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Mitochondrial oxidative metabolism and uncoupling proteins in the failing heart

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Abstract Despite significant progress in cardiovascular medicine, myocardial ischemia and infarction, progressing eventually to the final end point heart failure (HF), remain the leading cause of morbidity and mortality in the USA. HF is a complex syndrome that results from any structural or functional impairment in ventricular filling or blood ejection. Ultimately, the heart's inability to supply the body's tissues with enough blood may lead to death. Mechanistically, the hallmarks of the failing heart include abnormal energy metabolism, increased production of reactive oxygen species (ROS) and defects in excitation–contraction coupling. HF is a highly dynamic pathological process, and observed alterations in cardiac metabolism and function depend on the disease progression. In the early stages, cardiac remodeling characterized by normal or slightly increased fatty acid (FA) oxidation plays a compensatory, cardioprotective role. However, upon progression of HF, FA oxidation and mitochondrial oxidative activity are decreased, resulting in a significant drop in cardiac ATP levels. In HF, as a compensatory response to decreased oxidative metabolism, glucose uptake and glycolysis are upregulated, but this upregulation is not sufficient to compensate for a drop in ATP production. Elevated mitochondrial ROS generation and ROS-mediated damage, when they overwhelm the cellular antioxidant defense system, induce heart injury and contribute to the progression of HF. Mitochondrial uncoupling proteins (UCPs), which promote proton leak across the inner mitochondrial membrane, have emerged as essential regulators of mitochondrial membrane potential, respiratory activity and ROS generation. Although the physiological role of UCP2 and UCP3, expressed in the heart, has not been clearly established, increasing evidence suggests that these proteins by promoting mild uncoupling could reduce mitochondrial ROS generation and cardiomyocyte apoptosis and ameliorate thereby myocardial function. Further investigation on the alterations in cardiac UCP activity and regulation will advance our understanding of their physiological roles in the healthy and diseased heart and also may facilitate the development of novel and more efficient therapies.

Keywords Heart failure · Energy metabolism · Mitochondria · Reactive oxygen species · Proton leak · Uncoupling proteins

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Introduction

Despite significant progress in cardiovascular medicine, cardiovascular disease (CVD) remains the leading cause of combined morbidity and mortality in Western industrialized countries. According to the latest report from the American Heart Association, in the USA in 2008, CVD accounted for 32.8 % of all deaths. Major contributors are coronary artery disease, myocardial infarction (MI) and ischemic stroke, leading eventually to the final end point of heart failure (HF) [1, 2]. HF is a growing health problem, with a prevalence in the USA of almost 6 million, and is expected to reach 8.5 million by 2030 [3–6]. More than 1 million Americans with HF are hospitalized each year with a cost of approximately $40 billion/year [4]. Furthermore, the outcome for patients diagnosed with HF remains poor with approximately 50 % mortality within 4–5 years [7].

HF is 'a complex syndrome that results from any structural or functional impairment in ventricular filling or ejection of blood' [6]. Ultimately, this heart's inability to supply the body's tissues with enough blood may lead to death [8, 9]. Various types of heart damage, caused by myocardial ischemia, MI, pressure and work overload and genetic alterations, lead to cardiac remodeling progressing to HF [10–14]. The failing heart is characterized by impaired energy metabolism [15–20], increased production of reactive oxygen species (ROS) [21–24] and abnormal excitation–contraction coupling (ECC) [25–27].

HF is a highly dynamic pathological process, and observed alterations in substrate preference and energy metabolism depend on the disease progression [19, 20, 28–30]. In the early stages, cardiac remodeling plays an important compensatory, cardioprotective role with normal or slightly increased fatty acid (FA) β-oxidation (FAO), which provides 60–90 % of cardiac ATP production. However, upon progression of HF, FAO and mitochondrial respiratory activity decrease, resulting in cardiac ATP content reduction to 60–70 % of its physiological levels. As a compensatory response to decreased oxidative metabolism, glucose uptake and glycolysis are upregulated; however, this upregulation is not sufficient to compensate for the drop in ATP production [31–33].

Mitochondrial function is particularly important in the constantly energy demanding cardiomyocytes, in which mitochondria generate up to 90 % of cellular adenosine triphosphate (ATP) and occupy almost 1/3 of the cell volume of a cardiomyocyte [30, 34–37]. Over the past two decades, mitochondria have emerged not only as a powerhouse of the cell, but also as critical integrators of other essential cellular processes, such as cell death, contributing to health and disease [38–40]. Recent studies of cardiac mitochondria have convincingly demonstrated that the structural and functional alterations of these multifaceted organelles are implicated in the pathogenesis of various CVD, such as dysrhythmias, myocardial ischemia, cardiomyopathies and HF [15, 17, 41–47].

The double-membrane mitochondria use up to 90 % of O_2 consumed by the cell to mediate oxidative phosphorylation (OXPHOS), the process coupling the substrate oxidation to ATP synthesis. In this process, the electrons released upon oxidation of NADH (nicotinamide adenine dinucleotide, reduced) and $FADH_2$ (flavin adenine dinucleotide, reduced), the major products of the Krebs cycle, are transferred along the 'respiratory chain,' also known as the 'electron transport chain' (ETC), to O_2, the terminal electron acceptor [48–50]. The ETC consists of four multisubunit enzyme complexes I–IV, embedded in the inner mitochondrial membrane (IMM) and especially enriched in the cristae, and two soluble electron carriers, cytochrome *c* and coenzyme Q [51, 52]. According to Peter Mitchell's chemiosmotic theory, proposed more than 50 years ago, the electron transfer generates a proton gradient (ΔpH) across the IMM (protons outside and hydroxyl ions inside). ΔpH along with the electrical gradient ($\Delta\Psi_m$) forms the proton-motive force (Δp also denoted as $\Delta\mu_H$), which drives ATP synthesis from adenosine diphosphate (ADP) and inorganic phosphate (P_i) by the F_1F_0 ATP synthase (complex V) [53, 54].

However, OXPHOS is incompletely coupled, and protons can leak across the IMM and return to the mitochondrial matrix bypassing the F_1F_0 ATP synthase-mediated ATP production [54]. This proton leak can reach 20–70 % of the cellular metabolic rate in various cell types and depends on the presence of mitochondrial carrier proteins, the adenine nucleotide translocase (ANT) and uncoupling protein 1 (UCP1) in brown adipose tissue (BAT) [55–58].

Originally, UCP1-mediated proton conductance was believed to be a unique mechanism in BAT to generate heating, which Mitchell called 'protic heating,' evolutionarily acquired by mammals [54, 59–61]. However, it is currently well recognized that such proton leak uncoupling of OXPHOS occurs in other tissues, including the myocardium, and distinct UCP1 paralogues are present in various tissues and in all eukaryotic kingdoms: protists, fungi, plants and animals [62–65]. Growing evidence suggests that uncoupling proteins (UCPs) contribute to the regulation of mitochondrial ROS production associated with various disorders, including obesity, type 2 diabetes, insulin resistance, tumorigenesis, atherosclerosis, HF and aging [20, 64–67].

This review focuses on mitochondrial ROS generation in the healthy and failing heart and on the emerging role of UCPs in cardiac physiology and pathophysiology.

Oxidative stress and mitochondrial ROS production

In the heart, as one of the highest O_2-consuming organs, highly tuned balance between O_2 supply and consumption is vitally important to respond to physiological changes in workload and to pathological stresses, such as hypoxia, ischemia and excessive overload. ROS, including free radicals (e.g., superoxide [$O_2^{\cdot-}$] and hydroxyl [$^{\cdot}OH$]), and non-radical species (e.g., hydrogen peroxide [H_2O_2]), and reactive nitrogen species (RNS) (e.g., nitric oxide [NO] and peroxynitrite [$ONOO^-$]), are permanently generated from various intracellular sources [68–71].

