Altered mitochondrial metabolism in the insulin-resistant heart

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Abstract
Obesity-induced insulin resistance and type 2 diabetes mellitus can ultimately result in various complications, including diabetic cardiomyopathy. In this case, cardiac dysfunction is characterized by metabolic disturbances such as impaired glucose oxidation and an increased reliance on fatty acid (FA) oxidation. Mitochondrial dysfunction has often been associated with the altered metabolic function in the diabetic heart, and may result from FA-induced lipotoxicity and uncoupling of oxidative phosphorylation. In this review, we address the metabolic changes in the diabetic heart, focusing on the loss of metabolic flexibility and cardiac mitochondrial function. We consider the alterations observed in mitochondrial substrate utilization, bioenergetics and dynamics, and highlight new areas of research which may improve our understanding of the cause and effect of cardiac mitochondrial dysfunction in diabetes. Finally, we explore how lifestyle (nutrition and exercise) and pharmacological interventions can prevent and treat metabolic and mitochondrial dysfunction in diabetes.

KEYWORDS
diabetes, heart, lipotoxicity, mitochondria

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) has reached epidemic proportions; in 2014 around 422 million people had been diagnosed with T2DM, corresponding to about 8.5% of the global population of adults over 18 years of age. Obesity is a major risk factor for the development of T2DM, leading to an increased risk of cardiovascular disease, particularly coronary artery disease and stroke. Diabetic cardiomyopathy was first described in 1972, and since then, large cohort studies, such as the Framingham study and the Strong Heart Study have reported left ventricular hypertrophy in patients with T2DM, independent of hypertension. More recently, diabetic cardiomyopathy was described as a restrictive phenotype with concentric LV remodelling and diastolic LV dysfunction. These two phenotypes are not considered to be successive stages of diabetic cardiomyopathy, but instead each evolves independently to, respectively, heart failure with preserved left ventricular ejection fraction (HFPEF) or reduced left ventricular ejection fraction.

Individuals with pre-diabetes and patients with uncomplicated T2DM often suffer from circulating hyperglycaemia, hypertriglyceridaemia and elevated plasma levels of non-esterified fatty acids (FAs). This increased FA availability leads to increased myocardial FA uptake and further reduces insulin-mediated glucose uptake, shifting cardiac ATP production almost exclusively towards FA oxidation (FAO) both in early and late diabetes. Mouse studies suggest that the altered substrate preference precedes the development of cardiac dysfunction, implicating altered cardiac metabolism in the development of diabetic cardiomyopathy. Moreover, despite this relative increase in FAO, the excess supply of FAs results in the accumulation of lipid intermediates, which in turn play a major role in the pathophysiology of diabetic cardiomyopathy.

Here we initially consider these metabolic adaptations in the obese, insulin-resistant and ultimately type 2 diabetic heart, focusing on the loss of metabolic flexibility. We subsequently review the lipotoxicity-induced alterations in cellular and mitochondrial bioenergetic function of the diabetic heart. Finally, we explore how metabolic and mitochondrial alterations can be prevented by lifestyle and/or pharmacological intervention.

PATHOPHYSIOLOGY OF DISTURBANCES IN MITOCHONDRIAL METABOLISM IN T2DM

2.1 Metabolic inflexibility and myocardial substrate utilization

To maintain its high-energy demand, the heart utilizes multiple energy-providing substrates, primarily triglycerides, non-esterified FAs, carbohydrates (glucose and lactate) and to some extent also ketone bodies and amino acids. The contribution of these individual substrates to ATP production depends on substrate availability, hormonal status and energy demand, and the capacity of the normal heart to switch between the different energy substrates is referred to as ‘metabolic flexibility’. With the development of insulin resistance, however, the metabolic flexibility of the heart (as well as skeletal muscle) deteriorates, such that myocardial energy production becomes primarily dependent on FAO. This concept is coined metabolic inflexibility or loss of metabolic flexibility. In the 1960s, Sir Philip Randle performed landmark studies showing how products of increased FAO can inhibit glucose uptake in muscle. This mechanism, subsequently known as the Randle Cycle, underpins the ‘metabolic flexibility’ of healthy individuals, that is the capacity to switch between fuels, depending on nutrient composition and intake, as well as variations in insulin signalling. Cardiac metabolic flexibility is also linked to daily fasting-feeding cycles and the cellular circadian rhythm, which coordinate a vital interplay between food intake and metabolism. Recent data from humans and animal models suggest that disturbances in feeding and the circadian rhythm, for example as a result of jet-lag or shift-work, could lead to the development of insulin resistance (see also Section 4.2).

With the development of insulin resistance, however, the metabolic flexibility of the heart (as well as skeletal muscle) deteriorates, such that myocardial energy production becomes primarily dependent on FAO. The heart can use other substrates as metabolic fuel, such as branched-chain amino acids and ketone bodies, however, the relative contribution of these substrates to total ATP production is relatively low, and little is currently understood about their importance in insulin resistance and T2DM. The high supply of FAs exceeds mitochondrial FAO capacity, resulting in the accumulation of intermediates of FA metabolism in the cardiomyocytes and causing a state of lipotoxicity. Lipotoxicity can lead to cellular oxidative stress, impaired cytosolic and mitochondrial calcium homeostasis and mitochondrial dysfunction.

Diabetic cardiomyopathy is therefore initially characterized by metabolic disturbances and diastolic dysfunction (left ventricular stiffness and impaired relaxation). This condition can ultimately progress to cardiac hypertrophy and/ or systolic dysfunction when lipotoxicity and/or local perfusion heterogeneities result in cell death and fibrosis.

2.2 Increased myocardial oxygen consumption and impaired energetics

Landmark studies in the 1970s reported that canine myocardial oxygen consumption (MVO_2) increased markedly in response to acute elevations in the plasma concentration...
of FAs. Increased FA utilization and increased MVO$_2$ have also been reported in obese women with insulin resistance. The cellular and molecular mechanisms behind these metabolic alterations are not clear, although it has been suggested that uncoupling of oxidative phosphorylation (OXPHOS) and induction of energy-wasting triglyceride-FA$^{23,24}$ and Ca$^{2+}$ cycling$^{25}$ could contribute to this elevation in MVO$_2$. It was proposed that excess substrate supply might result in impaired transcriptional regulation of proteins constituting the pathways of cardiac energy metabolism. Indeed, in patients undergoing coronary artery bypass graft surgery, elevated plasma FA concentrations were associated with increased expression of cardiac mitochondrial uncoupling proteins (UCPs).$^{27}$ Moreover, an impaired cardiac energy reserve in patients with T2DM (as indicated by a lower myocardial phosphocreatine [PCr]/ATP ratio) correlated with fasting plasma FA concentration,$^{28}$ a finding which could also be explained by increased uncoupling of OXPHOS. Cardiac PCr/ATP ratios have also been found to be reduced during catecholamine stress$^{29}$ or exercise$^{30}$ in people with obesity and insulin resistance, although another study failed to confirm this latter observation.$^9$ Whether a lower myocardial PCr/ATP ratio in diabetic cardiomyopathy is a cause or effect of the progression to heart failure is currently unknown.$^{31}$

### 2.3 Cardiac efficiency

Cardiac efficiency is characterized by the relationship between the mechanical performance and energy consumption of the heart, whether measured as ATP utilization or oxygen consumption. Introduction of the conductance catheter allowed calculation of the total work performed by the heart during the cardiac cycle as pressure-volume area (PVA), and the relationship between MVO$_2$ and PVA allowed calculation of the oxygen used for mechanical activity vs oxygen consumption used for basal metabolism and excitation-contraction coupling (unloaded MVO$_2$)$^{32}$

Around the turn of the 21st century, Korvald et al$^{33}$ showed, for the first time, that the MVO$_2$-PVA relationship was significantly influenced by changes in myocardial substrate metabolism in pigs. Thus, a change in myocardial metabolism from glucose towards FAO shifted the in vivo MVO$_2$-PVA relationship upward in a parallel manner, indicating decreased cardiac efficiency, which could be ascribed to a higher unloaded MVO$_2$ (i.e., more oxygen used for basal metabolism and excitation-contraction coupling in the case of FAO). Similar observations were reported by How et al$^{34}$ using isolated perfused working mouse hearts exposed to different workloads. Here, elevating FA concentration in the perfusion buffer shifted the MVO$_2$-PVA relationship upward, resulting in a near 30% increase in unloaded oxygen cost (Figure 1).

