J Physiol 599.14 (2021) pp 3477–3493

The Journal of Physiology

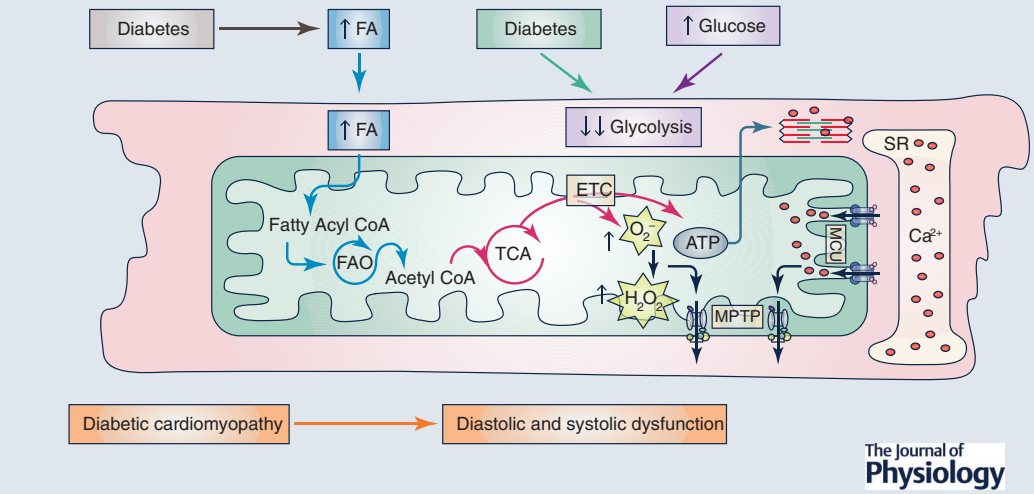
SYMPOSIUM REVIEW

The role of mitochondria in metabolic disease: a special emphasis on heart dysfunction

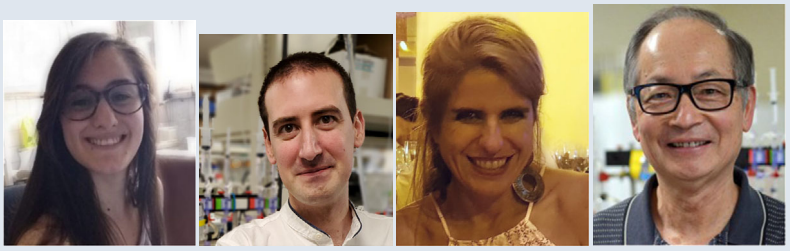
Marilen Federico[1], Sergio De la Fuente[2], Julieta Palomeque[1,3] and Shey-Shing Sheu[2]

[1]*Centro de Investigaciones Cardiovasculares, CCT-La Plata-CONICET, Facultad de Cs. Medicas, UNLP, La Plata, Argentina*
[2]*Department of Medicine, Center for Translational Medicine, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA, 19107, USA*
[3]*Centro de Altos Estudios en Ciencias Humanas y de la Salud, Universidad Abierta Interamericana, CABA, Argentina*

Edited by: Ian Forsythe & Livia Hool



Abstract Metabolic diseases (MetDs) embrace a series of pathologies characterized by abnormal body glucose usage. The known diseases included in this group are metabolic syndrome, pre-diabetes and diabetes mellitus types 1 and 2. All of them are chronic pathologies that present metabolic disturbances and are classified as multi-organ diseases. Cardiomyopathy has been extensively described in diabetic patients without overt macrovascular complications. The heart



Marilen Federico is a PhD student at Julieta Palomeque's Lab. Her work focuses on the mechanism of the apoptosis pathway in prediabetic heart emphasis on cytosolic Ca^{2+} and mitochondrial Ca^{2+} signalling. **Sergio de La Fuente** is a postdoctoral fellow at Dr Sheu's Lab. His research focuses on the mitochondrial Ca^{2+} fluxes within adult cardiomyocytes, with emphasis on the mitochondrial Ca^{2+} uniporter and the Na^{+}/Ca^{2+} exchanger. **Julieta Palomeque** is professor UNLP and Established Investigator at Cardiovascular Research Center, CONICET, UAI. Her research focuses on identifying molecular mechanisms in prediabetic hearts and high-glucose states related to CaMKII activity. **Shey-Shing Sheu** is William Wikoff Smith Professor of Cardiovascular Research and Associate Director at Center for Translational Medicine at Thomas Jefferson University in Philadelphia, Pennsylvania. His research has concentrated on understanding the mechanisms underlying mitochondrial ATP, Ca^{2+}, reactive oxygen species and fission–fusion dynamics in the heart.

This review was presented at the joint *Australian Physiological Society* and *Australian Society for Biophysics* Meeting symposium 'Unravelling the mysteries of mitochondria in health and disease' organised by Livia Hool (University of WA), which took place at the Australian National University, Acton Campus, Canberra on 2 December 2019.

DOI: 10.1113/JP279376

is severely damaged during the progression of the disease; in fact, diabetic cardiomyopathies are the main cause of death in MetDs. Insulin resistance, hyperglycaemia and increased free fatty acid metabolism promote cardiac damage through mitochondria. These organelles supply most of the energy that the heart needs to beat and to control essential cellular functions, including Ca^{2+} signalling modulation, reactive oxygen species production and apoptotic cell death regulation. Several aspects of common mitochondrial functions have been described as being altered in diabetic cardiomyopathies, including impaired energy metabolism, compromised mitochondrial dynamics, deficiencies in Ca^{2+} handling, increases in reactive oxygen species production, and a higher probability of mitochondrial permeability transition pore opening. Therefore, the mitochondrial role in MetD-mediated heart dysfunction has been studied extensively to identify potential therapeutic targets for improving cardiac performance. Herein we review the cardiac pathology in metabolic syndrome, prediabetes and diabetes mellitus, focusing on the role of mitochondrial dysfunctions.

(Received 31 December 2020; accepted after revision 18 March 2021; first published online 1 May 2021)
Corresponding author S.-S. Sheu: Center for Translational Medicine, Department of Medicine, Sidney Kimmel Medical College, Thomas Jefferson University, 1020 Locust Street, room 543D Philadelphia, PA 19107, USA. Email: shey-shing.sheu@jefferson.edu

Abstract figure legend Cardiac mitochondrial function in metabolic disease. Metabolic disease is characterized by decreased glycolysis due to insulin resistance and increased free fatty acid (FA) uptake that promotes FA oxidation (FAO) for ATP generation. Excessive FA accumulation leads to increases in superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) production over a threshold limit. High levels of reactive oxygen species (ROS) lead to uncoupling of the mitochondria electron transport chain (ETC), which reduces mitochondrial ATP production. In addition, the influx of Ca^{2+} through mitochondrial Ca^{2+} uniporter (MCU) increases excessively. These increases in ROS and Ca^{2+} eventually trigger mitochondrial permeability transition pore (mPTP) opening, leading to cardiomyocyte death. All these alterations promote diastolic and systolic dysfunction, which leads to diabetic cardiomyopathy.

Introduction

Metabolic diseases (MetDs), which result from disrupted normal glucose usage, have increased in incidence in recent years (World Health Organization, 2020). They are divided into three types – prediabetes, metabolic syndrome (MetS) and diabetes mellitus (DM) – according to the organ compromised and/or the severity of the disease itself.

MetDs affect several organs across the human body and raise the risk for multiple conditions including cardiovascular disease (CVD). In patients with DM, metabolism-mediated heart damage can affect heart structure and function, leading to diabetic cardiomyopathy (DCM) (Rubler *et al.* 1972), without the need for other associated co-morbidities. DCM manifests as persistent cardiac dysfunction that frequently leads to heart failure (HF), the principal cause of death in DM (Boonman-de Winter *et al.* 2012; Vasiliadis *et al.* 2014; Lee & Kim, 2017).

Among the factors that affect MetD aetiology, mitochondrial dysfunction has been shown to play a critical role. It is essential to elucidate the mitochondrial processes that lead to MetD progression, from the early stages to the most severe conditions. Improved understanding may guide the identification of potential therapeutic targets and eventually develops strategies to mitigate or even revert the DCM. The purpose of this review is to summarize the current knowledge about the mitochondrial role within several MetDs, including MetS, prediabetes, and DM, with a focus on the mechanisms of cardiac dysfunction.

Metabolic diseases

Prediabetes. Impaired glucose tolerance and impaired fasting glycaemia are conditions that define prediabetes. Without changes in their diet and exercise habits, half of the patients with prediabetes will develop type 2 DM (T2DM) (American Diabetes Association, 2019). Diagnosis for prediabetes can be made using the same tests as for T2DM (i.e. fasting glucose, glycated haemoglobin test and/or oral glucose tolerance test) but with a different cut-off (for prediabetes diagnosis: 100 mg/dL $\leq$ fasting glucose $<$ 126 mg/dL, 5.7% $\leq$ glycated haemoglobin $<$ 6.4%, and/or 140 mg/dL $\leq$ glucose after 2 h tolerance test $<$ 200 mg/dL). However, because prediabetes is clinically silent and its detection is usually random, the chances that the patient evolves to develop the more severe T2DM are high.

To study prediabetes, researchers use animal models in which glycaemia is altered without other relevant risk factors (such as obesity and hypertension). Mouse and

rat fed with a fructose-rich diet are proven models of prediabetes (Alzugaray *et al.* 2009; Mellor *et al.* 2011; Sommese *et al.* 2016; Federico *et al.* 2017; Szűcs *et al.* 2019), as well as fructose-rich diet + streptozotocin (STZ; to destroy pancreatic β-cells) (Koncsos *et al.* 2016), or a single dose of STZ (Mali *et al.* 2016).

Metabolic syndrome. According to the American Heart Association, MetS is diagnosed when an individual shows at least three of the following risk factors: hyperglycaemia, increased blood pressure, dyslipidaemia and abdominal obesity (Grundy *et al.* 2004; American Heart Association, 2016; Dommermuth & Ewing, 2018). In the USA the prevalence of MetS is around 35% (Moore *et al.* 2017), and the worldwide incidence is linearly associated with the degree of obesity and overweight (Saklayen, 2018).

In earlier studies of MetS, researchers used a transgenic mouse model, *ob*/*ob* (Ingalls *et al.* 1950; Enser, 1972), which shows hyperinsulinaemia, hyperglycaemia, obesity and associated cardiac complications. Currently, additional models are available to mimic MetS, including mice with deficient leptin receptor (*db*/*db*) (Hummel *et al.* 1966), transgenic mice that overexpress 11β-hydroxysteroid dehydrogenase type 1 to develop increased visceral obesity (Masuzaki *et al.* 2001), and mice fed with a high-sucrose plus high-fat diet (HFD) (Surwit *et al.* 1995) among others (for full review see Kennedy *et al.* 2010; Panchal & Brown, 2011; Fellmann *et al.* 2013).

Diabetes mellitus. DM is a well-studied chronic disease that is classified into three different types: type 1 diabetes mellitus (T1DM), T2DM and gestational diabetes. DM is characterized by elevated levels of blood glucose, which occur when the body becomes resistant to insulin or doesn't make enough insulin' (World Health Organization, 2020). T1DM can be an idiopathic disease or an autoimmune disease in which islet autoantibodies are produced against the pancreatic β-cells and therefore insulin production is defective. T2DM results from ineffective use of insulin and is often associated with modifiable factors like obesity and sedentarism (World Health Organization, 2020). An increase in blood pressure due to the impact of the underlying insulin resistance on the vasculature and kidney is also usually related to T2DM (Ferrannini & Cushman, 2012). Gestational diabetes is diabetes diagnosed for the first time during pregnancy, and it leads to an increased risk of developing DM in the future for both the mother and the child. Additionally, pregnancy and delivery complications are higher in individuals with gestational diabetes than in non-diabetic people (Alberti & Zimmet, 1998; American Diabetes Association, 2019; World Health Organization, 2020). The DM diagnosis can be made by measuring fasting glucose (fasting glucose >126 mg/dL), by using the glycated haemoglobin test (>6.4%), and/or by using the oral glucose tolerance test (glucose after 2 h tolerance test >200 mg/dL).