Upon reduction of O_2, one electron is added resulting in the formation of $O_2^{\cdot-}$; the addition of the second electron converts $O_2^{\cdot-}$ into non-radical H_2O_2. The reduction of H_2O_2 in the presence of endogenous Fe yields the generation of OH via the Fenton reaction. OH can also be generated

through electron exchange between $O_2^{\cdot-}$ and H_2O_2 in the Haber–Weiss reaction. $O_2^{\cdot-}$ can also react with NO, leading to the production of $ONOO^-$, the highly toxic lipid-soluble RNS capable to damage multiple molecules, leading eventually to cell dysfunction and death [70, 72–74]. At the molecular level, ROS/RNS mediate cysteine and methionine thiol oxidation, arginine and proline hydroxylation and tyrosine nitration of various proteins and modifications of other molecules affecting a variety of redox-sensitive process.

Under physiological conditions, ROS/RNS exert a critical role of second messengers, inducing multiple signaling pathways essential for cardiac function [23, 70, 75]. In cardiomyocytes, physiological levels of ROS/RNS can activate mitogen-activated protein kinases (MAPKs), such as the extracellular signal-regulated kinase 1/2 (ERK1/2), p38 and Jun N-terminal kinase (JNK) [76, 77], as well as other protective kinases, such as phosphatidylinositol 3-kinase (PI3K), protein kinase B/Akt and PKC [78], contributing to cardioprotection against ischemia/reperfusion (I/R) injury (IRI) [75, 79, 80].

However, when ROS/RNS generation is sharply increased and overwhelms the cellular antioxidant defense system, the condition known as oxidative stress (OS), they cause oxidative damage to a plethora of cellular macromolecules [23, 70, 75]. Among them are subunits of the ETC complexes I, III and V [81, 82], multiple myocardial proteins implicated in ECC, such as the ryanodine receptor 2 (RYR2) [83–86], myosin heavy chains [87], sarcoplasmic reticulum (SR) Ca^{2+}-ATPase 2a (SERCA2a) [88–90], Ca^{2+}/calmodulin-dependent kinase II (CaMKII) [91], cAMP-dependent protein kinase A (PKA) [92–94], cGMP-dependent protein kinase G 1α (PKG1α) [94, 95], and ion channels and transporters, such as L-type Ca channels, the plasmalemmal Ca^{2+}-ATPase, the Na^+/Ca^{2+} exchanger [96, 97]. S-nitrosylation of GAPDH and caspase-3 contributes to the initiation of hyperglycemia and cardiomyocyte death in the diabetic heart [98]. Oxidation of histone deacetylases (HDACs) and transcription factors nuclear factor-kB (NF-kB) and hypoxia-inducible factor 1 (HIF1) modulate the transcription in response to OS [99–102]. A recent list of ROS/RNS-modified key myocardial proteins found in diabetic cardiomyopathy includes 30 entries [103].

In addition to myocardial proteins, membrane lipids, cardiolipin (CL) in particular, are also subject to ROS-induced damage [104]. CL is a phospholipid predominantly localized in the IMM and implicated in assembly and function of the ETC, apoptotic signaling and mitochondrial protein import [105–107]. Oxidation of CL results in its pathogenic remodeling, affecting mitochondrial respiratory activity and triggering mitochondria-dependent apoptosis [106, 108–112]. Finally, excessive oxidative damage to mitochondrial DNA (mtDNA), if overwhelms the repair capacity, can cause severe mitochondrial dysfunction and eventually cardiomyocyte death [113–117]. Accumulation of oxidized and nitrated/nitrosylated proteins and lipids and oxidative lesions in mtDNA can lead to myocardial remodeling and dysfunction culminating eventually in HF (Fig. 1).

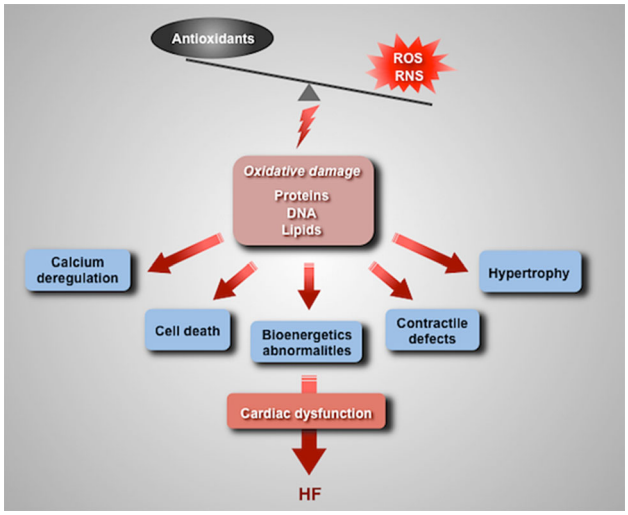

Fig. 1 Oxidative stress in the myocardium. Excessive generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which overwhelms the capacity of antioxidant system, results in oxidative damage to proteins, DNA and lipids. Oxidative damaged cellular molecules affect a variety of essential cardiac processes, including energy metabolism, excitation–contracting coupling, Ca^{2+} homeostasis and cardiomyocyte death. These detrimental alterations lead to myocardial remodeling and dysfunction eventually culminating in heart failure (HF)

In cardiomyocytes, the main sources of endogenously generated ROS are mitochondria, NADPH oxidases (NOXs), uncoupled NO synthases (NOSs) and xanthine oxidase (XO). This review focuses on mitochondria-generated ROS/RNS and their role in the pathogenesis of HF. Role of NOXs, NOSs and XO in myocardial physiology and pathophysiology has been discussed in detail in recent excellent reviews [23, 70, 75, 118].

Mitochondrial ROS

In cardiomyocytes, mitochondria are the major source of ROS, which are generated as by-product of electron flow through the ETC, predominantly at complexes I and III [64, 69, 119]. It is believed that complex I produces $O_2^{\cdot-}$ on the matrix side of the IMM, whereas complex III generates $O_2^{\cdot-}$ on both the matrix and intermembrane sides of the IMM (Fig. 2). Complex I (NADH:ubiquinone oxidoreductase), composed of approximately 45 subunits, promotes oxidation of NADH to NAD. The electrons from NADH are

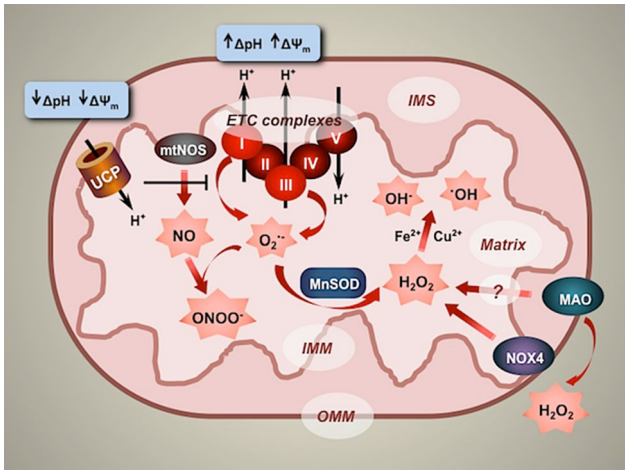

Fig. 2 Cardiac mitochondria are the main source of reactive oxygen species (ROS). Superoxide ($O_2^{\cdot-}$) is generated as by-product of electron flow through the electron transport chain (ETC), predominantly at complexes I and III. $O_2^{\cdot-}$ can further be converted spontaneously or by action of mitochondrial Mn^{2+}-dependent superoxide dismutase (MnSOD) to hydrogen peroxide (H_2O_2). NADPH oxidase 4 (NOX4), expressed in cardiomyocyte mitochondria, endoplasmic reticulum and nucleus, appears to generate predominantly H_2O_2 contributing to mitochondrial ROS. Monoamine oxidase (MAO), localized on the outer mitochondrial membrane (OMM), can also contribute to H_2O_2 production. The reduction of H_2O_2 in the presence of endogenous Fe^{2+} or Cu^{2+} yields the generation of hydroxyl radicals ($^{\cdot}OH$) and hydroxyl anions (OH^-). Mitochondrial nitric oxide synthase (mtNOS), embedded in the inner mitochondrial membrane (IMM), can generate nitric oxide (NO), which rapidly reacts with $O_2^{\cdot-}$ resulting in the production of peroxynitrite ($ONOO^-$). Mitochondrial uncoupling proteins (UCP), located in the IMM, promote proton (H^+) leak across the IMM from the intermembrane space (IMS) to the matrix, dissipating the proton gradient (ΔpH) and mitochondrial membrane potential ($\Delta\Psi_m$), generated by electron flow through the ETC, preventing excessive $O_2^{\cdot-}$ generation

accepted by the flavin mononucleotide (FMN) of complex I and are then transferred through a series of Fe–S clusters to ubiquinone (Q), resulting in its reduction to ubiquinol (QH_2) [120]. The FMN prosthetic group in the soluble arm and the Q-binding site is mainly responsible for $O_2^{\cdot-}$ generation by complex I [121–124].

