

Review Article

Glycolysis and fatty acid β -oxidation, which one is the culprit of ischemic reperfusion injury?

Qing Gao^{1*}, Hao Deng^{2*}, Huhu Li^{1*}, Chun Sun¹, Yingxin Sun¹, Bing Wei¹, Maojuan Guo¹, Xijuan Jiang¹

¹School of Integrative Medicine, ²First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, Tianjin 300193, China. *Equal contributors

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Abstract: Thrombolysis therapy and percutaneous coronary intervention are common methods in the treatment of acute myocardial infarction. These methods can recover the cardiac function in most cases. But in almost one-third circumstances, cardiac dysfunction and structural damage aggravated, which is known as ischemia-reperfusion injury. Normally, most ATP in cardiomyocytes was produced from fatty acid β -oxidation. However, both fatty acid β -oxidation and glycolysis accelerated due to AMPK activation during ischemic. Glycolysis uncoupled from oxidation results in intermediate metabolite accumulation, such as lactate, proton, succinate and NADH. During reperfusion, the recovering rate of fatty acid β -oxidation even exceed the rate under physiological condition due to the sudden influx of high concentration of oxygen. High rate of fatty acid β -oxidation inhibits glucose oxidation and results in proton and Ca^{2+} overload, especially huge amount of ROS production, which leads to mitochondria damage and cell death. Clearly, energy metabolism disorder result from the sudden change of oxygen supply during ischemic and reperfusion is the main cause of ischemic reperfusion injury. However, glycolysis and fatty acid β -oxidation, which one is the real culprit in ischemic reperfusion injury is controversial. In this review, we will discuss the process of glucose metabolism and fatty acid β -oxidation thoroughly, as well as the energy sensor AMPK signaling, in order to clarify how to modulate energy metabolism to reduce injury during ischemic and reperfusion.

Keywords: Glycolysis, fatty acid β -oxidation, intermediate metabolite, ischemic reperfusion injury

Introduction

Although cardiomyocytes were supplied by multiple energy sources, fatty acid and glucose are the main ones. Under physiological condition, most of its energy was produced from fatty acid β -oxidation (FAO) (All abbreviations are listed in **Table 1**), due to its high efficiency in ATP production. Free fatty acids (FFA) in cardiomyocytes generate fatty acyl-CoA following esterification reaction, the process of which is catalyzed by a family of fatty acyl-CoA synthase (FACS) enzymes [1]. The mitochondrial uptake of fatty acyl-CoA into its matrix is mediated by carnitine palmitoyl-transferase I and II (CPT-I, II), which are localized to the mitochondrial outer membrane and inner membrane respectively [2]. Once enter the mitochondrial matrix, fatty acyl-CoA are catalyzed via the process of fatty acid β -oxidation, eventually they were dismembered to acyl-CoA that were metabolized in TCA cycle [3, 4]. Both the level of circulating

FFA in the plasma and the intracellular level of malonyl-CoA can regulate the rate of fatty acid β -oxidation [5, 6]. Malonyl-CoA is synthesized from cytosolic acetyl-CoA via acetyl-CoA carboxylase (ACC), while it is degraded through malonyl-CoA decarboxylase (MCD) [7, 8]. Malonyl-CoA regulates fatty acid β -oxidation by inhibiting the activity of CPT-I, which is the rate limiting enzyme of mitochondrial fatty acid uptake, thereby it controls the rate of fatty acids entering into the mitochondria for subsequent oxidation [9, 10].

Since the well-known fact that fatty acid are normally the predominant fuel for cardiac energy production, aerobic glucose metabolism has been neglected in heart. Actually, it is responsible for 10%-40% of ATP production in cardiomyocytes [11]. Glucose transportation into cardiomyocytes was regulated by glucose transporter protein family members such as GLUT 1 and 4, which are predominantly expressed at

Table 1. List of abbreviations

Full name	Abbreviate
Fatty acid β -oxidation	FAO
Free fatty acids	FFA
Acyl-CoA synthase	FACS
Carnitine palmitoyl-transferase I and II	CPT-I, II
Malonyl-CoA decarboxylase	MCD
Acetyl-CoA carboxylase	ACC
Pyruvate decarboxylase	PDC
6-phosphofructo-1-kinase	PFK-1
Reactive oxygen species	ROS
Ischemic reperfusion	IR
Electron transport chain	ETC
Adenosine Monophosphate Activated Protein Kinase	AMPK
Nicotinamide adenine dinucleotide	NDAH
Reverse electron transport	RET
Coenzyme Q	CoQ
Carbohydrate binding module	CBM
Cystathionine-b-synthase	CBS
Liver kinase B1	LKB1

