Molecular mechanisms for myocardial mitochondrial dysfunction in the metabolic syndrome

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ABSTRACT

The metabolic syndrome represents a cluster of abnormalities, including obesity, insulin resistance, dyslipidaemia and Type 2 diabetes, that increases the risk of developing cardiovascular diseases, such as coronary artery disease and heart failure. The heart failure risk is increased even after adjusting for coronary artery disease and hypertension, and evidence is emerging that changes in cardiac energy metabolism might contribute to the development of contractile dysfunction. Recent findings suggest that myocardial mitochondrial dysfunction may play an important role in the pathogenesis of cardiac contractile dysfunction in obesity, insulin resistance and Type 2 diabetes. This review will discuss potential molecular mechanisms for these mitochondrial abnormalities.

INTRODUCTION

The metabolic syndrome represents a highly prevalent cluster of metabolic abnormalities that markedly increases the risk of developing atherosclerotic cardiovascular disease and Type 2 diabetes. Since the first descriptions in the 1920s [1], the terminology ( metabolic syndrome, syndrome X, insulin-resistance syndrome and deadly quartet) and clinical criteria have undergone various revisions (for review see [2]). However, the following risk factors are common to all current definitions of the metabolic syndrome: central obesity, dyslipidaemia [increased triacylglycerol (triglyceride) and low HDL-cholesterol (high-density lipoprotein cholesterol) concentrations], hypertension and impaired glucose regulation (increased fasting plasma glucose levels, insulin resistance or Type 2 diabetes mellitus). Recent studies indicate that over 40 % of U.S. citizens older than 60 years have metabolic syndrome when evaluated by ATP III (Adult Treatment Panel III) criteria [3,4]. The striking increase in the prevalence of the metabolic syndrome over the last two decades parallels the global epidemic of obesity and diabetes [5]. Weiss and co-workers [6] reported that the prevalence of the metabolic syndrome in the U.S.A. increased with the severity of obesity and reached 50 % in severely obese youths. Given the importance of obesity in the development of the metabolic syndrome and the interdependence of obesity, insulin resistance and Type 2 diabetes, this review will focus on the mechanisms of myocardial mitochondrial dysfunction in the setting of obesity, insulin resistance and Type 2 diabetes.

CARDIOVASCULAR DISEASE IN HUMANS WITH THE METABOLIC SYNDROME

Obesity, insulin resistance and diabetes increase the risk of developing cardiovascular disease [7–9]. Diabetes increases the risk of myocardial infarction 2–4-fold, and the clinical outcome after any acute cardiovascular event...
is poorer in diabetic compared with non-diabetic patients [10]. Diabetes and ischaemic heart disease interact to accelerate the progression of myocardial dysfunction [11]. These clinical associations have led to the widely held view that the major determinant of cardiovascular complications in the metabolic syndrome is coronary artery disease; however, much less appreciated and perhaps more controversial is the notion that metabolic alterations that occur in obesity and Type 2 diabetes can also affect cardiac structure and function, independently of hypertension or coronary artery disease.

Obesity is associated with an increased risk of heart failure [8]. Myocardial intracellular triacylglycerol content appears to increase progressively with BMI (body mass index) in otherwise healthy subjects and correlates with concentric left ventricular hypertrophy and subtle decreases in regional systolic performance [12]. A study by Tægtmeyer and co-workers [13] demonstrated a 5–6-fold increase in intramyocardial lipid levels in obese or Type 2 diabetic patients suffering from non-ischaemic heart failure. The lipid-overload of the failing human hearts was associated with a transcriptional profile similar to that of the ZDF (Zucker diabetic fatty) rat, an animal model of lipotoxicity and contractile dysfunction, suggesting that dysregulation of intramyocellular fatty acid metabolism in failing human hearts may contribute to contractile dysfunction. Although these studies suggest that perturbations in myocardial lipid metabolism may be involved in the development of non-ischaemic heart failure in obese patients, the association between myocardial lipid content and left ventricular performance in humans does not necessarily prove a direct causal link.

More than 30 years ago, Rubler et al. [14] described four diabetic patients suffering from heart failure with normal coronary arteries and no other obvious aetiologies of heart failure. This finding has been confirmed in subsequent studies [15,16]. Other studies have shown that the increased risk of developing heart failure persists in diabetic patients after adjusting for age, blood pressure, weight, cholesterol and coronary artery disease [17,18]. This has led to the use of the term ‘diabetic cardiomyopathy’, which has been defined as ventricular dysfunction occurring in diabetic patients in the absence of coronary artery disease and hypertension [16,19,20]. The term now includes diabetic individuals with diastolic dysfunction [19], the prevalence of which may be as high as 60% in well-controlled Type 2 diabetic patients. Thus subclinical left ventricular dysfunction may be a very common consequence in diabetes in addition to the increased prevalence of coronary heart disease [19,21].

**MYOCARDIAL DISEASE IN ANIMAL MODELS OF THE METABOLIC SYNDROME**

Most mechanistic insights into obesity-related cardiomyopathy and diabetic cardiomyopathy have come from rodent studies. The most widely investigated models are db/db mice (leptin receptor mutation), ob/ob mice (leptin deficiency) and ZDF rats (leptin receptor mutation). All of these models have obesity, insulin resistance and hyperglycaemia in common, although to varying degrees in each model [22,23]. In addition, there are no reports of spontaneous myocardial macrovascular disease or atherosclerosis in these particular rodent models, in part because rodents are relatively resistant to atherosclerosis in the absence of additional mutations that increase blood cholesterol levels [23,24]. Thus these models allow an evaluation of the effects of obesity, insulin resistance and Type 2 diabetes in the heart that are independent of coronary artery disease. Echocardiography of db/db mice revealed both sytolic and diastolic dysfunction [25]. db/db mice also have increased left ventricular end-diastolic pressure and reduced cardiac output and cardiac power in isolated working heart preparations [26,27]. Investigations at different ages established an age-dependent development of contractile dysfunction in db/db and ob/ob mice [28–31]. Other investigators demonstrated contractile dysfunction in isolated working heart preparations of obese Zucker rats, ZDF rats and the Type 2 diabetic Goto–Kakizaki rats [32–34]. Thus cardiac contractile dysfunction occurs in rodent models of obesity, insulin resistance and Type 2 diabetes, supporting the existence of an obesity-related cardiomyopathy and a diabetic cardiomyopathy.

