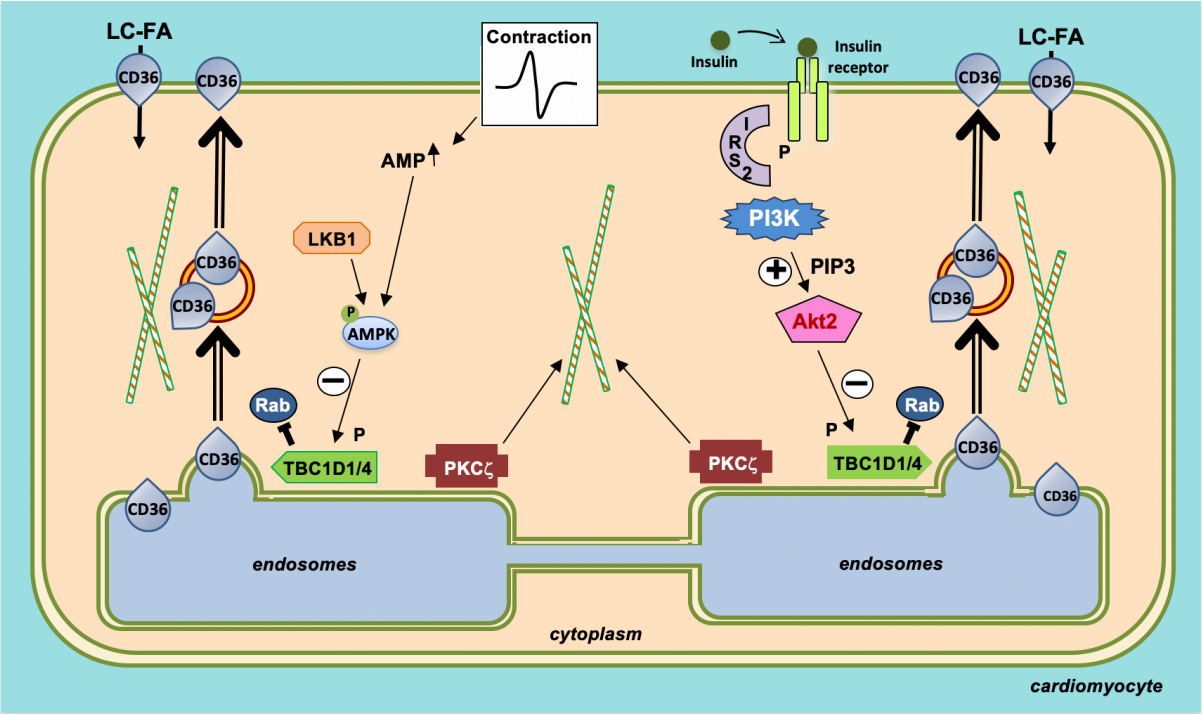


FIGURE 3. Signaling pathways involved in CD36 translocation. The (recycling) endosomes are the intracellular storage compartment for CD36, depicted as a tubulovesicular network consisting of specific vesicular compartments with different functions. It is speculated that one sub-compartment contains the contraction-responsive intracellular pool of CD36, while another sub-compartment harbors the insulin-responsive CD36 pool. The first event in contraction-induced CD36 translocation is the activation of AMPK by rising AMP levels upon increased workload. This induces a conformational change in this kinase making it accessible to phosphorylation by LKB1 as sole upstream kinase (149). Subsequently, AMPK places an inhibitory phosphorylation on TBC1D1 and/or TBC1D4, enabling the de-inhibition of specific Rab proteins to stimulate trafficking via GDP-GTP cycling (explained in section 4.3). Insulin is the major hormonal stimulus of CD36 translocation, and activates the canonical insulin signaling pathway consisting of IRS(2), a class-I phosphatidylinositol-3 kinase, producing phosphatidylinositol-(3,4,5)-trisphosphate (PIP3) leading to activation of Akt2 and phosphorylation of the same RabGAPs involved in contraction-induced CD36 translocation. Another point of convergence of contraction signaling and insulin signaling towards CD36 translocation is the atypical PKC isoform ζ (or λ), which basal activity is sufficient to allow CD36 translocation (150). There is evidence that atypical PKCs stimulate remodeling of actin filaments involved in CD36 translocation in L6 muscle cells, but also other filamentous networks may be activated by PKC-ζ/λ to enable CD36 translocation (see subsection 4.3).

4.2.3 CD36 and GLUT4 share similarities in the stimuli that induce their translocation

Glucose transporter type 4 (GLUT4) is a member of the family of GLUT proteins which enable the facilitated diffusion of glucose along its concentration gradient into various tissues. GLUT4 is predominantly expressed in cardiac and skeletal myocytes and in adipocytes and regulates glucose uptake by recycling between intracellular membrane compartments (GLUT4 storage vesicles) and the plasma membrane (151). CD36 and GLUT4 are united in their joint regulation by contraction and insulin, with both stimuli inducing CD36 and GLUT4 translocation resulting in enhanced fatty acid and glucose uptake rates, respectively (152). Hence, under these stimulatory conditions CD36 translocation occurs simultaneously with GLUT4 translocation. Circumstantial evidence suggests that CD36 and GLUT4 do not travel together on the same endosomally derived transport vesicles (153). Therefore, several studies have been undertaken to unravel the signaling pathways that are unique to CD36 and those that are unique to GLUT4. A key observation was that some stimuli, such as leptin and dipyridamole, induce CD36 translocation without affecting GLUT4 (**TABLE 1**), which confirms that both processes are separately regulated involving distinct sets of accessory proteins (153). Finally, selected stimuli were found also to increase the sarcolemmal contents of $FABP_{pm}$ and/or FATP1/FATP4 (**TABLE 1**), which suggests a cooperative involvement of CD36 and these fatty acid transporters in augmenting cellular fatty acid uptake (148).

4.3 Protein machinery involved in CD36 translocation

The process of translocation (i.e., exocytosis) of CD36 from the endosomal compartment to the plasma membrane follows the principles of vesicular trafficking, as explained in **FIGURE 4.**, thereby undergoing three major steps. *(i)* Fission: CD36 is released from the endosomes via excision of membranes thereby forming transport vesicles. *(ii)* Transport: CD36-containing transport vesicles move through the cytosol via cytoskeletal filaments. *(iii)* Fusion: these transport vesicles fuse with the correct region of the membrane to enable CD36' facilitatory function in fatty acid uptake. To allow for these sequential steps to occur a number of proteins are involved specifically in packaging, transport and fusion. In the retrograde transport of CD36 from the plasma membrane to the endosome (endocytosis) these same steps occur but in the opposite direction. The key proteins in the regulation of the translocation process for

CD36 are v-ATPase, coat proteins, specific Rab proteins, cytoskeletal elements, and SNAREs (e.g., VAMPs) (153). The current knowledge on their involvement is reviewed in **BOX 1**.

Although the trafficking machinery of CD36 has been unraveled to a large extent, still much work is needed for a full identification. Proteins uniquely dedicated to CD36 trafficking are of special interest because targeting of these proteins would enable us to specifically modulate CD36 localization at the cell surface without disturbing the subcellular recycling of other proteins such GLUT4. Such modulation of sarcolemmal CD36 localization would make it possible to specifically and precisely modulate the rate of fatty acid uptake into the heart without affecting glucose uptake, thereby offering treatment possibilities to cure cardiac metabolic diseases (see Chapter 6). Several interesting candidate trafficking proteins have already been found such as Rip11a and VAMP4 (see **BOX 1**). Unfortunately, at present trafficking proteins are not easily targetable by pharmacological approaches. For instance, SNARE proteins are cleaved by Botulinium and Tetanus toxins, but when using these toxins there is no apparent specificity towards distinct VAMP isoforms (such as VAMP4 to specifically inhibit CD36 translocation). Yet, using specific serotypes of these toxins, a greater degree of specificity of targeting of VAMP members can be achieved (154, 155), however specific targeting of each individual VAMP is not yet possible. Another approach would be to up or down regulate the expression of trafficking proteins by specific drugs, but, unfortunately virtually nothing is known about transcriptional regulation of VAMPs and of other trafficking protein families (156). Finally, CD36 translocation by other stimuli such as thyroid hormone may require other specific trafficking proteins.

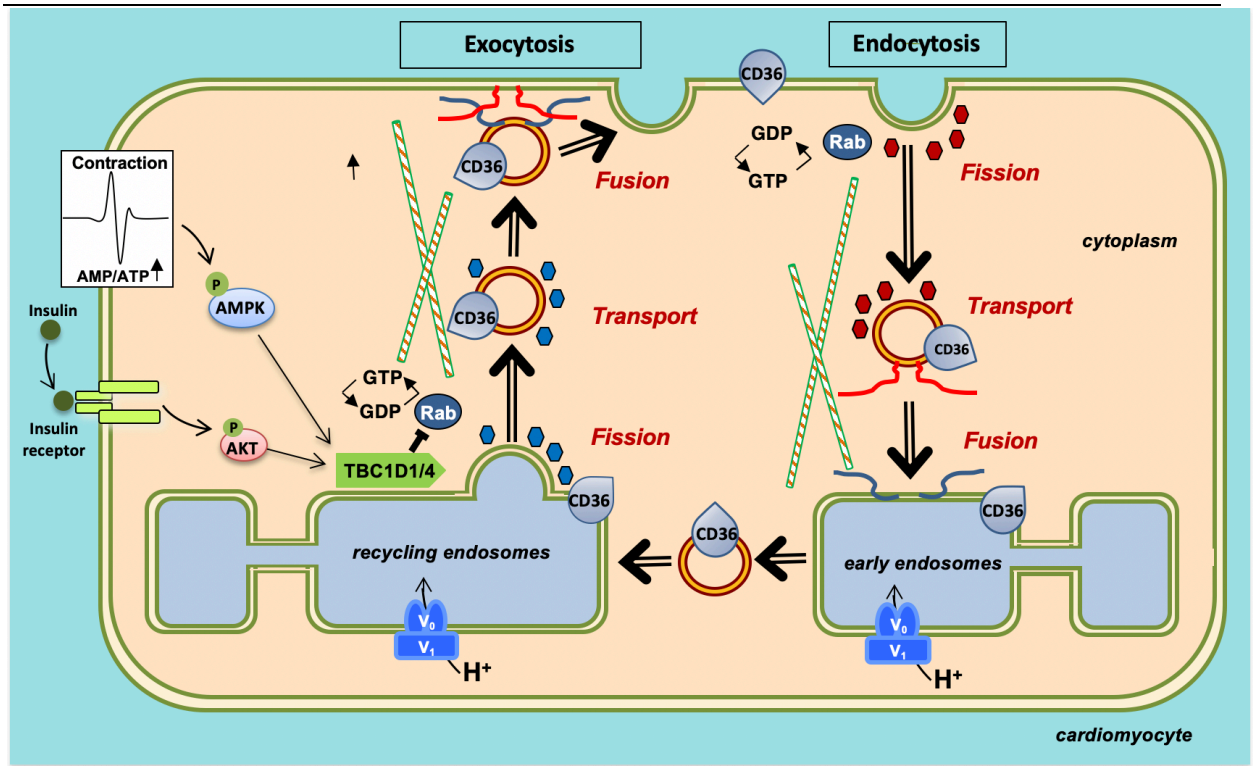


FIGURE 4. Schematic presentation of recycling of vesicles between endosomes and the plasma membrane and the general processes involved. The endosomes are depicted as a tubulovesicular network, similarly to FIGURE 3, but are now further subdivided into two separate compartments, being the recycling endosomes (*left*) and early endosomes (*right*). The recycling endosomes will provide a multitude of distinct trafficking pathways for individual cargos (such as CD36). The early endosomes are involved in internalization of cargo proteins and delivery to the recycling endosomes for further sorting. Other endosomal compartments are not included in this scheme, because they are likely not involved in CD36 translocation. Endosomes (both early and recycling) are acidic compartments with a pH < 6.0 (with some sub-compartments more acidic than others). This pH gradient with the cytoplasm is maintained mainly by v-ATPase, which operates as a proton pump. For budding of vesicles from the recycling endosomes, coat proteins (depicted with hexagonal shapes) are involved in packaging the newly formed vesicles, and the energy is provided by the GDP – GTP cycling of Rab proteins. These Rab proteins are kept in an inactivated state by RabGTPases (from the TBC1 domain family) to prevent random trafficking processes. Phosphorylation of the TBC1Ds via signaling pathways (FIGURE 3) results in their inhibition and subsequent de-inhibition of the Rabs, which is the start of the exocytotic pathway. The transport vesicles move towards the plasma membrane via a highway provided by cytoskeletal filaments. For fusion of these vesicles with the plasma membrane, the vesicles possess (spider leg-shaped) v-SNARE proteins which form a specific bond with cognate (spider leg-shaped) t-SNARE proteins (designated with a different color). For the endocytotic process the same trafficking steps are involved, and these trafficking steps are mediated by different members of the same protein families (depicted with the same shapes but different colors). This will ensure that exocytosis and endocytosis are differently regulated. A final remark, no RabGAP is indicated in the endosomal pathway. The latter is based on the assumption that this pathway will operate constitutively without regulation, in contrast to the exocytotic pathway which is heavily regulated.

BOX 1. Proteins involved in intracellular CD36 recycling

The trafficking machinery implicated in exocytosis and endocytosis of CD36 involves an estimated number of >50-60 proteins, analogous to GLUT4 translocation (157), several of which are generally involved in trafficking processes, and others more specifically dedicated to CD36 translocation, and which have been identified in the last two decades. Current knowledge on these proteins is reviewed below in the sequence at which they occur during CD36 translocation from the endosome, starting with the role of v-ATPase and followed by the role of coat proteins, Rab proteins, cytoskeletal filament proteins and SNARE proteins (**FIGURE 5**).

– v-ATPase. Endosomes are acidic compartments. The luminal acidification appears necessary for endosomes to function as storage compartment for CD36. Acidification of the endosomal lumen is mainly carried out by v-ATPase, a large protein complex consisting of two sub-complexes: the membrane-bound V_0 sub-complex comprising 6 subunits and containing the proton channel, and the cytoplasm-facing V_1 sub-complex comprising 8 subunits and containing the ATP-driven pump. Loss of luminal acidification either achieved by monensin (proton ionophore) or via bafilomycin-A (v-ATPase inhibitor) results in expulsion of CD36 from the endosomes and translocation to the cell surface (158). It is speculated that the low endosomal pH favours stabilization of the endosomal phospholipid bilayer, thereby impairing membrane curving events, such as budding of (CD36-containing) transport vesicles (153, 159). Under physiological conditions, the endosomal pH can be regulated via the degree of assembly of the V_0 and V_1 sub-complexes into the v-ATPase holo-complex (160). Only the assembled state of v-ATPase is compatible with optimal proton pump activity, while release of the V_1 complex into the cytoplasm leads to inhibition of proton pumping activity. A number of substrates and metabolites have been found to regulate the v-ATPase assembly state, and the list is still growing (161). The most relevant ones in the context of this review are glucose and fatty acids, which have opposing effects: increased glucose supply favors v-ATPase assembly and CD36 retention, whereas elevated fatty acid levels induce disassembly and CD36 release from endosomes (161). However, the effects of both substrates have not been studied in detail in short-term experiments (i.e., minutes) but rather upon overnight addition or withdrawal (discussed in greater detail in Chapter 5) (136, 162).

– Coat proteins. The formation of transport vesicles is dependent on coat proteins, which self-assemble onto the endosomes to assist in curving of the membranes and concentrating the cargo. There are various types of coat proteins, which are involved in different vesicle transport processes. Clathrin, coat protein-I (COP-I), and caveolins are generally involved in endocytotic processes, and COP-II (also named β-COP) assists in vesicle budding in the opposite direction (163). The fungal metabolite Brefeldin-A causes the release of β-COP from intracellular membranes such as the trans-Golgi network (TGN) and thereby blocks vesicular trafficking (164). Both glucose and fatty acid uptake as stimulated by insulin or contraction, are inhibited by Brefeldin-A treatment of cardiomyocytes, showing that GLUT4 and CD36 translocation by these stimuli is mediated by β-COP (158). While for GLUT4 translocation the involvement of vesicular trafficking has been well-established (151), the inhibitory action of Brefeldin-A on CD36 translocation provided for the first-time direct evidence that CD36 translocation was also a vesicle-mediated transport process. Yet, the inhibition of CD36 translocation by Brefeldin-A is partial (158), suggesting that other coat proteins also might contribute to CD36 translocation.

In line with the above, β-COP is not the only coat protein that has been associated with CD36 dynamics. Members of the family of caveolins also appear involved in CD36-mediated fatty acid uptake (107, 165). Caveolins are the defining proteins of caveolae, which are ~60 nm flask-shaped invaginations of the plasma membrane enriched in specific lipids (cholesterol, sphingolipids) and proteins involved in signaling and ion transport. In adipocytes, cell surface CD36 is predominantly present within caveolae, and disruption of caveolae leads to inhibition of CD36-mediated fatty acid uptake (166). Conversely, increased abundance of caveolae due to genetic ablation of EDH2 (a dynamin-related protein involved in internalization of caveolae) results in increased CD36-mediated fatty acid uptake (167). On the other hand, knock-out of muscle-specific isoform caveolin-3 in mice did not alter cardiac fatty acid uptake (168). Hence, further studies are needed to unravel the role of caveolins as a hub for CD36 translocation and CD36-mediated fatty acid uptake (166).

– Rab proteins and RabGAPs. Rab proteins are a member-rich family (>70 members) of small G proteins, which facilitate key steps in vesicular trafficking including vesicle formation, vesicle movement and membrane fusion by virtue of their GTPase activity. These Rabs provide specificity to vesicular trafficking, i.e., they are dedicated to trafficking routes between specific organelles, and also impose unidirectionality. To prevent unimpeded trafficking, Rab proteins are kept in an inactive state, which is mediated by Rab-specific GTPase-activating proteins (RabGAPs).

- **RabGAPs** - The RabGAP family of peripheral membrane proteins containing a Rab-inactivating TBC domain is also rich in members (>50), and confers further specificity to trafficking (169). Two RabGAPs have been associated with stimulus-induced GLUT4 translocation, being TBC1D1 and TBC1D4/AS160 (170, 171). Both proteins share a high degree of homology, especially within the TBC domain (>76%). Additionally, both proteins are directly phosphorylated on multiple Ser/Thr by various protein kinases including Akt2 and AMPK. This phosphorylation results in inhibition of their GTPase activity, which will lead to re-activation of Rabs and induction of GLUT4 translocation. Given that Akt2 as well as AMPK directly induce inhibitory phosphorylation on TBC1D1 and TBC1D4/AS160, it was concluded that both RabGAPs serve as a point of convergence of insulin and contraction signaling pathways to stimulate GLUT4 translocation in muscle cells (172, 173) . The question whether these RabGAPs fulfill unique or overlapping functions in GLUT4 translocation has not yet been fully answered. Both RabGAPs target a similar subset of Rab proteins in *in vitro* assays (174), while they fulfil analogous roles in GLUT4 translocation as demonstrated in studies in muscle cell lines using knockdown and overexpression of wild-type and mutant constructs (170). Among the subtle differences between both RabGAPs is that TBC1D4 might be more closely linked to insulin sensitivity (175). Another difference is the differential tissue distribution, with TBC1D4/AS160 being the major isoform in heart (with relative little TBC1D1 expression), whereas the relative expression of both RabGAPs differs in the various muscle fiber types (175).

With respect to CD36 translocation, studies in cardiomyocyte cell lines have shown that silencing of TBC1D4/AS160 results in CD36 translocation to the cell surface under non-stimulated conditions with no further increase in CD36 translocation upon stimulation of insulin or AMPK-mediated pathways (176). Similarly, overexpression of a phosphorylation-

deficient mutant of TBC1D4/AS160 abrogated the stimulation of CD36 translocation (176). Hence, TBC1D4/AS160 acts as a gatekeeper of CD36 retention within the endosomes, and when this RabGAP is phosphorylated by insulin or AMPK-mediated signaling, it can no longer fulfil this role. Skeletal muscle cells also have been used to silence TBC1D4/AS160 for studies of CD36 dynamics. In these studies, TBC1D4/AS160 knockdown resulted in increased fatty acid uptake concomitant with increased CD36 expression (177). Additionally, in skeletal muscle cells it was found that AMPK-mediated CD36 translocation was due to TBC1D1 involvement, and not to TBC1D4/AS160 (178). These studies indicate the importance of RabGAPs in CD36 translocation. The differences in involvement of the RabGAP isoforms upon stimulation of CD36 translocation could be due to differences in applied stimuli and/or cell models (178).

- **Rabs** - Studies to identify Rab proteins involved in CD36 translocation from endosomes to the cell surface have been scarce. Immuno-isolation of GLUT4-containing vesicles yielded the identification of numerous (~20) Rab proteins associated with these vesicles, of which at least Rab2a, 8a, 10 and 14 are potential substrates of TBC1D1 and TBC1D4/AS160 (179). Based on the similarities between stimulus-induced GLUT4 and CD36 translocation, it was argued that some of these Rabs are also involved in CD36 translocation. Silencing of Rab8a, but not of Rab10 and Rab14, in cardiomyocytes abrogated insulin-stimulated as well as AMPK-mediated CD36 translocation (176). More evidence that Rab8a is crucial for CD36 translocation was provided in that same study in which overexpression of this Rab isoform rescued stimulus-induced CD36 translocation in cardiomyocytes expressing the phosphorylation-deficient TBC1D4/AS160 mutant (176). Generally, Rab8 is known to be involved in multiple exocytotic pathways, such as GLUT4 translocation, and closely related membrane trafficking processes such as ciliogenesis (180).

Another Rab protein identified to be involved in CD36 translocation is Rab11a. It was found to be present on CD36-enriched subcellular (endosomal) vesicles (134). As already mentioned, Rab11 is the defining marker of the recycling endosomes, and is important in a large number of recycling processes, mostly related to exocytosis (181). Moreover, Rab11 acts as an upstream activator of Rab8a, as part of a conserved pathway also involving the motor protein myosin-Vb and an octameric complex called exocyst (182-184). Myosin-Vb moves endosomal

vesicles alongside actin filaments, and exocyst is involved in tethering of endosomal vesicles to the plasma membrane prior to membrane fusion. The cooperation between Rab11a and Rab8a also stimulates various other trafficking processes, such as ciliagenesis and tunneling nanotube formation (182, 185). Surprisingly, Rab11a knockdown leads to an increase in CD36 cell surface content in cardiac cells, suggesting that this Rab is involved in CD36 endocytosis rather than exocytosis (186). In line with this, stimulus-induced CD36 translocation was not affected by Rab11a silencing (186). Genetic Rab11a ablation also increased CD36 plasmalemmal presence in macrophages (187). Hence, it is currently unclear how Rab11a and Rab8a cooperate to regulate trafficking of CD36, as this seems to differ from other trafficking pathways. In conclusion, Rab8a and Rab11a carry out independent functions in CD36 trafficking, with Rab11 mediating stimulus-induced CD36 translocation, and Rab11a mediating CD36 endocytosis.

Rab11 interacts not only with Rab8a, but also with a number of other downstream effector proteins that can provide further specificity to Rab11 involvement in trafficking (188). Two other Rab11-interacting proteins are Rip11 and FIP2, and their silencing increases CD36 translocation, although not to the same extent as Rab11a (186). Yet their combined actions quantitatively matches the effect of Rab11, suggesting that both Rab11 effector proteins cooperate with Rab11 to mediate CD36 endocytosis. The involvement of Rab11 in the endocytotic process is not restricted to CD36, as also GLUT4 endocytosis is dependent on this Rab isoform (186). However, Rab11-mediated GLUT4 endocytosis can be fully accounted for by FIP2 and does not require Rip11a (186), making Rip11 a discriminator between CD36 and GLUT4 trafficking.

– Cytoskeletal elements. The cytoskeleton provides the intracellular highway for vesicular trafficking to move the vesicles from one compartment to the other to deliver their cargo. The movement of the vesicles alongside the cytoskeletal filaments is mediated by motor proteins, pushing or pulling the vesicles in a unidirectional manner driven by ATP hydrolysis. Among the filamentous systems are the actin filaments involved in a large number of trafficking processes including GLUT4 translocation/exocytosis. In insulin-stimulated GLUT4 translocation, GTP-loaded Rab8a binds to myosin-V motor proteins to initiate the trafficking of GLUT4 vesicles in muscle cells (189). The involvement of the actin filaments in the trafficking of CD36 vesicles is

still an open question. On the one hand, the actin polymerization inhibitor Latrunculin-B does not alter CD36 translocation in cardiomyocytes (158), but another actin inhibitor, i.e., cytochalasin-D, inhibited in L6 muscle cells CD36 co-localization with actin as well as CD36 translocation (178). In the last setting, actin polymerization and subsequent CD36 translocation was dependent on Rac1, a member of the Rho family of GTPases regulating various aspects of cytoskeletal plasticity (178). The discrepancy between both observations is not readily explained but probably the difference in applied stimuli (insulin/contraction) (158) versus palmitate (178) and in applied cell models. As a result, the role of actin in CD36 translocation needs further investigation.

The other two known filamentous networks comprise the microtubule and the intermediate filaments. The microtubule are likely not involved in CD36 (nor GLUT4) translocation, given that the microtubule inhibitor colchicine has no effect hereon (158). The involvement of the intermediate filaments such as desmin (highly expressed in muscle and heart) in CD36 translocation has not yet been investigated but these filaments are increasingly being recognized to be involved in vesicular trafficking (190).

