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Energy substrate metabolism and mitochondrial oxidative stress in cardiac ischemia/reperfusion injury

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ABSTRACT

The heart is the most metabolically flexible organ with respect to the use of substrates available in different states of energy metabolism. Cardiac mitochondria sense substrate availability and ensure the efficiency of oxidative phosphorylation and heart function. Mitochondria also play a critical role in cardiac ischemia/reperfusion injury, during which they are directly involved in ROS-producing pathophysiological mechanisms. This review explores the mechanisms of ROS production within the energy metabolism pathways and focuses on the impact of different substrates. We describe the main metabolites accumulating during ischemia in the glucose, fatty acid, and Krebs cycle pathways. Hyperglycemia, often present in the acute stress condition of ischemia/reperfusion, increases cytosolic ROS concentrations through the activation of NADPH oxidase 2 and increases mitochondrial ROS through the metabolic overloading and decreased binding of hexokinase II to mitochondria. Fatty acid-linked ROS production is related to the increased fatty acid flux and corresponding accumulation of long-chain acylcarnitines. Succinate that accumulates during anoxia/ischemia is suggested to be the main source of ROS, and the role of itaconate as an inhibitor of succinate dehydrogenase is emerging. We discuss the strategies to modulate and counteract the accumulation of substrates that yield ROS and the therapeutic implications of this concept.

1. Introduction

The heart intensively produces and consumes energy to maintain cardiac functionality and blood circulation. Fatty acids (FAs) and glucose are the primary fuels in the healthy adult heart [1]. However, extreme concentrations of both, hyperglycemia and dyslipidemia, likely contribute to cardiovascular disease and associated morbidity and mortality [2]. Energy metabolism in the heart is regulated by humoral factors, substrate availability, the AMP/ATP ratio, the redox state, and changes in gene expression. Under normal conditions, cardiac mitochondria sense substrate availability and ensure the efficiency of oxidative phosphorylation [3]. In the case of ischemia/reperfusion (I/R) injury, stress conditions alter systemic substrate levels, impact metabolic reactions, and lead to the accumulation of metabolic intermediates during ischemia. Here, we review the current knowledge of the main metabolic pathways and energy metabolites that drive the formation of mitochondrial reactive oxygen species (ROS) in reperfusion and exacerbate the impact of I/R-induced injury.

The heart has limited substrate reserves in the form of glycogen and triglycerides. The majority of ATP production requires a sufficient supply of oxygen for mitochondrial oxidative metabolism; therefore the heart highly depends on energy substrates from the circulation. In comparison to other organs, the heart is known as the most metabolically flexible when exposed to different energy-related challenges and substrate conditions, and it is better suited to oxidize FAs than other tissues [4]. FAs are the major substrate for energy production and contribute approximately 60–95% of the total oxidative phosphorylation [5]. However, the heart is capable of gaining energy from any available substrate suitable for energy production, including glucose, lactate, ketones, pyruvate, and amino acids. This ability ensures adaptation to changing conditions and uninterrupted energy production for

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Abbreviations

ACC	acetyl-CoA carboxylase
Acyl-CoA	acyl-coenzyme A
AMPK	5′ AMP-activated protein kinase
CAD	cis-aconitate decarboxylase
CaMKII	Ca^{2+}/calmodulin-dependent kinase II
CPT1	carnitine palmitoyltransferase 1
CPT2	carnitine palmitoyltransferase 2
cytoHKII	cytosolic hexokinase II
FA	fatty acids
G6P	glucose-6-phosphate
GLP-1	glucagon-like peptide-1
HKI, II	hexokinase I or II
I/R	ischemia/reperfusion
Irg1	Immune-Responsive Gene 1
LCAD	long-chain acyl-CoA dehydrogenase
LPS	lipopolysaccharide
MCD	malonyl-CoA decarboxylase
mitoHK	mitochondrial hexokinase
NADPH	nicotinamide-adenine dinucleotide phosphate
NOX2	NADPH oxidase 2
NRF2	nuclear factor erythroid 2–related factor 2
O-GlcNAcylation	O-linked-N-acetylglucosaminylation
PDH	Pyruvate dehydrogenase
PI3K-Akt	phosphoinositide-3 kinase/protein kinase B
PKCβ	β isoform of protein kinase C
RISK	reperfusion injury salvage kinase
RET	reverse electron transport
ROS	reactive oxygen species
SDH	succinate dehydrogenase
SMIT1	sodium-myoinositol cotransporter-1
SSO	sulfo-N-succinimidyl oleate
ψ_m	mitochondrial membrane potential

heart function; however, it requires tight regulation of substrate selection.

During myocardial ischemia, sympathetic overstimulation due to excessive concentrations of catecholamines induces systemic changes in energy metabolism. Stress-induced hyperglycemia and insulin resistance are often observed during acute myocardial infarction. In addition, catecholamine-stimulated triglyceride breakdown in adipocytes is suggested as a source of elevated FA levels in circulation [6]. In the heart, all major metabolic pathways are affected by ischemia (Fig. 1). In the ischemic heart, the shift from oxidative phosphorylation to anaerobic glycolysis results in significantly increased concentrations of glucose-6-phosphate (G6P) and lactate [7]. During cardiac arrest, mitochondrial dysfunction and changes in intracellular signalling pathways are associated with the downregulation of FA metabolism and accumulation of cytosolic long-chain FAs [8]. Limited mitochondrial metabolism is mostly caused by the absence of oxygen as an electron acceptor. In addition, in ischemic mitochondria, depletion of Krebs cycle intermediates and free CoA underlie limited mitochondrial metabolism. Thus, altered mitochondrial functionality increases the levels of the Krebs cycle intermediate succinate and the FA metabolic intermediates acylcarnitines and acyl-coenzymes A (acyl-CoAs). Currently, the accumulation of succinate is considered as a universal metabolic signature of ischemia and is suggested to be the main metabolite responsible for mitochondrial ROS production during reperfusion [9].

Mitochondria are both a target and a source of cellular ROS [10,11], producing both superoxide and, after its spontaneous or enzymatic dismutation, hydrogen peroxide [12–14]. In general, mitochondrial ROS generation decreases when [O_2] drops below 10 μM depending on the metabolic conditions [15]. Knowing that approximately 10% of mitochondria within the healthy heart *in vivo* already experience such oxygen tension [16], cardiac mitochondrial ROS production is likely partially controlled by oxygen. This suggests that ROS production is controlled by the interplay among metabolic processes, oxidative phosphorylation efficiency and oxygen availability. The lack of oxygen

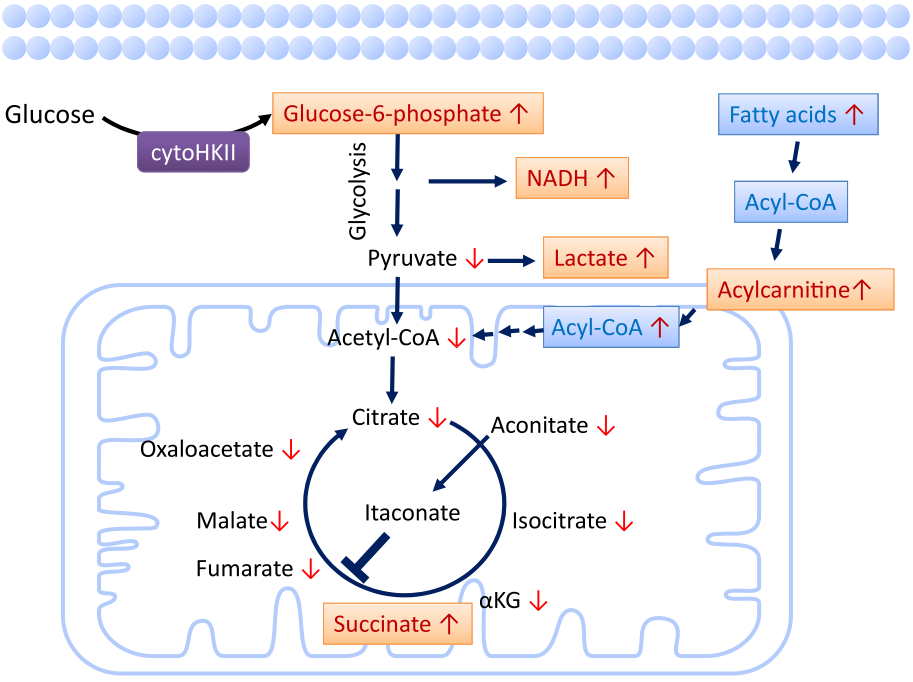

Fig. 1. Changes in cardiac content of metabolic intermediates during ischemia. Increased concentrations of glucose-6-phosphate, NADH, lactate, acylcarnitines, and succinate are observed. Abbreviations: Acyl-CoA = acyl-coenzyme A; cytoHKII = cytosolic hexokinase II; αKG = α-ketoglutarate.

