TOPICAL REVIEW

# Mitochondrial energetics and calcium coupling in the heart

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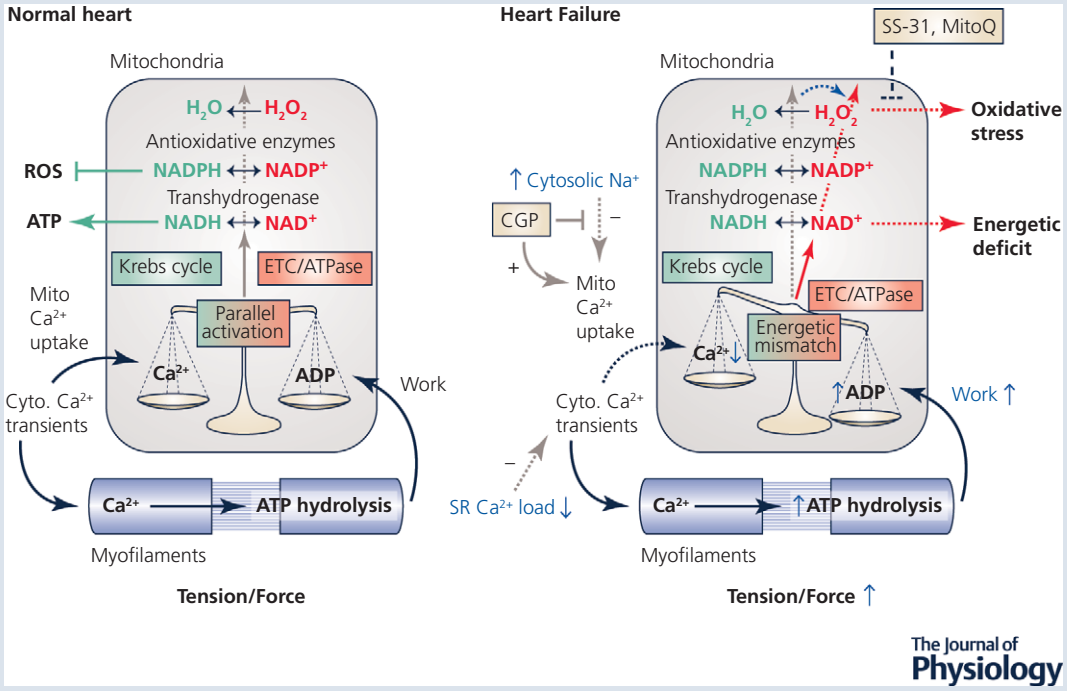


**Abstract** Contraction and relaxation of the heart consume large amounts of energy that need to be replenished by oxidative phosphorylation in mitochondria, and matching energy supply to demand involves the complimentary control of respiration through ADP and $Ca^{2+}$. In heart failure, an imbalance between ADP and $Ca^{2+}$ leads to oxidation of mitochondrial pyridine nucleotides, where NADH oxidation may limit ATP production and contractile function,

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while NADPH oxidation can induce oxidative stress with consecutive maladaptive remodelling. Understanding the complex mechanisms that disturb this finely tuned equilibrium may aid the development of drugs that could ameliorate the progression of heart failure beyond the classical neuroendocrine inhibition.

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**Abstract figure legend** Mitochondrial redox balance is under the control of $Ca^{2+}$ and ADP in the normal heart and heart failure. Under physiological conditions, parallel activation of energy consumption (via force transduction) and energy regeneration (via Krebs cycle stimulation) by $Ca^{2+}$ balances the redox states of NADH/$NAD^+$ and NADPH/$NADP^+$, maintaining reducing equivalents for ATP production and the antioxidative capacity. In heart failure, a mismatch of workload and mitochondrial $Ca^{2+}$ uptake induces a redox mismatch that results in oxidation of pyridine nucleotides and that accounts for energy deprivation and oxidative stress. Drugs directed against this mismatch and oxidative stress are indicated in pale brown (CGP-37157, an inhibitor of the mitochondrial $Na^+/Ca^{2+}$ exchanger; SS-31, a tetrapeptide binding to cardiolipin; MitoQ, an antioxidant accumulating in mitochondria). ATP, adenosine triphosphate; cyto, cytosolic; ETC, electron transport chain; mito, mitochondrial; ROS, reactive oxygen species; SR, sarcoplasmic reticulum.

**Abbreviations** $\beta$-AR, $\beta$-adrenergic receptor; DN, dominant negative; EC, excitation–contraction; ETC, electron transport chain; $H_2O_2$, hydrogen peroxide; KO, knock-out; MCU, mitochondrial $Ca^{2+}$ uniporter; MitoQ, mitoquinone; NNT, nicotinamide nucleotide transhydrogenase; ROS, reactive oxygen species; SR, sarcoplasmic reticulum.