– VAMP proteins. The final step in the vesicular trafficking process is the fusion of the transport vesicles with the target membrane, which is postulated to occur via the rules of the SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) hypothesis, developed in the early nineties (191). This hypothesis entails that there is specific pairing of a SNARE protein on the vesicles (v-SNARE) with a SNARE protein on the target membrane (t-SNARE). The specificity of the v-SNARE – t-SNARE complex is considered to contribute to the specificity of vesicular trafficking ensuring that the transport vesicles arrive at the appropriate address. SNARE proteins are a family of 20–30 kDa membrane-bound proteins with a 60–70 amino acid-long segment in their cytosolic domain called a SNARE motif. This motif contains heptad repeats that have the ability to form coiled-coil structures. The latter structures are formed in the assembly of SNARE proteins into a trans-SNARE complex which is needed to overcome the repulsive electrostatic forces between adjacent membranes so as to allow membrane fusion. Recently, the SNARE hypothesis has been slightly adjusted based on the observations that in cell-free studies with reconstituted SNAREs the bonding was far less specific, and that multiple v-SNARE – t-SNARE combinations gave rise to thermally stable

complexes. It is now accepted that more than one t-SNARE participates into the trans-SNARE complex and that accessory proteins are necessary to provide the required specificity of membrane fusion (192).

Investigations of the involvement of SNARE proteins in CD36 translocation have been restricted to v-SNAREs, which all belong to the family of VAMPs (vesicle-associated membrane proteins, numbered VAMP1 – VAMP8) (193). In a systematic study in which each of the VAMP members were silenced in cardiomyocytes, it appeared that three VAMPS are necessary in CD36 translocation, being VAMP2, VAMP3 and VAMP4 (194). Remarkably, it appears that for stimulus-induced CD36 translocation events two VAMP proteins are required. Insulin-stimulated CD36 translocation is dependent on the combined presence of VAMP2 and VAMP4, while AMPK-mediated CD36 translocation requires the combination of VAMP3 and VAMP4, indicating that VAMP4 is generally associated with CD36 translocation. VAMP2 appears specific for the insulin-stimulated translocation events, such as the well-described GLUT4 translocation, whereas VAMP3 specifically assists in AMPK-mediated translocation processes. Finally, VAMP2 appears also to fulfil a second function being involved in CD36 endocytosis (194). Importantly, the involvement of two VAMPs in stimulus-induced CD36 translocation may imply that CD36 exocytosis occurs in two steps that are separated by a hypothetical intermediate membrane compartment (153). This intermediate compartment likely contains v-ATPase and, therefore, is still part of the greater endosomal compartment. As a result, the first trafficking step would be entirely intra-endosomal. There is evidence that VAMP4 mediates the first trafficking step bringing CD36 to this intermediate compartment (153). Insulin stimulation leads to activation of VAMP2 which then brings CD36 from the intermediate compartment to the sarcolemma. In turn, activation of VAMP3 via AMPK signaling brings CD36 from the intermediate compartment to the sarcolemma. Hence, the subcellular localization of VAMP4 appears an indicator of CD36-specific intracellular compartments.

A systematic search for t-SNARE proteins involved in CD36 translocation awaits further studies and would be expected to provide additional information on the specificity of CD36 trafficking. This will be a more challenging undertaking given that the number of t-SNARE members exceeds the number of v-SNAREs by about 3-fold, and also being divided over different protein

families (including syntaxins and SNAPs). The only information available is that the syntaxin4-associated protein Munc18c is involved in GLUT4 translocation but not in CD36 translocation (195).

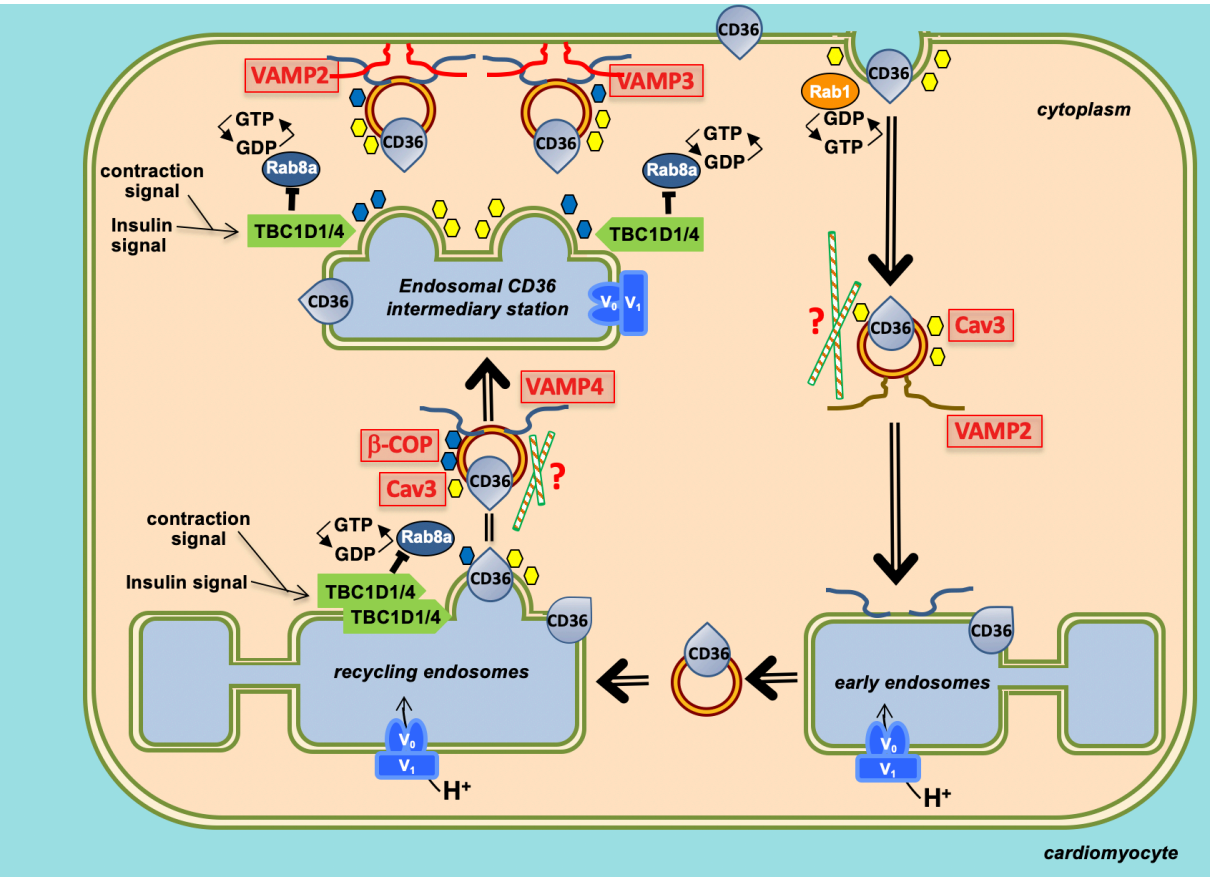


FIGURE 5. Application of the scheme of vesicular trafficking (see **FIGURE 4**) to CD36 translocation. CD36 is intracellularly stored in the recycling endosomes containing v-ATPase (V_0/V_1). Upon activation of contraction/insulin-responsive signaling routes (see **FIGURE 3**), the RapGAP proteins TBC1D1 and/or TBC1D4 are phosphorylated, leading to de-inhibition of Rab8a. Subsequently, CD36 budding is initiated via GTP/GDP cycling and involving at least the coat protein β-COP (brown hexagram). Given that β-COP cannot fully account for CD36 translocation (**BOX 1**), there is another coat protein involved, which could be caveolin-3 (yellow hexagram). Then the vesicle-mediated travel of CD36 to the cell surface starts, which is hypothesized to be a two-step process. The first travel part may bring CD36 to an intermediary compartment. This first track is mediated by VAMP4 (blue spider legs, and depicted to be on its way to fuse with an unknown t-SNARE; yellow spider legs). This intermediary CD36 station is endosomal, and contains v-ATPase. From this station, CD36 can be further recruited by either insulin signaling (mediated by VAMP2; purple spider legs, and a second unknown t-SNARE; red legs), or by contraction signaling (mediated by VAMP3; blue legs and a third unknown t-SNARE; red legs). At the sarcolemma, the fusion step is depicted by a completion of the SNARE complex formation (spider legs intertwined). Also this second travel event relies on Rab8a de-inhibition. This exocytotic part of the

CD36 recycling process is better characterized than the endocytotic part, which latter is at least known to be dependent on Rab11. Since both increased and decreased abundance of caveolae have been associated with increased fatty acid uptake (**BOX 1**), caveolin-3 might, next to its postulated involvement in CD36 exocytosis, also be involved in CD36 endocytosis. Besides caveolin-3, VAMP2 may be re-used for CD36 endocytosis to fuse with a yet another t-SNARE (green legs; located in the early endosomes). With respect to the involvement of cytoskeletal elements in CD36 trafficking, there is too much uncertainty whether such role could be fulfilled by actin filaments (**BOX 1**), which is why a question mark has been used here. The total CD36 trafficking machinery may encompass >60 proteins.

Chapter 5. CD36 as a receptor for lipid signaling

By virtue of its transport function for fatty acids, CD36 is not only a main regulator of fatty acid metabolism, but it also plays a key role in lipid signaling. As already mentioned, increased CD36-mediated fatty acid uptake leads to an increased activation by fatty acids of intracellular receptors such as the PPAR transcription factors (section 2.3), which will push cells to switch to a lipid metabolic gene program (53) . Besides this 'passive role' in lipid signaling, CD36 participates in signaling cascades directly by serving as a receptor for fatty acids upon which it is activated to form complexes with a number of membrane proteins/receptors (196). In this way, CD36 may be involved in multiple lipid signaling pathways. Below are the primary CD36 partners which may each induce specific signaling routes.

5.1 SRC kinases

Evidence for a role of CD36 in cellular signaling was obtained three decades ago when it was reported that in platelets, CD36, at that time referred to as GPIV, is associated with a number of Src kinases; a family of non-receptor tyrosine kinases, including Fyn and Lyn (197). Src kinases are broadly involved in key cellular processes such as growth, proliferation, migration, immune responses and invasion. Fatty acids binding to CD36 at the cell surface induce the phosphorylation of specific Src kinases and induction of signaling in various cell/tissue types including taste buds on the tongue (26), neurons in specialized brain regions (198) and enteroendocrine cells in the intestine (199). These signaling actions will give rise to the release of bioactive molecules in order to prepare the body to respond to ample lipid availability. Hence, CD36 is an important fatty acid receptor in whole body lipid sensing (199). Also in muscle cells, CD36 has been found associated with Src kinases, most notably Fyn, at least in a low lipid environment (200). Fyn, when bound to CD36, will phosphorylate LKB1, the main upstream kinase of AMPK in the heart. LKB1 phosphorylation will lead to its migration to the nucleus and reduced accessibility to AMPK. Lipid binding to CD36 will lead to the dissociation of Fyn from CD36, reduction of LKB1 phosphorylation, and subsequent AMPK activation by LKB1. This CD36 signaling action serves to channel fatty acids to mitochondrial β-oxidation during high lipid availability (200).

5.2 Toll-like receptors

Toll-like receptors (TLRs) form a family of single-pass membrane spanning receptors involved in recognition of molecular structures shared by pathogens. As such they play a key role in the innate immune response. TLRs can also recognize endogenous molecules, among which (saturated) long-chain fatty acids, which then trigger a 'sterile' response. It becomes increasingly recognized that TLR function can be enhanced via association with coreceptors that increase ligand recognition and subsequent signaling responses (201). In this context, binding of fatty acids or of other ligands such as oxLDL to CD36 induces the formation of a CD36–TLR4–TLR6 complex followed by inflammatory signaling responses mediated by adaptor proteins such as MyD88 (202, 203). Downstream of these adaptors, the MAP kinase pathways is activated and subsequently the IKK–NF-κB axis leading to induction of inflammatory proteins such as TNFα. CD36 can also act as co-receptor for a TLR2–TLR6 heterodimer (204). Finally, TLRs are not only present in immune cells, but also in many cell types of the cardiovascular system including cardiomyocytes (205). This makes the TLR-mediated inflammation signaling an integral part of the pleiotropy of lipid-induced CD36-dependent signaling responses operating in the heart.

5.3 Other receptors

CD36 may also bind to other plasma membrane receptors in cardiomyocytes to induce additional signaling routes. In taste bud cells, CD36 has been found to associate with members of the G protein-coupled receptors (GPCRs) as part of gustatory perception of dietary lipids (198). Whether lipid signaling following CD36–GPCR complex formation also occurs in the heart is likely but has not yet been studied. Finally, in adipocytes CD36 has been shown to be in complex with annexin-2 and prohibitin-1, the latter functioning as a chaperone-like protein involved in mitochondrial respiration (206), but the signaling consequences are not yet fully understood.

In summary, in response to lipid exposure CD36 has the ability to associate with a number of other membrane proteins/receptors to induce a wide array of signaling responses for preparing cardiomyocytes and other cells to optimally use these substrates for metabolic and other purposes. Yet, these mechanisms can derail upon chronic lipid exposure as detailed in the next section.

Chapter 6. Role of CD36 in the pathogenesis of metabolic diseases

Several chronic diseases are characterized by perturbed fatty acid or lipid metabolism. For instance, obesity is associated with disturbances in lipid metabolism resulting in deleterious triacylglycerol accumulation in ectopic tissues including heart, skeletal muscle and liver. This lipid accumulation is subsequently linked with the development of insulin resistance, cardiac and skeletal muscle contractile dysfunction, and non-alcoholic steatohepatitis (207). Given the key regulatory role of CD36 in lipid metabolism, it logically follows that CD36 is involved in the development and propagation of metabolic diseases, and may be a suitable target for metabolic modulation therapy. This is illustrated by findings in mice that CD36 is necessary for the development of high fat diet-induced cardiac lipotoxicity and dysfunction, and that CD36 deficiency rescues such disease development (208). Furthermore, a large number of CD36 genetic variants have been implicated in organ dysfunction and metabolic disease development (section 2.2) (reviewed in (33)). Because CD36 can integrate cell signaling and metabolic pathways through its dual functions, its role in disease development extends to a broad range of abnormalities including foam cell formation (atherosclerosis), tumor growth, and cardiovascular diseases (Chapter 8) (5, 209, 210).

Recently, the molecular mechanism underlying these pathological developments have been disclosed in great detail, at least in the heart, and confirms a key role for CD36. Namely, any chronic deviation from the rate of CD36-mediated fatty acid uptake occurring in the healthy heart, towards either an increased or a decreased fatty acid utilization, is commonly associated with a reciprocal shift to the utilization of other substrates (such as glucose). In most instances, these chronic changes in substrate preference are associated with cardiac disease (see section 6.4). Changes in either the expression or subcellular translocation/recycling of CD36 may underlie these altered fatty acid uptake rates.

As a result, CD36 has emerged as a pivotal protein both for the homeostatic control of cellular fatty acid uptake and utilization (and indirectly of other fuels), and the development of pathological changes leading to organ dysfunction (211). Distinction should be made between short-term effects, usually caused by altered subcellular CD36 recycling, and long-term effects, which are accompanied by chronic metabolic remodeling.

6.1 Ischemia-reperfusion of the heart

Studying the cellular response to an acute pathological challenge allows us to understand the hearts' endogenous attempts to restore homeostasis, but also may give insight into the longer-term adaptation if that pathological challenge continues. Acute ischemia and reperfusion resembles the pathological processes associated with myocardial infarction, and has revealed a role for CD36 in the early metabolic remodeling when blood supply is restricted. Low-flow global ischemia of the isolated perfused heart induces a rapid remodeling of cardiac metabolism within minutes, characterized by a decrease in fatty acid oxidation due to decreased oxygen delivery (hypoxia) to the mitochondrial electron transport chain (212, 213). This is accompanied by an acute upregulation of glycolysis to generate anaerobic ATP (212, 213). However, this compensation is insufficient to maintain overall cellular energetics (214), so that cardiac function is rapidly impaired.

In response to acute ischemia, CD36 rapidly relocates away from the sarcolemma into the intracellular storage pool (215). The internalization of CD36 decreases the capacity for fatty acid uptake under these oxygen-restricted conditions. This may be an attempt by the heart to protect itself from becoming overloaded with lipid, given that these lipids can no longer be directed to mitochondrial oxidation. This internalization event is specific for CD36, and not observed for the other fatty acid transporters, $FABP_{pm}$ and FATP1, and is maintained for at least 30 minutes of reperfusion. At the same time that CD36 is moving away from the sarcolemma, GLUT4 is moving towards the sarcolemma, demonstrating divergent regulation of these two predominant substrate transporters during myocardial ischaemia (**FIGURE 6**) (215). However, an unanswered question remains as to the mechanism driving CD36 (net) internalization, as during ischemia the decreased energetics activate AMPK, which would be predicted to stimulate CD36 translocation towards the sarcolemma, rather than away. During ischemia the heart experiences hypoxia as well as a decreased delivery of substrates and decreased removal of waste products (including lactic acid that is generated to much higher levels during ischaemia). Studies to investigate the hypoxic component in isolation have shown that short-term acute hypoxia in cardiac myoblasts increases CD36 sarcolemmal content within 5 minutes (216). This would indicate that the ischemia-induced CD36 internalization is not driven by the hypoxic component of ischemia but more likely by other

factors, potentially the change in intracellular pH (lactate) or in endosomal v-ATPase activity could be regulating intracellular CD36 trafficking.

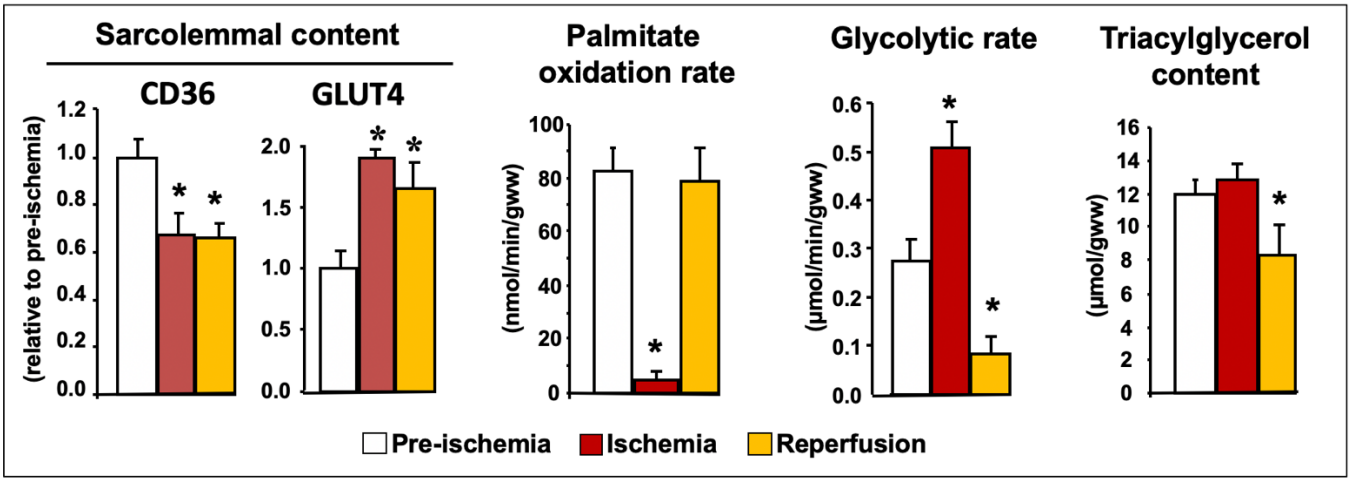


FIGURE 6. Divergent regulation of sarcolemmal CD36 and GLUT4 in response to acute (low-flow) ischemia in perfused rat heart. The relocations of CD36 and GLUT4 were associated with a shift of myocardial substrate metabolism from fatty acid oxidation to glycolysis while intramyocardial lipid accumulation was prevented. Data obtained from (215).

Genetic CD36 knock-out models have contributed significantly to our understanding of whether the presence of absence of CD36 is most beneficial during ischemia, however, the results from the global knockout mice have been contradictory. Some studies have found a deleterious outcome following ischaemia-reperfusion in the CD36 knockout mice, characterized by a decreased cardiac output and an increased end-diastolic pressure (indicative of myocardial contracture) (217). In contrast, others have reported a similar recovery when compared to wildtype mice with a significant improvement in reperfusion heart rate in the CD36 knock-out mice (218). A potential explanation for these conflicting findings may lay in small differences in the knock-out models and specific experimental conditions employed. More recently tamoxifen-inducible cardiomyocyte-specific CD36 knock-out mice have been challenged with ischaemia-reperfusion, and revealed improved reperfusion recovery driven by an increased force of contraction (219).

In summary, during myocardial ischemia and reperfusion the sarcolemmal CD36 content decreases, resulting in diminished CD36-mediated fatty acid uptake and oxidation, which protects the heart from lipotoxicity and promotes the metabolic shift towards glucose use, critical for post-ischemic cardiac efficiency and functional recovery.

6.2 Diabetic cardiomyopathy

Chronic caloric overconsumption is one of the main features of a Western lifestyle, and is a serious risk factor for development of type-2 diabetes. The leading cause of mortality in type-2 diabetic patients is heart failure, which is normally preceded by a relatively long latent phase in which the disease progression is largely asymptomatic. Diabetic cardiomyopathy refers to the changes that occur in cardiac function and morphology of patients with type 2 diabetes, independent of hypertension and coronary artery disease (220). Diabetic cardiomyopathy is associated with left ventricular diastolic dysfunction, myocardial fibrosis and often left ventricular hypertrophy (220-222). Metabolic changes are increasingly recognized to underlie these functional and structural changes in the diabetic heart, and include mitochondrial oxidative stress and insulin resistance as well as increased lipid handling (223). Rodent studies indicate that this latter change is notably the initial event in time and causally related to the former two (136, 224).

The pivotal role of lipid accumulation in the etiology of diabetic cardiomyopathy also pinpoints CD36 as an important early factor in this disease progression. Accordingly, pharmacological or genetic inhibition of CD36 *in vitro* in lipid-overloaded cardiomyocytes (117, 136) and *in vivo* in high fat diet-fed rodents (158, 208) protected against loss of contractile function. Hence, targeting CD36-mediated fatty acid uptake might be a valuable strategy to already attack the initial stage of disease development (see chapter 7). For this reason, it is important to understand the mechanism(s) by which CD36-mediated fatty acid uptake is increased early on in the development of diabetic heart disease. Theoretically, CD36-mediated fatty acid uptake could be increased at various levels including (*i*) an increase in CD36 expression; (*ii*) increased CD36 translocation from endosomes to the sarcolemma; (*iii*) altered post-translational modification.

6.2.1 CD36 expression and localization in the lipid-overloaded diabetic heart

Rodent and and HL-1 cardiomyocytes cultured chronically in media containing high amounts of fatty acids (most notably palmitate) display insulin resistance and contractile dysfunction. While the sarcolemmal CD36 content was markedly increased, no difference was observed in the total protein expression of CD36 (117, 136, 225). Muscle biopsies or myotubes derived from obese and type 2 diabetic patients also displayed increased sarcolemmal CD36 localization without alterations in total CD36 protein content (226-229). Hence, in these studies increased lipid uptake and accumulation must be due to increased CD36 translocation to and/or decreased internalization from the sarcolemma. In line with these *in vitro* findings were *in vivo* observations in various animal models of insulin resistance and diabetes. Specifically, hearts from rats (230) or mice (231) fed a high-fat diet, obese Zucker rats (232), and *db/db* mice (233) showed increased sarcolemmal CD36 but no changes in total CD36 protein expression compared to healthy control animals. Fructose-fed diabetic rats also showed increased sarcolemmal CD36 (234). Similar findings were observed in L6 myotubes (178) and in skeletal muscle from rodent models of lipid-induced insulin resistance (178, 235, 236).

In contrast, in several rodent models of type-2 diabetes others reported that the increased cardiac fatty acid uptake rate was associated with an increased total protein expression of CD36 (237-240). When the total cellular content of CD36 increases it can be expected that also the sarcolemmal CD36 content will increase (but in most studies this parameter was not assessed). Although it is unclear precisely why these studies have yielded different outcomes in these animal models of diabetes and high-fat feeding, one potential explanation relates to differences in disease severity. This has been shown to be the case in the type 1 diabetic heart, where there is a stepwise increase in CD36 protein content with increasing disease severity, as determined by the degree of hyperglycaemia (241).

Why in the type-2 diabetic heart, CD36 expression is not upregulated or only in severe disease states is not yet understood and may feel counterintuitive. Namely, the increased influx of lipids is expected to activate PPAR members, at least PPARα, which would lead to upregulation of CD36 and other genes with PPREs. This topic may need further investigation.

6.2.2 CD36 translocation in the lipid-overloaded diabetic heart

Increased CD36 translocation, rather than increased expression, appears an early mechanism by which lipids are taken up and progressively accumulate in the heart during conditions of lipid oversupply. The first evidence for such an increased CD36 translocation to the cell surface was found in hearts of obese Zucker rats in which subcellular fractionation showed that there was a major depletion of CD36 from a low-density microsomal fraction containing endosomes and a concomitant increase (>1.5-fold) in cell surface CD36 (232, 235). These same observations then were made in hearts from high fat diet-fed rats (230). This CD36 translocation appears to be a very early change preceding other lipid-induced maladaptive changes in skeletal muscle and heart (136, 236), including changes in glucose utilization and the onset of insulin resistance.

