From fat to FAT (CD36/SR-B2)

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From fat to FAT (CD36/SR-B2): Understanding the regulation of cellular fatty acid uptake☆

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ABSTRACT

The molecular mechanisms underlying the cellular uptake of long-chain fatty acids and the regulation of this process have been elucidated in appreciable detail in the last decades. Two main players in this field, each discovered in the early 1990s, are (*i*) a membrane-associated protein first identified in adipose ('fat') tissue and referred to as putative fatty acid translocase (FAT)/CD36 (now officially designated as SR-B2) which facilitates the transport of fatty acids across the plasma membrane, and (*ii*) the family of transcription factors designated peroxisome proliferator-activated receptors (PPARα, PPARγ, and PPARβ/δ) for which fatty acids and fatty acid metabolites are the preferred ligand. CD36/SR-B2 is the predominant membrane protein involved in fatty acid uptake into intestinal enterocytes, adipocytes and cardiac and skeletal myocytes. The rate of cellular fatty acid uptake is regulated by the subcellular vesicular recycling of CD36/SR-B2 from endosomes to the plasma membrane. Fatty acid-induced activation of PPARs results in the upregulation of the expression of genes encoding various proteins and enzymes involved in cellular fatty acid utilization. Both CD36/SR-B2 and the PPARs have been implicated in the derangements in fatty acid and lipid metabolism occurring with the development of pathophysiological conditions, such as high fat diet-induced insulin resistance and diabetic cardiomyopathy, and have been suggested as targets for metabolic intervention. In this brief review we discuss the discovery and current understanding of both CD36/SR-B2 and the PPARs in metabolic homeostasis.

1. Introduction

Long-chain fatty acids (for convenience referred to as 'fatty acids') are a group of nutritional compounds that serve pivotal roles in various functions of the body. Fatty acids are (*i*) a main fuel for cellular energy production, especially in liver, muscle and heart, (*ii*) are part of phospholipids constituting biological membranes, (*iii*) may covalently be attached to specific proteins to regulate their function, and (*iv*) act in selected signal transduction events, for example in fat taste perception or in regulation of protein phosphorylation, and (*v*) to modify gene expression. In addition, storage of fatty acids as part of triacylglycerols in adipose tissue is an efficient form of creating a reserve energy source. Because of their amphipathic nature, fatty acids also may exert detergent-like activities such as causing (acute) cellular injury. In addition, this physicochemical nature of fatty acids limits their aqueous solubility and, therefore, dictates specific requirements to their presence and transport in aqueous compartments [1]. In blood plasma and the interstitium fatty acids are avidly bound by albumin (68 kDa) or are present as fatty esters in lipoproteins. Intracellularly, fatty acids are bound by (tissue-specific) cytoplasmic fatty acid-binding proteins ($FABP_c$, 14–15 kDa), which act as an intracellular counterpart of plasma albumin [2,3]. How fatty acids traverse the plasma membrane to enter the soluble cytoplasm, however, is not yet fully understood. Specifically, there is debate on the rate-limiting step in the overall fatty acid uptake process and if, and to what extent, one or more membrane-associated proteins could facilitate and/or regulate cellular fatty acid uptake [4–9].

A major contribution to our current understanding of cellular fatty acid uptake has been made by Dr. Paul A. Grimaldi

☆ This article is dedicated to the memory of dr. Paul A. Grimaldi who was involved in the identification of CD36/SR-B2 as facilitator of membrane fatty acid transport whereafter our laboratory adopted this finding to start elucidating the functioning of CD36/SR-B2 in cardiac and skeletal muscle lipid metabolism as main research topic.