## Introduction

Chronic heart failure is the most common cause of hospital admissions in Western countries, and its prevalence is expected to increase further. The current medical treatments target in particular the excessive activation of the sympathetic nervous system and the renin–angiotensin–aldosterone system. Beyond these neuroendocrine interventions, few novel targets to treat heart failure have been identified. Over recent years, metabolic aspects to heart failure have gained increasing interest, since in patients with heart failure, the energy stores of the heart are depleted, with decreased ratios of phosphocreatine to adenosine triphosphate (ATP) predicting an adverse outcome (Neubauer, 2007). More recent work suggests that while depletion of phosphocreatine *per se* may limit maximal exercise capacity, maladaptive remodelling – typically closely associated with the prognosis – is not affected by such an energetic deficit (Lygate *et al.* 2013). Instead, the accumulation of metabolic intermediates of glycolysis and fatty acid oxidation as well as oxidative stress, which can all induce maladaptive signalling in their own right, may be the more relevant triggers for disease progression than the mere depletion of ATP (or phosphocreatine) stores (Chatham & Young, 2012; Nickel *et al.* 2013).

In the plasma and hearts of patients with heart failure, the levels of oxidative stress are increased and correlate with left ventricular dysfunction (Belch *et al.* 1991; Maack *et al.* 2003). Oxidative stress is an imbalance between the production and detoxification of reactive oxygen species (ROS), and their major sources in the heart are NADPH oxidases, uncoupled nitric oxide synthases and mitochondria (Burgoyne *et al.* 2012; Nickel *et al.* 2014). Mitochondria are considered to be the main source of ROS in most cell types and in particular in cardiac myocytes by most (Turrens, 2003; Adam-Vizi, 2005; Balaban *et al.* 2005; Hool *et al.* 2005; Murphy, 2009) but not all authors (Brown & Borutaite, 2012). Oxidative stress impairs excitation–contraction (EC) coupling in cardiac myocytes, causes arrhythmias (Wagner *et al.* 2013; Yang *et al.* 2015), activates pro-hypertrophic signalling (Ago *et al.* 2008; Erickson *et al.* 2008) and induces apoptotic and/or necrotic cell death through activation of the permeability transition pore (Halestrap, 2005). In particular, *mitochondrial* ROS play a causal role in the development and progression of heart failure in response to various pathological stimuli, such as ischaemia–reperfusion, pressure overload and angiotensin II (Matsushima *et al.* 2006; Dai *et al.* 2011, 2012). Moreover, mitochondria appear to amplify ROS production from other ROS sources, such as NADPH oxidase, by a mechanism termed ROS-induced ROS release (Zorov *et al.* 2000; Aon *et al.* 2003; Brandes, 2005; Kimura *et al.* 2005; Dai *et al.* 2011; Maack & Böhm, 2011). Therefore, understanding the regulation of mitochondrial ROS is key to eventually developing treatments that may prevent or ameliorate heart failure development beyond the inhibition of neuroendocrine activation.

In the past 10 years, we (Nickel *et al.* 2014, 2015) and others (Aon *et al.* 2010; Gauthier *et al.* 2013; Kembro *et al.* 2013) have developed a concept in which ROS emission from cardiac mitochondria is dynamically regulated by $Ca^{2+}$ and ADP, with the redox state of mitochondrial pyridine nucleotides as a central level of control. According to this concept, a mismatch between decreased mitochondrial $Ca^{2+}$ uptake and increased cardiac workload induces net oxidation of NADH,

which also oxidizes NADPH and thereby depletes the anti-oxidative capacity of the matrix, giving rise to excessive emission of $H_2O_2$ (Fig. 1). In the following, we will discuss the pathophysiological basis of this concept and address current controversies, future directions of research and potential points of intervention.