**MITOCHONDRIAL DYSFUNCTION IN THE METABOLIC SYNDROME**

Mitochondria are the major consumers of oxygen in the body. Two decades ago, it was shown that Type 2 diabetics have decreased whole-body aerobic capacity [35]. A study by Wisløff and co-workers [36] demonstrated an association between low expression of key proteins required for mitochondrial function with the occurrence of multiple cardiovascular risk factors (insulin resistance, hyperlipidaemia and hypertension) in skeletal muscle of rats bred to have low oxidative capacity. Two other independent studies have used DNA microarray analysis to identify genes that are down-regulated in skeletal muscle of Type 2 diabetic patients [37,38]. Both groups found a co-ordinated down-regulation of genes regulated by PGC-1α [PPAR (peroxisome-proliferator-activated receptor) γ co-activator 1α], a master regulator of mitochondrial biogenesis and mitochondrial gene expression. In addition, one of these studies [37] also showed that this expression pattern correlated with whole-body aerobic capacity. Additional support for an association between mitochondrial dysfunction and the metabolic syndrome comes from studies investigating actual mitochondrial function and mitochondrial morphology in various tissues in prediabetic and diabetic
states. Shulman and co-workers [39,40] found a 30% reduction in ATP synthesis in severely insulin-resistant offspring of Type 2 diabetics, which could be attributed to a 38% reduction in muscle mitochondrial content. Kelley et al. [41] reported impaired mitochondrial NADH:O₂ oxidoreductase activity, reduced citrate synthase activity and smaller average size of mitochondria in skeletal muscle. Another study [42] also revealed impaired mitochondrial respiration, reduced mitochondrial number and reduced OXPHOS (oxidative phosphorylation) gene expression in adipocytes of diabetic db/db mice. It is not yet clear whether these mitochondrial abnormalities represent a genetic predisposition to the metabolic syndrome or represent acquired defects [43,44].

**MYOCARDIAL MITOCHONDRIAL DYSFUNCTION IN THE METABOLIC SYNDROME**

Very few studies have attempted to examine mitochondrial function in the human heart in obesity and Type 2 diabetes. In humans, no direct measurement of mitochondrial respiratory function has been published to date, presumably because of the intrinsic difficulties in generating appropriate human heart samples. However, a few indirect studies indicate that myocardial mitochondrial function is altered in obesity and diabetes. Peterson and co-workers [45] investigated obese and insulin-resistant women and found that an increase in BMI was associated with an increase in myocardial Vo₂ (oxygen consumption), reduced cardiac efficiency (ratio of cardiac work to VO₂) and impaired glucose tolerance correlating with increased fatty acid utilization. Since both VO₂ and fatty acid oxidation occur in mitochondria, this study [45] suggests that a perturbation of mitochondrial energy metabolism may contribute to the impaired cardiac contractility observed in obese subjects. More direct support for the existence of cardiac mitochondrial dysfunction in Type 2 diabetics has come from studies using ³¹P NMR (nuclear magnetic resonance) spectroscopy. Type 2 diabetic patients have reduced cardiac phosphocreatine/ATP ratios, indicating impaired high-energy phosphate metabolism and suggesting a cardiac energy deficit [46,47]. Similarly, phosphocreatine/ATP ratios are decreased in failing hearts of other aetologies, and mitochondrial dysfunction has also been reported for these hearts, supporting the assumption that mitochondrial dysfunction may occur in the human diabetic heart [48–50]. In addition, it was demonstrated that plasma NEFA (non-esterified fatty acid; ‘free fatty acid’) concentrations correlated negatively with phosphocreatine/ATP ratios in diabetics [47]. Since increased fatty acid delivery usually results in increased myocardial fatty acid uptake, oxidation and also increased expression of UCPs (uncoupling proteins), it is thought that increased plasma NEFA levels in diabetics may lead to decreased phosphocreatine/ATP ratios by increasing the expression or activity of cardiac UCPs. These changes in UCP expression are thought to increase mitochondrial uncoupling, thereby reducing the efficiency of ATP production and leading to reduced phosphocreatine/ATP ratios. Moreover, a study by Sharma et al. [13] showed increased lipid deposition in hearts of Type 2 diabetic and obese subjects suffering from heart failure. In this study [13], fatty acid uptake may have exceeded mitochondrial fatty acid oxidative capacity, thus resulting in increased lipid storage instead of oxidation, thereby exerting a lipotoxic effect. Finally, it was shown that human diabetic heart tissue cannot be preconditioned [51]. The lack of a decrease in mitochondrial membrane potential by diazoxide treatment of diabetic heart mitochondria suggests that mitochondrial dysfunction may be involved in the failure to precondition the diabetic heart, possibly due to dysfunctional mitochondrial KATP channels.

In contrast with human studies, mitochondrial function has been directly investigated in several animal studies. The first studies on mitochondrial function in the Type 2 diabetic rodent heart that can be found in PubMed were conducted in the 1980s. Kuo et al. [52,53] demonstrated reduced State 3 respiration of mitochondria isolated from db/db mouse hearts both with pyruvate and palmitoyl carnitine as a substrate. More recently, Boudina et al. [54] demonstrated reduced State 3 respiration and ATP synthesis in ob/ob mice using several respiratory substrates. In addition, expression of respiratory chain complexes was decreased in ob/ob hearts. Duncan et al. [55] also identified a decrease in mitochondrial ATP synthesis in UCP-DTA mice, a transgenic model of obesity and insulin resistance caused by partial ablation of brown adipose tissue via tissue-specific targeting of a diphtheria toxigene. Besides functional impairment, mitochondrial structural defects and mitochondrial proliferation occur in ob/ob and UCP-DTA mice [55–57]. Furthermore, expression of mitochondrial UCP2 and UCP3 can be increased in hearts of db/db mice or ZDF rats [58,59]. Taken together, these studies indicate that myocardial mitochondrial function is impaired in animal models of obesity, insulin resistance and Type 2 diabetes. In the next sections, the molecular mechanisms that may account for this mitochondrial dysfunction will be discussed.

**MOLECULAR MECHANISMS OF MYOCARDIAL MITOCHONDRIAL DYSFUNCTION IN THE METABOLIC SYNDROME**

Many studies that investigated myocardial mitochondrial function in diabetes have been performed in animal models of Type 1 diabetes. Although the pathogenesis of Type 1 and Type 2 diabetes are distinct, the cardiac alterations show remarkable similarities between the models, and some of the underlying mechanisms may be identical.
Fatty acid oxidation, cardiac efficiency and mitochondrial uncoupling

