Fatty Acid Oxidation Inhibitors in the Management of Chronic Complications of Atherosclerosis

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Ischemic heart disease is characterized by a modification of the normal energy balance of the heart. During and following an ischemic event, circulating fatty acids are elevated, resulting in the acceleration of fatty acid oxidation at the expense of glucose oxidation. Despite the reduction in glucose oxidation, the rate of glycolysis increases, leading to an uncoupling of glucose metabolism. This results in the accumulation of metabolic byproducts, which leads to a decrease in cardiac efficiency. A novel therapeutic strategy involves improving the efficiency of oxygen utilization by the ischemic heart by the modulation of energy metabolism. This can be achieved by a reduction in the levels of circulating fatty acids using β-blockers, glucose-insulin-potassium infusions, and nicotinic acid. Alternatively, fatty acid oxidation can be directly inhibited using trimetazidine, ranolazine, or glucose oxidation directly activated using dichloroacetate, which significantly improves the efficiency of the heart.

Introduction

Myocardial ischemia occurs when the oxygen requirement of the heart exceeds the oxygen supplied to the heart by the coronary circulation. Common manifestations of ischemia are angina pectoris and acute myocardial infarction (AMI), most often caused by decreased blood flow to the heart due to obstruction of large coronary vessels by atherosclerotic plaques and/or clot formation, or due to a diminished ability to increase coronary flow in response to an increase in metabolic demand. The result of ischemia is modifications to the metabolism, structure, and function of the ischemic zone.

The classic treatment of ischemic heart disease consists of strategies that correct the oxygen supply-demand imbalance either by increasing oxygen delivery or decreasing oxygen demand. Therapeutic strategies to increase oxygen delivery include invasive procedures such as coronary bypass grafting and balloon angioplasty, as well as pharmacologic agents including vasodilators, thrombolytics, and antiplatelet drugs. Decreasing oxygen demand can be achieved noninvasively by the use of pharmacologic agents including organic nitrates, calcium channel blockers, or β-adrenoceptor antagonists. Despite these treatment options, ischemic heart disease remains a major cause of morbidity and mortality in Western society, emphasizing the need to develop novel therapeutic strategies, such as improving the efficiency of oxygen utilization in the ischemic and reperfused heart. One such approach is the use of metabolic modulation to optimize myocardial energy metabolism to both decrease the symptoms of ischemia and also reduce the damage resulting from an acute coronary event. This review discusses how ischemia alters myocardial metabolism, how these changes in metabolism depress cardiac efficiency, and how strategies that modify metabolism can improve efficiency and be used as a therapeutic approach to treating ischemic heart disease.

Aerobic Myocardial Energy Metabolism

In order to meet the high energy demands of contraction and ionic homeostasis, the heart must produce an abundant supply of ATP (between 3.5 and 5 kg/d) [1]. To meet this high demand, the heart acts as a metabolic omnivore, metabolizing a variety of carbon substrates, including carbohydrates (glucose, lactate, and pyruvate), fatty acids, and ketone bodies [2,3,4•]. Under normal aerobic conditions, the heart preferentially metabolizes fatty acids, which contribute between 60% and 80% of the required ATP [5,6], with carbohydrates contributing the residual 20% to 40%. This ratio is influenced by a number of conditions, which include alterations in hormonal control, workload, energy substrate supply, and oxygen supply to the heart. Despite producing more ATP than carbohydrates, fatty acids are not as oxygen efficient, requiring approximately 10% more oxygen to produce an equivalent

amount of ATP [6]. This observation is of particular importance during times of ischemic stress, when oxygen is the limiting factor for oxidative metabolism.