and respiratory substrates inhibits pyruvate and FA β-oxidation and oxidative phosphorylation. When oxygen is reintroduced rapidly, changes in mitochondrial metabolism lead to a collapse of the membrane potential, Ca^{2+} overloading, swelling of mitochondria, cytochrome *c* release, disruption of cellular membranes, and finally, cell necrosis [17]. In various cardioprotective strategies, including protection of mitochondrial functionality (mitoprotection) [18], myocardial conditioning, and regulation of energy metabolism, the preservation of mitochondrial function is central in the reducing the severity of I/R injury [19,20]. The optimal mitochondrial bioenergetics based on alternative substrate availability and maintained cardiomyocyte redox homeostasis should counteract mitochondrial dysfunction and cardiac tissue damage in I/R.

2. Role of glucose metabolism in oxidative stress

Derangements in glucose metabolism, induced by either chronic (e.g., diabetes), or acute (e.g., stress) conditions, present during I/R can contribute to cardiac oxidative stress through various mechanisms. Chronic alterations in glucose metabolism and their impact on cardiac I/R are discussed in separate reviews within this special issue of the journal. In the present review, we mainly focus on how acute changes in glucose substrate channelling into the cell and glucose cellular metabolism contribute to the level of oxidative stress during an I/R episode. We discuss the two main features of glucose metabolism affecting ROS during I/R that have become apparent: hyperglycemia and the roles of mitochondrial hexokinase and glucose-6-phosphate (G6P).

2.1. *Hyperglycemia increases cardiac ROS*

Stress hyperglycemia is often present during acute cardiac I/R episodes because of elevated stress hormones such as cortisol and catecholamines, which provoke insulin resistance, impair insulin release and stimulate glucagon release from the pancreas (e.g. Refs. [21,22]). Most studies have demonstrated that hyperglycemia per se worsens the outcomes of a cardiac I/R insult (recently summarized in Ref. [23]).

Hyperglycemia increases the cytosolic and mitochondrial ROS levels in several different cell types through various cellular mechanisms [24]. It has been reported that elevated glucose levels can increase the mitochondrial superoxide concentration via mitochondrial metabolic overloading that triggers an increased mitochondrial membrane potential (ψ_m) in endothelial cells [25] or increase mitochondrial ROS levels by disrupting ATP synthase through increased calpain-1 activity in cardiomyocytes [26]. Alternatively, knowing that hyperglycemia increases the cytosolic [Na^+] [27], high glucose can also generate mitochondrial superoxide through Na^+-induced impairment of inner mitochondrial membrane fluidity [28]. Finally, high glucose can also increase ψ_m through the detachment of hexokinase II (HKII) from mitochondria, a process induced by an increase in cellular G6P levels due to high glucose [29]. Normally, mitochondrially bound HKII at voltage-dependent anion channels has preferential access to mitochondrially produced ATP, thereby facilitating the exchange of ADP and ATP through the inner mitochondrial membrane and maintaining ψ_m at a lower level. Dislodging of HKII from mitochondria will therefore raise ψ_m [29]. Hyperglycemia was also shown to increase cytosolic ROS through the activation of NADPH oxidase 2 (NOX2). Several pathways, which are not mutually exclusive, have been suggested to underlie how elevated glucose activates NOX2 (Fig. 2). Glucose transport through sodium myoinositol cotransporter-1 (SMIT1) was shown to be required for hyperglycemia-induced NOX2 activation [30]. Interestingly, glucose brought in through SMIT1 did not facilitate the NOX2 activation, indicating that the extracellular metabolic signal (glucose) transduced into an intracellular ionic signal activating NOX2 [31]. Intracellular Na^+ and Ca^{2+} are likely candidates because previous research showed that increases in extracellular glucose acutely increased concentrations of these ions in cardiomyocytes [27].

High glucose-induced ROS production in cardiomyocytes was also shown to be dependent on the β isoform of protein kinase C (PKCβ) activation, resulting in NOX2 activation [32]. Recent work has suggested that the O-GlcNAcylation of Ca^{2+}/calmodulin-dependent kinase

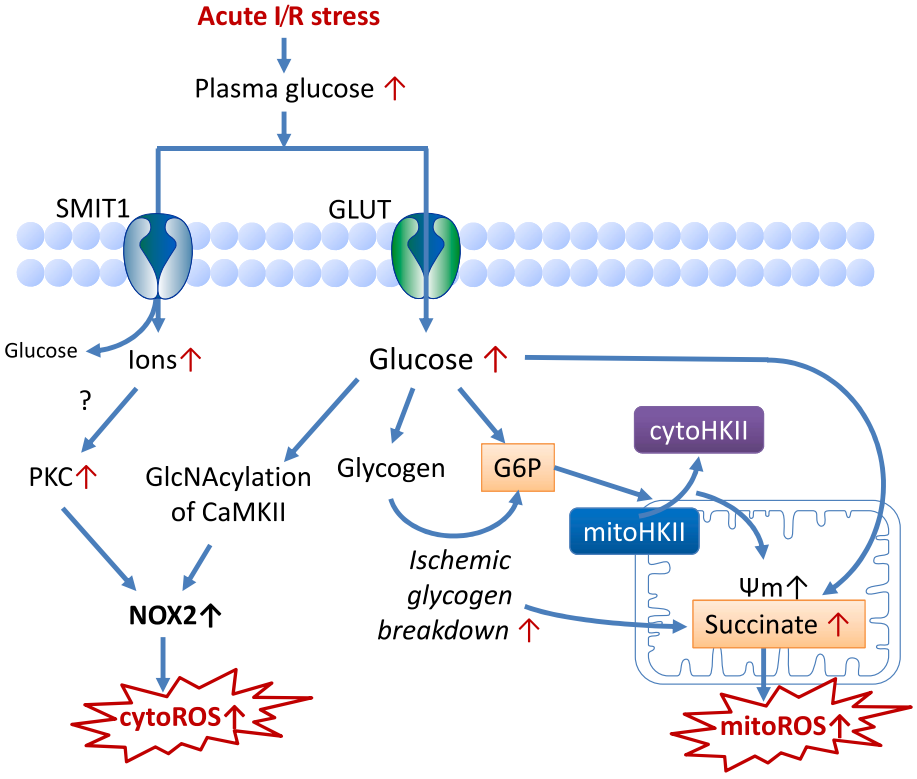

Fig. 2. Hyperglycemia increases cytosolic and mitochondrial ROS levels during I/R. High glucose-induced ROS production in the cardiomyocyte cytosol depends on the PKC activation and O-GlcNAcylation of CaMKII, resulting in NOX2 activation. Increased glucose uptake and glycogen breakdown during ischemia increase the content of G6P and induce the dislodgement of HKII from mitochondria, resulting in the accumulation of succinate and enhanced mitochondrial ROS production. Abbreviations: AcylCoA = acyl-coenzyme A; CaMKII = Ca^{2+}/calmodulin-dependent protein kinase II; cytoHKII = cytosolic hexokinase II; cytoROS = cytosolic ROS; mitoHKII = mitochondria-bound hexokinase II; mitoROS = mitochondrial ROS; GLUT = glucose transporter; G6P = glucose-6-phosphate; NOX2 = NADPH oxidase 2; PKC = protein kinase C; SMIT1 = sodium-myoinositol cotransporter-1.