Complex III (ubiquinol:cytochrome *c* oxidoreductase), composed of 11 subunits, passes electrons from the ubiquinol produced by complex I and II to cytochrome *c* [125, 126]. The Q_0 site of complex III is the major site responsible for ROS production [127]. Although the electron cycling process referred to the Q-cycle, in which lone electrons are reused to produce QH_2, prevents to utilize these electrons for $O_2^{\cdot-}$ production, complex III still remains the main source of ROS generation [127–130].

$O_2^{\cdot-}$ produced by complex I and III can further be converted spontaneously or by action of mitochondrial Mn^{2+}-dependent superoxide dismutase (MnSOD) or cytosolic Cu/ZnSOD to H_2O_2 [69, 71]. H_2O_2 is more stable than $O_2^{\cdot-}$ and can cross membranes and oxidize glutathione (GSH) and thiol residues on various proteins, including kinases, phosphatases and other enzymes, as well as transcription factors [131]. Thus, H_2O_2 can modulate multiple signaling pathways essential for cell adaptation. However, excessively produced H_2O_2 results in the generation of highly reactive OH, which causes cell damage contributing to IRI [21].

It has recently been shown that NOX4, which is expressed in cardiomyocytes and generates predominantly H_2O_2, is located in the endoplasmic reticulum, nucleus and mitochondria and therefore can be consider as a source of mitochondrial ROS [75, 132]. Myocardial ischemia and chronic pressure overload have activated NOX4 expression in mouse hearts [133–135]. Transgenic mice with cardiac-specific NOX4 overexpression have exhibited compromised left-ventricular function and elevated apoptosis and fibrosis upon aging [136]. Consistently, cardiac-specific $Nox4^{-/-}$ mice have shown the reduced ROS levels in the heart and attenuated apoptosis associated with improved mitochondrial and cardiac function upon pressure overload compared with wild-type animals [137]. Importantly, preclinical studies of inhibitors specific for NOX4 and other NOX isoforms for treatment various cardiovascular conditions are in progress [138, 139].

In addition to the ETC-generated ROS on the IMM, monoamine oxidases (MAOs), which appear to be localized on the outer mitochondrial membrane (OMM), represent another potential mitochondrial source of ROS (Fig. 2) [140]. These enzymes, present in two isoforms, MAO-A and MAO-B, produce H_2O_2 and catalyze oxidative deamination of catecholamines and biogenic amines (e.g., epinephrine, norepinephrine and serotonin) [141]. Although the role of MAOs in the pathogenesis of human HF remains yet to be determined, it has recently been reported that MAO-A appears to contribute to adverse cardiac remodeling in a mouse pressure-overload HF model [142]. Functional role of MAOs in mitochondrial ROS production and the pathogenesis of HF has to be addressed in the future studies.

The presence of NOS in mitochondria was first reported by Bates et al. [143] and subsequently confirmed by other laboratories [144–147]. Despite some initial controversy regarding the identity of mitochondrial NOS (mtNOS) [148–152], current evidence suggests that mtNOS is a splicing variant of nNOS, embedded into the IMM (Fig. 2) [153–156]. Furthermore, the dependence of mtNOS activity on the function of the ETC complex I suggests their association [157, 158]. Consistent with this hypothesis, it has more recently been shown that inhibition of either complex I or II or mtNOS has led to reduction in ROS production in HF cardiomyocytes [159].

mtNOS generates NO that reacts rapidly with $O_2^{\cdot-}$, resulting in the production of $ONOO^-$, a highly reactive short-lived peroxide, which can modify and inactivate several key mitochondrial proteins [160, 161]. $ONOO^-$ nitrates and inhibits activity of mitochondrial aconitase, an enzyme of Krebs cycle [162–164], and complexes I, II and V (ATP synthase), compromising mitochondrial bioenergetics [165, 166]. $ONOO^-$-mediated nitration of tyrosine 34 (Tyr-34) on MnSOD inactivates this essential antioxidant amplifying mitochondrial OS [167–169]. MnSOD Tyr-34 nitration has been detected in various CVD [170, 171]. Nitration of Tyr-74 on cytochrome *c* has resulted in its translocation to the cytosol and nucleus and might be related to apoptotic response [172, 173]. Importantly, nitrated subunits of complex I and V and oxidized/nitrated MnSOD have been detected in diabetic failing hearts [174–176].

Antioxidant systems

In the heart, non-enzymatic and enzymatic antioxidative defense systems are involved in the control of ROS/RNS levels [70]. Non-enzymatic antioxidants include β-carotene (a precursor of vitamin A), vitamins C (ascorbic acid) and E (α-tocopherol), GSH, lipoic acid, ubiquinol (coenzyme Q-10), urate, polyamines and polyphenols and other substances [70, 177, 178]. Ascorbic acid and GSH are the main aqueous non-enzymatic scavengers playing a key role in cellular redox homeostasis. Intracellular GSH provides efficient protection against $ONOO^-$ and $O_2^{\cdot-}$, and cellular susceptibility to $ONOO^-$ largely depends on GSH abundance [73, 177].

Cytosolic and mitochondrial SODs, catalase (CAT) and the mitochondrial thioredoxin (Trx)/peroxiredoxin (Prx)/thioredoxin reductase and GSH/glutathione peroxidase (GPx) systems represent the best characterized antioxidant enzymes protecting the myocardium against OS [70, 179].

Superoxide dismutases (SODs)

SODs are key antioxidant enzymes, which catalyze the very fast conversion ($2 \times 10^9\ M^{-1}\ s^{-1}$) of $O_2^{\cdot-}$ into molecular O_2 and H_2O_2 [180–182]. In humans, three types of SODs with regard to the metal cofactor they contain are known: Cu/ZnSOD, MnSOD and FeSOD. They have distinct structure and intracellular localization: Cu/ZnSOD is a homodimeric enzyme located in the cytosol (also known as SOD1 in humans) and in the extracellular space (also known as extracellular SOD or SOD3), while MnSOD (also known as SOD2) and FeSOD are homotetrameric enzymes located in the mitochondrial matrix and peroxisomes and in the extracellular space, respectively [183]. Mitochondrial MnSOD accounts for up to 90 % of total SOD activity in cardiomyocytes [70, 184].

Cu/ZnSOD deficiency in $Sod1^{-/-}$ mice has resulted in high levels of oxidative damage associated with a significant decrease in lifespan compared with wild-type animals [185, 186]. Importantly, mutations in human gene encoded Cu/ZnSOD have been shown to be associated with neurodegenerative disorder amyotrophic lateral sclerosis (also known as Lou Gehrig's disease) [187–189].

The essential role of mitochondrial MnSOD has been highlighted by studies on transgenic mice. Homozygous deletion of the *Sod2* gene encoded MnSOD in mice has led to death within the first week of life with cardiomyopathy, degeneration of neurons, lipid accumulation in the liver and oxidative mitochondrial damage [190–193]. The most severely affected tissues have been the high-energy demanding heart and brain. Heterozygous $Sod2^{+/-}$ mice have exhibited reduced MnSOD activity, elevated oxidative mtDNA damage in the heart and have developed cardiomyopathy during aging [114, 194]. Consistently, MnSOD overexpression has mediated cardioprotective effect against OS-induced cell death [195]. Furthermore, MnSOD deficiency has been shown to be associated with mtDNA damage and accelerate the development of atherosclerosis in $ApoE^{-/-}$ mice [196]. However, in contrast to data obtained from transgenic mice, current data on dynamics of cardiac SOD activity in patients with HF are controversial. Sam et al. [197] have reported decreased SOD activity associated with elevated ROS generation in the human failing heart, whereas others have failed to detect any significant changes in myocardial SOD levels or activities in patients with HF [198–200].