Prevalence of DM has been increasing in recent years and is currently 8.6% on average worldwide, although it varies widely by country (World Health Organization, 2019). Several non-biological factors (e.g. socioeconomic, demographics, environmental), as well as the increase in human population age and obesity (Unwin & International Diabetes Federation, 2009; International Diabetes Federation, 2019), contribute substantially to the increasing prevalence. The most frequent type of DM is T2DM, which is *per se* a risk for heart disease and can run with other vascular co-morbidities such as increased blood pressure, microangiopathy or kidney disease (among others), enhancing a vicious cycle to increase the risk for heart damage (Boonman-de Winter *et al.* 2012; Shah *et al.* 2012; Chen *et al.* 2018).

To study DM, several models are available. For T1DM, the most widely used is the induction of pancreatic β-cell destruction by STZ injection (McNeill, 2018). However, the potential of STZ to cause non-specific effects has been a major criticism of this model. Other animal models are based on genetic manipulation, such as the diabetic BB rat (Mordes *et al.* 2005), non-obese diabetic mice (Li *et al.* 2008), and the Otsuka Long–Evans Tokushima fatty rat (Karakikes *et al.* 2009; for review see Yorek, 2016). Distinguishing DM, MetS and prediabetes can be difficult since MetS and prediabetes are closely related to DM as the common endpoint of the disease progression. For T2DM, several researchers adopt *ob*/*ob* and *db*/*db* mice since they present hyperinsulinaemia and hyperglycaemia (Han *et al.* 2017; Lee *et al.* 2018), but both models present hyperlipidaemia and obesity, worsening the cardiovascular risk. Another widespread model of T2DM is based on a HFD (Surwit *et al.* 1988; Namekawa *et al.* 2017; Li *et al.* 2020) in which animals are obese and develop T2DM. Recently, a HFD plus low doses of STZ have been used to mimic T2DM (Guo *et al.* 2018).

The genetic models of T2DM have an advantage in that they can be used at an early age, but the HFD models are more representative of the human disease. The decision of which model to use for experiments should be made carefully, taking into account that T2DM and MetS present different features that could interfere with the interpretation of the results.

Cardiac pathology in metabolic diseases

Heart disease is a major concern in MetD because it is the primary cause of death in these patients. An extensive study in patients from the Netherlands with T2DM showed that the prevalence of unknown HF was 27.7%, which was higher than in patients with increased body mass index and patients treated for arterial

hypertension (Boonman-de Winter *et al.* 2012). The presence of prediabetes or MetS also enhances the probability of developing T2DM and its progression to DCM and HF (Grundy *et al.* 2004).

The triggers that lead to DCM include hyperglycaemia, hyperlipidaemia and hyperinsulinemia, but the molecular mechanisms are not completely understood (Battiprolu *et al.* 2010). Several harmful processes occur together in DCM, including left ventricular hypertrophy, interstitial fibrosis, cell death, diastolic and systolic dysfunction, impaired contractility, changes in Ca^{2+} homeostasis, altered substrate utilization, myocardial lipotoxicity and increased reactive oxygen species (ROS) production, and several of these are consequences of mitochondrial dysfunction (Battiprolu *et al.* 2010). Not all of these deleterious alterations develop at the same time and some of them are the cause or consequence of another. Indeed, to study the MetD model, it is critical to consider not only the election of the model but also the time course of disease progression for conducting the research. In normal conditions, cardiac mitochondria use fatty acids (FAs) to generate approximately 70% of the ATP required by the working heart. In DCM, decreases in glucose transporter type 4 (GLUT4) cause excessive mitochondrial FA uptake, which enhances ROS generation to toxic levels leading to subsequent oxidative stress damage (Boudina & Abel, 2010). The cellular redox environment is one of the major post-translational modulators of protein activity, such as that of the Ca^{2+} handling proteins responsible for excitation–contraction coupling. Sarcoplasmic reticulum (SR) Ca^{2+} uptake and release proteins are subject to oxidative modulations (for review see Federico *et al.* 2020). For instance, oxidative conditions generally increase the ryanodine receptor 2 (RyR2) open probability, which can lead to cardiac arrhythmias and HF (Xu *et al.* 1998; Sun *et al.* 2008). Moreover, the kinases and phosphatases are also subject to oxidation. Lastly, the decreased rate of glycolysis generates glucose accumulation and advanced glycation end products (AGEs). AGE complexes can compromise several enzyme activities, altering cardiac contraction (Shao & Tian, 2015).

Taken together, the metabolic imbalance between FA oxidation and glycolysis is critical in the pathogenesis of MetD-mediated cardiac pathology (Wang *et al.* 2006). The disproportionate mitochondrial FA uptake subsequently leads to disturbances in mitochondrial functions that have a direct impact on cardiac performance.

Mitochondrial role in MetD-mediated cardiac dysfunction

The heart obtains most of its energy from FA oxidation (FAO) and switches to the glycolysis pathway under pathological conditions (Stanley *et al.* 2005; Shao & Tian, 2015). In MetD, the heart is forced to use FAs almost exclusively for generating ATP and this overburdens mitochondria and subjects them to oxidative stresses and injury (Christoffersen *et al.* 2003; Hall *et al.* 2014). On the contrary, it has also been shown that FA can regulate mitochondrial biogenesis by modulating the activity of the peroxisome proliferator-activated receptor-γ (PPARγ) and PPARγ-coactivator 1 α (PGC1α) (Lehman *et al.* 2000; Finck *et al.* 2002; Arany *et al.* 2005).

The morphology of mitochondria is directly related to their functions including ATP and ROS production. It has been described that there are subpopulations of mitochondria defined by their spatial location: subsarcolemmal mitochondria, located immediately beneath the plasma membrane, perinuclear mitochondria, surrounding the nucleus, and intermyofibrillar (IMF) mitochondria, embedded within the myofibrillar networks and making up the most abundant population (Palmer & Hoppel, 1977). The IMF mitochondria in the adult heart are densely compacted between sarcomeres, but their morphology can be changed by the fission–fusion process (Kane & Youle, 2010) and tunnelling connection (Lavorato *et al.* 2017). Although mitochondrial fission–fusion processes in adult cardiomyocytes are infrequent in physiological conditions, it has been described in pathological situations such as MetDs (Galloway & Yoon, 2015). Furthermore, mitochondrial distribution is altered in MetS and prediabetes (Federico *et al.* 2017; Yuan *et al.* 2018) affecting SR–mitochondrial communication, impairing mitochondrial Ca^{2+} and ADP exchange. Therefore, not only is mitochondrial distribution important for the adequate traffic of molecules from and to the SR, but also the expression of several key proteins that tether both organelles and maintain the optimal distance to ensure privileged signal transduction (e.g. Ca^{2+}) between them (Seidlmayer *et al.* 2019). Since three enzymes (2-oxoglutarate dehydrogenase, pyruvate dehydrogenase, and NAD^+-isocitrate dehydrogenase) of the tricarboxylic acid (TCA) cycle are regulated by Ca^{2+} in the mitochondrial matrix, it is important to maintain proper Ca^{2+} communication between these two organelles to have an efficient excitation–contraction–bioenergetic coupling (Brookes *et al.* 2004).

Taken together, there are multifaceted changes in mitochondrial energy metabolism, shape, Ca^{2+} signalling, connections to SR, ROS generation and quality control that can contribute to the pathogenesis of MetD-mediated heart dysfunction.

Mitochondrial energy metabolism in MetD-mediated heart dysfunction. To carry out blood-pumping activities, the human heart requires 6 kg of ATP per day (Neubauer, 2007). To meet this high energy demand, the

cardiomyocytes possess a higher number of mitochondria when compared to other cell types. ATP production is coupled to the O_2 consumption at the electron transport chain (ETC). H^+ is pumped into the intermembrane space during the electron transfer process, which generates an inwardly directed proton motive force that will be used later on by the ATP synthase to produce ATP (Mitchell, 1972).

The alterations in O_2 consumption and ATP production rate of MetD hearts remain controversial and may depend on the degree of disease progression. Pham *et al.* have demonstrated that in the rat STZ model, the decrease in O_2 consumption is associated with a decrease in ATP production, either stimulating complex I with glutamate/malate/pyruvate or complex II with succinate. The authors conclude that diabetic hearts have an overall depression of respiration capacity and ATP production with a significantly decreased P/O ratio (ATP production per O_2 consumed) (Pham *et al.* 2014). As mentioned above, mitochondria can be divided into different subpopulations according to their location. In patients with T2DM, the O_2 consumption was decreased in states 3 and 4 in subsarcolemmal mitochondria either with glutamate/malate or FA/malate as substrates, without changes in IMF mitochondria.

Additional experiments showed a decrease in ATP production rate in rat models with one dose of STZ (Bombicino *et al.* 2017) or HFD+STZ (Fang *et al.* 2018). Mitochondrial oxidative phosphorylation (OXPHOS) alterations were also studied by measuring the activity of enzymatic complexes from the ETC, TCA cycle enzyme activity and proteomics. The analysed data showed that several of the essential proteins required for a normal mitochondrial function, such as PGC1α, complex I, II, III and IV, among others, were downregulated in MetD (Yan *et al.* 2013; Szűcs *et al.* 2019; Wang *et al.* 2020).

However, How *et al.* showed increased O_2 consumption in isolated mitochondria from *db*/*db* mice in state 3 when the substrate was palmitoyl-carnitine and detected no changes when pyruvate was used, suggesting an enhanced FAO and a decreased glucose oxidation. The increased O_2 consumption did not translate into an enhancement of cardiac output, indicating inefficiency in cardiac performance, and this may contribute to contractile dysfunction in the diabetic heart (How *et al.* 2006).

These changes in energy metabolism observed in diabetic animal models are also found in humans. It has been shown that mitochondria in atrial tissue of T2DM patients show a decrease in respiration with glutamate and FA as substrates. Furthermore, the atrial tissue from diabetic patients shows increased mitochondrial H_2O_2 emission and decreased glutathione (GSH) levels. These data support the role of mitochondrial dysfunction and oxidative stress in the pathogenesis of HF in diabetic patients (Anderson *et al.* 2009). Another study also showed decreases in complex I and IV activity in mitochondria isolated from right atrial appendages of diabetic patients compared to non-diabetic (Croston *et al.* 2014). Finally, Montaigne *et al.* reported that heart tissue from patients with T2DM has reduced complex II and III activity, and decreased state 3 respiration, supported by FA, pyruvate or succinate. In contrast, heart tissue from obese patients, associated with less pronounced contractile dysfunction than T2DM, did not show any significant perturbation of mitochondrial function or oxidative stress (Montaigne *et al.* 2014). From these results it can be concluded that the worsening intrinsic myocardial contraction in the transition from obesity to DM is likely related to the impairment of cardiac mitochondrial function (Montaigne *et al.* 2014).

Therefore, using different models of MetD, the majority of studies show an altered O_2 consumption (Table 1) suggesting the importance of mitochondrial energy metabolism in the pathogenesis of MetD-mediated heart dysfunction. Results obtained from human heart tissues (Anderson *et al.* 2009; Montaigne *et al.* 2014) were similar to those from animal models (Yan *et al.* 2013; Wang *et al.* 2020), endorsing the translation of animal studies to humans.

Mitochondrial dynamics in MetD-mediated heart dysfunction. Mitochondrial dynamics encompasses fusion, fission, selective degradation and transport processes (Chan, 2020). Fission and fusion are in balance in physiological conditions and maintain normal mitochondrial mass, shape, network, biogenesis and turnover. The fusion process generates a bigger mitochondrion from two smaller ones, merging the contents of both original mitochondria, which helps to mitigate the mitochondrial damage in addition to establishing a mitochondrial network. On the opposite side, fission creates two new mitochondria from a single one, contributing to mitochondrial turnover and facilitating apoptosis during high levels of cellular stress (Kane & Youle, 2010).