## Energy supply and demand matching

During EC coupling, $Ca^{2+}$ influx via L-type $Ca^{2+}$ channels triggers an even greater release of $Ca^{2+}$ from the sarcoplasmic reticulum (SR), and this $Ca^{2+}$ binds to troponin C and thereby induces contraction. During diastole, $Ca^{2+}$ is taken back up into the SR by the SR $Ca^{2+}$-ATPase or exported across the cell membrane via the $Na^+/Ca^{2+}$ exchanger (Bers, 2006). A physiological increase in workload is triggered by $\beta$-adrenergic stimulation, elevating the rate and amplitude of cytosolic $Ca^{2+}$ transients and thereby increasing force generation at the myofilaments. Through this increase of work, ATP is hydrolysed to adenosine diphosphate (ADP), and after shuttling into mitochondria via the adenine nucleotide transporter, ADP activates the $F_1F_o$-ATPase to regenerate ATP (Fig. 1*A*). This hastens electron flux along the electron transport chain (ETC), oxidizing NADH to $NAD^+$ (so-called 'pull' condition; Fig. 1*A*). At the same time, $Ca^{2+}$ is taken up into mitochondria via the mitochondrial $Ca^{2+}$ uniporter (MCU), where it activates Krebs cycle dehydrogenases to adapt NADH regeneration to ETC-induced oxidation ('push' condition). This dual role of $Ca^{2+}$, i.e. increasing both energy consumption ('pull' on electrons along the ETC) and regeneration ('push' of electrons from the Krebs cycle into the ETC), is termed 'parallel activation' (Fig. 1*A*) (Balaban, 2002; Cortassa *et al.* 2006).

The discovery of the molecular identity of the MCU (Baughman *et al.* 2011; De Stefani *et al.* 2011) has led to the consecutive identification of its various regulatory proteins, such as MICU1, MICU2, MCUR and EMRE, together forming the mitochondrial $Ca^{2+}$ 'uniplex' (Finkel *et al.* 2015; Kamer & Mootha, 2015) (Kwong (2017), in this issue). Since the affinity of the MCU for $Ca^{2+}$ is rather low (i.e. in the micromolar range), the close association of mitochondria to the SR creates a $Ca^{2+}$ microdomain between these two organelles that allows efficient mitochondrial $Ca^{2+}$ uptake and privileges this pathway over trans-sarcolemmal $Ca^{2+}$ influx (Kohlhaas & Maack, 2010, 2013). This microdomain is governed by physical tethers that link both organelles, one of these being mitofusin 2 (Mfn-2) (de Brito & Scorrano, 2008; Chen *et al.* 2012; Naon *et al.* 2016) (Fig. 1*A*). $Ca^{2+}$ is exported from mitochondria by a $Na^+/Ca^{2+}$ ($Li^+$) exchanger (Palty *et al.* 2010), whose kinetics are much slower than those of uptake via the MCU and therefore $Ca^{2+}$ accumulates in mitochondria when amplitude and/or rate of cytosolic $Ca^{2+}$ transients increase (Di Lisa *et al.* 1993; Maack *et al.* 2006).

The absolute amounts of $Ca^{2+}$ taken up by mitochondrial, and whether these are consequential for cytosolic $Ca^{2+}$ handling, is currently not fully resolved yet. While in other, non-cardiomyocyte cell types, mitochondrial $Ca^{2+}$ uptake commonly buffers cytosolic $Ca^{2+}$ (Rizzuto & Pozzan, 2006), this has been suggested to be the case in cardiac myocytes as well in some models, but refuted by others, as previously reviewed (O'Rourke & Blatter, 2009; Kohlhaas & Maack, 2013; Williams *et al.* 2013). These controversies are related to technical challenges, but also potential species differences. In this context, it should be considered that in humans, cardiac output during exertion can increase 4- to 6-fold (Chapman *et al.* 1960; Grimby *et al.* 1966), while in the mouse, baseline heart rate is already ~600 beats per minute and increases by a factor of only ~1.5 during maximal exertion with no increase in blood pressure (Desai *et al.* 1997; Georgakopoulos & Kass, 2001), indicating that workload variance requiring energetic adaptations is clearly much smaller in mice than in humans. Since mitochondrial $Ca^{2+}$ uptake is required to match energy supply to demand, particularly during $\beta$-adrenergic stimulation, it is likely that in humans, mitochondrial $Ca^{2+}$ uptake may play a more important role than in the mouse. In fact, when comparing MCU current density in various organs within the mouse, it is lowest in the heart (Fieni *et al.* 2012). However, no study has systematically compared cardiac MCU current densities between different species so far. Therefore, the interesting results obtained in mice with genetic inactivation of the MCU (Pan *et al.* 2013; Kwong *et al.* 2015; Luongo *et al.* 2015; Rasmussen *et al.* 2015; Wu *et al.* 2015), which will be discussed in more detail further below, have to be extrapolated with some care and may to some extent underestimate the importance of mitochondrial $Ca^{2+}$ uptake in the human situation.