The molecular mechanism underlying increased CD36 translocation upon lipid overload relates to endosomal acidification being crucial for keeping CD36 intracellularly (section 4.3). Accordingly, loss of endosomal acidification induced by proton ionophores or v-ATPase inhibition leads to rapid CD36 translocation to the cell surface. Indeed, in the hearts of high fat diet-fed rodents *in vivo* or in palmitate exposed cardiomyocyte cultures *in vitro*, there is a loss in endosomal acidification (136, 225). The source of endosomal de-acidification can be traced back to an impairment of v-ATPase activity, leading to reduced proton pumping into the endosomal lumen. The main regulatory mechanism of v-ATPase includes cycles of assembly and disassembly of the two sub-complexes V_0 and V_1 (section 4.3). Long-chain fatty acids (palmitate, oleate) have the ability to induce the disassembly of v-ATPase, and this effect is apparent after a short period of exposure (within 1 hour) (136), indicating that dissipation of the pH gradient between endosomes and the cytoplasm is a very early general hallmark on a route towards lipid-induced insulin resistance (**FIGURE 7)**.

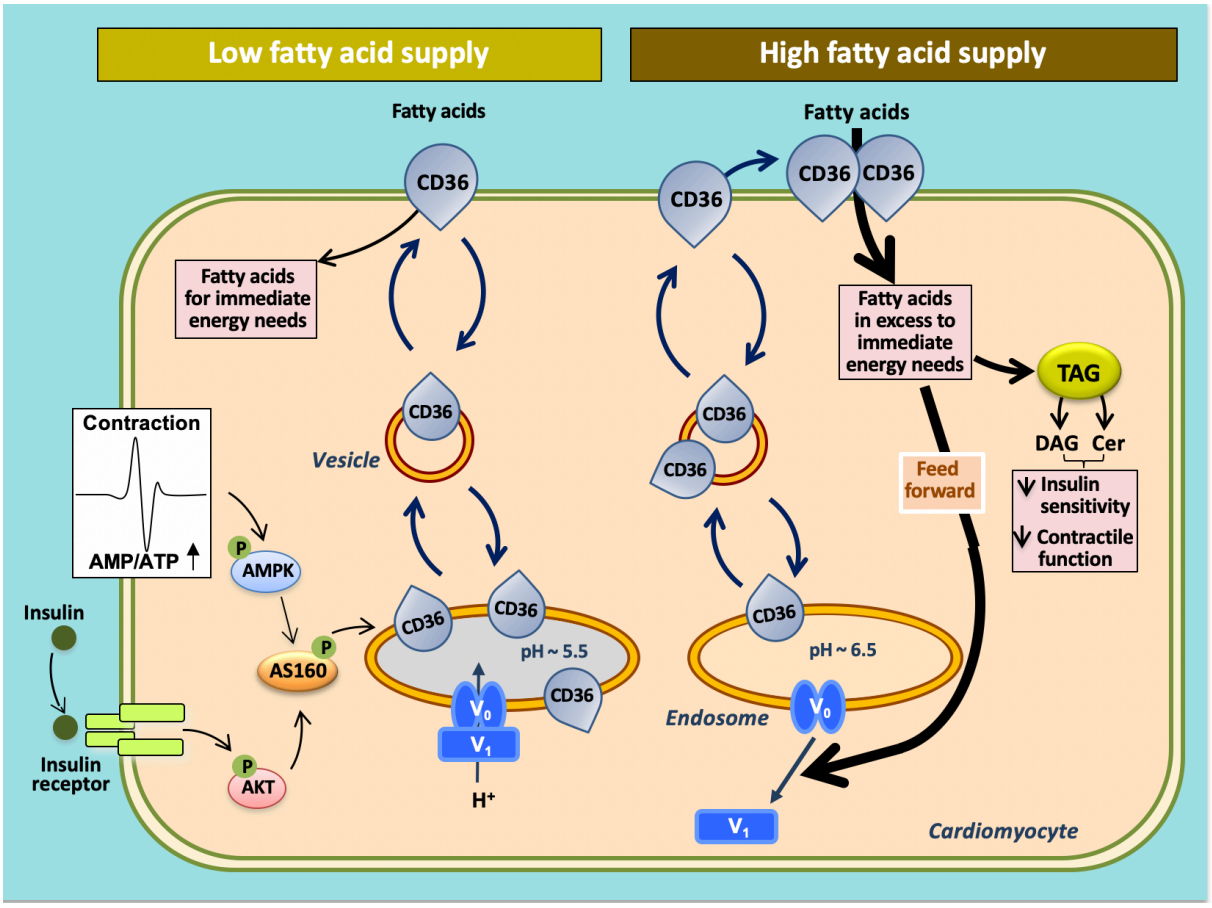


FIGURE 7. Mechanism of lipid-induced v-ATPase inhibition and loss of insulin sensitivity in cardiac myocytes upon lipid over-supply. When the supply of long-chain fatty acids is low, CD36 is primarily found in endosomes. Furthermore, the v-ATPase V_0 subcomplex, which is integral to the endosomal membrane, is assembled with the cytosolic V_1 subcomplex, allowing for acidification of the endosomal lumen. In this situation, the available fatty acids are metabolized to meet the immediate energy demand of the myocyte. Elevated extracellular fatty acids supply triggers a series of events: (1) increased CD36-mediated fatty acid uptake results in elevated intramyocellular levels causing the V_1 subcomplex to dissociate; (2) the V_1 subcomplex is shifted toward the cytoplasm; (3) the disassembly of v-ATPase leads to endosomal alkalinization; (4) increased endosomal pH triggers the translocation of CD36 vesicles to the sarcolemma. Upon chronic lipid over-supply, when fatty acid uptake surpasses the metabolic needs, further processes are set in motion: (5) the lipid-induced increase in sarcolemmal CD36 feeds forward to further fatty acid uptake and progressive lipid storage (triacylglycerol, TAG); (6) excess formation of diacylglycerol (DAG) and ceramides (Cer) culminates into impairment of the insulin signaling cascade and, therefore, loss of insulin sensitivity.

The fatty acid-induced disassembly of v- ATPase is not only observed on short-term, but is also maintained *in vitro* upon much longer exposure times (>48h). The mechanism behind this lipid-induced v-ATPase disassembly has not yet been elucidated but might include a palmitoylation of the a-subunit of v-ATPase. Specifically, in neuronal cells from mice with a deletion in the de-palmitoylating enzyme CLN1, hyper-palmitoylation of the a1 subunit of v-ATPase leads to its disassembly and subsequent elevation of lysosomal pH, followed by neurodegeneration (242). In a greater perspective, v-ATPase disassembly might play a role in several neurodegenerative diseases, such as Alzheimers disease, in which defective regulation of lysosomal pH has been observed (243). It is not known whether a palmitate-driven hyper-palmitoylation of v-ATPase might also occur in the lipid overloaded heart.

In conclusion, in the lipid-overloaded heart there is an increase in 'basal' CD36 translocation driven by lipid-induced v-ATPase disassembly. In addition, CD36 translocation becomes unresponsive to insulin (117, 230, 232, 236). Hence, on top of the increase in basal CD36 translocation, insulin fails to induce further CD36 translocation. Kinetically, this suggests that the increase in basal CD36 translocation is due to expulsion of CD36 from a sub-compartment of the endosomes that is responsive to insulin. Taken together, increased basal CD36 translocation is tightly linked to impairment in insulin-stimulated CD36 translocation.

6.2.3 Downstream effects resulting from CD36 translocation in the lipid-overloaded diabetic heart

As a result of increased CD36 translocation, cardiac fatty acid uptake will be increased which will lead to progressive myocellular lipid accumulation. As a result, the lipid-overexposed heart displays a massive increase in triacylglycerols (244). These neutral lipids are considered inert, however, more bioactive lipid species will also increase, such as diacylglycerols and ceramides, both of which set the heart on a road to insulin resistance (245). Importantly, increased basal CD36 translocation upon lipid overexposure is a very early rapid event, both *in vitro* (136) and *in vivo* (236), while the onset of insulin resistance follows much later with a considerable time gap in between (236). This time gap may be needed to progressively build up triacylglycerol stores from which fatty acids will be liberated for conversion to bio-active

fatty acid metabolites (246, 247). When certain threshold levels of these metabolites are exceeded, insulin signaling may be increasingly inhibited and insulin resistance develops.

Besides at the level of v-ATPase assembly and the canonical insulin signaling pathway, there are also further downstream levels at which lipids might have maladaptive effects on CD36 translocation, such as at the level of the CD36 trafficking machinery. For instance, lipid droplets and the CD36 trafficking machinery share certain SNARE proteins such as VAMP4 (248), allowing the possibility that upon lipid overload the growing lipid droplet population might hijack these SNAREs from the CD36 trafficking machinery (153). Furthermore, studies on the effect of lipid overload on expression and dynamics of trafficking proteins are scarce. Conversely, more work has been done to rescue lipid-induced insulin resistance by knock-out, knock-down, or overexpression, of specific trafficking proteins. For example, VAMP3 overexpression prevents the loss of insulin-stimulated GLUT4 translocation in cardiomyocytes (135). Nonetheless, a high fat diet has been reported to alter expression of several SNARE proteins and Rab proteins in muscle, such as syntaxin-4 expression, which is downregulated upon lipid oversupply (249, 250). Although the involvement of this t-SNARE in CD36 translocation has not been investigated, it has been identified as a specific binding partner for VAMP2 (251), which operates as v-SNARE in insulin-stimulated CD36 translocation (section 4.3). For future research, It would be interesting to observe whether such lipid-changes in these CD36-dedicated trafficking proteins will further aggravate lipid-induced insulin resistance, or alternatively that they present a compensatory mechanism.

6.3 Cardiac hypertrophy

In response to a rise in blood pressure, the heart has to increase its contractility to preserve cardiac output. Chronic hypertension results in cardiac growth, enabled by increased protein synthesis and hypertrophy of the cardiac myocytes. At the macroscopical level, there is an increase in left ventricular wall thickness leading to a concentric hypertrophy eventually transitioning in an eccentric hypertrophy culminating into severe dilatation of the heart (252, 253). During the progression through all these stages, there is a continuous further loss of contractile function. At the molecular level there are multiple alterations, including oxidative stress, inflammation and fibrosis. One of the first of these molecular changes is alterations in

substrate uptake within the cardiomyocytes, with CD36 playing an important role herein (254-256).

6.3.1 Observations in rodent models of cardiac hypertrophy

Experimental studies on pressure overload-induced cardiac hypertrophy most often have been performed on rodents subjected to transverse aortic constriction (TAC) or on spontaneously hypertensive (SH) rats. These two rodent models show essentially the same changes in metabolic and functional parameters. For instance, TAC induces a consistent decrease in myocardial CD36 expression (both at mRNA and protein level) as well as a decrease in cellular fatty acid uptake rate (257-260), while CD36 expression also decreases in SH rats (50). These same SH rats show that in heart and muscle, plasmalemmal contents of CD36 decrease to the same extent as that of total protein levels suggesting that there is little room for CD36 traffic to contribute to decreased myocellular fatty acid uptake (50). Besides this observation, there is little information as to whether decreased CD36 translocation contributes to the observed decrease in fatty acid uptake. Additionally, there is a consistent decrease in fatty acid oxidation in the pressure-overloaded heart (261-263), which is accompanied by mitochondrial dysfunction (264, 265). Conversely, the triacylglycerol (TAG) content tends to rise in the hypertrophied heart (260, 265, 266). It has been suggested that the decrease in fatty acid uptake serves to prevent the accumulation of toxic fatty acid metabolites which would otherwise accumulate due to decreased fatty acid oxidation (256). The increase in TAG seems paradoxical in view of the decreased fatty acid uptake. Potential explanations could relate to changes in either cardiac triglyceride lipolysis or changes in systemic fatty acid concentrations. For the former, the increased TAG content could result from decreased levels of cardiac lipolysis relative to esterification, which is in line with decreased expression of lipolytic enzymes (260, 265). For the latter, increased circulating concentrations of lipids in pressure-overload hypertrophy (267) could change the concentration gradient of fatty acids across the sarcolemma.

The role of CD36 in pressure overload-induced cardiac hypertrophy has also been studied by using various CD36 knockout mouse models. Global loss of CD36 decreases survival post-TAC surgery (268), increases hypertrophy and fibrosis, and decreases fractional shortening over the weeks following TAC surgery (268-270). This deleterious response to pressure overload-

induced hypertrophy was maintained when the inducible cardiomyocyte specific CD36 knockout underwent TAC surgery, demonstrating the occurrence of a primary cardiac response rather than systemic consequences of CD36 loss (257). Evidence points towards a decline in metabolic flexibility and energy starvation in the CD36 knockout hearts driving the deleterious response to pressure overload hypertrophy. In contrast to a wild-type heart, in the global knock-out model post-TAC there is no metabolic shift away from fat metabolism towards increased glucose metabolism (269). Data from dietary studies suggest energy starvation may be the underlying cause of the increased mortality, as feeding a high fat diet to CD36 knockout mice prior to TAC surgery resulted in improved fractional shortening and decreased hypertrophy, relative to the null mice fed a chow diet prior to TAC (270). Therefore, while loss of cardiac CD36 is protective for the diabetic heart (section 6.2) and the heart following acute ischemia-reperfusion, it is deleterious for the heart in response to chronic pressure overload hypertrophy.

In models of heart failure induced by *in vivo* myocardial infarction, changes in CD36 were reported to occur in synchrony with the other cardiac fatty acid transporters (271). Six months after coronary artery ligation, failing hearts had decreased fatty acid oxidation rates and decreased intracellular TAG content, metabolic changes that were proportional to the function of the heart. These metabolic changes were accompanied by decreased CD36 total protein levels, along with decreased levels of $FABP_{pm}$, $FABP_c$, FATP1 and FATP6 (271). Thus, in chronic heart failure, the whole range of proteins involved in fatty acid uptake is downregulated, with this suppression being proportional to how severely cardiac function is compromised.

6.3.2 Human cardiac hypertrophy

Little information is available on changes in CD36 expression in human cardiac hypertrophy. In patients with aortic stenosis, CD36 total protein content decreased proportionally with the extent of cardiac hypertrophy, with the more severe aortic stenosis characterised by the lowest levels of CD36 protein (272). In contrast, in patients with atrial fibrilliation, left atrial CD36 plasma membrane content is significantly upregulated, likely caused by overactivation of AMPK (273). Investigations of platelets from patients with hypertrophic cardiomyopathy (HCM) demonstrated a lack of CD36 on the platelets of 37% of the HCM patients (274).

6.3.3 Mechanisms driving CD36 downregulation in hypertrophy

The molecular mechanisms explaining the decreased CD36 expression in cardiac hypertrophy have been at least partly identified. There is consensus that decreased CD36 levels could be due to downregulation of the expression/activity of PPARα and its master regulator PGC1α in the pressure overloaded heart (261, 275). Similarly, in heart failure models the decrease in CD36 was accompanied by decreases in other PPARα target genes (271). Increased hypertrophic signaling as a response to the increased workload is expected to negatively impact on PPARα activation (276). However, hypertrophic signaling has a very complex signature with simultaneous stimulation of many signaling branches including Ca^{2+}-calcineurin/NFAT signaling, cyclic GMP signaling, mTORC1/PI3K signaling, GPCR signaling, stress signaling (MAPK/ERK), *et cetera* (276, 277), and some of these signaling pathways may directly decrease the transcriptional activity of PPARα. Additionally, PPARα has been shown to be suppressed by low oxygen via the hypoxia-inducible factor 1 (278). PPARα deactivation may also be more indirect, i.e., as a result of increased glucose uptake by the hypertrophic heart as part of a switch to a fetal program (279), which, in turn, may be in part due to increased O-GlcNAcylation (280). Indeed, protein O-GlcNAcylation is increased in cardiac hypertrophy, which may contribute to the decline in contractile function (281). In addition, PPARs were found to be O-GlcNAcylated which was accompanied by a decrease of their trans-activator function (282). A link between cardiac hypertrophy and the substrate switch towards glucose is provided by the kinase PKD1 which after TAC surgery is upregulated by GPCRs (254) to stimulate glucose uptake via induction of GLUT4 translocation (231).

Taken together, the cause of a decrease in CD36 expression in hypertrophy is a complicated research topic requiring extensive further research. Moreover, the above-mentioned suggested mechanisms operating in the hypertrophic heart inducing the switch from fatty acid to glucose utilization may be far from complete, and additional mechanisms may be involved, as further discussed in other reviews (279, 283).

6.3.4 Contribution of CD36 alterations in non-cardiac cells to impaired functioning of the hypertrophic heart

Given the expression of CD36 also in other cell types in the heart, such as macrophages and endothelial cells, malfunctioning of CD36 is these cell types also may contribute to changes in cardiac metabolism and function. For instance, bone marrow transplantation from CD36 knockout mice into wild-type mice prior to coronary artery ligation led to increased infarct size and decreased ejection fraction, demonstrating that bone marrow-derived CD36 expressing cells are needed to acutely limit infarct size (284). Loss of CD36 in this study also resulted in decreased clearing of dead cardiomyocytes and a delayed emergence of cardiac macrophages within the heart following myocardial infarction (284). Pharmacological approaches have confirmed a role for CD36 in macrophage phagocytic capacity post-myocardial infarction (285). Finally, endothelial-specific CD36 knockout mice have implicated a role for this transporter in atherosclerosis and angiogenesis. Ablation of endothelial CD36 in mice on a pro-atherosclerotic background have decreased aortic lesions, demonstrating that endothelial CD36 contributes to atherosclerosis development (286). In endothelial-specific CD36 knockout mice the response to chronic ischemia has been studied in a hindlimb ischemia model. Laser Doppler imaging demonstrated a decreased blood flow at 21 days post hindlimb surgery, compared with wild-type controls, indicating a role for CD36 in the pro-angiogenic response to skeletal muscle ischemia (287).

6.4 CD36 and the fatty acid-glucose substrate balance for homeostatic control

Optimal cardiac contractile performance is dependent on a balanced utilization of metabolic substrates, especially with regard to long-chain fatty acids and glucose (288). Under physiological conditions the heart utilizes fatty acids and glucose simultaneously, with each substrate contributing between 30 and 60% to overall energy provision (depending on substrate, oxygen availability, and hormonal milieu), with other substrates (lactate, ketone bodies, amino acids) covering only up to 10% of energy provision (122). The utilization of fatty acids and glucose in a distinct and finely tuned balance allows a rapid switching between these substrates, elicited by endogenous and exogenous factors such as diet and exercise. This

virtually instantaneous switching between substrates is designated as 'metabolic flexibility'. In addition, homeostatic control requires the ample availability of both fatty acids and glucose as they also serve subsidiary metabolic pathways and are key substrates for post-translational modification of proteins (289, 290).

Chronic fuel shifts towards a single type of substrate appear to be linked with cardiac dysfunction. Examples are increased glucose utilization, at the expense of fatty acids, being associated with cardiac hypertrophy, and increased fatty acid utilization, at the expense of glucose, being linked with insulin resistance and diabetic cardiomyopathy (122). The corollary is that re-balancing a tilted substrate balance toward an appropriate mix of fatty acids and glucose may result in recovery of cardiac contractile function. Indeed, there is convincing evidence for this concept (reviewed in (288)), as part of the notion that metabolic modulation, either alone or as adjunct to other medication, is an effective strategy to treat cardiac disease (291).

For instance, in PPARα-overexpressing mice, the fuel shift in the heart towards predominant fatty acid utilization which elicits lipid accumulation and contractile dysfunction could be rescued by ablation of CD36 and re-balancing of fatty acid and glucose utilization (208). Conversely, mice overexpressing protein kinase-D1, which leads to increased glucose utilization and cardiac hypertrophy, could be rescued by feeding a high fat diet (231). Taken together, due to its key role in cellular lipid metabolism, CD36 is involved in the pathogenesis of (cardio)metabolic diseases, and, therefore, forms a suitable therapeutic target to treat the failing heart.

Chapter 7. CD36 as a target for metabolic intervention therapy in heart

The accumulating catalogue of studies implicating CD36 in metabolic disease convincingly demonstrates its role in the pathogenesis and propagation of (cardio)metabolic diseases. This has led to a focused interest in developing compounds that target CD36, as a therapeutic route to prevent or reverse metabolic disease.

7.1 Advantages of targeting CD36

Cardiometabolic disease is commonly associated with changes in expression and activity of several proteins within the fatty acid metabolic pathway. However, CD36 sits in a privileged position from the point in view of a therapeutic target for a number of reasons. Firstly, within the pathway of fatty acid metabolism, CD36 is one of the primary steps in the pathway. Thus, its regulation impacts on everything that occurs intracellularly within fatty acid metabolism. This means that by regulating CD36 there is the capacity to impact pathways related to both fat oxidation and fat storage, for instance to rectify both arms that are abnormal in diabetes. Secondly, the added advantage of targeting a protein at the onset of the pathway is that this will limit intracellular accumulation of metabolites upstream of the point of regulation. As an example, targeting mitochondrial fatty acyl-CoA uptake would correct mitochondrial fatty acid oxidation but the lipid intermediates upstream of the mitochondria would still accumulate, which could have deleterious effects for other processes within the cell. Finally, the location within the vicinity of the sarcolemma makes CD36 and its regulatory proteins a relatively easily accessible target for pharmacological manipulation, compared with fatty acid metabolic proteins within the cell or within the mitochondrial matrix.

As our understanding of the role of CD36 in cellular homeostasis progresses, modulation of its involvement may be directed more precisely to one (or more) of the five levels that determine the functioning of CD36 (**FIGURE 8**). A number of proof-of-concept approaches have been investigated to pharmacologically target CD36 in various tissues, including modified non-metabolisable lipids, naturally occurring plant extracts and synthetic peptides. These will be discussed in detail below.

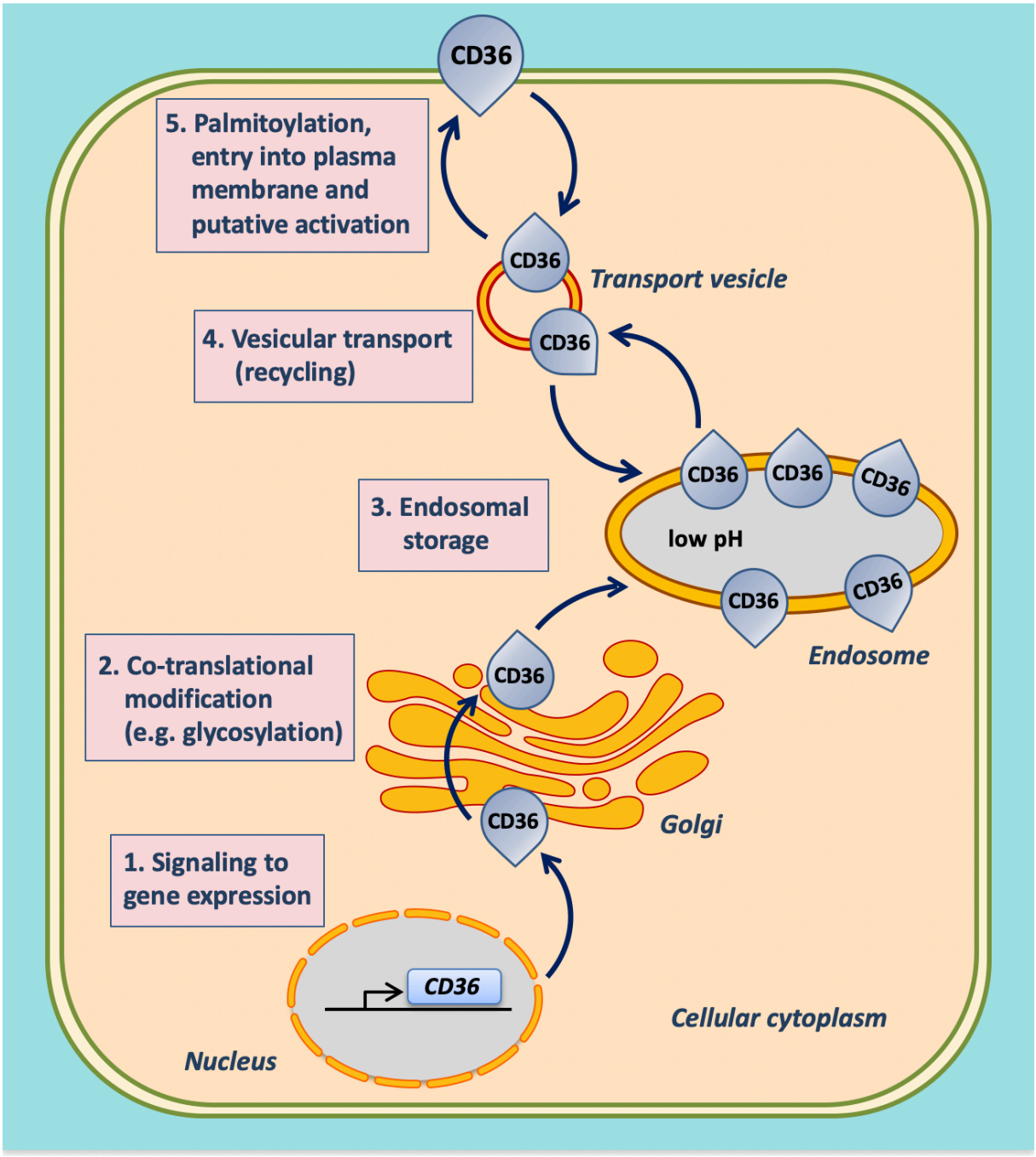


FIGURE 8. Schematic presentation of the five distinct levels at which the functioning of CD36 in cellular fatty acid uptake can be modulated. **1**. Intervention of signaling pathways affecting CD36 gene expression. **2**. Intervention in co-translational modification of CD36, in particular its glycosylation. **3.** Manipulation of the endosomal storage of CD36. **4.** Modulation of the reversible vesicular transport of CD36 between an endosomal storage compartment and the plasma membrane (subcellular recycling). **5.** Influencing CD36 palmitoylation, its insertion into the membrane bilayer, and putative activation (e.g., interaction with other membrane-associated proteins).