II (CaMKII) mediates the activation of NOX2 upon hyperglycemic treatment of cardiomyocytes [33], although previous work in adult rat cardiomyocytes was unable to detect changes in overall O-GlcNAc residues [31].

2.2. Glucose metabolism reduces ROS through mitochondrial hexokinase (mitoHK)

As soon as glucose enters the cells, it is phosphorylated by hexokinase I or II (HKI, II), thereby trapping ionized glucose within the cell. HKI is predominantly localised at the outer membrane of mitochondria, whereas HKII is either cytosolic or mitochondrially bound [34]. HKII is mainly present in insulin-sensitive tissues (heart, skeletal muscle, adipose tissue), whereby insulin stimulation translocates HKII from the cytosol to the mitochondria [35]. Intact mitochondria within tissue always have HKI/II bound to them, indicating the importance of this glycolytic enzyme for mitochondrial homeostasis. Mitochondria-bound HK (mitoHK) is a determinant of ROS produced by mitochondria [29]. The decreased binding of HK (and creatine kinase) to mitochondria was recently proposed to contribute to the oxidative stress associated with ageing [36]. HK binding to mitochondria results in mild depolarization of the inner mitochondrial potential, and thereby diminishes mitochondrial ROS production [29,36].

Mitochondrial HKII (mitoHK) plays a major role in mitigating cardiac I/R injury and ROS production. It was shown that 1) cardiac protection against I/R injury by ischemic preconditioning is, at least partly, mediated through the maintenance of HKII at mitochondria during ischemia and reperfusion [37–40]; 2) detaching HKII from mitochondria increases ROS production during reperfusion and exacerbates cardiac I/R injury [41,42]; and 3) glucose metabolism is required for the mitoHK induced protection [43]. The essential role of glucose in mitoHK-induced protection may also explain why glucose is required for ischemic preconditioning to be protective [44,45]. Furthermore, it has been shown that the addition of glucose to isolated brain mitochondria lowers the generation of hydrogen peroxide, an effect not seen in mitoHK-depleted mitochondria [29].

Thus, glucose metabolism needs to be active/present to diminish oxidative stress during the cardiac reperfusion and to facilitate cardioprotection by ischemic preconditioning. Excessive inhibition of glucose metabolism, e.g., through elevated FA metabolism within cardiac cells (the Randle effect), can contribute to the increased production of ROS during reperfusion. In addition, increased FA levels may also lead to the dissociation of HK from the mitochondria [46], indicating how FAs may indirectly contribute to increased mitochondrial ROS production.

Glucose metabolism during ischemia, i.e., glycolysis derived from glycogen, is a double-edged sword for cardiac I/R injury and ROS production (Fig. 2). If glycogen is not fully depleted, ischemic contracture does not develop, and cardiac I/R injury is minor. However, when glycogen is fully depleted, the breakdown products of anaerobic glycolysis, protons and G6P, contribute to cardiac I/R injury [47]. A likely explanation is that a drop in the cytosolic pH along with increased G6P accumulation dislodges HKII from mitochondria during ischemia, resulting in increased ROS production during reperfusion [39]. The drop in cytosolic pH will also translate into mitochondrial acidification resulting in decreased mitochondrial calcium loading [48]. However, although mitochondrial acidification may be beneficial for the prevention of I/R-induced mitochondrial calcium overloading, current research indicates that the effects of mitochondrial acidification are mostly neutral for mitochondrial ROS production [49]. In summary, loading the preischemic heart with glycogen (for example, by providing high levels of insulin), or increasing glycolysis during ischemia, will contribute to increased ROS when glycogen becomes fully depleted, due to the dislodgement of HKII from mitochondria [20]. Additionally, glycogen breakdown may also enhance the production of mitochondrial ROS early during reperfusion through its contribution to the ischemic accumulation of succinate [50] (Fig. 2).

3. Role of FA metabolism in oxidative stress

Both the increase in β-oxidation and the accumulation of FA and their metabolites, especially long-chain acyl-CoAs and acylcarnitines, are associated with increased ROS production and subsequent oxidative stress [51–54]. FA-linked ROS production is associated with mitochondrial dysfunction, which includes inhibition of the electron transfer system and support of the reverse electron transport (RET); FAs and/or their metabolites act as protonophores/uncouplers, and alter mitochondrial membrane fluidity (reviewed in Ref. [55]). However, it is unclear to what extent mitochondrial β–oxidation or the accumulation of FAs and/or their metabolites (acyl-CoAs and/or acylcarnitines) impact I/R-associated oxidative stress.

3.1. FA metabolism in the heart

Increased plasma concentrations of FAs are associated with increased FA flux to the heart, which further shifts cardiac energy metabolism towards FA oxidation [56]. Elevated levels of circulating FAs have been found in the fasted state [57] as well as during early reperfusion, reaching concentrations of approximately 0.8 mM, but not at later time points (concentration is maintained in the 0.4–0.6 mM range) [58] if measured in appropriately collected samples [59]. Interestingly, despite the significant increase in FA concentrations observed in circulation in a fasted versus a fed state, no such increase was observed in the cardiac FA concentration [60]. The cardiac contents of activated FAs, acyl-CoAs and acylcarnitines, however, are increased in the fasted state [60,61]. Long-chain FA metabolism is controlled by interactions between carnitine palmitoyltransferase 1 (CPT1) and acetyl-CoA carboxylase (ACC) via intracellular malonyl-CoA levels (Fig. 3). CPT1 activity is stimulated when the malonyl-CoA-synthesizing enzyme ACC is inactivated through phosphorylation by 5′ AMP-activated protein kinase (AMPK). At the same time, malonyl-CoA decarboxylase (MCD) catalyzes the reverse reaction, the conversion of malonyl-CoA to acetyl-CoA. In the fasted state, AMPK activity ensures a low concentration of malonyl-CoA, high activity of CPT1, and a substantially elevated long-chain acylcarnitine production rate. In the fed state, activation of insulin signalling induces the dephosphorylation (activation) of ACC and a lower long-chain acylcarnitine production rate. These mechanisms are crucial for the regulation of long-chain acylcarnitine levels and FA oxidation rates to ensure the adaptation of cardiac metabolism to the substrate availability and nutritional state.

During ischemia, due to no or limited blood flow, the myocardial triglyceride stores are broken down to provide FAs for further oxidation. However, during ischemia, FA metabolism is not coupled with oxidation in mitochondria, leading to the accumulation of long-chain acylcarnitines in mitochondria [62]. Ischemic stress-induced insulin resistance and changes in the AMP/ATP ratio strongly activate AMPK, which blocks malonyl-CoA synthesis to facilitate FA metabolism [63]. In turn, CPT1 generates tremendous amounts of long-chain acylcarnitines that cannot be metabolized by mitochondria due to the lack of oxygen [62]. In the mitochondrial matrix, the anaplerosis/depletion of Krebs cycle substrates results in a limited availability of free CoA, which is necessary for CPT2 and corresponding β-oxidation. Thus, long-chain acylcarnitine accumulation is a result of stimulated synthesis and a highly decreased CPT2-dependent β-oxidation rate [62]. Additionally, because of the relative deficiency of the mitochondrial carnitine content [64], long-chain acylcarnitines cannot be transferred from the intermembrane space into the mitochondrial matrix via the carnitine-acylcarnitine translocase. Collectively, all of these mechanisms contribute to long-chain acylcarnitine accumulation on the mitochondrial inner membrane and in the intermembrane space.