Fatty acid metabolism

Long-chain fatty acids are supplied to the heart as either triglycerides in chylomicrons (CM) and very low-density lipoproteins (VLDL) or as fatty acids bound to albumin [5]. Lipoprotein lipase, bound to the capillary endothelium through a herparin sulphate proteoglycan bond, removes fatty acids from CMs and VLDL, which allows them access to the cardiomyocytes. These fatty acids are then taken up either by way of simple diffusion across the sarcolemmal membrane or by way of a carrier-mediated process utilizing transport proteins such as CD36/FAT, and FABPpm [6,7]. Alternatively, fatty acids may also be taken up by way of the VLDL receptor, which is highly expressed in the heart [8]. Following uptake, the fatty acids are activated by way of acyl coenzyme A (acyl-CoA) synthetase to acyl-CoAs, which may be taken up by the mitochondria (Fig. 1).

Fatty acid oxidation is tightly controlled at a number of steps, but the uptake of fatty acyl-CoAs into the mitochondria is arguably one of the most important. The key regulatory enzyme in this process is carnitine palmitoyl-CoA transferase 1 (CPT-1), which catalyzes the conversion of fatty acyl-CoAs (which cannot cross the mitochondrial membrane) to fatty acyl-carnitines, which can then cross the inner mitochondrial membrane by way of the carnitine shuttle (Fig. 1). Malonyl-CoA is a potent endogenous inhibitor of CPT-1 and is a major determinant of long-chain fatty acyl-CoAs' flux into the mitochondria [5,6]. Malonyl-CoA concentrations are under the control of two enzymes: acetyl-CoA carboxylase (ACC), which synthesizes malonyl-CoA, and malonyl-CoA decarboxylase (MCD), which degrades malonyl-CoA. Alterations in malonyl-CoA levels are an important determinant of ischemic-induced alterations in fatty acid oxidation.

Fatty acyl-carnitines transported across the inner mitochondrial membrane by way of an acyl-carnitine translocase are subsequently converted back to fatty acyl-CoAs by CPT-2 and are metabolized in the β-oxidation spiral (Fig. 1). Within the tricaboxylic acid (TCA) cycle, acetyl-CoA produced from β-oxidation undergoes further metabolism, resulting in the production of reduced electron donors for the electron transport chain. The electron transport chain is a series of sequentially acting electron carriers with increasing reduction potentials, linked to proton extrusion from the mitochondria. The final electron acceptor is molecular oxygen; thus it is essential for oxidative metabolism. The proton-motive force produced by the pumping of protons is used to produce the energy for the synthesis of ATP.

Carbohydrate metabolism

Glucose is the principal carbohydrate metabolized by the heart. The majority of glucose is derived from the blood, and its uptake is facilitated by glucose transporters (GLUT) such as GLUT1, which sustains basal glucose uptake, and GLUT4, which translocates from an intracellular pool in response to insulin and AMP-activated protein kinase (AMPK) (Fig. 1) [9]. Alternatively, glucose-6-phosphate can be obtained by mobilizing endogenous glycogen stores. Subsequent glucose metabolism can be separated into two major components: glycolysis and glucose oxidation. Glycolysis consists of the initial sequence of events that activate glucose-6-phosphate to fructose-1,6-bisphosphate, followed by its breakdown to pyruvate. This sequence of reactions yields less than 10% of the total ATP produced by the aerobically perfused heart [2].

The majority of ATP from carbohydrate sources is produced in the second part of this pathway, termed glucose oxidation, wherein pyruvate from glycolysis and exogenous lactate are converted to acetyl-CoA, which is oxidized in the TCA cycle. The pyruvate dehydrogenase (PDH) complex catalyzes the rate-limiting step of glucose oxidation, which is decarboxylation of pyruvate to acetyl-CoA. The PDH complex is tightly regulated by an upstream kinase (PDH kinase), which phosphorylates and inactivates the complex, and an upstream phosphatase (PDH phosphatase), which dephosphorylates and activates the complex [2]. PDH kinase and phosphatase are also under allosteric regulation. The kinase is positively regulated by acetyl-CoA and NADH and negatively regulated by pyruvate, CoA, and NAD^+, whereas PDH phosphatase is positively regulated by calcium and magnesium ions. The negative feedback inhibition of PDH is important, as acetyl-CoA derived from both carbohydrates and fatty acids may activate the PDH kinase and inactivate glucose oxidation. This phenomenon, which was originally described by Randle *et al.* [10], is of particular importance when hearts are exposed to elevated levels of fatty acids, which stimulate fatty acid oxidation that in turn suppresses glucose oxidation.