Actually, the heart is an organ that can exert maximum function when it apply different energy sources simultaneously [11]. In aerobic condition, high rate of fatty acid β -oxidation can inhibit glucose oxidation in cardiomyocytes. This phenomenon is based on the Randle cycle [20], in which fatty acid-derived acetyl CoA can decrease the production of glucose-derived acetyl CoA via inhibition of the pyruvate dehydrogenase complex. On the other side, under anoxic condition, energy-provision way switch into the more efficient way, glycolysis but brings harmful metabolites. Any alterations in energy metabolism can contribute to development of heart diseases, including IR injury. Optimizing energy metabolism in the heart is a feasible and important approach to treat IRI. Under this concept, we recapitulate myocardial energy metabolism and its relevance to IR injury.

the surface of adult cardiomyocytes [12]. Intracellular glucose is rapidly phosphorylated and becomes a substrate for the glycolytic pathway, glycogen synthesis, and ribose synthesis [13, 14]. Once entering the glycolytic pathway, the process will be examined by key enzymes such as hexokinase, 6-phosphofructo-1-kinase (PFK-1) and pyruvate kinase [15]. Pyruvate enters the mitochondria via a monocarboxylate carrier, and becomes a cross point for several metabolic pathways. For example, it can produce lactate glycolysis; it can convert to acetyl-CoA by pyruvate decarboxylase (PDC), and transform into oxaloacetate [16].

Although ischemic treatment such as coronary bypass surgery, thrombolysis, and percutaneous coronary intervention achieved significant accomplishment, ischemic reperfusion (IR) injury is still to be solved [17]. Reactive oxygen species (ROS) and Ca^{2+} overload are the main culprits as supported by many researchers [18]. But the resources of ROS and Ca^{2+} are still under debates. During ischemic and reperfusion process, oxygen supply in cardiomyocytes changed suddenly, which cause energy metabolism disorder and further damage [19]. The heart has a very high energy demand, and of course, oxygen demand. Energy metabolism pathway changes with oxygen concentration.

Alterations of glycolysis and fatty acid β -oxidation

Ischemic

During ischemia, ATP production from electron transport chain (ETC) is almost terminated without oxygen. It results in AMP accumulated, which activate Adenosine Monophosphate Activated Protein Kinase (AMPK) signaling (**Figure 1**) [21]. Activated AMPK can accelerate both glucose and fatty acid β -oxidation through relocating GLUT4 and FAT/CD36 to sarcolemma as well as phosphorylating PFK-1 and inhibiting ACC [22]. Subsequently, malonyl-CoA decrease thus relieves the inhibition of CPT-1 [23]. However, Krebs cycle cannot disposal the huge amount of Acetyl-CoA from glucose and fatty acid oxidation with blocked ETC [24]. As a consequence, Acetyl-CoA produced from fatty acid β -oxidation will inhibit PDC, i.e. Randle cycle as previously described, and results in glycolysis that uncoupled with ATP production [25]. In addition, fatty acid accumulate in cytoplasm under both prandial state and catecholamine discharge. Catecholamine discharge was up-regulated in the ischemic stress, together with plasma norepinephrine levels [26]. Catecholamines stimulate adipose tissue lipolysis, decrease pancreatic insulin release, and des-

