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Targeting metabolic disturbance in the diabetic heart

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Abstract

Diabetic cardiomyopathy is defined as ventricular dysfunction initiated by alterations in cardiac energy substrates in the absence of coronary artery disease and hypertension. In addition to the demonstrated burden of cardiovascular events associated with diabetes, diabetic cardiomyopathy partly explains why diabetic patients are subject to a greater risk of heart failure and a worse outcome after myocardial ischemia. The raising prevalence and accumulating costs of cardiovascular disease in diabetic patients underscore the deficiencies of tertiary prevention and call for a shift in medical treatment. It is becoming increasingly clearer that the effective prevention and treatment of diabetic cardiomyopathy require measures to regulate the metabolic derangement occurring in the heart rather than merely restoring suitable systemic parameters. Recent research has provided deeper insight into the metabolic etiology of diabetic cardiomyopathy and numerous heart-specific targets that may substitute or reinforce current strategies. From both experimental and translational perspectives, in this review we first discuss the progress made with conventional therapies, and then focus on the need for prospective metabolic targets that may avert myocardial vulnerability and functional decline in next-generation diabetic care.

Keywords: Diabetic cardiomyopathy, Metformin, Dipeptidyl peptidase-4, Glucagon-like protein-1, Advanced glycation end-products, Statins, Peroxisome proliferator activated receptor agonists, Fatty acid translocase/cluster of differentiation-36, Toll-like receptor-4, Nod-like receptor-3

Introduction

As of 2013, one in ten individuals living in Western countries was diagnosed with diabetes mellitus (DM), and three in ten were obese [1]. Epidemiological studies have long recognized diabetes to be an independent risk factor for heart failure (HF) and a worse prognosis after myocardial infarction (MI) [2]. Further, imaging studies have shown that diabetic patients are prone to ventricular hypertrophy, diastolic dysfunction, and decreased myocardial strain.

Diabetic cardiomyopathy (DCM) encompasses the group of diabetic patients who, while being otherwise free of cardiovascular events, display echocardiographic abnormalities ranging from mild diastolic impairment to overt heart failure, and prevalence of DCM may be over 50% among patients in clinical and preclinical stages [3,4]. Triggered by insulin inefficiency, hyperglycemia and hyperlipidemia, DCM brings out the damage of the myocardium due to

cell hypertrophy, steatosis, apoptosis, oxidative stress, inflammation, fibrosis, and microtubular derangement [5,6]. The existence of cardiac sinusoids representing fetal vascular pattern support that the pathophysiological mechanism of DCM initiates in the coronary microcirculation due to alterations in cardiac metabolism [6]. Although this phenotype has been described in both type-I (T1DM) and type-II (T2DM) diabetic patients, hyperglycemia-induced myocardial fibrosis may be predominant in T1DM hearts, while cardiomyocyte hypertrophy and steatosis are more related to the insulin resistance and dyslipidemia of T2DM [7]. In addition, the earlier onset of ventricular remodelling and down-regulation of pro-survival factors in experimental female rats but not yet in male counterparts suggests gender differences in DCM progression [8].

Intensive glycemic goals (HbA1c <7%) have failed to prevent cardiac complications in long-term diabetic patients or have even increased cardiovascular mortality [9]. The failure of the therapy approaches employed to date calls for accelerating efforts towards the development of

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new therapeutic strategies capable of preserving heart function while contributing to the overall care of diabetes. Growing experimental research emphasizes the prospective utility of correcting the metabolic imbalance that occurs in the diabetic heart.

In this work we aim to provide an updated, metabolism-oriented overview of the main therapeutic findings on therapy approaches for DCM. A comprehensive search of the PubMed/MEDLINE and EMBASE databases was conducted for articles published up to and including 2014 using the terms "diabetic cardiomyopathy" and "diabetes heart" in combination with the different animal models, drugs and related pathogenic factors to retrieve available literature. All types of articles (e.g., original studies, randomized controlled trials, review articles) published in English and reporting on studies in animals and humans were included.