Myocardial energy metabolism during ischemia and reperfusion

Inadequate supply of oxygen during ischemia results in a striking reduction in the oxidative metabolism of both carbohydrates and fatty acids and an impairment of ATP production, the degree of which is dependent on the severity of ischemia [1,2]. During severe ischemia, coronary blood flow (and the oxygen and nutrients carried in it) is significantly reduced or even halted; thus all oxidative metabolism effectively ceases and the primary source of ATP is glycolysis of glycogen-derived glucose [1,2]. As glucose oxidation is inhibited, pyruvate normally metabolized in the mitochondria is converted to lactate to preserve sufficient NAD^+ to sustain flux through glycolysis. This uncoupling of glycolysis from glucose oxidation is associated with an increase in cytosolic protons, resulting in an intracellular acidosis because of the inability to remove these protons due to the reduction in coronary blood flow [3,11,12].

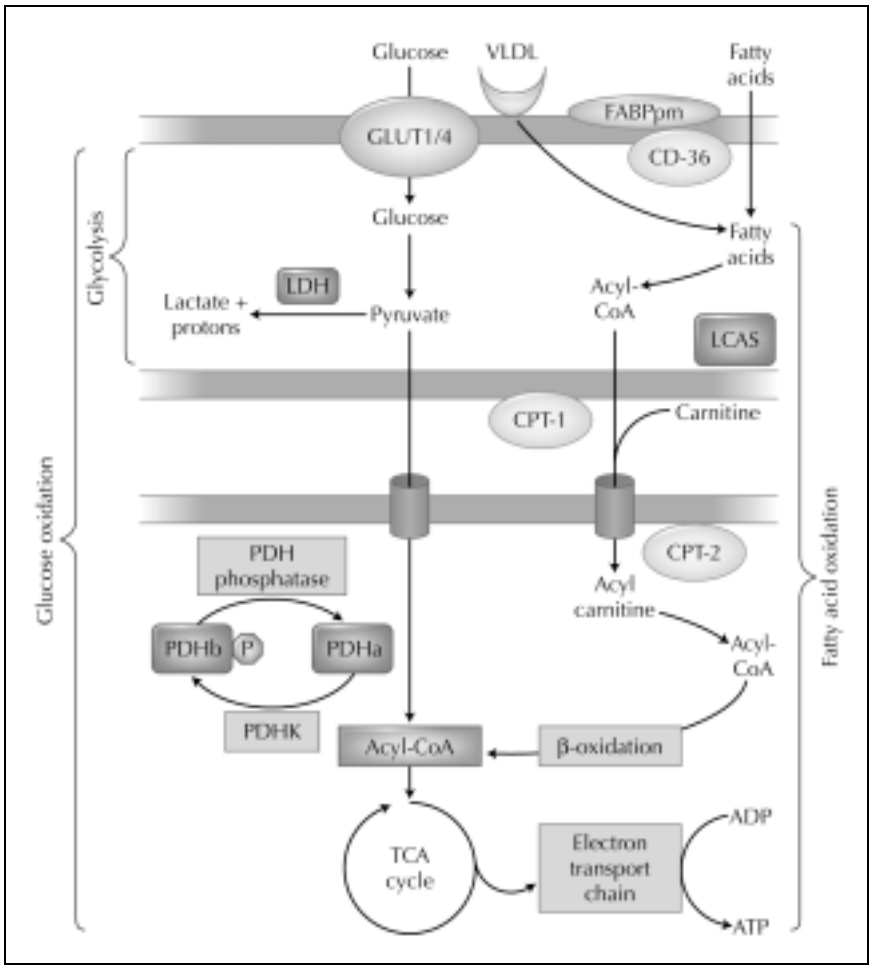

Figure 1. The major pathways of fatty acid and glucose metabolism in the heart. Downstream of acetyl-CoA (acyl-CoA), the pathways for both glucose and fatty acid oxidative metabolism are the same. However, under aerobic conditions, glucose can still pass through glycolysis without further oxidation in the mitochondria. (CPT—carnitine palmitoyl transferase; FABPpm—plasma membrane fatty acid–binding protein; GLUT—glucose transporter; LCAS—long-chain fatty acyl-CoA synthetase; LDH—lactate dehydrogenase; PDHa—active form of pyruvate dehydrogenase; PDHb—inactive form of pyruvate dehydrogenase; PDHK—pyruvate dehydrogenase kinase; TCA cycle— tricarboxylic acid cycle; VLDL—very low-density lipoprotein.)

When reperfusion of reversibly injured myocardium occurs, a rapid recovery of oxygen consumption and TCA cycle activity leads to a replenishment of the supply of ATP [11,13,14]. This is associated with a recovery of mechanical function once Ca^{2+} levels have normalized. During this reperfusion period, fatty acids can provide over 90% of the myocardium's energy requirement [14,15]. This excessive use of fatty acids is due, in part, to increased levels of circulating fatty acids as a result of a hormonal