## Regulation of mitochondrial ROS emission

During respiration, the superoxide anion radical ($\cdot O_2^-$) is generated at complexes I and III of the ETC, and is dismutated to hydrogen peroxide ($H_2O_2$) by the $Mn^{2+}$-dependent superoxide dismutase (Balaban *et al.* 2005; Murphy, 2009; Chen & Zweier, 2014; Zorov *et al.* 2014; Murphy *et al.* 2016). $H_2O_2$ is then detoxified by glutathione peroxidase (GPX) and the thioredoxin/peroxiredoxin systems that all require reduced NADPH (Fig. 1*A*). The regeneration of NADPH is governed by enzymes that derive their substrates from the Krebs cycle, in particular, isocitrate dehydrogenase and nicotinamide nucleotide transhydrogenase (NNT) (Ying, 2008; Nickel *et al.* 2015) (Fig. 1*A*). Therefore, $Ca^{2+}$-induced stimulation of the Krebs cycle not only matches energy supply to demand, but also regenerates

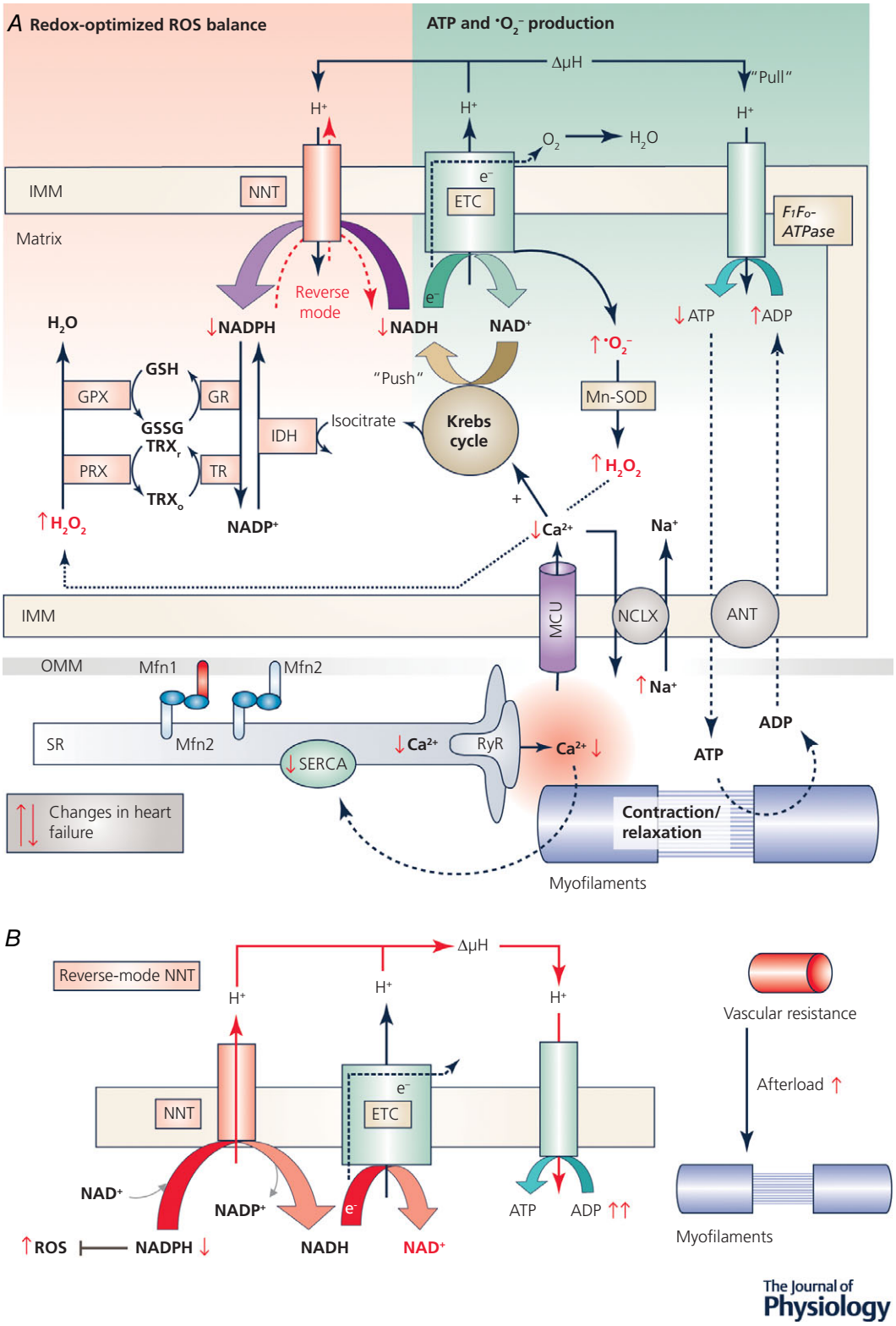


**Figure 1. Regulation of mitochondrial respiration and redox state by $Ca^{2+}$ and ADP**
*A*, integration of mitochondrial ROS production with ROS elimination and the control through ion handling. Abbreviations: $\Delta\mu_H$, proton motive force; ANT, adenine nucleotide translocator; GPX, glutathione peroxidase; GR, glutathione reductase; GSH/GSSG, reduced/oxidized glutathione; IDH, isocitrate dehydrogenase; IMM, inner mitochondrial membrane; MCU, mitochondrial $Ca^{2+}$ uniporter; Mfn, mitofusin; Mn-SOD, $Mn^{2+}$-dependent