7.2 CD36 targeting: evidence from cell-based studies

Sulfo-*N*-succinimidyl esters of the long chain fatty acids palmitate and oleate have been used most extensively to demonstrate the benefit of CD36 inhibition. Sulfo-*N*-succinimidyl oleate (SSO) was used in the initial identification of CD36 in the seminal work by Harmon and Abumrad (292, 293). SSO works by irreversibly inhibiting CD36 via modification of a lysine residue within the fatty acid binding site (294). The formation of this oleoyl-amide bond in the binding pocket inhibits CD36's function as a fatty acid transporter. Studies in isolated cardiomyocytes demonstrated that SSO decreases long chain fatty acid uptake, oxidation and lipid esterification by similar percentages, associated with a compensatory increase in glucose metabolism (295, 296). By using giant sarcolemmal vesicles, it has been demonstrated that the site of SSO inhibition was specifically at the sarcolemma. SSO not only affects basal fatty acid uptake in cardiomyocytes, but has been critical in demonstrating signaling pathways both upstream and downstream of CD36. The inhibition of fatty acid uptake by SSO was not further enhanced when another transporter ($FABP_{pm}$) was additionally inhibited, indicating that the mechanism of action of these transporters was within a linear pathway and not a parallel pathway (247). Insulin and AMPK activation both drive increased fatty acid uptake at the sarcolemma, which is blunted by SSO. This demonstrates the CD36-dependence of these signals, and provides the mechanistic link between these metabolic regulators and their downstream metabolic effects (297). Similarly, SSO has been used to elucidate the downstream targets of fatty acids within the cardiomyocyte. For instance, fatty acids block the activation of the Hypoxia Inducible Factor 1α (HIF1α) in response to hypoxia, which could be restored by blocking CD36 with SSO (298). The challenges associated with SSO as a therapeutic agent relate to its instability, resulting in a short half-life in solution, and its hydrophobicity (296). There is some evidence that when applied to isolated mitochondria, SSO can also inhibit respiration via complex III of the electron transport chain (299, 300). There is also recent evidence suggesting that SSO can interact with other proteins (301). However, it is currently the most studied inhibitor of CD36, with a greater specificity than other inhibitors investigated.

The synthetic amphipathic helical peptide L37pA has been shown to bind to CD36 (in addition to other class B scavenger receptors) and antagonize its activity, resulting in decreased

endothelial cell permeability in response to pro-inflammatory stimulation (302). Monoclonal antibodies to CD36 have demonstrated very similar findings to those shown for chemical inhibition of CD36, suppressing fatty acid metabolism and promoting glucose metabolism in cardiomyocytes (**FIGURE 9**) (117). In addition, monoclonal antibodies to CD36 were able to prevent the development of insulin resistance in response to high fat exposure in cells.

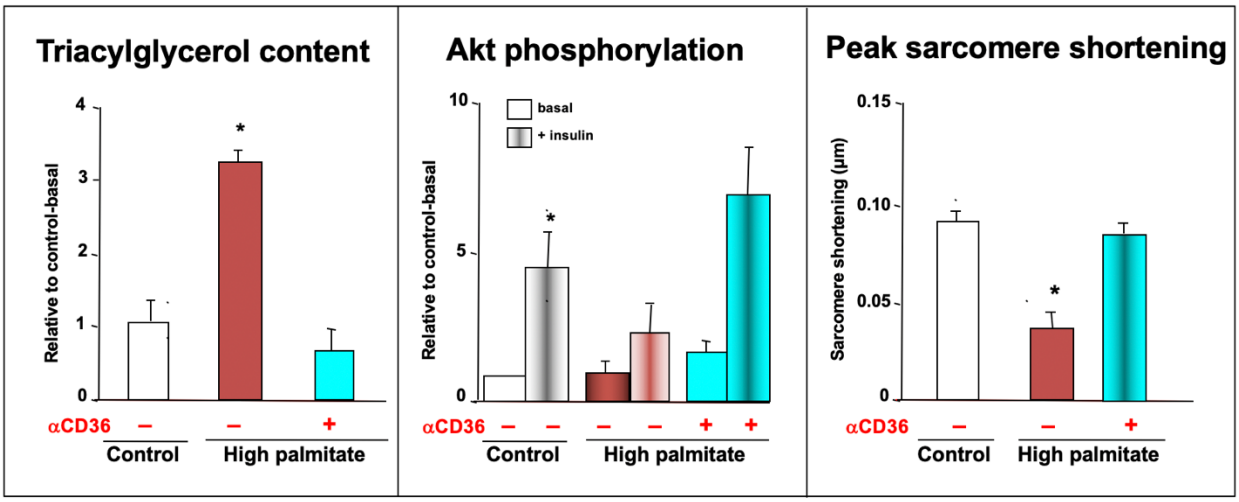


FIGURE 9. CD36 inhibition prevents lipid accumulation, insulin resistance, and contractile dysfunction as illustrated in an *in vitro* experiment with isolated rat cardiomyocytes. Cells were incubated in a medium with either low (20 µM) or high (200 µM) palmitate, with or without insulin, and in the absence or presence of an anti-CD36 antibody. Data obtained from (117).

7.3 *Ex vivo* and *in vivo* applications of CD36 regulators

Following on from cell studies involving CD36 inhibition, there have been a limited number of studies in intact tissue or *in vivo*. Infusion of SSO into an intact contracting heart perfused *ex vivo* demonstrated an acute decrease in fatty acid metabolism together with an increase in glucose metabolism without compromising cardiac function (303, 304). When infused into hearts from type-2 diabetic rats, SSO was able to improve the metabolic imbalance, decrease lipid deposition within the heart and improve functional recovery following a hypoxic challenge (304). Administering sulfo-*N*-succinimidyl palmitate (SSP) *in vivo* to healthy rats for 12 weeks elicited an increase in heart weight and cardiomyocyte hypertrophy, but no other effects on physiological measurements or plasma metabolites (305).

Through chemical library screening, two compounds, AP5055 and AP5258, were identified as potent inhibitors of CD36-mediated fatty acid uptake (306). *In vivo* treatment with AP5055 decreased atherosclerotic plaque size in a genetically susceptible mouse model. In addition, both compounds decreased circulating triacylglycerols and improved glucose tolerance in insulin resistant rat models. Thus, these small molecules show clear benefit for the heart by targeting both the vasculature (vascular endothelium) and the myocardium, correcting atherogenic dyslipidaemia as well as type 2 diabetes.

EP80317 is a synthetic peptide ligand of CD36, and was developed from earlier work showing that hexarelin, a synthetic growth hormone-releasing peptide, could bind both CD36 and growth hormone secretagogue receptor 1 a (GHS-R1a) (307, 308). EP80317 has reduced binding affinity for the GHS-R1a but still binds CD36, and has no effect in $CD36^{-/-}$ mice. Pretreatment with EP803177 decreased infarct size following *in viv*o myocardial infarction and improved cardiac contractile function. A refined ligand, CP-3(iv) also demonstrated beneficial cardiovascular effects following myocardial infarction (309). These findings could be linked mechanistically with effects on adipose lipolysis, mediated via adipose CD36 and its downstream effects on adiponectin secretion. Thus, CD36 inhibition prior to myocardial infarction may have beneficial effects for the heart via both cardiac and systemic fatty acid modulation. However, it remains to be determined whether this adipose-driven mechanism is specific to this modified growth hormone-releasing peptide ligand, or is also true for other molecules that modulate CD36.

The approaches described above target CD36, whereas there have been numerous studies that regulate the fatty acid metabolic pathway as a unit, of which CD36 is a component. Under these conditions CD36 protein levels change along with many other enzymes involved in metabolism. A number of natural occurring plant-based compounds have received attention for their ability to modulate CD36 (310). In a genetic model of cardiolipotoxicity, puerarin *in vivo* decreased CD36 protein levels and fatty acid metabolism within the heart. The decrease in CD36 was seen at both the mRNA and protein levels, suggesting that this compound chronically decreased the production of CD36, though the transcription factor driving this effect was not identified (310). In cells, the plant-derived alkaloid berberine decreases CD36 expression via modulation of the PPARγ transcription factor (311). Many of these plant-based

compounds have been explored within the setting of non-alcoholic fatty liver disease (reviewed in (7)).

Other studies have modulated CD36 location pharmacologically by affecting the intracellular distribution of CD36 between endosomes and the sarcolemma, without influencing the total CD36 content. This occurs by targeting the recycling process to stimulate CD36 translocation or inhibit its re-internalization. However, the approaches taken to date appear to target proteins that regulate multiple metabolic pathways. Examples include the glucagon-like peptide 1 (GLP1) analogue exenatide, which decreased sarcolemmal CD36 content, via a p38γ MAPK dependent pathway (312), but also increased GLUT4 translocation to the membrane. Similarly, the AMPK agonist AICAR increased CD36 sarcolemmal content in perfused hearts (313) but also induces GLUT4 translocation to the membrane (141). It is unclear from these studies whether the pharmacological agents are targeting proteins shared between both fatty acid and glucose transporter translocation, or if they are targeting just one substrate uptake pathway with the subsequent effect on the other transporter via a Randle cycle-mediated restoration of homeostasis (314).

As the machinery involved in CD36 trafficking continues to be elucidated, more specific targets for therapeutic modulation of CD36 activity will become apparent. Recent studies to identify the enzymes involved in the palmitoylation and de-palmitoylation of CD36 thereby regulating its cellular location have started to identify the kinases driving these signaling pathways. Studies in adipose tissue have implicated the tyrosine kinases LYN and SYK in the regulation of CD36 cellular location, by regulating the de-palmitoylation of the fatty acid transporter (82). *In vivo* studies using anticancer drugs (bafetinib and entospletinib) that target these kinases involved in endocytosis have shown decreased body weight gain on high fat diet and decreased adiposity in a CD36-dependent manner (Chapter 8). Nutraceutical strategies have also been investigated to regulate the trafficking machinery involved in CD36 translocation. Supplementing lysine, leucine and arginine into a high fat diet has been shown to restore the vATPase assembly (critical for CD36 intracellular retention), resulting in decreased lipotoxicity and improved wall thickness in hearts from high fat fed rats (225). These compounds are far from specific for CD36 regulation, but provide proof-of-concept that targeting the regulatory enzymes for CD36 is feasible.

7.4 Future strategies for targeting CD36 to improve specificity

Going forward, some of the major hurdles with regard to targeting CD36 involve the specificity towards CD36 and the specificity towards the heart over other organs. While targeting CD36 in the heart to reverse metabolic disease is desirable, targeting CD36 in adipose may be undesirable, as this could impair lipid storage. Finding a way to selectively reach CD36 in a specific tissue may require strategies that don't directly target CD36 but instead target the regulatory enzymes that govern CD36 localization, as many of these show more obvious tissue specificity. In addition, if targeting the regulatory enzymes, it will be essential to target enzymes that have specificity to CD36 translocation without also regulating trafficking of other substrate transporters, such as GLUT4. These could include targeting the VAMP proteins specific to CD36 translocation (BOX 1) or the palmitoyl acyl transferases expressed within the cardiomyocytes (section 2.4).

A corollary of the requirement of the heart for a balanced mixture of fatty acids and glucose so as to secure optimal contractile performance (section 6.4), is that the healthy heart exists within a "Goldilocks zone" for fatty acid metabolism: too much fatty acid metabolism drives the lipotoxic phenotype seen in diseases such as type 2 diabetes, but too little fatty acid metabolism is also deleterious for the heart (315). Inhibiting fatty acid metabolism too much may result in energetic insufficiency that impairs cardiac function. Thus, pharmacological inhibition of CD36 in cardiometabolic disease needs to rebalance fatty acid metabolism back to that healthy position, without inhibiting fatty acid oxidation too much resulting in energy starvation and possible contractile dysfunction.

Chapter 8 – CD36 beyond the heart in metabolic disease

In metabolic disease, changes in CD36 have been identified in many organs, and play pivotal roles in disease development. Due to cross-talk among multiple tissues, primary changes in non-cardiac tissue can drive changes within the heart, and *vice versa*. Below we briefly discuss how CD36 is involved in metabolic disease in other tissues.

8.1 CD36 in skeletal muscle in metabolic disease

Skeletal muscle plays a fundamental role in the development of metabolic syndrome and type 2 diabetes, due to it being the largest insulin-sensitive tissue. Given the critical role of skeletal muscle as a key site for insulin-stimulated glucose disposal, changes in skeletal muscle metabolism can have profound knock-on effects on circulating substrates supplied to other tissues in the body, such as the heart. CD36 protein is found at lower concentrations in muscle than in heart, however, in many instances muscle displays a similar profile of change in metabolic disease as seen in heart.

A short-term high fat diet was sufficient to upregulate CD36 mRNA expression in both rodents and humans (316, 317), and an elegant transcriptomic study (318) demonstrated that CD36 mRNA expression is normally suppressed by insulin's action on skeletal muscle. In long term obese animal studies, changes in sarcolemmal CD36 were present in skeletal muscle, heart and adipose tissue of the same animals, suggesting a similar time frame for systemic changes in these obese animals (235). In red muscles from both obese Zucker *(fa/fa)* rats and obese individuals, sarcolemmal CD36 is increased along with fatty acid uptake, without an increase in CD36 total protein (226, 227, 235, 319, 320). Metabolic disease is not a static phenomenon and changes in metabolism occur in a time-dependent fashion both with regard to the development and progression of metabolic disease. In a "Herculean" study involving 827 rats, the time frame of changes in skeletal muscle fatty acid transporters, transport and insulin sensitivity from day 1 through to 6 weeks following induction of a high fat diet was studied (236). They showed that increased sarcolemmal CD36 and fatty acid uptake occurred as early as two days into the high fat diet, at the same time as an increase in the circulating insulin concentrations. Changes in sarcolemmal CD36 were accompanied by increased intramuscular triacylglyceroles, and, of note, total CD36 muscle content did not change over the 6-week

period. However, markers of glucose tolerance and muscle insulin sensitivity were not affected until three weeks into the high fat diet (236). This study clearly demonstrates that within muscle changes in CD36 precede changes in insulin sensitivity and abnormal glucose metabolism in response to a high fat diet, and that this is driven by increased translocation of CD36 to the membrane.

8.2 CD36 in the liver in non-alcoholic fatty liver disease (NAFLD)

CD36 global knockout mice have systemic hyperlipidaemia and hypoglycaemia due to metabolic reprogramming, however, the metabolic profile in skeletal muscle and liver are divergent (321). Whereas *in vivo* fatty acid uptake is decreased into muscle tissue in CD36 knockout mice, it is increased into the liver, indicating that under baseline conditions, uptake of fatty acids into the liver is much less dependent on CD36 than in other insulin-sensitive oxidative tissues. In agreement, in the liver of healthy rodents, CD36 expression is low compared to heart and muscle (3, 247).

Non-alcoholic fatty liver disease (NAFLD) is often associated with obesity and type 2 diabetes, and spans a range of conditions characterized by excessive accumulation of fat within the liver. Additionally, NAFLD is an independent risk factor for cardiovascular disease, potentially via hepatic-driven dyslipidaemia and production of inflammatory cytokines (322). Despite CD36 being lowly expressed in the liver under healthy conditions, upon high fat feeding, CD36 expression is substantially upregulated, along with hepatic triacylglycerols, genes involved in fatty acid synthesis as well as in fatty acid oxidation (section 2.3) (323). In patients with NAFLD, CD36 is upregulated at both mRNA and protein levels, and correlates positively with liver fat percentage and the grade of steatosis, indicating that the higher the CD36 expression the more severe the hepatic pathology (324, 325). In addition to transcriptional control, in the lipid-overloaded liver CD36 becomes hyper-palmitoylated, which anchors this protein to the plasma membrane, thereby increasing fatty acid uptake (326). Consequently, pharmacological inhibition of palmitoylation prevented excessive fatty acid uptake and the development of non-alcoholic steatohepatitis (NASH) (326). As mentioned in section 2.5, CD36 has also been identified to exist in a soluble form in plasma, denoted as sCD36 (31) which was increased in patients with NAFLD (327, 328), correlating with disease severity. Further work is needed to determine whether sCD36 could serve as a biomarker for NAFLD diagnosis and progression.

8.3 CD36 in immunity and the development of atherosclerosis

CD36 is expressed on both macrophages and platelets, contributing to immune regulation under healthy conditions. In metabolic disease, CD36-expressing macrophages and platelets play pivotal roles in atherosclerotic plaque development and thrombus formation following plaque rupture. CD36 is responsible for uptake of oxidised low-density lipoprotein (oxLDL) into macrophages, leading to the formation of foam cells within the intima. CD36-mediated oxLDL uptake drives a reprogramming of the macrophages, upregulating markers of oxidative stress, inflammation and retention of the macrophages within the plaque (329, 330). CD36 knockout in an atherosclerotic prone mouse model resulted in decreased lesion formation, with macrophages from these knockout mice displaying a decreased pro-inflammatory phenotype (331). In addition, CD36 knockout decreased platelet activation and aggregation (332). Thus, CD36 contributes to the chronic inflammation within the vessel wall and plaque formation driven by these lipid-loaded foam cells in metabolic disease, predisposing to vessel plaque rupture and thrombosis.

8.4 CD36 in adipose tissue in metabolic disease

CD36 protein is increased in adipose tissue from type 2 diabetic patients (333), displaying a linear relationship between CD36 content and palmitate storage in subcutaneous adipose tissue (334). Similarly, CD36 mRNA is increased in adipose tissue of *ob/ob* mice (21). Just as there is CD36-dependent cross-talk between the immune system and the heart, there is also a similar CD36-dependent interaction between macrophages and adipose tissue. Deletion of CD36 decreases the pro-inflammatory phenotype of adipose tissue in response to a high fat diet, decreasing macrophage infiltration and increasing adipose tissue insulin sensitivity (335).

In adipose tissue CD36 has been shown to be palmitoylated by the palmitoyl acyltransferases DHHC4 and DHHC5, driving CD36 localisation to the adipocyte membrane and upregulating fatty acid uptake (336). There is evidence in adipose tissue that CD36 may function as part of a complex with annexin-2 and prohibitin-1 to mediate fatty acid uptake via endocytosis of the fatty acid-CD36 complex, thereby removing the CD36 molecule away from the membrane and towards the lipid droplet each time it facilitates the uptake of a fatty acid molecule (82, 337, 338). To what extent this mechanism contributes to the overall rate of fatty acid uptake is not

yet known. What remains to be studied is whether the physiological rate of fatty acid uptake can be accommodated by the time frame proposed for an endocytic model for fatty acid uptake, and whether the cellular content of CD36 is sufficient to accommodate this mode of fatty acid uptake. Studies have suggested that the interaction between CD36 and the lipid droplet might not only drive fatty acid uptake into storage under anabolic conditions, but might also regulate the reciprocal process under catabolic conditions, by moving the lipid droplet-derived fatty acid up to the plasma membrane for release into the circulation in response to lipolytic stimuli (338).

8.5 CD36 in cancer

Metabolic disease is associated with increased risk of developing certain cancers, including cancers of the breast, liver, pancreas, colon and bladder (339). The significance of lipid metabolism for cancer cells has only relatively recently been identified, and it is becoming apparent that many forms of cancer are dependent not only on *de novo* lipogenesis but also exogenous fatty acid uptake from the circulation (340). There is growing evidence that CD36 fuels lipid metabolism in cancer cells, as inhibition of CD36 decreases fatty acid uptake into prostate cancer cells, thereby decreasing proliferation and progression (341). Upregulation of CD36 has been shown to be associated with increased mitochondrial mass and oxidative phosphorylation in leukemia (342). In addition to fueling fatty acid oxidation, upregulation of CD36 has been linked to the increased production of oncogenic lipid signals, such as lysophospholipids, which contribute to tumor progression (341).

Studies have demonstrated that CD36-mediated fatty acid uptake plays a key role in regulating metastases (343), by promoting the epithelial-to-mesenchymal transition (344), with CD36 more upregulated in metastatic compared with primary cancers (345). In ovarian cancer pharmacological inhibition of CD36-mediated lipid import lead to lipid deficiency associated with reduced proliferation (346). CD36 has also been implicated in drug resistance of breast cancer cells, as expression of CD36 has been shown to correlate with poor prognosis following tamoxifen-inhibited cell growth (347) or following anti-HER2 therapy (348). Likewise, cross talk between immune cells and cancer cells within the tumor microenvironment (TME) has

implicated CD36 as a potential target for therapy. CD36 is upregulated in regulatory T cells (T_{reg} cells) within the TME, along with other genes involved in lipid metabolism, and is implicated in the ability of the tumor to evade immune attack and suppress the antitumor response (349). Strategies to decrease T_{reg} CD36 expression decreased tumor growth and improved survival in mouse models of melanoma. Thus, there is growing interest in using CD36 expression as a biomarker for metastatic prognosis as well as the potential to target CD36 as a therapeutic strategy. In conclusion, while the precise role of CD36 in linking metabolic disease to cancer development and progression remains to be disclosed, targeting lipid metabolism, in particular CD36, appears a promising therapeutic strategy for cancer (9).

Chapter 9. Concluding remarks and perspective

Research performed in recent years has clearly established a pivotal role for the membrane protein CD36 in cellular lipid metabolism and signaling. Now that the underlying molecular mechanisms of CD36 functioning have been largely disclosed and that, in particular in cardiac and skeletal muscle, proof of concept has been obtained that its manipulation alters lipid metabolism in an effective and tissue-specific manner, further studies are warranted to explore such therapeutic approaches in broader sense. For instance, various alternative manners of manipulating CD36 plasma membrane presence and function are feasible, such as targeting tissue- and vesicle-specific trafficking proteins or targeting post-translational modification of CD36. In addition, now that the protein structure of CD36 is known, chemical compounds could be generated to specifically influence CD36 functioning (to replace small peptides applied so far but which were found marginally effective). Finally, given that researchers often study a single type of tissue, the insights obtained for a certain tissue should be advocated to be explored for their applicability to other tissues as well as other conditions associated with altered metabolism such as cancer.

A typical characteristic of CD36 is that its regulation occurs at multiple levels, that is, gene expression, co-translational and post-translational modification, storage in endosomes, reversible intracellular transport in vesicles (recycling), entry into the plasma membrane, and putative interaction with other (membrane) proteins. A corollary is that studying the involvement of CD36 requires investigating each of these levels, with special reference to cellular localization, an aspect that is not covered when focusing on CD36 mRNA or protein expression alone. Another corollary is that manipulation of CD36 may involve each of these levels, suggesting that such manipulation may be exerted on either a more global or cell-specific aspect. Therefore, the notion arises that CD36 is a major target for treatment of metabolic disease in general, and specifically to mend a failing heart.