Fission and fusion are also related to mitochondrial OXPHOS. Stressed mitochondria or defective OXPHOS promotes mitochondrial fragmentation (Sauvanet *et al.* 2010). The fission process is regulated by several proteins, including dynamin-related protein 1 (DRP1) (Herskovits *et al.* 1993), Fis-1, which connects DRP1 to the outer mitochondrial membrane, and other described adaptors such as MFF, MiD49 and MiD51 (Yoon *et al.* 2003; Otera *et al.* 2010; Palmer *et al.* 2011). Mitochondrial fusion is controlled by two major proteins: optic atrophy protein (OPA1), located in the inner mitochondrial membrane, and mitofusin 1 and 2 (Mfn1/2), located in the outer mitochondrial membrane (Cipolat *et al.* 2004, 2006). The balance between mitochondrial fusion and

Table 1. O_2 consumption rate in different models

O_2 Consumption	Substrate	Model	Disease	Reference
↓↓	FA/malate	Human	Diabetic patients (right atrial appendages)	Croston *et al.* (2014)
↑	FA	*db/db*	Genetic model of T2DM	How *et al.* (2006)
↔	Pyruvate	*db/db*	Genetic model of T2DM	
↓↓	FA/glutamate	Human	Diabetic patients (right atrial appendages)	Anderson *et al.* (2009)
↓↓	FA/pyruvate/succinate	Human	Diabetic patients (right atrial appendages)	Montaigne *et al.* (2014)
↓↓	Pyruvate/malate	OVE26	Genetic model of T1DM	Shen *et al.* (2004)
↔	Glutamate/malate/succinate	HFD	Dietary model of T2DM	Koncsos *et al.* (2016)

FA, fatty acids; HFD, high-fat diet; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus. Note, the references mentioned in this table are cited in the main text.

fission is essential in mammals, and even mild defects in mitochondrial dynamics are associated with disease aetiology. In general, a tip of the balance toward fission is usually associated with deleterious processes and toward fusion is associated with compensatory mechanisms (Chen *et al.* 2003; Ishihara *et al.* 2009).

In the adult myocardium, fission events are challenging to detect in part due to the abundance and immobility of mitochondria, and therefore the data available related to the mitochondrial dynamics in MetD-mediated heart dysfunction are limited. Transmission electron microscopy images of the heart tissue have shown that in the early stages of cardiac MetD pathogenesis, such as pre-diabetes induced by a fructose-rich diet, mitochondria are smaller and more spherical in shape compared with control animals (Federico *et al.* 2017), indicating that fission processes might occur. Koncsos *et al.* similarly described a decrease in area, perimeter and sphericity of mitochondria in the prediabetic rat model with HFD and STZ treatments (Koncsos *et al.* 2016). Flow cytometry showed that mitochondrial size and cristae complexity were decreased in diabetic IMF mitochondria in mouse models with one dose of STZ (Williamson *et al.* 2010). In another paper, the same research group reported that cardiac mitochondrial density was increased and mitochondrial area was decreased, showing unbalanced mitochondrial dynamics towards the fission processes in this diabetic model (Dabkowski *et al.* 2010).

Montaigne *et al.* explore the role of impaired mitochondrial dynamics in myocardial contractile dysfunction in patients with T2DM without obesity. The mitochondria of the heart tissues from these patients, harvested during cardiopulmonary bypass, showed no difference in density but a significant decrease in size. The authors also analysed the amounts of proteins related to the mitochondrial dynamics such as Mfn2, Mfn1, OPA1, DRP1 and Fis1. They only found a large decrease in the expression of the mitochondrial fusion related protein Mfn1, which may account for mitochondrial fragmentation. These changes in mitochondrial morphology were associated with impaired complex I, II and III activity, decreased respiratory control ratio and increased oxidative stress, further confirming the close relationship between mitochondrial shape and function (Montaigne *et al.* 2014).

Mitochondrial calcium signalling in MetD-mediated heart dysfunction. The mitochondria are juxtaposed with the SR in the cardiomyocytes and participate in taking up a fraction of the Ca^{2+} during each heart-beat (Beutner *et al.* 2005). Localized Ca^{2+} released from the SR creates a high Ca^{2+} microdomain in the SR mitochondria contact sites to stimulate mitochondrial Ca^{2+} uptake. The mitochondrial calcium uniporter (MCU) at the inner mitochondrial membrane, a highly Ca^{2+}-selective ion channel (Kirichok *et al.* 2004), is responsible for the bulk of Ca^{2+} uptake from microdomains to the mitochondrial matrix (Csordás *et al.* 2006; de Brito & Scorrano, 2008). In addition to the MCU, other mitochondrial Ca^{2+} influx mechanisms such as mitochondrial ryanodine receptor 1 (Beutner *et al.* 2005) and the rapid mode of Ca^{2+} uptake (RaM) (Buntinas *et al.* 2001) have also been identified. The extrusion of Ca^{2+} from the matrix to the cytosol is carried out by the $Na^+/Ca^{2+}/Li^+$ exchanger (NCLX) (Li *et al.* 1992) and the Ca^{2+}/H^+ exchanger (Gunter *et al.* 1991).

The kinetics of mitochondrial Ca^{2+} uptake and extrusion in the beating adult cardiomyocytes is still under debate (De la Fuente & Sheu, 2019). Some authors describe that the amount of Ca^{2+} taken up by mitochondria in each heartbeat is modest and the Ca^{2+} gradually accumulates inside the mitochondrial matrix throughout the heartbeat until a new steady state is reached, in which the uptake and extrusion are balanced (Miyata *et al.* 1991). Other authors propose that mitochondria can follow the cytosolic Ca^{2+} oscillations and take up and release Ca^{2+} on a beat-to-beat basis (Murgia *et al.* 2009; Andrienko *et al.* 2009). It has been proposed that mitochondria may function as a Ca^{2+} buffer due to their capacity in taking up a large amount of Ca^{2+} through the MCU, and as such they can modulate the amplitude of cytosolic Ca^{2+} transients (Drago *et al.* 2012). This will require that mitochondria can take up

Ca^{2+} on a beat-to-beat basis, which is still unclear at present. The most accepted role for the mitochondrial Ca^{2+} in cardiomyocytes is associated with the regulation of cardiac energy production. The mitochondrial Ca^{2+} regulates the activity of the TCA cycle, as mentioned above (Duchen, 1992; Kohlhaas *et al.* 2017; De la Fuente & Sheu, 2019). Interestingly, this function has been recently challenged due to the lack of energetic phenotype in the normal beating heart of a germline MCU-knockout mouse model (Pan *et al.* 2013). However, under intense β-adrenergic stimulation, it was reported that Ca^{2+} influx through the MCU is a requisition for the fight or flight response (Wu *et al.* 2015).

In pathological conditions, the mitochondrial Ca^{2+} uptake and overload have detrimental consequences for energy production and possibly promote DCM complications (Federico *et al.* 2017; Wu *et al.* 2019). Several studies have attempted to monitor Ca^{2+} signalling in isolated cardiomyocytes from different models of MetD. In cardiomyocytes isolated from 5-week STZ-induced diabetic rats, diastolic Ca^{2+} concentration and Ca^{2+} spark frequency significantly increased in comparison to age-matched control rats. The amplitude of Ca^{2+} transients was significantly decreased and the duration was prolonged (Yaras *et al.* 2005). In cardiomyocytes isolated from a prediabetic model, increased spontaneous Ca^{2+} oscillations in the cytosol were associated with spontaneous contractions, which were prevented by KN-93, a Ca^{2+}–calmodulin kinase II (CaMKII) inhibitor, or the addition of Tempol, a ROS scavenger, in the diet (Sommese *et al.* 2016). The increases in Ca^{2+} spark frequency and spontaneous Ca^{2+} transients indicate that these animals are prone to cardiac arrhythmias. In another study, the increment in the frequency of sparks, which also depends on ROS and CaMKII, promoted apoptosis that was linked to increased mitochondrial swelling and decreased mitochondrial membrane potential (Federico *et al.* 2017).

A small number of studies have measured mitochondrial Ca^{2+} regulation in the cardiomyocytes of MetD animal models. Suarez *et al.* observed decreased MCU expression, low glucose usage and high FAO in the heart of an STZ mouse model, which led to a decrease in respiratory control ratio, ATP production and mitochondrial membrane depolarization (Suarez *et al.* 2018). The mitochondrial Ca^{2+} concentration was monitored with a mitochondria-targeted Ca^{2+} probe, pericam, and was found to be decreased in diabetic heart. Moreover, when the MCU was restored by AVV9-MCU injection, mitochondrial metabolism recovered to normal levels (Suarez *et al.* 2018). Also, mitochondrial Ca^{2+} was found to be decreased in neonatal cardiomyocytes (Suarez *et al.* 2008) and adult cardiomyocytes exposed to high glucose (HG) (Diaz-Juarez *et al.* 2016). Besides, Diaz-Juarez *et al.* showed decreased expression of MCU in the HG condition, and recovered MCU expression improved mitochondrial Ca^{2+} handling (Diaz-Juarez *et al.* 2016), proving that optimal MCU expression reverts the mitochondrial metabolic and functional changes in MetD.

Despite the importance of mitochondrial Ca^{2+} in regulating heart function, very few studies have monitored mitochondrial Ca^{2+} dynamics in MetD hearts (Yaras *et al.* 2005; Sommese *et al.* 2016; Federico *et al.* 2017; Suarez *et al.* 2018). Several additional mitochondria-targeted Ca^{2+} probes are currently available, such as genetically expressed CEPIA and MitoCam (Lu Xiyuan *et al.* 2013; Kanemaru *et al.* 2020), and chemical dyes such as Rhod-2AM (Chen Chen *et al.* 2012; Fernandez-Sanz *et al.* 2014), for measuring spatiotemporal aspects of the mitochondrial Ca^{2+} signalling in the heart of MetD models. These measurements will help in our understanding of the role of mitochondrial Ca^{2+} dynamics in MetD-mediated heart dysfunction.

ROS in MetD-mediated heart dysfunction. The mitochondrion is the principal organelle involved in ROS production (Jensen, 1966). ROS are free radical oxidants, such as superoxide (O_2^-) and the hydroxyl radical ($^\bullet OH$), and non-radical oxidants, such as singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2). ROS generation is tightly related to ATP production, O_2 consumption and mitochondrial Ca^{2+} signalling (Brookes *et al.* 2004). A sustained net increase of ROS will eventually damage the cell (Wang *et al.* 2008; Zorov *et al.* 2014; Nickel *et al.* 2014; Korge *et al.* 2017) and therefore both ROS generating and eliminating systems exist in cells. Under physiological conditions, mitochondria produce O_2^- from O_2 oxidation by complex I or complex III, which is dismutated to H_2O_2 by manganese-dependent superoxide dismutase (MnSOD). The H_2O_2 will be further eliminated in the mitochondrial matrix by antioxidant systems, glutathione peroxidase and peroxiredoxin. These systems are coupled with NADPH production. Thus, increased cytosolic ROS can be a cause of increased production and/or decreased elimination (Nickel *et al.* 2014). Zorov *et al.* described a process named ROS-induce ROS-release in which the ROS produced by a single mitochondrion can be transferred to an adjacent mitochondrion, leading to a chain reaction in which many consecutive mitochondria produce a massive amount of ROS, which causes subsequent cell injury (Zorov *et al.* 2000, 2014).

In the early stages of MetD, such as prediabetes and hyperglycaemia, morphological and functional changes in mitochondria have been associated with an increase in oxidative stress. Koncsos *et al.* (2016) showed an increased H_2O_2 and nitrotyrosine production in DCM. As mentioned above, excessive Ca^{2+} traffic from SR/ER to the mitochondria can cause mitochondrial Ca^{2+} overload that

results in ETC uncoupling and excessive ROS production. Koncsos *et al.* attributed the increase in ROS production to an enhanced SR/ER–mitochondria connection through Mfn2 due to their reported overexpression of the Mfn2 without any changes in other fusion proteins (Koncsos *et al.* 2016). In a prediabetic model, we found an increase of ROS as well as lipid peroxidation in cardiac homogenates, which promoted CaMKII-mediated arrhythmias and apoptosis. These effects could be prevented either by ROS scavenging or by CaMKII inhibition (Sommese *et al.* 2016; Federico *et al.* 2017). Phosphorylation of RyR2 by CaMKII enhances the open probability of the channel, increasing SR Ca^{2+} leak. Therefore, preventing RyR2 activation by CaMKII avoids not only mitochondrial swelling but also mitochondrial membrane depolarization induced by prediabetes. We also reported a decreased distance between SR and mitochondria in the prediabetic heart, which would further augment ROS-mediated CaMKII activation (Federico *et al.* 2017).