The heart depends on continuous oxidative metabolism for ATP generation to maintain contractile function. This is reflected by the high content of mitochondria in cardiac muscle tissue, which accounts for approx. 40% of cardiomyocyte volume. Although the heart is able to oxidize a broad variety of substrates for ATP production, the normal heart generates ATP mainly from the mitochondrial oxidation of fatty acids (60–70% of ATP generated) and to a lesser extent from glucose, lactate and other substrates (30–40%). In contrast, hearts of diabetic and obese animals use relatively more fatty acids to generate ATP while glucose oxidation rates are decreased [29,30]. Isolated working heart perfusions of db/db mouse hearts showed that increased fatty acid oxidation and decreased glucose oxidation occur as early as 4 weeks of age [29]. Similarly, increased fatty acid oxidation has been observed in ZDF rats [33]. The increased myocardial fatty acid oxidative capacity in obesity and diabetes are mediated, in part, by increased activity of PPARs (in particular PPARα). PPARα has been shown to be a central regulator of fatty acid oxidation in the heart. PPARα increases the expression of genes involved in virtually every step of cardiac fatty acid utilization [60]. Studies using PPARα−/− mice showed decreased expression of genes involved in fatty acid utilization and reduced cardiac fatty acid uptake and oxidation [61,62]. Cardiac-specific overexpression (MHC-PPARα mice; where MHC is α-myosin heavy chain) resulted in increased expression of fatty acid utilization genes and increased fatty acid oxidation [63]. In turn, expression of genes encoding glucose utilization was reduced, and glucose uptake and oxidation were decreased. Thus mice with cardiac overexpression of PPARα mimicked the metabolic phenotype of the diabetic heart, thereby implicating PPARα in the regulation of cardiac metabolism in the diabetic heart. Indeed, cardiac PPARα expression is increased in ZDF rats [13]. In 10-week-old db/db mice, PPARα expression was not increased, but PPARα target gene expression was increased [63]. In these animals, increased PPARα activity may be explained by increased activation of these receptors by their putative ligands, fatty acids, and by increased expression of PGC1α, a potent transcriptional co-activator of PPARα that would amplify the transcriptional activity of PPARα.

However, in some strains, increased fatty acid oxidation cannot be entirely accounted for by the activation of PPARα signalling. Buchanan et al. [29] demonstrated that fatty acid oxidation was increased at early ages (4 weeks) in ob/ob and db/db mice, but expression of both PPARα and its target genes was not increased at this age. Increased PGC-1α expression appeared not to amplify PPARα activity, since PPARα target gene expression was unaltered. Interestingly, in the same study [29], the expression of PPARα and its target genes was found to be increased at 15 weeks of age. Thus additional mechanisms may increase myocardial fatty acid oxidation at early stages in Type 2 diabetes, independent of increased PPARα activity or PPARα-regulated gene expression. However, increased PPARα signalling may sustain elevated rates of fatty acid oxidation in the heart as diabetes persists.

It has been known since the early 1970s that increasing cardiac fatty acid uptake by lipid infusion results in higher oxygen extraction in healthy dogs [64,65]. In these studies, myocardial contractility remained unaltered, thus resulting in reduced cardiac efficiency. This phenomenon can similarly be observed in hearts of Type 2 diabetics. Several studies from our group and others have demonstrated that myocardial VO2 is increased in obese and diabetic ob/ob and db/db mice that are also characterized by increased fatty acid oxidation and reduced cardiac efficiency [29,30,54,66,67]. In addition, such an association has also been observed in hearts of obese humans [45]. Thus reduced cardiac efficiency is a hallmark in obesity and Type 2 diabetes in both rodents and humans. In contrast with mouse models, the ZDF rat does not have increased myocardial VO2 when substrate preference changes toward fatty acid oxidation, and another study demonstrated that cardiac efficiency is unchanged in these animals [33,34]. However, the mechanisms for these differences may be species-related or unique to the ZDF rat model.

Theoretical calculations of the yield of ATP per oxygen atom consumed show that fatty acids are a less efficient fuel when compared with glucose [68]. It is calculated that shifting from 100% palmitate to 100% glucose would increase the ATP yield per molecule of oxygen consumed by 12–14%. Thus increased fatty acid oxidation in the diabetic heart may be energetically detrimental because of the higher oxygen cost to produce ATP. The higher oxygen cost and the decrease in cardiac efficiency may contribute to the development of contractile dysfunction in the metabolic syndrome. Cardiac energy depletion may become even more pronounced by the coexistence of hypertension (a common co-morbidity in the metabolic syndrome), which increases the energy demand for the heart. In addition, these mechanisms may also contribute to the increased susceptibility to ischaemic damage and poorer outcomes after myocardial infarction.

The mechanisms for increased myocardial VO2 and decreased cardiac efficiency are incompletely understood. Our findings [54] suggest increased mitochondrial
uncoupling as one underlying mechanism. We observed that \textit{ob/ob} mice, perfused with glucose and palmitate, consume more oxygen without a concomitant increase in contractile performance when compared with hearts perfused with glucose only. This fatty-acid-induced increase in myocardial $V_o_2$ occurred despite decreased expression of OXPHOS Complex I, III, and V, indicating that fatty acids in the perfusion medium were able to increase basal $V_o_2$ despite a global mitochondrial defect. Since ATP/oxygen ratios were decreased and mitochondrial State 4 respiration rates were increased, these findings suggest that increased $V_o_2$ in \textit{ob/ob} hearts after addition of fatty acids to the perfusion medium may be the consequence of fatty-acid-mediated mitochondrial uncoupling. This uncoupling may increase $V_o_2$ without proportionately increasing mitochondrial ATP production. The resulting energy deficit may explain the lack of increase in cardiac contractile function, resulting in reduced cardiac efficiency. We obtained similar results in a study performed in \textit{db/db} mice [66], supporting the hypothesis that fatty-acid-induced uncoupling may be a general phenomenon observed in models of obesity, insulin resistance and Type 2 diabetes. Furthermore, Duncan et al. [55] reported reduced ATP/oxygen ratios in cardiac mitochondria of insulin-resistant prediabetic UCP-DTA mice. Although the expression of PPAR$\alpha$ and fatty acid oxidation genes was elevated, fatty-acid-driven mitochondrial respiration was not increased. Fatty acid oxidation, myocardial $V_o_2$ and contractile function in intact hearts were not investigated in this study [55]. Taken together, at least in the \textit{ob/ob} and \textit{db/db} mouse models, mitochondrial uncoupling represents a probable mechanism that underlies the increase in myocardial $V_o_2$ and the decrease in cardiac efficiency. What might the mechanisms be that lead to cardiac mitochondrial uncoupling in Type 2 diabetes?