Catalase (CAT)

Mammalian CAT is a homotetrameric enzyme, which promotes the reduction of H_2O_2 to H_2O and O_2 [201–203]. As mammalian CAT is located in the peroxisomes and utilizes H_2O_2 generated during FAO in these organelles, it is thought to be not directly implicated in mitochondrial function. However, in some reports CAT has been found in cardiac mitochondria [204, 205]. Treatment of cells with H_2O_2 activates the Abelson (Abl) family of non-receptor tyrosine kinases, c-Abl and Arg, that phosphorylate CAT leading to its activation [206, 207]. To explore a role for CAT in OS response transgenic mice, which overexpress human CAT targeted to mitochondria (mCAT), have been generated [208]. mCAT animals have displayed reduced ROS production, oxidative mtDNA damage and deletion accumulation and extended life spans. Importantly, cardiac age-related alterations, including accumulation of mitochondrial protein oxidation, decreased cardiac SERCA2, increased mtDNA mutations and deletions and

mitochondrial biogenesis, increased ventricular fibrosis, and enlarged myocardial fiber size, have significantly been attenuated in mCAT mice [209–211]. Intriguingly, CAT-deficient mice display no marked abnormalities, leaving question on the precise role of CAT in OS response open to debate [212].

Peroxiredoxins (Prxs) and glutathione peroxidases (GPxs)

Both Prxs and GPxs play an important role in redox state regulation by catalyzing the reduction of H_2O_2 to H_2O and limiting thereby H_2O_2-induced OS [179, 213–215]. Among six mammalian Prx isoforms Prx3 and Prx5 are located in mitochondria. Prxs function through H_2O_2 mediated oxidation of its active cysteine site with subsequent reduction of the active site by Trx enabling Prxs to act as a H_2O_2 sensor [214, 215].

Mammalian cells express eight GPx isoforms, among them GPx1 is the predominant isoform and it is expressed in the myocardium. GPxs are tetrameric enzymes containing seleno-cysteine in the active site, which catalyze the reduction of H_2O_2 to H_2O via oxidation of GSH into its disulfide form (GSSH) [179]. Similar to CAT, GPx1 can be phosphorylated and activated by c-Abl and Arg [216]. Ablation of GPx1 in mice causes increased susceptibility to H_2O_2-mediated OS and to myocardial IRI [217, 218]. Furthermore, GPx1 deficiency accelerates atherosclerotic progression in ApoE$^{-/-}$ mice [219].

The role of Prxs and GPxs in ROS scavenging depends on their relative abundance within mitochondria and on levels of ROS. Highly abundant Prxs appear to be responsible for conversion of low (nanomolar) levels of H_2O_2 under physiological conditions, while similarly active but less abundant GPxs can compete with Prxs at elevated H_2O_2 concentrations upon OS [214].

Uncoupling proteins: structure, regulation and function

Mammalian cells have evolved multiple mechanisms to tightly regulate levels of mitochondrial ROS. In addition to ROS scavengers as the first line of defense, inducible mitochondrial H^+ leak across the IMM controlled by UCPs has emerged as an essential modulator of mitochondrial function. UCPs located in the IMM promote proton transport from the intermembrane space to the mitochondrial matrix dissipating ΔpH. This UCP-regulated mild uncoupling plays an important physiological role to avoid oversupply of electrons into the ETC adjusting energy metabolism and preventing excessive mitochondrial ROS generation [63–66, 220–222].

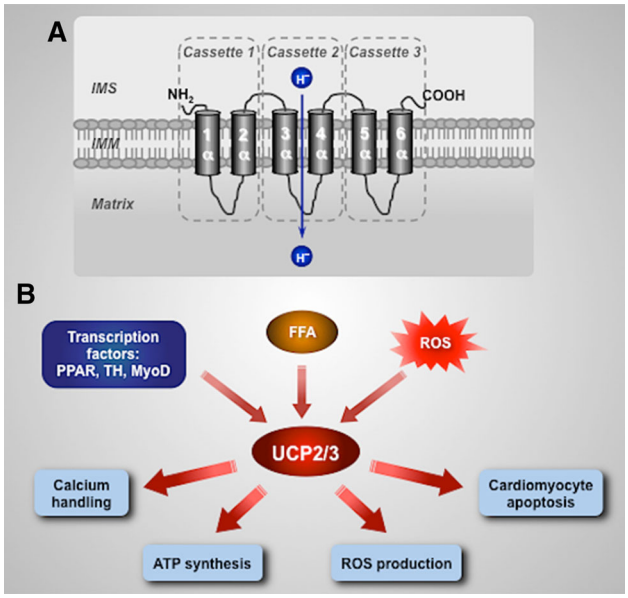

Fig. 3 Schematic representation of molecular structure and suggested physiological role of cardiac uncoupling protein 2 and 3 (UCP2/3). *a* Human UCPs share similar molecular structure. Six hydrophobic α-helices (1α through 6α), which span the inner mitochondrial membrane (IMM), are arranged into three cassettes. The N- and C-termini and two loops, which connect cassettes, are oriented toward the intermembrane space (IMS), while three long loops that connect α-helices within each cassette face the matrix. UCP α-helices appear to be arranged to create a channel within the IMM mediating proton (H^+) leak across the IMM, while the loops control access to the channel. *b* Transcription factors (e.g., peroxisome proliferator-activated receptor [PPAR], thyroid hormone [TH], myogenic differentiation antigen [MyoD]), free fatty acids (FFA) and reactive oxygen species (ROS) regulate UCP expression in the heart. Although the physiological role of cardiac UCPs is not clearly defined, growing evidence suggests that they are involved in the regulation of energy metabolism, ROS generation, Ca^{2+} handling and cardiomyocyte apoptosis contributing to cardiac physiology and pathophysiology. See text for details

The UCP family

UCPs constitute a subfamily of the mitochondrial solute carrier 25 (SLC25) protein family, which contains over 40 members that mediate transport of broad range of molecules [223]. These ubiquitous eukaryotic trans-membrane proteins with molecular masses of 31–34 kDa are metabolite transporters, sharing similar molecular structure. UCP molecule is composed of six hydrophobic membrane-spanning α-helices, arranged into three cassettes. The amino and carboxyl termini and two loops, which connect cassettes, are oriented toward the intermembrane space, while three long loops that connect α-helices within each cassette face the matrix (Fig. 3) [63, 65, 224–227]. UCP α-helices appear to be arranged to create a channel within the IMM, while the loops are implicated in control of access to the channel [228]. Furthermore, UCPs contain

also a binding site for purine nucleotides, which inhibit their uncoupling activity. Three arginine residues, Arg82, Arg182 and Arg276, conserved in UCP paralogues appear to play the crucial role in purine nucleotide binding [229].

The first UCP, UCP1 (SLC25A7), was identified in BAT more than 30 years ago and has since become the canonical most characterized UCP [60, 230, 231]. UCP1 dissipates ΔpH to produce heat required to maintain body temperature in mammals [58, 232, 233]. Subsequently, four paralogues of UCP1, UCP2 through UCP5, have been indentified in fungal, plant and animal kingdoms [63, 226, 227, 234]. Mammalian cells express all five UCP paralogues, which share sequence similarities, but have different tissue distribution. UCP1 is predominantly expressed in BAT and upon hyperglycemia is also expressed in white adipose tissue, skeletal muscle, retinal cells and pancreatic β cells [58, 59, 235, 236]. Human UCP2 (SLC25A48) and UCP3 (SLC25A9) have high sequence identity with UCP1: 59 and 57 %, respectively, and ∼70 % identity with each other [234]. UCP2 is widely expressed in various tissues, including central nervous system, kidney, macrophages, pancreas, spleen and thymus [237–239]; its expression in the heart is the issue of debate [240, 241]. UCP3 is predominantly expressed in skeletal muscle and BAT; it is also present in the heart albeit at lower levels compared with skeletal muscle [242–244]. Despite high sequence similarity of UCP2 and UCP3 with UCP1, they are not implicated in adaptive thermogenesis and their physiological role is largely unknown [63, 245–247].