Finally, hearts from the diabetic $db/db$ mouse show metabolic shifts towards a predominant FA utilization, and the MVO$_2$-PVA relationships obtained from these hearts were also shifted upward relative to those of normal mouse hearts.$^{35}$ These results therefore demonstrate that acute elevations in myocardial FAO, but also more chronic dependence on FA as oxidative fuel for the heart such as in T2DM, results in decreased cardiac efficiency. It should be noted that the FA-induced elevation in MVO$_2$ can by no means be explained by the switch in metabolism from glucose to FA, since the differences in phosphorylation-to-oxidation (P/O) ratios between FA and glucose oxidation (2.33 vs 2.58 respectively) could account for a maximum increase in oxygen consumption of 11%. Other mechanisms, for example uncoupling of OXPHOS and induction of futile cycles, as discussed in Section 2.2 above, could explain the high MVO$_2$ during predominant FA utilization.

In conclusion, the healthy heart is characterized by a high metabolic flexibility, whereby metabolic supply and demand are optimally matched. The cardiac muscle from patients with insulin resistance and diabetes cannot effectively switch from FA to glucose metabolism in the post-prandial state and are therefore metabolically less flexible in adapting fuel preference to altered energy supply and demand. When relying primarily on FAO for energy production, the heart uses more oxygen for a given workload, compared with a heart oxidizing a mixture of FA and glucose. The FA-induced elevation in MVO$_2$ is due to increased oxygen use for non-contractile processes, such as basal metabolism and excitation-contraction coupling.
2.4 | FA metabolism and cellular lipotoxicity

Lipid metabolism is a complex process, involving lipid intake, synthesis, transport and metabolism. Fatty acids are major components of all lipid species, and thus the lipid content of plasma and tissues depends upon FA availability. FAs also influence multiple intracellular processes through mechanisms that include the activation of peroxisome proliferator-activated receptor (PPAR)α and PPAR gamma coactivator 1 α (PGC-1α), leading to the upregulation of genes involved in FA metabolism and the biogenesis of peroxisomes and mitochondria. Reports have suggested that excessive FAs might augment inflammation through activation of toll-like receptor (TLR) signalling and following activation of nuclear factor kappa-light-chain enhancer of activated B-cells (NF-κB). There is increasing evidence that FA availability is an independent predictor of metabolic disorders including insulin resistance and T2DM. It appears likely that FA accumulation results in increased levels of FA intermediates, such as long-chain acylcarnitines, which underpin lipotoxic effects in heart mitochondria. Notably, however, in contrast to saturated long-chain FAs, polyunsaturated FAs at reasonable amounts are cardioprotective rather than detrimental to the heart and mitochondrial function.

2.4.1 | FA-induced uncoupling of OXPHOS

It has been proposed that FA-induced mitochondrial uncoupling contributes to the higher MVO₂ and impaired ATP synthesis capacity in the T2DM heart. Indeed, the higher leak respiration and lower ADP/O ratio observed in mitochondria isolated from hearts of ob/ob mice suggest that mild mitochondrial uncoupling is one of the causes for the reduced OXPHOS capacity. Proton leak across the inner mitochondrial membrane, mediated by proteins such as the adenine nucleotide translocase (ANT) and UCPs have been proposed to increase the respiratory rate and decrease the proton electrochemical gradient. This would significantly affect the cellular metabolic rate in various cell types, with consequent impairment of ATP synthesis. The shift towards long-chain acylcarnitine accumulation has been shown to be regulated primarily by PPARx, whereas cardiac UCP2 expression is regulated in part by a FA-dependent, PPARx-independent mechanism. Increased expression of UCP3 has been described in the hearts of animals with streptozotocin-induced diabetes. Other studies have demonstrated an association with UCP3 and enhanced myocardial FAO during insulin resistance and diabetes, and in humans increased concentrations of circulating free FAs correlate with expression of both UCP2 and UCP3. However, FA-induced leak respiration can occur without alterations in UCP3 protein content (eg as in ob/ob hearts). This suggests a role for other mechanisms that may also mediate proton leak, independent of UCPs. Notably, recent observations suggest that mitochondrial ADP/ATP carriers, also activated by FA, may be responsible for FA-induced increase in leak respiration.

There does not seem to be a role for UCP3 as a mechanism to transport FA out of the mitochondria during elevated FA supply, as has been suggested previously. However, enhanced UCP3 expression has been associated with the mitigation of oxidative stress, and in line with this there is evidence to suggest a relationship between increased mitochondrial ROS and UCP3 deficiency. In intact cell systems, mild mitochondrial uncoupling, due to a decrease in ΔΨm, has been proposed to be a protective strategy under conditions of oxidative stress such as diabetes and obesity. However, this situation may only apply at the extremes of high redox potential, which is further elaborated within the R-ORB hypothesis (Redox-Optimized ROS Balance).

The debate regarding the capacity of UCPs to uncouple mitochondria in the heart and the extent to which UCP3 is involved in the prevention of ROS formation remains unsettled. However, the correlation between UCP3 levels and FAO in the heart under obese/diabetic conditions does support a role for UCP3 under conditions of perturbed cardiac energy balance. In line with this, UCPs and the mechanistic basis of mitochondrial uncoupling in the obese and T2DM heart remains an area that requires further study.

2.4.2 | Long-chain acylcarnitine-induced lipotoxicity

Several steps are needed to ensure long-chain FA transport into the mitochondria. The first step of long-chain FA metabolism is the synthesis of long-chain acyl-CoA in the outer mitochondrial membrane catalysed by acyl-CoA synthase. Next, the synthesis of long-chain acylcarnitine is catalysed by carnitine palmitoyltransferase I (CPT1) to allow FA to cross the mitochondrial inner membrane. Long-chain FAO rate is therefore regulated by the cytosolic concentration of malonyl-CoA, which is an allosteric inhibitor of CPT1. Activation of insulin signalling stimulates malonyl-CoA synthesis and inhibits CPT1, providing an important mechanism for the regulation of FAO and adaptation of cardiac metabolism to substrate availability and nutritional state.

The shift towards long-chain acylcarnitine accumulation is a result of unbalanced acylcarnitine synthesis and mitochondrial oxidation rates, which leads to accumulation of long-chain acylcarnitines in mitochondria—often referred to in the literature as incomplete FAO. In this case, the highest concentrations of long-chain acylcarnitines are found in the mitochondrial inner membrane and the intermembrane space, but long-chain acylcarnitines can also escape from mitochondria and inhibit the insulin signalling cascade.
upstream of protein kinase b (Akt) phosphorylation, favouring FA metabolism at the expense of glucose/pyruvate metabolism. Meanwhile, in cardiac mitochondria, long-chain acylcarnitines inhibit pyruvate and lactate metabolism even at physiological concentrations. At elevated levels, the accumulation of long-chain acylcarnitines inhibits OXPHOS, inducing mitochondrial membrane hyperpolarization and stimulating ROS production. Thus, in patients with insulin resistance and T2DM, the high mitochondrial content of long-chain acylcarnitines could increase the risk of mitochondrial and cardiac damage, particularly in conditions of cardiac ischaemia, while mild uncoupling of mitochondria might prove to be a useful strategy. Interestingly, the accumulation of long-chain acylcarnitines per se and altered PI3K signalling likely have additional, less studied, consequences for cardiomyocyte function, namely electrophysiological alterations, predisposing the cardiomyocytes to cellular arrhythmias. This may help to explain why patients with T2DM also have an increased risk of life-threatening arrhythmias. Overall long-chain acylcarnitines are physiologically important substrates for energy metabolism during the fasted state, however, their accumulation in insulin-resistant subjects might result in disturbances of energy metabolism and elevated risk of cardiac damage.