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Abbreviations

ACS	acyl-CoA synthetase
AMPK	AMP-activated kinase
CD36	cluster of differentiation 36
C/EBPα	CCAAT/enhancer-binding protein α
DHA	docosahexaenoic acid (22:6 n−3)
EPA	eicosapentaenoic acid (20:5 n−3)
FAAR	fatty acid-activated receptor
$FABP_c$	cytoplasmic fatty acid-binding protein
$FABP_{pm}$	plasma membrane fatty acid-binding protein
FAT	(putative) fatty acid translocase (CD36/SR-B2)
FATP	fatty acid-transport protein
GLUT4	glucose transporter-4
HIF-1	hypoxia-inducible factor-1
PPAR	peroxisome proliferator-activated receptor
PPRE	peroxisome proliferator-activated receptor response element
RXR	retinoid X receptor
SR-B2	scavenger receptor B2
VAMP	vesicle-associated membrane protein
v-ATPase	vacuolar-type H^+-ATPase

(1956–2016) [10]. Studying fatty acid homeostasis in adipose tissue, he discovered in 1992 that fatty acid uptake into adipose cells is important for adipogenesis, especially through fatty acid-induced expression of aP2 (the adipocyte-specific $FABP_c$) [11]. Subsequently, in 1993 Dr. Nada Abumrad together with Dr. Raafat el-Maghrabi (at that time in Stony Brook, NY, U.S.A.) cloned the protein implicated in adipocyte fatty acid uptake identifying it as the multifunctional membrane protein CD36 (now officially designated as scavenger receptor B2, SR-B2; Ref. [12]). Dr. Grimaldi together with Dr. Ez-Zoubir Amri contributed to this work by establishing that CD36/SR-B2 is crucial for preadipocyte differentiation [13]. In the present short review we will focus on the discovery of CD36/SR-B2 as 'putative membrane fatty acid translocase' (FAT) and its involvement in fatty acid metabolism in both physiological and pathophysiological conditions.

2. Mechanism of cellular fatty acid uptake: historical perspective

From the early 1980s onwards there has been dispute as to the involvement, or not, of membrane-associated proteins in the uptake of fatty acids by parenchymal cells. Several researchers reported cellular fatty acid uptake to show saturation kinetics, to be sensitive to universal inhibitors of protein-mediated plasma membrane transport processes such as phloretin, and to be sensitive to competitive inhibition. While these observations are in favor of protein-mediated transmembrane fatty acid transport, it could be argued that saturation of fatty acid transport can be explained alternatively as saturation of metabolism in combination with passive diffusional uptake [5]. In addition, the used inhibitors may act on fatty acid uptake indirectly via effects on structural organization of the membrane bilayer, while the fatty acid competition experiments may be explained by competition at the level of fatty acid binding to albumin and/or $FABP_c$ rather than a membrane-associated protein. From a physiological perspective, however, having fatty acids moving in or out of cells without control at the membrane would be undesirable as this may seriously hamper coordination of intracellular availability of fatty acids with changing metabolic needs [8].

Starting in the mid-1980s several groups reported on the identification of peripheral and integral membrane proteins putatively involved in the cellular uptake of fatty acids. These include plasma membrane fatty acid-binding protein ($FABP_{pm}$; 40–43 kDa), fatty acid transport proteins 1–6 (FATP1–6; 63 kDa), caveolin-1 (21–24 kDa), and fatty acid translocase (FAT; 88 kDa) [for review 14,15]. Of these, the FATP's were found to be enzymes, i.e., acyl-CoA synthetases, and function in cellular fatty acid uptake by converting incoming fatty acids directly into their acyl-CoA ester, resulting in metabolic trapping of fatty acids [15]. With respect to nomenclature it should be mentioned that, for convenience, these proteins commonly are referred to as 'fatty acid transporters' despite the remaining uncertainty as to the exact mechanism by which any of these proteins participate in the fatty acid transport process within the plasma membrane. After all, these proteins merely share the feature of facilitating – not necessarily transporting – the transmembrane translocation of (long-chain) fatty acids.