UCP4 (SLC25A27) and UCP5 (SLC25A14; also known as BMCP1) have less sequence identity with UCP1: ∼30 and 33 %, respectively; these UCPs are expressed mainly in the brain [63, 234, 248–252]. It has been hypothesized that the *UCP4* and *UCP5* genes are more closely related to the ancestral gene, which possibly encoded a primitive ADP/ATP transporter. Highly homologous to each other UCP1, UCP2 and UCP3 have evolved later during evolution [253]. Similar to UCP2 and UCP3, the physiological functions of human neuronal UCP4 and UCP5 remain yet largely uncertain [254].

UCP activity and its regulation

All mitochondrial UCPs promote H^+ conductance dissipating ΔpH and affecting thereby mitochondrial function, although the precise mechanism of their action is still not fully understood. UCP1, which is highly abundant in BAT and implicated in the regulation of non-shivering thermogenesis, represents the archetypical UCP and our knowledge of the mechanism of the UCP-mediated proton conductance has mainly derived from studies of this protein. UCP1 is tightly regulated at several levels, including acute regulation of its uncoupling activity, transcriptional control of the *Ucp1* gene expression and control of its degradation [65, 67].

Small molecules, such as FAs, ROS and purine nucleoside di- and tri-phosphates, exert acute regulation of UCP1 activity. Although the precise mechanism remains to be determined, three models of FA activation of the UCP-mediated proton leak have been proposed. According to the first 'cofactor' model, UCP1 functions as a H^+ translocator, while anionic FAs (FA^-) act as cofactors by associating with UCP1 and forming a H^+-conducting channel [255–257]. In the second model, called the FA-cycling or flip-flop model, UCP1 functions as FA^- carrier exporting FA^- from the mitochondrial matrix. In the intermembrane space, exported FA^- are protonated and diffuse back into the mitochondrial matrix, where the accepted protons are released [258]. Although according to this model UCP1 does not directly translocate H^+, this cycle leads to a net H^+ uptake into the matrix. Finally, given that the effect of FAs and purine nucleotides on H^+ leak can be described by a simple competitive kinetics, the competition model suggests that FAs are not directly implicated in the H^+ translocation, but rather act as allosteric activators influencing UCP1 conformation [259, 260].

At the molecular level, UCP1 activity is acutely stimulated not only by free FAs, but also by ROS ($O_2^{\cdot-}$ and lipid peroxidation products) and inhibited by purine nucleoside di- and tri-phosphates, such as ADP, ATP, GDP and GTP [65–67, 261]. The mechanism of the acute ROS-mediated activation of UCPs is unclear and is currently debated. It has been suggested that $O_2^{\cdot-}$ promotes peroxidation of membrane phospholipids resulting in the formation of 4-hydroxy-2-nonenal (4-HNE), which acts as a proximal activator of UCPs [262–265]. However, this model is not widely accepted and controversial observations exist [67, 247, 266–268].

Similarly, the precise mechanisms underlying inhibition of uncoupling activity by purine nucleotides remain to be determined. High affinity of UCPs to purine nucleotides with binding constants in the micromolar range and the millimolar concentrations of purine nucleotides in the cell raise the question how FAs can overcome this inhibition in vivo [63, 65, 260, 262, 269, 270].

More recently, distinct regulatory mechanisms for UCP1 and UCP2 and 3 have been suggested [64, 246]. Elevated ROS levels stimulate proton leak mediated by UCP2 and UCP3 leading subsequently to attenuation of ROS generation and creating a negative feedback loop [271, 272]. UCP1 is mainly activated by elevated free FAs in response to sympathetic neuronal stimulation resulting in mitochondria-promoted ROS generation [272, 273]. Furthermore, in vitro studies have shown that the reversible glutathionylation of UCP2 and UCP3

contributes to the regulation of mitochondrial ROS production [271]. Under OS condition, high ROS levels result in the glutathionylation of UCP2 and UCP3 inhibiting their uncoupling activity, while lower physiological levels of ROS lead to deglutathionylation of these proteins activating proton leak and attenuating thereby ROS production. Two cysteine residues Cys^{25} and Cys^{259}, located in the first transmembrane region and the last loop facing the matrix, respectively, are main sites for the regulation of UCP3 function by glutathionylation. Given high similarities in amino acid sequence between UCP2 and UCP3, it has been suggested that UCP2 is regulated in a similar fashion [271]. Consistently, ROS-induced proton leak has been observed in the primary cells from wild-type but not from $UCP2^{-/-}$ or $UCP3^{-/-}$ mice [271]. Although UCP1 and UCP2/3 share high homology in cysteine residues, UCP1 activity appears to be not regulated by glutathionylation and is not involved in the control of mitochondrial ROS generation [271]. However, a physiological significance of this novel regulatory mechanism remains to be determined.

UCP levels are also highly regulated at the transcriptional level. In response to cold acclimation or chronic overfeeding, BAT sympathetic neurons release catecholamines (e.g., noradrenaline), which engage β_3-adrenoceptors. Induced β_3-adrenergic signaling activates adenylyl cyclase to produce cyclic AMP (cAMP). Elevated cAMP activates in turn PKA. PKA-promoted phosphorylation and activation of triacylglycerol lipase results in stimulation of lipolysis leading to the elevation of FA levels [274, 275]. Furthermore, cAMP-mediated signaling cascades control expression of the *Ucp1* gene. A cAMP-responsive enhancer region upstream of the *Ucp1* gene has binding sites for transcription factors belonging to the nuclear receptor family, such as the peroxisome proliferator-activated receptor γ (PPARγ), retinoic acid receptor (RXR) and thyroid hormone receptor (TR) [276–278].

The *Ucp2* gene expression is also induced by PPARs as well as the sterol regulatory element-binding protein-1c (SREBP-1c) and forkhead transcription factors [279–283]. Furthermore, *Ucp2* expression is upregulated by FAO and ROS contributing to cellular defense against OS [284–286]. Similarly, *Ucp3* translation is stimulated by free FAs mediated through PPARs and the myogenic regulatory factor MyoD, thyroid hormone, retinoic acid and tumor necrosis factor α [287–291]. Furthermore, starvation-induced *Ucp3* expression appears to be conserved in vertebrates including mammals [292, 293]. Finally, SIRT1, a protein involved in metabolic stress resistance, suppresses expression of both *Ucp2* and *Ucp3* genes and activates insulin secretion [294, 295].

Ucp2 and *Ucp3* expression is also controlled at the translational level. The 5′ untranslated region of *Ucp2* mRNA contains an inhibitory upstream open reading frame (ORF). Glutamine, an amino acid involved in insulin secretion, overcomes inhibitory effect of the ORF and upregulates *Ucp2* mRNA translation [239, 296, 297]. Although *Ucp3* mRNA translation is currently less studied, the 5′ untranslated region of *Ucp3* mRNA also contains pseudo-start codons that can affect its translation [67].

Intracellular levels of UCPs are also regulated by proteolytic degradation. In BAT, UCP1 is relatively slowly degraded with a half-life on the order of days. Furthermore, noradrenergic stimulation upregulates not only UCP1 synthesis but also significantly extends its half-life [298, 299]. Although the precise mechanisms of the UCP1 turnover are largely unknown, its half-life is similar to those of other mitochondrial proteins, mirrors whole mitochondrial turnover and is extended upon lysosomal inhibition [299, 300].

In contrast to UCP1 and other mitochondrial carriers, including ANT, UCP2 and UCP3 are characterized by rapid degradation with very short half-lives of approximately 1 and 1–4 h for UCP2 and UCP3, respectively [239, 286, 301, 302]. Experiments with isolated energized mitochondria have suggested that non-mitochondrial factors are needed for rapid proteolytic degradation of these UCPs. Indeed, the cytosolic proteosome promotes rapid degradation of UCP2 and UCP3, while UCP1 and ANT are not degraded by this machinery [302, 303]. In fact, this has been the first demonstration that the IMM proteins are degraded by the cytosolic ubiquitin-proteosome system. Similar to highly coordinated control of UCP1 synthesis and degradation, cellular levels of UCP2 and UCP3 may be also regulated in a concerted manner [63, 67]. However, the precise mechanisms of these regulatory processes remain to be determined.