TABLE 1. Overview of short-term (< 1 h) stimuli inducing CD36 translocation in myocytes

Stimulus	Model system	Effect of stimulus		Signaling pathway involved	Translocation of other transporters*	Reference
		CD36 translocation (fold)	Fatty acid uptake (fold)			
• *In vitro* studies						
Contraction	Rat cardiomyocytes	1.3 – 1.6	1.5 – 1.9	LKB1/AMPK	GLUT4	Luiken et al, 2003 (141)
Insulin	Rat cardiomyocytes	1.6	1.5	PI3K/Akt2	GLUT4	Luiken et al., 2002 (143)
Oligomycin	Rat cardiomyocytes	1.6	2.0	LKB1/AMPK	GLUT4	Luiken et al., 2003 (141)
Dipyridamole	Rat cardiomyocytes	1.5	1.8	n.d.	Not GLUT4	Luiken et al., 2004 (350)
AICAR	Rat/mouse cardiomyocytes	1.4	1.4 – 1.9	LKB1/AMPK	$FABP_{pm}$	Chabowski et al., 2006 (351) Habets et al., 2009 (149)
Leptin	HL-1 cells	1.8 – 2.0	1.6 – 1.8	AMPK	FATP1	Palanivel et al., 2006 (352) Momken et al., 2017 (353) Jain et al., 2009 (148)
Adiponectin	Rat cardiomyocytes	1.5	1.6	APPL1/AMPK	n.d.	Fang et al., 2010 (354)
Palmitate	Rat cardiomyocytes/L6 myoblasts	1.4 – 1.5	1.3 – 1.5	AMPK and PKCζ	n.d.	Liu et al., 2017 (136) Zhu et al., 2020 (178)
• *In vivo* studies						
Contraction	Rat skeletal muscle	1.6	1.5	AMPK	GLUT4, $FABP_{pm}$ FATP1, FATP4	Bonen et al., 2000 (133) Jain et al., 2009 (148)
Insulin	Rat skeletal muscle	1.5	1.5	PI3K/Akt2	GLUT4, $FABP_{pm}$ FATP1, FATP4	Luiken et al., 2002 (142) Jain et al., 2009 (148)
Caffeine	Mouse skeletal muscle	1.3	1.5	AMPK	n.d.	Lally et al., 2012 (355)
Thyroid hormone (T2/T3)	Rat skeletal muscle	1.8 – 2.1	n.d.	AMPK (?)	n.d.	Lombardi et al., 2012 (129)

* Translocation of other substrate transporters as far as reported.
n.d., not determined

REFERENCES

1. **Clemetson KJ, Pfueller SL, Luscher EF, and Jenkins CS**. Isolation of the membrane glycoproteins of human blood platelets by lectin affinity chromatography. *Biochim Biophys Acta* 464: 493-508, 1977.
2. **Endemann G, Stanton LW, Madden KS, Bryant CM, White RT, and Protter AA**. CD36 is a receptor for oxidized low density lipoprotein. *J Biol Chem* 268: 11811-11816, 1993.
3. **Abumrad NA, el-Maghrabi MR, Amri EZ, Lopez E, and Grimaldi PA**. Cloning of a rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during preadipocyte differentiation. Homology with human CD36. *J Biol Chem* 268: 17665-17668, 1993.
4. **Tian K, Xu Y, Sahebkar A, and Xu S**. CD36 in Atherosclerosis: Pathophysiological Mechanisms and Therapeutic Implications. *Curr Atheroscler Rep* 22: 59, 2020.
5. **Chen Y, Zhang J, Cui W, and Silverstein RL**. CD36, a signaling receptor and fatty acid transporter that regulates immune cell metabolism and fate. *J Exp Med* 219: 2022.
6. **Rada P, Gonzalez-Rodriguez A, Garcia-Monzon C, and Valverde AM**. Understanding lipotoxicity in NAFLD pathogenesis: is CD36 a key driver? *Cell Death Dis* 11: 802, 2020.
7. **Feng Y, Sun W, Sun F, Yin G, Liang P, Chen S, Liu X, Jiang T, and Zhang F**. Biological Mechanisms and Related Natural Inhibitors of CD36 in Nonalcoholic Fatty Liver. *Drug Des Devel Ther* 16: 3829-3845, 2022.
8. **Munir R, Lisec J, Swinnen JV, and Zaidi N**. Too complex to fail? Targeting fatty acid metabolism for cancer therapy. *Prog Lipid Res* 85: 101143, 2022.
9. **Ruan C, Meng Y, and Song H**. CD36: an emerging therapeutic target for cancer and its molecular mechanisms. *J Cancer Res Clin Oncol* 148: 1551-1558, 2022.
10. **Zaidi NE, Shazali NAH, Leow TC, Osman MA, Ibrahim K, Cheng WH, Lai KS, and Nik Abd Rahman NMA**. CD36-Fatty Acid-Mediated Metastasis via the Bidirectional Interactions of Cancer Cells and Macrophages. *Cells* 11: 2022.
11. **Guerrero-Rodriguez SL, Mata-Cruz C, Perez-Tapia SM, and Velasco-Velazquez MA**. Role of CD36 in cancer progression, stemness, and targeting. *Front Cell Dev Biol* 10: 1079076, 2022.
12. **Shaw S**. Characterization of human leukocyte differentiation antigens. *Immunol Today* 8: 1-3, 1987.
13. **Asch AS, Barnwell J, Silverstein RL, and Nachman RL**. Isolation of the thrombospondin membrane receptor. *J Clin Invest* 79: 1054-1061, 1987.
14. **Ockenhouse CF, and Chulay JD**. Plasmodium falciparum sequestration: OKM5 antigen (CD36) mediates cytoadherence of parasitized erythrocytes to a myelomonocytic cell line. *J Infect Dis* 157: 584-588, 1988.
15. **Prabhudas M, Bowdish D, Drickamer K, Febbraio M, Herz J, Kobzik L, Krieger M, Loike J, Means TK, Moestrup SK, Post S, Sawamura T, Silverstein S, Wang XY, and El Khoury J**. Standardizing scavenger receptor nomenclature. *J Immunol* 192: 1997-2006, 2014.
16. **Van Nieuwenhoven FA, Verstijnen CP, Abumrad NA, Willemsen PH, Van Eys GJ, Van der Vusse GJ, and Glatz JF**. Putative membrane fatty acid translocase and cytoplasmic fatty acid-binding protein are co-expressed in rat heart and skeletal muscles. *Biochem Biophys Res Commun* 207: 747-752, 1995.
17. **Neculai D, Schwake M, Ravichandran M, Zunke F, Collins RF, Peters J, Neculai M, Plumb J, Loppnau P, Pizarro JC, Seitova A, Trimble WS, Saftig P, Grinstein S, and Dhe-Paganon S**. Structure of LIMP-2 provides functional insights with implications for SR-BI and CD36. *Nature* 504: 172-176, 2013.
18. **Luiken JJ, Chanda D, Nabben M, Neumann D, and Glatz JF**. Post-translational modifications of CD36 (SR-B2): Implications for regulation of myocellular fatty acid uptake. *Biochim Biophys Acta* 1862: 2253-2258, 2016.
19. **Hsieh FL, Turner L, Bolla JR, Robinson CV, Lavstsen T, and Higgins MK**. The structural basis for CD36 binding by the malaria parasite. *Nat Commun* 7: 12837, 2016.
20. **May SC SD**. Now in 3D! Novel insights into CD36 structure and function. *Annals of Blood* 6: 33, 2021.
21. **Memon RA, Fuller J, Moser AH, Smith PJ, Grunfeld C, and Feingold KR**. Regulation of putative fatty acid transporters and Acyl-CoA synthetase in liver and adipose tissue in ob/ob mice. *Diabetes* 48: 121-127, 1999.

22. **Zhou J, Febbraio M, Wada T, Zhai Y, Kuruba R, He J, Lee JH, Khadem S, Ren S, Li S, Silverstein RL, and Xie W**. Hepatic fatty acid transporter Cd36 is a common target of LXR, PXR, and PPARgamma in promoting steatosis. *Gastroenterology* 134: 556-567, 2008.
23. **Kim TT, and Dyck JR**. The role of CD36 in the regulation of myocardial lipid metabolism. *Biochim Biophys Acta* 1861: 1450-1460, 2016.
24. **Silverstein RL, and Febbraio M**. CD36, a scavenger receptor involved in immunity, metabolism, angiogenesis, and behavior. *Sci Signal* 2: re3, 2009.
25. **Dobri AM, Dudau M, Enciu AM, and Hinescu ME**. CD36 in Alzheimer's Disease: An Overview of Molecular Mechanisms and Therapeutic Targeting. *Neuroscience* 453: 301-311, 2021.
26. **Laugerette F, Passilly-Degrace P, Patris B, Niot I, Febbraio M, Montmayeur JP, and Besnard P**. CD36 involvement in orosensory detection of dietary lipids, spontaneous fat preference, and digestive secretions. *J Clin Invest* 115: 3177-3184, 2005.
27. **Niot I, and Besnard P**. Appetite control by the tongue-gut axis and evaluation of the role of CD36/SR-B2. *Biochimie* 136: 27-32, 2017.
28. **Pelsers MM, Lutgerink JT, Nieuwenhoven FA, Tandon NN, van der Vusse GJ, Arends JW, Hoogenboom HR, and Glatz JF**. A sensitive immunoassay for rat fatty acid translocase (CD36) using phage antibodies selected on cell transfectants: abundant presence of fatty acid translocase/CD36 in cardiac and red skeletal muscle and up-regulation in diabetes. *Biochem J* 337 (Pt 3): 407-414, 1999.
29. **Koonen DP, Febbraio M, Bonnet S, Nagendran J, Young ME, Michelakis ED, and Dyck JR**. CD36 expression contributes to age-induced cardiomyopathy in mice. *Circulation* 116: 2139-2147, 2007.
30. **Zhang X, Fitzsimmons RL, Cleland LG, Ey PL, Zannettino AC, Farmer EA, Sincock P, and Mayrhofer G**. CD36/fatty acid translocase in rats: distribution, isolation from hepatocytes, and comparison with the scavenger receptor SR-B1. *Lab Invest* 83: 317-332, 2003.
31. **Handberg A, Levin K, Hojlund K, and Beck-Nielsen H**. Identification of the oxidized low-density lipoprotein scavenger receptor CD36 in plasma: a novel marker of insulin resistance. *Circulation* 114: 1169-1176, 2006.
32. **Bhattacharya A, Ashouri R, Fangman M, Mazur A, Garett T, and Dore S**. Soluble Receptors Affecting Stroke Outcomes: Potential Biomarkers and Therapeutic Tools. *Int J Mol Sci* 22: 2021.
33. **Xu X, Zheng X, and Zhu F**. CD36 gene variants and their clinical relevance: a narrative review. *Annals of Blood* 6: 2021.
34. **Corpeleijn E, van der Kallen CJ, Kruijshoop M, Magagnin MG, de Bruin TW, Feskens EJ, Saris WH, and Blaak EE**. Direct association of a promoter polymorphism in the CD36/FAT fatty acid transporter gene with Type 2 diabetes mellitus and insulin resistance. *Diabet Med* 23: 907-911, 2006.
35. **Fukuchi K, Nozaki S, Yoshizumi T, Hasegawa S, Uehara T, Nakagawa T, Kobayashi T, Tomiyama Y, Yamashita S, Matsuzawa Y, and Nishimura T**. Enhanced myocardial glucose use in patients with a deficiency in long-chain fatty acid transport (CD36 deficiency). *J Nucl Med* 40: 239-243, 1999.
36. **Tanaka T, Nakata T, Oka T, Ogawa T, Okamoto F, Kusaka Y, Sohmiya K, Shimamoto K, and Itakura K**. Defect in human myocardial long-chain fatty acid uptake is caused by FAT/CD36 mutations. *J Lipid Res* 42: 751-759, 2001.
37. **Nozaki S, Tanaka T, Yamashita S, Sohmiya K, Yoshizumi T, Okamoto F, Kitaura Y, Kotake C, Nishida H, Nakata A, Nakagawa T, Matsumoto K, Kameda-Takemura K, Tadokoro S, Kurata Y, Tomiyama Y, Kawamura K, and Matsuzawa Y**. CD36 mediates long-chain fatty acid transport in human myocardium: complete myocardial accumulation defect of radiolabeled long-chain fatty acid analog in subjects with CD36 deficiency. *Mol Cell Biochem* 192: 129-135, 1999.
38. **Ma X, Bacci S, Mlynarski W, Gottardo L, Soccio T, Menzaghi C, Iori E, Lager RA, Shroff AR, Gervino EV, Nesto RW, Johnstone MT, Abumrad NA, Avogaro A, Trischitta V, and Doria A**. A common haplotype at the CD36 locus is associated with high free fatty acid levels and increased cardiovascular risk in Caucasians. *Hum Mol Genet* 13: 2197-2205, 2004.
39. **Love-Gregory L, and Abumrad NA**. CD36 genetics and the metabolic complications of obesity. *Curr Opin Clin Nutr Metab Care* 14: 527-534, 2011.
40. **Keller KL, Liang LC, Sakimura J, May D, van Belle C, Breen C, Driggin E, Tepper BJ, Lanzano PC, Deng L, and Chung WK**. Common variants in the CD36 gene are associated with oral fat perception, fat preferences, and obesity in African Americans. *Obesity (Silver Spring)* 20: 1066-1073, 2012.

41. **Du Y, Chen K, Liu E, Wang X, Li F, Liu T, Zheng X, Li G, and Che J**. Gender-specific associations of CD36 polymorphisms with the lipid profile and susceptibility to premature multi-vessel coronary artery heart disease in the Northern Han Chinese. *Gene* 753: 144806, 2020.
42. **Yazdanpanah Z, Mozaffari-Khosravi H, Mirzaei M, Sheikhha MH, and Salehi-Abargouei A**. A systematic review and meta-analysis on the association between CD36 rs1761667 polymorphism and cardiometabolic risk factors in adults. *Sci Rep* 12: 5916, 2022.
43. **Kintaka T, Tanaka T, Imai M, Adachi I, Narabayashi I, and Kitaura Y**. CD36 genotype and long-chain fatty acid uptake in the heart. *Circ J* 66: 819-825, 2002.
44. **Hames KC, Vella A, Kemp BJ, and Jensen MD**. Free fatty acid uptake in humans with CD36 deficiency. *Diabetes* 63: 3606-3614, 2014.
45. **Zhao L, Li Y, Ding Q, Li Y, Chen Y, and Ruan XZ**. CD36 Senses Dietary Lipids and Regulates Lipids Homeostasis in the Intestine. *Front Physiol* 12: 669279, 2021.
46. **Dramane G, Akpona S, Simonin AM, Besnard P, and Khan NA**. Cell signaling mechanisms of gustatory perception of lipids: can the taste cells be the target of anti-obesity agents? *Curr Med Chem* 18: 3417-3422, 2011.
47. **Chamoun E, Mutch DM, Allen-Vercoe E, Buchholz AC, Duncan AM, Spriet LL, Haines J, Ma DWL, and Guelph Family Health S**. A review of the associations between single nucleotide polymorphisms in taste receptors, eating behaviors, and health. *Crit Rev Food Sci Nutr* 58: 194-207, 2018.
48. **Aitman TJ, Cooper LD, Norsworthy PJ, Wahid FN, Gray JK, Curtis BR, McKeigue PM, Kwiatkowski D, Greenwood BM, Snow RW, Hill AV, and Scott J**. Malaria susceptibility and CD36 mutation. *Nature* 405: 1015-1016, 2000.
49. **Aitman TJ, Glazier AM, Wallace CA, Cooper LD, Norsworthy PJ, Wahid FN, Al-Majali KM, Trembling PM, Mann CJ, Shoulders CC, Graf D, St Lezin E, Kurtz TW, Kren V, Pravenec M, Ibrahimi A, Abumrad NA, Stanton LW, and Scott J**. Identification of Cd36 (Fat) as an insulin-resistance gene causing defective fatty acid and glucose metabolism in hypertensive rats. *Nat Genet* 21: 76-83, 1999.
50. **Bonen A, Han XX, Tandon NN, Glatz JF, Lally J, Snook LA, and Luiken JJ**. FAT/CD36 expression is not ablated in spontaneously hypertensive rats. *J Lipid Res* 50: 740-748, 2009.
51. **Motojima K, Passilly P, Peters JM, Gonzalez FJ, and Latruffe N**. Expression of putative fatty acid transporter genes are regulated by peroxisome proliferator-activated receptor alpha and gamma activators in a tissue- and inducer-specific manner. *J Biol Chem* 273: 16710-16714, 1998.
52. **Neels JG, and Grimaldi PA**. Physiological functions of peroxisome proliferator-activated receptor beta. *Physiol Rev* 94: 795-858, 2014.
53. **Nakamura MT, Yudell BE, and Loor JJ**. Regulation of energy metabolism by long-chain fatty acids. *Prog Lipid Res* 53: 124-144, 2014.
54. **Teboul L, Febbraio M, Gaillard D, Amri EZ, Silverstein R, and Grimaldi PA**. Structural and functional characterization of the mouse fatty acid translocase promoter: activation during adipose differentiation. *Biochem J* 360: 305-312, 2001.
55. **Tontonoz P, Nagy L, Alvarez JG, Thomazy VA, and Evans RM**. PPARgamma promotes monocyte/macrophage differentiation and uptake of oxidized LDL. *Cell* 93: 241-252, 1998.
56. **Nisoli E, Carruba MO, Tonello C, Macor C, Federspil G, and Vettor R**. Induction of fatty acid translocase/CD36, peroxisome proliferator-activated receptor-gamma2, leptin, uncoupling proteins 2 and 3, and tumor necrosis factor-alpha gene expression in human subcutaneous fat by lipid infusion. *Diabetes* 49: 319-324, 2000.
57. **Chen M, Yang Y, Braunstein E, Georgeson KE, and Harmon CM**. Gut expression and regulation of FAT/CD36: possible role in fatty acid transport in rat enterocytes. *Am J Physiol Endocrinol Metab* 281: E916-923, 2001.
58. **Alexander Aguilera A, Hernandez Diaz G, Lara Barcelata M, Angulo Guerrero O, and Oliart Ros RM**. Induction of Cd36 expression elicited by fish oil PUFA in spontaneously hypertensive rats. *J Nutr Biochem* 17: 760-765, 2006.
59. **Montaigne D, Butruille L, and Staels B**. PPAR control of metabolism and cardiovascular functions. *Nat Rev Cardiol* 18: 809-823, 2021.
60. **Andersen M, Lenhard B, Whatling C, Eriksson P, and Odeberg J**. Alternative promoter usage of the membrane glycoprotein CD36. *BMC Mol Biol* 7: 8, 2006.

61. **Sato O, Kuriki C, Fukui Y, and Motojima K**. Dual promoter structure of mouse and human fatty acid translocase/CD36 genes and unique transcriptional activation by peroxisome proliferator-activated receptor alpha and gamma ligands. *J Biol Chem* 277: 15703-15711, 2002.
62. **Steger DJ, Lefterova MI, Ying L, Stonestrom AJ, Schupp M, Zhuo D, Vakoc AL, Kim JE, Chen J, Lazar MA, Blobel GA, and Vakoc CR**. DOT1L/KMT4 recruitment and H3K79 methylation are ubiquitously coupled with gene transcription in mammalian cells. *Mol Cell Biol* 28: 2825-2839, 2008.
63. **Mikkelsen TS, Xu Z, Zhang X, Wang L, Gimble JM, Lander ES, and Rosen ED**. Comparative epigenomic analysis of murine and human adipogenesis. *Cell* 143: 156-169, 2010.
64. **Sato O, Takanashi N, and Motojima K**. Third promoter and differential regulation of mouse and human fatty acid translocase/CD36 genes. *Mol Cell Biochem* 299: 37-43, 2007.
65. **Chanda D, Luiken JJ, and Glatz JF**. Signaling pathways involved in cardiac energy metabolism. *FEBS Lett* 590: 2364-2374, 2016.
66. **Li H, Fan J, Zhao Y, Zhang X, Dai B, Zhan J, Yin Z, Nie X, Fu XD, Chen C, and Wang DW**. Nuclear miR-320 Mediates Diabetes-Induced Cardiac Dysfunction by Activating Transcription of Fatty Acid Metabolic Genes to Cause Lipotoxicity in the Heart. *Circ Res* 125: 1106-1120, 2019.
67. **Li X, Zhang H, Wang Y, Li Y, Wang Y, Zhu J, and Lin Y**. Chi-Circ_0006511 Positively Regulates the Differentiation of Goat Intramuscular Adipocytes via Novel-miR-87/CD36 Axis. *Int J Mol Sci* 23: 2022.
68. **Lin HY, Wang FS, Yang YL, and Huang YH**. MicroRNA-29a Suppresses CD36 to Ameliorate High Fat Diet-Induced Steatohepatitis and Liver Fibrosis in Mice. *Cells* 8: 2019.
69. **Smolka C, Schlosser D, Hohnloser C, Bemtgen X, Janich C, Schneider L, Martin J, Pfeifer D, Moser M, Hasselblatt P, Bode C, Grundmann S, and Pankratz F**. MiR-100 overexpression attenuates high fat diet induced weight gain, liver steatosis, hypertriglyceridemia and development of metabolic syndrome in mice. *Mol Med* 27: 101, 2021.
70. **Aebi M**. N-linked protein glycosylation in the ER. *Biochim Biophys Acta* 1833: 2430-2437, 2013.
71. **Hoosdally SJ, Andress EJ, Wooding C, Martin CA, and Linton KJ**. The Human Scavenger Receptor CD36: glycosylation status and its role in trafficking and function. *J Biol Chem* 284: 16277-16288, 2009.
72. **Hart GW, Housley MP, and Slawson C**. Cycling of O-linked beta-N-acetylglucosamine on nucleocytoplasmic proteins. *Nature* 446: 1017-1022, 2007.
73. **Laczy B, Fulop N, Onay-Besikci A, Des Rosiers C, and Chatham JC**. Acute regulation of cardiac metabolism by the hexosamine biosynthesis pathway and protein O-GlcNAcylation. *PLoS One* 6: e18417, 2011.
74. **Lauzier B, Vaillant F, Merlen C, Gelinas R, Bouchard B, Rivard ME, Labarthe F, Dolinsky VW, Dyck JR, Allen BG, Chatham JC, and Des Rosiers C**. Metabolic effects of glutamine on the heart: anaplerosis versus the hexosamine biosynthetic pathway. *J Mol Cell Cardiol* 55: 92-100, 2013.
75. **Chu LY, and Silverstein RL**. CD36 ectodomain phosphorylation blocks thrombospondin-1 binding: structure-function relationships and regulation by protein kinase C. *Arterioscler Thromb Vasc Biol* 32: 760-767, 2012.
76. **Hatmi M, Gavaret JM, Elalamy I, Vargaftig BB, and Jacquemin C**. Evidence for cAMP-dependent platelet ectoprotein kinase activity that phosphorylates platelet glycoprotein IV (CD36). *J Biol Chem* 271: 24776-24780, 1996.
77. **Lynes M, Narisawa S, Millan JL, and Widmaier EP**. Interactions between CD36 and global intestinal alkaline phosphatase in mouse small intestine and effects of high-fat diet. *Am J Physiol Regul Integr Comp Physiol* 301: R1738-1747, 2011.
78. **Guthmann F, Maehl P, Preiss J, Kolleck I, and Rustow B**. Ectoprotein kinase-mediated phosphorylation of FAT/CD36 regulates palmitate uptake by human platelets. *Cell Mol Life Sci* 59: 1999-2003, 2002.
79. **Schianchi F, Glatz JFC, Navarro Gascon A, Nabben M, Neumann D, and Luiken J**. Putative Role of Protein Palmitoylation in Cardiac Lipid-Induced Insulin Resistance. *Int J Mol Sci* 21: 2020.
80. **Tao N, Wagner SJ, and Lublin DM**. CD36 is palmitoylated on both N- and C-terminal cytoplasmic tails. *J Biol Chem* 271: 22315-22320, 1996.
81. **van Oort MM, Drost R, Janbetaen L, Van Doorn JM, Kerver J, Van der Horst DJ, Luiken JJ, and Rodenburg KC**. Each of the four intracellular cysteines of CD36 is essential for insulin- or AMP-activated protein kinase-induced CD36 translocation. *Arch Physiol Biochem* 120: 40-49, 2014.