Studies on fatty acid uptake into isolated rat adipocytes by Abumrad and colleagues had revealed that prior incubation of the cells with diisothiocyanodisulfonic acid (DIDS) or with sulfo-*N*-succinimidyl derivates of long-chain fatty acids (in particular oleate) led to a marked (about 70%) and irreversible inhibition of the rate of fatty acid uptake [16,17]. Both inhibitors reacted covalently with a membrane protein of about 85–88 kDa, suggesting this protein to be involved in membrane permeation of (long-chain) fatty acids. Subsequent cloning of this protein, then referred to as putative fatty acid translocase (FAT), revealed it to be the rat homologue of the transmembrane glycoprotein CD36 (cluster of differentiation 36), a ubiquitous protein originally identified in platelets [13]. CD36 is also identical to GP IIIb or PAS IV, a protein that is enriched in the apical membranes of lipid-secreting mammary cells during lactation. The role of FAT/CD36 in facilitating fatty acid uptake by adipocytes was further supported by superimposed time courses of FAT/CD36 expression and oleate uptake rates during adipose differentiation, and by parallel induction of FAT/CD36 expression and oleate transport in preadipocytes following treatment with the glucocorticoid dexamethasone [13]. This 1993-publication by Abumrad, Grimaldi and colleagues served as a landmark paper that initiated research into the role of FAT/CD36 in fatty acid and lipid metabolism in health and disease, not only in adipose tissue but also in other tissues in particular cardiac and skeletal muscle [18]. Recently, the nomenclature of CD36 and of other scavenger receptors with two transmembrane domains has been standardized; CD36 now officially is designated as scavenger receptor B2 (SR-B2) [12]. As a result, whereas the abbreviation FAT/CD36 or just CD36 has been used in the literature from 1993 to 2015, the newly recommended designation is CD36/SR-B2.

Convincing evidence for a role of CD36/SR-B2 in cellular fatty acid uptake was obtained when studying mice with a targeted deletion of CD36/SR-B2. Compared to wild-type mice, CD36/SR-B2 knock-out mice showed reduced fatty acid uptake rates *in vivo* in heart (−50 to −80%), skeletal muscle (−40 to −75%) and adipose tissue (−60 to −70%), but not in liver [19]. The latter is consistent with the finding that in liver CD36/SR-B2 expression is absent or very low [20]. These reductions in fatty acid uptake also contributed to altered rates of fatty acid metabolism, especially with regard to fatty acid oxidation in working hearts and skeletal muscles [21].

CD36/SR-B2 is currently known as the predominant membrane protein facilitating fatty acid transport in adipocytes, enterocytes, cardiac myocytes and skeletal myocytes [22–24]. With respect to the molecular mechanism of cellular fatty acid uptake, at the extracellular site CD36/SR-B2 most likely functions as an acceptor for fatty acids to promote the partitioning of the fatty acids into the

outer leaflet of the lipid bilayer, most likely in plasma membrane lipid rafts [25] (*adsorption step*). Subsequently, the fatty acids make their way from the outer to the inner leaflet of the membrane, a process referred to as 'flip-flop' (the polar carboxyl group of the fatty acid moves through the bilayer interior and re-positions at the opposite interface) (*translocation step*). This latter process occurs very fast and would not need assistance from membrane proteins [6]. At the inner site of the membrane the fatty acid will move into the aqueous phase to be bound to cytoplasmic FABP (*desorption step*). Desorption from the membrane has been suggested to be the rate-limiting step of overall transmembrane transport [6]. At the intracellular site CD36/SR-B2 may facilitate the transport by providing a docking site for $FABP_c$ or for enzymes that act on fatty acids such as acyl-CoA synthetase. Taken together, CD36/SR-B2 is viewed to function in sequestering fatty acids in the membrane, and help organize them within specific membrane domains (presumably lipid rafts) so as to make the fatty acids readily available for subsequent aqueous transport and/or enzymic conversion (Fig. 1). Interestingly, at the extracellular site CD36/SR-B2 shows protein-protein interaction with $FABP_{pm}$, suggesting that clusters of membrane proteins function in facilitating and modulating cellular fatty acid uptake. In line with this, intracellularly the presence of cytoplasmic FABP is required for proper functioning of CD36/SR-B2, because transfection of CD36/SR-B2 in a rat heart muscle cell line (H9c2) devoid of cytoplasmic FABP did not result in increased rates of fatty acid uptake [26].

Whether CD36/SR-B2 would preferentially bind specific (long-chain) fatty acid types has remained elusive. This is due, at least in part, to the complexity of appropriate experimental approaches which would need to consider a.o. differences among fatty acid types in aqueous solubility and differences in interaction with soluble proteins (such as albumin) and with biological membranes. Of note, there is circumstantial evidence that the cellular uptake of fatty acid species from marine oils, in particular eicosapentaenoic acid (EPA, 20:5 n−3) and docosahexaenoic acid (DHA, 22:6 n−3), also is facilitated by CD36/SR-B2 [1,27], suggesting that the beneficial health effects of n−3 long-chain polyunsaturated fatty acids are dependent on the sarcolemmal presence and proper functioning of CD36/SR-B2.