Additionally, in advanced stages of MetD, an increase in ROS, malondialdehyde or 4-hydroxy-2-nonenal has been reported, as well as changes in O_2 consumption, MnSOD activity and/or NADPH oxidase activity (Santos *et al.* 2003; Csont *et al.* 2007; Rajesh *et al.* 2010; Suzuki *et al.* 2015). Furthermore, increases in mitochondrial superoxide flashes and ROS generation have been described in the STZ model (Ni *et al.* 2016*b*).

Either increased production or decreased antioxidant capacity can cause net increased ROS levels. Anderson *et al.* showed human atrial tissue from T2DM had enhanced H_2O_2 production and decreased GSH/GSSG ratio (Ghosh *et al.* 2005; Anderson *et al.* 2009). Dabkowski *et al.* showed the *ob*/*ob* model had increased malondialdehyde and 4-hydroxyalkenal (both products of oxidation of polyunsaturated FAs), coupled with decreased peroxiredoxin-V (Dabkowski *et al.* 2010). Accordingly, Shen *et al.* showed that intensifying the ROS scavenger systems, such as overexpression of MnSOD, was beneficial in preventing DCM (Shen *et al.* 2006).

Taken together, ROS imbalance appears to be one of the most damaging factors in cardiometabolic pathologies (Shen *et al.* 2006; Suzuki *et al.* 2015). Therefore, antioxidant treatments could be a reasonable approach to deter the harmful ROS effects on the heart (Qin *et al.* 2012; Fang *et al.* 2018). Mito-TEMPO, a scavenger of mitochondrial ROS, has been shown to prevent mitochondrial ROS-mediated damage and to mitigate the diastolic dysfunction in DCM (Ni *et al.* 2016*a*). The search for effective candidates, including SOD mimetics and ROS scavengers among others, to relieve oxidative stress in MetD is ongoing (see Kiyuna *et al.* 2018). However, only limited studies have proven the benefits of the antioxidant approach in humans. Coenzyme Q10 (CoQ10) has been reported to improve cardiac function in patients with DM and HF (Mortensen *et al.* 2014). CoQ10 is a component of the ETC, mediating the electron transport from complexes I and II to complex III. Ubiquinol is a reduced form of CoQ10 that acts as an antioxidant inside mitochondria (Kelso *et al.* 2001). Since optimal ROS concentrations are critical in carrying out physiological signalling, future studies will be needed to identify compounds that will lessen the pathological oxidative stresses while preserving physiological redox signalling.

Mitochondrial permeability transition pore in MetD-mediated heart dysfunction. The mitochondrial permeability transition pore (mPTP) is a non-selective pore in the mitochondrial membrane that allows any solute up to 1.5 kDa to pass through (Hunter & Haworth, 1979). The mPTP has multi-conductance that suggests the molecular nature is a multi-subunit complex oligomerizing to varying degrees (Hunter *et al.* 1976). The molecular identity of the mPTP has been studied for years and is still a matter of debate. One of the first models proposed that the mPTP is composed of Bcl-2 associated-X-protein (Bax), voltage-dependent anion channel (VDAC), the peripheral benzodiazepine receptor (translocator protein, TSPO) and hexokinase II (HKII) in the outer mitochondrial membrane; mitochondrial creatine kinase (mtCK) in the inter-membrane space; adenine nucleotide transporter (ANT) in the inner mitochondrial membrane; and mitochondrial cyclophilin D (CypD) bound to ANT in the matrix (Halestrap & Davidson, 1990; Kinnally *et al.* 1993; Beutner *et al.* 1997; Marzo *et al.* 1998). The phosphate carrier model proposed the following composition of mPTP: Bax, VDAC, TSPO, HKII, mtCK, ANT and CypD, bound to the phosphate inorganic carrier (PiC) (Kokoszka *et al.* 2004). Recent studies have provided new insights about several potential candidates for the molecular identity of the mPTP, which include multiple subtypes of ANT (Bround *et al.* 2020), F-ATP synthase c-subunit (Mnatsakanyan & Jonas, 2020), and the dimer (tetramer) of F-ATP synthase (Carraro *et al.* 2020). The idea that more than one protein may act as mPTPs offers a rational clarification for current disagreements in the field. Intriguingly, these candidate proteins are already well-known for their role in catalysing ATP generation. Therefore, mPTPs appear to have two opposite functions: controlling cell life and death through their participation in energy metabolism and apoptosis/necrosis. The mPTP can be regulated or inhibited by different compounds. The most well-known is cyclosporin A, which blocks CypD (Fournier *et al.* 1987). Other inhibitors include Mg^{2+} and Mn^{2+} (by competing with Ca^{2+} to bind), adenine nucleotides, and matrix acidic pH. The primary activator of the mPTP is mitochondrial Ca^{2+} overload (Baumgartner *et al.* 2009); nevertheless, ROS (Seidlmayer *et al.* 2015), reactive nitrogen species, mitochondrial morphology and

inorganic phosphate can also modulate its activity (Hurst *et al.* 2017).

The transient opening of the mPTP may have a physiological role by serving as a releasing valve for Ca^{2+} efflux to prevent mitochondrial Ca^{2+} overload. Under pathological situations, the pore is opened more sustainably, which leads to the dissipation of proton force, loss of metabolites, mitochondrial swelling, and cytochrome *c* release. All of these processes will eventually lead to cell death (Halestrap, 2009*a*,*b*).

Studies using the STZ model have reported that Ca^{2+} retention capacity is decreased, which is a sign of increased propensity for mPTP opening, coupled to a decreased O_2 consumption (Oliveira *et al.* 2003; Ma *et al.* 2016). Similar results were found when the cardiac myoblast cell line H9c2 was exposed to HG (Diao *et al.* 2019). The mPTP opening can also be determined by Ca^{2+}-induced swelling of isolated cardiac mitochondria. Some studies have shown increased mitochondrial swelling in mitochondria isolated from prediabetic or diabetic animals, demonstrating susceptibility to the Ca^{2+} overload (Federico *et al.* 2017; Guo *et al.* 2018). Finally, when Anderson *et al.* tested the mPTP opening by Ca^{2+} retention capacity assays in human atrial fibres, they found, once more, an increase in mPTP opening sensitivity. The authors noted that during prolonged metabolic changes and oxidative stress (as happens in DM), the components that can activate the mPTP opening, such as CypD, may be overexpressed (Anderson *et al.* 2011).

Mitochondrial biogenesis in MetD-mediated heart dysfunction. Mitochondrial biogenesis has been described as a process that includes mitochondrial division and growth. The mitochondrion has its own DNA (mtDNA) which encodes 13 subunits of ETC proteins (Robin & Wong, 1988; Dorn *et al.* 2015). Initially, the nDNA controls mtDNA quality and biogenesis by regulating mtDNA replication. The critical regulator of mitochondrial biogenesis is PGC1α (Wu *et al.* 1999; Ventura-Clapier *et al.* 2008). PGC1α can regulate nuclear factors 1 and 2, as well as transcription factor A mitochondrial (TFAM) (Wu *et al.* 1999). It has been described that when the PGC1α level is reduced, there is a loss of mtDNA, and when it is overexpressed, there is an increase in mitochondrial biogenesis, OXPHOS, FAO and glycolysis (Lehman *et al.* 2000; Arany *et al.* 2005; Lin *et al.* 2004). In organs that require high energy for their functional performance, such as skeletal or heart muscle, the PGC1α level is higher in comparison with other tissues (Garnier *et al.* 2003). Multiple mechanisms regulate PGC1α, including epigenetic regulation, post-transcriptional modifications and post-translational modifications (Duncan *et al.* 2007; Oka *et al.* 2020).

When mitochondrial biogenesis declines, heart function is eventually compromised. Bombicino *et al.* showed in an STZ-diabetic rat model that there was an enhancement in PGC1α expression. The authors proposed that the H_2O_2 and nitric oxide are responsible for the PGC1α activation (Bombicino *et al.* 2017). Similar results were shown previously by Finck *et al.*, where PPARγ and PGC1α were increased in an STZ model (Finck *et al.* 2002). In contrast, Yan *et al.* described decreased PGC1α function in the heart in the *ob*/*ob* model, due to acetylation by AMP-activated protein kinase, which is activated by dephosphorylation for adiponectin activity (Yan *et al.* 2013).

In an OVE26 mouse model of T1DM, mRNA levels of TFAM and two mitochondrially encoded proteins were increased, suggesting that mitochondrial biogenesis was augmented (Shen *et al.* 2004). Using HFD as a model of T2DM, it was found that mtDNA, PGC1α expression and nuclear factor expression were all decreased (Fang *et al.* 2018). The same result was confirmed by Duncan *et al.* (2007) in an insulin-resistance model.

Even though several studies have measured the amount of PGC1α in the MetD mouse models, the data obtained are still controversial. The different MetD models used may underlie these discrepancies, where some studies detect a decrease in PGC1α while others see an increase. The expression of PGC1α has never been correlated with the PPARγ expression and mtDNA changes in MetD. Therefore, divergent outcomes from these studies show that the regulation of the expression or PGC1α activity is multifaceted. Further studies would be required to fully understand the whole process of mitochondrial biogenesis in MetD (Nisoli *et al.* 2007; Ren *et al.* 2010).

Conclusions

The prevalence of MetDs has been increasing in recent years. Several research groups around the world have focused on determining the mechanisms of disease pathogenesis with a common goal to prevent, delay or revert MetD. The impact of MetDs on the heart is highly relevant to human health due to their propensity to cause life-threatening cardiac arrhythmias and HF.

The heart has extremely high energy demands. Heart mitochondria consume glucose and FAs to produce the necessary ATP (Fig. 1, left), but since the usage of glucose is reduced in MetD, FAO by mitochondria is increased. This leads to a shift in the balance of mitochondrial energy metabolism favouring cardiac dysfunction.

In this review, we have summarized what is known about the mitochondrial changes occurring in heart tissue during the progression of MetD. We have described the mechanisms by which metabolic stress causes mitochondrial dysfunction, including disturbances

in energetics, changes in dynamics, Ca^{2+} signalling impairment, increased oxidative stress, mPTP opening and mitochondrial biogenesis (Fig. 1, right). The mitochondrial alterations described are not exclusive for MetD and DCM. Similar phenotypes have been also seen in the pathogenesis of other heart diseases like hypertension, coronary artery disease and hypertrophy. As in MetD these mitochondrial alterations can also lead to heart failure in many cases (Graham *et al.* 2009; Ardanaz *et al.* 2010; Bhatt *et al.* 2011; Hollander *et al.* 2014; Ait-Aissa *et al.* 2019). However, the origin of mitochondria dysfunction is different and pivotal in designing therapeutic strategies, i.e. for MetD the fuel for ATP production is the initial mitochondrial signal disturbing its function. A confluence point in several heart pathologies in which mitochondria are compromised is increased ROS generation, the most important component of mitochondrial dysfunction. As brief examples, Graham *et al.* (2009) showed that inhibition of ROS production by mitoQ prevents hypertension development. Similarly, the use of resveratrol avoids NO generation and hypertension (Bhatt *et al.* 2011). Finally, in coronary arterial disease, altered mitochondrial dynamics due to alteration in DRP1 levels as well as alterations in ETC complex activity have been reported. Thereby general mitochondrial function and metabolism are affected in coronary arterial disease (Ait-Aissa *et al.* 2019).