superoxide dismutase; NCLX, mitochondrial $Na^+/Ca^{2+}$ (and $Li^+$) exchanger; NNT, nicotinamide nucleotide transhydrogenase; OMM; outer mitochondrial membrane; PRX, peroxiredoxin; TR, thioredoxin reductase; $TRX_{r/o}$, reduced/oxidized thioredoxin; RyR, ryanodine receptor; SERCA, SR $Ca^{2+}$ ATPase; SR, sarcoplasmic reticulum. *B*, reverse-mode of the nicotinamide nucleotide transhydrogenase (NNT). Through an increase in cardiac afterload, NADH is consumed by the ETC, and the NNT reverses, oxidizing NADPH to regenerate NADH and ATP, but at the cost of the antioxidative capacity, giving rise to ROS emission.

the antioxidative capacity of the matrix to prevent the emission of $H_2O_2$ during transitions of workload (Kohlhaas *et al.* 2010). Together with studies from isolated mitochondria, this helped to establish the concept of a 'redox-optimized ROS balance' (Aon *et al.* 2010), in which the physiological steady state in cardiac mitochondria is tuned to an intermediate redox state that on the one hand prevents excessive formation of ROS at the ETC under highly reduced conditions (Starkov & Fiskum, 2003; Balaban *et al.* 2005), and on the other prevents depletion of the antioxidative capacity under highly oxidized conditions (Aon *et al.* 2010; Kohlhaas *et al.* 2010; Gauthier *et al.* 2013; Kembro *et al.* 2013).

## Pathological alterations in heart failure

In systolic heart failure, contractile dysfunction is the result of decreased systolic $Ca^{2+}$ transients, and this is primarily related to decreased SR $Ca^{2+}$ load secondary to lowered SR $Ca^{2+}$-ATPase activity and leaky ryanodine receptors (Bers, 2006) (Fig. 1*A*). On the other hand, the cytosolic $Na^+$ concentration is elevated, which activates the reverse-mode of the sarcolemmal $Na^+/Ca^{2+}$ exchanger to contribute to cytosolic $Ca^{2+}$ influx during the action potential (Armoundas *et al.* 2003; Weber *et al.* 2003) (Fig. 1*A*). While this partly compensates for decreased SR $Ca^{2+}$ release (Weisser-Thomas *et al.* 2003), the rather slow trans-sarcolemmal $Na^+/Ca^{2+}$ exchanger-mediated $Ca^{2+}$ influx (Sipido *et al.* 1997) is less efficient for mitochondrial $Ca^{2+}$ uptake (Kohlhaas & Maack, 2010). Furthermore, elevated cytosolic $Na^+$ accelerates mitochondrial $Ca^{2+}$ efflux via the mitochondrial $Na^+/Ca^{2+}$ ($Li^+$) exchanger (Maack *et al.* 2006; Liu & O'Rourke, 2008; Kohlhaas *et al.* 2010) (Fig. 1*A*). Finally, in human cardiac mitochondria from patients with heart failure, the open probability of the MCU is decreased (Michels *et al.* 2009). Therefore, deterioration of EC coupling in heart failure compromises the well-tuned mitochondrial $Ca^{2+}$ uptake machinery. This has consequences for both energy supply-and-demand matching as well as the anti-oxidative capacity: in isolated cardiac myocytes from a guinea-pig model of systolic heart failure, NADH and NADPH oxidized, decreasing the amount of reducing equivalents for ATP production at the ETC and provoking the emission of ROS (Fig. 1*A*) (Liu & O'Rourke, 2008; Kohlhaas *et al.* 2010). Since the inhibition of the mitochondrial $Na^+/Ca^{2+}$ ($Li^+$) exchanger prevented NAD(P)H oxidation in myocytes (Liu & O'Rourke, 2008) and the development of heart failure *in vivo* (Liu *et al.* 2014), the $Na^+$-induced redox and energetic mismatch may play a causal role for heart failure progression and possibly represent a potential novel therapeutic target for patients with heart failure. However, this approach has not been followed up by large animal or clinical studies yet.