Controversy exist regarding whether FA oxidation during early reperfusion is increased, decreased, or unaltered (e.g. Refs. [65–69]). In

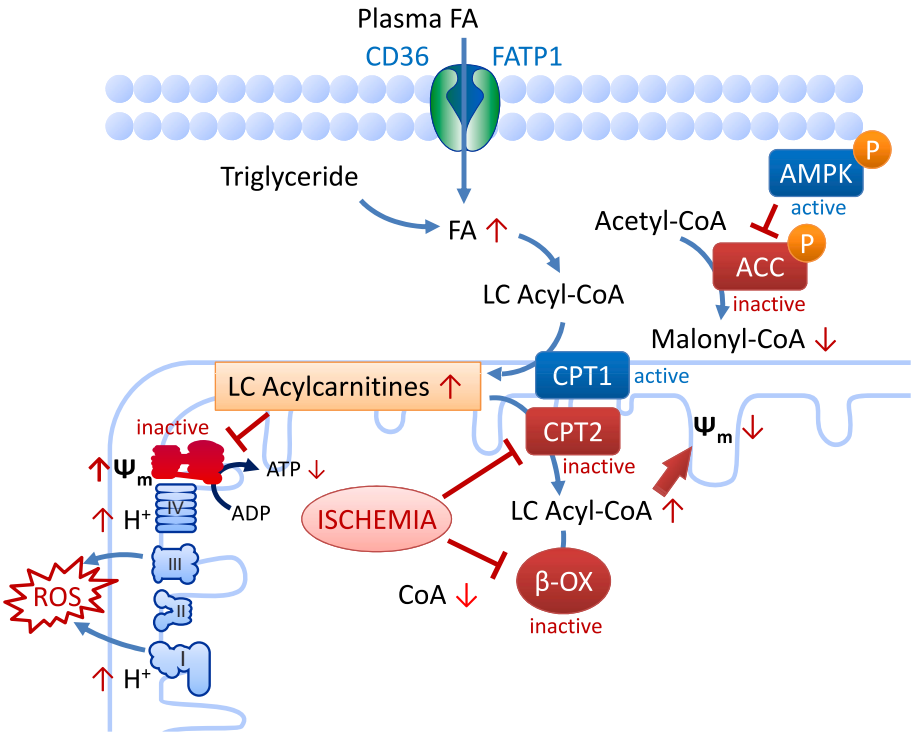

Fig. 3. Accumulation of fatty acids and their intermediates, long-chain acyl-CoAs and acylcarnitines, in the ischemic myocardium. During ischemia, the FA metabolism in mitochondria is reduced, leading to the accumulation of long-chain FA acylcarnitines and acyl-CoAs. Ischemic stress-induced insulin resistance and activated AMPK block malonyl-CoA synthesis to stimulate the production of long-chain acylcarnitines. In the mitochondrial matrix, the depletion of Krebs cycle substrates results in the reduced activity of CPT2 and corresponding β-oxidation. Long-chain acylcarnitine accumulation results from stimulated synthesis by CPT1 and a limited CPT2-dependent β-oxidation rate. Long-chain acylcarnitines in cardiac mitochondria inhibit oxidative phosphorylation and thus induce mitochondrial membrane hyperpolarization and the subsequent stimulation of ROS production. Abbreviations: I, II, III, IV = mitochondrial respiratory complexes; ACC = acetyl coenzyme A carboxylase; AMPK = AMP-activated protein kinase; β-OX = β-oxidation; CPT1 = carnitine palmitoyltransferase 1; CPT2 = carnitine palmitoyltransferase 2; CoA = Coenzyme A; H^+ = hydrogen ion; FA = fatty acids, CD36 = fatty acid translocase; FATP1 = fatty acid transport protein 1; LC = long-chain; Ψm = mitochondrial membrane potential; P = phosphorylated protein.

reperfusion, if the plasma levels of FAs are increased as a result of rising catecholamines or the use of heparin, higher plasma levels will likely increase FA uptake in cardiac cells. Due to AMPK activation and ACC inhibition, CPT1 is activated, thus facilitating long-chain acylcarnitine synthesis. In turn, long-chain acylcarnitines inhibit pyruvate metabolism [60] and, as a result, FA metabolism prevails over pyruvate/glucose oxidation. Nevertheless, FA metabolites still accumulate in mitochondria during reperfusion and continuously stimulate ROS production [62,66]. Overall, increased FA-linked ROS production is related to the increased FA flux and corresponding accumulation of FAs and their metabolites.

3.2. FA oxidation and mitochondrial ROS production

In general, in the heart, mitochondrial β-oxidation is associated with higher levels of ROS formation than the oxidation of other energy substrates [51]. The main sites of superoxide and hydrogen peroxide production by β-oxidation are acyl-CoA dehydrogenase and the electron-transferring flavoprotein complex [51,70,71]. However, when β-oxidation is coupled to oxidative phosphorylation (direct ATP production), the rate of ROS production is comparable to that of other metabolic pathways [52]. Thus, it is more likely that the increase in mitochondrial ROS production associated with the increased β-oxidation rate occurs when oxidation is not coupled with energy (ATP) production, and the oxidative phosphorylation coupling efficiency is somewhat limited [72,73]. In this case, ROS are formed as side/waste products of β-oxidation. On the other hand, an increase in ROS is also observed when mitochondrial β-oxidation is impaired [74–79]. The inhibition of β-oxidation in the case of ischemia is associated with the accumulation of FAs and FA metabolites, long-chain acyl-CoAs and acylcarnitines [62,80,81], suggesting that ROS production is also linked to the accumulation of metabolites.

Intracellular FAs have a dual effect on mitochondrial ROS production [55,82]. It has been shown that FAs promote superoxide generation in the forward mode of electron transport by inhibiting the rate of electron flow through complexes I and III and between complexes III and IV [53,83–85]. On the other hand, FAs can act as uncouplers and strongly decrease superoxide production in mitochondria due to their protonophoric action [83]. Long-term FA overloading has been associated with the upregulation of uncoupling proteins, ensuring protection against excessive ROS production [86]. Thus, it is not clear whether the accumulation of FAs can directly promote mitochondrial ROS formation and induce oxidative stress. Since FAs are water-insoluble molecules per se, in cells, they are bound to proteins. However, high concentrations of FAs decrease the capacity of the protein to bind other metabolites. Thus, FA-binding proteins could be overwhelmed, and more active metabolites (such as acyl-CoAs and acylcarnitines) remain unbound and induce oxidative stress. Such a situation could occur in the fasted state. It has been shown that due to higher amounts of FAs and their metabolites in the cytosol and to the limited capacity of proteins to bind the excess metabolites, heart mitochondria are not protected from their damaging action [62]. Moreover, the addition of FAs to the cytosol prepared from fed hearts abolished the protection against FA metabolite mitochondrial damage [62]. Similar effects were observed after hypoxia, when the content of FAs in the tissue was increased [87]. Overall, we can conclude that while FAs may not directly promote mitochondrial ROS formation, their accumulation reduces the capacity of cellular defence system to neutralize/protect against metabolite-induced mitochondrial damage.

During ischemia, CPT1 activity is increased while carnitine palmitoyltransferase 2 (CPT2) activity is decreased, which leads to the accumulation of long-chain acylcarnitines in the mitochondrial intermembrane space [62]. In addition, the accumulation of NADH and $FADH_2$ in ischemic mitochondria leads to the inhibition of several enzyme reactions in β-oxidation [56,88], which results in the accumulation of long-chain acyl-CoAs in the mitochondrial matrix. After ischemia, both FA intermediates, acyl-CoAs and acylcarnitines, accumulate and contribute to mitochondrial oxidative stress by different mechanisms [54,62] (Fig. 3). First, acyl-CoAs and acylcarnitines accumulate at different sites of mitochondria. Acyl-CoAs accumulate in the matrix, while the majority of acylcarnitines accumulate in the intermembrane space. Thus, their mechanisms of action at different ROS production sites could differ. It has been shown that the addition of long-chain acylcarnitines to cardiac mitochondria inhibits oxidative phosphorylation, thus inducing mitochondrial membrane

hyperpolarization and subsequently stimulating superoxide and hydrogen peroxide production [62]. Moreover, the accumulation of acylcarnitines in the intermembrane space could alter the mitochondrial membrane fluidity and further promote superoxide formation. In addition, in contrast to acylcarnitines, long-chain acyl-CoAs induce mitochondrial membrane depolarization and cause only small and transient (for approximately 2–3 min) ROS production [54]. Furthermore, the amounts superoxide and hydrogen peroxide produced via acyl-CoA are significantly lower than those produced by acylcarnitines [54]. Second, acyl-CoAs are considered to be more damaging FA metabolites [89,90] than long-chain acylcarnitines, since long-chain acyl-CoAs are approximately 3 times more toxic to mitochondria [62]. However, the concentrations of long-chain acyl-CoAs in ischemic cardiac mitochondria are approximately 50 times lower than those of long-chain acylcarnitines and do not reach levels that are capable of inducing mitochondrial damage [62]. The observed concentrations of long-chain acylcarnitines in the ischemic myocardium, on the other hand, are sufficient to promote ROS production and induce mitochondrial damage [62,81]. Taken together, it is likely that the accumulation of long-chain acylcarnitines, and not long-chain acyl-CoAs, in ischemic mitochondria, is the main player in ROS production.