Importantly, the components involved in the insulin secretion pathway in the pancreatic β-cells are also under control of the cytosolic ubiquitin-proteosome system [304–306]. Therefore, rapid turnover of UCP2 and UCP3 expressed in the pancreatic β-cells may serve for rapid response to changes in nutrients in coordinated fashion with other proteins implicated in the same pathway [63, 67, 239]. Moreover, upregulation of UCP2, which has been detected in type 2 diabetes mellitus, may be linked to the proteosome system dysfunction [63, 307]. Physiological role and regulation of UCP3 and UCP2, which is particularly highly expressed in the pancreatic β-cells in type 2 diabetes mellitus, have been discussed in the several recent reviews [308–311].

Taken together, emerging distinct mechanisms underlying the regulation of UCP1 compared with UCP2 and UCP3 highlight the divergent physiological roles, which these proteins play. Function of UCP1 in the regulation of adaptive thermogenesis is well documented, whereas

complex roles of UCP2 and UCP3 in the control of mitochondrial ROS generation and ROS signaling have to be further explored.

UCP 2 and UCP3 in the healthy and failing heart

It has already been discussed that HF as the terminal point of various CVD is characterized by abnormal bioenergetics and profound mitochondrial dysfunction, associated with excessive ROS production. Therefore, it is not surprising that several studies have focused on UCPs as regulators of these processes in the HF setting as well as on their use as potential therapeutic targets in treatment of HF. However, number of reports on UCP in various CVD is relatively limited and different laboratories have reached controversial conclusions regarding the functional roles of UCPs in cardiac physiology and pathophysiology.

In contrast to UCP1 present predominantly in BAT, highly homologous UCP2, found in various mammalian tissues, and UCP3 are expressed in the mammalian myocardium [237, 238, 240, 242, 312–314]. Cardiac UCP2 expression appears to be species specific; in the mouse heart, higher levels of UCP2 have been observed compared with those present in the human heart [237, 240]. UCP3 is a major UCP isoform expressed in skeletal muscle and in the heart, although its levels in the latter are lower [315]. The physiological role of UCP2 and UCP3 in the heart is not yet clearly defined; however, emerging evidence suggests that they are implicated in the regulation of cardiac energetics, mitochondrial ROS production, Ca^{2+} handling and cardiomyocyte death [241, 316, 317].

Several observations link cardiac UCPs, UCP3 in particular, and cardiac energy metabolism. Fasting and caloric restriction have been shown to modulate cardiac UCP3 expression. Fasting has induced a significant (1.5- to 3-fold) increase in UCP3 transcript levels in rat hearts [318, 319]. Similarly, caloric restriction has been associated with alterations in transcriptional profiles including upregulation of UCP3 expression in mouse hearts [320].

Thyroid hormone influences cardiac metabolism and bioenergetics [321]. It has been reported that thyroid hormone induces UCP2 and UCP3 expression at both mRNA and protein levels [317, 322–324]. Intriguingly, in the heart, thyroid hormone has upregulated UCP3 and possibly UCP2, but this upregulation has not been associated with increased respiratory uncoupling and inhibition of ATP production [325, 326]. Thus, the role of cardiac UCPs in the thyroid hormone-mediated heart energy metabolism remains to be determined.

Analyses of alterations in UCP2 and UCP3 expression in various cellular and animal models as well as in patients with CVD have provided to a certain degree contradictory data (Table 1). Some studies have demonstrated that both UCP2 and UCP3 are downregulated in the rat and human failing heart. In a rat model of HF induced by pressure overload, levels of UCP2 and UCP3 transcripts have been significantly decreased compared with control rats [319]. Furthermore, transcript levels of two other regulators of FAO and mitochondrial metabolism, pyruvate dehydrogenase kinase 4 (PDK4), malonyl-CoA decarboxylase (MCD), have also been reduced [327]. Expression of all these proteins are regulated by PPARα, consistently PPARα has been downregulated in hypertrophic and failing hearts [319, 327, 328]. The authors have suggested that downregulation of PPARα axis plays an adaptive cardioprotective role to prevent severe cardiac contractile dysfunction [327].

Metabolic expression profile has been analyzed in human fetal, non-failing (donor) and failing hearts. In failing hearts, the expression of UCP2 and to some extent UCP3 along with glucose transporter GLUT1 and GLUT4, energy metabolism enzymes (e.g., carnitine palmitoyl transferase, citrate synthase, pyruvate dehydrogenase kinase) and myosin heavy chains have been downregulated to the levels observed in fetal hearts [343]. Intriguingly, in the subsequent study, significantly reduced mRNA levels of UCP3, but not UCP2, have been detected in patients with HF awaiting heart transplantation upon placement and subsequent removal of a left ventricular assist device (LVAD) [344]. Importantly, UCP3 expression has been normalized with mechanical unloading after the LVAD treatment. Unfortunately, UCP2 and UCP3 protein levels have not been analyzed; therefore, it is not clear whether observed alterations in mRNA levels are translated into changes in protein concentrations.

Using a rat HF model caused by aortic regurgitation (AR), more complex dynamics of UCP2 expression in the failing heart has been shown. Initial decrease in UCP2 mRNA levels has changed to a significant increase in UCP2 expression in the late stage of HF [335]. Furthermore, upregulation of proinflammatory cytokine TNF-α has been detected in the late, but not early, stage of the development of HF. Authors have hypothesized that elevated TNF-α might be responsible for the induction of cardiac UCP2 expression in the late, chronic phase of HF. At the chronic stage of HF, increase in the cardiac expression of UCP2 has been accompanied by significant reduction of the high-energy phosphate, creatine phosphate (CrP), implying a decrease in energy efficiency in failing hearts [335]. In the follow-up study, authors have reported that elevated cardiac UCP2 expression, observed in the chronic stage of AR-induced HF, could be suppressed by the angiotensin-converting enzyme (ACE) inhibitor perindopril. Importantly, perindopril has also normalized CrP levels [336]. Unfortunately, as in previous studies, dynamics of UCP2 protein levels has been not analyzed.

Table 1 UCP2 and UCP3 in cellular and animal models for myocardial ischemia and heart failure

Model	UCP2/3 alterations	Effects/comments	References
Rat neonatal cardiomyocytes	UCP2 overexpression	Prevention of H_2O_2-induced mitochondrial potential loss, attenuation of Ca^{2+} overload and apoptosis	[329]
Rat cardiac H9c2 cells	UCP2 and 3 depletion by RNAi	UCP2, but not UCP3, depletion alone reduces tolerance to IRI due to increased ROS; however, the highest reduction was upon UCP2 and 3 depletion	[330]
Rat adult cardiomyocytes	UCP2 overexpression	Reduction in ATP production, increase in pro-apoptotic protein BNIP3 associated with lower survival after IRI	[331]
$UCP2^{-/-}$ mice	Loss of UCP2	UCP2 expression depends on PPARγ-PGC1α axis. Chronic PPARγ stimulation upregulates UCP2, leading to mild mitochondrial membrane depolarization and reduced ROS generation	[332]
Cardiac $UCP2^{-/-}$ and $UCP3^{-/-}$ mice	Loss of UCP2 or UCP3	$UCP2^{-/-}$, but not $UCP3^{-/-}$, hearts produce more ROS during I/R, display impaired cardiac energetics and poor recovery during IRI	[333]
$UCP3^{-/-}$ mice	Loss of UCP3	$UCP3^{-/-}$ hearts exhibit increased oxidative coupling efficiency, ROS generation, apoptosis, and larger IRI-induced infarct size	[334]
Rat pressure-overload HF	Reduced UCP2 mRNA and 3	UCP2 and 3 protein levels have not been assessed. Other PPARα-controlled regulators of FAO have also been downregulated	[319, 327]
Rat aortic regurgitation HF	Initial decrease in mRNA UCP2 then its increase at the late stage of HF	Elevated UCP2 expression has been accompanied by reduction of CrP. Increase in UCP2 transcription may be mediated by elevated TNF-α, observed also at the late stage of HF. Alterations in both UCP2 and CrP could be normalized by perindopril	[335, 336]
Rat doxorubicin-induced HF	Reduced UCP2 and 3	Mitochondria isolated from failing hearts have exhibited greater coupling and ATP synthesis and increased ROS generation	[337]
Rat myocardial ischemia	Increased UCP2 and 3	Isolated mitochondria have had increased proton leak and reduced ROS production and infarct size	[330]
Rat Dahl salt-sensitive HF	Increased UCP2 mRNA	Reduced ATP production, upregulated pro-apoptotic protein BNIP3 associated with augmented cardiomyocyte death	[331]
Rat chronic HF	Increased UCP3	Increased UCP3 may be induced by elevated FA. Mitochondria have exhibited greater uncoupling and reduced efficiency	[338]
Rat acute myocardial IRI	Increased UCP2 in ischemic area; increased UCP3 in ischemic and non-ischemic area	UCP2 has been increased at protein level only, while UCP3 has been increased at both mRNA and protein level. I/R-induced UCP2 upregulation can be suppressed by losartan and ramiprilat	[339, 340]
Porcine chronic myocardial ischemia	Increased UCP2, unchanged UCP3	Isolated mitochondria have exhibited mild membrane depolarization and reduced ROS generation	[341]
Dog acute myocardial ischemia	Increased UCP3	Elevated UCP3 has been found in the non-ischemic wall. Mitochondria within this area have been uncoupled, ROS have been increased	[342]