3. Short-term regulation of cellular fatty acid uptake by CD36/SR-B2

Besides serving as a facilitator of transmembrane fatty acid transport, CD36/SR-B2 was found to be involved in the regulation of the rate of cellular fatty acid uptake. This notion was triggered by the observation that CD36/SR-B2 not only is present on the cell membrane but also in intracellular compartments, notably endosomes. Regulation of fatty acid transport occurs by the reversible translocation of CD36/SR-B2 from endosomes to the plasma membrane to increase fatty acid uptake. For instance, in cardiac and skeletal muscle, an increase in muscle contraction or the presence of insulin each stimulate, within a few minutes, the translocation of CD36/SR-B2 from the endosomal compartment to the sarcolemma which increases fatty acid uptake up to two-fold [28–30]. The contraction-induced CD36/SR-B2 translocation is mediated by AMP-activated kinase (AMPK). The insulin-induced translocation is mediated by phosphoinositide 3 (PI3)-kinase [14]. Insulin and contraction signaling operate independently to induce CD36 translocation, but converge at the level of the Rab GTPase-activating protein AS160 via inactivating phosphorylation. This leads to de-inhibition of Rab 8a, which is then allowed to use the accelerated GDP/GTP cycling for the benefit of CD36 translocation [31]. Remarkably, this mechanism of regulation of cellular fatty acid

