Biochimica et Biophysica Acta xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbalip

Review

CD36 actions in the heart: Lipids, calcium, inflammation, repair and more?☆

Nada A. Abumrad [a,*], Ira J. Goldberg [b]

[a] Departments of Medicine and Cell Biology, Washington University, St. Louis, MO, United States.
[b] Division of Endocrinology, Diabetes and Metabolism, New York University School of Medicine, New York, NY, United States.

ARTICLE INFO

Article history:
Received 16 February 2016
Received in revised form 14 March 2016
Accepted 15 March 2016
Available online xxxx

Keywords:
Fatty acid
Uptake
Signaling
Mitochondria
AMPK
LKB1

ABSTRACT

CD36 is a multifunctional immuno-metabolic receptor with many ligands. One of its physiological functions in the heart is the high-affinity uptake of long-chain fatty acids (FAs) from albumin and triglyceride rich lipoproteins. CD36 deletion markedly reduces myocardial FA uptake in rodents and humans. The protein is expressed on endothelial cells and cardiomyocytes and at both sites is likely to contribute to FA uptake by the myocardium. CD36 also transduces intracellular signaling events that influence how the FA is utilized and mediate metabolic effects of FA in the heart. CD36 transduced signaling regulates AMPK activation in a way that adjusts oxidation to FA uptake. It also impacts remodeling of myocardial phospholipids and eicosanoid production, effects exerted via influencing intracellular calcium (iCa^{2+}) and the activation of phospholipases. Under excessive FA supply CD36 contributes to lipid accumulation, inflammation and dysfunction. However, it is also important for myocardial repair after injury via its contribution to immune cell clearance of apoptotic cells. This review describes recent progress regarding the multiple actions of CD36 in the heart and highlights those areas requiring future investigation. This article is part of a Special Issue entitled: Heart Lipid Metabolism edited by G.D. Lopaschuk.

1. Introduction: Diverse pathways coordinate energy regulation

At the heart of the cell's most basic functions is the need for energy and this is particularly true of the cardiomyocyte. Numerous cellular pathways are normally dedicated to energy procurement or usage and to regulating interactions with the extracellular environment and other cells in order to maintain energy homeostasis. The surface receptor cluster of differentiation 36 (CD36) has a regulatory role in energy metabolism through its ability to recognize long chain fatty acids (FAs), promoting accumulation of intracellular free FA (FFAs) [2]. In addition CD36-mediated signaling facilitates, in a cell type or context dependent manner, FA storage or usage [3]. CD36 binds multiple ligands and resides within transmembrane or membrane-associated functional protein clusters [4–6]. Ligand interaction with CD36 can differentially induce changes in protein–protein interactions within these molecular clusters and consequently alter downstream signaling. This way FAs induce CD36-mediated signals that influence FA oxidation [1] and/or raise cytosolic calcium, triggering production of mediators that impact behavior of distant cells [7–9]. Some CD36 ligands, e.g. extracellular matrix components, influence cell-environment responses and processes such as cell adhesion/migration and angiogenesis [5] that indirectly relate to tissue energetics [10]. The protein is also a receptor for modified lipid moieties [11], contributing to uptake of oxidized low density lipoproteins (oxLDL), [12] or to apoptotic cell clearance [13,14]. It functions as a co-receptor for Toll-like Receptors (TLRs) in recognizing pathogen-associated lipids and facilitating the acute inflammation that is part of the innate immune response [15]. But it also contributes to inflammation resolution [13]. This review will focus on only those CD36-mediated processes that appear to affect heart metabolism and function and will attempt to highlight controversy and biology that require future investigation.

2. Heart FA uptake: metabolic flexibility and the evolving role of CD36

Chronically active muscles including the heart utilize large amounts of ATP, much of which is acquired from circulating lipids. Predominantly this includes FFAs associated with albumin as well as FFAs released from very low density lipoproteins (VLDL) or from intestinally derived chylomicrons via lipoprotein lipase (LpL)-mediated enzymatic cleavage of the triacylglycerol (TG) ester bonds [2,16]. During fasting, as circulating

Abbreviations: CD36, cluster of differentiation 36; FFA, unesterified fatty acids; TG, triglycerides; LpL, Lipoprotein lipase; VLDL, very low density lipoproteins; ^{123}I-BMIPP, 15-(p-iodophenyl)-3-(R, S)-methyl pentadecanoic acid; AMPK, adenosine monophosphate-activated protein kinase; LKB1, serine–threonine liver kinase B1; SERCA, SR/ER calcium ATPase; CaMK, calmodulin kinase; CPTI, carnitine palmitoyltransferase I; ACSL1, Acyl-CoE A synthase for long chain FA 1; oxLDL, oxidized low density lipoproteins.