There are still many questions and challenges that need to be addressed to further understand the role of mitochondria in MetD-mediated heart dysfunction. For instance, there is a lack of reliable direct measurements of mitochondrial functional parameters such as ATP, fission and fusion events, Ca^{2+} and ROS concentrations in live cardiomyocytes isolated from the DCM heart to elucidate the dynamics and specific role of these parameters in the

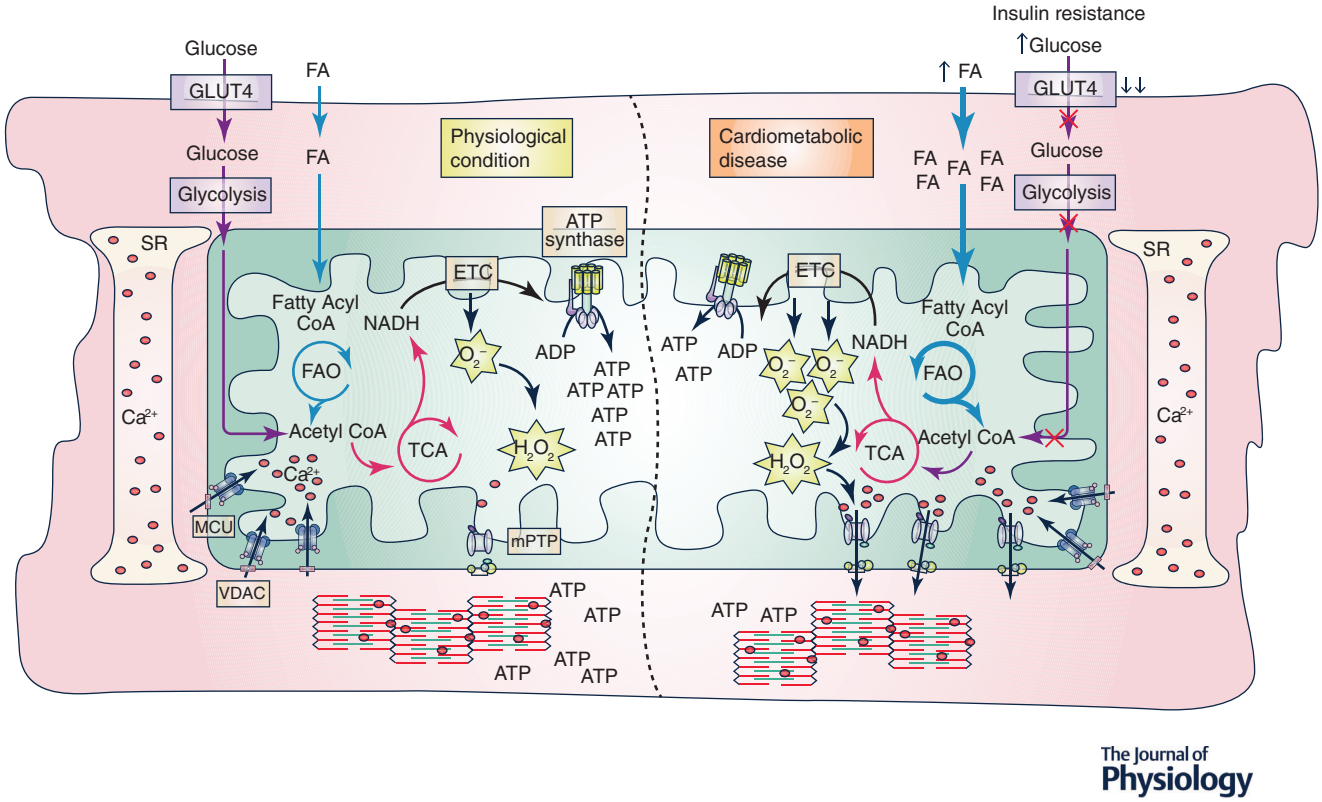


Figure 1. Mitochondrial metabolism in the physiological condition *vs*. metabolic disease
In the physiological condition (left), the cardiomyocytes fuel up mainly with free fatty acid (FA) but also supplement this from glycolysis. FA oxidation (FAO), the tricarboxylic acid (TCA) cycle, the electron transport chain (ETC), and ATP synthase produce the ATP required for muscle contraction. The ATP production is coupled with O_2 consumption and superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) production in healthy amounts. Metabolic disease (right) presents elevated free FA and insulin resistance. Therefore, decreased expression of GLUT4 favours FA uptake and oxidation. FAO requires higher levels of O_2 than glucose to produce an equal amount of ATP, decreasing the efficiency of energy production. Under this condition, reactive oxygen species (ROS) production increases up to pathological levels, triggering mitochondrial permeability transition pore (mPTP) opening and oxidation of other proteins for normal cell function. The whole process decreases the cardiomyocyte's performance and favours the pathogenesis of diabetic cardiomyopathy that finally leads to heart failure.

pathogenesis of MetD-mediated heart dysfunction. These measurements will also provide information about the mechanisms of crosstalk signalling among these interconnected functions such as the role of mitochondrial Ca^{2+} signalling in the regulation of mitochondrial energy metabolism and ROS homeostasis. Future advances in experimental technology and theoretical concepts will help to resolve these challenges. One such progress is the recent discovery of multiple molecular identities in the formation of the mPTP complex, which provides a new opportunity for using genetic approaches to decipher the role mPTP opening in MetD-linked cell injury and death.

All of the MetD animal models and heart samples from MetD patients show increased ROS production. Thus, ROS appear to be key players in the development of the MetD-mediated heart dysfunction, although the mechanisms of ROS-mediated downstream effects are still to be elucidated. It is plausible that the ROS-mediated signalling pathways are potential drug targets for the treatment of MetD-mediated heart dysfunction.

Findings of MetD-related changes in heart mitochondrial biogenesis are still contradictory. While some studies show an increase in PGC1α, others show no changes in this protein. Furthermore, other components involved in mitochondrial biogenesis have been barely studied in DCM. Therefore, additional experiments are required to elucidate the specific role of other mitochondrial biogenesis regulators in MetD.

Finally, the comparative studies between animal models and heart samples from human patients (including induced pluripotent stem cells differentiated into cardiomyocytes) will be useful for translating basic mechanisms into clinical practice. Collectively, this new knowledge will be useful for the development of new and effective therapeutic interventions for treating these devastating disorders.

References

Ait-Aissa K, Blaszak SC, Beutner G, Tsaih S-W, Morgan G, Santos JH, Flister MJ, Joyce DL, Camara AKS, Gutterman DD, Donato AJ, Porter GA & Beyer AM (2019). Mitochondrial oxidative phosphorylation defect in the heart of subjects with coronary artery disease. *Sci Rep* **9**, 7623.

Alberti KGMM & Zimmet PZ (1998). Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO Consultation. *Diabet Med* **15**, 539–553.

Alzugaray ME, García ME, Del Zotto HH, Raschia MA, Palomeque J, Rossi JPFC, Gagliardino JJ & Flores LE (2009). Changes in islet plasma membrane calcium-ATPase activity and isoform expression induced by insulin resistance. *Arch Biochem Biophys* **490**, 17–23.

American Diabetes Association (2019). Standards of medical care in diabetes—2019 abridged for primary care providers. *Clinical Diabetes* **37**, 11–34.

American Heart Association (2016). About metabolic syndrome. https://www.heart.org/en/health-topics/metabolic-syndrome/about-metabolic-syndrome.

Anderson EJ, Kypson AP, Rodriguez E, Anderson CA, Lehr EJ & Neufer PD (2009). Substrate-specific derangements in mitochondrial metabolism and redox balance in the atrium of the type 2 diabetic human heart. *J Am Coll Cardiol* **54**, 1891–1898.

Anderson EJ, Rodriguez E, Anderson CA, Thayne K, Chitwood WR & Kypson AP (2011). Increased propensity for cell death in diabetic human heart is mediated by mitochondrial-dependent pathways. *Amn J Physiol Heart Circ Physiol* **300**, H118–H124.

Andrienko TN, Picht E & Bers DM (2009). Mitochondrial free calcium regulation during sarcoplasmic reticulum calcium release in rat cardiac myocytes. *J Mol Cell Cardiol* **46**, 1027–1036.

Arany Z, He H, Lin J, Hoyer K, Handschin C, Toka O, Ahmad F, Matsui T, Chin S, Wu P-H, Rybkin II, Shelton JM, Manieri M, Cinti S, Schoen FJ, Bassel-Duby R, Rosenzweig A, Ingwall JS & Spiegelman BM (2005). Transcriptional coactivator PGC-1α controls the energy state and contractile function of cardiac muscle. *Cell Metab* **1**, 259–271.

Ardanaz N, Yang XP, Cifuentes ME, Haurani MJ, Jackson KW, Liao TD, Carretero OA, Pagano PJ (2010). Lack of glutathione peroxidase 1 accelerates cardiac-specific hypertrophy and dysfunction in angiotensin ii hypertension. *Hypertension* **55**, 116–123.

Battiprolu PK, Gillette TG, Wang ZV, Lavandero S & Hill JA (2010). Diabetic cardiomyopathy: mechanisms and therapeutic targets. *Drug Discov Today Dis Mech* **7**, e135–e143.

Baumgartner HK, Gerasimenko JV, Thorne C, Ferdek P, Pozzan T, Tepikin AV, Petersen OH, Sutton R, Watson AJM & Gerasimenko OV (2009). Calcium elevation in mitochondria is the main Ca^{2+} requirement for mitochondrial permeability transition pore (mPTP) opening. *J Biol Chem* **284**, 20796–20803.

Beutner G, Rück A, Riede B & Brdiczka D (1997). Complexes between hexokinase, mitochondrial porin and adenylate translocator in brain: regulation of hexokinase, oxidative phosphorylation and permeability transition pore. *Biochem Soc Trans* **25**, 151–157.

Beutner G, Sharma VK, Lin L, Ryu S-Y, Dirksen RT & Sheu S-S (2005). Type 1 ryanodine receptor in cardiac mitochondria: Transducer of excitation–metabolism coupling. *Biochim Biophys Acta Bioenerg* **1717**, 1–10.

Bhatt SR, Lokhandwala MF & Banday AA (2011). Resveratrol prevents endothelial nitric oxide synthase uncoupling and attenuates development of hypertension in spontaneously hypertensive rats. *Eur J Pharmacol* **667**, 258–264.

Bombicino SS, Iglesias DE, Rukavina-Mikusic IA, Buchholz B, Gelpi RJ, Boveris A & Valdez LB (2017). Hydrogen peroxide, nitric oxide and ATP are molecules involved in cardiac mitochondrial biogenesis in diabetes. *Free Radic Biol Med* **112**, 267–276.

Boonman-de Winter LJM, Rutten FH, Cramer MJM, Landman MJ, Liem AH, Rutten GEHM & Hoes AW (2012). High prevalence of previously unknown heart failure and left ventricular dysfunction in patients with type 2 diabetes. *Diabetologia* **55**, 2154–2162.

Boudina S & Abel ED (2010). Diabetic cardiomyopathy, causes and effects. *Rev Endocr Metab Disord* **11**, 31–39.

Brookes PS, Yoon Y, Robotham JL, Anders MW & Sheu S-S (2004). Calcium, ATP, and ROS: a mitochondrial love-hate triangle. *Am J Physiol Cell Physiol* **287**, C817–C833.

Bround MJ, Bers DM & Molkentin JD (2020). A 20/20 view of ANT function in mitochondrial biology and necrotic cell death. *J Mol Cell Cardiol* **144**, A3–A13.

Buntinas L, Gunter KK, Sparagna GC & Gunter TE (2001). The rapid mode of calcium uptake into heart mitochondria (RaM): comparison to RaM in liver mitochondria. *Biochim Biophys Acta Bioenerg* **1504**, 248–261.

Carraro M, Carrer A, Urbani A & Bernardi P (2020). Molecular nature and regulation of the mitochondrial permeability transition pore(s), drug target(s) in cardioprotection. *J Mol Cell Cardiol* **144**, 76–86.

Chan DC (2020). Mitochondrial dynamics and its involvement in disease. *Annu Rev Pathol* **15**, 235–259.

Chen C-M, Juan S-H & Chou H-C (2018). Hyperglycemia activates the renin-angiotensin system and induces epithelial-mesenchymal transition in streptozotocin-induced diabetic kidneys: *J Renin Angiotensin Aldosterone Syst* **19**, 147032031880300.

Chen H, Detmer SA, Ewald AJ, Griffin EE, Fraser SE & Chan DC (2003). Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. *J Cell Biol* **160**, 189–200.

Chen Y, Csordás G, Jowdy C, Schneider TG, Csordás N, Wang W, Liu Y, Kohlhaas M, Maxie M, Bergem S, Nerbonne JM, Dorn GW & Maack C (2012). Mitofusin 2-containing mitochondrial-reticular microdomains direct rapid cardiomyocyte bioenergetic responses via interorganelle Ca^{2+} crosstalk. *Circ Res* **111**, 863–875.