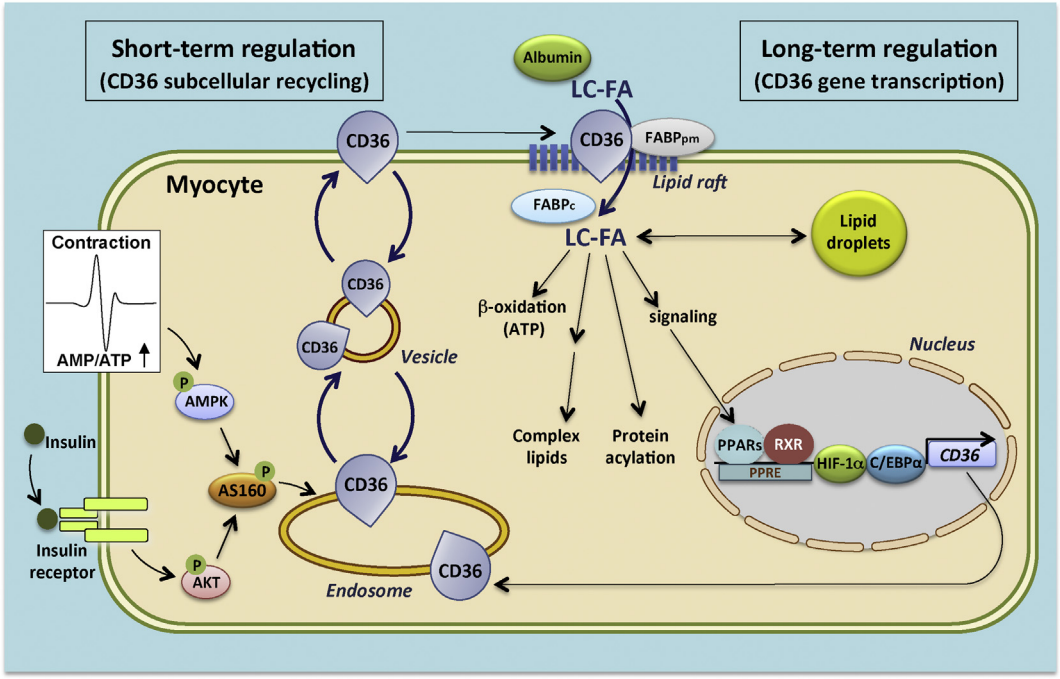

Fig. 1. Schematic presentation of both the facilitatory and regulatory roles of CD36/SR-B2 in (long-chain) fatty acid uptake into cardiac and skeletal myocytes. At sarcolemmal lipid rafts, CD36/SR-B2, most likely in interaction with the peripheral membrane protein $FABP_{pm}$ and, at the intracellular site, with $FABP_c$, facilitates the entry of fatty acids into the cell (*middle part of figure*). Short-term regulation (i.e., minutes) of the rate of cellular fatty acid uptake occurs by reversible intracellular recycling (vesicular transport) of CD36 from an endosomal storage compartment to the sarcolemma, which is triggered by either changes in the frequency of muscle contraction or by plasma insulin. These latter triggers are mediated by the AMPK-activated and insulin signaling cascades, respectively, which converge at AS160 (*left part of figure*). Long-term regulation of cellular fatty acid uptake occurs via changes in CD36 gene transcription, mediated a.o. by fatty acid-induced PPAR activation, HIF-1, and C/EBPα (*right part of figure*). See text for further explanation.

uptake by recycling of CD36/SR-B2 is very similar to the well-known regulation of cellular glucose uptake which in cardiac and skeletal muscle involves the translocation of the glucose transporter GLUT4 from an intracellular storage depot to the sarcolemma [32]. Upon increased muscle contraction or insulin stimulation, both CD36/SR-B2 and GLUT4 are recruited to the sarcolemma within the same time frame resulting in increased uptake rates for both fatty acids and glucose [14] (Fig. 1). CD36/SR-B2 recycling has been confirmed to occur also in human skeletal muscle [33,34] and in adipose tissue [35].

Current research is aimed at further deciphering the signaling pathways, and vesicular trafficking and cytoskeletal network proteins that control the short-term subcellular recycling of CD36/SR-B2, especially in relation to that of GLUT4. For instance, it has been reported that in cardiac muscle, specific vesicle-associated membrane proteins (VAMPs) are necessary for both CD36/SR-B2 and GLUT4 translocation while one isoform (VAMP4) is specifically involved in CD36/SR-B2 traffic and another isoform (VAMP7) in GLUT4 traffic [36]. More recently, the notion has arisen that the facilitatory action of CD36/SR-B2 not only is controlled by intracellular recycling of the protein but also by various types of post-translational modification, in particular palmitoylation, *N*-glycosylation and ubiquitination [37,38]. In addition, CD36/SR-B2 has been proposed as target of O-linked *N*-acetylglucosamine (O-GlcNac) which modification was observed to induce translocation of CD36/SR-B2 to cardiac sarcolemma and subsequently increase both the rate of myocellular fatty acid uptake and fatty acid oxidation [39].

4. Transcriptional control of CD36/SR-B2 expression

The occurrence of CD36/SR-B2 in various tissues follows its role as facilitator of fatty acid transport in tissues with a high capacity for fatty acid metabolism, and fluctuates depending on developmental, hormonal, and environmental conditions [40]. CD36/SR-B2 expression is high in segments of the intestine where most lipid absorption occurs, in adipose tissue where fatty acids are stored in neutral lipids, and in cardiac and (oxidative) skeletal muscle where fatty acids constitute the main substrate for energy production. CD36/SR-B2 is also found in endothelial cells, lung pneumocytes, platelets, and macrophages, where its expression likewise has been associated to fatty acid uptake and to binding of oxidized low-density lipoproteins (oxLDL) [40].

CD36/SR-B2 expression is regulated by agonists of the nuclear peroxisome proliferator-activated receptors (PPAR) in a tissue-specific manner. PPARs are a family of transcription factors activated by a diversity of molecules including fatty acids and fatty acid metabolites, and regulate the transcription of a large variety of genes implicated in metabolism, inflammation, proliferation, and differentiation in different cell types [41]. While PPARα is expressed mostly in tissues exhibiting high rates of fatty acid oxidation (liver, cardiac and skeletal muscle, kidney and brown adipose tissue) and regulates the expression of proteins involved in fatty acid catabolism, PPARγ is expressed predominantly in white adipose tissue and is a key regulator of adipogenesis. The third family member, PPARβ/δ, shows a more widespread tissue distribution but in particular is highly expressed in skeletal muscle during fasting and endurance exercise [41]. PPARs can directly regulate the transcription of target genes by forming a heterodimer with the retinoid X receptor (RXR) and binding to peroxisome proliferator response element (PPRE) sequences in the promotor- or transcribed-regions of target genes through DNA binding domains [41].