## Mitochondrial transhydrogenase: the yin and yang of antioxidative capacity

Besides contractile dysfunction of the heart, cardiac haemodynamics are further compromised by an elevation of cardiac afterload due to increased systemic vascular resistance (Mason *et al.* 1964) as a result of the neuroendocrine activation that triggers vasoconstriction (Francis *et al.* 1984). We recently identified a mechanism in which the mere increase in cardiac afterload provokes mitochondrial ROS emission (Nickel *et al.* 2015). The NADH and NADPH pools are directly linked by NNT, catalysing the reaction $NADH + NADP^+ \leftrightarrow NADPH + NAD^+$, which is coupled to the protonmotive force ($\Delta\mu_H$; Fig. 1*A*). In energized mitochondria, the forward NNT reaction towards NADPH regeneration is strongly favoured and therefore NNT is considered a key anti-oxidative enzyme (Rydstrom, 2006). Intriguingly, the most commonly used mouse strain, C57BL/6J, but not C57BL/6N, has a loss-of-function mutation in the gene encoding NNT (*Nnt*), which causes oxidative stress and impairs ATP production in pancreatic islet cells, leading to glucose intolerance in this strain (Toye *et al.* 2005; Freeman *et al.* 2006). Furthermore, the *Nnt* mutation sensitized BL/6J hearts to develop cardiomyopathy upon deletion of $Mn^{2+}$-dependent superoxide dismutase (Huang *et al.* 2006; Kim *et al.* 2010). In contrast to this *anti*-oxidative role, we observed that in response to an increase in cardiac afterload, NNT can reverse its direction when oxidation of NADH at the ETC outweighs its Krebs cycle-mediated regeneration, consuming NADPH towards NADH and ATP regeneration, but at the cost of the anti-oxidative capacity (Nickel *et al.* 2015) (Fig. 1*B*). The ensuing oxidative stress accounts for necrosis, left ventricular dysfunction and death during pressure overload, which was prevented in BL/6J mice (with inactivated NNT) or when BL/6N mice (with intact NNT) were treated with SS-31 (Nickel *et al.* 2015), a tetrapeptide that accumulates ~1000-fold in mitochondria and ameliorates mitochondrial ROS production (Szeto, 2014). While initial experiments suggested that SS-31 was a ROS scavenger (Zhao *et al.* 2004), more recent work suggests

that it does not have direct anti-oxidative effects (Brown *et al.* 2014), but binds to cardiolipin, an essential phospholipid of the inner mitochondrial membrane (Szeto, 2014). This interaction with SS-31 protects cardiolipin from oxidation and dysfunction, preventing disassembly of the ETC supercomplexes and thereby energetic deficit and mitochondrial ROS production (Szeto, 2014). SS-31 also improved left ventricular function in the short and long term in a dog model of heart failure (Sabbah *et al.* 2016) and is currently being tested in phase II clinical trials on patients with systolic (NCT02788747, NCT02914665) and diastolic heart failure (NCT02814097).

Although the reverse-mode of NNT mediates oxidative stress during pathological cardiac workload, targeting NNT itself may not be a valuable pharmacological concept since the forward mode of NNT is required in most cells and conditions for anti-oxidative capacity (Rydstrom, 2006), and C57BL/6J mice, which lack a functional NNT, have impaired glucose tolerance and thereby are more prone to develop diabetes. Furthermore, mutations of *Nnt* in humans are associated with familial glucocorticoid deficiency (Meimaridou *et al.* 2012). Therefore, inhibiting NNT in humans globally may cause more harm than benefit.

An alternative therapeutic approach to reduce mitochondrial ROS is targeting the antioxidant electron acceptor ubiquinone to mitochondria using lipophilic cations, as with mitoquinone (MitoQ) (Murphy, 2016). MitoQ reduced infarct size after cardiac ischaemia–reperfusion (Adlam *et al.* 2005) and ameliorated cardiac remodelling in hypertensive rats (Graham *et al.* 2009). In clinical trials, MitoQ ameliorated hepatic necroinflammation in patients with hepatitis C (Gane *et al.* 2010), but had neutral results in patients with Parkinson's disease (Snow *et al.* 2010). MitoQ has not yet been tested in patients with cardiovascular diseases.