Under physiological conditions, mitochondrial $V_o_2$ is relatively tightly coupled to ATP synthesis. Reducing equivalents, resulting from energy substrate oxidation, deliver electrons to the mitochondrial electron transport chain and Type 2 diabetes. The energy resulting from electron transfer to oxygen atoms (thereby reducing $O_2$ to water) is used to generate an electrochemical gradient by pumping protons ($H^+$) from the mitochondrial matrix into the intermembrane space. Under physiological conditions, the bulk of $H^+$ re-enter the matrix via the $F_2F_1$-ATPase, which uses the energy to regenerate ATP from ADP (so-called coupling of oxidative phosphorylation). In addition, a small proportion of $H^+$ can bypass the $F_2F_1$-ATPase, so that $V_o_2$ is not coupled to ATP synthesis (so-called mitochondrial uncoupling). In the 1970s, an $H^+$-translocase was identified in the inner mitochondrial membrane of brown adipose tissue mitochondria and named after its function: UCP (or UCP1) [69–71]. UCP1 serves a role in non-shivering thermogenesis in brown fat tissue [71]. Within the last decade, four more UCP homologues have been identified [UCP2, UCP3, UCP4 and UCP5/BCMP1 (brain mitochondrial carrier protein 1)] [72]. Because of the high degree of homology of UCP2 and UCP3 with UCP1, it has been proposed that these proteins also mediate proton leak across the mitochondrial membrane. Indeed, overexpression of UCP2 and UCP3 in yeast leads to uncoupling of oxidative phosphorylation and decreased membrane potential [73,74]. For UCP3, several studies have demonstrated uncoupling activity in skeletal muscle [75,76]. Brand and co-workers [77,78] have also demonstrated that both fatty acids and superoxide can activate UCP3 protein activity in skeletal muscle. Superoxides can activate UCP3 activity either by direct interaction with the protein or indirectly by generating lipid peroxidation products, which then activate UCP3. Since ROS (reactive oxygen species) production increases with increasing membrane potential, UCP-mediated uncoupling has been proposed to play a role in decreasing mitochondrial ROS production and may represent one mechanism by which mitochondria might protect themselves from oxidative damage [79].

Both UCP2 and UCP3 are expressed in the heart, but their roles are still unclear [58,80]. Besides a potential role in ROS detoxification, UCPs might also be involved in the regulation of cardiac fatty acid metabolism. Conditions that increase plasma NEFA concentrations, such as high-fat feeding, fasting and streptozotocin-induced diabetes, increase UCP expression in the heart and skeletal muscle [59,81,82]. Similarly, circulating NEFA levels correlate with the expression of UCP2 and UCP3 in human heart, suggesting that plasma NEFA concentrations may regulate cardiac UCP expression [80]. Since long-chain fatty acids are ligands for PPAR$\alpha$ and UCP genes contain PPAR$\alpha$-response elements in their promoter regions, it is thought that NEFA levels regulate cardiac UCP expression in a PPAR$\alpha$-dependent fashion [83–86]. A study by Murray et al. [58] demonstrated the dependence of cardiac UCP3 expression on PPAR$\alpha$ signalling, whereas the expression of UCP2 appeared to be regulated both by PPAR$\alpha$-dependent and –independent mechanisms. On the basis of their differential regulation, different physiological roles have been suggested for UCP2 and UCP3 in the heart. However, many questions remain, and the relative importance of putative roles of UCPs in mitochondrial uncoupling, ROS detoxification and regulation of fatty acid metabolism and ways in which these mechanisms are involved in the pathogenesis of cardiac diseases remain relatively under explored.

In a recent study [66], we have directly demonstrated that mitochondrial uncoupling occurs in hearts of diabetic \textit{db/db} mice and that this uncoupling is mainly mediated by increased UCP activity. We observed that mitochondria from \textit{db/db} mouse hearts preperfused with glucose and palmitate exhibit increased respiration in the presence of oligomycin, decreased ATP synthesis and decreased ATP/oxygen ratios compared with similarly perfused wild-type mice. In addition, preperfusion of
*db/db* hearts with glucose and palmitate increased mitochondrial ADP-stimulated VO₂ compared with hearts that were perfused with glucose only. These findings suggest the presence of fatty-acid-induced mitochondrial uncoupling in *db/db* mouse hearts. In addition and more importantly, we were also able to demonstrate that proton leak was increased in *db/db* mitochondria and that the addition of GDP, an inhibitor of UCPs, restored proton leak to wild-type levels. Interestingly, UCP3 protein levels were unchanged between the groups, suggesting a mechanism that increases uncoupling by activation of UCPs, independent of changes in the expression levels. However, this does not exclude that increases in cardiac UCP expression, as observed under conditions of increased fatty acid delivery and utilization, also result in increased or maybe even additional uncoupling activity. Increased research in this area has the hope of elucidating the complex role of cardiac UCPs in the regulation of myocardial energy metabolism, particularly in the context of obesity and diabetes.

Another mechanism by which mitochondrial respiration can be uncoupled from ATP synthesis is proton leak via the ANT (adenine nucleotide translocator). In rodents, two isoforms are expressed (ANT1 and ANT2). This protein was shown to mediate uncoupling by fatty acids and to lower mitochondrial membrane potential in heart and skeletal muscle [87,88]. It was also demonstrated that ANT1-deficient mice have a 50% decrease in proton conductance in skeletal muscle [89]. In the heart, 4-hydroxy-2-nonenal-induced proton leak can be inhibited by the ANT inhibitor GDP, suggesting ANT as an uncoupler of mitochondrial respiration in the heart. Indeed, Boudina et al. [66] found in their recent study that a small component of the proton leak in *db/db* heart mitochondria was sensitive to inhibition with atracyloside, suggesting that the large part of mitochondrial uncoupling was mediated by UCPs, but that a small part of proton leak was also mediated by ANT activity.

**Oxidative stress**

Mitochondria are the principal source of ROS in cells. During normal metabolism, electrons derived from substrate oxidation are funneled through the redox carriers of the respiratory chain to the final electron acceptor, molecular oxygen, thereby reducing O₂ to water. However, even during normal metabolism, some electrons leak from the respiratory chain, resulting in the generation of reactive incompletely reduced forms of oxygen, such as superoxide and hydroxyl anions. The primary factor governing mitochondrial ROS generation is the redox state of the respiratory chain [90,91]. If the membrane potential across the inner mitochondrial membrane rises above a certain threshold value, a large stimulation of ROS generation occurs [92]. Such a rise can occur as a consequence of augmented delivery of electrons to the respiratory chain. Both increased electron delivery from increased glucose oxidation or increased fatty acid oxidation have been shown to increase mitochondrial ROS generation [93,94]. ROS can severely harm the cell by several mechanisms. ROS can directly damage proteins by oxidation, or they oxidize lipids to yield reactive lipid peroxidation products, such as 4-hydroxy-2-nonenal and malondialdehyde, which can, in turn, induce oxidative damage to proteins or phospholipids. DNA is another major site of ROS-induced damage, and mitochondrial DNA has been proposed to be particularly susceptible to oxidative damage [95]. In addition, superoxide can generate reactive nitrogen species from NO, leading to intracellular nitrosylation, for example protein tyrosine nitration [96]. Oxidative stress can result either from an overproduction of ROS and/or decreased efficiency of inhibitory scavenger systems. The major antioxidant systems in the cell are SOD (superoxide dismutase) isoforms present in the cytosol (Cu/Zn-SOD) or within mitochondria (Mn-SOD), which convert superoxide into H₂O₂ that is subsequently detoxified to water by the activity of catalase and glutathione peroxidase respectively. This system is supported by the presence of non-enzymatic antioxidants, such as tocopherols, carotenoids, ascorbate and several others.