### 2.4.3 Diacylglycerol- and ceramide-induced lipotoxicity

Other lipid metabolism intermediates, namely diacylglycerols (DAGs) and ceramides, have been shown to interact with the insulin signalling pathway (Figure 2), and their accumulation might lead to metabolic disturbances. The accumulation of DAGs increases protein kinase C (PKC)-θ and PKC-ε translocation in heart, following the reduction of Akt phosphorylation and decreased expression of mitochondrial fusion mediators. Ceramides inhibit Akt signalling via increased protein phosphatase 2 activity and activation of atypical PKC-ζ. In addition, in isolated rat heart mitochondria, ceramides perturb mitochondrial membrane structure, inhibit mitochondrial complex I and III, and increase ROS production.

The relative contribution of these lipid intermediates to insulin resistance and altered mitochondrial function remains to be elucidated, and in particular it is not clear whether lipid intermediates accumulate in cardiac tissues at sufficient levels to induce insulin resistance and alter mitochondrial function. It was recently reported, however, that the diabetic heart exhibits a decreased mitochondrial capacity for β-oxidation and increased accumulation of intracellular lipids, even in the absence of contractile failure. Depending on nutritional status and metabolic state, concentrations of lipid intermediates vary significantly. The DAG content in control animal hearts varies from 50 to 800 nmol/g. Genetic manipulation, diabetes and chronic lipid overload might increase cardiac DAG content several fold, however, when manipulating lipid content and signalling pathways by dietary, genetic or pharmacological means, it is not possible to influence the content of a single lipid intermediate in isolation of other upstream or downstream intermediates. This has lead to controversial observations in vitro in various animal and human studies. For example myriocin, a pharmacological tool used to limit ceramide accumulation-induced insulin resistance, has been shown to alter energy balance, weight gain and ectopic lipid accumulation in multiple models of obesity. Very recently, however, it has been suggested that highly insulin-sensitive, endurance-trained athletes have elevated intramuscular lipid contents (triglycerides, DAG and ceramides) similar to those of insulin-resistant obese and T2DM subjects (known as the athlete’s paradox). The mechanistic basis behind this observation is currently unknown, but likely relates to the intrinsically high mitochondrial function (and FA flux).

### 2.5 Systemic inflammation and cardiac mitochondrial function in T2DM

Systemic low-grade inflammation has been highlighted as a possible link between obesity, insulin resistance and metabolic disorders in T2DM. Secretion of pro-inflammatory cytokines from obese adipose tissue is thought to result in dysregulation of adipocyte metabolism with increased release of non-esterified FAs, which over time leads to ectopic fat deposition, including in the form of epicardial fat. The latter contributes to a local pro-inflammatory environment of adjacent cardiomyocytes with a substantial increase in macrophage infiltration. The development of inflammation in T2DM has been extensively reviewed, and here we focus on the key concepts of how a low-grade systemic inflammation in T2DM affects mitochondrial metabolism.

Circulating inflammatory markers, but also non-esterified FAs and high glucose, are known to activate TLRs on the myocardial cell membrane (particularly TLR2 and TLR4), increasing the transcriptional activity of NF-κB inside cardiomyocytes. In addition, C-Jun-N-terminal kinase (JNK) activity is higher in cardiomyocytes of patients with obesity or T2DM than in healthy individuals, and this is probably due to circulating pro-inflammatory cytokines such as tumour necrosis factor-α and interleukin-6. The subsequent NF-κB- and JNK-mediated inhibition of insulin receptor substrate 1 and PI3K-Akt results in the removal of glucose transporter 4 from the plasma membrane. This process exacerbates the inhibition of cardiac glucose uptake and contributes to enhanced FAO seen in T2DM.

Moreover, pro-inflammatory cytokines and caspases are produced intracellularly by the NF-κB and JNK-pathways.
Together with long-chain saturated FAs (e.g., palmitate), ceramides, modified low-density lipoprotein and glycaemia (which are all elevated in T2DM), this can activate the cardiac NLRP3 inflammasome, although it remains uncertain whether FAs alone can also promote activation of the NLRP3 inflammasome. By a currently unknown cellular process, likely involving additional factors such as transforming growth factor beta-1, the NLRP3 receptor binds to mitochondria, increasing ATP hydrolysis and ROS production. Meanwhile, mitochondria can also promote NLRP3 inflammasome activation through local ROS production, cytosolic mitophagy-induced mtDNA accumulation and binding to cardiolipin. What the causes and consequences are of this mitochondrial binding is currently unknown.

3 | MITOCHONDRIAL STRUCTURE AND FUNCTION IN THE DIABETIC HEART

Numerous studies have suggested that lipotoxicity affects mitochondrial respiration, however, the altered mitochondrial
metabolism in the diabetic heart cannot fully be explained by the accumulation of lipids and FA intermediates per se, suggesting the influence of additional factors. A variety of changes in cardiac mitochondrial morphology, structure and function have also been observed in insulin resistance and T2DM. However, there are conflicting reports of changes in mitochondrial number/content in the diabetic heart, and it remains unclear whether mitochondria have a smaller size or are more fragmented. Increased mitochondrial mass, area and number were observed in hearts from diabetic mice whereas no differences in mitochondrial content were found in ob/ob mice or high-fat diet-induced diabetic mice. Adding to the complexity, a lower mitochondrial content was seen in the hearts of fructose-fed rats with T2DM, but this was not associated with a loss in respiratory capacity per mitochondrial mass. Of note, however, even if T2DM is associated with a higher cardiac mitochondrial density, this would not necessarily result in a higher OXPHOS capacity. In fact, a lower mitochondrial OXPHOS capacity is commonly seen in diabetes, for example in human atrial tissue from patients with T2DM and metabolic syndrome. In experimental mouse models of insulin resistance and diabetes, reduced cardiac function is frequently associated with lower maximal oxidative capacities compared with lean controls, using pyruvate, glutamate and FA substrates. The picture is not clear, however, and elevated FAO in the diabetic heart, whereas, activity of pyruvate dehydrogenase is diminished. In mice. As such, the higher FAO measured in the isolated diabetic heart does not necessarily correspond to higher ex vivo mitochondrial respiration rates using FA substrates. In support of this, Wang et al did not find increased maximal respiration with FAs in permeabilized cardiac fibres following high-fat feeding of ob/ob mice. Activity of mitochondrial complexes I, II and IV have been reported to be low in patients with diabetes and in the hearts of insulin-resistant mice. Although protein levels of mitochondrial complexes were reportedly unchanged in the db/db mouse heart, lower content of the α subunit of ATP synthase was associated with increased ROS production and oxidative stress. Transcriptional activity of PPARα and PGC-1α are reported to be upregulated in the diabetic heart, whereas, activity of pyruvate dehydrogenase is diminished. Spectrophotometric assessment of mitochondrial complex activity or analysis of protein levels does not provide a complete picture of mitochondrial function though, and instead this should ideally be assessed in functionally intact respiring mitochondria. Moreover, in addition to enzymatic activities and transporter levels, OXPHOS is regulated by mitochondrial dynamics (fusion/fission), and supercomplex organization. Furthermore, a wide range of post-translational modifications of mitochondrial proteins contributes to the regulation of pathways responsible for mitochondrial ROS and redox conditions, as well as for substrate metabolism, where lysine acetylation has emerged as an important modulator of cardiac metabolism. In the diabetic myocardium enhanced acetylation of mitochondrial proteins has been reported to diminish complex I function and efficiency of ATP production, as well as NADH-linked respiration. Meanwhile, increasing evidence has highlighted how mitochondrial shape and cristae remodelling is influenced by obesity and insulin resistance, which in turn regulate mitochondrial metabolism. Here, we discuss the recent advances in these fields, with a particular focus on insulin resistance and T2DM.