Pathobiology of diabetic cardiomyopathy

Chronic hyperglycemia is primarily elicited by insulin deficiency and/or resistance and damages the myocardium by reducing the expression of glucose transporters, by increasing the expression of the pyruvate decarboxylase inhibitor pyruvate dehydrogenase kinase-4 (PDK4), and by raising the flux of glucose into hexosamine, pentose and polyol pathways. Such metabolic changes increase the burden of advanced glycation end-products (AGEs) and restrict energy production to fatty acid (FA) [5]. The harmful effects of AGEs are related to their ability to either promote collagen cross-linking and myocardial fibrosis or activate AGE receptor (RAGE)-mediated oxidative stress and pro-inflammatory nuclear factor-κB (NF-κB) signaling. Also, derangement of lipid metabolism is widely accepted as the most deleterious impact of diabetes on the heart. FA uptake may exceed the capacity for cardiac utilization and promote the deposition of triacylglycerols (TAGs) and ceramides, thus leading to cardiomyocyte steatosis and hypertrophy. Concurrently, increased FA oxidation leads to higher rates of oxygen consumption, reactive oxygen and nitrogen species (ROS/RNS) overproduction, endoplasmic reticulum (ER) stress and mitochondrial uncoupling [5]. Subsequent mitochondrial damage and increased ROS seem to be the main triggers for calcium mishandling, cardiomyocyte apoptosis, and the chronic low-grade inflammation occurring in DCM [10,11]. Moreover, the pro-oxidant status may be accentuated by defects in copper trafficking leading to myocardial copper deficiency [12]. All these pathogenic factors, in conjunction with many others (for further detail, see [5]) eventually converge to generate the progressive impairment of ventricular contractility, and thus provide valuable therapeutic substrates for the diabetic heart.

Progress and limitations of conventional antihyperglycemic therapies

DCM may account for a substantial fraction of idiopathic HF diagnoses in T2DM patients, and also contribute to a worse prognosis of HF after myocardial ischemia in these patients. The United Kingdom Prospective Diabetes Study 34 (UKPDS 34) evaluated the outcomes of overweight T2DM patients following tight glycemic control with metformin, and is the only clinical study to show reduced cardiovascular mortality following intensive blood-glucose control [13]. However, there is long-standing controversy regarding the safety and efficacy of metformin in the setting of HF. A recent systematic review of observational studies involving 34,000 patients concluded that metformin is at least as safe as other glucose-lowering treatments even in the presence of reduced ejection fraction and chronic kidney disease [14]. Ongoing trials will soon provide specific evidence on the role of metformin in T2DM patients with HF (e.g. NCT01690091 - ClinicalTrials.gov). A variety of preclinical studies have pointed out the cardioprotective benefits of metformin on both T1DM and T2DM animal models of DCM, an effect likely associated with its ability to prevent cardiomyocyte oxidative stress and apoptosis by inhibiting the respiratory complex I and modulating specific ER stress signaling pathways (Table 1) [15-22]. Yet, the positive outcome on T1DM models seems more related to a dramatic reduction of plasma glucose and TAGs than to a myocardial-specific response (Table 2) [15]. Although metformin is not prescribed for most T1DM patients, it is widely recommended as the first-line oral therapy for T2DM. Unexpectedly, in T2DM animals cardiac fibrosis and contractile performance together with molecular markers of inflammation were not consistently improved or even aggravated. Such effects challenge our knowledge of the impact on metformin in the heart and question its utility against DCM. Of other conventional antihyperglycemic agents, only sulfonylureas have been proven to exert some benefit against experimental DCM (Table 1) [23]. However, sulfonylureas have been associated with an excess risk of MI and cardiovascular events [13], with the only possible exception of gliclazide [24]. Further, the use of sulfonylureas has been clearly restricted given their tendency to trigger hypoglycemias and weight gain (Table 2).

New directions in therapy against glucose-associated injury

Current trends in the care of the diabetic heart have downgraded the importance of HbA1c and other classical glycemic markers. However, abrogation of myocardial-specific insulin resistance and glucose toxicity could still be a valid strategy against DCM. Some of the most intuitive genetic interventions with therapeutic interest have targeted GLUT4, the most prevalent insulin-regulated glucose transporter in the heart, PDK4 and insulin-like

Table 1 Progress on the effects of conventional metabolic therapies in experimental DCM

	Type 1 diabetes			Type 2 diabetes				
	STZ	OVE26	Akita	*ob/ob*	*db/db*	ZDF	GK	DIO[†]
Metformin	↑Glucose utilization ↑Cardiac function	↓Apoptosis ↑Cardiac function		↓Hypertrophy ~Fibrosis ↑Cardiac function		~Hypertrophy ↑Fibrosis ↓Steatosis	↓/~Hypertrophy ↓Fibrosis ↓Apoptosis ~Oxidative stress ↑Cardiac function	
Sulfonylureas	↑Cardiac function							
DPP-4 inhibitors			↑Hypertrophy ↑Cardiac function		~Hypertrophy ↓Fibrosis ↓Oxidative stress ~Cardiac function		↓Hypertrophy ↓Fibrosis ↓Apoptosis ↑Cardiac function	
GLP-1R agonists								↑Glucose utilization ↓Hypertrophy ↓Apoptosis ↓Inflammation ↑Cardiac function
Statins	↓Fibrosis ↓Oxidative stress ↓Inflammation ↑Cardiac function							↓Hypertrophy ↓Fibrosis ↓Inflammation ↑Cardiac function
PPARα agonists				↑Glucose utilization ↓Steatosis ~Cardiac function	~Hypertrophy ↓Fibrosis ↓Steatosis			↑Glucose utilization ↓ER stress ↓Inflammation ↑Cardiac function
PPARγ agonists	↓Hypertrophy ↓Apoptosis ↓Steatosis ↓Oxidative stress ↑Cardiac function		↑Hypertrophy	↑Glucose utilization ~ Cardiac function		↑Glucose utilization		
Reference	[15,16,23,25-27]	[17]	[28,29]	[18]	[30-32]	[19,33]	[20,21]	[34-38]