Glycolysis and fatty acid β -oxidation disorder in IR

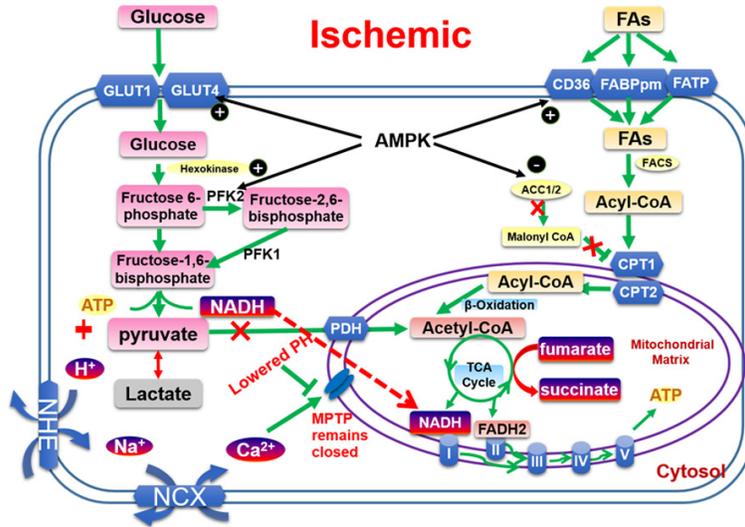


Figure 1. Alterations of glucose and fatty acid β -oxidation metabolism during ischemic. AMP accumulation during ischemic leads to AMPK activation, which accelerate both glucose and fatty acid β -oxidation. High rate of fatty acid β -oxidation results in glycolysis uncoupled from oxidation. Then the intermediate of glycolysis accumulated, including lactate, protons and NDAH. Intracellular acidosis impair the activity of Na^+/K^+ ATPase, which extrudes 3Na^+ ions in exchange for 2K^+ ions, leads to intracellular Na^+ overload, subsequently activation of $\text{Na}^+/\text{Ca}^{2+}$ exchangers, results in intracellular Ca^{2+} overload. Excess NDAH in Cytoplasm enter the mitochondria membrane through malate/aspartate shuttle. Moreover, ischemic succinate accumulation arises from reversal of succinate dehydrogenase, which is driven by fumarate overflow from purine nucleotide break down and partial reversal of the malate/aspartate shuttle. Green arrow means normal physiological process. Red arrow means pathophysiological process. Red X mark means the pathway is inhibited.

ensitize peripheral insulin [27-29]. Meanwhile, plasma levels of hydrocortisone elevate, which also desensitize insulin [30]. All these effects promote adipose tissue lipolysis that leads to increase plasma concentrations of FFA and increase delivery of FFA to the myocardium. The increased delivery of FFA to the myocardium can alter fatty acid utilization during both ischemia, and reperfusion following ischemia.

Although ATP production from glycolysis may be sufficient to maintain ionic homeostasis during mild to moderate ischemia, the hydrolysis of ATP derived from glycolysis uncoupled from subsequent pyruvate oxidation results in the increased generation of lactate, protons and nicotinamide adenine dinucleotide (NDAH) [31-33]. Intracellular acidosis impair the activity of Na^+/K^+ ATPase [34], which leads to intracellular Na^+ overload and subsequent activation of $\text{Na}^+/\text{Ca}^{2+}$ exchangers. Also, impaired activity of the sarcolemmal and sarcoplasmic Ca^{2+} ATPase which are responsible for the extrusion and

reuptake of Ca^{2+} into the sarcoplasmic reticulum, respectively, leads to intracellular Ca^{2+} overload, which in turn increases the production of free radicals. This also changes the affinity of different proteins, such as enzymes and troponin C, to Ca^{2+} [35], modifies tertiary protein structures, inhibits enzymes and disrupts the function of sarcoplasmic pumps and carriers [36-38]. Low dose of $\text{Na}^+/\text{Ca}^{2+}$ exchange inhibitor protects ischemic reperfusion injury in rat hearts [39]. In general, glycolysis generates ATP for cardiomyocytes utilization, but also results in intracellular acidosis, NDAH and Ca^{2+} overload during ischemic.

Reperfusion

At reperfusion, the rates of glucose oxidation, cardiac efficiency, and mechanical function remain depressed [40-41]. The accelerated rates of glycolysis during ischemic period can resolve during reperfusion, but still remain uncoupled from glucose oxidation (Figure 2) [42-44]. Furthermore, as reperfusion rapidly normalizes extracellular pH, it generates a large trans-sarcolemmal proton gradient that increases Na^+/H^+ exchange and exacerbates intracellular Na^+ overload followed ischemia. In return, Na^+ overload promotes reverse activation of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger, thereby contributing to intracellular Ca^{2+} overload and aggravation during reperfusion [45].

These disturbances in ionic homeostasis during ischemia and during reperfusion contribute to the deficits in both cardiac function and cardiac efficiency, both of which can be improved by therapies that correct myocardial energy production. By activating the PDC, by products accumulation can be limited, and cardiac functional recovery is improved. Improving the coupling of glucose metabolism by stimulating glucose oxidation accelerates the recovery of PH and improves both cardiac mechanical function and cardiac efficiency [44].