stimulation of lipolysis in adipose tissue [16,17]. A decrease in the myocardial malonyl-CoA levels also contributes to these high fatty acid oxidation rates, due to less inhibition of CPT-1 and an associated increase in mitochondrial fatty acid uptake and oxidation [13–16,18]. The reduction in malonyl-CoA is due to the ischemia-induced activation of AMPK, a so-called "fuel gauge" of the cell [19]. AMPK can phosphorylate the heart isoform of ACC on Ser227, resulting in an inactivation of the enzyme [20]. As MCD activity is preserved during reperfusion, there is a reduction in malonyl-CoA levels, a stimulation of fatty acid oxidation, and consequent impairment in the recovery of glucose oxidation. AMPK activation by ischemia also exacerbates the uncoupling of glucose metabolism, as it can stimulate the translocation of GLUT4 to the plasma membrane and increase glucose uptake [21]. AMPK can also stimulate glycolysis by the phosphorylation (Ser466) and activation of phosphofructokinase-2 (PFK-2) which increases the concentration of fructose-2,6-bisphosphate, a potent stimulator of PFK-1 [22]. A combination of high glycolytic rates and low glucose oxidation rates, secondary to high fatty acid oxidation rates, puts an excessive proton burden on the heart.

Intracellular acidosis is reduced by a number of pathways, including two Na^+-dependent mechanisms: the Na^+-H^+ exchanger 1 (NHE1) [23] and the Na^+-HCO_3^- cotransporter (NBC1, 3, or 4) [24]. Both these mechanisms lead to an intracellular Na^+ overload and activation of the reverse mode of the Na^+-Ca^{2+}exchanger (NCX) [25]. Activation of NCX results in the ischemia-induced Ca^{2+} overload that is associated with reversible injury such as arrhythmias and stunning, and irreversible injury such as apoptosis and necrosis. During both ischemia and reperfusion, there is an increased need for ATP to correct these ionic imbalances, which shunts ATP away from contractile function and results in a decrease in contractile efficiency. The use of metabolic modulation to inhibit fatty acid oxidation and stimulate glucose oxidation is a novel therapeutic approach that can be utilized to improve cardiac efficiency. This approach is discussed in the following section.