Conventional treatments against DCM have been assessed in different animal models of type-I [STZ, streptozotocin-treated mice/rats; OVE26, calmodulin transgenic mice; Akita, insulin-2 deficient mice] and type-II [*ob/ob*, leptin deficient mice; *db/db*, leptin receptor deficient mice; ZDF, Zucker Diabetic Fatty, heterozygous leptin receptor deficient rats; GK, Goto-Kakizaki rats; DIO, diet-induced obesity] diabetes. ↑, ↓ and ~ stand for increased, decreased or not modified effect, respectively. [†]For the sake of simplicity, evidence from low-dose STZ- plus diet-induced T2DM models are displayed in the DIO column. References: [34] and [37].

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Table 2 Limitations and drawbacks for the use of conventional metabolic therapies against DCM

Therapy	Limitation/Drawback	Reference
Metformin	Dependence on plasma glucose reduction	[15,19]
	Inconsistent recovery of cardiac function	
	Potential induction of myocardial fibrosis	
Sulfonylureas	Higher risk of total cardiovascular events (*except gliclazide*)	[13,23]
	Hypoglycemia	
	Weight gain	
DPP-4 inhibitors	Higher risk of subclinical cardiac dysfunction and HF hospitalization	[28,30,39,40]
	Inconsistent recovery of cardiac function and remodelling	
	Higher risk of myocardial hypertrophy with effective dose	
GLP-1R agonists	Lack of data from large clinical trials on cardiovascular outcome	
Statins	Dependence on plasma lipid reduction and vascular remodelling	[41,42]
	Adverse impact on insulin production and sensitivity	
	Higher risk of total cardiovascular events (*torcetrapib*)	
PPARα agonists	Dependence on plasma lipid reduction	[31,38,43]
	Inconsistent recovery of cardiac function (*fenofibrate*)	
	DCM-like phenotype by experimental PPARα agonism	
PPARγ agonists	Dependence on plasma lipid reduction	[29,44,45]
	Higher risk of HF (*pioglitazone*)	
	Higher risk of total cardiovascular events (*rosiglitazone*)	
	Inconsistent recovery of cardiac function (*rosiglitazone*)	
	Potential induction of myocardial hypertrophy (*rosiglitazone*)	
	DCM-like phenotype by experimental PPARγ agonism	

growth factor-1 (IGF-1) (Table 3) [46-48]. Yet, these approaches face the potential threat of inducing intracellular glucose excess and associated toxicity. In this scenario, incretins may be advantageous. Although drugs modulating the incretin system are conventional agents in the management of diabetes, they may have more cardioprotective properties than those currently known (Table 1) [20,28,30,34-36,49]. The cardinal role of incretins (i.e. glucagon-like peptide 1, GLP-1) is the regulation of postprandial glycemia by stimulating insulin secretion in a glucose-dependent manner. Such endogenous modulation provides an exciting advantage over conventional antihyperglycemic drugs. Moreover, GLP-1 receptor (GLP-1R)