Christoffersen C, Bollano E, Lindegaard MLS, Bartels ED, Goetze JP, Andersen CB & Nielsen LB (2003). Cardiac lipid accumulation associated with diastolic dysfunction in obese mice. *Endocrinology* **144**, 3483–3490.

Cipolat S, de Brito OM, Dal Zilio B & Scorrano L (2004). OPA1 requires mitofusin 1 to promote mitochondrial fusion. *Proc Natl Acad Sci U S A* **101**, 15927–15932.

Cipolat S, Rudka T, Hartmann D, Costa V, Serneels L, Craessaerts K, Metzger K, Frezza C, Annaert W, D'Adamio L, Derks C, Dejaegere T, Pellegrini L, D'Hooge R, Scorrano L & De Strooper B (2006). Mitochondrial rhomboid PARL regulates cytochrome c release during apoptosis via OPA1-dependent cristae remodeling. *Cell* **126**, 163–175.

Croston TL, Thapa D, Holden AA, Tveter KJ, Lewis SE, Shepherd DL, Nichols CE, Long DM, Olfert IM, Jagannathan R & Hollander JM (2014). Functional deficiencies of subsarcolemmal mitochondria in the type 2 diabetic human heart. *Am J Physiol Heart Circ Physiol* **307**, H54–H65.

Csont T, Bereczki E, Bencsik P, Fodor G, Gorbe A, Zvara A, Csonka C, Puskas L, Santha M & Ferdinandy P (2007). Hypercholesterolemia increases myocardial oxidative and nitrosative stress thereby leading to cardiac dysfunction in apoB-100 transgenic mice. *Cardiovasc Res* **76**, 100–109.

Csordás G, Renken C, Várnai P, Walter L, Weaver D, Buttle KF, Balla T, Mannella CA & Hajnóczky G (2006). Structural and functional features and significance of the physical linkage between ER and mitochondria. *J Cell Biol* **174**, 915–921.

Dabkowski ER, Baseler WA, Williamson CL, Powell M, Razunguzwa TT, Frisbee JC & Hollander JM (2010). Mitochondrial dysfunction in the type 2 diabetic heart is associated with alterations in spatially distinct mitochondrial proteomes. *Am J Physiol Heart Circ Physiol* **299**, H529–H540.

de Brito OM & Scorrano L (2008). Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature* **456**, 605–610.

De la Fuente S & Sheu S-S (2019). SR-mitochondria communication in adult cardiomyocytes: a close relationship where the Ca^{2+} has a lot to say. *Arch Biochem Biophys* **663**, 259–268.

Diao J, Wei J, Yan R, Fan G, Lin L & Chen M (2019). Effects of resveratrol on regulation on UCP2 and cardiac function in diabetic rats. *J Physiol Biochem* **75**, 39–51.

Diaz-Juarez J, Suarez J, Cividini F, Scott BT, Diemer T, Dai A & Dillmann WH (2016). Expression of the mitochondrial calcium uniporter in cardiac myocytes improves impaired mitochondrial calcium handling and metabolism in simulated hyperglycemia. *Am J Physiol Cell Physiol* **311**, C1005–C1013.

Dommermuth R & Ewing K (2018). Metabolic syndrome: systems thinking in heart disease. *Prim Care* **45**, 109–129.

Dorn GW, Vega RB & Kelly DP (2015). Mitochondrial biogenesis and dynamics in the developing and diseased heart. *Genes Dev* **29**, 1981–1991.

Drago I, Stefani DD, Rizzuto R & Pozzan T (2012). Mitochondrial Ca^{2+} uptake contributes to buffering cytoplasmic Ca^{2+} peaks in cardiomyocytes. *Proc Natl Acad Sci U S A* **109**, 12986–12991.

Duchen MR (1992). Ca^{2+}-dependent changes in the mitochondrial energetics in single dissociated mouse sensory neurons. *Biochem J* **283**, 41–50.

Duncan JG, Fong JL, Medeiros DM, Finck BN & Kelly DP (2007). Insulin-resistant heart exhibits a mitochondrial biogenic response driven by the peroxisome proliferator-activated receptor-α/PGC-1α gene regulatory pathway. *Circulation* **115**, 909–917.

Enser M (1972). Clearing-factor lipase in obese–hyperglycaemic mice (*ob/ob*). *Biochem J* **129**, 447–453.

Fang W, Wang C, He Y, Zhou Y, Peng X & Liu S (2018). Resveratrol alleviates diabetic cardiomyopathy in rats by improving mitochondrial function through PGC-1α deacetylation. *Acta Pharmacol Sin* **39**, 59–73.

Federico M, Portiansky EL, Sommese L, Alvarado FJ, Blanco PG, Zanuzzi CN, Dedman J, Kaetzel M, Wehrens XHT, Mattiazzi A & Palomeque J (2017). Calcium-calmodulin-dependent protein kinase mediates the intracellular signalling pathways of cardiac apoptosis in mice with impaired glucose tolerance. *J Physiol* **595**, 4089–4108.

Federico M, Valverde CA, Mattiazzi A & Palomeque J (2020). Unbalance between sarcoplasmic reticulum Ca^{2+} uptake and release: a first step toward Ca^{2+} triggered arrhythmias and cardiac damage. *Front Physiol* **10**, 1630.

Fellmann L, Nascimento AR, Tibiriça E & Bousquet P (2013). Murine models for pharmacological studies of the metabolic syndrome. *Pharmacol Ther* **137**, 331–340.

Fernandez-Sanz C, Ruiz-Meana M, Miro-Casas E, Nuñez E, Castellano J, Loureiro M, Barba I, Poncelas M, Rodriguez-Sinovas A, Vázquez J & Garcia-Dorado D (2014). Defective sarcoplasmic reticulum–mitochondria calcium exchange in aged mouse myocardium. *Cell Death Dis* **5**, e1573–e1573.

Ferrannini E & Cushman WC (2012). Diabetes and hypertension: the bad companions. *Lancet* **380**, 601–610.

Finck BN, Lehman JJ, Leone TC, Welch MJ, Bennett MJ, Kovacs A, Han X, Gross RW, Kozak R, Lopaschuk GD & Kelly DP (2002). *J Clin Invest* **109**, 121–130.

Fournier N, Ducet G & Crevat A (1987). Action of cyclosporine on mitochondrial calcium fluxes. *J Bioenerg Biomembr* **19**, 297–303.

Galloway CA & Yoon Y (2015). Mitochondrial Dynamics in Diabetic Cardiomyopathy. *Antioxid Redox Signal* **22**, 1545–1562.

Garnier A, Fortin D, Deloménie C, Momken I, Veksler V & Ventura-Clapier R (2003). Depressed mitochondrial transcription factors and oxidative capacity in rat failing cardiac and skeletal muscles. *J Physiol* **551**, 491–501.

Ghosh S, Pulinilkunnil T, Yuen G, Kewalramani G, An D, Qi D, Abrahani A & Rodrigues B (2005). Cardiomyocyte apoptosis induced by short-term diabetes requires mitochondrial GSH depletion. *Am J Physiol Heart Circ Physiol* **289**, H768–H776.

Graham D, Huynh NN, Hamilton CA, Beattie E, Smith RAJ, Cochemé HM, Murphy MP & Dominiczak AF (2009). Mitochondria-targeted antioxidant MitoQ10 improves endothelial function and attenuates cardiac hypertrophy. *Hypertension* **54**, 322–328.

Grundy SM, Brewer HB, Cleeman JI, Smith SC & Lenfant C (2004). Definition of metabolic syndrome: report of the national heart, lung, and blood institute/American Heart Association Conference on scientific issues related to definition. *Circulation* **109**, 433–438.

Gunter KK, Zuscik MJ & Gunter TE (1991). The Na^+-independent Ca^{2+} efflux mechanism of liver mitochondria is not a passive $Ca^{2+}/2H^+$ exchanger. *J Biol Chem* **266**, 21640–21648.

Guo X-X, Wang Y, Wang K, Ji B-P & Zhou F (2018). Stability of a type 2 diabetes rat model induced by high-fat diet feeding with low-dose streptozotocin injection. *J Zhejiang Univ Sci B* **19**, 559–569.

Halestrap AP (2009*a*). Mitochondria and reperfusion injury of the heart—a holey death but not beyond salvation. *J Bioenerg Biomembr* **41**, 113–121.

Halestrap AP (2009*b*). What is the mitochondrial permeability transition pore? *J Mol Cell Cardiol* **46**, 821–831.

Halestrap AP & Davidson AM (1990). Inhibition of Ca^{2+}-induced large-amplitude swelling of liver and heart mitochondria by cyclosporin is probably caused by the inhibitor binding to mitochondrial-matrix peptidyl-prolyl *cis-trans* isomerase and preventing it interacting with the adenine nucleotide translocase. *Biochem J* **268**, 153–160.

Hall ME, Maready MW, Hall JE & Stec DE (2014). Rescue of cardiac leptin receptors in *db/db* mice prevents myocardial triglyceride accumulation. *Am J Physiol Endocrinol Metabol* **307**, E316–E325.

Han X, Tao Y, Deng Y, Yu J, Sun Y & Jiang G (2017). Metformin accelerates wound healing in type 2 diabetic db/db mice. *Mol Med Rep* **16**, 8691–8698.

Herskovits J, Burgess C, Obar R & Vallee R (1993). Effects of mutant rat dynamin on endocytosis. *J Cell Biol* **122**, 565–578.

Hollander JM, Thapa D & Shepherd DL (2014). Physiological and structural differences in spatially distinct subpopulations of cardiac mitochondria: influence of cardiac pathologies. *Am J Physiol Heart Circ Physiol* **307**, H1–H14.

How O-J, Aasum E, Severson DL, Chan WYA, Essop MF & Larsen TS (2006). Increased myocardial oxygen consumption reduces cardiac efficiency in diabetic mice. *Diabetes* **55**, 466–473.

Hummel KP, Dickie MM & Coleman DL (1966). Diabetes, a new mutation in the mouse. *Science* **153**, 1127–1128.

Hunter DR & Haworth RA (1979). The Ca^{2+}-induced membrane transition in mitochondria: I. The protective mechanisms. *Arch Biochem Biophys* **195**, 453–459.

Hunter DR, Haworth RA & Southard JH (1976). Relationship between configuration, function, and permeability in calcium-treated mitochondria. *J Biol Chem* **251**, 5069–5077.

Hurst S, Hoek J & Sheu S-S (2017). Mitochondrial Ca^{2+} and regulation of the permeability transition pore. *J Bioenerg Biomembr* **49**, 27–47.

Ingalls AM, Dickie MM & Snell GD (1950). Obese, a new mutation in the house mouse. *J Hered* **41**, 317–318.

International Diabetes Federation (2019). IDF Atlas 9th edition and other resources. https://diabetesatlas.org/en/resources/

Ishihara N, Nomura M, Jofuku A, Kato H, Suzuki SO, Masuda K, Otera H, Nakanishi Y, Nonaka I, Goto Y, Taguchi N, Morinaga H, Maeda M, Takayanagi R, Yokota S & Mihara K (2009). Mitochondrial fission factor Drp1 is essential for embryonic development and synapse formation in mice. *Nat Cell Biol* **11**, 958–966.

Jensen PK (1966). Antimycin-insensitive oxidation of succinate and reduced nicotinamide-adenine dinucleotide in electron-transport particles II. Steroid effects. *Biochim Biophys Acta* **122**, 167–174.

Kane LA & Youle RJ (2010). Mitochondrial fission and fusion and their roles in the heart. *J Mol Med* **88**, 971–979.

Kanemaru K, Suzuki J, Taiko I & Iino M (2020). Red fluorescent CEPIA indicators for visualization of Ca^{2+} dynamics in mitochondria. *Sci Rep* **10**, 2835.

Karakikes I, Kim M, Hadri L, Sakata S, Sun Y, Zhang W, Chemaly ER, Hajjar RJ & Lebeche D (2009). Gene remodeling in type 2 diabetic cardiomyopathy and its phenotypic rescue with SERCA2a. *PLoS One* **4**, e6474.

Kelso GF, Porteous CM, Coulter CV, Hughes G, Porteous WK, Ledgerwood EC, Smith RAJ & Murphy MP (2001). Selective targeting of a redox-active ubiquinone to mitochondria within cells: Antioxidant and antiapoptotic properties. *J Biol Chem* **276**, 4588–4596.