82. **Hao JW, Wang J, Guo H, Zhao YY, Sun HH, Li YF, Lai XY, Zhao N, Wang X, Xie C, Hong L, Huang X, Wang HR, Li CB, Liang B, Chen S, and Zhao TJ**. CD36 facilitates fatty acid uptake by dynamic palmitoylation-regulated endocytosis. *Nat Commun* 11: 4765, 2020.
83. **Jochen A, and Hays J**. Purification of the major substrate for palmitoylation in rat adipocytes: N-terminal homology with CD36 and evidence for cell surface acylation. *J Lipid Res* 34: 1783-1792, 1993.
84. **Xu Y, Shi Z, and Bao L**. An Expanding Repertoire of Protein Acylations. *Mol Cell Proteomics* 21: 100193, 2022.
85. **Main A, and Fuller W**. Protein S-Palmitoylation: advances and challenges in studying a therapeutically important lipid modification. *FEBS J* 289: 861-882, 2022.
86. **Smith J, Su X, El-Maghrabi R, Stahl PD, and Abumrad NA**. Opposite regulation of CD36 ubiquitination by fatty acids and insulin: effects on fatty acid uptake. *J Biol Chem* 283: 13578-13585, 2008.
87. **Kim KY, Stevens MV, Akter MH, Rusk SE, Huang RJ, Cohen A, Noguchi A, Springer D, Bocharov AV, Eggerman TL, Suen DF, Youle RJ, Amar M, Remaley AT, and Sack MN**. Parkin is a lipid-responsive regulator of fat uptake in mice and mutant human cells. *J Clin Invest* 121: 3701-3712, 2011.
88. **Glatz JFC, and Luiken J**. Dynamic role of the transmembrane glycoprotein CD36 (SR-B2) in cellular fatty acid uptake and utilization. *J Lipid Res* 59: 1084-1093, 2018.
89. **Pownall HJ, and Hamilton JA**. Energy translocation across cell membranes and membrane models. *Acta Physiol Scand* 178: 357-365, 2003.
90. **Hamilton JA**. New insights into the roles of proteins and lipids in membrane transport of fatty acids. *Prostaglandins Leukot Essent Fatty Acids* 77: 355-361, 2007.
91. **Kampf JP, and Kleinfeld AM**. Is membrane transport of FFA mediated by lipid, protein, or both? An unknown protein mediates free fatty acid transport across the adipocyte plasma membrane. *Physiology (Bethesda)* 22: 7-14, 2007.
92. **Bonen A, Chabowski A, Luiken JJ, and Glatz JF**. Is membrane transport of FFA mediated by lipid, protein, or both? Mechanisms and regulation of protein-mediated cellular fatty acid uptake: molecular, biochemical, and physiological evidence. *Physiology (Bethesda)* 22: 15-29, 2007.
93. **Pownall H, and Moore K**. Commentary on fatty acid wars: the diffusionists versus the translocatists. *Arterioscler Thromb Vasc Biol* 34: e8-9, 2014.
94. **Glatz JFC, and Luiken J**. Time for a detente in the war on the mechanism of cellular fatty acid uptake. *J Lipid Res* 61: 1300-1303, 2020.
95. **Kleinfeld AM**. Lipid phase fatty acid flip-flop, is it fast enough for cellular transport? *J Membr Biol* 175: 79-86, 2000.
96. **Vorum H, Brodersen R, Kragh-Hansen U, and Pedersen AO**. Solubility of long-chain fatty acids in phosphate buffer at pH 7.4. *Biochim Biophys Acta* 1126: 135-142, 1992.
97. **Glatz JF, and van der Vusse GJ**. Cellular fatty acid-binding proteins: their function and physiological significance. *Prog Lipid Res* 35: 243-282, 1996.
98. **Storch J, and Corsico B**. The emerging functions and mechanisms of mammalian fatty acid-binding proteins. *Annu Rev Nutr* 28: 73-95, 2008.
99. **Stremmel W, Strohmeyer G, Borchard F, Kochwa S, and Berk PD**. Isolation and partial characterization of a fatty acid binding protein in rat liver plasma membranes. *Proc Natl Acad Sci U S A* 82: 4-8, 1985.
100. **Schaffer JE, and Lodish HF**. Expression cloning and characterization of a novel adipocyte long chain fatty acid transport protein. *Cell* 79: 427-436, 1994.
101. **Trigatti BL, Anderson RG, and Gerber GE**. Identification of caveolin-1 as a fatty acid binding protein. *Biochem Biophys Res Commun* 255: 34-39, 1999.
102. **Glatz JF, Luiken JJ, and Bonen A**. Membrane fatty acid transporters as regulators of lipid metabolism: implications for metabolic disease. *Physiol Rev* 90: 367-417, 2010.
103. **Kazantzis M, and Stahl A**. Fatty acid transport proteins, implications in physiology and disease. *Biochim Biophys Acta* 1821: 852-857, 2012.
104. **Mallick R, Basak S, and Duttaroy AK**. Fatty acids and evolving roles of their proteins in neurological, cardiovascular disorders and cancers. *Prog Lipid Res* 83: 101116, 2021.
105. **Chabowski A, Gorski J, Luiken JJ, Glatz JF, and Bonen A**. Evidence for concerted action of FAT/CD36 and FABPpm to increase fatty acid transport across the plasma membrane. *Prostaglandins Leukot Essent Fatty Acids* 77: 345-353, 2007.

106. **Turcotte LP, Swenberger JR, Tucker MZ, Yee AJ, Trump G, Luiken JJ, and Bonen A**. Muscle palmitate uptake and binding are saturable and inhibited by antibodies to FABP(PM). *Mol Cell Biochem* 210: 53-63, 2000.
107. **Ring A, Le Lay S, Pohl J, Verkade P, and Stremmel W**. Caveolin-1 is required for fatty acid translocase (FAT/CD36) localization and function at the plasma membrane of mouse embryonic fibroblasts. *Biochim Biophys Acta* 1761: 416-423, 2006.
108. **Shvets E, Ludwig A, and Nichols BJ**. News from the caves: update on the structure and function of caveolae. *Curr Opin Cell Biol* 29: 99-106, 2014.
109. **Nickerson JG, Alkhateeb H, Benton CR, Lally J, Nickerson J, Han XX, Wilson MH, Jain SS, Snook LA, Glatz JFC, Chabowski A, Luiken J, and Bonen A**. Greater transport efficiencies of the membrane fatty acid transporters FAT/CD36 and FATP4 compared with FABPpm and FATP1 and differential effects on fatty acid esterification and oxidation in rat skeletal muscle. *J Biol Chem* 284: 16522-16530, 2009.
110. **Holloway GP, Nickerson JG, Lally JSV, Petrick HL, Dennis K, Jain SS, Alkhateeb H, and Bonen A**. Co-overexpression of CD36 and FABPpm increases fatty acid transport additively, not synergistically, within muscle. *Am J Physiol Cell Physiol* 322: C546-C553, 2022.
111. **Garcia NA, Gonzalez-King H, Grueso E, Sanchez R, Martinez-Romero A, Javega B, O'Connor JE, Simons PJ, Handberg A, and Sepulveda P**. Circulating exosomes deliver free fatty acids from the bloodstream to cardiac cells: Possible role of CD36. *PLoS One* 14: e0217546, 2019.
112. **Abumrad NA, Cabodevilla AG, Samovski D, Pietka T, Basu D, and Goldberg IJ**. Endothelial Cell Receptors in Tissue Lipid Uptake and Metabolism. *Circ Res* 128: 433-450, 2021.
113. **Habets DD, Coumans WA, Voshol PJ, den Boer MA, Febbraio M, Bonen A, Glatz JF, and Luiken JJ**. AMPK-mediated increase in myocardial long-chain fatty acid uptake critically depends on sarcolemmal CD36. *Biochem Biophys Res Commun* 355: 204-210, 2007.
114. **Abumrad NA, and Goldberg IJ**. CD36 actions in the heart: Lipids, calcium, inflammation, repair and more? *Biochim Biophys Acta* 1861: 1442-1449, 2016.
115. **Coburn CT, Knapp FF, Jr., Febbraio M, Beets AL, Silverstein RL, and Abumrad NA**. Defective uptake and utilization of long chain fatty acids in muscle and adipose tissues of CD36 knockout mice. *J Biol Chem* 275: 32523-32529, 2000.
116. **Luiken JJ, Willems J, van der Vusse GJ, and Glatz JF**. Electrostimulation enhances FAT/CD36-mediated long-chain fatty acid uptake by isolated rat cardiac myocytes. *Am J Physiol Endocrinol Metab* 281: E704-712, 2001.
117. **Angin Y, Steinbusch LK, Simons PJ, Greulich S, Hoebers NT, Douma K, van Zandvoort MA, Coumans WA, Wijnen W, Diamant M, Ouwens DM, Glatz JF, and Luiken JJ**. CD36 inhibition prevents lipid accumulation and contractile dysfunction in rat cardiomyocytes. *Biochem J* 448: 43-53, 2012.
118. **Son NH, Basu D, Samovski D, Pietka TA, Peche VS, Willecke F, Fang X, Yu SQ, Scerbo D, Chang HR, Sun F, Bagdasarov S, Drosatos K, Yeh ST, Mullick AE, Shoghi KI, Gumaste N, Kim K, Huggins LA, Lhakhang T, Abumrad NA, and Goldberg IJ**. Endothelial cell CD36 optimizes tissue fatty acid uptake. *J Clin Invest* 128: 4329-4342, 2018.
119. **Goldberg IJ, Cabodevilla AG, Samovski D, Cifarelli V, Basu D, and Abumrad NA**. Lipolytic enzymes and free fatty acids at the endothelial interface. *Atherosclerosis* 329: 1-8, 2021.
120. **Keizer HA, Schaart G, Tandon NN, Glatz JF, and Luiken JJ**. Subcellular immunolocalisation of fatty acid translocase (FAT)/CD36 in human type-1 and type-2 skeletal muscle fibres. *Histochem Cell Biol* 121: 101-107, 2004.
121. **Li LO, Klett EL, and Coleman RA**. Acyl-CoA synthesis, lipid metabolism and lipotoxicity. *Biochim Biophys Acta* 1801: 246-251, 2010.
122. **Stanley WC, Recchia FA, and Lopaschuk GD**. Myocardial substrate metabolism in the normal and failing heart. *Physiol Rev* 85: 1093-1129, 2005.
123. **Lee K, Kerner J, and Hoppel CL**. Mitochondrial carnitine palmitoyltransferase 1a (CPT1a) is part of an outer membrane fatty acid transfer complex. *J Biol Chem* 286: 25655-25662, 2011.
124. **Glatz JF, Nabben M, Heather LC, Bonen A, and Luiken JJ**. Regulation of the subcellular trafficking of CD36, a major determinant of cardiac fatty acid utilization. *Biochim Biophys Acta* 1861: 1461-1471, 2016.

125. **Luiken JJ, Niessen HE, Coort SL, Hoebers N, Coumans WA, Schwenk RW, Bonen A, and Glatz JF**. Etomoxir-induced partial carnitine palmitoyltransferase-I (CPT-I) inhibition in vivo does not alter cardiac long-chain fatty acid uptake and oxidation rates. *Biochem J* 419: 447-455, 2009.
126. **Lopaschuk GD, Ussher JR, Folmes CD, Jaswal JS, and Stanley WC**. Myocardial fatty acid metabolism in health and disease. *Physiol Rev* 90: 207-258, 2010.
127. **Luiken JJ, Koonen DP, Coumans WA, Pelsers MM, Binas B, Bonen A, and Glatz JF**. Long-chain fatty acid uptake by skeletal muscle is impaired in homozygous, but not heterozygous, heart-type-FABP null mice. *Lipids* 38: 491-496, 2003.
128. **Holloway GP, Luiken JJ, Glatz JF, Spriet LL, and Bonen A**. Contribution of FAT/CD36 to the regulation of skeletal muscle fatty acid oxidation: an overview. *Acta Physiol (Oxf)* 194: 293-309, 2008.
129. **Lombardi A, De Matteis R, Moreno M, Napolitano L, Busiello RA, Senese R, de Lange P, Lanni A, and Goglia F**. Responses of skeletal muscle lipid metabolism in rat gastrocnemius to hypothyroidism and iodothyronine administration: a putative role for FAT/CD36. *Am J Physiol Endocrinol Metab* 303: E1222-1233, 2012.
130. **Holloway GP, Bezaire V, Heigenhauser GJ, Tandon NN, Glatz JF, Luiken JJ, Bonen A, and Spriet LL**. Mitochondrial long chain fatty acid oxidation, fatty acid translocase/CD36 content and carnitine palmitoyltransferase I activity in human skeletal muscle during aerobic exercise. *J Physiol* 571: 201-210, 2006.
131. **Smith BK, Bonen A, and Holloway GP**. A dual mechanism of action for skeletal muscle FAT/CD36 during exercise. *Exerc Sport Sci Rev* 40: 211-217, 2012.
132. **King KL, Stanley WC, Rosca M, Kerner J, Hoppel CL, and Febbraio M**. Fatty acid oxidation in cardiac and skeletal muscle mitochondria is unaffected by deletion of CD36. *Arch Biochem Biophys* 467: 234-238, 2007.
133. **Bonen A, Luiken JJ, Arumugam Y, Glatz JF, and Tandon NN**. Acute regulation of fatty acid uptake involves the cellular redistribution of fatty acid translocase. *J Biol Chem* 275: 14501-14508, 2000.
134. **Muller H, Deckers K, and Eckel J**. The fatty acid translocase (FAT)/CD36 and the glucose transporter GLUT4 are localized in different cellular compartments in rat cardiac muscle. *Biochem Biophys Res Commun* 293: 665-669, 2002.
135. **Schwenk RW, Angin Y, Steinbusch LK, Dirkx E, Hoebers N, Coumans WA, Bonen A, Broers JL, van Eys GJ, Glatz JF, and Luiken JJ**. Overexpression of vesicle-associated membrane protein (VAMP) 3, but not VAMP2, protects glucose transporter (GLUT) 4 protein translocation in an in vitro model of cardiac insulin resistance. *J Biol Chem* 287: 37530-37539, 2012.
136. **Liu Y, Steinbusch LKM, Nabben M, Kapsokalyvas D, van Zandvoort M, Schonleitner P, Antoons G, Simons PJ, Coumans WA, Geomini A, Chanda D, Glatz JFC, Neumann D, and Luiken J**. Palmitate-Induced Vacuolar-Type H(+)-ATPase Inhibition Feeds Forward Into Insulin Resistance and Contractile Dysfunction. *Diabetes* 66: 1521-1534, 2017.
137. **Liu Y, Rodriguez-Calvo R, Wang S, Zhu X, Broers JLV, Glatz JFC, Luiken J, and Neumann D**. Fluorescent labelling of membrane fatty acid transporter CD36 (SR-B2) in the extracellular loop. *PLoS One* 14: e0210704, 2019.
138. **Jeppesen J, Mogensen M, Prats C, Sahlin K, Madsen K, and Kiens B**. FAT/CD36 is localized in sarcolemma and in vesicle-like structures in subsarcolemma regions but not in mitochondria. *J Lipid Res* 51: 1504-1512, 2010.
139. **Casey JR, Grinstein S, and Orlowski J**. Sensors and regulators of intracellular pH. *Nat Rev Mol Cell Biol* 11: 50-61, 2010.
140. **Taguchi T**. Emerging roles of recycling endosomes. *J Biochem* 153: 505-510, 2013.
141. **Luiken JJ, Coort SL, Willems J, Coumans WA, Bonen A, van der Vusse GJ, and Glatz JF**. Contraction-induced fatty acid translocase/CD36 translocation in rat cardiac myocytes is mediated through AMP-activated protein kinase signaling. *Diabetes* 52: 1627-1634, 2003.
142. **Luiken JJ, Dyck DJ, Han XX, Tandon NN, Arumugam Y, Glatz JF, and Bonen A**. Insulin induces the translocation of the fatty acid transporter FAT/CD36 to the plasma membrane. *Am J Physiol Endocrinol Metab* 282: E491-495, 2002.
143. **Luiken JJ, Koonen DP, Willems J, Zorzano A, Becker C, Fischer Y, Tandon NN, Van Der Vusse GJ, Bonen A, and Glatz JF**. Insulin stimulates long-chain fatty acid utilization by rat cardiac myocytes through cellular redistribution of FAT/CD36. *Diabetes* 51: 3113-3119, 2002.

144. **Tokarz VL, MacDonald PE, and Klip A**. The cell biology of systemic insulin function. *J Cell Biol* 217: 2273-2289, 2018.
145. **Abel ED**. Insulin signaling in the heart. *Am J Physiol Endocrinol Metab* 321: E130-E145, 2021.
146. **Jain SS, Luiken JJ, Snook LA, Han XX, Holloway GP, Glatz JF, and Bonen A**. Fatty acid transport and transporters in muscle are critically regulated by Akt2. *FEBS Lett* 589: 2769-2775, 2015.
147. **Habets DD, Luiken JJ, Ouwens M, Coumans WA, Vergouwe M, Maarbjerg SJ, Leitges M, Bonen A, Richter EA, and Glatz JF**. Involvement of atypical protein kinase C in the regulation of cardiac glucose and long-chain fatty acid uptake. *Front Physiol* 3: 361, 2012.
148. **Jain SS, Chabowski A, Snook LA, Schwenk RW, Glatz JF, Luiken JJ, and Bonen A**. Additive effects of insulin and muscle contraction on fatty acid transport and fatty acid transporters, FAT/CD36, FABPpm, FATP1, 4 and 6. *FEBS Lett* 583: 2294-2300, 2009.
149. **Habets DD, Coumans WA, El Hasnaoui M, Zarrinpashneh E, Bertrand L, Viollet B, Kiens B, Jensen TE, Richter EA, Bonen A, Glatz JF, and Luiken JJ**. Crucial role for LKB1 to AMPKalpha2 axis in the regulation of CD36-mediated long-chain fatty acid uptake into cardiomyocytes. *Biochim Biophys Acta* 1791: 212-219, 2009.
150. **Luiken JJ, Ouwens DM, Habets DD, van der Zon GC, Coumans WA, Schwenk RW, Bonen A, and Glatz JF**. Permissive action of protein kinase C-zeta in insulin-induced CD36- and GLUT4 translocation in cardiac myocytes. *J Endocrinol* 201: 199-209, 2009.
151. **Klip A, McGraw TE, and James DE**. Thirty sweet years of GLUT4. *J Biol Chem* 294: 11369-11381, 2019.
152. **Luiken JJ, Coort SL, Koonen DP, van der Horst DJ, Bonen A, Zorzano A, and Glatz JF**. Regulation of cardiac long-chain fatty acid and glucose uptake by translocation of substrate transporters. *Pflugers Arch* 448: 1-15, 2004.
153. **Luiken J, Nabben M, Neumann D, and Glatz JFC**. Understanding the distinct subcellular trafficking of CD36 and GLUT4 during the development of myocardial insulin resistance. *Biochim Biophys Acta Mol Basis Dis* 1866: 165775, 2020.
154. **Yamamoto H, Ida T, Tsutsuki H, Mori M, Matsumoto T, Kohda T, Mukamoto M, Goshima N, Kozaki S, and Ihara H**. Specificity of botulinum protease for human VAMP family proteins. *Microbiol Immunol* 56: 245-253, 2012.
155. **Kumar R, Feltrup TM, Kukreja RV, Patel KB, Cai S, and Singh BR**. Evolutionary Features in the Structure and Function of Bacterial Toxins. *Toxins (Basel)* 11: 2019.
156. **Wang T, Li L, and Hong W**. SNARE proteins in membrane trafficking. *Traffic* 18: 767-775, 2017.
157. **Larance M, Ramm G, Stockli J, van Dam EM, Winata S, Wasinger V, Simpson F, Graham M, Junutula JR, Guilhaus M, and James DE**. Characterization of the role of the Rab GTPase-activating protein AS160 in insulin-regulated GLUT4 trafficking. *J Biol Chem* 280: 37803-37813, 2005.
158. **Steinbusch LK, Wijnen W, Schwenk RW, Coumans WA, Hoebers NT, Ouwens DM, Coumans WA, Hoebers NT, Diamant M, Bonen A, Glatz JF, and Luiken JJ**. Differential regulation of cardiac glucose and fatty acid uptake by endosomal pH and actin filaments. *Am J Physiol Cell Physiol* 298: C1549-1559, 2010.
159. **Yeagle PL**. Lipid regulation of cell membrane structure and function. *FASEB J* 3: 1833-1842, 1989.
160. **Forgac M**. Vacuolar ATPases: rotary proton pumps in physiology and pathophysiology. *Nat Rev Mol Cell Biol* 8: 917-929, 2007.
161. **Wang S, Han Y, Nabben M, Neumann D, Luiken J, and Glatz JFC**. Endosomal v-ATPase as a Sensor Determining Myocardial Substrate Preference. *Metabolites* 12: 2022.
162. **Sautin YY, Lu M, Gaugler A, Zhang L, and Gluck SL**. Phosphatidylinositol 3-kinase-mediated effects of glucose on vacuolar H+-ATPase assembly, translocation, and acidification of intracellular compartments in renal epithelial cells. *Mol Cell Biol* 25: 575-589, 2005.
163. **Hurley JH, Boura E, Carlson LA, and Rozycki B**. Membrane budding. *Cell* 143: 875-887, 2010.
164. **Donaldson JG, Lippincott-Schwartz J, Bloom GS, Kreis TE, and Klausner RD**. Dissociation of a 110-kD peripheral membrane protein from the Golgi apparatus is an early event in brefeldin A action. *J Cell Biol* 111: 2295-2306, 1990.
165. **Pohl J, Ring A, Ehehalt R, Schulze-Bergkamen H, Schad A, Verkade P, and Stremmel W**. Long-chain fatty acid uptake into adipocytes depends on lipid raft function. *Biochemistry* 43: 4179-4187, 2004.
166. **Pohl J, Ring A, Korkmaz U, Ehehalt R, and Stremmel W**. FAT/CD36-mediated long-chain fatty acid uptake in adipocytes requires plasma membrane rafts. *Mol Biol Cell* 16: 24-31, 2005.

167. **Matthaeus C, Lahmann I, Kunz S, Jonas W, Melo AA, Lehmann M, Larsson E, Lundmark R, Kern M, Bluher M, Olschowski H, Kompa J, Brugger B, Muller DN, Haucke V, Schurmann A, Birchmeier C, and Daumke O**. EHD2-mediated restriction of caveolar dynamics regulates cellular fatty acid uptake. *Proc Natl Acad Sci U S A* 117: 7471-7481, 2020.
168. **Augustus AS, Buchanan J, Addya S, Rengo G, Pestell RG, Fortina P, Koch WJ, Bensadoun A, Abel ED, and Lisanti MP**. Substrate uptake and metabolism are preserved in hypertrophic caveolin-3 knockout hearts. *Am J Physiol Heart Circ Physiol* 295: H657-666, 2008.
169. **Frasa MA, Koessmeier KT, Ahmadian MR, and Braga VM**. Illuminating the functional and structural repertoire of human TBC/RABGAPs. *Nat Rev Mol Cell Biol* 13: 67-73, 2012.
170. **Sakamoto K, and Holman GD**. Emerging role for AS160/TBC1D4 and TBC1D1 in the regulation of GLUT4 traffic. *Am J Physiol Endocrinol Metab* 295: E29-37, 2008.
171. **Mafakheri S, Chadt A, and Al-Hasani H**. Regulation of RabGAPs involved in insulin action. *Biochem Soc Trans* 46: 683-690, 2018.
172. **Cartee GD, and Wojtaszewski JF**. Role of Akt substrate of 160 kDa in insulin-stimulated and contraction-stimulated glucose transport. *Appl Physiol Nutr Metab* 32: 557-566, 2007.
173. **Thong FS, Bilan PJ, and Klip A**. The Rab GTPase-activating protein AS160 integrates Akt, protein kinase C, and AMP-activated protein kinase signals regulating GLUT4 traffic. *Diabetes* 56: 414-423, 2007.
174. **Roach WG, Chavez JA, Miinea CP, and Lienhard GE**. Substrate specificity and effect on GLUT4 translocation of the Rab GTPase-activating protein Tbc1d1. *Biochem J* 403: 353-358, 2007.
175. **Espelage L, Al-Hasani H, and Chadt A**. RabGAPs in skeletal muscle function and exercise. *J Mol Endocrinol* 64: R1-R19, 2020.
176. **Samovski D, Su X, Xu Y, Abumrad NA, and Stahl PD**. Insulin and AMPK regulate FA translocase/CD36 plasma membrane recruitment in cardiomyocytes via Rab GAP AS160 and Rab8a Rab GTPase. *J Lipid Res* 53: 709-717, 2012.
177. **Miklosz A, Lukaszuk B, Zendzian-Piotrowska M, Kurek K, and Chabowski A**. The Effects of AS160 Modulation on Fatty Acid Transporters Expression and Lipid Profile in L6 Myotubes. *Cell Physiol Biochem* 38: 267-282, 2016.
178. **Zhu B, Li MY, Lin Q, Liang Z, Xin Q, Wang M, He Z, Wang X, Wu X, Chen GG, Tong PC, Zhang W, and Liu LZ**. Lipid oversupply induces CD36 sarcolemmal translocation via dual modulation of PKCzeta and TBC1D1: an early event prior to insulin resistance. *Theranostics* 10: 1332-1354, 2020.
179. **Sano H, Roach WG, Peck GR, Fukuda M, and Lienhard GE**. Rab10 in insulin-stimulated GLUT4 translocation. *Biochem J* 411: 89-95, 2008.
180. **Peranen J**. Rab8 GTPase as a regulator of cell shape. *Cytoskeleton (Hoboken)* 68: 527-539, 2011.
181. **Welz T, Wellbourne-Wood J, and Kerkhoff E**. Orchestration of cell surface proteins by Rab11. *Trends Cell Biol* 24: 407-415, 2014.
182. **Lim YS, Chua CE, and Tang BL**. Rabs and other small GTPases in ciliary transport. *Biol Cell* 103: 209-221, 2011.
183. **Khandelwal P, Prakasam HS, Clayton DR, Ruiz WG, Gallo LI, van Roekel D, Lukianov S, Peranen J, Goldenring JR, and Apodaca G**. A Rab11a-Rab8a-Myo5B network promotes stretch-regulated exocytosis in bladder umbrella cells. *Mol Biol Cell* 24: 1007-1019, 2013.
184. **Essid M, Gopaldass N, Yoshida K, Merrifield C, and Soldati T**. Rab8a regulates the exocyst-mediated kiss-and-run discharge of the Dictyostelium contractile vacuole. *Mol Biol Cell* 23: 1267-1282, 2012.
185. **Zhu S, Bhat S, Syan S, Kuchitsu Y, Fukuda M, and Zurzolo C**. Rab11a-Rab8a cascade regulates the formation of tunneling nanotubes through vesicle recycling. *J Cell Sci* 131: 2018.
186. **Schwenk RW, Luiken JJ, and Eckel J**. FIP2 and Rip11 specify Rab11a-mediated cellular distribution of GLUT4 and FAT/CD36 in H9c2-hIR cells. *Biochem Biophys Res Commun* 363: 119-125, 2007.
187. **Jiang C, Liu Z, Hu R, Bo L, Minshall RD, Malik AB, and Hu G**. Inactivation of Rab11a GTPase in Macrophages Facilitates Phagocytosis of Apoptotic Neutrophils. *J Immunol* 198: 1660-1672, 2017.
188. **Horgan CP, and McCaffrey MW**. The dynamic Rab11-FIPs. *Biochem Soc Trans* 37: 1032-1036, 2009.
189. **Sun Y, Chiu TT, Foley KP, Bilan PJ, and Klip A**. Myosin Va mediates Rab8A-regulated GLUT4 vesicle exocytosis in insulin-stimulated muscle cells. *Mol Biol Cell* 25: 1159-1170, 2014.
190. **Margiotta A, and Bucci C**. Role of Intermediate Filaments in Vesicular Traffic. *Cells* 5: 2016.