has been detected in large amounts in the sarcolemma of isolated cardiomyocytes, thus suggesting that the cardiac effects of GLP-1 may be partially driven in a myocardial, insulin-independent, and specific fashion [20]. GLP-1 has been shown to enhance glucose uptake and limit FA consumption and lipotoxicity, likely by increasing GLUT-1 translocation and down-regulating the main cardiac FA transporter FAT/CD36 [30,50,51]. Further, we described anti-apoptotic/fibrotic effects of exogenous GLP-1 and its insulinotropic inactive metabolite GLP-1(9–36) in cardiomyocytes under diabetic conditions [20]. The first set of drugs to be developed, dipeptidyl peptidase-4 (DPP-4) inhibitors, increases GLP-1 levels in a physiological range and are weight neutral (Table 1). However, palliation of cardiac architectural and functional alterations was inconsistent among different models of T2DM exposed to sitagliptin [20,28,30]. In addition, the Saxagliptin Assessment of Vascular Outcomes Recorded in Patients with Diabetes Mellitus (SAVOR)-Thrombolysis in Myocardial Infarction (TIMI) 53 (SAVOR-TIMI 53) trial reported a 27% increase in hospitalization due to HF among diabetic patients who received saxagliptin as compared with placebo, and found no significant reductions on any macrovascular endpoint [39]. No clear explanation has been provided for these heterogeneous results. In this regard, DPP-4 inhibitors may increase the circulating half-life of DPP-4, which has been independently associated with a higher risk of cardiac dysfunction in T2DM patients [40]. This may be explained by the ability of DPP-4 to inactivate non-incretin molecules such as the brain natriuretic peptide, several chemokines (i.e., SDF1), and energy-related molecules (i.e., peptide-YY) [52]. Further, dosage might also be a relevant drawback for at least the use of sitagliptin, given that the improvement of glycemic control required higher doses which in turn induced cardiac hypertrophy in experimental models (Table 2) [28]. Despite the disappointing results, DPP-4 inhibition has also elicited positive myocardial outcomes and thus further research on its cardioprotective actions is warranted. For instance, sitagliptin has been shown to increase myocardial glucose uptake and ejection fraction in cohorts of non-diabetic patients with non-ischemic cardiomyopathy and stable coronary artery disease, respectively [53,54].

GLP-1R agonists may withstand some of the controversy concerning DPP-4 inhibitors (Table 1). Exenatide was recently shown to ameliorate heart performance by increasing glucose uptake and, consistent with Randle's hypothesis, down-regulating FAT/CD36 in a DCM-like model [49]. Similarly, liraglutide improved cardiac outcome in diet- plus streptozotozin (STZ)-induced T2DM animals independent of restoration of fasting blood glucose [34]. Nevertheless, the mechanisms underlying such functional recovery remain elusive and the attenuation of molecular markers of myocardial remodeling

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Table 3 Genetic manipulation of key metabolic mediators of DCM with prospective therapeutic relevance

Mechanism	Model	Year	Genetic engineering	Myocardial outcome	Reference
Glucose-associated injury	*db/db*	2002	Transgenic GLUT4	↑Glucose utilization, ↑Cardiac function	[46]
	DIO	2011	Transgenic PDK4	↓Steatosis	[47]
	STZ	2001	Transgenic IGF-1	↓Hypertrophy, ↓Apoptosis, ↓Oxidative stress, ↑Cardiac function	[48]
	STZ	2009	RAGE knockdown	↑Mitochondrial function, ↑Cardiac function	[55]
	STZ	2013	Transgenic GLO-1	↓Fibrosis, ↓Oxidative stress, ↓Inflammation	[56]
	STZ	2005	Transgenic O-GlcNAcase	↑Ca^{2+} mobilization, ↑Cardiac function	[57]
	MHC-PPARγ	2010	PPARα knockout	↓Hypertrophy, ↓ Apoptosis, ~Steatosis, ↓Oxidative stress, ↑Mitochondrial function, ↑Cardiac function	[58]
Lipid-associated injury	DIO	2007	Transgenic PPARβ/δ	↓Steatosis	[59]
	MHC-PPARα	2007	FAT/CD36 knockout	↓Steatosis, ↑Glucose utilization, ~Hypertrophy, ↑Ca^{2+} mobilization, ↑Cardiac function	[60]
	MHC-PPARα	2010	LPL knockout	↓Hypertrophy, ↓Steatosis, ↑Glucose utilization, ↑Mitochondrial function, ↑Cardiac function	[61]
	STZ	2014	Perilipin-5 knockout	↓Steatosis, ↓Oxidative stress, ↑Cardiac function	[62]
	Akita	2013	Transgenic ATGL	↓Steatosis, ↑Glucose utilization, ↑Mitochondrial function, ↑Cardiac function	[63]
	DIO	2014	Transgenic ATGL	↓Steatosis, ↑Diastolic/Systolic function	[64]
	STZ	2008	Transgenic HSL	↓Fibrosis, ↓Steatosis, ↓Oxidative stress	[65]
	STZ	2014	Arachidonate 12/15-lypoxygenase	↓Fibrosis, ↓Oxidative stress, ↓Inflammation	[66]
	DIO	2012	Transgenic SCD1	↑Steatosis, ↑Glucose utilization, ↓Oxidative stress, ↓Apoptosis	[67]
	MHC-PPARγ	2012	Transgenic DGAT-1	~Steatosis, ↑Glucose utilization, ↑Cardiac function	[68]
	MHC-ACS	2009	Transgenic DGAT-1	↑Steatosis, ↓Apoptosis, ↑Glucose utilization, ↓Oxidative stress, ↑Mitochondrial function, ↑Cardiac function	[69]
	DIO	2014	Transgenic Adiponectin R1	↓Hypertrophy, ↓Steatosis, ↓Oxidative stress	[70]
	DIO	2013	Transgenic APPL1	↓Steatosis, ↑Glucose utilization, ↑Cardiac function	[71]
	MHC-LPL^{GPI}	2004	Transgenic human apoB	↓Steatosis, ↑Glucose utilization	[72]
Metabolism-associated inflammation	STZ	2010/13	TLR4 knockdown	↓Hypertrophy, ↓Fibrosis, ↓Apoptosis, ↓Oxidative stress, ↑Cardiac function	[73,74]
	NOD	2012	TLR4 knockout	↓Steatosis, ↑Cardiac function	[75]
	STZ	2014	HMGB1 knockdown	↓Hypertrophy, ↓Fibrosis, ↑Cardiac function	[76]
	DIO + STZ	2013	NLRP3 knockdown	↓Hypertrophy, ↓Fibrosis, ↑Cardiac function	[37]