Table 1. Metabolic modulators that reduce levels of circulating fatty acids

Metabolic agent	Metabolic action	Clinical use
Glucose-insulin-potassium solution	Increase glucose uptake and glycolysis Reduce circulating fatty acids	Reduction in post-reperfusion mortality
β-Blockers	Decrease myocardial oxygen consumption Blunt catecholamine release Reduce circulating fatty acids	Long-established benefit in acute coronary syndromes Improved short- and long-term survival
Nicotinic acid	Antilipolytic Reduce circulating fatty acids Precursor for NAD^+ synthesis	Decreased mortality in hyperlipidemic patients No clinical studies for reperfusion

Table 2. Metabolic modulators that specifically inhibit fatty acid oxidation or stimulate glucose oxidation

Metabolic agent	Metabolic action	Clinical use
Trimetazidine	Inhibition of fatty acid oxidation by inhibiting 3-ketoacylcoenzyme A thiolase	Approved for use as antianginal agent in 80 countries
Ranolazine	Partial fatty acid oxidation inhibitor	Potential application as antianginal agent Approval for clinical use pending
Dichloroacetate	Inhibits pyruvate dehydrogenase kinase Stimulates glucose oxidation	Experimental only, has short half-life

Metabolic Modulation: Inhibition of Fatty Acid Oxidation

Modification of myocardial metabolism is one of the key consequences of ischemia and reperfusion that can contribute to reversible or irreversible ischemia-reperfusion injury. It is now becoming clear that agents that can reduce fatty acid oxidation and improve the coupling of glucose metabolism can alleviate the dysregulation of ion homeostasis and the associated impairment of contractile function that occur during and following ischemia. These agents can be divided into two classes: those that lower circulating free fatty acids (and indirectly modify fatty acid oxidation) (Table 1) and those that directly modify fatty acid oxidation or glucose oxidation (Table 2).

Reduction of circulating free fatty acids

This class of agents includes compounds such as β-adrenoceptor antagonists (β-blockers), glucose-insulin-potassium (GIK) infusions, and nicotinic acid. One of the hypothesized modes of action of these compounds is the reduction in circulating free fatty acids, which reduce fatty acid supply to the mitochondria and would, therefore, be expected to decrease fatty acid oxidation rates.

The use of β-blockers has long been associated with improved survival of patients following AMI, which has primarily been attributed to a direct effect on contractility as well as an antiarrhythmic effect [26]. β-Blockers are also effective at reducing the adverse effects of the catecholamine surge that is associated with ischemia, including the reduction of catecholamine-induced mobilization of fatty acids from adipocytes. As a result, in addition to reducing myocardial oxygen demand, an additional metabolic component of β-blockers may be associated with the lowering of plasma free fatty acid levels.

The concept of infusing GIK solutions during ischemia was originally introduced by Sodi-Pallares *et al.* [27] in 1969 to reduce electrocardiogram abnormalities associated with AMI. Recently GIK therapy has seen renewed interest in its use as a metabolic treatment for AMI, and a meta-analysis of previous trials reported a 28% reduction of proportional in-hospital mortality [28]. Data from the Estudios Cardiologicos Lantinoamerica Collaborative Group [29] reported a significant reduction of in-hospital mortality from AMI. A large-scale trial is presently addressing whether GIK therapy can reduce mortality following AMI.

The effectiveness of GIK therapy may be explained by several metabolic mechanisms. Typically, the beneficial effects are ascribed to its ability to increase glucose uptake (by way of insulin-induced translocation of GLUT4 to the plasma membrane) and an acceleration of glycolytic ATP production. However, GIK therapy also has the potential to increase the production of deleterious metabolic byproducts of glycolysis, namely lactate and protons. An alternative mechanism for the benefits of GIK therapy may be due to insulin-mediated inhibition of fatty acid release from adipocytes, thereby reducing plasma free fatty acid levels. This possibility is presently being investigated.