191. **Sollner T, Bennett MK, Whiteheart SW, Scheller RH, and Rothman JE**. A protein assembly-disassembly pathway in vitro that may correspond to sequential steps of synaptic vesicle docking, activation, and fusion. *Cell* 75: 409-418, 1993.
192. **Yoon TY, and Munson M**. SNARE complex assembly and disassembly. *Curr Biol* 28: R397-R401, 2018.
193. **Rossi V, Picco R, Vacca M, D'Esposito M, D'Urso M, Galli T, and Filippini F**. VAMP subfamilies identified by specific R-SNARE motifs. *Biol Cell* 96: 251-256, 2004.
194. **Schwenk RW, Dirkx E, Coumans WA, Bonen A, Klip A, Glatz JF, and Luiken JJ**. Requirement for distinct vesicle-associated membrane proteins in insulin- and AMP-activated protein kinase (AMPK)-induced translocation of GLUT4 and CD36 in cultured cardiomyocytes. *Diabetologia* 53: 2209-2219, 2010.
195. **Jain SS, Snook LA, Glatz JF, Luiken JJ, Holloway GP, Thurmond DC, and Bonen A**. Munc18c provides stimulus-selective regulation of GLUT4 but not fatty acid transporter trafficking in skeletal muscle. *FEBS Lett* 586: 2428-2435, 2012.
196. **Samovski D, Jacome-Sosa M, and Abumrad NA**. Fatty Acid Transport and Signaling: Mechanisms and Physiological Implications. *Annu Rev Physiol* 85: 317-337, 2023.
197. **Huang MM, Bolen JB, Barnwell JW, Shattil SJ, and Brugge JS**. Membrane glycoprotein IV (CD36) is physically associated with the Fyn, Lyn, and Yes protein-tyrosine kinases in human platelets. *Proc Natl Acad Sci U S A* 88: 7844-7848, 1991.
198. **Gilbertson TA, and Khan NA**. Cell signaling mechanisms of oro-gustatory detection of dietary fat: advances and challenges. *Prog Lipid Res* 53: 82-92, 2014.
199. **Sundaresan S, and Abumrad NA**. Dietary Lipids Inform the Gut and Brain about Meal Arrival via CD36-Mediated Signal Transduction. *J Nutr* 145: 2195-2200, 2015.
200. **Samovski D, Sun J, Pietka T, Gross RW, Eckel RH, Su X, Stahl PD, and Abumrad NA**. Regulation of AMPK activation by CD36 links fatty acid uptake to beta-oxidation. *Diabetes* 64: 353-359, 2015.
201. **Medzhitov R**. Recognition of microorganisms and activation of the immune response. *Nature* 449: 819-826, 2007.
202. **Stewart CR, Stuart LM, Wilkinson K, van Gils JM, Deng J, Halle A, Rayner KJ, Boyer L, Zhong R, Frazier WA, Lacy-Hulbert A, El Khoury J, Golenbock DT, and Moore KJ**. CD36 ligands promote sterile inflammation through assembly of a Toll-like receptor 4 and 6 heterodimer. *Nat Immunol* 11: 155-161, 2010.
203. **Rocha DM, Caldas AP, Oliveira LL, Bressan J, and Hermsdorff HH**. Saturated fatty acids trigger TLR4-mediated inflammatory response. *Atherosclerosis* 244: 211-215, 2016.
204. **Stuart LM, Deng J, Silver JM, Takahashi K, Tseng AA, Hennessy EJ, Ezekowitz RA, and Moore KJ**. Response to Staphylococcus aureus requires CD36-mediated phagocytosis triggered by the COOH-terminal cytoplasmic domain. *J Cell Biol* 170: 477-485, 2005.
205. **Bezhaeva T, Karper J, Quax PHA, and de Vries MR**. The Intriguing Role of TLR Accessory Molecules in Cardiovascular Health and Disease. *Front Cardiovasc Med* 9: 820962, 2022.
206. **Rupert JE, and Kolonin MG**. Fatty acid translocase: a culprit of lipid metabolism dysfunction in disease. *Immunometabolism (Cobham)* 4: e00001, 2022.
207. **Abel ED, Litwin SE, and Sweeney G**. Cardiac remodeling in obesity. *Physiol Rev* 88: 389-419, 2008.
208. **Yang J, Sambandam N, Han X, Gross RW, Courtois M, Kovacs A, Febbraio M, Finck BN, and Kelly DP**. CD36 deficiency rescues lipotoxic cardiomyopathy. *Circ Res* 100: 1208-1217, 2007.
209. **Karunakaran U, Elumalai S, Moon JS, and Won KC**. CD36 Signal Transduction in Metabolic Diseases: Novel Insights and Therapeutic Targeting. *Cells* 10: 2021.
210. **Glatz JFC, Wang F, Nabben M, and Luiken J**. CD36 as a target for metabolic modulation therapy in cardiac disease. *Expert Opin Ther Targets* 25: 393-400, 2021.
211. **Glatz JFC, Nabben M, and Luiken J**. CD36 (SR-B2) as master regulator of cellular fatty acid homeostasis. *Curr Opin Lipidol* 33: 103-111, 2022.
212. **Scheuer J**. Myocardial metabolism in cardiac hypoxia. *Am J Cardiol* 19: 385-392, 1967.
213. **Opie LH**. Acute metabolic response in myocardial infarction. *Br Heart J* 33: Suppl:129-137, 1971.
214. **Kerr M, Dennis K, Carr CA, Fuller W, Berridge G, Rohling S, Aitken CL, Lopez C, Fischer R, Miller JJ, Clarke K, Tyler DJ, and Heather LC**. Diabetic mitochondria are resistant to palmitoyl CoA inhibition of respiration, which is detrimental during ischemia. *FASEB J* 35: e21765, 2021.
215. **Heather LC, Pates KM, Atherton HJ, Cole MA, Ball DR, Evans RD, Glatz JF, Luiken JJ, Griffin JL, and Clarke K**. Differential translocation of the fatty acid transporter, FAT/CD36, and the glucose

transporter, GLUT4, coordinates changes in cardiac substrate metabolism during ischemia and reperfusion. *Circ Heart Fail* 6: 1058-1066, 2013.

216. **Chen YP, Hsu HH, Baskaran R, Wen SY, Shen CY, Day CH, Ho TJ, Vijaya Padma V, Kuo WW, and Huang CY**. Short-Term Hypoxia Reverses Ox-LDL-Induced CD36 and GLUT4 Switching Metabolic Pathways in H9c2 Cardiomyoblast Cells. *J Cell Biochem* 118: 3785-3795, 2017.
217. **Irie H, Krukenkamp IB, Brinkmann JF, Gaudette GR, Saltman AE, Jou W, Glatz JF, Abumrad NA, and Ibrahimi A**. Myocardial recovery from ischemia is impaired in CD36-null mice and restored by myocyte CD36 expression or medium-chain fatty acids. *Proc Natl Acad Sci U S A* 100: 6819-6824, 2003.
218. **Kuang M, Febbraio M, Wagg C, Lopaschuk GD, and Dyck JR**. Fatty acid translocase/CD36 deficiency does not energetically or functionally compromise hearts before or after ischemia. *Circulation* 109: 1550-1557, 2004.
219. **Nagendran J, Pulinilkunnil T, Kienesberger PC, Sung MM, Fung D, Febbraio M, and Dyck JR**. Cardiomyocyte-specific ablation of CD36 improves post-ischemic functional recovery. *J Mol Cell Cardiol* 63: 180-188, 2013.
220. **Rubler S, Dlugash J, Yuceoglu YZ, Kumral T, Branwood AW, and Grishman A**. New type of cardiomyopathy associated with diabetic glomerulosclerosis. *Am J Cardiol* 30: 595-602, 1972.
221. **Ritchie RH, and Abel ED**. Basic Mechanisms of Diabetic Heart Disease. *Circ Res* 126: 1501-1525, 2020.
222. **Marwick TH, Ritchie R, Shaw JE, and Kaye D**. Implications of Underlying Mechanisms for the Recognition and Management of Diabetic Cardiomyopathy. *J Am Coll Cardiol* 71: 339-351, 2018.
223. **Boudina S, and Abel ED**. Diabetic cardiomyopathy, causes and effects. *Rev Endocr Metab Disord* 11: 31-39, 2010.
224. **Glatz JFC, Luiken J, and Nabben M**. CD36 (SR-B2) as a Target to Treat Lipid Overload-Induced Cardiac Dysfunction. *J Lipid Atheroscler* 9: 66-78, 2020.
225. **Wang S, Schianchi F, Neumann D, Wong LY, Sun A, van Nieuwenhoven FA, Zeegers MP, Strzelecka A, Col U, Glatz JFC, Nabben M, and Luiken J**. Specific amino acid supplementation rescues the heart from lipid overload-induced insulin resistance and contractile dysfunction by targeting the endosomal mTOR-v-ATPase axis. *Mol Metab* 53: 101293, 2021.
226. **Bonen A, Parolin ML, Steinberg GR, Calles-Escandon J, Tandon NN, Glatz JF, Luiken JJ, Heigenhauser GJ, and Dyck DJ**. Triacylglycerol accumulation in human obesity and type 2 diabetes is associated with increased rates of skeletal muscle fatty acid transport and increased sarcolemmal FAT/CD36. *FASEB J* 18: 1144-1146, 2004.
227. **Aguer C, Mercier J, Man CY, Metz L, Bordenave S, Lambert K, Jean E, Lantier L, Bounoua L, Brun JF, Raynaud de Mauverger E, Andreelli F, Foretz M, and Kitzmann M**. Intramyocellular lipid accumulation is associated with permanent relocation ex vivo and in vitro of fatty acid translocase (FAT)/CD36 in obese patients. *Diabetologia* 53: 1151-1163, 2010.
228. **Aguer C, Foretz M, Lantier L, Hebrard S, Viollet B, Mercier J, and Kitzmann M**. Increased FAT/CD36 cycling and lipid accumulation in myotubes derived from obese type 2 diabetic patients. *PLoS One* 6: e28981, 2011.
229. **Chen Q, Rong P, Xu D, Zhu S, Chen L, Xie B, Du Q, Quan C, Sheng Y, Zhao TJ, Li P, Wang HY, and Chen S**. Rab8a Deficiency in Skeletal Muscle Causes Hyperlipidemia and Hepatosteatosis by Impairing Muscle Lipid Uptake and Storage. *Diabetes* 66: 2387-2399, 2017.
230. **Ouwens DM, Diamant M, Fodor M, Habets DDJ, Pelsers M, El Hasnaoui M, Dang ZC, van den Brom CE, Vlasblom R, Rietdijk A, Boer C, Coort SLM, Glatz JFC, and Luiken J**. Cardiac contractile dysfunction in insulin-resistant rats fed a high-fat diet is associated with elevated CD36-mediated fatty acid uptake and esterification. *Diabetologia* 50: 1938-1948, 2007.
231. **Dirkx E, van Eys GJ, Schwenk RW, Steinbusch LK, Hoebers N, Coumans WA, Peters T, Janssen BJ, Brans B, Vogg AT, Neumann D, Glatz JF, and Luiken JJ**. Protein kinase-D1 overexpression prevents lipid-induced cardiac insulin resistance. *J Mol Cell Cardiol* 76: 208-217, 2014.
232. **Coort SL, Hasselbaink DM, Koonen DP, Willems J, Coumans WA, Chabowski A, van der Vusse GJ, Bonen A, Glatz JF, and Luiken JJ**. Enhanced sarcolemmal FAT/CD36 content and triacylglycerol storage in cardiac myocytes from obese zucker rats. *Diabetes* 53: 1655-1663, 2004.
233. **Carley AN, Atkinson LL, Bonen A, Harper ME, Kunnathu S, Lopaschuk GD, and Severson DL**. Mechanisms responsible for enhanced fatty acid utilization by perfused hearts from type 2 diabetic db/db mice. *Arch Physiol Biochem* 113: 65-75, 2007.

234. **Lou PH, Lucchinetti E, Scott KY, Huang Y, Gandhi M, Hersberger M, Clanachan AS, Lemieux H, and Zaugg M**. Alterations in fatty acid metabolism and sirtuin signaling characterize early type-2 diabetic hearts of fructose-fed rats. *Physiol Rep* 5: 2017.
235. **Luiken JJ, Arumugam Y, Dyck DJ, Bell RC, Pelsers MM, Turcotte LP, Tandon NN, Glatz JF, and Bonen A**. Increased rates of fatty acid uptake and plasmalemmal fatty acid transporters in obese Zucker rats. *J Biol Chem* 276: 40567-40573, 2001.
236. **Bonen A, Jain SS, Snook LA, Han XX, Yoshida Y, Buddo KH, Lally JS, Pask ED, Paglialunga S, Beaudoin MS, Glatz JF, Luiken JJ, Harasim E, Wright DC, Chabowski A, and Holloway GP**. Extremely rapid increase in fatty acid transport and intramyocellular lipid accumulation but markedly delayed insulin resistance after high fat feeding in rats. *Diabetologia* 58: 2381-2391, 2015.
237. **Greenwalt DE, Scheck SH, and Rhinehart-Jones T**. Heart CD36 expression is increased in murine models of diabetes and in mice fed a high fat diet. *J Clin Invest* 96: 1382-1388, 1995.
238. **Ge F, Hu C, Hyodo E, Arai K, Zhou S, Lobdell Ht, Walewski JL, Homma S, and Berk PD**. Cardiomyocyte triglyceride accumulation and reduced ventricular function in mice with obesity reflect increased long chain Fatty Acid uptake and de novo Fatty Acid synthesis. *J Obes* 2012: 205648, 2012.
239. **Mansor LS, Gonzalez ER, Cole MA, Tyler DJ, Beeson JH, Clarke K, Carr CA, and Heather LC**. Cardiac metabolism in a new rat model of type 2 diabetes using high-fat diet with low dose streptozotocin. *Cardiovasc Diabetol* 12: 136, 2013.
240. **Cai H, Chen S, Liu J, and He Y**. An attempt to reverse cardiac lipotoxicity by aerobic interval training in a high-fat diet- and streptozotocin-induced type 2 diabetes rat model. *Diabetol Metab Syndr* 11: 43, 2019.
241. **Luiken JJ, Arumugam Y, Bell RC, Calles-Escandon J, Tandon NN, Glatz JF, and Bonen A**. Changes in fatty acid transport and transporters are related to the severity of insulin deficiency. *Am J Physiol Endocrinol Metab* 283: E612-621, 2002.
242. **Bagh MB, Peng S, Chandra G, Zhang Z, Singh SP, Pattabiraman N, Liu A, and Mukherjee AB**. Misrouting of v-ATPase subunit V0a1 dysregulates lysosomal acidification in a neurodegenerative lysosomal storage disease model. *Nat Commun* 8: 14612, 2017.
243. **Nixon RA**. The role of autophagy in neurodegenerative disease. *Nat Med* 19: 983-997, 2013.
244. **Sharma S, Adrogue JV, Golfman L, Uray I, Lemm J, Youker K, Noon GP, Frazier OH, and Taegtmeyer H**. Intramyocardial lipid accumulation in the failing human heart resembles the lipotoxic rat heart. *FASEB J* 18: 1692-1700, 2004.
245. **Shulman GI**. Cellular mechanisms of insulin resistance. *J Clin Invest* 106: 171-176, 2000.
246. **Dagenais GR, Tancredi RG, and Zierler KL**. Free fatty acid oxidation by forearm muscle at rest, and evidence for an intramuscular lipid pool in the human forearm. *J Clin Invest* 58: 421-431, 1976.
247. **Luiken JJ, Turcotte LP, and Bonen A**. Protein-mediated palmitate uptake and expression of fatty acid transport proteins in heart giant vesicles. *J Lipid Res* 40: 1007-1016, 1999.
248. **Bostrom P, Andersson L, Rutberg M, Perman J, Lidberg U, Johansson BR, Fernandez-Rodriguez J, Ericson J, Nilsson T, Boren J, and Olofsson SO**. SNARE proteins mediate fusion between cytosolic lipid droplets and are implicated in insulin sensitivity. *Nat Cell Biol* 9: 1286-1293, 2007.
249. **Merz KE, Hwang J, Zhou C, Veluthakal R, McCown EM, Hamilton A, Oh E, Dai W, Fueger PT, Jiang L, Huss JM, and Thurmond DC**. Enrichment of the exocytosis protein STX4 in skeletal muscle remediates peripheral insulin resistance and alters mitochondrial dynamics via Drp1. *Nat Commun* 13: 424, 2022.
250. **Pei J, Prasad M, Mohamed Helal G, El-Sherbiny M, Abdelmonem Elsherbini DM, Rajagopal P, Palanisamy CP, Veeraraghavan VP, Jayaraman S, and Surapaneni KM**. Beta-Sitosterol Facilitates GLUT4 Vesicle Fusion on the Plasma Membrane via the Activation of Rab/IRAP/Munc 18 Signaling Pathways in Diabetic Gastrocnemius Muscle of Adult Male Rats. *Bioinorg Chem Appl* 2022: 7772305, 2022.
251. **Ishiki M, and Klip A**. Minireview: recent developments in the regulation of glucose transporter-4 traffic: new signals, locations, and partners. *Endocrinology* 146: 5071-5078, 2005.
252. **Nakamura M, and Sadoshima J**. Mechanisms of physiological and pathological cardiac hypertrophy. *Nat Rev Cardiol* 15: 387-407, 2018.
253. **Yildiz M, Oktay AA, Stewart MH, Milani RV, Ventura HO, and Lavie CJ**. Left ventricular hypertrophy and hypertension. *Prog Cardiovasc Dis* 63: 10-21, 2020.

254. **Abdurrachim D, Nabben M, Hoerr V, Kuhlmann MT, Bovenkamp P, Ciapaite J, Geraets IME, Coumans W, Luiken J, Glatz JFC, Schafers M, Nicolay K, Faber C, Hermann S, and Prompers JJ**. Diabetic db/db mice do not develop heart failure upon pressure overload: a longitudinal in vivo PET, MRI, and MRS study on cardiac metabolic, structural, and functional adaptations. *Cardiovasc Res* 113: 1148-1160, 2017.
255. **Geraets IME, Glatz JFC, Luiken J, and Nabben M**. Pivotal role of membrane substrate transporters on the metabolic alterations in the pressure-overloaded heart. *Cardiovasc Res* 115: 1000-1012, 2019.
256. **Shu H, Peng Y, Hang W, Nie J, Zhou N, and Wang DW**. The role of CD36 in cardiovascular disease. *Cardiovasc Res* 118: 115-129, 2022.
257. **Sung MM, Byrne NJ, Kim TT, Levasseur J, Masson G, Boisvenue JJ, Febbraio M, and Dyck JR**. Cardiomyocyte-specific ablation of CD36 accelerates the progression from compensated cardiac hypertrophy to heart failure. *Am J Physiol Heart Circ Physiol* 312: H552-H560, 2017.
258. **Iemitsu M, Miyauchi T, Maeda S, Sakai S, Fujii N, Miyazaki H, Kakinuma Y, Matsuda M, and Yamaguchi I**. Cardiac hypertrophy by hypertension and exercise training exhibits different gene expression of enzymes in energy metabolism. *Hypertens Res* 26: 829-837, 2003.
259. **Dong D, Li L, Gu P, Jin T, Wen M, Yuan C, Gao X, Liu C, and Zhang Z**. Profiling metabolic remodeling in PP2Acalpha deficiency and chronic pressure overload mouse hearts. *FEBS Lett* 589: 3631-3639, 2015.
260. **Dobrzyn P, Pyrkowska A, Duda MK, Bednarski T, Maczewski M, Langfort J, and Dobrzyn A**. Expression of lipogenic genes is upregulated in the heart with exercise training-induced but not pressure overload-induced left ventricular hypertrophy. *Am J Physiol Endocrinol Metab* 304: E1348-1358, 2013.
261. **Kaimoto S, Hoshino A, Ariyoshi M, Okawa Y, Tateishi S, Ono K, Uchihashi M, Fukai K, Iwai-Kanai E, and Matoba S**. Activation of PPAR-alpha in the early stage of heart failure maintained myocardial function and energetics in pressure-overload heart failure. *Am J Physiol Heart Circ Physiol* 312: H305-H313, 2017.
262. **Riehle C, Wende AR, Zaha VG, Pires KM, Wayment B, Olsen C, Bugger H, Buchanan J, Wang X, Moreira AB, Doenst T, Medina-Gomez G, Litwin SE, Lelliott CJ, Vidal-Puig A, and Abel ED**. PGC-1beta deficiency accelerates the transition to heart failure in pressure overload hypertrophy. *Circ Res* 109: 783-793, 2011.
263. **Kolwicz SC, Jr., Olson DP, Marney LC, Garcia-Menendez L, Synovec RE, and Tian R**. Cardiac-specific deletion of acetyl CoA carboxylase 2 prevents metabolic remodeling during pressure-overload hypertrophy. *Circ Res* 111: 728-738, 2012.
264. **Guo Y, Wang Z, Qin X, Xu J, Hou Z, Yang H, Mao X, Xing W, Li X, Zhang X, and Gao F**. Enhancing fatty acid utilization ameliorates mitochondrial fragmentation and cardiac dysfunction via rebalancing optic atrophy 1 processing in the failing heart. *Cardiovasc Res* 114: 979-991, 2018.
265. **Bednarski TK, Duda MK, and Dobrzyn P**. Alterations of Lipid Metabolism in the Heart in Spontaneously Hypertensive Rats Precedes Left Ventricular Hypertrophy and Cardiac Dysfunction. *Cells* 11: 2022.
266. **Wang C, Yuan Y, Wu J, Zhao Y, Gao X, Chen Y, Sun C, Xiao L, Zheng P, Hu P, Li Z, Wang Z, Ye J, and Zhang L**. Plin5 deficiency exacerbates pressure overload-induced cardiac hypertrophy and heart failure by enhancing myocardial fatty acid oxidation and oxidative stress. *Free Radic Biol Med* 141: 372-382, 2019.
267. **Tham YK, Bernardo BC, Huynh K, Ooi JYY, Gao XM, Kiriazis H, Giles C, Meikle PJ, and McMullen JR**. Lipidomic Profiles of the Heart and Circulation in Response to Exercise versus Cardiac Pathology: A Resource of Potential Biomarkers and Drug Targets. *Cell Rep* 24: 2757-2772, 2018.
268. **Nakatani K, Masuda D, Kobayashi T, Sairyo M, Zhu Y, Okada T, Naito AT, Ohama T, Koseki M, Oka T, Akazawa H, Nishida M, Komuro I, Sakata Y, and Yamashita S**. Pressure Overload Impairs Cardiac Function in Long-Chain Fatty Acid Transporter CD36-Knockout Mice. *Int Heart J* 60: 159-167, 2019.
269. **Umbarawan Y, Syamsunarno M, Koitabashi N, Obinata H, Yamaguchi A, Hanaoka H, Hishiki T, Hayakawa N, Sano M, Sunaga H, Matsui H, Tsushima Y, Suematsu M, Kurabayashi M, and Iso T**. Myocardial fatty acid uptake through CD36 is indispensable for sufficient bioenergetic metabolism to prevent progression of pressure overload-induced heart failure. *Sci Rep* 8: 12035, 2018.
270. **Steinbusch LK, Luiken JJ, Vlasblom R, Chabowski A, Hoebers NT, Coumans WA, Vroegrijk IO, Voshol PJ, Ouwens DM, Glatz JF, and Diamant M**. Absence of fatty acid transporter CD36 protects against Western-type diet-related cardiac dysfunction following pressure overload in mice. *Am J Physiol Endocrinol Metab* 301: E618-627, 2011.