It is noteworthy to mention that P.A. Grimaldi and Ez-Zoubir Amri were the first to clone from preadipocytes the cDNA encoding this third PPAR isoform [42]. Searching for a protein that mediates transcriptional effects of fatty acids in preadipocytes, they designated it accordingly as 'fatty acid-activated receptor' (FAAR). Interestingly, during adipose differentiation FAAR expression is induced markedly (2–4 days) earlier than that of PPARγ [42]. Later FAAR was renamed as PPARβ (which, in turn, appeared identical to PPARδ). Although from a physiological perspective the term FAAR would have been more appropriate for this entire subfamily of nuclear receptors, Grimaldi's designation was not adopted, so that the name PPAR remains to be in use for the three isoforms PPARα, PPARγ, and PPARβ/δ.

Fatty acids, especially long-chain fatty acids, are considered to be the natural ligands for PPARs [43]. In addition, various fatty acid metabolites, such as acyl-CoA esters, oxidized fatty acids and eicosanoids, also activate PPARs [44]. Activation of PPARα in heart and PPARβ/δ in skeletal muscle results in the coordinated increased expression of various proteins and enzymes involved in mitochondrial β-oxidation of fatty acids, including an upregulation of proteins involved in cellular fatty acid uptake, i.e., CD36/SR-B2 and $FABP_c$. In this way, by facilitating the entry and thus promoting the intracellular availability of fatty acids CD36/SR-B2 positively influences its own *de novo* synthesis (feed-forward cycle) (Fig. 1). In cardiac muscle the pivotal roles of both CD36/SR-B2 and the nuclear receptor PPARα in governing cardiac fatty acid utilization is evident from the observations that (*i*) PPARα-null mice display a decreased capacity of cardiac fatty acid utilization [45,46], (*ii*) cardiac specific overexpression of PPARα in mice results in enhanced fatty acid uptake and utilization [47], while (*iii*) deletion of CD36/SR-B2 in PPARα-overexpressing mice prevents an increase in cardiac fatty acid utilization [48].

Besides PPARs, also other transcriptional activators have been implicated in the regulation of CD36/SR-B2 expression, including CCAAT/enhancer-binding protein-a and hypoxia-inducible factor-1 (for review see Ref. [49]). The CD36/SR-B2 gene has a complicated promoter structure with alternative transcription start sites [50]. Following the initial studies showing that the CD36/SR-B2 gene promoter is regulated by PPARs [51,52], the subsequently reported absence of PPRE in the upstream promoter region suggested that transcriptional activation of the gene by PPAR ligands would be indirectly dependent on PPAR [53]. However, in more recent studies the PPRE sites have been identified and validated in the distal part of the CD36/SR-B2 promoter [54,55].

5. CD36/SR-B2 and pathophysiology of lipid metabolism

Several chronic diseases are characterized by a perturbed fatty acid or lipid metabolism. For instance, obesity is associated with disturbances in the control of glucose and lipid metabolism resulting in an atherogenic plasma lipid profile and deleterious triacylglycerol accumulation in non-adipose tissues like liver, heart and muscle (ectopic lipid accumulation). Consequently, obese individuals are at high risk of developing insulin resistance which may further lead to type 2 diabetes and cardiovascular complications such as acute myocardial infarction and diabetic cardiomyopathy [56–58]. Because of its key role in the alterations in lipid metabolism upon fatty acid oversupply, CD36/SR-B2 has been implicated in the etiology of obesity-induced or high fat diet-induced ectopic lipid accumulation and insulin resistance, and has been suggested as target for therapeutic intervention [14,59].

The molecular mechanism whereby high fat diet induces lipid accumulation in cardiac and skeletal muscle, and subsequently insulin resistance and contractile dysfunction, has been unraveled in much detail and shows a key role for CD36/SR-B2. Chronic oversupply of fatty acids to the heart or muscle triggers changes in the subcellular recycling of CD36/SR-B2 resulting in a permanent re-location of this transporter from endosomes to the sarcolemma.