Kennedy AJ, Ellacott KLJ, King VL & Hasty AH (2010). Mouse models of the metabolic syndrome. *Dise Models Mech* **3**, 156–166.

Kinnally KW, Zorov DB, Antonenko YN, Snyder SH, McEnery MW & Tedeschi H (1993). Mitochondrial benzodiazepine receptor linked to inner membrane ion channels by nanomolar actions of ligands. *Proc Natl Acad Sci U S A* **90**, 1374–1378.

Kirichok Y, Krapivinsky G & Clapham DE (2004). The mitochondrial calcium uniporter is a highly selective ion channel. *Nature* **427**, 360–364.

Kiyuna LA, Albuquerque RPE, Chen C-H, Mochly-Rosen D & Ferreira JCB (2018). Targeting mitochondrial dysfunction and oxidative stress in heart failure: Challenges and opportunities. *Free Radic Biol Med* **129**, 155–168.

Kohlhaas M, Nickel AG & Maack C (2017). Mitochondrial energetics and calcium coupling in the heart: Mitochondrial energetics and calcium coupling in the heart. *J Physiol* **595**, 3753–3763.

Kokoszka JE, Waymire KG, Levy SE, Sligh JE, Cai J, Jones DP, MacGregor GR & Wallace DC (2004). The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. *Nature* **427**, 461–465.

Koncsos G, Varga ZV, Baranyai T, Boengler K, Rohrbach S, Li L, Schlüter K-D, Schreckenberg R, Radovits T, Oláh A, Mátyás C, Lux Á, Al-Khrasani M, Komlódi T, Bukosza N, Máthé D, Deres L, Barteková M, Rajtík T, Adameová A, Szigeti K, Hamar P, Helyes Z, Tretter L, Pacher P, Merkely B, Giricz Z, Schulz R & Ferdinandy P (2016). Diastolic dysfunction in prediabetic male rats: Role of mitochondrial oxidative stress. *Am J Physiol Heart Circ Physiol* **311**, H927–H943.

Korge P, Calmettes G, John SA & Weiss JN (2017). Reactive oxygen species production induced by pore opening in cardiac mitochondria: The role of complex III. *J Biol Chem* **292**, 9882–9895.

Lavorato M, Iyer VR, Dewight W, Cupo RR, Debattisti V, Gomez L, De la Fuente S, Zhao Y-T, Valdivia HH, Hajnóczky G & Franzini-Armstrong C (2017). Increased mitochondrial nanotunneling activity, induced by calcium imbalance, affects intermitochondrial matrix exchanges. *Proc Natl Acad Sci U S A* **114**, E849–E858.

Lee VK, Hosking BM, Holeniewska J, Kubala EC, Lundh von Leithner P, Gardner PJ, Foxton RH & Shima DT (2018). BTBR *ob/ob* mouse model of type 2 diabetes exhibits early loss of retinal function and retinal inflammation followed by late vascular changes. *Diabetologia* **61**, 2422–2432.

Lee W-S & Kim J (2017). Diabetic cardiomyopathy: where we are and where we are going. *Korean J Intern Med* **32**, 404–421.

Lehman JJ, Barger PM, Kovacs A, Saffitz JE, Medeiros DM & Kelly DP (2000). Peroxisome proliferator-activated receptor γ coactivator-1 promotes cardiac mitochondrial biogenesis. *J Clin Invest* **106**, 847–856.

Li J, Wu H, Liu Y & Yang L (2020). High fat diet induced obesity model using four strains of mice: Kunming, C57BL/6, BALB/c and ICR. *Exp Anim* **69**, 326–335, https://doi.org/10.1538/expanim.19-0148.

Li Q, Xu B, Michie SA, Rubins KH, Schreriber RD & McDevitt HO (2008). Interferon- initiates type 1 diabetes in nonobese diabetic mice. *Proc Natl Acad Sci* **105**, 12439–12444.

Li W, Shariat-Madar Z, Powers M, Sun X, Lane RD & Garlid KD (1992). Reconstitution, identification, purification, and immunological characterization of the 110-kDa Na^+/Ca^{2+} antiporter from beef heart mitochondria. *J Biol Chem* **267**, 17983–17989.

Lin J, Wu PH, Tarr PT, Lindenberg KS, St-Pierre J, Zhang CY, Mootha VK, Jäger S, Vianna CR, Reznick RM, Cui L, Manieri M, Donovan MX, Wu Z, Cooper MP, Fan MC, Rohas LM, Zavacki AM, Cinti S, Shulman GI, Lowell BB, Krainc D & Spiegelman BM (2004). Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1α null mice. *Cell* **119**, 121–135.

Lu X, Ginsburg KS, Kettlewell S, Bossuyt J, Smith GL & Bers DM (2013). Measuring local gradients of intramitochondrial $[Ca^{2+}]$ in cardiac myocytes during sarcoplasmic reticulum Ca^{2+} release. *Circ Res* **112**, 424–431.

Ma J, Banerjee P, Whelan SA, Liu T, Wei A-C, Ramirez-Correa G, McComb ME, Costello CE, O'Rourke B, Murphy A & Hart GW (2016). Comparative proteomics reveals dysregulated mitochondrial O-GlcNAcylation in diabetic hearts. *J Proteome Res* **15**, 2254–2264.

Mali VR, Pan G, Deshpande M, Thandavarayan RA, Xu J, Yang X-P & Palaniyandi SS (2016). Cardiac mitochondrial respiratory dysfunction and tissue damage in chronic hyperglycemia correlate with reduced aldehyde dehydrogenase-2 activity. *PLoS One* **11**, e0163158.

Marzo I, Brenner C, Zamzami N, Susin SA, Beutner G, Brdiczka D, Rémy R, Xie Z-H, Reed JC & Kroemer G (1998). The permeability transition pore complex: a target for apoptosis regulation by caspases and Bcl-2-related proteins. *J Exp Med* **187**, 1261–1271.

Masuzaki H, Paterson J, Shinyama H, Morton NM, Mullins JJ, Seckl JR & Flier JS (2001). A transgenic model of visceral obesity and the metabolic syndrome. *Science* **294**, 2166–2170.

McNeill JH (2018). *Experimental Models of Diabetes*. Routledge.

Mellor KM, Bell JR, Wendt IR, Davidoff AJ, Ritchie RH & Delbridge LMD (2011). Fructose modulates cardiomyocyte excitation-contraction coupling and Ca^{2+} handling in vitro. *PLoS One* **6**, e25204.

Mitchell P (1972). Chemiosmotic coupling in energy transduction: A logical development of biochemical knowledge. *J Bioenerg* **3**, 5–24.

Miyata H, Silverman HS, Sollott SJ, Lakatta EG, Stern MD & Hansford RG (1991). Measurement of mitochondrial free Ca^{2+} concentration in living single rat cardiac myocytes. *Am J Physiol Heart Circ Physiol* **261**, H1123–H1134.

Mnatsakanyan N & Jonas EA (2020). ATP synthase c-subunit ring as the channel of mitochondrial permeability transition: Regulator of metabolism in development and degeneration. *J Mol Cell Cardiol* **144**, 109–118.

Montaigne D, Marechal X, Coisne A, Debry N, Modine T, Fayad G, Potelle C, El Arid J-M, Mouton S, Sebti Y, Duez H, Preau S, Remy-Jouet I, Zerimech F, Koussa M, Richard V, Neviere R, Edme J-L, Lefebvre P & Staels B (2014).

Myocardial contractile dysfunction is associated with impaired mitochondrial function and dynamics in type 2 diabetic but not in obese patients. *Circulation* **130**, 554–564.

Moore JX, Chaudhary N & Akinyemiju T (2017). Metabolic syndrome prevalence by race/ethnicity and sex in the United States, National Health and Nutrition Examination Survey, 1988–2012. *Prevent Chronic Dis* **14**, E24.

Mordes JP, Guberski DL, Leif JH, Woda BA, Flanagan JF, Greiner DL, Kislauskis EH & Tirabassi RS (2005). LEW.1WR1 rats develop autoimmune diabetes spontaneously and in response to environmental perturbation. *Diabetes* **54**, 2727–2733.

Mortensen SA, Rosenfeldt F, Kumar A, Dolliner P, Filipiak KJ, Pella D, Alehagen U, Steurer G, Littarru GP & Investigators Q-SS (2014). The effect of coenzyme Q_{10} on morbidity and mortality in chronic heart failure: results from Q-SYMBIO: a randomized double-blind trial. *JACC Heart Fail* **2**, 641–649.

Murgia M, Giorgi C, Pinton P & Rizzuto R (2009). Controlling metabolism and cell death: at the heart of mitochondrial calcium signalling. *J Mol Cell Cardiol* **46**, 781–788.

Namekawa J, Takagi Y, Wakabayashi K, Nakamura Y, Watanabe A, Nagakubo D, Shirai M & Asai F (2017). Effects of high-fat diet and fructose-rich diet on obesity, dyslipidemia and hyperglycemia in the WBN/Kob-*Lepr^{fa}* rat, a new model of type 2 diabetes mellitus. *J Vet Med Sci* **79**, 988–991.

Neubauer S (2007). The failing heart—an engine out of fuel. *N Engl J Med* **356**, 1140–1151.

Ni R, Cao T, Xiong S, Ma J, Fan G-C, Lacefield JC, Lu Y, Tissier SL & Peng T (2016*a*). Therapeutic inhibition of mitochondrial reactive oxygen species with mito-TEMPO reduces diabetic cardiomyopathy. *Free Radic Biol Med* **90**, 12–23.

Ni R, Zheng D, Xiong S, Hill DJ, Sun T, Gardiner RB, Fan G-C, Lu Y, Abel ED, Greer PA & Peng T (2016*b*). Mitochondrial calpain-1 disrupts ATP synthase and induces superoxide generation in type-1 diabetic hearts: a novel mechanism contributing to diabetic cardiomyopathy. *Diabetes* **65**, 255–268.

Nickel A, Kohlhaas M & Maack C (2014). Mitochondrial reactive oxygen species production and elimination. *J Mol Cell Cardiol* **73**, 26–33.

Nisoli E, Clementi E, Carruba MO & Moncada S (2007). Defective mitochondrial biogenesis. *Circ Res* **100**, 795–806.

Oka S, Sabry AD, Cawley KM & Warren JS (2020). Multiple levels of PGC-1α dysregulation in heart failure. *Front Cardiovasc Med* **7**, 2.

Oliveira PJ, Seiça R, Coxito PM, Rolo AP, Palmeira CM, Santos MS & Moreno AJM (2003). Enhanced permeability transition explains the reduced calcium uptake in cardiac mitochondria from streptozotocin-induced diabetic rats. *FEBS Lett* **554**, 511–514.

Otera H, Wang C, Cleland MM, Setoguchi K, Yokota S, Youle RJ & Mihara K (2010). Mff is an essential factor for mitochondrial recruitment of Drp1 during mitochondrial fission in mammalian cells. *J Cell Biol* **191**, 1141–1158.

Palmer CS, Osellame LD, Laine D, Koutsopoulos OS, Frazier AE & Ryan MT (2011). MiD49 and MiD51, new components of the mitochondrial fission machinery. *EMBO Rep* **12**, 565–573.

Palmer W & Hoppel L (1977). Properties of subsarcolemmal and mitochondria isolated from rat cardiac muscle. *J Biol Chem* **252**, 8731–8739.

Pan X, Liu J, Nguyen T, Liu C, Sun J, Teng Y, Fergusson MM, Rovira II, Allen M, Springer DA, Aponte AM, Gucek M, Balaban RS, Murphy E & Finkel T (2013). The physiological role of mitochondrial calcium revealed by mice lacking the mitochondrial calcium uniporter. *Nat Cell Biol* **15**, 1464–1472.

Panchal SK & Brown L (2011). Rodent models for metabolic syndrome research. *J Biomed Biotechnol* **2011**, 1–14.

Pham T, Loiselle D, Power A & Hickey AJR (2014). Mitochondrial inefficiencies and anoxic ATP hydrolysis capacities in diabetic rat heart. *Am J Physiol Cell Physiol* **307**, C499–C507.