Glycolysis and fatty acid β -oxidation disorder in IR

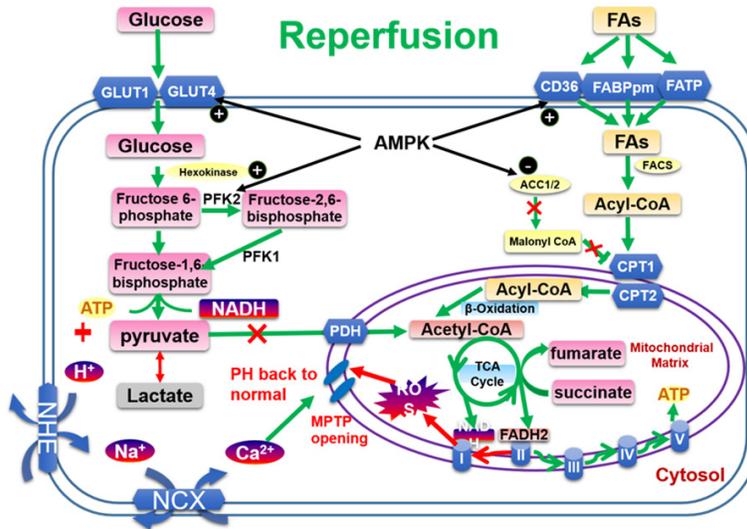


Figure 2. Alterations of glucose and fatty acid β -oxidation metabolism in reperfusion. At reperfusion, fatty acid β -oxidation is rapidly recovered due to the sudden restore of oxygen, with rates exceeding pre-ischemic levels. The combination of high-circulating fatty acid levels and a decrease in malonyl CoA control of mitochondrial fatty acid uptake results in the preferential use of fatty acids as an oxidative substrate over glucose at reperfusion. High fatty acid oxidation rates still inhibit glucose oxidation. Most importantly, the accumulated succinate is rapidly re-oxidized by succinate dehydrogenase, driving extensive ROS generation by reverse electron transport at mitochondrial complex I in the first few minutes. MPTP opening because of ROS burst and Ca^{2+} overload. Green arrow means normal physiological process. Red arrow means pathophysiological process. Red X mark means the pathway is inhibited.

Paradoxically, fatty acid β -oxidation is rapidly recovered at reperfusion, due to the sudden restore of oxygen, with its rates exceeding pre-ischemic levels [46]. The combination of high-circulating fatty acid levels with decreased malonyl CoA mitochondrial fatty acid uptake results in the preferential use of fatty acids as an energy source over glucose at reperfusion. High fatty acid oxidation rates also inhibit glucose oxidation. Because the regulation of malonyl CoA levels is central in administration of the rate of fatty acid β -oxidation in the heart, MCD may play a major role in controlling the extent of ischemic injury by promoting glucose oxidation. This hypothesis was proved by the study that MCD inhibitors can increase myocardial malonyl CoA levels, decrease fatty acid β -oxidation, accelerate glucose oxidation in both *in vitro* and *in vivo* [47]. This suggests that switching in energy substrate preference improves cardiac function during and after ischemia. Perhexiline, trimetazidine, ranolazine, and etomoxir, all stimulate glucose metabolism while down regulate free-fatty-acid metabolism. Therefore,

they enhance oxygen efficiency during myocardial ischemic [48, 49].

Succinate accumulates and reverse electron transport chain

Accumulation of the Krebs cycle intermediate succinate is a universal metabolic marker of ischemia due to its role in tissues arrangement and mitochondrial ROS production during reperfusion. Most importantly, succinate accumulation arises from reversal of succinate dehydrogenase under ischemic conditions, which in turn is driven by fumarate overflow from purine nucleotide break down and partial reversal of the malate/aspartate shuttle [50]. Elevated NADH level in the cytoplasm that traveled out from mitochondria during glycolysis relies on malate/aspartate shuttle in cardiomyocyte [51]. Deamination of aspartate to

oxaloacetate transfers electrons from NADH in the cytosol to form malate. The malate in the cytosol is then exchanged with mitochondrial succinate through dicarboxylate carrier [52]. Once in the matrix, the succinate fails to form succinyl CoA due to the lack of CoA and GTP during ischemia. In parallel, AMPs, which accumulates during ischemia, was metabolized into fumarate through purine nucleotide cycle [53]. This fumarate can then be hydrolyzed to malate by cytosolic fumarate hydratase.