### 3.1 Mitochondrial fission, fusion and biogenesis

Recent studies have highlighted a key role for altered mitochondrial quality control in diabetic cardiomyopathy. Mitochondria undergo structural changes in architecture through the process of fusion and fission dynamics. Interruption of fusion/fission has been associated with impaired mitophagy, contributing to the development of cardiomyopathy. Therefore, altered mitochondrial dynamics negatively affects mitochondrial respiration and increases ROS generation, however, this may in turn be a consequence of abrogated quality control within the mitochondrial network.

Increased mitochondrial fragmentation and the downregulation of mitochondrial fusion proteins were found in atrial tissue from patients with T2DM. Correspondingly, in a mouse model of cardiac lipotoxicity more fragmented mitochondria were seen, and this was attributed to enhanced mitochondrial fission (via DRP1) and reduced fusion. The observation that nutrient overload results in mitochondrial fission is of particular interest in the context of T2DM. Although fragmentation can occur under conditions of nutrient overload, it remains unclear whether this is due to diet-induced oxidative stress or to specific toxic effects of high glucose and/or FAs. Proteins controlling mitochondrial dynamics are clearly sensitive to ROS, and in line with this, altering the redox state through over-expression of superoxide dismutase and/or use of a superoxide dismutase mimetic reduced mitochondrial fragmentation.

In cardiomyocytes, insulin can acutely regulate mitochondrial metabolism through a mechanism that depends on increased mitochondrial fusion via Opa-1. Opa-1, located in the mitochondrial inner-membrane, is a main regulator of mitochondrial fusion and participates in cristae remodelling. Higher Opa-1 levels due to increased insulin signalling were
associated with higher mitochondrial membrane potential, ATP production and OXPHOS capacity,\textsuperscript{123} and may also contribute to the stabilization of mitochondrial supercomplexes\textsuperscript{128,129} (see Section 3.2). Thus, impaired insulin signalling may also directly contribute to mitochondrial structural remodelling in the heart.

Adult cardiomyocytes have a regionally interconnected mitochondrial subnetwork that is thought to limit the cellular consequences of mitochondrial dysfunction by disconnecting damaged mitochondria within seconds, essentially serving as a local power grid protection.\textsuperscript{130} It is conceivable that mitochondrial fragmentation (and the consequent lowering of mitochondrial membrane potential) may protect the remaining mitochondria from the damage of energy overload\textsuperscript{115} or ROS.\textsuperscript{131} However, the role of mitochondrial dynamics in the diabetic heart remains to be fully explored, particularly the importance of dynamics in the regulation of ATP production and mitophagy.

3.2 | Mitochondrial supercomplex function in T2DM

Assembly of mitochondrial protein complexes into supercomplexes is an important factor in optimizing OXPHOS function (Figure 3). However, the exact composition and functional role of the supercomplexes are still unclear.\textsuperscript{132-138} Preliminary evidence suggests that mitochondrial membrane lipid composition and peroxidation may influence supercomplex organization.\textsuperscript{139} In particular, cardiolipin is considered to be an important factor anchoring the supercomplex in the mitochondrial inner-membrane.\textsuperscript{140} Furthermore, cristae morphology may influence supercomplex formation and stability.\textsuperscript{128} Supercomplex formation can facilitate changes in OXPHOS capacity without necessarily altering the expression of individual protein complexes. Indeed, in the non-diabetic failing dog heart, Rosca et al\textsuperscript{141} reported a decrease in cardiac respiration rate, without any reduction in the enzymatic activity of individual mitochondrial complexes, whereas the formation of supercomplexes was lower and the number of isolated individual mitochondrial complexes increased.\textsuperscript{141}

Limited data are available on supercomplex function and composition in the diabetic heart. In skeletal muscle fibres of overweight women with T2DM, a reduction in OXPHOS capacity was associated with a significant decrease in complex I-III-IV containing supercomplexes compared with controls.\textsuperscript{118} More recently, the same group reported lower OXPHOS capacity, lower supercomplex assembly and more oxidative damage to proteins in the atrial tissue of patients with T2DM and atrial fibrillation.\textsuperscript{119} Interestingly, a high-fat diet did not alter mitochondrial supercomplex formation in cardiac muscle of C57BL/6 mice, although remodelling of cardiolipin acyl chains was observed.\textsuperscript{142} In addition, dramatic loss of cardiolipin content and remodelling of acyl chains were observed in very early stages of streptozotocin-induced diabetes and in ob/ob mouse hearts.\textsuperscript{143} It has been proposed that lyso-cardiolipin acyltransferase 1 is upregulated by oxidative stress and determines cardiolipin-remodelling by catalysing the synthesis of cardiolipin species that are highly sensitive to oxidative damage.\textsuperscript{144} It should be noted, however, that although no significant changes in mitochondrial function have been reported after short-time (5 days) exposure to streptozotocin,\textsuperscript{143} the remodelling of cardiolipin could promote alterations in mitochondrial function by destabilizing supercomplexes during onset of diabetes. Interestingly, some impairment of cardiolipin synthesis may be tissue-specific and even have a protective effect. In Tafazzin knockout mice, there was a decrease in the cardiolipin level in heart and skeletal muscle, but not in liver, where higher synthesis rates preserved the cardiolipin level.\textsuperscript{145} As a result, hypermetabolism in liver protected these animals from high-fat diet-induced weight gain and glucose intolerance.

Another mitochondrial supercomplex, known as the mitochondrial interactosome (Figure 3B), is comprised of the mitochondrial ATP synthase, ANT, inorganic phosphate carrier, the mitochondrial creatine kinase (mtCK) and voltage-dependent anion channel (VDAC).\textsuperscript{146,147} In the oxidative muscle cells the diffusion of adenine nucleotides through the VDAC is impeded, but the movement of PCr and Cr through the channel is not restricted. In the intermembrane space the phosphoryl group is transferred from ATP to Cr by mtCK, whereas formed ADP is moved back to matrix via ANT. The mitochondrial interactosome supercomplex enhances the transfer of energy via the CK/PCr pathway from the site of production to the sites of utilization, and increases effectiveness of ATP synthesis in mitochondria.\textsuperscript{148} The function of mitochondrial interactosome is altered in ageing,\textsuperscript{149} but it is currently unknown whether changes in the interactosome contribute to the mitochondrial alterations observed in obesity and T2DM. The observation by Scheuermann-Freestone et al that patients with diabetes have a significantly impaired PCr/ATP ratio may suggest a role for the mitochondrial interactosome in the pathophysiology of T2DM.\textsuperscript{28}

The discovery of mitochondrial supercomplexes and the interactosome add complexity to our understanding of mitochondrial physiology. While the current hypothesis suggests that the dynamic assembly of supercomplexes contributes to increased efficiency of electron transport and lower ROS production, it remains unknown in the context of obese and/or diabetic heart. As supercomplexes were associated with skeletal muscle adaptation to exercise, and was shown to improve skeletal muscle strength in sedentary humans,\textsuperscript{150} there is also reason to believe that altered assembly of these complexes can contribute to the progression of heart disease.
3.3 Production of reactive oxygen species in obesity and T2DM

The cellular redox environment ensures the balance between ROS production and the efficiency of ROS scavenging systems. When the balance is shifted towards more ROS production, and failure of the antioxidant systems to lower oxidative stress, cellular damage will occur. Approximately 90% of cellular ROS is produced in the mitochondria mainly from complexes I and III. Increased H₂O₂ resulting from superoxide (O₂⁻) production at complex I has been observed in cardiac mitochondria from obese mice. During ADP-driven respiration (coupled OXPHOS), H₂O₂ production was found to be higher in atrial tissue from patients with T2DM, and in obese mice with T2DM compared with non-diabetic controls. In contrast, lower ROS production was reported in high-fat-high-sugar diet fed rats. While there are discrepancies in findings relating to ROS production, there are consistent observations of myocardial oxidative stress in obesity/insulin resistance.