Oxidative stress is widely accepted as a key player in the development and progression of diabetes and its complications [97–99], including cardiac pathologies [100,101]. It has been proposed by several workers that, in diabetes, ROS may be predominantly derived from mitochondria as opposed to cytosolic origins [93,102]. Brownlee and co-workers [93,103] have provided strong evidence that ROS from mitochondria activate pathological pathways that induce diabetic complications. Since ROS have a very short half-life, they are believed to cause damage close to their origin. Thus mitochondria would not only be the origin, but also the target of oxidative stress. Indeed, a proteomic approach by Türko et al. [96] revealed tyrosine nitration of several cardiac mitochondrial proteins in alloxan-induced Type 1 diabetic rats, including proteins involved in energy metabolism (succinyl-CoA:3-oxoacid CoA transferase and creatine kinase) and antioxidant defence (peroxiredoxin 3). Such tyrosine nitration can alter the structure and function of proteins and may prevent tyrosine phosphorylation [104,105].

Most studies investigating the effect of ROS on mitochondrial function in diabetic hearts have been performed in Type 1 diabetic models. A study by Lashin et al. [106] showed that 4-hydroxy-2-nonenal is produced within cardiac mitochondria, forms an adduct with Complex II of the respiratory chain and that this modification is associated with decreased succinate-supported respiration and Complex II enzymatic activity in streptozotocin-induced Type 1 diabetic rats. Ye et al. [107] demonstrated that catalase overexpression restored impaired mitochondrial morphology and cardiomyocyte
contractility in Type 1 diabetic OVE26 mice. It was shown further that incubation of cardiomyocytes from OVE26 mice in high-glucose medium results in increased ROS production, and that this increase could be prevented either by catalase overexpression or by inhibition of electron transport at Complex I or II, indicating a mitochondrial source for ROS production in OVE26 mouse hearts. Interestingly, incubation of wild-type cardiomyocytes in high-glucose medium did not increase ROS production, suggesting that diabetes induced mitochondrial changes that increased the propensity of OVE26 cardiac mitochondria to ROS overproduction following exposure to glucose. Another recent report from this group [108] demonstrated that decreases in OVE26 cardiomyocyte contractility could be completely reversed, and that impaired mitochondrial State 3 respiration could, at least in part, be restored by the overexpression of the mitochondrial SOD isoform (Mn-SOD). The accompanying alterations in mitochondrial morphology (swelling, mottled matrix and broken mitochondrial membrane) and the increase in mitochondrial biogenesis could be reversed as well. Taken together, these studies demonstrate varied effects of oxidative stress on mitochondria and implicate oxidative stress as a cause of mitochondrial dysfunction in Type 1 diabetic hearts. Indeed, for all of the these animal models, cardiac mitochondrial respiratory dysfunction has been demonstrated and, in some studies, improving the antioxidant defense was able to at least partially, if not completely, restore mitochondrial respiratory function [106–109].

It is possible that the mechanisms by which ROS causes mitochondrial damage are similar in Type 2 diabetes, as supported by limited observations in Type 2 diabetic models. For example, lipid peroxidation is increased in hearts of Goto–Kakizaki rats [110], Ye et al. [107] showed that overexpression of catalase in insulin-resistant and obese Ay mice restored impaired cardiomyocyte contractility, and obese Zucker rats have increased oxidative stress and induction of the antioxidant defence system [111,112]. Furthermore, we reported recently [66] that db/db mice have increased mitochondrial H$_2$O$_2$ production, increased levels of malondialdehyde and 4-hydroxy-2-nonenal protein adducts, and increased Mn-SOD expression in the heart.

Taken together, increased mitochondrial ROS production and oxidative stress may represent important mediators of cardiac mitochondrial dysfunction in obesity and diabetes. ROS can induce mitochondrial uncoupling, and we reported an association of fatty-acid-induced mitochondrial uncoupling by UCPs and ANT, increased mitochondrial ROS generation and reduced cardiac efficiency in hearts of db/db mice [66]. Therefore we propose a model in which ROS-induced mitochondrial uncoupling may reduce cardiac efficiency in the metabolic syndrome (Figure 1). The increased cardiac fatty acid uptake and oxidation that characterizes many models of diabetes increases the delivery of reducing equivalents to the electron transport chain. Increased electron transport chain flux would result in increased generation of ROS and lipid peroxides, which may increase UCP and ANT activity. Increased mitochondrial uncoupling would in turn lead to increased cardiac V_o2 and may thereby increase fatty acid oxidation rates further. However, the increase in V_o2 would not be accompanied by an appropriate increase in ATP synthesis. Therefore cardiac work would not increase and cardiac efficiency would be decreased. Ultimately, mitochondrial uncoupling may result in decreased myocardial high-energy reserves and thereby contribute to the development of cardiac contractile dysfunction in obesity, insulin resistance and Type 2 diabetes. In addition, ROS-mediated mitochondrial dysfunction may ultimately result in decreased ATP production. Thus it appears likely that ROS plays a central role in impairing mitochondrial energy metabolism, on the one hand by participating in mitochondrial uncoupling, thereby reducing cardiac efficiency, and on the other hand by directly damaging mitochondrial proteins (see Figure 1). Both mechanisms probably contribute to a deficit in energy reserve, thereby contributing to the development of contractile dysfunction.

**Mitochondrial calcium handling**

Cardiomyocyte contraction relies upon intricate interactions between ATP and Ca$^{2+}$, both of which need to be present in adequate amounts. In the relaxed state, ATP is bound to myosin cross-bridges, and the cross-bridges are not attached to actin filaments. Subsequent ATP hydrolysis energizes the cross-bridges for eventual contraction, but, without the actomyosin interaction, contraction will not occur. Excitation of the cardiomyocyte results in increased Ca$^{2+}$ influx via L-type Ca$^{2+}$ channels, which, in turn, triggers the release of significant amounts of Ca$^{2+}$ from the sarcoplasmic reticulum. Binding of Ca$^{2+}$ to troponin C on actin filaments induces a conformational change in contractile-regulatory proteins, which results in exposure of active sites on actin that interact with myosin cross-bridges. This promotes interactions between myosin cross-bridges and actin filaments, the release of ADP and the performance of mechanical work. At the end of contraction, ATP binds to the cross-bridges, which allows the dissociation of the actomyosin interaction prior to the start of another cycle.