4. Succinate as an energy substrate and its role in oxidative stress in the heart

Succinate is an intermediate of the Krebs cycle that is produced via α-ketoglutarate by α-ketoglutarate dehydrogenase and succinyl-CoA synthetase and is oxidized by succinate dehydrogenase (SDH) through which electrons from succinate are transferred into the mitochondrial electron transfer system, thus stimulating mitochondrial respiration. For this reason, SDH is also considered to be complex II of the electron transfer system. Complex II (SDH) is different from other electron transfer system complexes in that it does not generate protonmotive force directly. By transferring electrons from succinate to coenzyme Q in the membrane, complex II also supplies hydrogen atoms for the respiratory system and thus makes this metabolic system irreversible. This is an important function because it has an impact on RET and the associated production of ROS [91].

4.1. Extramitochondrial sources of succinate

In addition to being produced intramitochondrially, extramitochondrial succinate can be used as an energy substrate. This was demonstrated decades ago using isolated mitochondria, and this approach, stimulation of mitochondrial respiration by succinate, is now widely used for investigating mitochondrial pathophysiological functions. First, Chappel and Haarhoff [92] demonstrated that isolated mitochondria are permeable to succinate, which then stimulates mitochondrial respiration [93]. The rate of respiration has been found to be controlled by succinate uptake, particularly at low submilimolar concentrations [93]. Later, cytosolic succinate was shown to be transferred into the mitochondrial matrix by the dicarboxylate carrier [94–96]. However, the expression level of this carrier in muscle mitochondria is very low [97]. Therefore it is thought that succinate is transported into muscle (including the heart) mitochondria via the α-ketoglutarate carrier [98,99] or other mitochondrial anion transfer systems such as the inner membrane anion channel [100,101].

External succinate may come from dietary sources, such as mushrooms, seafood, meat, broccoli, rhubarb, sugar beets, and various cheeses. Succinic acid and its sodium salt have been found in these foods and are considered to contribute to their umami taste [102]; for a review see Ref. [103]. Succinate is also used as a food additive to contribute to taste and as a preservative.

It is not clear whether dietary or extramitochondrial succinate is a significant contributor to oxidative phosphorylation under physiological conditions in the heart. Under normal conditions, the permeability of the cardiomyocyte plasma membrane to succinate is rather poor. However, it may increase as a result of anoxia which has been shown to lead to increased succinate-stimulated oxygen consumption in isolated cardiomyocytes [104]. This suggests that succinate is an important energy substrate under certain conditions, such as heart I/R, when complex I of the mitochondrial electron transfer system is inhibited and when succinate accumulates in the tissues and mitochondria.

4.2. Accumulation of succinate in the heart during ischemia

In tissues, including the heart, the levels of succinate are low (in the range 0.2–1.0 mM) [10,105] but can increase under pathological conditions such as hypoxia/ischemia [106]. In humans, succinate accumulates in skeletal muscle during physical exercise [107], possibly due to tissue hypoxia. *In vitro* perfusion of hypoxic rat hearts with α-ketoglutarate, malate or fumarate results in up to a fourfold elevation in succinate levels in myocardial tissue [108]. It has also been observed that as short as a 10 min period of total ischemia in canine hearts can significantly increase the levels of succinate in tissues [106]. Succinate accumulation during anoxia/ischemia has been suggested to be caused by the reversed SDH reaction in relation to the activation of the malate-aspartate shuttle rather than to the inhibition of SDH [9]. However, this hypothes remains debatable, as only approximately 2–15% of mitochondrial succinate is suggested to originate via this route, and the rest is produced by α-ketoglutarate via canonical Krebs cycle reactions [109]. The study by Zhang and colleagues [50] found that pre-existing α-ketoglutarate is the main precursor for succinate accumulation during heart ischemia. Cytosolic aspartate and glutamine contribute to the elevation of mitochondrial α-ketoglutarate resulting in succinate accumulation through corresponding transamination reactions [50] (Fig. 4A). Glycogen-derived glucose may also play a small role in ischemia-induced succinate accumulation, although the mechanism does not involve pyruvate entering the mitochondria, but, again, requires the participation of aminotransferases leading to the formation of α-ketoglutarate [50]. The formation of succinate via the α-ketoglutarate route may be beneficial during the first few min of ischemia as it involves GTP generation by substrate-level phosphorylation of succinyl-CoA synthetase, thereby delaying ischemia-induced ATP depletion in cardiomyocytes (Fig. 4A).

4.3. Succinate and ROS production

In early studies, it was thought that the accumulation of succinate in the myocardium during ischemic period may be beneficial for the heart as succinate may serve as a respiratory substrate during reoxygenation [108]. However, this paradigm has changed as it was shown that during reperfusion succinate may be rapidly oxidized by SDH resulting in ROS production by complex I of the mitochondrial electron transfer system [9]; for reviews see Refs. [110,111].

The first observations that succinate may contribute to oxidative stress came from studies on the isolated brain and skeletal muscle mitochondria showing the production of ROS in the presence of millimolar concentrations of succinate [112,113]. Moreover, succinate has been shown to enhance the generation of ROS in isolated mitochondria respiring on NADH-dependent substrates (glutamate and malate) – conditions that better resemble the physiological state [114]. The mechanism of ROS production has been suggested to involve RET at the level of complex I producing superoxide (Fig. 4B). Under physiological conditions and at rest, ROS generation from succinate is minimal, possibly due to both the low levels of succinate in mitochondria and SDH inhibition by intrinsic oxaloacetate and malate [91]. The detailed mechanisms of ROS production in the ischemic heart are reviewed by other authors in this issue of the journal. Some investigators question the proposed role of succinate-driven ROS production in I/R injury and suggest that the opening of the mitochondrial permeability transition pore is the primary cause of ROS production later in reperfusion [115].