☆ This article is part of a Special Issue entitled: Heart lipid metabolism edited by G.D. Lopaschuk.

* Corresponding author at: Department of Medicine, Center for Human Nutrition, Campus Box 8031, 660 S. Euclid Ave, St. Louis, MO 63110-1093, United States.
E-mail address: nabumrad@wustl.edu (N.A. Abumrad).

http://dx.doi.org/10.1016/j.bbalip.2016.03.015
1388-1981/

insulin decreases, FFA levels markedly increase in the bloodstream reflecting activation within adipocytes of two intracellular lipases, adipose TG lipase (ATGL) and hormone sensitive lipase (HSL). Uptake of FFAs by tissues increases in parallel with the rise in FFA levels. In particular, muscle and heart reduce their glucose use and rely primarily on FA catabolism for energy.

2.1. Cellular uptake of FFA by cardiomyocytes

FFAs can rapidly move across the plasma membrane [17] but whether this process is sufficient to provide the high rates of FA uptake necessary for cells active in FA metabolism has been questioned [18]. Studies in many mammalian cell types using FA complexed to albumin at increasing molecular ratios to vary concentration of unbound FA, yielded evidence for two distinct pathways of FA uptake, a low capacity saturable component and a high capacity non-saturable component. The saturable pathway has kinetics consistent with protein-facilitation: saturability, high affinity for long chain FAs (Km around 10 nanomolar within the concentration range of unbound FA in the circulation) and sensitivity to protein modifying agents [19]. The non-saturable pathway would operate at high ratios of FA: albumin and unbound FA concentrations above those found in the circulation, for example such as occurs with hydrolysis of TG-rich lipoproteins by LpL along the capillary surface or following hydrolysis of dietary TG by pancreatic lipase in the small intestinal lumen [20,21].

In cardiomyocytes, high affinity saturable FA uptake supporting a protein mediated process has been described [22–24]. One study that monitored appearance of intracellular FAs as a function of exogenous FA levels suggested that FA uptake is an energy consuming process that can occur against the FA concentration gradient [25].

2.2. Heart FA uptake from albumin and TG rich lipoproteins

CD36 was identified as a cellular FA transporter in 1993 based on work with isolated adipocytes [26], and physiological relevance of this function, especially as related to heart metabolism, has been documented by numerous studies [2,16,27]. CD36 was shown to traffic between the cell surface and intracellular compartments and is recruited to the sarcolemma by insulin and AMPK in a vesicle-mediated process [27,28] controlled by the Akt substrate 160 (AS160) Rab GTPase-activating protein (RabGAP AS160) and its target GTPase, Rab8a [29].

Uptake of FFAs into the heart has been studied using isolated hearts and whole animals. Several lines of evidence support the hypothesis that CD36 modulates cellular FFA transfer and accumulation in vivo. When CD36 was knocked out in mice the phenotype included increased circulating levels of FFAs and a 50–80% reduction in heart uptake of the slowly oxidized palmitate analog ^{123}I-BMIPP (15-(p-iodophenyl)-3-(R, S)-methyl pentadecanoic acid) or of iodinated (15-(p-iodophenyl) pentadecanoic acid (IPPA), both injected intravenously. Reduced tracer accumulation was also observed in skeletal muscle and adipose tissues but not in liver [30]. Activity of LpL in plasma was not altered but that of heart tissue was increased indicating no defect in hydrolysis of TG from lipoproteins in CD36 deficiency. When myocardial FA uptake from VLDL and chylomicrons was compared in mice lacking CD36, heart LpL or both, LpL was found important for uptake from both particle types while CD36 was only important for FA uptake from VLDL (Fig. 1). In the case of chylomicrons, the effect of CD36 deletion on FA uptake was negligible [31]. Since lipolysis of chylomicron TG would result in especially high local release of FFAs, these findings are consistent with CD36 facilitation of a high affinity low capacity process that is quantitatively marginal at very high FA levels.