To allow cardiac muscle relaxation, Ca$^{2+}$ is rapidly removed from the cytosol during diastole. Although some Ca$^{2+}$ is exported via the sarcolemmal membrane, the bulk of Ca$^{2+}$ is re-sequestered in the sarcoplasmic reticulum by the activity of SERCA2a (sarcoplasmic/endoplasmic reticulum Ca$^{2+}$-ATPase 2a). SERCA2a activity refills the depleted sarcoplasmic reticulum Ca$^{2+}$ stores after every contraction, and intact Ca$^{2+}$ cycling between the sarcoplasmic reticulum and the cytosol is critical to maintain contractile force from beat to beat. In diabetes,
cardiac sarcoplasmic reticulum function is impaired [113–115]. Several studies have observed the decreased expression of SERCA2a in streptozotocin-induced diabetic animals [116–118], and transgenic overexpression of SERCA2a in these diabetic animals has been shown to improve or normalize contractile function [119,120]. Thus contractile dysfunction in the Type 1 diabetic heart has been proposed to be the consequence of abnormalities in sarcoplasmic reticulum Ca$^{2+}$ handling and has been specifically attributed to the decreased expression of SERCA2a. In addition, other mechanisms, such as an altered phospholamban–SERCA2a interaction, free-radical-induced damage and reduced glycolytic ATP supply to SERCA2a, may contribute to impaired Ca$^{2+}$ handling in diabetic hearts [121–124].

Besides its role in contraction mechanics, Ca$^{2+}$ can also regulate substrate metabolism, i.e. ATP production. After a cytosolic increase in Ca$^{2+}$ concentration, intramitochondrial Ca$^{2+}$ concentration rises as well. There is growing evidence that the mitochondrial Ca$^{2+}$ concentration can track cytosolic Ca$^{2+}$ concentrations during the cardiac cycle, although the rapid influx and efflux mechanisms participating in Ca$^{2+}$ exchange between the cytosol and mitochondria are still unclear [125–127]. Ca$^{2+}$ exchange between these subcellular compartments is believed to provide a mechanism for matching energy production to energy demand under physiological conditions or increased workload and is termed the ‘parallel activation model’ [125]. Ca$^{2+}$ can up-regulate the activity of several Ca$^{2+}$-sensitive metabolic enzymes, including the mitochondrial dehydrogenases pyruvate dehydrogenase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase. Although pyruvate dehydrogenase activity is increased indirectly via the Ca$^{2+}$ activation of pyruvate dehydrogenase phosphatases, α-ketoglutarate dehydrogenase and isocitrate dehydrogenase appear to be activated by direct binding of Ca$^{2+}$ to enzyme subunits [128,129]. Activation of intramitochondrial Ca$^{2+}$-sensitive dehydrogenases by Ca$^{2+}$ has been directly documented in isolated cardiac mitochondria [130]. Such Ca$^{2+}$-mediated activation of oxidative metabolism would result in increased energy substrate oxidation and ATP production. Indeed, mitochondrial membrane potential, ATP generation and
tricarboxylic acid cycle activity (measured by increased mitochondrial NADH content) all increase following an elevation in mitochondrial [Ca\(^{2+}\)]\(_{\text{mito}}\) [131–133]. In addition to mitochondrial dehydrogenases, the F\(_{\text{o}}\)F\(_{\text{i}}\)-ATPase may also be regulated by Ca\(^{2+}\) and participate in Ca\(^{2+}\)-mediated regulation of energy substrate metabolism. Territo et al. [134] demonstrated in isolated cardiac mitochondria that, in the absence of Ca\(^{2+}\), F\(_{\text{o}}\)F\(_{\text{i}}\)-ATPase could not produce ATP despite an adequate supply of NADH and a high mitochondrial membrane potential. After addition of Ca\(^{2+}\), the F\(_{\text{o}}\)F\(_{\text{i}}\)-ATPase was able to generate ATP, and the Ca\(^{2+}\) \(K_m\) for this activation was in the physiological range. This study [134] also estimated that the activation of F\(_{\text{o}}\)F\(_{\text{i}}\)-ATPase may have been responsible for greater than 60\% of the Ca\(^{2+}\)-induced activation of oxidative phosphorylation, whereas the contribution of Ca\(^{2+}\)-sensitive dehydrogenases accounted for approx. 40\%. Thus Ca\(^{2+}\) exchange between the cytosol and the mitochondria has been suggested to provide a simple, but elegant, way to co-ordinate the rate of ATP production with its use for cardiac contraction [135].

Given the importance of the regulatory interactions between mitochondrial energy metabolism and Ca\(^{2+}\) handling, it is possible that impaired mitochondrial Ca\(^{2+}\) handling may contribute to the development of contractile dysfunction in the metabolic syndrome. The existence and importance of impaired cardiac mitochondrial Ca\(^{2+}\) dynamics in the regulation of ATP production and development of contractile dysfunction in Type 2 diabetes is incompletely understood, as only a few studies have addressed this issue to date. However, studies have demonstrated decreased intracellular Ca\(^{2+}\) release upon electrical stimulation, a slowed intracellular Ca\(^{2+}\) decay rate and impaired mitochondrial Ca\(^{2+}\) handling in ob/ob mice [57,136]. In addition, Ca\(^{2+}\) transients from isolated myocytes revealed lower systolic and diastolic Ca\(^{2+}\) levels in db/db mice, and the decay rate of Ca\(^{2+}\) transients was also reduced [137]. In the same study, increased Ca\(^{2+}\) leakage from the sarcoplasmic reticulum was observed, and the impairment in sarcoplasmic reticulum Ca\(^{2+}\) handling was attributed to a decline in sarcoplasmic reticulum Ca\(^{2+}\) activity, probably due to a small decrease in SERCA2a expression and a large increase in phospholamban expression [137]. Mitochondrial Ca\(^{2+}\) handling in this model remains to be investigated. These initial observations suggest that perturbations in cardiac cytosolic and mitochondrial Ca\(^{2+}\) handling may occur in models of Type 2 diabetes, insulin resistance and obesity. For a more complete understanding of potential pathophysiological consequences in the heart, and since the observations in Type 2 diabetic animals parallel observations in Type 1 diabetic animals, it would be worthwhile to review the findings from animal models of Type 1 diabetes, in which a larger number of studies have been performed.

In streptozotocin-induced diabetes, the rate of Ca\(^{2+}\) uptake into rat heart mitochondria is low compared with normal rats, and these changes occur very shortly after the onset of hyperglycaemia [138–140]. Flarsheim et al. [138] demonstrated that cardiac mitochondrial Ca\(^{2+}\) uptake was reduced and was accompanied by a decrease in \(\alpha\)-ketoglutarate-supported State 3 respiration in isolated mitochondria. In contrast, succinate-supported respiration, which is initiated by the Ca\(^{2+}\)-insensitive enzyme succinate dehydrogenase, was unaffected. Since the investigators were able to improve, but only partially restore, mitochondrial respiratory function with \(\alpha\)-ketoglutarate after insulin treatment, additional and irreversible alterations in oxidative phosphorylation might already have been present. In the same study, it was also observed that cardiac relaxation was impaired in response to increased workload, whereas systolic contraction was mainly unaffected. As it has been suggested that diastolic relaxation is more sensitive to a moderate decrease in ATP than is ventricular contraction [141], the authors proposed that the impaired relaxation during increased workload in this study may be the consequence of the failure to appropriately augment ATP synthesis. Such an observation would be in line with the general view that diastolic dysfunction may be the earliest manifestation of contractile dysfunction in diabetic cardiomyopathy.