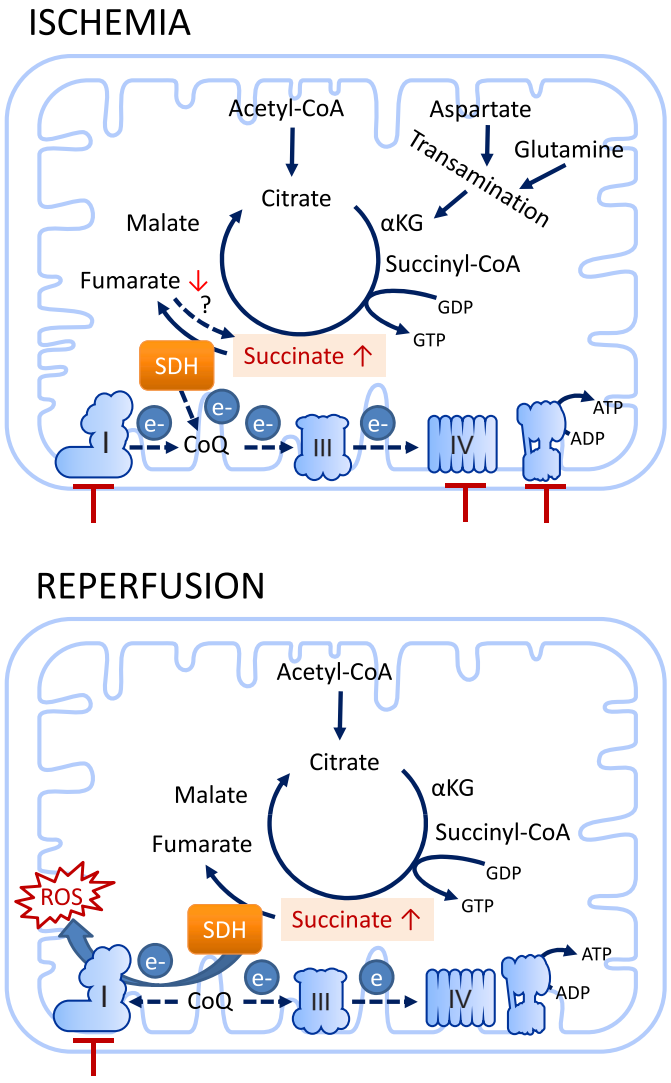

Fig. 4. Succinate accumulation and the effects on ischemia (A) and reperfusion (B)
(A) During ischemia when oxidative phosphorylation is blocked due to a lack of oxygen and complex I injury, succinate accumulation is driven by α-ketoglutarate. Aspartate and glutamine contribute to α-ketoglutarate production via transamination. Reversal of the SDH reaction to produce succinate from fumarate is unlikely. Substrate phosphorylation at succinyl-CoA synthetase produces GTP. (B) During reperfusion, the accumulated succinate is rapidly oxidized by SDH and generates ROS by reverse electron transfer at complex I. Abbreviations: I, III, IV = mitochondrial respiratory complexes; Acetyl-CoA = acetyl-coenzyme A; α-KG = α-ketoglutarate; CoQ = Coenzyme Q; e- = electrons; SDH = Succinate dehydrogenase, or respiratory complex II.

In addition, a succinate-driven burst of ROS can only occur during the first few min of reperfusion [38], whereas I/R-induced ROS are present for at least the first 30 min of reperfusion [50]. Thus, although the accumulation of succinate in mitochondria under ischemia is a well-established fact, its pathophysiological role needs to be further investigated.

Notably, only some of the succinate that accumulates in the heart during ischemia is oxidized under reperfusion. A significant amount of succinate has been detected in the circulation of patients after myocardial infarction [116]. In the circulatory system, succinate may stimulate inflammatory reactions involving ROS production, thus further exacerbating reperfusion injury [117]. The detailed mechanism by which succinate is released from the myocardium has not yet been determined.

4.4. Itaconate as an endogenous SDH inhibitor

Itaconic acid (2-methylidenebutanedioic acid; methylene succinic acid) is an unsaturated dicarboxylic acid. For a long time, itaconate was best known as a widely used reagent in chemical synthesis or as an antimicrobial agent. Recent metabolomic analysis has revealed that human and mouse macrophages exposed to the bacterial endotoxin lipopolysaccharide (LPS) produce high levels of itaconate [118,119]. This discovery prompted a new interest in this compound and its physiological functions. Itaconate is a diverted derivative from the Krebs cycle that is synthesized from cis-aconitate by the enzyme cis-aconitate decarboxylase, which is encoded by the *Irg1* gene (Immune responsive gene 1) [120]. The expression of *Irg1* has been observed in macrophages, microglial cells, lung tissue, and neurons of the cortex and cerebellum (for review see Ref. [121]). The generation of itaconate in macrophages can be triggered by various agonists of Toll-like receptors (TLRs), LPS, and interferons [120,122]. From mitochondria, itaconate is transported into the cytosol most likely by a dicarboxylate carrier, and in LPS-stimulated macrophages, the intracellular concentrations of itaconate can reach as high as 8 mM [120]. The release of itaconate from the cells is slow and extracellular concentrations usually do not reach the millimolar range. However, exogenous itaconate reportedly enter the cellular cytoplasm, albeit slowly, thus requiring longer incubation times (48–72 h) *in vitro* [123].

Itaconate has been shown to act as a competitive inhibitor of SDH leading to accumulation of succinate and limiting the reprogramming of proinflammatory macrophages into a more anti-inflammatory phenotype [124,125]. Although the detailed mechanism of macrophage reprogramming is not yet clear, in addition to SDH inhibition, it may involve the activation of nuclear factor erythroid 2–related factor 2 (NRF2), which regulates the expression of antioxidant genes and leads to reduced intracellular ROS and interleukin-1β production [126] (Fig. 5). The itaconate-induced accumulation of succinate is not limited to immune cells but has also been observed in pulmonary adenocarcinoma cell lines [127]. However, to date, there are no data on itaconate production or *Irg1* expression in cardiomyocytes. Nevertheless, a protective

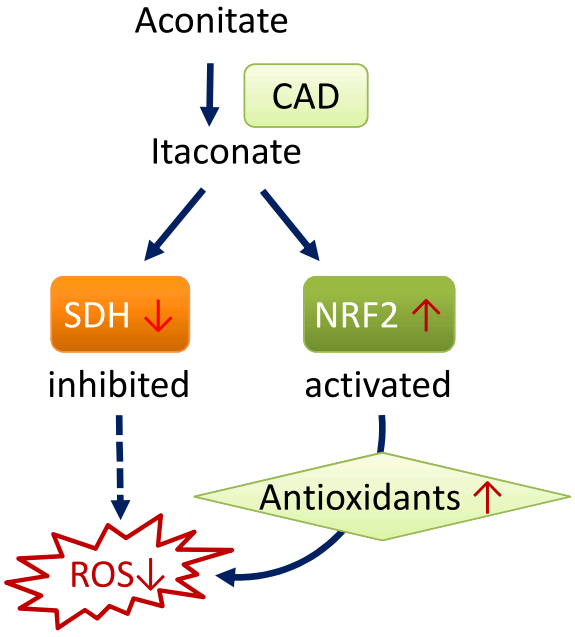

Fig. 5. Mechanisms of ROS suppression by itaconate. Itaconate is produced from aconitate by CAD. Itaconate inhibits SDH, thus preventing ROS production by mitochondria. Itaconate also is involved in the activation of NRF2, which promotes the expression of antioxidants leading to decreased ROS. Abbreviations: CAD = cis-aconitate decarboxylase; NRF2 = nuclear factor erythroid 2–related factor 2; SDH = succinate dehydrogenase.

effect of dimethylitaconate (a cell-permeable derivative of itaconate) against doxorubicin-induced ROS-mediated cardiotoxicity has been reported and involves the increased expression of NRF2-dependent antioxidants and reduced oxidative stress [128]. In a recent study on brain I/R injury, the infusion of itaconate into mice before ischemia decreased the SDH activity in brain cells and reduced the ROS levels during reperfusion [129]. An important observation was that itaconate-induced SDH inhibition gradually decreased during reperfusion (due to the clearance of itaconate from plasma). This allowed a gradual return of the mitochondrial electron transfer system to levels of normal function, thus avoiding the burst of ROS early during reperfusion and oxidative stress-induced brain injury with neurological outcomes and allowing full recovery. Such observations raise the possibility that the application of itaconate or its more cell-permeable derivatives (such as dimethyl-itaconate and octanoyl-itaconate) might have beneficial effects against myocardial I/R injury by reducing succinate-induced ROS production or limiting the proinflammatory activity of macrophages that invade the infarcted myocardium. For the latter, stimulation of exogenous itaconate production in macrophages may also be beneficial, though this is more difficult to achieve. An important advantage of using itaconate as a cardioprotective therapeutic is that this compound is endogenously produced at relatively high concentrations, therefore, the side effects might be less damaging. Before clinical applications, however, more preclinical studies on the effects of itaconate in the heart are needed.

5. Interplay among mitochondrial energy substrates in ROS production

Each substrate can contribute to mitochondrial ROS production; however, it remains unclear which substrate/metabolic pathway is the main source of ROS. In isolated mitochondrial systems under normoxic conditions, in the state when respiration is driven by individual substrate metabolism and when ADP is not present (LEAK state or state 2), succinate metabolism activates RET and drives higher levels of superoxide and hydrogen peroxide production compared to those achieved by pyruvate or FA (palmitoylcarnitine) metabolism (Fig. 6). In an oxidative phosphorylation-dependent state, when ADP is available and forces forward electron transfer, the production of ROS decreases [130,131]. Moreover, no difference in the hydrogen peroxide production rates are observable among pyruvate-, succinate- and FA-dependent pathways (Fig. 6), and the H_2O_2/O ratio is dramatically decreased [52,132,133], indicating that less oxygen is wasted for ROS production. Moreover, stimulation of other pathways by the addition of pyruvate and long-chain acylcarnitines, thus ensuring the activation of all mitochondrial energy pathways, does not change the ROS production rate (Fig. 6) [15,83].