Qin F, Siwik DA, Luptak I, Hou X, Wang L, Higuchi A, Weisbrod RM, Ouchi N, Tu VH, Calamaras TD, Miller EJ, Verbeuren TJ, Walsh K, Cohen RA & Colucci WS. (2012). The polyphenols resveratrol and S17834 prevent the structural and functional sequelae of diet-induced metabolic heart disease in mice. *Circulation* **125**, 1757–1764.

Rajesh M, Mukhopadhyay P, Bátkai S, Patel V, Saito K, Matsumoto S, Kashiwaya Y, Horváth B, Mukhopadhyay B, Becker L, Haskó G, Liaudet L, Wink DA, Veves A, Mechoulam R & Pacher P (2010). Cannabidiol attenuates cardiac dysfunction, oxidative stress, fibrosis, and inflammatory and cell death signaling pathways in diabetic cardiomyopathy. *J Am Coll Cardiol* **56**, 2115–2125.

Ren J, Pulakat L, Whaley-Connell A & Sowers JR (2010). Mitochondrial biogenesis in the metabolic syndrome and cardiovascular disease. *J Mol Med* **88**, 993–1001.

Robin ED & Wong R (1988). Mitochondrial DNA molecules and virtual number of mitochondria per cell in mammalian cells. *J Cell Physiol* **136**, 507–513.

Rubler S, Dlugash J, Yuceoglu YZ, Kumral T, Branwood AW & Grishman A (1972). New type of cardiomyopathy associated with diabetic glomerulosclerosis. *Am J Cardiol* **30**, 595–602.

Saklayen MG (2018). The global epidemic of the metabolic syndrome. *Curr Hypertens Rep* **20**, 12.

Santos DL, Palmeira CM, Seiça R, Dias J, Mesquita J, Moreno AJ & Santos MS (2003). Diabetes and mitochondrial oxidative stress: A study using heart mitochondria from the diabetic Goto-Kakizaki rat. In *Vascular Biochemistry*, ed. Zahradka P, Wigle J & Pierce GN, pp. 163–170. Springer US, Boston, MA. http://link.springer.com/10.1007/978-1-4615-0298-2_23

Sauvanet C, Duvezin-Caubet S, di Rago J-P & Rojo M (2010). Energetic requirements and bioenergetic modulation of mitochondrial morphology and dynamics. *Semin Cell Dev Biol* **21**, 558–565.

Seidlmayer LK, Juettner VV, Kettlewell S, Pavlov EV, Blatter LA & Dedkova EN (2015). Distinct mPTP activation mechanisms in ischaemia–reperfusion: contributions of Ca^{2+}, ROS, pH, and inorganic polyphosphate. *Cardiovasc Res* **106**, 237–248.

Seidlmayer LK, Mages C, Berbner A, Eder-Negrin P, Arias-Loza PA, Kaspar M, Song M, Dorn GW, Kohlhaas M, Frantz S, Maack C, Gerull B & Dedkova EN (2019). Mitofusin 2 is essential for IP_3-mediated SR/mitochondria metabolic feedback in ventricular myocytes. *Front Physiol* **10**, 733.

Shah AM, Shin SH, Takeuchi M, Skali H, Desai AS, Køber L, Maggioni AP, Rouleau JL, Kelly RY, Hester A, Keefe D, McMurray JJV, Pfeffer MA & Solomon SD (2012). Left ventricular systolic and diastolic function, remodelling, and clinical outcomes among patients with diabetes following myocardial infarction and the influence of direct renin inhibition with aliskiren. *Eur J Heart Fail* **14**, 185–192.

Shao D & Tian R (2015). Glucose transporters in cardiac metabolism and hypertrophy. *Compr Physiol* **6**, 331–351.

Shen X, Zheng S, Metreveli NS & Epstein PN (2006). Protection of cardiac mitochondria by overexpression of MnSOD reduces diabetic cardiomyopathy. *Diabetes* **55**, 798–805.

Shen X, Zheng S, Thongboonkerd V, Xu M, Pierce WM, Klein JB & Epstein PN (2004). Cardiac mitochondrial damage and biogenesis in a chronic model of type 1 diabetes. *Am J Physiol Endocrinol Metabol* **287**, E896–E905.

Sommese L, Valverde CA, Blanco P, Castro MC, Rueda OV, Kaetzel M, Dedman J, Anderson ME, Mattiazzi A & Palomeque J (2016). Ryanodine receptor phosphorylation by CaMKII promotes spontaneous Ca^{2+} release events in a rodent model of early stage diabetes: The arrhythmogenic substrate. *Int J Cardiol* **202**, 394–406.

Stanley WC, Recchia FA & Lopaschuk GD (2005). Myocardial substrate metabolism in the normal and failing heart. *Physiol Rev* **85**, 1093–1129.

Suarez J, Cividini F, Scott BT, Lehmann K, Diaz-Juarez J, Diemer T, Dai A, Suarez JA, Jain M & Dillmann WH (2018). Restoring mitochondrial calcium uniporter expression in diabetic mouse heart improves mitochondrial calcium handling and cardiac function. *J Biol Chem* **293**, 8182–8195.

Suarez J, Hu Y, Makino A, Fricovsky E, Wang H & Dillmann WH (2008). Alterations in mitochondrial function and cytosolic calcium induced by hyperglycemia are restored by mitochondrial transcription factor A in cardiomyocytes. *Am J Physiol Cell Physiol* **295**, C1561–C1568.

Sun J, Yamaguchi N, Xu L, Eu JP, Stamler JS & Meissner G (2008). Regulation of the cardiac muscle ryanodine receptor by O_2 tension and *S*-nitrosoglutathione. *Biochemistry* **47**, 13985–13990.

Surwit RS, Feinglos MN, Rodin J, Sutherland A, Petro AE, Opara EC, Kuhn CM & Rebuffe-Scrive M (1995). Differential effects of fat and sucrose on the development of obesity and diabetes in C57BL/6J and AJ mice. *Metabolism* **44**, 645–651.

Surwit RS, Kuhn CM, Cochrane C, McCubbin JA & Feinglos MN (1988). Diet-induced type II diabetes in C57BL/6J mice. *Diabetes* **37**, 1163–1167.

Suzuki H, Kayama Y, Sakamoto M, Iuchi H, Shimizu I, Yoshino T, Katoh D, Nagoshi T, Tojo K, Minamino T, Yoshimura M & Utsunomiya K (2015). Arachidonate 12/15-lipoxygenase-induced inflammation and oxidative stress are involved in the development of diabetic cardiomyopathy. *Diabetes* **64**, 618–630.

Szűcs G, Sója A, Péter M, Sárközy M, Bruszel B, Siska A, Földesi I, Szabó Z, Janáky T, Vígh L, Balogh G & Csont T (2019). Prediabetes induced by fructose-enriched diet influences cardiac lipidome and proteome and leads to deterioration of cardiac function prior to the development of excessive oxidative stress and cell damage. *Oxid Med Cell Longev* **2019**, 3218275.

Unwin N & International Diabetes Federation (2009). *IDF diabetes atlas*. IDF Executive Office, Brussels. http://www.diabetesatlas.org/

Vasiliadis I, Kolovou G, Mavrogeni S, Nair DR & Mikhailidis DP (2014). Sudden cardiac death and diabetes mellitus. *J Diabetes Complications* **28**, 573–579.

Ventura-Clapier R, Garnier A & Veksler V (2008). Transcriptional control of mitochondrial biogenesis: the central role of PGC-1α. *Cardiovasc Res* **79**, 208–217.

Wang J, Song Y, Wang Q, Kralik PM & Epstein PN (2006). Causes and characteristics of diabetic cardiomyopathy. *Rev Diabet Stud* **3**, 108–108.

Wang SY, Zhu S, Wu J, Zhang M, Xu Y, Xu W, Cui J, Yu B, Cao W & Liu J (2020). Exercise enhances cardiac function by improving mitochondrial dysfunction and maintaining energy homoeostasis in the development of diabetic cardiomyopathy. *J Mol Med* **98**, 245–261.

Wang W, Fang H, Groom L, Cheng A, Zhang W, Liu J, Wang X, Li K, Han P, Zheng M, Yin J, Wang W, Mattson MP, Kao JPY, Lakatta EG, Sheu S-S, Ouyang K, Chen J, Dirksen RT & Cheng H (2008). Superoxide flashes in single mitochondria. *Cell* **134**, 279–290.

Williamson CL, Dabkowski ER, Baseler WA, Croston TL, Alway SE & Hollander JM (2010). Enhanced apoptotic propensity in diabetic cardiac mitochondria: influence of subcellular spatial location. *Am J Physiol-Heart Circ Physiol* **298**, H633–H642.

World Health Organization (2019). Diabetes prevalence (% of population ages 20 to 79. https://data.worldbank.org/indicator/SH.STA.DIAB.ZS?view=map

World Health Organization (2020). Diabetes. https://www.who.int/news-room/fact-sheets/detail/diabetes

Wu S, Lu Q, Ding Y, Wu Y, Qiu Y, Wang P, Mao X, Huang K, Xie Z & Zou M-H (2019). Hyperglycemia-driven inhibition of AMP-activated protein kinase α2 induces diabetic cardiomyopathy by promoting mitochondria-associated endoplasmic reticulum membranes in vivo. *Circulation* **139**, 1913–1936.

Wu Y, Rasmussen TP, Koval OM, Joiner MA, Hall DD, Chen B, Luczak ED, Wang Q, Rokita AG, Wehrens XHT, Song L-S & Anderson ME (2015). The mitochondrial uniporter controls fight or flight heart rate increases. *Nat Commun* **6**, 6081.

Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC & Spiegelman BM (1999). Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* **98**, 115–124.

Xu L, Eu JP, Meissner G & Stamler JS (1998). Activation of the cardiac calcium release channel (ryanodine receptor) by poly-*S*-nitrosylation. *Science* **279**, 234–237.

Yan W, Zhang H, Liu P, Wang H, Liu J, Gao C, Liu Y, Lian K, Yang L, Sun L, Guo Y, Zhang L, Dong L, Lau WB, Gao E, Gao F, Xiong L, Wang H, Qu Y & Tao L (2013). Impaired mitochondrial biogenesis due to dysfunctional adiponectin-AMPK-PGC-1α signaling contributing to increased vulnerability in diabetic heart. *Basic Res Cardiol* **108**, 329.

Yaras N, Ugur M, Ozdemir S, Gurdal H, Purali N, Lacampagne A, Vassort G & Turan B (2005). Effects of diabetes on ryanodine receptor Ca release channel (RyR2) and Ca^{2+} homeostasis in rat heart. *Diabetes* **54**, 3082–3088.

Yoon Y, Krueger EW, Oswald BJ & McNiven MA (2003). The mitochondrial protein hFis1 regulates mitochondrial fission in mammalian cells through an interaction with the dynamin-like protein DLP1. *Mol Cell Biol* **23**, 5409–5420.

Yorek MA (2016). Alternatives to the Streptozotocin-diabetic rodent. *Int Rev Neurobiol* **127**, 89–112.

Yuan F, Woollard JR, Jordan KL, Lerman A, Lerman LO & Eirin A (2018). Mitochondrial targeted peptides preserve mitochondrial organization and decrease reversible myocardial changes in early swine metabolic syndrome. *Cardiovasc Res* **114**, 431–442.

Zorov DB, Filburn CR, Klotz L-O, Zweier JL & Sollott SJ (2000). Reactive oxygen species (ROS-induced) ROS release: a new phenomenon accompanying induction of the mitochondrial permeability transition in cardiac myocytes. *J Exp Med* **192**, 1001–1014.

Zorov DB, Juhaszova M & Sollott SJ (2014). Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol Rev* **94**, 909–950.

Additional information

Competing interests

None.

Author contributions

M.F. and S.D.la.F. wrote the first draft, J.P. and S.-S.S. reviewed and revised the manuscript. All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Funding

This work was supported by NIH R01HL093671, R01HL137266, R01HL142864 and R01HL122124 (to S.-S.S.); and by PICT 2015–3009 and PS-1 (UAI Argentina). M.F. is a doctoral fellow of CONICET.

Acknowledgements

We thank Jennifer Wilson for the English language editing on the manuscript and Gyorgy Csordas for comments on the content of the manuscript.

Keywords

diabetic cardiomyopathy, heart, metabolic diseases, mitochondria, oxidative stress