Mitochondrial Ca\(^{2+}\) concentrations in isolated cardiomyocytes from streptozotocin-diabetic rats do not increase to the same extent as normal cardiomyocytes following electrical stimulation [130]. As concentrations are in the range where modulation of dehydrogenase activation occurs, decreased flux through Ca\(^{2+}\)-sensitive dehydrogenases may indeed contribute to impaired ATP generation in these hearts. The lower mitochondrial concentrations of Ca\(^{2+}\) may be the consequence of the smaller systolic transients in cytosolic Ca\(^{2+}\) concentrations, which have been observed in several studies in myocytes from Type 1 diabetic hearts [116,142]. As mentioned above, this may be the consequence of decreased Ca\(^{2+}\) loading of the sarcoplasmic reticulum and decreased activity of SERCA2a in these hearts [113,143]. Another potential contributor to impaired mitochondrial Ca\(^{2+}\) accumulation in Type 1 diabetic hearts may be increased susceptibility for the opening of MPTPs (mitochondrial permeability transition pores). Increased permeability transition is usually triggered by stressors such as oxidative stress and mitochondrial Ca\(^{2+}\) overload, leading to mitochondrial dysfunction and eventually cell death. In a study by Oliveira et al. [144], it was observed that, in response to a Ca\(^{2+}\) stimulus, mitochondria from both wild-type and streptozotocin-induced diabetic rats accumulated the same amount of Ca\(^{2+}\), suggesting intact mitochondrial Ca\(^{2+}\) uptake mechanisms. However, mitochondria from the diabetic hearts were not able to retain the accumulated Ca\(^{2+}\) unless the MPTP inhibitor cyclosporin was present. Thus it was suggested that mitochondria from Type 1 diabetic hearts
have a depressed capacity to accumulate Ca\(^{2+}\) because of an enhanced sensitivity to the induction of MPTP opening.

Taken together, these findings support the hypothesis that impaired mitochondrial Ca\(^{2+}\) handling may compromise cardiac energy metabolism and thereby contribute to the development of contractile dysfunction in Type 1 diabetes. Although few studies have been performed in Type 2 diabetes, the few available studies have found similar alterations to those observed in Type 1 diabetes. Thus it is tempting to speculate that lower cytosolic Ca\(^{2+}\) levels may reduce mitochondrial Ca\(^{2+}\) transients, which may result in reduced Ca\(^{2+}\)-sensitive dehydrogenase activities and therefore impaired ATP production. Impaired ATP synthesis and reduced cytosolic Ca\(^{2+}\) transients may both contribute to the development of contractile dysfunction in the metabolic syndrome (Figure 2). Although further conclusions are not possible due to the lack of studies addressing this issue, mitochondrial Ca\(^{2+}\) handling may be another mechanism that may amplify the negative effect of increased mitochondrial uncoupling and increased ROS production on cardiac energy metabolism in Type 2 diabetes.

**Mitochondrial biogenesis**

It has recently been demonstrated that mitochondrial biogenesis occurs in hearts of obese and insulin-resistant animals [55,56]. UCP-DTA mice have increased mitochondrial volume density, mitochondrial DNA content and OXPHOS gene expression. In contrast, ADP-stimulated mitochondrial respiration was not increased and ATP synthesis was decreased [55]. Similarly, we observed [54,56,66] that mitochondrial volume density and mitochondrial DNA content are increased in ob/ob and db/db mice, but these alterations were also accompanied by impairment in ADP-stimulated respiration and ATP synthesis. Thus cardiac mitochondrial biogenesis occurs in obesity and insulin resistance, but is not accompanied by a co-ordinate increase in mitochondrial function. These observations raise the question as to whether mitochondrial biogenesis is adaptive or maladaptive in the metabolic syndrome.
A major regulator of mitochondrial biogenesis is PGC-1α [145–147]. PGC-1α is a highly inducible transcriptional co-activator that docks to a variety of transcription factors to amplify their activity and their target gene expression [148]. Among others, such transcription factors include the PPAR family of nuclear hormone receptors (PPARα, PPARβ/δ and PPARγ) and transcription factors that regulate the expression of mitochondrial genes, such as ERRα (oestrogen-related receptor α) and NRFs (nuclear respiratory factors; NRF1 and NRF2). PGC-1α can also increase the expression of NRFs and the mitochondrial transcription factor A [147]. When ectopically expressed in fat or muscle cells, PGC-1α robustly increases mitochondrial DNA content, increases the expression of a large set of nuclear- and mitochondrial-encoded mitochondrial genes, and increases mitochondrial respiration [147]. PGC-1α is also involved in the control of energy metabolic pathways in the heart, such as fatty acid oxidation, the regulation of hepatic gluconeogenesis, the regulation of fibre-type composition and glucose oxidation in skeletal muscle, and secretion of insulin from pancreatic β-cells. Thus, besides promotion and co-ordination of mitochondrial biogenesis, PGC-1α also exhibits tissue-specific functions in metabolic regulation.

PGC-1α is highly expressed in the heart. Hearts of mice with cardiomyocyte-specific overexpression of PGC-1α (MHC-PGC-1α) exhibit a marked increase in cardiomyocyte mitochondrial biogenesis and, ultimately, develop overt heart failure [149]. In a cardiomyocyte-specific tetracycline-inducible transgenic mouse, induction of PGC-1α in neonatal mice triggered a dramatic expansion of mitochondria within cardiomyocytes, whereas activation of PGC-1α expression in adult mice resulted in a modest biogenic response that still led to the development of a cardiomyopathy and mitochondrial ultrastructural abnormalities [150]. Interestingly, knockout of PGC-1α in the heart results in reduced expression of fatty acid oxidation, tricarboxylic acid cycle and OXPHOS genes, and baseline cardiac contractile dysfunction, but mitochondrial volume density was unaltered, suggesting that PGC-1α might not be essential for the fundamental process of mitochondrial biogenesis or that other related molecules such as PGC-1β might compensate in its absence [151,152]. Studies showing a positive correlation between PGC-1α expression and conditions of increased fatty acid oxidation in the heart, and the fact that PGC-1α can increase fatty acid oxidation gene expression via co-activation of PPARα, suggests a role in the regulation of energy substrate utilization [149]. Since PGC-1α expression is down-regulated in pressure-overload hypertrophy and heart failure, which is associated with reduced fatty acid oxidation and OXPHOS gene expression and impaired mitochondrial respiration, reduced expression of PGC-1α during the development of heart failure has been suggested to contribute to its pathogenesis [153–155]. Thus altered PGC-1α activity may be linked to cardiac dysfunction.