Despite the fact that the highest levels of ROS formation could be due to succinate accumulation resulting in RET, several aspects should be considered when analyzing the main ROS-driving metabolic pathway during ischemia-reperfusion damage in the heart. First, it has been shown that in the heart, ROS production increases after 2–3 min of reperfusion [134]. The accumulation of FA metabolites, namely, long-chain acylcarnitines, still occurs in the heart at least 30 min after reperfusion [62], while it has been shown that succinate is washed out from the heart during the first 3–5 min of reperfusion [9,50]. Thus, the dynamics of substrate influx and efflux during reperfusion should be taken into account. Second, complex I is inhibited/damaged after I/R injury [135], and inhibition of complex I limits the ROS production driven by succinate (Fig. 6). Since the I/R-induced inhibition of complex I depends on the duration of ischemia [136], in the case of prolonged ischemia (≥30 min), ROS formation during reperfusion will be only partially related to RET via complex I. Third, during ischemia, the simultaneous accumulation of several substrates/intermediates occurs [9,62]. Thus, ROS production during reperfusion is simultaneously driven by several metabolic pathways, e.g., FA and succinate metabolism. Since FA metabolism decreases the mitochondrial generation of ROS during RET driven by succinate accumulation (Fig. 6) [83], it is unlikely that ROS production during reperfusion is mainly driven by succinate accumulation-induced RET. Last, at the end of ischemia, lactate, long-chain acylcarnitines and succinate simultaneously accumulate [9,62,137]. Therefore, there is no single metabolic pathway driving ROS formation during reperfusion in the heart, and the availability of each substrate together with their metabolic interplay determines the ROS production rate. It was also confirmed in isolated mitochondria that there is no difference in the ROS production rate [15,83], regardless of the substrate chosen to initiate electron transfer, in an oxidative phosphorylation-dependent state when all metabolic pathways are activated, i.e., all main energy substrates are present (Fig. 6). Overall, the assumption that succinate accumulation and the resulting RET are the main sources of ROS during reperfusion is still under debate. At the same time, it is clear that the contribution of long-chain acylcarnitines to ROS generation and the role of FA oxidation in oxidative stress in cardiac I/Ri damage are important and merit further investigation.

6. Targeting substrate energy metabolism pathways and cardiac ROS

A myriad of events in the pathogenesis of I/R injury are suggested to be druggable targets for the prevention or treatment of I/R-induced cardiac damage [138]. Current therapeutic approaches affecting the

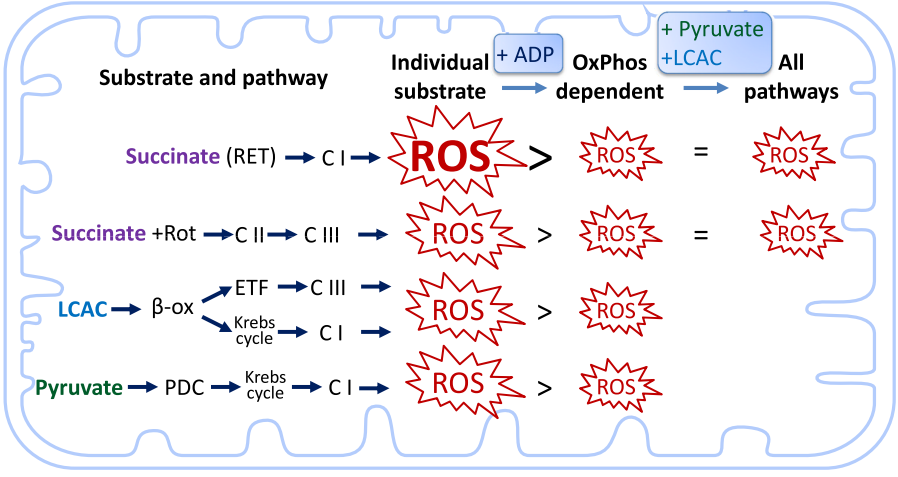

Fig. 6. Comparison of specific substrate-linked ROS production under different conditions/pathway interplay scenarios. Succinate-induced reverse electron transfer (RET) via complex I (CI) results in a massive increase in the ROS production rate, which decreases on the addition of ADP and on the activation of other pathways (addition of pyruvate and long-chain acylcarnitines, LCAC). In the presence of rotenone, succinate-induced ROS production is driven by forward electron transfer via complex III, and the rate of ROS production is comparable to that achieved via the fatty acid (LCAC) and pyruvate metabolism-linked pathways. Further, in the presence of ADP, in an oxidative phosphorylation-dependent state (addition of ADP), the ROS production rate does not differ between the substrate-dependent pathways. Moreover, the activation of other energetic pathways does not induce significant changes in the ROS production rate.

intracellular redox status integrate numerous considerations, such as the prevention of metabolic intermediate accumulation during ischemia, decrease in ROS production during reperfusion, scavenging of free radicals during reperfusion, induction of antioxidative defence systems, mitochondrial protection against ROS damage (e.g., by inhibiting mitochondrial permeability transition pore opening or applying mitochondria-targeted antioxidants) and blocking of proapoptotic pathways (Fig. 7).

The synergistic inhibition of multiple deleterious pathways using a combination of agents represents an attractive strategy. Various events appear at different time points during ischemia and reperfusion, and it could be assumed that targeting later events would offer a longer time window for a pharmacological agent to reach the target in the heart and mitochondria. However, the benefit of targeting early events could prevent various irreversible changes in cardiac mitochondria. In clinical settings, patients hospitalized for acute myocardial infarction have already developed ischemia and pre-ischemic treatments are not applicable.

The cardiac accumulation of metabolic intermediates starts at the beginning of ischemia. Some of the metabolites might already be at increased levels even before ischemia and are thus associated with a higher risk of cardiac damage. The modulation of energy metabolism to prevent the accumulation of harmful intermediates can be achieved intrinsically by affecting the target cells or extrinsically by altering the systemic metabolic status (Table 1). Hyperglycemia and hyperlipidemia are well-known conditions of systemic insulin resistance that are linked to the inability of insulin signalling to reduce lipid flux and inhibit FA metabolism. In insulin-resistant individuals, higher baseline levels of cardiac FA metabolites are an additional risk factor leading to a higher risk of cardiac damage [139,140]. Improvement of insulin sensitivity by insulin sensitizers such as glucagon-like peptide-1 (GLP-1) receptor agonists (long- and short-acting Table 1) reduces the risk of cardiac damage in experimental and clinical settings [141–143]. In contrast, reductions in the concentrations of circulating glucose and FAs might not be beneficial for cardiac survival during I/R [144]. Additionally, reperfusion injury salvage kinase (RISK) is a pathway known to activate prosurvival protein kinases that protect against I/R injury [145]. One of the two RISK cascades activated during conditioning is the phosphoinositide-3 kinase/protein kinase B (PI3K-Akt) pathway. In the context of the metabolic status, the protective effects of PI3K-Akt pathway activators can also be attributed to the lower mitochondrial content of long-chain acylcarnitines and acyl-CoA. It must be noted that the fed state is characterized by activated insulin signalling, lower levels of FA metabolic intermediates, and a cardioprotective phenotype [5].