Similarly, the up or downregulation of enzymatic antioxidant systems such as glutathione peroxidase, thioredoxin, catalase and superoxide dismutase and the non-enzymatic antioxidant glutathione, have all been associated with altered mitochondrial energetics. Impaired thioredoxin-2 signalling occurred in combination with lower mitochondrial capacity, increased ROS production and cardiac dysfunction in db/db mice and in humans with T2DM. In contrast, the thioredoxin and catalase systems were upregulated in the hearts of other experimental obese/insulin resistance models. It is possible that antioxidant upregulation is a compensatory mechanism to offset increased ROS production, however, as the contribution of thioredoxin is greater when FAs are used as substrates, this additionally
suggests a substrate-dependent effect. Glutathione is thought to be the major thiol antioxidant within cells, and lower levels of reduced glutathione or a lower reduced/oxidized glutathione ratio (GSH/GSSG) have been associated with mitochondrial dysfunction in humans, and in db/db mice and mice on a high-fat-high-sucrose diet. While the supplementation of antioxidants in the context of heart disease has generally shown little benefit, recent evidence suggests that targeting mitochondrial ROS can improve energetics and maladaptation in the obese/insulin-resistant heart (see Section 4.4.2). Increased expression of mitochondrial catalase in response to high-fat diet was shown to prevent oxidative stress and rescue diet-induced mitochondrial dysfunction. Furthermore, mitochondrial ROS scavenging was shown to improve cardiac insulin signalling and mitochondrial energetics. Perhaps focused studies to elucidate changes in redox buffering systems (ie thiorodoxin and glutathione systems) in the heart associated with substrate availability and utilization could reveal origins of oxidative stress. Furthermore, post-translational mechanisms resulting from ROS over-production may also contribute to diminished redox buffering capacity in the obese and T2DM heart.

### 3.4 Impaired mitochondrial calcium handling

MitochondrialOXPHOS is regulated by the Ca\(^{2+}\) concentration in the matrix. Accordingly, mitochondrial ATP production rate matches cardiac ATP utilization rate, independent of ADP feedback by a process called excitation-energetics coupling. Mitochondrial Ca\(^{2+}\) uptake and extrusion occur with each excitation-contraction cycle, owing to the vicinity of mitochondria to the sarcoplasmic reticulum (SR) and their interaction through well-coordinated processes. Ca\(^{2+}\) uptake is mediated via the mitochondrial Ca\(^{2+}\) uniporter (MCU) system and occurs primarily within specialized microdomains between the SR and the mitochondria, where local changes in the Ca\(^{2+}\) concentration trigger opening of the MCU. Mitochondrial Ca\(^{2+}\) efflux in the heart is slower than uptake, and is regulated primarily by the Na\(^+\)/Ca\(^{2+}\) exchanger (NCLX).

Ca\(^{2+}\) accumulation in the mitochondrial matrix, which occurs during increased heart rate, is a key trigger to increase the activity of three important regulatory enzymes of the TCA cycle, including pyruvate, α-ketoglutarate and iso-citrate dehydrogenase, all of which contribute to regenerate the redox state of the pyridine nucleotides (NADH/NAD\(^+\) and FADH\(_2\)/FAD) and enhance antioxidant capacity. Activation of the pyruvate dehydrogenase complex (PDC) also stimulates glucose oxidation, which due to the higher P/O ratio of glucose, contributes to cardiac efficiency at higher workloads. In contrast, blunted mitochondrial Ca\(^{2+}\) uptake results in the oxidation of NADH/NAD\(^+\) and FADH\(_2)/FAD\) and hinders the supply of electrons for the ETS. As such, Ca\(^{2+}\) can directly modulate the activity of the entire OXPHOS cascade.

Elevated intracellular Na\(^{+}\) in the failing heart increases NCLX-mediated Ca\(^{2+}\) efflux and may negatively affect the matching of energy demand and supply. Likewise, myocardial intracellular Na\(^{+}\) levels are aggravated in diabetes, due to the upregulation of the sodium-glucose cotransporter 1, thus driving mitochondrial Ca\(^{2+}\) efflux through the NCLX and reducing mitochondrial calcium levels. This can impede key steps in the TCA cycle and in turn limit the supply of electrons to the respiratory complexes and lead to a shortfall in ATP synthesis. In the db/db mouse heart a key component of the MCU (MICU1) was reported to be down-regulated, whereas targeting mitochondrial Ca\(^{2+}\) uptake by overexpression of MICU1 rescued cardiac function, lowered mitochondrial ROS, improved the NADPH antioxidant system and resulted in less apoptosis mediated by oxidative stress in these diabetic hearts. Mice with streptozotocin-induced diabetes also exhibited reduced mitochondrial Ca\(^{2+}\), and restoration of the Ca\(^{2+}\) concentration by MCU overexpression in this model resulted in increased PDC activity, a shift in metabolism towards glucose oxidation, and improved mitochondrial membrane potential and respiratory efficiency. Recently, mitochondrial Ca\(^{2+}\) handling in intact cardiomyocytes from ZSF1-obese rats, a model for diabetic cardiomyopathy, was assessed. At similar extracellular Ca\(^{2+}\) and Na\(^{+}\) concentrations, both cytosolic and mitochondrial Ca\(^{2+}\) concentrations were higher in isolated cardiomyocytes from diabetic animals, because of alterations in cytosolic Ca\(^{2+}\) handling and mild mitochondrial dysfunction. Furthermore, isolated mitochondria from these hearts were more prone to mitochondrial swelling, suggestive that the elevation in Ca\(^{2+}\) concentration made these mitochondria more prone to membrane permeability pore opening and apoptosis.

Although the cause for the discrepancies in mitochondrial Ca\(^{2+}\) levels is not clear, we can speculate that differences in the severity of the disease model, multifactorial progression of the disease, or technical discrepancies may be factors involved. Importantly, increased mitochondrial Ca\(^{2+}\) levels observed in cardiomyocytes from obese mice may reflect an early adaptation to the diabetic condition, whereas low levels of mitochondrial Ca\(^{2+}\) might occur as cardiomyopathy and cytosolic calcium alterations develop. Despite the importance of Ca\(^{2+}\) in modulating cardiac energy homeostasis and apoptosis, it is currently unclear if the SR/mitochondria interaction is altered in the diabetic heart, and whether alterations in mitochondrial calcium homeostasis directly influence cardiac metabolism or reflect an adaptation to altered energy metabolism in the T2D heart.
4 | LIFESTYLE AND PHARMACOLOGICAL INTERVENTIONS TO TARGET MITOCHONDRIAL METABOLISM IN THE DIABETIC HEART

Type 2 diabetes mellitus is primarily a lifestyle-related disease that is progressive over time (Figure 4). Metabolic alterations in diabetic hearts are associated with lipotoxicity and changes in mitochondrial function. Two strategies might therefore be used to improve metabolism function in the diabetic heart, namely reduction in lipotoxicity and improvement of mitochondrial function. Alternatively altering the whole-body response to diabetes might indirectly improve cardiac metabolic function (Figure 4). The adoption of a healthy lifestyle or the adoption of pharmacological interventions targeting lipotoxicity and/or mitochondrial function (Figure 5) are generally associated with improvements in cardiac function in T2DM. Some of these interventions are often also effective in reducing the age-related decline in cardiometabolic function in obesity and T2DM.105 Here, we focus on approaches which have a mechanistic basis involving modification of cardiac mitochondrial function or content.