Given the role of PGC-1α in mitochondrial biogenesis, it is tempting to speculate that mitochondrial biogenesis in UCP-DTA, ob/ob and db/db mice may be mediated by increased PGC-1α signalling. Indeed, mitochondrial biogenesis in UCP-DTA mice was accompanied by a significant increase in PGC-1α and OXPHOS gene expression, suggesting that PGC-1α signalling may drive mitochondrial biogenesis in these mice [55]. However, PGC-1α expression was not increased and even decreased at later ages in ob/ob mice that developed decreased OXPHOS protein content, suggesting that mitochondrial proliferation may have occurred via a PGC-1α-independent pathway [29,54]. Moreover, in db/db mice, although mitochondrial biogenesis was shown to be associated with increased expression of PGC-1α expression, there was no co-ordinate up-regulation in OXPHOS subunit gene expression [66]. The discrepancy between PGC-1α and OXPHOS expression argues against the notion that an increase in PGC-1α may represent a unifying underlying hypothesis for the regulation of cardiac mitochondrial biogenesis in the metabolic syndrome, or could suggest that activity of alternative co-regulators of OXPHOS gene expression might be deficient in the hearts of obese, insulin-resistant or diabetic animals. The question also remains whether mitochondrial biogenesis is beneficial or detrimental in these hearts. It is possible that the mitochondrial biogenic response occurs as a compensatory mechanism since mitochondrial function is impaired. If true, then this mechanism represents a partial adaptation because overall respiratory capacity was not increased, and was even impaired, in mitochondria from UCP-DTA, ob/ob and db/db mice. It has also been suggested that failure to up-regulate a certain subset of metabolic genes in parallel with mitochondrial number could lead to a state of energy depletion under conditions of profoundly increased PGC-1α expression [66,150]. However, the modest change in PGC-1α expression in UCP-DTA and db/db mice, and the absence of any increase in PGC-1α expression in ob/ob mice, suggests that other possible mechanisms exist. It is plausible, for example, that other mechanisms regulating mitochondrial proliferation and biogenesis may be involved, which limits the biogenesis of functionally competent mitochondria. An alternative interpretation of these results is that mitochondrial dysfunction is not the trigger, but instead the consequence, of increased mitochondrial biogenesis. It may be possible that a maladaptive proliferative signal may trigger mitochondrial biogenesis, but this mechanism may not be able to activate the full programme of mitochondrial biogenesis. Thus mitochondrial proliferation and biogenesis is not well orchestrated with an increase in OXPHOS subunit content, resulting in dysfunctional mitochondria. Until other regulators of mitochondrial...
biogenesis and proliferation are identified, this argument remains speculative. Thus additional studies are required to clarify the role of PGC-1α in cardiac mitochondrial biogenesis in the metabolic syndrome.

**Insulin resistance**

Obesity, dyslipidaemia, hyperglycaemia and hyper-insulinaemia are characteristics of most rodent models that mimic the metabolic syndrome. These models also exhibit insulin resistance in the heart and myocardial contractile dysfunction [29,30]. Thus the question arises as to whether cardiac insulin resistance may contribute to the development of contractile dysfunction. As all of these models are characterized by systemic metabolic alterations, the evaluation of the contribution of insulin resistance to contractile dysfunction is challenging. Thus we generated mice with a cardiomyocyte-restricted deletion of the insulin receptor (CIRKO mice) [156]. These mice allow the investigation of impaired myocardial insulin signalling without confounding effects of systemic metabolic alterations and obesity. CIRKO mice have reduced insulin-stimulated glucose uptake and also have a modest decrease in contractile function, thereby implicating insulin resistance as a contributing factor in the development of contractile dysfunction in the metabolic syndrome. Interestingly, CIRKO mice have a decrease in the oxidation of glucose and fatty acids as they age and as contractile dysfunction occurs [156]. As oxidation of both substrates occurs in the mitochondria, these results indirectly point towards a general mitochondrial oxidative defect in CIRKO mice. This is also supported by decreased expression of mitochondrial fatty acid oxidation genes in CIRKO mice. In other studies, insulin has been shown to up-regulate mitochondrial gene expression, and conditions of insulin resistance are associated with decreased mitochondrial gene expression [157–159]. Thus it appears plausible that the chronic lack of insulin action via the insulin receptor in CIRKO mouse hearts may lead to decreased mitochondrial gene expression, which may limit oxidative capacity, thereby impairing mitochondrial energetics and contractile function. If this hypothesis is correct, then one would expect that CIRKO hearts may be prone to injury when subjected to increased energy demands. Indeed, CIRKO mice subjected to pressure overload using transverse aortic banding or following chronic β-adrenergic stimulation resulted in left ventricular dysfunction, left ventricular dilation and increased interstitial fibrosis relative to similarly treated controls [160,161]. Thus impaired insulin signalling in CIRKO mice may impair mitochondrial oxidative capacity, which may result in modest baseline contractile dysfunction and significant impairment in the cardiac adaptation to haemodynamic stress. Further studies from our laboratory [54,66] have identified decreased OXPHOS expression and impairment in ADP-stimulated mitochondrial VO₂ with various substrates in ob/ob and db/db mice, which are severely insulin-resistant at the organismal level and in the heart. These findings therefore support the notion that insulin resistance may play a role in the development of contractile dysfunction in the metabolic syndrome, and impaired myocardial mitochondrial oxidative capacity due to reduced insulin action could be an underlying mechanism.

**CONCLUSIONS**

In this review, we have discussed several potential mechanisms that may contribute to myocardial mitochondrial dysfunction in the metabolic syndrome. It is likely that no one single mechanism, but rather the combination of several mechanisms, may lead to mitochondrial dysfunction. We propose that the resultant mitochondrial dysfunction will compromise cardiac ATP generation, ultimately leading to contractile dysfunction. Findings have been presented that suggest roles for altered mitochondrial substrate flux, increased ROS and activation of UCPs as important contributors to cardiac dysfunction in obesity, diabetes and insulin-resistant states. Thus novel treatments that are targeted to these abnormalities might lead to new therapeutic avenues for the prevention of cardiac dysfunction. Given the dramatic increase in the prevalence of the metabolic syndrome, it might be predicted that our societies might face an explosive increase in heart disease. Thus a compelling case can be made for additional mechanistic studies in humans and increased emphasis on therapeutic strategies that will address fundamental underlying pathophysiological mechanisms.

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