At least in rats, this change is very rapid, i.e., within 2–3 days after the start of high fat feeding, and is accompanied by a concomitant increase in the rate of myocellular fatty acid uptake [60]. Fatty acids then not only become the main metabolic substrate for energy production, but also lead to excess intramyocellular formation of triacylglycerols, diacylglycerols and ceramides. The latter two fatty acid metabolites inhibit insulin signaling and impair the translocation of glucose transporter GLUT4 from endosomes to the sarcolemma, causing lowered glucose uptake and decreased incorporation of glucose into glycogen. At that stage, the heart or muscle has become insulin resistant and shows impaired contractile function [24,60]. In turn, this creates a vicious cycle of increased fatty acid uptake, lipid-induced insulin resistance and further impairment of cardiac function. In conclusion, CD36/SR-B2 plays an essential role already in an early stage of the development of insulin resistance due to lipid overload. Indeed, specific inhibition of CD36/SR-B2 by sulfo-*N*-succinimidyl oleate [61] or deletion of CD36/SR-B2 [62] protects against high fat diet-induced insulin resistance and cardiac contractile dysfunction.

It has not yet been elucidated why increased fatty acid delivery to heart or muscle results in a dysbalance of the subcellular recycling of CD36/SR-B2 with most of the protein permanently residing on the sarcolemma so that the rate of incoming fatty acids is not tuned anymore to the metabolic needs of the myocyte. Recent studies, however, suggest that increased CD36/SR-B2 translocation to the sarcolemma is caused by alkalinization of endosomes due to inhibition of the proton pumping activity of vacuolar-type H^+-ATPase (v-ATPse). Endosomal alkalinization was observed in rats fed a high fat diet and appeared an early lipid-induced event preceding the onset of insulin resistance. The mechanism of lipid-induced v-ATPase inhibition involves disassembly of the ATPase-containing sub-complex (V1) from the membrane-bound proton channel sub-complex (V0) and its subsequent migration into the soluble cytoplasm [Y. Liu, J.J.F.P. Luiken, unpublished observations]. These new insights suggest that activation of endosomal v-ATPase could be an effective approach to treat lipid-induced diabetic cardiomyopathy.

6. Concluding remarks

In the last 25 years the notion has arisen that the importance of (long-chain) fatty acids extends beyond their well-known functions as metabolic substrate and constituent of complex lipids (e.g. in biological membranes) in that fatty acids also modulate protein function and gene expression. Important steps in this recognition were made (*i*) with the identification of membrane-associated and cytoplasmic proteins functioning in the uptake and transport of fatty acids [14], and (*ii*) with the discovery of various isoforms of the nuclear receptor subfamily of PPARs which are activated by fatty acids and selected fatty acid metabolites as primary ligands [cf. 36,52]. In both these fields, dr. Paul A. Grimaldi has made significant contributions in that with his team he was the first to show the important role of the membrane protein CD36/SR-B2 in adipocyte differentiation [13] and to demonstrate the existence of a third isoform of the PPAR family, i.e., PPARβ/δ [42]. Both discoveries were made in the early 90s and have triggered much research activity in various preclinical and clinical laboratories. Specifically, both CD36/SR-B2 and PPARβ/δ have emerged as potential therapeutic targets in a range of metabolic diseases, in particular for metabolic syndrome [41,63,64].

Targeting CD36/SR-B2 could help normalize fatty acid metabolism in cardiac and skeletal muscle of obese and/or insulin resistant subjects thereby limiting the cardiovascular complications associated with these conditions [24]. Activation of PPARβ/δ by synthetic agonists has been found to promote fatty acid oxidation in several tissues such as skeletal muscle and brown adipose tissue [65]. However, reported positive actions of PPAR agonists on blood lipids, whole-body insulin resistance, and obesity show appreciable side-effects, although less for PPARβ/δ agonists than for agonists of the isoforms PPARα and PPARγ. Taken together, additional insight into the functioning of the PPARs and the cellular uptake, transport and, hence, biological availability of their primary ligands, i.e., long-chain fatty acids, under physiological and pathophysiological circumstances is needed in order to better design strategies for therapeutic intervention.

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