Nicotinic acid has also been shown to reduce fatty acid release from adipocytes, as well as inhibit the release of VLDL from the liver [30]. It has been also been shown to be efficacious in lowering serum lipid levels, resulting in a decrease in mortality from cardiovascular causes [31]. Nicotinic acid also has direct effects on myocardial metabolism and causes a two- to threefold increase in glucose oxidation rates that is associated with a reduction in fatty

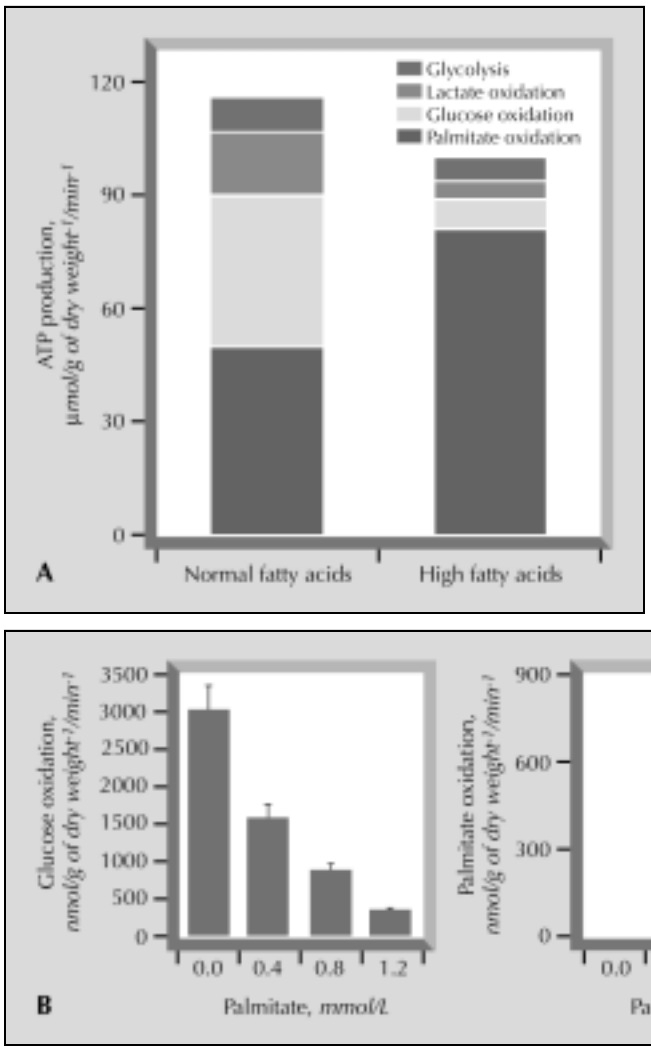

Figure 2. A, Relative contribution of various substrates to total ATP production in the isolated working rat heart. Isolated working rat hearts were perfused with 11 mmol/L of glucose and either 0.4 or 1.2 mmol/L of palmitate. Under these conditions, fatty acid contributes almost 85% of the total ATP production. **B,** The effect of increasing fatty acids on glucose and fatty acid metabolism. Isolated working rat hearts were perfused with 5 mmol/L of glucose and increasing concentrations of palmitate bound to 3% albumin.

acid oxidation and a protective effect on contractility [32]. A protective effect of nicotinic acid has also been seen in isolated rat hearts, but these data must be interpreted cautiously because the hearts were perfused in the absence of fatty acids (Fig. 2).

Inhibition of β-oxidation

One approach to treat ischemic heart disease is the inhibition of fatty acid oxidation, because high rates of fatty acid oxidation markedly reduce glucose oxidation rates and uncouple glucose metabolism. This strategy has been proven as an efficacious treatment for both acute ischemia and heart failure. Clinically, this approach has been shown with a class of piperazine derivatives, including trimetazidine and ranolazine.