↑, ↓ and ~ stand for increased, decreased or not modified effect, respectively.

still requires further support from histological examination [35,36]. In the clinical setting, exenatide achieved greater benefits in glycemic control, bodyweight and heart pressure as compared to sitagliptin or pioglitazone when all three were used in combination with metformin [77]. It is expected that the Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results (LEADER) trial will for the first time provide insight into the cardiovascular impact of GLP-1R-agonists in T2DM patients. To fully assess the potential utility of incretin-based drugs against DCM, along with their safe contribution to the overall care of diabetes, more evidence is needed on its clinical use and a deeper

understanding of the direct impact on cardiomyocytes must be attained.

Decreasing the excess of harmful AGEs is also a main goal of glycemic control in DCM. AGEs are well-described mediators of hyperglycemia-associated cellular injury caused by increased reactive substrate availability. Although there is clinical evidence that AGE disruption may improve diastolic impairment [78], no data on such an effect in DCM patients are available. Consistently, RAGE ablation or cross-linking disruption in STZ-treated animals has been shown to effectively revert cardiac dysfunction [55,79]. Main strategies to palliate AGE-induced dysfunction are depicted in Table 3 [56,57] and may make

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the case for targeting AGEs for prevention of DCM. Overall, facilitating glucose transport across the sarcolemma in a glucose-dependent manner and avoiding its conversion into toxic alternate products may mark a major success against DCM.

Progress and limitations of conventional antihyperlipidemic therapies

The unsuccessful prevention of cardiac complications in long-term T2DM patients by glycemia-oriented therapies has prompted a shift in the care of the diabetic heart. Various clinical studies have reported an outstanding decrease of cardiovascular risk in T2DM patients who undergo intensive lipidemic control [80-82]. It is known that hyperlipidemia may lead to FA accumulation and lipotoxicity in the diabetic myocardium. Therapeutic standards for diabetic patients include the prescription of lipid modifying agents, mainly HMG-CoA reductase inhibitors (i.e., statins) and peroxisome proliferator-activated receptor (PPAR) agonists (i.e., fibrates, glitazones), in both primary and secondary prevention. While statins are one of the most prescribed drugs worldwide due to their effective and safe impact on cholesterol levels, they do not seem to affect the natural history of DCM and their repercussion on the diabetic myocardium is currently being investigated. Most animal evidence is based on STZ-treated animals, and it has been shown that a variety of statins successfully reverted the DCM phenotype (Table 1) [25,26,37]. Yet it remains unclear whether the improvement of cardiac performance is due mostly to decreased vascular remodeling and heart workload rather than to a direct cardiac effect. Major clinical shortcomings in need of explanation include the recently reported adverse impact of statins on insulin sensitivity and β-cell function [41] and the increase of overall cardiovascular events induced by other cholesterol-modulating therapies such as the HDL raiser torcetrapib (Table 2) [42]. Nevertheless, current data do not support discontinuing statin therapy in diabetic patients [41].