## Lessons learned from novel MCU-deficient mouse models for bioenergetic feedback coupling

After the molecular identity of the MCU was resolved, three different mouse models with either constitutive global (Pan *et al.* 2013; Holmström *et al.* 2015) or conditional cardiac myocyte-specific MCU knock-out (KO) (Kwong *et al.* 2015; Luongo *et al.* 2015) or overexpression of a dominant negative (DN-) MCU (Rasmussen *et al.* 2015; Wu *et al.* 2015) were generated (reviewed by Kwong (2017) in this issue). They all have in common that although mitochondria isolated from hearts are unable to rapidly accumulate $Ca^{2+}$, their cardiac and/or cardiomyocyte function is normal in the absence of $\beta$-adrenergic receptor ($\beta$-AR) stimulation, indicating that MCU-mediated mitochondrial $Ca^{2+}$ uptake is required during physiological increases of workload, but not to sustain cardiac function at rest. Interestingly, mice with cardiomyocyte-specific MCU-KO had limited capacity to sprint at high speed (Kwong *et al.* 2015), which was attributed to a delay and/or blunting of the positive inotropic, but not chronotropic, effect of catecholamines *in vivo* (Kwong *et al.* 2015; Luongo *et al.* 2015). In DN-MCU mice, both the positive inotropic *and* the chronotropic responses to $\beta$-AR stimulation were blunted (Rasmussen *et al.* 2015; Wu *et al.* 2015). In both models, cytosolic $Ca^{2+}$ concentrations in isolated cardiac myocytes were unaffected at baseline, but increased during systole in response to $\beta$-AR stimulation in the absence of an MCU (Luongo *et al.* 2015; Rasmussen *et al.* 2015), in agreement with a study on neonatal cardiac myocytes with siRNA-induced silencing of the MCU. This may suggest that, at least to some extent, mitochondrial $Ca^{2+}$ uptake may contribute to cytosolic $Ca^{2+}$ buffering, which, however, is a subject of continual debate (O'Rourke & Blatter, 2009; Williams *et al.* 2013). Rasmussen *et al.* (2015) argue that this increase in cytosolic $Ca^{2+}$ may increase ATP demand through the activation of EC coupling.

As a metabolic consequence, and in agreement with our previous results on guinea pig cardiac myocytes, in which we acutely inhibited the MCU with Ru360 (Kohlhaas *et al.* 2010), the $\beta$-AR-induced stimulation of Krebs cycle-mediated NAD(P)H production is blunted also by genetic MCU inactivation (Luongo *et al.* 2015; Wu *et al.* 2015). In quiescent cells, in which little ATP is consumed and thereby only a small ADP-induced 'pull' on electrons along the ETC occurs, $\beta$-AR stimulation increases NAD(P)H, while MCU inactivation blunts this increase (Fig. 2*B*) (Luongo *et al.* 2015; Wu *et al.* 2015). In contrast, in working hearts of wild-type animals, the redox states of NADH/$NAD^+$ and NADPH/$NADP^+$ remained balanced after $\beta$-AR stimulation (Luongo *et al.* 2015), in agreement with the concept of the 'parallel activation' of 'push' and 'pull' conditions (Fig. 2*A*). In MCU-KO hearts, NADH/$NAD^+$ and NADPH/$NADP^+$ oxidized after $\beta$-AR stimulation (Luongo *et al.* 2015), indicating that 'pull' (through ATP consumption) now outweighs the 'push' (in the absence of $Ca^{2+}$-induced and Krebs cycle-mediated NADH production; Figs 1*A* and 2*B*). These data are in agreement with our previous results on beating guinea pig cardiac myocytes (Kohlhaas *et al.* 2010).

In experiments on the MCU-KO model, $Ca^{2+}$ activated respiration and ATP production in wild-type mitochondria that respired maximally in the presence of ADP ('state 3' respiration) (Kwong *et al.* 2015; Luongo *et al.* 2015). However, computational modelling predicted that during state 3 respiration, when respiratory control is dominated by the ADP-induced acceleration of electron flux along the ETC ('pull'), $Ca^{2+}$-induced activation of the Krebs cycle with increased NADH generation would only increase ATP production by 5% (Cortassa *et al.* 2003). In addition to this $Ca^{2+}$-mediated 'push' effect on NADH,

however, $Ca^{2+}$ can also directly activate the $F_1F_o$-ATPase by ~2-fold (Territo *et al.* 2000). Therefore, the observed $Ca^{2+}$-induced (and MCU-sensitive) acceleration of $O_2$ consumption rates (Kwong *et al.* 2015; Luongo *et al.* 2015) are therefore presumably related to both $Ca^{2+}$-induced activation the Krebs cycle and of the $F_1F_o$-ATPase (arrow in Fig. 2*A*).