After reperfusion, the accumulated succinates are rapidly re-oxidized by succinate dehydrogenase, which drives extensive ROS generation by reverse electron transport (RET) at mitochondrial complex I in the first few minutes [54, 55]. Under physiological condition, transportation of electron across the differences in reduction potential between the NAD^+/NADH and the Coenzyme Q (CoQ) pool across complex I (ΔE_h) has to be sufficient to pump protons across the mitochondrial inner membrane against the proton motive force, Δp [56]. As

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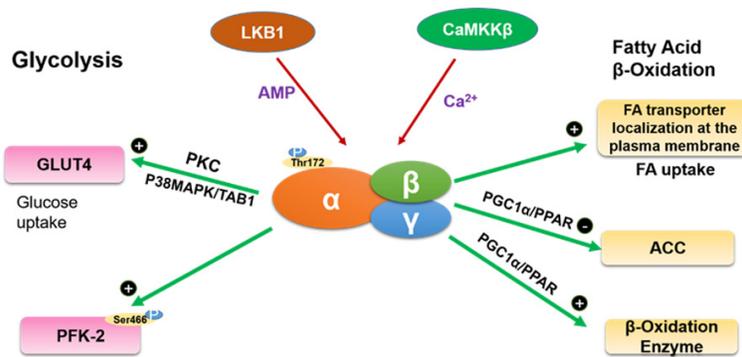


Figure 3. Role of AMPK in ischemic reperfusion injury. Ca²⁺-dependent pathway mediated by CaMKK β and an AMP-dependent pathway mediated by liver kinase B1 (LKB1) can activate AMPK. Firstly, activated AMPK promote glucose utilization. AMPK stimulate glucose uptake by translocating GLUT4-containing intracellular vesicles across the plasma membrane by phosphorylating PKC and p38MAPK/TAB1 pathway. Besides, AMPK promotes glycolysis by increasing PFK2 activity. Secondly, AMPK inhibits FA synthesis while increase Fatty acid oxidation through PGC1 α /PPAR pathway.

four protons are pumped for every two electrons that pass through complex I, $2\Delta E_h > 4\Delta p$ is requirement for the forward reaction to occur [57]. Electrons can be driven backward from the CoQ pool onto the FMN of complex I, reduces the FMN which can donate a pair of electrons to NAD⁺ to form NADH, or pass one electron to oxygen to generate superoxide. The condition to be met for RET to occur is $4\Delta p > 2\Delta E_h$ [58]. The rapid oxidation of the succinate that accumulates during ischemia favors reduction of the CoQ pool, thereby maintaining a large ΔE_h [59]. The reduced CoQ pool also favors proton pumping by complexes III and IV helping maintain a large Δp upon reperfusion [60]. In addition, the degradation of adenine nucleotides during ischemia limits ADP availability upon reperfusion that would otherwise diminish Δp by stimulating ATP synthesis. In this scenario accumulated succinate act as electron sink during ischemia, which is then used to drive ROS by RET at complex I upon reperfusion. Excessive ROS will bring further damage to the mitochondrial, such as Mitochondrial Permeability Transition Pore (MPTP) opening.

AMPK signaling in IR

AMPK, known as energy sensor, has been reported to be related to ischemic reperfusion injury. Intrinsic modulation of AMPK is critical to prevent irreversible mitochondrial damage and myocardial injury [61, 62]. However, the

specific pathway through which AMPK works seems ambiguous in the previous literatures. AMPK is a heterotrimeric complex, and composed of a catalytic α -subunit comprising a typical Ser/Thr kinase domain and regulatory β and γ subunits [63]. Each subunit has multiple isoforms (α_1 and α_2 , β_1 and β_2 , and γ_1 , γ_2 , and γ_3) with tissue-specific distribution. In heart, all isoforms have been reported to be expressed [64]. The α -subunit of AMPK is a 63-kDa protein that exhibits catalytic activity. The α_1 isoform is widely expressed, whereas the α_2 isoform has its highest

levels of expression in liver, heart, and skeletal muscle. The β and γ subunits appear to be important in substrate specificity and maintenance of heterotrimer stability [65]. The β -subunit acts as a scaffold for the binding of the α - and γ -subunits and, by virtue of having a carbohydrate binding module (CBM), also function in the regulation of glycogen metabolism [66]. The γ -subunit containing four cystathionine- β -synthase (CBS) domains, that serve to bind adenine nucleotides and has also been implicated in regulating glycogen metabolism, since mutations in the CBS domains lead to altered glycogen metabolism in both skeletal muscle and heart.