CrP creatine phosphate, *FA* fatty acid, *FAO* FA oxidation, *HF* heart failure, *I/R* ischemia/reperfusion, *IRI* ischemia/reperfusion injury, *PGC1α* peroxisome proliferator-activated receptor γ coactivator 1α, *PPARγ* peroxisome proliferator-activated receptor γ, *UCP* uncoupled protein

More recently, in rat HF induced with intraperitoneal injections of chemotherapeutic agent doxorubicin, significant reduction in both UCP2 and UCP3 protein levels have been demonstrated [337]. Consistently, mitochondria isolated from failing hearts are characterized by greater coupling between citric acid cycle flux and ATP production. However, the beneficial effects of UCP2 and UCP3 downregulation on mitochondrial bioenergetics have been counteracted by augmented ROS generation observed in this HF model [337]. The mechanism responsible for UCP2 and UCP3 downregulation is also unknown.

It is well known that myocardial ischemia and infarction leading eventually to HF are associated with significant augmentation of circulating free FA levels [29, 345]. In contrast to UCP2 and UCP3 downregulation in HF observed in cited above reports, several studies have shown that elevated plasma free FA levels in HF are associated with upregulation of UCP2 and UCP3 in the failing heart. In animal models and in patients with HF, increased plasma long-chain FAs have activated PPARα leading to increased cardiac UCP mRNA and protein concentrations and reduced glucose transporter GLUT4 [240, 346–348]. Consistent with the regulatory role of PPARα, increased FA levels have been associated with UCP3 upregulation in wild-type mice but not in PPARα-deficient animals [348]. In the failing heart, elevated UCP2 and UCP3 increase mitochondrial uncoupling to attenuate ATP synthesis, while reduced GLUT4 downregulates glucose uptake. In addition, FAs may also act as activators of UCP activity further increasing mitochondrial uncoupling [349]. Similarly, in animal models of diabetic cardiomyopathy, elevated plasma free FA levels have been associated with significant upregulation of FA transporters (e.g., FATP and CD36) and UCP3 [325, 348, 350–354]. Moreover, significant increase in UCP2 mRNA levels, associated with reduced ATP production, has been detected in the Dahl salt-sensitive rat HF model [331].

In a rat model for chronically infarcted heart, UCP3 alterations along with mitochondrial respiration and efficiency of the isolated working heart have been measured [338]. Increased UCP3 levels in the failing heart have positively correlated with FA concentrations in the plasma. UCP3 upregulation has been associated with greater mitochondrial respiratory uncoupling and low efficiency in the failing heart [338]. Although augmented UCP-mediated respiratory uncoupling appears to underlie mitochondrial and cardiac dysfunction, it is not clear whether these alterations play adaptive or detrimental role in the development of HF.

Myocardial ischemia and infarction leading to HF are characterized by IRI, which affect cardiac energy metabolism, induce ROS generation, Ca^{2+} overload, acidosis and cardiomyocyte death [355, 356]. Mild mitochondrial uncoupling through UCP-mediated proton leak can play a protective role against myocardial IRI. One of the first direct evidence of a role of UCP3 in cardioprotection against I/R has recently been reported [333]. Ex vivo induced IRI in *UPC3*$^{-/-}$ mouse hearts has resulted in poorer recovery of LV contractile function compared with wild-type mouse hearts under I/R conditions. Interestingly, isolated *UPC2*$^{-/-}$ and wild-type mouse hearts have displayed a similar recovery of LV function, suggesting that UCP2 function is less essential for protection against cardiac IRI [333]. Using in vivo occlusion of the left coronary artery, these authors have further demonstrated that *UPC3*$^{-/-}$ mice have twofold larger infarct size and higher incidence of I/R dysrhythmias than wild-type animals. Moreover, I/R has induced more severe alterations in cardiac energetics associated with more prominent increase in ROS generation in *UPC3*$^{-/-}$ hearts compared with wild-type hearts. Pretreatment of *UPC3*-deficient hearts with the uncoupling drug carbonyl cyanide *p*-(trifluoromethoxy) phenylhydrazone has ameliorated recovery after IRI. Finally, ischemic preconditioning has been completely abolished in *UPC3*-deficient hearts further confirming an essential role of UCP3 in cardioprotection against IRI [333].

In a porcine model of chronic myocardial ischemia, UCP2 protein levels have been found to be significantly increased within ischemic region, while UCP3 protein levels have not been changed. Importantly, mitochondria isolated from ischemic myocardium have displayed stress-resistant state characterized by mild uncoupling and reduced ROS production [341]. More recently, these authors using transgenic mouse model have demonstrated that UCP2 upregulation depends on the PPARγ-PGC1α axis. Chronic stimulation of PPARγ with its agonist pioglitazone has resulted in twofold increase in nuclear-located PGC1α and UCP2 levels [332]. Furthermore, isolated cardiac mitochondria with PPARγ-mediated UCP2 upregulation have displayed mild IMM depolarization and reduced ROS generation. These beneficial effects have not been detected in *UPC2*-deficient mice, suggesting that observed cardioprotection against IRI depends on UCP2 [332].

Acute MI in dog has led to significant upregulation (~1.7- and 3-fold 6 and 24 h post-infarction, respectively) of levels of UCP3 protein, the main cardiac UCP in dog [357], in the non-ischemic wall of the right ventricle (RV) [342]. Mitochondria within the non-ischemic wall have been uncoupled and levels of ROS have significantly been elevated. Authors have hypothesized that an increase in ROS induced by acute MI is responsible for the adaptive UCP3 upregulation [342].

It is well established that the heart can be protected against IRI by ischemic conditioning—brief repetitive cycles of ischemia delivered before or after the ischemic event [358–362]. Emerging evidence suggests that myocardial UCPs may be implicated in this complex process. It has recently been shown that cardiac ischemic preconditioning triggers upregulation of UCP2 and UCP3 on both mRNA and protein levels [330]. Mitochondria from preconditioned hearts have displayed increased proton leak associated with decreased ROS production. Significantly, UCP upregulation has been associated with reduced infarct size in preconditioned rat hearts. Furthermore, UCP depletion by RNA interference (RNAi) in rat cardiac H9c2

cells has resulted in attenuation of preconditioning of these cells and augmentation of ROS generation [330]. Similar ischemic preconditioning-induced upregulation of UCP2 have been demonstrated in the brain, where elevated UCP2 levels protect against neuronal ischemic injury possibly through attenuation of ROS generation [363, 364].