PPARs are critical regulators of lipid transport, storage and utilization mainly by inducing the expression of FAT/CD36, FA oxidation enzymes, and PDK4. Importantly, PPARs are differentially located in organic tissues: PPARα is abundant in the heart while PPARγ is preferentially found in the adipose tissue. In diabetic animals (Table 1), pharmacological agonism of PPARα or PPARγ alleviated the DCM phenotype, likely by normalizing myocardial insulin-stimulated glucose uptake and reducing myocardial FA misuse and accumulation [19,27,31-33,38]. However, most studies in animal models do not provide consistent evidence that these drugs can rescue cardiac contractile function, or whether the cardiac benefits are rather a partial consequence of their lipid-lowering action (Table 2) [27,29,31,32,38]. From a clinical perspective, the

relative safety of widely prescribed fibrates contrasts with the fact that PPARγ-based strategies have faced important drawbacks. Fibrates have proved to reduce the incidence of non-fatal MI and cardiovascular deaths in diabetic patients [43], although their contribution was milder than expected and further effort is needed to elucidate their mechanistic rationale. The Prospective Pioglitazone Clinical Trial in Macrovascular Events (PROactive) observed reduced total cardiovascular events, but detected an increase in HF episodes [83]. Rosiglitazone, however, was associated with a two-fold increase of incidence of cardiovascular events as compared with metformin, which eventually led to its withdrawal from the market [44]. Consistent with their role as transcriptional regulators of lipid metabolism, the transgenic enhancement of both PPARα and PPARγ induced a DCM-like phenotype [58,84]. Such effects have been explained by the reciprocal repression of glucose uptake and utilization in a setting of exacerbated lipid use, and may underlie the harmful response to the aforementioned PPARγ agonists. Taken as a whole, PPARα and PPARγ agonisms do not seem to cause a clinically meaningful benefit on the cardiac outcome of T2DM patients, and may even aggravate the condition.

New directions against lipid-associated injury

The critical role of PPARs in the regulation of lipid metabolism should not be overlooked, and further research on different isoforms may suggest future tools against DCM (Table 3). PPARβ/δ is the most prevalent isoform in the myocardium and, unlike PPARα, its expression level is not increased in experimental T2DM [59]. Both genetic manipulation and selective agonism in mouse hearts and human cardiac cells provided evidence of the therapeutic potential of PPARβ/δ against cardiac steatosis, inflammation and dysfunction [59,85,86]. Such effects may depend on the ability of PPARβ/δ to up-regulate FA oxidation enzymes and GLUT4 while limiting FA uptake and TAG synthesis [59]. Experimental PPARβ/δ activation also ameliorated dyslipidemia and insulin resistance and prevented the development of obesity [87]. Thus, it is plausible that PPARβ/δ-based drugs provide an advantage over current PPARα agonists in the treatment of the diabetic heart.

Aside from PPARs, several molecular targets are found modulating lipid metabolism. The genetic manipulation of molecules involved in the initial steps of lipid absorption and accumulation has revealed cardioprotective properties and suggested potential strategies against DCM (Table 3) [60-62]. From this group, FAT/CD36 is the most studied molecule and currently has aroused the highest expectations due to its crucial role in lipotoxicity [88]. Although FAT/CD36 may be responsible for up to 60% of cardiac FA uptake, the mechanism of FA trafficking across the sarcolemma is only partially understood. Similar to

GLUT4, FAT/CD36 molecules are found stored inside endosomal vesicles and can be translocated upon stimulation by insulin and by contraction. The redistribution of FAT/CD36 to the sarcolemma is one of the earliest alterations occurring in the heart during diet-induced diabetes, and has been observed in ZDF rats and in STZ mice in proportion to the severity of insulin deficiency [89,90]. Consistently, ablation of FAT/CD36 rescued a PPARα over-expression-induced DCM phenotype [60]. The feasibility of modulating FAT/CD36 was recently addressed by Glatz et al. in diabetic cardiomyocytes, indicating the benefit of interfering with FAT/CD36 trafficking [91]. Interestingly, FAT/CD36 was recently shown to be down-regulated by sitagliptin in *db/db* mice [30], thus suggesting that myocardial lipid uptake could also depend upon GLP-1R signaling. Therefore, modulation of FAT/CD36

may deserve further attention as a prospective etiological treatment for the diabetic heart.