The mechanism of AMPK activation involves two distinct signals: a Ca²⁺-dependent pathway mediated by CaMKK β and an AMP-dependent pathway mediated by liver kinase B1 (LKB1) (Figure 3) [67]. The upstream kinases phosphorylated at Thr172 on α subunit, stimulate allosteric effect upon binding of AMP within the CBS domain of the γ subunit, thereby maintaining the enzyme in the activated state, as well as protecting Thr172 from dephosphorylation [68]. Thus, AMPK serves as a unique metabolic control node as it senses cellular energy status through modulation of its activities via phosphorylation and allosteric activation by AMP [69]. Numerous pathological processes have been shown to stimulate AMPK, including conditions that lead to alterations of the intracellular AMP/ATP ratio (e.g., hypoxia, glucose

deprivation) and calcium overload, which are the notable symptoms in ischemic reperfusion injury [70, 71].

Once AMPK activated, cardiomyocytes conducted energy consumption rather than reservation, which means catabolism overwhelms anabolism. On one hand, AMPK promotes glucose utilization. AMPK stimulates glucose uptake by translocating GLUT4-containing intracellular vesicles across the plasma membrane [72]. AMPK involves phosphorylation and activation of Akt substrate of 160 kDa [73], protein kinase C [74], endothelial nitric oxide synthase [75] and p38 mitogen-activated protein kinase/transforming growth factor β -activated protein complex 1 [76], regulate GLUT4 translocation to the plasma membrane. Besides, AMPK promotes glycolysis by increasing phosphofructokinase 2 activity [77, 78], which produces fructose 2, 6-bisphosphate, a potent stimulator of glycolysis. On the other hand, AMPK inhibits FA synthesis while increase Fatty acid oxidation. The phosphorylation process of acetyl-coA carboxylase 1, which catalyzes the rate-limiting step in fatty acid synthesis and sterol regulatory element-binding protein 1c, a transcription factor that promotes the expression of multiple lipogenic enzymes were inhibited. While fatty acid uptake is increased by promoting the translocation of fatty acid transporter CD36 to the plasma membrane, the mechanism underlying this is unclear. In cytoplasm, fatty acids are transported into the mitochondria for β -oxidation by CPT-1. AMPK activation enhances PGC-1 α transcription [79], which increases CPT-1 activity and activates fatty acid β -oxidation by inhibiting phosphorylation of ACC2. ACC2 is localized to the outer membrane of the mitochondria near CPT-1 where it inhibits production of malonyl-CoA, a potent allosteric inhibitor of CPT-1.

During ischemic, high AMP/ATP rate activates AMPK, which exerts catabolism to produce more ATP for cardiomyocytes utilization. Glycolysis dominates the main ATP production way due to lack of oxygen. In this point, AMPK presents beneficial effect for cardiomyocytes. However, during reperfusion, AMPK also promotes high rate of fatty acid β -oxidation, since oxygen content suddenly recover. In return, the increased rate of fatty acid β -oxidation can inhibit glucose oxidation, which leads to increase lactate and proton production and decrease cardiac efficiency. In this situation,

AMPK activation is harmful for cardiomyocytes. Since high rates of fatty acid oxidation can contribute to ischemic damage by inhibiting glucose oxidation, it is important to maintain proper control of fatty acid oxidation both during and following ischemic.

Conclusion and future perspective

In the present review, we analyzed the alterations of glucose and fatty acid β -oxidation during IR, as well as the role of AMPK signaling. In brief, glucose uncoupled from oxidation due to high rates of fatty acid β -oxidation result in intermediate metabolites, which are the main pathogenic factor of reperfusion during ischemic. In this case, glycolysis is the leading cause of IR injury. For this reason, the inhibition of glycolysis during ischemia will lessen lactate and proton production, and improve cardiac efficiency [80]. Previous clinical studies focused on inhibiting fatty acid oxidation and increasing glucose oxidation during ischemic heart disease, but barely no study paid attention to the metabolites of glycolysis. Since AMPK signaling has dual effect in regulating glucose and fatty acid metabolism, we need to elucidate the precise molecular pathway when we want to inhibit glycolysis and promote glucose oxidation. Also, decreasing ischemic succinate accumulation by pharmacological inhibition is sufficient to ameliorate *in vivo* IR injury in murine models of heart attack and stroke. In the future, we hope more studies will focus on the bad influence of glycolysis and try everything to eliminate it in the ischemic reperfusion process.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Xijuan Jiang, School of Integrative Medicine, Tianjin University of Traditional Chinese Medicine, 88th Yuquan Road, Nankai District, Tianjin, China. Tel: +86-22-59596287; E-mail: xijuanjiang@foxmail.com

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Glycolysis and fatty acid β -oxidation disorder in IR

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Glycolysis and fatty acid β -oxidation disorder in IR

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