271. **Heather LC, Cole MA, Lygate CA, Evans RD, Stuckey DJ, Murray AJ, Neubauer S, and Clarke K**. Fatty acid transporter levels and palmitate oxidation rate correlate with ejection fraction in the infarcted rat heart. *Cardiovasc Res* 72: 430-437, 2006.
272. **Heather LC, Howell NJ, Emmanuel Y, Cole MA, Frenneaux MP, Pagano D, and Clarke K**. Changes in cardiac substrate transporters and metabolic proteins mirror the metabolic shift in patients with aortic stenosis. *PLoS One* 6: e26326, 2011.
273. **Lenski M, Schleider G, Kohlhaas M, Adrian L, Adam O, Tian Q, Kaestner L, Lipp P, Lehrke M, Maack C, Bohm M, and Laufs U**. Arrhythmia causes lipid accumulation and reduced glucose uptake. *Basic Res Cardiol* 110: 40, 2015.
274. **Tanaka T, Sohmiya K, and Kawamura K**. Is CD36 deficiency an etiology of hereditary hypertrophic cardiomyopathy? *J Mol Cell Cardiol* 29: 121-127, 1997.
275. **Karam CN, Warren CM, Henze M, Banke NH, Lewandowski ED, and Solaro RJ**. Peroxisome proliferator-activated receptor-alpha expression induces alterations in cardiac myofilaments in a pressure-overload model of hypertrophy. *Am J Physiol Heart Circ Physiol* 312: H681-H690, 2017.
276. **Rohini A, Agrawal N, Koyani CN, and Singh R**. Molecular targets and regulators of cardiac hypertrophy. *Pharmacol Res* 61: 269-280, 2010.
277. **Warren JS, Oka SI, Zablocki D, and Sadoshima J**. Metabolic reprogramming via PPARalpha signaling in cardiac hypertrophy and failure: From metabolomics to epigenetics. *Am J Physiol Heart Circ Physiol* 313: H584-H596, 2017.
278. **Narravula S, and Colgan SP**. Hypoxia-inducible factor 1-mediated inhibition of peroxisome proliferator-activated receptor alpha expression during hypoxia. *J Immunol* 166: 7543-7548, 2001.
279. **Taegtmeyer H, Sen S, and Vela D**. Return to the fetal gene program: a suggested metabolic link to gene expression in the heart. *Ann N Y Acad Sci* 1188: 191-198, 2010.
280. **Hu Y, Suarez J, Fricovsky E, Wang H, Scott BT, Trauger SA, Han W, Hu Y, Oyeleye MO, and Dillmann WH**. Increased enzymatic O-GlcNAcylation of mitochondrial proteins impairs mitochondrial function in cardiac myocytes exposed to high glucose. *J Biol Chem* 284: 547-555, 2009.
281. **Lunde IG, Aronsen JM, Kvaloy H, Qvigstad E, Sjaastad I, Tonnessen T, Christensen G, Gronning-Wang LM, and Carlson CR**. Cardiac O-GlcNAc signaling is increased in hypertrophy and heart failure. *Physiol Genomics* 44: 162-172, 2012.
282. **Brunmeir R, and Xu F**. Functional Regulation of PPARs through Post-Translational Modifications. *Int J Mol Sci* 19: 2018.
283. **Kolwicz SC, Jr., and Tian R**. Glucose metabolism and cardiac hypertrophy. *Cardiovasc Res* 90: 194-201, 2011.
284. **Dehn S, and Thorp EB**. Myeloid receptor CD36 is required for early phagocytosis of myocardial infarcts and induction of Nr4a1-dependent mechanisms of cardiac repair. *FASEB J* 32: 254-264, 2018.
285. **DeLeon-Pennell KY, Tian Y, Zhang B, Cates CA, Iyer RP, Cannon P, Shah P, Aiyetan P, Halade GV, Ma Y, Flynn E, Zhang Z, Jin YF, Zhang H, and Lindsey ML**. CD36 Is a Matrix Metalloproteinase-9 Substrate That Stimulates Neutrophil Apoptosis and Removal During Cardiac Remodeling. *Circ Cardiovasc Genet* 9: 14-25, 2016.
286. **Rekhi UR, Omar M, Alexiou M, Delyea C, Immaraj L, Elahi S, and Febbraio M**. Endothelial Cell CD36 Reduces Atherosclerosis and Controls Systemic Metabolism. *Front Cardiovasc Med* 8: 768481, 2021.
287. **Bou Khzam L, Son NH, Mullick AE, Abumrad NA, and Goldberg IJ**. Endothelial cell CD36 deficiency prevents normal angiogenesis and vascular repair. *Am J Transl Res* 12: 7737-7761, 2020.
288. **Glatz JFC, Nabben M, Young ME, Schulze PC, Taegtmeyer H, and Luiken J**. Re-balancing cellular energy substrate metabolism to mend the failing heart. *Biochim Biophys Acta Mol Basis Dis* 1866: 165579, 2020.
289. **Des Rosiers C, Labarthe F, Lloyd SG, and Chatham JC**. Cardiac anaplerosis in health and disease: food for thought. *Cardiovasc Res* 90: 210-219, 2011.
290. **Csizmok V, and Forman-Kay JD**. Complex regulatory mechanisms mediated by the interplay of multiple post-translational modifications. *Curr Opin Struct Biol* 48: 58-67, 2018.
291. **Noordali H, Loudon BL, Frenneaux MP, and Madhani M**. Cardiac metabolism - A promising therapeutic target for heart failure. *Pharmacol Ther* 182: 95-114, 2018.

292. **Harmon CM, Luce P, Beth AH, and Abumrad NA**. Labeling of adipocyte membranes by sulfo-N-succinimidyl derivatives of long-chain fatty acids: inhibition of fatty acid transport. *J Membr Biol* 121: 261-268, 1991.
293. **Harmon CM, and Abumrad NA**. Binding of sulfosuccinimidyl fatty acids to adipocyte membrane proteins: isolation and amino-terminal sequence of an 88-kD protein implicated in transport of long-chain fatty acids. *J Membr Biol* 133: 43-49, 1993.
294. **Kuda O, Pietka TA, Demianova Z, Kudova E, Cvacka J, Kopecky J, and Abumrad NA**. Sulfo-N-succinimidyl oleate (SSO) inhibits fatty acid uptake and signaling for intracellular calcium via binding CD36 lysine 164: SSO also inhibits oxidized low density lipoprotein uptake by macrophages. *J Biol Chem* 288: 15547-15555, 2013.
295. **Luiken JJ, van Nieuwenhoven FA, America G, van der Vusse GJ, and Glatz JF**. Uptake and metabolism of palmitate by isolated cardiac myocytes from adult rats: involvement of sarcolemmal proteins. *J Lipid Res* 38: 745-758, 1997.
296. **Coort SL, Willems J, Coumans WA, van der Vusse GJ, Bonen A, Glatz JF, and Luiken JJ**. Sulfo-N-succinimidyl esters of long chain fatty acids specifically inhibit fatty acid translocase (FAT/CD36)-mediated cellular fatty acid uptake. *Mol Cell Biochem* 239: 213-219, 2002.
297. **Chabowski A, Coort SL, Calles-Escandon J, Tandon NN, Glatz JF, Luiken JJ, and Bonen A**. The subcellular compartmentation of fatty acid transporters is regulated differently by insulin and by AICAR. *FEBS Lett* 579: 2428-2432, 2005.
298. **Dodd MS, Sousa Fialho MDL, Montes Aparicio CN, Kerr M, Timm KN, Griffin JL, Luiken J, Glatz JFC, Tyler DJ, and Heather LC**. Fatty Acids Prevent Hypoxia-Inducible Factor-1alpha Signaling Through Decreased Succinate in Diabetes. *JACC Basic Transl Sci* 3: 485-498, 2018.
299. **Holloway GP, Jain SS, Bezaire V, Han XX, Glatz JF, Luiken JJ, Harper ME, and Bonen A**. FAT/CD36-null mice reveal that mitochondrial FAT/CD36 is required to upregulate mitochondrial fatty acid oxidation in contracting muscle. *Am J Physiol Regul Integr Comp Physiol* 297: R960-967, 2009.
300. **Drahota Z, Vrbacky M, Nuskova H, Kazdova L, Zidek V, Landa V, Pravenec M, and Houstek J**. Succinimidyl oleate, established inhibitor of CD36/FAT translocase inhibits complex III of mitochondrial respiratory chain. *Biochem Biophys Res Commun* 391: 1348-1351, 2010.
301. **Jay AG, Simard JR, Huang N, and Hamilton JA**. SSO and other putative inhibitors of FA transport across membranes by CD36 disrupt intracellular metabolism, but do not affect FA translocation. *J Lipid Res* 61: 790-807, 2020.
302. **Bocharov AV, Wu T, Baranova IN, Birukova AA, Sviridov D, Vishnyakova TG, Remaley AT, Eggerman TL, Patterson AP, and Birukov KG**. Synthetic Amphipathic Helical Peptides Targeting CD36 Attenuate Lipopolysaccharide-Induced Inflammation and Acute Lung Injury. *J Immunol* 197: 611-619, 2016.
303. **Tanaka T, and Kawamura K**. Isolation of myocardial membrane long-chain fatty acid-binding protein: homology with a rat membrane protein implicated in the binding or transport of long-chain fatty acids. *J Mol Cell Cardiol* 27: 1613-1622, 1995.
304. **Mansor LS, Sousa Fialho MDL, Yea G, Coumans WA, West JA, Kerr M, Carr CA, Luiken J, Glatz JFC, Evans RD, Griffin JL, Tyler DJ, Clarke K, and Heather LC**. Inhibition of sarcolemmal FAT/CD36 by sulfo-N-succinimidyl oleate rapidly corrects metabolism and restores function in the diabetic heart following hypoxia/reoxygenation. *Cardiovasc Res* 113: 737-748, 2017.
305. **Kusaka Y, Tanaka T, Okamoto F, Terasaki F, Matsunaga Y, Miyazaki H, and Kawamura K**. Effect of sulfo-N-succinimidyl palmitate on the rat heart: myocardial long-chain fatty acid uptake and cardiac hypertrophy. *J Mol Cell Cardiol* 27: 1605-1612, 1995.
306. **Geloen A, Helin L, Geeraert B, Malaud E, Holvoet P, and Marguerie G**. CD36 inhibitors reduce postprandial hypertriglyceridemia and protect against diabetic dyslipidemia and atherosclerosis. *PLoS One* 7: e37633, 2012.
307. **Bodart V, Febbraio M, Demers A, McNicoll N, Pohankova P, Perreault A, Sejlitz T, Escher E, Silverstein RL, Lamontagne D, and Ong H**. CD36 mediates the cardiovascular action of growth hormone-releasing peptides in the heart. *Circ Res* 90: 844-849, 2002.
308. **Bessi VL, Labbe SM, Huynh DN, Menard L, Jossart C, Febbraio M, Guerin B, Bentourkia M, Lecomte R, Carpentier AC, Ong H, and Marleau S**. EP 80317, a selective CD36 ligand, shows cardioprotective effects against post-ischaemic myocardial damage in mice. *Cardiovasc Res* 96: 99-108, 2012.

309. **Huynh DN, Bessi VL, Menard L, Piquereau J, Proulx C, Febbraio M, Lubell WD, Carpentier AC, Burelle Y, Ong H, and Marleau S**. Adiponectin has a pivotal role in the cardioprotective effect of CP-3(iv), a selective CD36 azapeptide ligand, after transient coronary artery occlusion in mice. *FASEB J* 32: 807-818, 2018.
310. **Chen XF, Wang L, Wu YZ, Song SY, Min HY, Yang Y, He X, Liang Q, Yi L, Wang Y, and Gao Q**. Effect of puerarin in promoting fatty acid oxidation by increasing mitochondrial oxidative capacity and biogenesis in skeletal muscle in diabetic rats. *Nutr Diabetes* 8: 1, 2018.
311. **Chen Y, Li Y, Wang Y, Wen Y, and Sun C**. Berberine improves free-fatty-acid-induced insulin resistance in L6 myotubes through inhibiting peroxisome proliferator-activated receptor gamma and fatty acid transferase expressions. *Metabolism* 58: 1694-1702, 2009.
312. **Zheng A, Cao L, Qin S, Chen Y, Li Y, and Zhang D**. Exenatide Regulates Substrate Preferences Through the p38gamma MAPK Pathway After Ischaemia/Reperfusion Injury in a Rat Heart. *Heart Lung Circ* 26: 404-412, 2017.
313. **Chabowski A, Momken I, Coort SL, Calles-Escandon J, Tandon NN, Glatz JF, Luiken JJ, and Bonen A**. Prolonged AMPK activation increases the expression of fatty acid transporters in cardiac myocytes and perfused hearts. *Mol Cell Biochem* 288: 201-212, 2006.
314. **Randle PJ, Garland PB, Hales CN, and Newsholme EA**. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* 1: 785-789, 1963.
315. **Kerr M, Dodd MS, and Heather LC**. The 'Goldilocks zone' of fatty acid metabolism; to ensure that the relationship with cardiac function is just right. *Clin Sci (Lond)* 131: 2079-2094, 2017.
316. **Hegarty BD, Cooney GJ, Kraegen EW, and Furler SM**. Increased efficiency of fatty acid uptake contributes to lipid accumulation in skeletal muscle of high fat-fed insulin-resistant rats. *Diabetes* 51: 1477-1484, 2002.
317. **Jordy AB, Serup AK, Karstoft K, Pilegaard H, Kiens B, and Jeppesen J**. Insulin sensitivity is independent of lipid binding protein trafficking at the plasma membrane in human skeletal muscle: effect of a 3-day, high-fat diet. *Am J Physiol Regul Integr Comp Physiol* 307: R1136-1145, 2014.
318. **Yechoor VK, Patti ME, Ueki K, Laustsen PG, Saccone R, Rauniyar R, and Kahn CR**. Distinct pathways of insulin-regulated versus diabetes-regulated gene expression: an in vivo analysis in MIRKO mice. *Proc Natl Acad Sci U S A* 101: 16525-16530, 2004.
319. **Chabowski A, Chatham JC, Tandon NN, Calles-Escandon J, Glatz JF, Luiken JJ, and Bonen A**. Fatty acid transport and FAT/CD36 are increased in red but not in white skeletal muscle of ZDF rats. *Am J Physiol Endocrinol Metab* 291: E675-682, 2006.
320. **Bonen A, Holloway GP, Tandon NN, Han XX, McFarlan J, Glatz JF, and Luiken JJ**. Cardiac and skeletal muscle fatty acid transport and transporters and triacylglycerol and fatty acid oxidation in lean and Zucker diabetic fatty rats. *Am J Physiol Regul Integr Comp Physiol* 297: R1202-1212, 2009.
321. **Goudriaan JR, Dahlmans VE, Teusink B, Ouwens DM, Febbraio M, Maassen JA, Romijn JA, Havekes LM, and Voshol PJ**. CD36 deficiency increases insulin sensitivity in muscle, but induces insulin resistance in the liver in mice. *J Lipid Res* 44: 2270-2277, 2003.
322. **Targher G, Byrne CD, and Tilg H**. NAFLD and increased risk of cardiovascular disease: clinical associations, pathophysiological mechanisms and pharmacological implications. *Gut* 69: 1691-1705, 2020.
323. **Wilson CG, Tran JL, Erion DM, Vera NB, Febbraio M, and Weiss EJ**. Hepatocyte-Specific Disruption of CD36 Attenuates Fatty Liver and Improves Insulin Sensitivity in HFD-Fed Mice. *Endocrinology* 157: 570-585, 2016.
324. **Greco D, Kotronen A, Westerbacka J, Puig O, Arkkila P, Kiviluoto T, Laitinen S, Kolak M, Fisher RM, Hamsten A, Auvinen P, and Yki-Jarvinen H**. Gene expression in human NAFLD. *Am J Physiol Gastrointest Liver Physiol* 294: G1281-1287, 2008.
325. **Miquilena-Colina ME, Lima-Cabello E, Sanchez-Campos S, Garcia-Mediavilla MV, Fernandez-Bermejo M, Lozano-Rodriguez T, Vargas-Castrillon J, Buque X, Ochoa B, Aspichueta P, Gonzalez-Gallego J, and Garcia-Monzon C**. Hepatic fatty acid translocase CD36 upregulation is associated with insulin resistance, hyperinsulinaemia and increased steatosis in non-alcoholic steatohepatitis and chronic hepatitis C. *Gut* 60: 1394-1402, 2011.
326. **Zhao L, Zhang C, Luo X, Wang P, Zhou W, Zhong S, Xie Y, Jiang Y, Yang P, Tang R, Pan Q, Hall AR, Luong TV, Fan J, Varghese Z, Moorhead JF, Pinzani M, Chen Y, and Ruan XZ**. CD36 palmitoylation disrupts

free fatty acid metabolism and promotes tissue inflammation in non-alcoholic steatohepatitis. *J Hepatol* 69: 705-717, 2018.

327. **Garcia-Monzon C, Lo Iacono O, Crespo J, Romero-Gomez M, Garcia-Samaniego J, Fernandez-Bermejo M, Dominguez-Diez A, Rodriguez de Cia J, Saez A, Porrero JL, Vargas-Castrillon J, Chavez-Jimenez E, Soto-Fernandez S, Diaz A, Gallego-Duran R, Madejon A, and Miquilena-Colina ME**. Increased soluble CD36 is linked to advanced steatosis in nonalcoholic fatty liver disease. *Eur J Clin Invest* 44: 65-73, 2014.
328. **Heeboll S, Poulsen MK, Ornstrup MJ, Kjaer TN, Pedersen SB, Nielsen S, Gronbaek H, and Handberg A**. Circulating sCD36 levels in patients with non-alcoholic fatty liver disease and controls. *Int J Obes (Lond)* 41: 262-267, 2017.
329. **Park YM, Febbraio M, and Silverstein RL**. CD36 modulates migration of mouse and human macrophages in response to oxidized LDL and may contribute to macrophage trapping in the arterial intima. *J Clin Invest* 119: 136-145, 2009.
330. **Lara-Guzman OJ, Gil-Izquierdo A, Medina S, Osorio E, Alvarez-Quintero R, Zuluaga N, Oger C, Galano JM, Durand T, and Munoz-Durango K**. Oxidized LDL triggers changes in oxidative stress and inflammatory biomarkers in human macrophages. *Redox Biol* 15: 1-11, 2018.
331. **Kuchibhotla S, Vanegas D, Kennedy DJ, Guy E, Nimako G, Morton RE, and Febbraio M**. Absence of CD36 protects against atherosclerosis in ApoE knock-out mice with no additional protection provided by absence of scavenger receptor A I/II. *Cardiovasc Res* 78: 185-196, 2008.
332. **Podrez EA, Byzova TV, Febbraio M, Salomon RG, Ma Y, Valiyaveettil M, Poliakov E, Sun M, Finton PJ, Curtis BR, Chen J, Zhang R, Silverstein RL, and Hazen SL**. Platelet CD36 links hyperlipidemia, oxidant stress and a prothrombotic phenotype. *Nat Med* 13: 1086-1095, 2007.
333. **Bonen A, Tandon NN, Glatz JF, Luiken JJ, and Heigenhauser GJ**. The fatty acid transporter FAT/CD36 is upregulated in subcutaneous and visceral adipose tissues in human obesity and type 2 diabetes. *Int J Obes (Lond)* 30: 877-883, 2006.
334. **Koutsari C, Ali AH, Mundi MS, and Jensen MD**. Storage of circulating free fatty acid in adipose tissue of postabsorptive humans: quantitative measures and implications for body fat distribution. *Diabetes* 60: 2032-2040, 2011.
335. **Kennedy DJ, Kuchibhotla S, Westfall KM, Silverstein RL, Morton RE, and Febbraio M**. A CD36-dependent pathway enhances macrophage and adipose tissue inflammation and impairs insulin signalling. *Cardiovasc Res* 89: 604-613, 2011.
336. **Wang J, Hao JW, Wang X, Guo H, Sun HH, Lai XY, Liu LY, Zhu M, Wang HY, Li YF, Yu LY, Xie C, Wang HR, Mo W, Zhou HM, Chen S, Liang G, and Zhao TJ**. DHHC4 and DHHC5 Facilitate Fatty Acid Uptake by Palmitoylating and Targeting CD36 to the Plasma Membrane. *Cell Rep* 26: 209-221 e205, 2019.
337. **Salameh A, Daquinag AC, Staquicini DI, An Z, Hajjar KA, Pasqualini R, Arap W, and Kolonin MG**. Prohibitin/annexin 2 interaction regulates fatty acid transport in adipose tissue. *JCI Insight* 1: 2016.
338. **Daquinag AC, Gao Z, Fussell C, Immaraj L, Pasqualini R, Arap W, Akimzhanov AM, Febbraio M, and Kolonin MG**. Fatty acid mobilization from adipose tissue is mediated by CD36 posttranslational modifications and intracellular trafficking. *JCI Insight* 6: 2021.
339. **Esposito K, Chiodini P, Colao A, Lenzi A, and Giugliano D**. Metabolic syndrome and risk of cancer: a systematic review and meta-analysis. *Diabetes Care* 35: 2402-2411, 2012.
340. **Snaebjornsson MT, Janaki-Raman S, and Schulze A**. Greasing the Wheels of the Cancer Machine: The Role of Lipid Metabolism in Cancer. *Cell Metab* 31: 62-76, 2020.
341. **Watt MJ, Clark AK, Selth LA, Haynes VR, Lister N, Rebello R, Porter LH, Niranjan B, Whitby ST, Lo J, Huang C, Schittenhelm RB, Anderson KE, Furic L, Wijayaratne PR, Matzaris M, Montgomery MK, Papargiris M, Norden S, Febbraio M, Risbridger GP, Frydenberg M, Nomura DK, and Taylor RA**. Suppressing fatty acid uptake has therapeutic effects in preclinical models of prostate cancer. *Sci Transl Med* 11: 2019.
342. **Farge T, Saland E, de Toni F, Aroua N, Hosseini M, Perry R, Bosc C, Sugita M, Stuani L, Fraisse M, Scotland S, Larrue C, Boutzen H, Feliu V, Nicolau-Travers ML, Cassant-Sourdy S, Broin N, David M, Serhan N, Sarry A, Tavitian S, Kaoma T, Vallar L, Iacovoni J, Linares LK, Montersino C, Castellano R, Griessinger E, Collette Y, Duchamp O, Barreira Y, Hirsch P, Palama T, Gales L, Delhommeau F, Garmy-Susini BH, Portais JC, Vergez F, Selak M, Danet-Desnoyers G, Carroll M, Recher C, and Sarry JE**. Chemotherapy-Resistant Human Acute Myeloid Leukemia Cells Are Not Enriched for Leukemic Stem Cells but Require Oxidative Metabolism. *Cancer Discov* 7: 716-735, 2017.

343. **Pascual G, Avgustinova A, Mejetta S, Martin M, Castellanos A, Attolini CS, Berenguer A, Prats N, Toll A, Hueto JA, Bescos C, Di Croce L, and Benitah SA**. Targeting metastasis-initiating cells through the fatty acid receptor CD36. *Nature* 541: 41-45, 2017.
344. **Corbet C, Bastien E, Santiago de Jesus JP, Dierge E, Martherus R, Vander Linden C, Doix B, Degavre C, Guilbaud C, Petit L, Michiels C, Dessy C, Larondelle Y, and Feron O**. TGFbeta2-induced formation of lipid droplets supports acidosis-driven EMT and the metastatic spreading of cancer cells. *Nat Commun* 11: 454, 2020.
345. **Nath A, and Chan C**. Genetic alterations in fatty acid transport and metabolism genes are associated with metastatic progression and poor prognosis of human cancers. *Sci Rep* 6: 18669, 2016.
346. **Lemberger L, Wagner R, Heller G, Pils D, and Grunt TW**. Pharmacological Inhibition of Lipid Import and Transport Proteins in Ovarian Cancer. *Cancers (Basel)* 14: 2022.
347. **Liang Y, Han H, Liu L, Duan Y, Yang X, Ma C, Zhu Y, Han J, Li X, and Chen Y**. CD36 plays a critical role in proliferation, migration and tamoxifen-inhibited growth of ER-positive breast cancer cells. *Oncogenesis* 7: 98, 2018.
348. **Feng WW, Wilkins O, Bang S, Ung M, Li J, An J, Del Genio C, Canfield K, DiRenzo J, Wells W, Gaur A, Robey RB, Guo JY, Powles RL, Sotiriou C, Pusztai L, Febbraio M, Cheng C, Kinlaw WB, and Kurokawa M**. CD36-Mediated Metabolic Rewiring of Breast Cancer Cells Promotes Resistance to HER2-Targeted Therapies. *Cell Rep* 29: 3405-3420 e3405, 2019.
349. **Wang H, Franco F, Tsui YC, Xie X, Trefny MP, Zappasodi R, Mohmood SR, Fernandez-Garcia J, Tsai CH, Schulze I, Picard F, Meylan E, Silverstein R, Goldberg I, Fendt SM, Wolchok JD, Merghoub T, Jandus C, Zippelius A, and Ho PC**. CD36-mediated metabolic adaptation supports regulatory T cell survival and function in tumors. *Nat Immunol* 21: 298-308, 2020.
350. **Luiken JJ, Coort SL, Willems J, Coumans WA, Bonen A, and Glatz JF**. Dipyridamole alters cardiac substrate preference by inducing translocation of FAT/CD36, but not that of GLUT4. *Mol Pharmacol* 65: 639-645, 2004.
351. **Chabowski A, Gorski J, Calles-Escandon J, Tandon NN, and Bonen A**. Hypoxia-induced fatty acid transporter translocation increases fatty acid transport and contributes to lipid accumulation in the heart. *FEBS Lett* 580: 3617-3623, 2006.
352. **Palanivel R, Eguchi M, Shuralyova I, Coe I, and Sweeney G**. Distinct effects of short- and long-term leptin treatment on glucose and fatty acid uptake and metabolism in HL-1 cardiomyocytes. *Metabolism* 55: 1067-1075, 2006.
353. **Momken I, Chabowski A, Dirkx E, Nabben M, Jain SS, McFarlan JT, Glatz JF, Luiken JJ, and Bonen A**. A new leptin-mediated mechanism for stimulating fatty acid oxidation: a pivotal role for sarcolemmal FAT/CD36. *Biochem J* 474: 149-162, 2017.
354. **Fang X, Palanivel R, Cresser J, Schram K, Ganguly R, Thong FS, Tuinei J, Xu A, Abel ED, and Sweeney G**. An APPL1-AMPK signaling axis mediates beneficial metabolic effects of adiponectin in the heart. *Am J Physiol Endocrinol Metab* 299: E721-729, 2010.
355. **Lally JSV, Jain SS, Han XX, Snook LA, Glatz JFC, Luiken J, McFarlan J, Holloway GP, and Bonen A**. Caffeine-stimulated fatty acid oxidation is blunted in CD36 null mice. *Acta Physiol (Oxf)* 205: 71-81, 2012.