In humans there are data to support existence of a higher affinity uptake process, possibly facilitated by CD36 at lower circulating FFA levels in humans [32]. In individuals with CD36 deficiency (2–6% prevalence in Asians and African–Americans), positron emission tomography (PET) studies usually in fasted subjects showed that myocardial accumulation of ^{123}I-BMIPP was undetectable while liver accumulation tended to increase [33–35]. A recent PET study examined [^{11}C]-palmitate uptake as a function of plasma FFA concentration by heart, muscle and adipose tissue in individuals with either partial or total CD36 deficiency. Two experimental protocols were used to create conditions with low (breakfast plus glucose intake) and high (overnight fast) circulating FAs. The findings indicated that CD36 is important for normal myocardial FFA uptake at both low and high circulating FFA levels while it is only important for muscle and adipose tissue uptake at low FFA levels [36]. Thus the human myocardium but not adipose tissue or muscle appears dependent on the CD36-mediated pathway for FFA uptake under both fed and fasted states.

2.3. Cardiac metabolic flexibility

A feature that is a characteristic of the CD36 deficient myocardium in rodents [37,38] and humans [39] is its increased reliance on glucose metabolism. The myocardium of the *Cd36* deficient mouse does not reduce its glucose utilization during fasting as occurs with the CD36 sufficient heart (Fig. 2). There is good evidence to support the interpretation that CD36 is important to the ability of the heart and muscle to accomplish substrate switching. This ability reflects the function of the protein in cellular FA uptake [2] and its role in regulating the activation of FOXO1 [38] and AMPK (see following sections), both important metabolic sensors. The increased reliance of the CD36 deficient heart on glucose and possibly ketones would help preserve myocardial energy [40]. Under baseline physiologic conditions heart function is normal and might even be improved in aged mice that are protected from excess lipid accumulation [41]. Based on its role in FFA uptake, targeting CD36 has been used to reduce the myocardial accumulation of intracellular lipids and the associated oxidative toxicity often observed in the diabetic heart. *Cd36* deficiency effectively reversed the myocyte TG accumulation and cardiac dysfunction of the MHC-PPARalpha mouse with cardiac specific overexpression of PPARalpha [42] and a similar rescue was observed with deficiency of cardiac LpL [43].

Tolerance to ischemia/reperfusion was studied in isolated working *Cd36*$^{-/-}$ hearts and the seemingly contradictory outcomes observed with one study showing impairement [44] while better recovery was observed in the second study [45] are instructive. In the latter study which used glucose and high insulin in the perfusate, *Cd36* deficiency led to reduced injury presumably because high glucose utilization requires less oxygen to create ATP from glucose rather than FFAs. In contrast, with more limited glucose delivery due to no insulin inclusion *Cd36* deficiency was deleterious, but injury was reduced when short chain FFAs were supplied as an alternative energy source [44]. Which of these experiments mimic in vivo actions of CD36 in animals or humans is currently unclear and likely to depend on the nutritional and/or activity state. Endogenous TG stores in the *Cd36*$^{-/-}$ heart are low and are markedly depleted after an overnight fast when stores in the CD36 sufficient heart are normally doubled [1,46]. The inability of the *Cd36*$^{-/-}$ myocardium to accomplish the metabolic adpatation of switching to more FA use during fasting makes the mouse susceptible to death when the fast exceeds 20 h [47] or when it is combined with a brief cold exposure [48].

2.4. CD36 and endothelial transport

The most obvious phenotype uncovered with CD36 deficiency is an increase in circulating FFAs levels, reduced FFA uptake into heart, skeletal muscle and adipose tissues, and enhanced cardiac glucose oxidation. While these observations support the importance of CD36 in parenchymal FFA uptake, the rapidity of normal blood FFA clearance is consistent with a defect in the first tissue cells required for removal of circulating FFAs. CD36 is highly expressed in capillary endothelial cells of the same tissues noted to have uptake defects in *Cd36*$^{-/-}$ mice: heart, skeletal muscle, and adipose tissue [49], including brown adipose. In the

Please cite this article as: N.A. Abumrad, I.J. Goldberg, CD36 actions in the heart: Lipids, calcium, inflammation, repair and more?, Biochim. Biophys. Acta (2016), http://dx.doi.org/10.1016/j.bbalip.2016.03.015