FAT/CD36 interference may also yield interesting effects on metabolism-associated inflammation given its role as Toll-like receptor-4 (TLR4) cofactor [92]. TLRs are pattern recognition receptors implicated in tailoring the innate immune cascade through conserved inflammatory pathways. Major cardiac isoforms, TLR4 and TLR2 represent an emerging standpoint for inflammatory and metabolic coupling in the diabetic heart, where they function as upstream inducers of NF-κB and regulate insulin resistance. Notably, ablation of TLR4 and associated ligands successfully reverted architectural aberrations and restored cardiac dysfunction in STZ-treated and non-obese T1DM (NOD) mice (Table 3) [73-76]. Moreover, pharmacological intervention of TLR4 has already caused
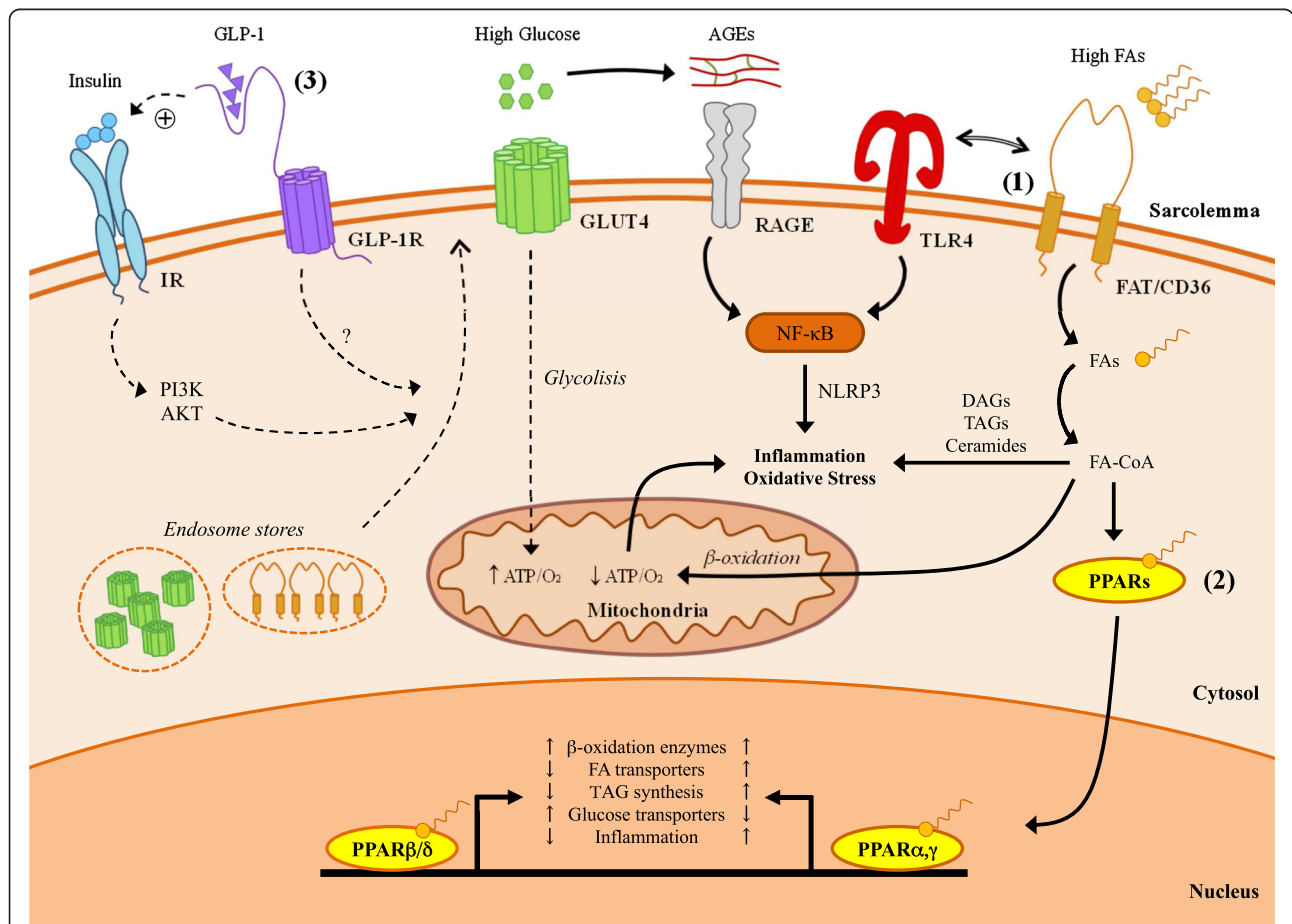

Figure 1 Metabolic disturbance in the diabetic heart and prospective therapeutic targets. Thickened lines denote activated pathways, and dotted lines denote reduced pathway. **(1)** The therapeutic reduction of FAT/CD36 activity may attenuate myocardial steatosis, inflammation and oxidative stress, and further improve the energetic yield by shifting metabolism to glucose utilization. **(2)** Induction of specific PPAR isoforms such as PPARβ/δ may provide cardioprotection by down-regulating FA transporters and TAG synthesis and up-regulating GLUT4, β-oxidation enzymes, and anti-inflammatory transcripts. **(3)** The elevation of incretin signaling by GLP-1 agonists (or DPP-4 inhibitors) may also control insulin resistance and hyperlipidemia. GLP-1R-dependent actions may include the regulation of glucose and FA receptors trafficking to the sarcolemma, and the amelioration of apoptosis and fibrosis. IR, insulin receptor; FA-CoA, fatty acid-coenzyme A.