4.1 | Dietary interventions

Several dietary interventions have led to improved health in patients or animal models with T2DM. Caloric restriction (CR) is one such intervention that has shown promising results, improving insulin sensitivity and reducing the risk of T2DM, in addition to enhancing lifespan in a wide range of animal models.174-179 Several studies have demonstrated that CR decreases the production of ROS and thereby limits oxidative damage in various tissues, including the heart.109,190,181 This CR-induced reduction in ROS generation occurs without altering mitochondrial oxygen consumption in the heart.181 Since the lower ROS production following CR was detected in permeabilized fibres in the presence of pyruvate and malate, but not with succinate and rotenone, the source of these ROS was suggested to be mitochondrial complex I.181 In the Otsuka Long-Evans Tokushima Fatty rat model for T2DM, CR lowered haemoglobin A1c, blood glucose, cholesterol, triglycerides and circulating FAs, and lowered UCP2 expression and mitochondrial ROS production in the heart and aorta.182 CR for 6 weeks improved the metabolic phenotype of rats on a high-fat diet, lowering obesity, insulin resistance and left ventricular dysfunction, as well as cardiac mitochondrial ROS production, membrane depolarization and swelling.183 Of note, these improvements were even more pronounced when exercise was combined with CR.183

Signalling targets known to be activated by CR (and inhibited by a high-fat diet) include sirtuins (Sirt) 1 and 3.174 Sirt1 is located in the nucleus, whereas Sirt3 is located in the mitochondria. Both are involved in mitochondrial function and biogenesis and the regulation of oxidative stress.184-186 The deletion of Sirt1 expression in the heart results in a phenotype similar to diabetic cardiomyopathy and includes mitochondrial dysfunction in association with acetylation of PGC-1α.187,188 Furthermore, drugs causing dual PPARα/γ activation have been shown to induce cardiac dysfunction due to Sirt1-PGC1α inhibition and decreased mitochondrial number.189 In the hearts of fructose-fed rats, Sirt1 activity decreased early in the progression to T2DM and was also associated with decreased in mitochondrial content and lower FAO capacity in the mitochondria.106 Recent evidence suggests that the cardioprotective effect of CR in the diabetic heart operates via Sirt1 and PGC-1α, increasing OXPHOS capacity and reducing cardiomyocyte oxidative stress and inflammation.190 Caloric restriction was also associated with an increase in Sirt3 activity in cardiac mitochondria191 without changes in expression level.192 The change in Sirt3 activity might be mediated via changes in the NAD+/NADH ratio, which decreases as a result of over-nutrition associated with obesity and T2DM (causing Sirt3 inactivation), and is increased by CR (causing Sirt3 activation).193 Sirt3 deacetylates and activates many mitochondrial enzymes including respiratory complexes and pyruvate dehydrogenase.193 A study using a combined in vivo and in vitro approach showed that the mitochondrial dysfunction and increased ROS production associated with T2DM could be prevented by ALDH2 activation, acting on PGC-1α function through Sirt3-mediated deacetylation.194

One further aspect of the metabolic syndrome and T2DM that might be targeted through dietary means is the link between nitric oxide (NO) bioavailability, tissue metabolism and cardiovascular health. In humans, polymorphisms in the gene encoding endothelial NO synthase (eNOS) give rise to insulin resistance and T2DM,195,196 whereas patients with T2DM have lower myocardial eNOS protein expression than healthy controls,197 and a lower systemic capacity for NO synthesis.198,199 While the attenuated bioavailability of NO is commonly recognized to be a causative factor driving endothelial dysfunction, NO also mediates signalling effects at the cellular level through the activation of soluble guanylyl cyclase (sGC), increasing cyclic guanosine monophosphate (cGMP) levels and activating protein kinase G (PKG).200 The NO/cGMP/PKG pathway has been proposed as a possible therapeutic target in heart failure with preserved ejection fraction (HFpEF),200,201 and may hold promise in the specific case of diabetic cardiomyopathy. Of note, cGMP levels are lower in the hearts of both Zucker Diabetic Fatty rats and db/db mice in comparison with lean controls. In mice, eNOS deficiency results in a metabolic syndrome-like...
phenotype, including hypertension, weight gain, dyslipidaemia and insulin resistance, and in these mice the mitochondrial biogenesis response to CR is attenuated. Treatment with the phosphodiesterase 5 inhibitor tadalafil enhanced Sirt1-PGC1α signalling, thereby attenuating mitochondrial dysfunction in hearts of type 2 diabetic db/db mice. More recently, restoration of the sGC-cGMP-PKG pathway was seen in the hearts of db/db mice following treatment with empagliflozin, in association with improvements in systolic and diastolic function, whereas inhibition of sGC using siRNA prevented these protective effects. For more on empagliflozin see Section 4.4.2.

In addition to the endogenous route of NO synthesis, NO bioavailability can be increased by dietary supplementation with stable nitrogen oxides, for example nitrate (NO$_3^-$) or nitrite (NO$_2^-$) and their sequential reduction in vivo to NO. Dietary inorganic nitrate is principally acquired through the consumption of leafy, green vegetables and improves mitochondrial function and human health. Nitrate is reduced to nitrite via oral nitrate reductase in commensal bacteria. Nitrite is then converted to NO in the stomach by acid disproportionation and is absorbed into the bloodstream. In eNOS deficient mice, dietary supplementation with inorganic nitrate elevated plasma and tissue levels of nitrogen oxides, and reversed features of the metabolic syndrome, lowering body weight, plasma triglycerides, visceral adiposity, fasting blood glucose, arterial blood pressure and haemoglobin A1c, whereas improving whole-body insulin sensitivity.

The link between nitrate supplementation, NO bioavailability and tissue insulin sensitivity may involve changes in the expression of genes involved in FAO and the control of mitochondrial content. Dietary nitrate increases plasma levels of cGMP in humans, and enhanced FAO capacity in rat skeletal muscle in a mechanism that depended upon PPARα activation by cGMP. Additionally,
Mitochondrial biogenesis occurred with higher doses of nitrate supplementation via the activation of PGC-1α. Similarly, nitrate supplementation increased FAO capacity in rodent hearts in a PPARα-dependent manner. Owing to its effects on mitochondrial FAO capacity, dietary nitrate supplementation might be beneficial in metabolic syndrome and T2DM both systemically and to the heart in particular. This may be the case even when the primary cause of the metabolic condition is not deficient expression/activity of eNOS, and this deserves further investigation in models of T2DM beyond the eNOS knockout mouse.

While the mechanisms behind the beneficial effects of dietary modifications on T2DM are not fully understood, many of the reported explanations support the involvement of cardiac mitochondria. Protecting the heart against mitochondrial dysfunction and oxidative stress could potentially exert strong effects on the development of cardiovascular defects associated with T2DM and ageing.

4.2 Interplay between circadian rhythm and myocardial function

While food quantity and quality can impact on metabolic pathways and health in T2DM, the timing of food intake in relation to the circadian rhythm might also deserve consideration. In the heart approximately 6% of protein-encoding genes show a rhythmic expression throughout the day, whereas various measures of cardiac metabolism also show diurnal variations. For example ex vivo experiments on rat heart demonstrated that oxidation of exogenous glucose showed significant diurnal
variation, whereas no such variations were seen in the oxidation of exogenous oleate.\textsuperscript{219,220} Mice that have a misalignment between the internal clock and the external environment (ie housed under a day/night cycle of 20 hours or 30 hours instead of the normal 24 hour cycle) showed altered daily patterns of energy expenditure and carbohydrate and fat oxidation, leading to longer QT intervals.\textsuperscript{221} The metabolic alterations were associated with a lower cardiac mitochondrial content.\textsuperscript{222} Furthermore, expression of \textit{Pgc1a}, \textit{Mfn1} and \textit{Opal} was downregulated in mice with disturbed circadian rhythms, suggesting that the molecular clock plays a role in the regulation of cardiac mitochondrial dynamics. Of note, cardiac mitochondrial respiratory function appeared to be more affected in the subsarcolemmal population of mitochondria, in comparison with the inter-myofibrillar population.\textsuperscript{223}

Time-restricted feeding has been shown to prevent and even cure diabetes in rodent models of T2DM (reviewed by \textsuperscript{224}) even without invoking CR.\textsuperscript{225} In nocturnal rodents, time-restricted feeding during the dark (ie active) period was associated with improved metabolic health, whereas time-restricted feeding during the light (ie inactive) period was associated with adverse metabolic outcomes.\textsuperscript{226} Rats on a time-restricted feeding regimen have lower GSSG, protein carbonyl and malondialdehyde levels, all of which are indicative of substantial protection against cardiac oxidative damage in comparison with mice fed ad libitum.\textsuperscript{227} Data on the effects of time-restricted feeding on cardiac mitochondrial metabolism are somewhat scarce, but research on other tissues indicates that time-restricted feeding could impact on mitochondrial respiratory function. For example livers of mice fed only during the active phase showed enhanced expression rhythms of mitochondrial encoding genes compared with ad libitum fed mice.\textsuperscript{228} The time-restricted feeding paradigm has been further studied in other model organisms. For example fruit flies that were put on time-restricted feeding during their active phase showed improved cardiac physiology, as indicated by ex vivo heart period and arrhythmia index, together with altered cardiac gene expression, with circadian clock and electron transfer system (ETS) genes ranking as top clusters.\textsuperscript{229} The beneficial effects of time-restricted feeding on the heart are understood to be at least partly mediated by ATP-dependent chaperonin and mitochondrial ETS components.\textsuperscript{230} Nineteen different genes which encode the mitochondrial ETS were downregulated by 10%-20%, whereas seven out of eight components of an ATP-dependent chaperonin were upregulated in the hearts of rats on a time-restricted diet.\textsuperscript{230}

Interestingly, the relationship between circadian rhythm and insulin signalling appears to be reciprocal, with several clock genes in cardiac tissue showing a phase shift in rats with a streptozotocin-induced diabetes.\textsuperscript{231} In the hearts of these diabetic rats, the daily rhythms of seven molecular clock genes peaked about 3 hours earlier during a 24 hour cycle (phase advance) compared with non-diabetic rats.