One conflicting observation from studies on genetically modified mice deserves further considerations. While in both the conditional MCU-KO and the DN-MCU model, MCU inactivation *increased* cytosolic $Ca^{2+}$ transients in unloaded cardiac myocytes, it *decreased* (and/or delayed) the inotropic response to $\beta$-AR stimulation or an increase in stimulation rate in whole hearts (Kwong *et al.* 2015; Luongo *et al.* 2015; Rasmussen *et al.* 2015). It is unlikely that a decrease of myofilament $Ca^{2+}$ affinity accounts for this effect, since under baseline conditions, contractility was similar in wild-type and MCU-deficient mouse hearts. It may therefore be assumed that the more cardiac workload increases, NADH oxidation may actually limit electron supply of the ETC and thereby ATP production. This would further demand that decreases in ATP affect contraction and relaxation. In fact, the SR $Ca^{2+}$-ATPase is the enzyme that is most sensitive to a decrease in the free energy of ATP (Tian & Ingwall, 1996). Furthermore, the parallel oxidation of NADPH/$NADP^+$ may provoke excessive emission of $H_2O_2$ from mitochondria (Kohlhaas *et al.* 2010; Nickel *et al.* 2015), which may additionally hamper EC coupling (Wagner *et al.* 2013; Yang *et al.* 2015). This, however, remains to be addressed by future studies.

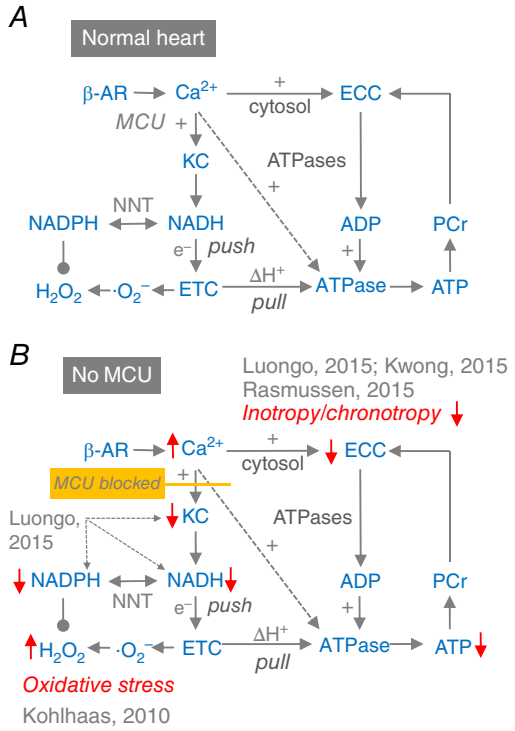


**Figure 2. The impact of deficient mitochondrial $Ca^{2+}$ uptake on mitochondrial redox state and energetics**
*A*, control of respiration from upstream by $Ca^{2+}$, which increases the availability of NADH through the Krebs cycle (KC) to 'push' electrons into the electron transport chain (ETC), and from downstream by ADP, which 'pulls' electrons ($e^-$) down the ETC to regenerate the proton gradient ($\Delta H^+$) that fuels the $F_1F_o$-ATPase. *B*, changes that occur in the control of respiration and redox state, according to experimental results by Luongo *et al.* 2015, Kwong *et al.* 2015, Rasmussen *et al.* 2015 and Kohlhaas *et al.* 2010. Abbreviations: ATPases, energy consumption by myosin ATPase, SR $Ca^{2+}$-ATPase, $Na^+/K^+$-ATPase and other ATP-consuming enzymes; ECC, excitation–contraction coupling; NNT, nicotinamide nucleotide transhydrogenase; PCr, phosphocreatinine. Red arrows indicate increases or decreases.

## Conclusions

There is now ample evidence for the tight and bidirectional interplay between cytosolic ion handling, mitochondrial redox regulation and ATP production in cardiac myocytes. Mitochondrial $Ca^{2+}$ uptake and its impact on the redox state of pyridine nucleotides is a central element of this interplay (Fig. 1*A*), and disease conditions that interfere at this level are likely to induce catastrophic events that induce contractile dysfunction, arrhythmias and maladaptive remodelling through oxidative stress and energetic deficit. The data of the novel mouse models have aided in further characterization of this interplay, but since the dynamic range of cardiac workloads is much smaller in the mouse than in humans, and mitochondrial $Ca^{2+}$ uptake is required for matching ATP supply to demand particularly during these variations, these results may still underestimate the true effects that may occur in humans when mitochondrial $Ca^{2+}$ uptake is impaired. Our growing understanding of the pathophysiology of these processes in heart failure may aid the development of novel mitochondria-directed treatment options to ameliorate disease progression in these patients.

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## Additional information

### Competing interests

None.

### Author contributions

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