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a benefit in several forms of non-diabetic myocardial dysfunction [93]. It is also known that FAT/CD36 cooperates with TLR4 to prime the inflammasome, a group of multimeric protein complexes of which Nod-like receptor-3 (NLRP3) is the most common form, leading to caspase-1-mediated IL-1β processing and release [94,95]. Besides contributing to multiple pro-inflammatory and pro-fibrotic pathways, NLRP3 has also been shown to promote insulin resistance. Consistently, NLRP3 knockdown reduced cardiac hypertrophy and fibrosis and restored cardiac function in STZ-induced T2DM mice (Table 3) [37]. This emerging inflammatory perspective of FAT/CD36 actions reinforces the expectations for its targeting against DCM.

Genetic engineering has uncovered numerous other key molecules of lipid metabolism with potential use against DCM. This group includes rate-limiting lipases of TAG hydrolysis [63-65], arachidonate lypoxygenases [66], and mitochondrial enzymes involved in FA synthesis [67-69]. A practical summary of their impact in several animal models of DCM is shown in Table 3. There is also growing interest for the pharmacological utility of specific peptides regulating energy homeostasis such as adiponectin and ghrelin. In this regard, genetic enhancement of upstream components of adiponectin signalling ameliorated DCM phenotype, glucose uptake and cardiac function in diet-induced T2DM [70,71]. Similar results were recently described in *db/db* mice administered desacyl ghrelin, the non-octanoylated form of this "hunger hormone" [96]. Remarkably, the intervention of some of these factors achieved functional recovery despite inconsistent lowering of cardiac TAG content. In sum, since the increase of FA oxidation is tightly coupled with higher rates of oxygen consumption and ROS/RNS generation, it would seem reasonable to assume that therapies against lipid uptake will provide advantage over those enhancing intracellular metabolism. We would also argue that prospective therapies should not focus on reducing the content of cardiac TAGs but rather prioritize the burden of derived molecules with greater toxicity such as diacylglycerides and ceramides.

Conclusions

As therapeutic resources currently allow diabetic patients to manage glucose homeostasis, reach near-normal life expectancy and delay microvascular complications, it is unacceptable that heart-risk reductions are not yet properly achieved. By targeting DCM we may prevent cardiac functional decline and improve the response to potentially lethal coronary events. Although the fine-tune mechanisms leading to DCM remain partially opaque, restoring cardiac energy metabolism seem to be a cornerstone. In light of the experimental data provided here, we may outline a potential therapeutic strategy focused on correcting the etiological imbalance between lipids and glucose as fuel substrates (Figure 1). Excessive FA uptake, accumulation, and utilization may be attenuated by down-modulating cardiac FAT/CD36 and/or stimulating its transcriptional regulator PPARβ/δ. Subsequent up-regulation of GLUT4 and glycolitic enzymes would elevate the ATP/O_2^- consumption ratio and reduce hyperglycemia and AGE generation. Insulin resistance and hyperlipidemia may be also controlled by statins, and perhaps more prospectively by incretin-based drugs, which would balance the content of sarcolemmal glucose and FA transporters and exert anti-apoptotic/fibrotic benefits in the cardiomyocyte. In addition, FAT/CD36 interference may provide further protection against myocardial inflammation and stiffness through the attenuation of TLR4-NLRP3 signaling. The design of these cardioprotective plans, however, may lead to difficulties concerning the possibility of provoking other end-organ complications associated with glucotoxicity. To overcome these hurdles, the assessment of differential traits, stages and pathogenic factors in each diabetic patient becomes a clinical priority.

Abbreviations

DM: Diabetes mellitus; HF: Heart failure; DCM: Diabetic cardiomyopathy; T1DM/T2DM: Type 1 or Type 2 Diabetes Mellitus; PDK4: Pyruvate dehydrogenase kinase-4; AGEs: Advanced glycation end-products; FA: Fatty acid; RAGE: AGE receptor; NF-κB: Nuclear factor-κB; TAGs: Triacylglycerols; ROS/RNS: Reactive oxygen and nitrogen species; IGF-1: Insulin-like growth factor-1; GLP-1: Glucagon-like peptide-1; DPP-4: Dipeptidyl peptidase-4; PPAR: Peroxisome proliferator-activated receptor; TLR4: Toll-like receptor-4; NLRP3: Nod-like receptor-3; HMGB1: High-mobility group box-1.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

JF-A and OL wrote and edited the manuscript. BP and ER researched the data. JE and JT reviewed the manuscript. All authors read and approved the final manuscript.

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