### 4.3 Exercise training and cardiometabolic health

Physical activity is associated with protection against cardiovascular disease,\textsuperscript{232} with development of the athletic heart characterized by normal or slightly enhanced diastolic function\textsuperscript{233}; however, the effects of exercise training on cardiac metabolism in humans remain somewhat unclear. In rodents, endurance training promotes angiogenesis\textsuperscript{234} and decreases ROS generation\textsuperscript{235,236} with no increase in fibrosis.\textsuperscript{237} Several studies have investigated the cardiometabolic effects of treadmill-running or swim-training in rodents.\textsuperscript{238} It is commonly reported that training increases myocardial FAO capacity in rodents.\textsuperscript{239,244} Moreover, swim-training in mice increased cardiac citrate synthase (CS) activity (a putative marker of mitochondrial density\textsuperscript{245}) alongside increased PGC-1α expression.\textsuperscript{244} Of note, moderate intensity training (MIT) elicited different effects to high-intensity interval training (HIIT) in mice when treadmill-running at matched distances.\textsuperscript{246,247} While both regimens increased skeletal muscle CS activity, only HIIT resulted in increased cardiac CS, alongside increased mitochondrial respiratory capacity and improved cardiac efficiency (work/O\textsubscript{2} consumed).\textsuperscript{246}

The metabolic effects of endurance training are likely to benefit the obese/diabetic heart, through improvements in substrate metabolism, lipid handling, mitochondrial function and antioxidant capacity.\textsuperscript{248} Indeed in mice with diet-induced obesity, both MIT and HIIT prevented concentric left ventricle remodelling and improved systolic and diastolic function, while lowering fibrosis and oxidative stress.\textsuperscript{19} Moreover, both exercise modalities were associated with changes in myocardial substrate utilization with increased work-adjusted rates of glucose oxidation and decreased work-adjusted rates of palmitate oxidation in isolated, perfused hearts from diet-induced obese mice, suggesting a partial restoration in substrate balance towards that of non-obese mice.\textsuperscript{15} The authors further reported a normalization of mitochondrial respiratory function in the diet-induced obese heart following HIIT, with increased OXPHOS capacity and improved efficiency (higher P/O ratios) in isolated cardiac mitochondria supplied with glutamate and malate as substrates for the N-Pathway via Complex I.\textsuperscript{19} Similarly, both in rats fed an obesogenic high-fat, high-sucrose diet for 12 weeks and controls on a non-obesogenic diet, HIIT enhanced mitochondrial oxygen consumption rates supported by palmitoylcarnitine and malate, suggesting enhanced FAO capacity following training.\textsuperscript{\textsuperscript{110}} High-intensity interval training was also found to elicit cardioprotective effects in high-fat fed mice, with decreased infarct sizes following myocardial ischaemia alongside lower work-independent oxygen consumption and lower ROS production in comparison with sedentary controls, suggesting at least a partial normalization of mitochondrial function in these hearts.\textsuperscript{249}
Studies in the overtly diabetic db/db mouse have also suggested beneficial effects of training on cardiac mitochondrial function. For instance 15 weeks of endurance training lowered fibrosis and enhanced mitochondrial content in db/db mice (7 weeks of age at the start of the training protocol), with increased expression of a number of mtDNA-encoded genes. Mechanistically, this was attributed to exercise-induced activation of Akt and PGC-1α signalling, which was otherwise inhibited in the diabetic heart. Similarly, in a separate study 5 weeks of MIT on a treadmill lowered fibrosis and improved ATP levels in the hearts of db/db mice at 4 months of age, alongside improved oxygen consumption rates and improved contractile function in isolated cardiomyocytes. In addition to effects on mitochondrial content and respiratory capacity, exercise may also influence mitochondrial dynamics, with decreased expression of DRP1 relative to that of the mitochondrial fusion factor Mfn1 seen following exercise in db/db mice. The protective effects of exercise may be more limited in the older diabetic heart, however. In 8-month-old db/db mice, 3 weeks of endurance training resulted in increased myocardial expression of PGC-1α alongside increased mtDNA density, but decreased ETS complex expression and increased mitochondrial fission, which the authors attributed to increased oxidative stress following exercise in older diabetic hearts.

Beyond direct effects on the myocardium, endurance training is likely to have further beneficial effects for the metabolically diseased heart. Skeletal muscle is the largest insulin-sensitive tissue in the body, and improvements in muscle mitochondrial function and FAO capacity via training may prevent accumulation of lipotoxic intermediates associated with insulin resistance improving whole-body glycaemic control. Moreover, weight loss is itself associated with improved myocardial energetics and diastolic function and may result from a sustained increase in physical activity.

### 4.4 | Pharmacological targeting of mitochondria in insulin resistance

#### 4.4.1 | Compounds to reduce lipotoxicity

The accumulation of lipid intermediates advances myocar-dial insulin resistance, therefore targeting this lipid overload is a possible strategy to improve insulin sensitivity and mitochondrial function in the diabetic heart. Several treatment approaches have been trialled in animal models and clinical settings, however, for various reasons, use of mitochondria-protective metabolic modulators against lipid overload is currently limited in clinical practice. The main targets for preventing accumulation of FAO intermediates are related to long-chain acylcarnitines (Figure 5). These include the pharmacological reduction of CPT1 activity using direct or indirect inhibitors of CPT1 and the use of carnitine-lowering compounds. Direct inhibitors of CPT1 (etomoxir, perhexiline, oxifenicine, teglicar) have been shown to decrease long-chain acylcarnitine concentration, inhibit FAO (at least partially) and increase glucose oxidation in heart. It has been shown that etomoxir and perhexiline improve cardiac function in patients with heart failure or cardiomyopathy, however, CPT1 inhibitors are not currently prescribed due to hepatotoxic and cardiotoxic effects which may relate to unspecific side effects in mitochondria. An alternative approach is to increase the concentration of malonyl-CoA, an endogenous CPT1 inhibitor, using malonyl-CoA decarboxylase (MCD) inhibitors (eg CBM-301106, CBM-301940). MCD inhibition decreases long-chain acylcarnitines and FAO, thereby promoting glucose oxidation and improving insulin sensitivity. Studies in animal models have suggested that MCD is a possible drug target for the treatment of diabetic cardiomyopathy, however, further studies are necessary to prove the therapeutic efficacy of pharmacological inhibition of MCD.

Treatment with inhibitors of carnitine biosynthesis and its transport into tissues (eg with the clinically prescribed drug meldonium (3-(2,2,2-trimethylhydraziniumyl) propionate) or experimental candidate methyl-GBB (4-ethyl(dimethyl) ammonio]butanoate), is protective against cardiometabolic disease. These two compounds decrease long-chain acylcarnitine content in cardiac tissues and mitochondria and therefore prevent acylcarnitine-induced mitochondrial damage as well as impaired insulin signalling. Both compounds protect cardiac mitochondria against ischaemia-reperfusion injury, reduce infarct size in healthy and diabetic animals and improve insulin sensitivity in experimental models of insulin resistance.