More recently, levels of cardiac UCP2 have been measured following acute cardiac I/R in rats. Acute myocardial I/R has induced significant increase in UCP2 protein levels in the ischemic area of the left ventricle (LV) but not in the RV [339]. Interestingly, the angiotensin type 1 receptor blocker losartan and the ACE inhibitor ramiprilat could suppress UCP2 expression induced by myocardial I/R protecting the heart against IRI. In the follow-up study, these authors have extended their analyses to UCP3 in cardiac I/R setting. Protein levels of both UCP2 and UCP3 have been significantly increased as an early response to acute myocardial I/R in rats [340]. However, the mechanism underplaying this upregulation appears to be different for UCP2 and UCP3. First, UCP2 has been upregulated only in ischemic area of the LV, while UCP3 has been increased in both ischemic area of the LV and non-ischemic region of the RV [340]. Authors have hypothesized that local upregulation of both UCP2 and UCP3 in ischemic region may be caused mainly by elevated ROS. A more global cardiac upregulation of UCP3 might be due to a higher responsiveness of UCP3 expression to elevated circulating free FAs observed in rats with HF [338]. Second, I/R-induced UCP2 upregulation has been shown on protein level, but not on UCP2 mRNA level, while upregulation of both UCP3 mRNA and protein has been detected [340].

Myocardial ischemia leading eventually to HF causes cardiomyocyte death resulting in significant cardiomyocyte loss, which represents the main prognostic parameter in the disease progression [365, 366]. Cardioprotective roles of UCPs against IRI may be linked to their involvement in cardiomyocyte death. UCP1 overexpression in cultured heart-derived H9c2 cells has limited ROS generation after I/R and prevented ROS-induced cell death preserving mitochondrial structure and function [367]. Consistently, using adenovirus-mediated transfection of cultured neonatal rat cardiomyocytes, it has been demonstrated that the overexpression of human UCP2 protects these cells from OS induced by H_2O_2 [329]. UCP2 overexpression has prevented mitochondrial membrane potential loss, ROS generation and Ca^{2+} overload. In addition, elevated UCP2 levels have attenuated the appearance of mitochondria-mediated apoptosis markers, such as TUNEL positivity, phosphatidyl serine exposure, propidium iodide uptake, and caspase-3 cleavage [329].

On the other hand, the overexpression of UCP2 in primary adult rat cardiomyocytes has led to controversial results [331]. Although UCP2 overexpression in these cells has resulted in significant decrease in ATP production and acidosis as well as in upregulation of pro-apoptotic protein BNIP3, cell survival at baseline has not been affected. However, UCP2-overexpressing cells have displayed lower survival after I/R compared with control cardiomyocytes. Furthermore, authors have demonstrated using a rat HF model the significant upregulation of UCP2 and BNIP3 in failing hearts [331]. Authors have concluded that under used experimental conditions UCP2 plays a detrimental role in cardiomyocyte survival in HF. However, they have suggested that it might have a protective effect under different conditions and/or in other species.

More recently, role of UCP3 in cell death induced by myocardial ischemia leading to HF has been studied using $UPC3^{-/-}$ mice [334]. $UPC3^{-/-}$ mouse embryonic fibroblasts and cardiomyocytes have displayed mitochondrial dysfunction, increased ROS generation and apoptosis under hypoxia. Infarct size has been larger in $UPC3^{-/-}$ mice and these animals have exhibited lower survival compared with wild-type animals. Treatment with the antioxidant agent α-tocopherol has decreased infarct size in $UPC3^{-/-}$ hearts to values found in wild-type hearts. $UPC3^{-/-}$ hearts have been characterized by elevated of oxidative damage markers (e.g., TUNEL positive nuclei, p53 and cleaved caspase-3 levels). Finally, mitochondrial structural and functional abnormalities and elevated ROS production in ischemic $UPC3^{-/-}$ hearts have occurred despite a normal UCP2 upregulation at mRNA and protein level [334]. Thus, these findings suggest a cardioprotective role of UCP3 in the ischemic heart.

Cardiac mitochondria play a critical role in Ca^{2+} handling in cardiomyocytes, which is vital for myocardial ECC and for proper cardiac function [368–370]. Data on the involvement of UCP2 and UCP3 in mitochondrial Ca^{2+} uniport activity in non-cardiac cells have been controversial [371, 372]. Using adenovirus-mediated UCP2 delivery into neonatal rat cardiomyocytes, Turner et al. [373] have demonstrated that UCP2 overexpression has suppressed mitochondrial Ca^{2+} uptake exerting thereby detrimental effects on beat-to-beat Ca^{2+} handling and ECC. Authors suggest that UCP2 upregulation observed in HF may enhance dysrhythmogenic potential and exacerbate contractile dysfunction contributing to the progression of the disease [373].

Finally, UCP2 protein may play a protective role against atherosclerosis, although data are so far limited. It has been reported that transplantation of bone marrow from $UCP2^{-/-}$ mice into irradiated low-density lipoprotein receptor deficient ($LDLR^{-/-}$) mice has led to significantly increased atherosclerotic lesion size when animals have been fed an atherogenic diet [374]. In addition, $UCP2^{-/-}$ transplanted mice have displayed elevated levels of OS

markers and the plaques from these animals have shown higher apoptosis. Consistent with a protective role of UCP2 in atherogenesis, the 866G/A and of a 45nt-del/ins polymorphism in the 3′-untranslated region of the *UCP2* gene have been identified to be associated with carotid atherosclerosis in female study participants [375].

Conclusions

Despite great progress in our understanding of the molecular mechanisms underlying HF, this devastating disease represents a true challenge. Hallmarks of the failing heart are abnormal energy metabolism, increased production of ROS and defects in ECC. HF is a highly dynamic pathological process, and observed alterations in cardiac metabolism and function depend on the disease progression.

Mitochondrial ROS generation and ROS-mediated damage clearly contribute to the development and progression of HF. Cardiac UCP2 and UCP3 promoting proton leak across the IMM have emerged as essential regulators of mitochondrial membrane potential and respiratory function. However, unlike the well established role of their homolog UCP1 in adaptive thermogenesis in BAT, the physiological function of UCP2 and UCP3 in the heart is not clearly understood. Growing evidence suggests that cardiac UCPs are able to promote mild uncoupling reducing excessive mitochondrial ROS generation and ameliorating thereby myocardial function.

Controversial data have been reported regarding alterations in the expression of UCP2 and UCP3 seen in different animal HF models. Poorly understood variability in animal models of HF combined with species-specific UCP expression pattern might be responsible at least in part for these controversies. Unfortunately, it is even less known the alterations in UCP2 and UCP3 expression in patients with HF. Nevertheless, more recent studies suggest an upregulation of UCP2 or UCP3, leading to mitochondrial uncoupling and reduced ROS generation especially in ischemia-induced HF (Table 1). Since UCP2 and/or UCP3 can limit excessive mitochondrial ROS generation reducing the efficiency of ATP synthesis, it is unclear under which conditions their function would be protective or deleterious in the development of HF.

Similarly, although emerging evidence suggests that cardiac UCPs contribute to triggering cardiomyocyte death and ECC dysfunction in the failing heart, the underlying mechanisms remain largely undefined. For example, upregulated cardiac UCPs appear to trigger the expression of several apoptotic markers; however, this does not necessarily affect cardiomyocyte survival. Finally, it remains possible that the observed alterations in cardiac UCPs levels and/or activity are secondary to pathological cardiac remodeling. Future research efforts should address these critical issues.

High-throughput screening of mutations and polymorphisms in the genes encoding these proteins associated with increased risk for CVD is a novel promising approach. Recent identification of several polymorphisms in the *UCP1*, *UCP2* and *UCP3* genes associated with diabetes mellitus represents a successful example of such approach [311]. As discussed above, this approach has successfully been applied to identifying polymorphisms in the *UCP3* gene associated with atherosclerosis.

In summary, further research on UCPs activity and regulation will be necessary to advance our understanding of their function in the healthy and diseased heart. Moreover, the combined effort of molecular and clinical cardiologists is needed before we can use cardiac UCPs as targets to treat HF.

Conflict of interest This manuscript has not been, nor will be published elsewhere, has been read and is submitted with the approval of all authors, all of which participated in the writing of the manuscript with no conflict of interest in its publication in Heart Failure Reviews.

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