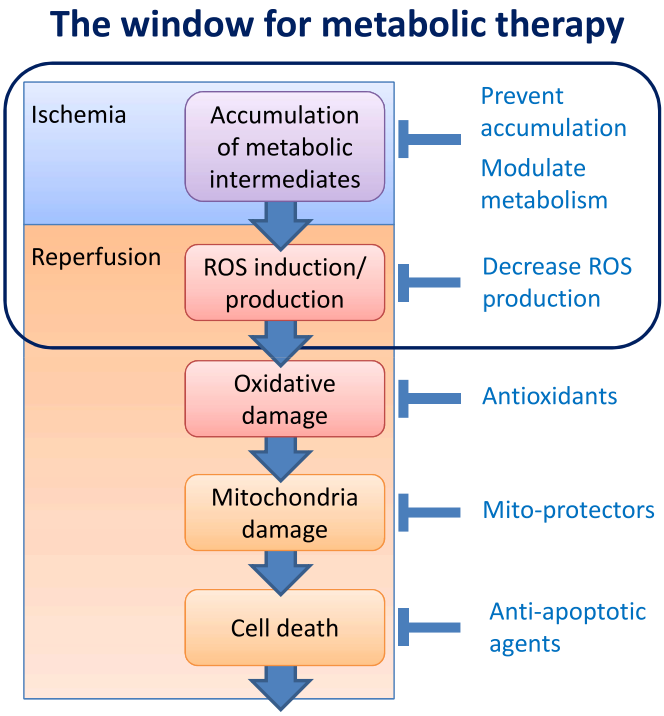

Fig. 7. Treatment strategies for the amelioration of cardiac damage by ROS.

The mechanism by which the manipulation of FA metabolism improves cardiac function in the postischemic heart is incompletely understood. Whether the effects depend on the decreased levels of long-chain intermediates or the enhancement of glucose oxidation is still being investigated. Manipulation of myocardial FA metabolism is beneficial to the prevention of myocardial ischemia, particularly during situations of controlled ischemia and reperfusion (Table 1). In experimental studies, inhibition of FA metabolism always results in decreases in the concentrations of harmful metabolic intermediates such as long-chain acylcarnitines [146]. Thus, it is hard to discriminate whether the effect of metabolic modulators, e.g., trimetazidine, sulfo-N-succinimidyl oleate (SSO), and methyl-GBB are more attributable to facilitated glucose metabolism or to the reduction in FA metabolism [146–148]. Since acylcarnitine accumulation plays an important role in ROS production [54,62], a substantial benefit can be ascribed to the lowered acylcarnitine content. Given the important role of FAs as energy substrates for cardiac functioning, it would be reasonable to reduce the concentrations of FA intermediates while maintaining ATP production from FAs. Currently, multiple targets are suggested to affect FA metabolism and the accumulation of FA intermediates before ischemia, while the possibilities for the reduction of acylcarnitines in reperfusion should be addressed in future studies.

Stimulation of FA metabolism in mitochondria as the main energy source of the heart has been considered for the treatment of mitochondrial dysfunction and related cardiac dysfunction in heart failure. A similar mechanism of action would be beneficial during the treatment of the heart shortly after ischemia to reduce the acylcarnitine mitochondrial content at reperfusion. A recent study indicated that treatment with empagliflozin protects mitochondria from dietary lipid overload-induced damage and helps to maintain FA metabolism [149]. Accordingly, the benefit of sustained FA oxidation might be extended to lower levels of ROS production during reperfusion [150]. Different regulatory mechanisms for mitochondrial FA oxidation are mediated by sirtuins via the acetylation/deacetylation of mitochondrial proteins [151]. The mitochondrial enzyme long-chain acyl-CoA dehydrogenase (LCAD) is hyperacetylated in the absence of Sirtuin 3 (SIRT3) and results in increased levels of long-chain acylcarnitines [152]. Similarly, SIRT5 ablation results in impaired β-oxidation and the accumulation of medium- and long-chain acylcarnitines in the liver and muscles of *Sirt5* KO mice [153]. Mice lacking sirtuins exhibit hallmarks of FA oxidation disorders, including reduced ATP levels and intolerance to cold exposure. Thus, treatment with sirtuin activators might be beneficial during reperfusion. Overall, the stimulation of mitochondrial FA metabolism could be a critical mechanism for the restoration of cardiac function in reperfusion.

The accumulation of succinate and RET-mediated ROS production have emerged as drug targets for the treatment of myocardial infarction. The Krebs cycle intermediate malate and the succinate competitor malonate are known SDH (complex II) inhibitors [154,155] and thus have demonstrated cardioprotective properties in experimental settings of myocardial infarction. Malonate effects were demonstrated in an isolated mouse heart model if administered before ischemia [9] or at the onset of reperfusion [156]. Intracoronary administration of malonate in a swine model of transient coronary occlusion prevented excessive ROS production and limited the infarct size [157]. The difficulties of malonate treatment are related to its systemic toxicity and relatively low bioavailability which requires high doses [157]. Dimethyl malonate, which is more membrane permeable, also showed less ischemic succinate accumulation and subsequently lower ROS production [9,110,

Table 1
Effect of cardiometabolic drugs on ROS generation during I/R injury.

Drug/compound	Mechanism/target	Cardioprotective effect	Effect on ROS	Ref.
Liraglutide Exenatide	GLP1 receptor agonists PI3K-Akt pathway	↓ infarct size	↓ ROS	[163–166]
Trimetazidine	shifting FA oxidation to glucose oxidation during reperfusion;	↓ infarct size	↓ oxidative stress	[147]
Methyl-GBB	↓Long-chain acylcarnitines	↓ infarct size; improved cardiac functions;	↓ long-chain acylcarnitines, ↓ ROS?	[62,146]
SSO	CD36 inhibitor ↓ FA oxidation rate; ↑ glycolytic rate, ↑PDH activity	Prevent cardiac dysfunction	ROS?	[148]
Carvedilol	adrenergic receptor blocker; modulator of cardiac AMPK signaling pathway	↓ infarct size improved cardiac functions; ↑ glucose uptake& oxidation, ↓ FA oxidation during I/R	↓ ROS production upon complex I injury	[167,168]
Dapagliflozin Empaglifozin	SGLT2 inhibitors, ↑ FA oxidation	↓ infarct size mitoprotection	↓ROS	[149,150,169,170]
Itaconate	↓SDH activity, ↓ succinate	↓ cerebral I/R injury, heart?	↓ ROS	[129]
Malonate Dimethyl-malonate	↓SDH activity	↓ infarct size	↓ ROS	[156,157,171]
Diazoxide	ATP-sensitive K (K_{ATP}) channel opener; ↓SDH activity	↓ I/R injury	↓*ROS?*	[159,160]

Abbreviations: AMPK = AMP-activated protein kinase; CD36 = fatty acid translocase; FA = fatty acid; GLP1 = glucagon-like peptide-1; Methyl-GBB = methyl-γ-butyrobetaine; PI3K-Akt = phosphoinositide-3 kinase/protein kinase B; PDH = pyruvate dehydrogenase; SGLT2 = sodium-glucose co-transporter-2; SSO = Sulfo-N-succinimidyl oleate.

158]. Diazoxide is another SDH inhibitor that mitigates ROS generation by RET [159,160]. However, diazoxide is also an ATP-sensitive potassium channel (K_{ATP} channel) opener [161,162], and likely also exerts cardioprotective effects independent of SDH or RET inhibition. Since SDH is localized in the mitochondrial matrix, the effectiveness of the compounds is highly dependent on their timely delivery to cardiac mitochondria. Considering that a major portion of succinate is metabolized or eliminated from the heart within 3–5 min of reperfusion beginning [9,50], it would be a challenge to manage compound delivery in a clinical setting in such a short time frame. Importantly, succinate plays a pivotal role in oxidative metabolism in the mitochondria, and inhibition of this pathway might compromise cardiac recovery at later stages of reperfusion. Therefore, potential pharmacological inhibitors of SDH must be reversible, ensuring dissociation of the inhibitor from the enzyme.

7. Conclusions

The studies summarized in this review clearly indicate that the mechanisms of cardiac ROS formation in reperfusion are multifactorial and are driven by the ischemia-induced accumulation of energy substrates. Under hyperglycemic conditions, NOX2 activation, an increased ψ_m and decreased mitoHKII activity result in ROS production. In the FA metabolism pathway, the accumulation of long-chain acylcarnitines in ischemic mitochondria is the main player in ROS production. Succinate accumulation during ischemia may exert some beneficial effects, but during reperfusion, it leads to ROS production and exacerbates I/R injury. Therapies that target energy metabolism pathways by regulating substrate concentrations, prevent ROS production, and protect the heart from I/R injury. These results emphasize the importance of strategies to modulate and counteract substrate accumulation for the attenuation of ROS production in cardiac I/R injury.

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