Trimetazidine and ranolazine are clinically used as antianginal agents. Growing evidence suggests that trimetazidine treatment can reduce the severity of adverse cardiovascular events in both experimental models of insulin resistance and in patients with T2DM. It was first proposed that trimetazidine inhibits FA metabolism in cardiac mitochondria by inhibiting long-chain 3-ketoacyl CoA thiolase (the last enzyme in the β-oxidation pathway). More recently, however, it has been demonstrated that trimetazidine does not alter metabolic substrate oxidation in the cardiac mitochondria of T2DM patients. The antianginal effects of ranolazine are achieved by blocking the late sodium channels, thereby preventing a downstream rise in cytosolic Ca2+ concentrations. In addition, ranolazine may be useful in the management of stable ischaemic heart disease with diabetes, as indicated by a fall in circulating haemoglobin A1c.
4.4.2 Improving mitochondrial function

While the targeting of mitochondria is not a novel approach for the treatment of metabolic disorders or cardiovascular disease, studies of the efficacy of mitochondria-targeted compounds in the diabetic heart are somewhat limited (Figure 5). One such strategy might include treatment with mitochondrial-targeted antioxidants, such as MitoQ and MitoTEMPO. These compounds comprise a lipophilic cation (tetraphenylphosphonium) conjugated to an antioxidant component.

MitoQ treatment has been shown to decrease H$_2$O$_2$ formation and improve mitochondrial respiratory capacity in the heart following ischaemia-reperfusion and in pressure overload-induced heart failure. In experimental models of diabetes, MitoQ treatment reduced diabetic nephropathy by ameliorating mitophagy and the production of excess ROS and modulated oxidative stress in leucocytes isolated from T2DM patients. The efficacy of MitoQ has not, however, been assessed in the diabetic heart.

MitoTEMPO prevents mitochondrial permeability transition pore opening, necrosis and mitochondrial apoptosis after ATP depletion. Treatment of hyperglycaemic and/or hyperinsulinaemic animals with MitoTEMPO improved cardiac function. Moreover, enhanced mitochondrial antioxidant capacity following MitoTEMPO treatment improved insulin sensitivity and preserved cardiovascular function in animals with metabolic syndrome or diabetes, as well as in aged animals. Analysis of ROS-sensitive networks, particularly those associated with MitoTEMPO treatment, highlighted that increased mitochondrial ROS in the metabolically diseased heart disrupts the normal coupling between cytosolic signals and nuclear gene programs underpinning mitochondrial respiratory function, antioxidant capacity, Ca$^{2+}$ handling and action potential repolarization.

Resveratrol, a natural polyphenol, is a multitarget compound and has been widely studied in the context of diabetes. In addition to its antioxidant properties, treatment with resveratrol improves mitochondrial OXPHOS and biogenesis by activating Sirt1 and Sirt3 in experimental models of insulin resistance. Although treatment with resveratrol has shown promising results in preclinical studies, no effect of resveratrol was observed on different markers of cardiovascular disease risk in ageing and obese individuals. Recent studies have demonstrated that resveratrol supplementation improves skeletal muscle mitochondrial function, however, it does not improve insulin sensitivity in people at risk of or with T2DM. Further studies aiming to understand resveratrol metabolism and pharmacokinetics, may therefore be required to realize the therapeutic potential of this approach.

Elamipretide (SS-31) is a mitochondrial-targeted tetrapeptide that interacts with cardiolipin. It has been proposed that elamipretide stabilizes cardiolipin, thereby reducing ROS production and preserving ETS function under stressed conditions, and might improve supercomplex stability. Elamipretide improved calcium retention capacity in cardiac mitochondria, and reduced infarct size in the hearts of STZ-treated rats. Moreover elamipretide improved mitochondrial organization and attenuated oxidative stress in a swine model of metabolic syndrome.

One group of drugs that has received significant attention in recent years are inhibitors of the renal sodium-glucose cotransporter (SGLT2). High-capacity, low-affinity SGLT2 are located in the renal proximal tubular epithelium and reabsorb filtered glucose. SGLT2 inhibitors lower blood sugar, and have been associated with loss of body weight in patients with T2D, attributable to the induction of a negative caloric balance. In addition, it is suggested that SGLT2 inhibition promotes production of ketone bodies, which can act as an efficient substrate for myocardial energy production. One such compound, empagliflozin, has been shown to improve cardiac outcome and reduce mortality in diabetic patients. The mechanism by which empagliflozin improves cardiac outcome is a matter of current debate, although improved cytosolic and mitochondrial Ca$^{2+}$ and sodium homeostasis has been suggested to play a role.

Cardioprotection in rats with metabolic syndrome following treatment with dapagliflozin, another SGLT2 inhibitor was associated with changes in fusion/fission proteins towards control values, as well as lower cellular ROS production.

5 CONCLUDING REMARKS

The alterations in metabolism in insulin-resistant and T2DM hearts can be studied at various levels, from long-term treatment of cultured cells with high concentrations of glucose, through to studies of the whole heart or at the whole body level in diabetic animal or human subjects. Many of the findings discussed in this review were derived from human or animal studies, and arguably the pathophysiology of diabetic cardiomyopathy is best understood by studying the interactions between various contributing factors in humans.

Human studies are intrinsically limited, however, in part due to the practical difficulties and ethical implications associated with obtaining cardiac tissue from living patients, but also owing to uncertainties about the time course of the disease and the difficulty of inferring cause vs effect in observational studies. Moreover, most patients with T2DM are over 50 years old, and suffer from comorbidities, such as obesity, hypertension, atherosclerosis or inactivity-related metabolic alterations. As such, studying T2DM in animal models has proven to be valuable for our understanding of the pathophysiology of diabetic cardiomyopathy. The choice of animal model varies depending on the specific research question, cost and ease-of-use (see 324 for an overview of animal models). The time course of disease and nature of the T2DM model
(genetic, diet-induced or diet plus streptozotocin-induced) can influence the severity of metabolic and mitochondrial alterations, as well as the progression of cardiac dysfunction, and might explain discrepancies between findings across different studies. Comorbidities are often overlooked in studies using rodents, complicating the translation of results to the human setting. Future work is therefore needed to understand the interplay between factors such as age, inactivity and cardiac mitochondrial dysfunction in T2DM. New animal models with a slower disease progression, such as the Nile rat, could play an important role in understanding the role of cardiac mitochondrial dysfunction in T2DM.

Regardless of whether the altered mitochondrial metabolism observed in the diabetic heart is a cause or a consequence of myocardial insulin resistance, the mitochondria are central to the maintenance of cardiac metabolism and contractile function. Thus, targeting mitochondrial function to delay or reverse diabetic cardiomyopathy is an attractive, but underexplored avenue for future treatment options. Reducing mitochondrial lipotoxicity and oxidative stress seem effective in experimental animal models, but such strategies have not yet been translated to the clinic. On the other hand, the promising effects of treatment with the renal SGLT2 inhibitor empagliflozin may include a cardiac mitochondrial component. Likewise, nutritional interventions and physical exercise both have proven benefits for the contractile function of the heart, at least in part due to improved cardiac mitochondrial function. However, to what extent these effects occur through improvements in whole-body insulin sensitivity vs heart metabolic function in particular remain to be determined.

In conclusion, we have highlighted how T2DM alters glucose and FAO and can lead to FA-induced lipotoxicity and mitochondrial dysfunction. The alterations observed in mitochondrial substrate utilization, bioenergetic function and fission/fusion indicate that mitochondrial function is significantly altered in T2DM. Future work needs to focus on the regulation of FA metabolism, the dynamic assembly/organization of the ETS complexes and the regulation of fusion/fission in obesity and diabetes, as well as the role of circulating inflammatory markers in response to physiological changes. Finally, the improvement of metabolic and mitochondrial function through lifestyle (nutrition and exercise) and pharmacological interventions could be an important strategy to improve cardiovascular performance in diabetes.

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We confirm that all authors have no competing interests.

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