Trimetazidine is an inhibitor of long-chain 3-ketoacyl-coenzyme A thiolase (the final enzyme in the β-oxidation spiral) that reduces fatty acid oxidation and increases glucose oxidation via the Randle cycle both during and following ischemia [34•,35•]. It is the first widely used antianginal drug with a mechanism of action that can be attributed to the optimization of energy metabolism. In vivo studies have shown that trimetazidine reduces infarct size in dog and rabbit models of cardiac ischemia, and reduces ST-segment elevation in regionally ischemic rabbit hearts [36]. This compound has also been shown to be cardioprotective in in vitro models of ischemia by reducing acidosis and the accumulation of intracellular Na^+ [37–39]. These experimental observations have also been confirmed in a number of clinical studies, with the antianginal efficacy of trimetazidine being equivalent to that of nifedipine and propranolol, but occurring without hemodynamic alterations such as changes in coronary blood flow or rate-pressure product [40,41]. Additionally, these antianginal effects are additive with the effects of diltiazem [42]. Beneficial effects have been seen in various clinical endpoints, such as improved ergometric exercise duration and time to 1-mm ST-segment depression in effort angina [43,44], a 50% reduction in anginal attack frequency, and a reduction in nitroglycerin requirement in patients with chronic stable angina. Trimetazidine has also proven beneficial by reducing the acute ischemic changes that occur during coronary angioplasty [45]. To date, this pharmaceutical has been approved for clinical use throughout Europe and in over 80 countries worldwide.

The second compound of this class, ranolazine, also appears to exert its anti-ischemic effect through the direct inhibition of fatty acid oxidation [46]. At clinically relevant concentrations, ranolazine partially inhibits fatty acid oxidation in both isolated rat hearts and skeletal muscle, and thus has been termed a partial fatty acid oxidation inhibitor [47,48]. Decreased fatty acid oxidation is associated with a reciprocal increase in glucose oxidation that is accompanied by an increase in PDH activity [46]. Several experimental models have shown that these metabolic changes are associated with improved contractile function during and following ischemia [47–49]. Although ranolazine is not currently clinically approved for the treatment of angina, clinical trials show that it is an effective antianginal agent, which exerts its metabolic effects independent of changes in hemodynamics [50]. Further phase III clinical trials have shown beneficial effects in angina-limited exercise as both a monotherapy and combination therapy [51•,52•].

Direct stimulation of glucose oxidation

Experimental studies have shown that direct stimulation of glucose oxidation both during and following an ischemic insult can benefit the heart. The prototype of this class, dichloroacetate (DCA), acts by way of the inhibition of PDH kinase, thus ultimately activating PDH [53]. This improves the coupling of glycolysis to glucose oxidation, resulting in a reduction in the buildup of glycolytic byproducts and an improvement of cardiac efficiency [54]. The anti-ischemic effects of DCA are associated with improved functional recovery during reperfusion in the isolated working rat heart [11,12,55] and a reduction in epicardial ST-segment elevation during in vivo coronary artery occlusion in dogs [56]. In a small clinical study of only nine patients with coronary artery disease, DCA was shown to augment stroke volume and enhance myocardial efficiency, possibly due to a stimulation of myocardial lactate utilization [57]. Although these results are encouraging, the clinical use of DCA is complicated by its short half-life and the necessity of high-dose intravenous administration.

Conclusions

During and following acute ischemic events, accelerated rates of fatty acid oxidation and the subsequent reduction in glucose oxidation are associated with a worsening of contractile function. Despite the reduction of oxidative glucose metabolism, glycolysis is accelerated, resulting in a mismatch in the coupling of glycolysis to glucose metabolism. This mismatch produces an increase in the accumulation of metabolic byproducts, particularly protons that can directly contribute to a reduction in mechanical function and cardiac efficiency. A number of pharmacologic agents are now available that modulate myocardial metabolism, including those that reduce circulating free fatty acids (that causes a secondary decrease in fatty acid oxidation) and those that direct inhibit fatty acid oxidation or stimulate glucose oxidation. Importantly, unlike β-blockers and the Ca^{2+} entry blocker, metabolic modulators can elicit their beneficial effects without causing concomitant hemodynamic alterations. Evidence based on both basic and clinical studies has shown that these agents provide a very important and novel approach to treat ischemic heart disease, both as a monotherapy and as an adjunct to existing therapies.

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