# MINIREVIEW

# Metabolic Cardioprotection by Pyruvate: Recent Progress

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**Pyruvate, a natural metabolic fuel and antioxidant in myocardium and other tissues, exerts a variety of cardioprotective actions when provided at supraphysiological concentrations. Pyruvate increases cardiac contractile performance and myocardial energy state, bolsters endogenous antioxidant systems, and protects myocardium from ischemia-reperfusion injury and oxidant stress. This article reviews and discusses basic and clinically oriented research conducted over the last several years that has yielded fundamental information on pyruvate's inotropic and cardioprotective mechanisms. Particular attention is placed on pyruvate's enhancement of sarcoplasmic reticular $Ca^{2+}$ transport, its antioxidant properties, and its ability to mitigate reversible and irreversible myocardial injury. These research efforts are establishing the essential foundation for clinical application of pyruvate therapy in numerous settings including cardiopulmonary bypass surgery, cardiopulmonary resuscitation, myocardial stunning, and cardiac failure.** Exp Biol Med 230:435–443, 2005

**Key words:** antioxidant; bypass surgery; cardiac stunning; cardiopulmonary resuscitation; ethyl pyruvate; infarction; myocardial ischemia; phosphorylation potential; reactive oxygen species

## Introduction

Pyruvate, a natural aliphatic carbohydrate and intermediary metabolite in mammalian cells, has become recognized as an intervention capable of protecting the myocardium from the ravages of ischemia-reperfusion and oxidant stress. This article discusses recent advances in the use of exogenous pyruvate as a cardioprotective intervention and the energetic and antioxidant mechanisms responsible for its cardioprotective character. Special attention is focused on work published in the five years since publication of an earlier review of this topic (1). This article emphasizes research using exogenous pyruvate and its chemical derivatives. Although a comprehensive review of the intermediary metabolism of endogenous pyruvate is beyond the scope of this article, the reader is referred to the earlier review (1) and several recent, authoritative reports on that topic (2–6).

## Metabolic Mechanisms of Pyruvate Cardioprotection

Mammalian myocardium has a high energy demand but limited energy reserves; consequently, oxidation of exogenous fuels is essential to generate the energy required to sustain cardiac contractile performance. The myocardium is capable of consuming myriad fuels, including fatty acids, glucose, lactate, amino acids, and ketone acids. The myocardium's metabolic versatility provides opportunities to enhance cardiac contractile performance by modifying the heart's fuel supply. Endogenous pyruvate concentrations in arterial plasma are between 0.1 and 0.2 m*M* in overnight-fasted dogs (7), pigs (2, 8, 9), and human subjects (10, 11). Pyruvate normally is not an important bloodborne myocardial fuel because of its low, submillimolar plasma concentrations, but the heart is responsive, functionally and metabolically, to exogenous pyruvate.

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**Pyruvate Enhancement of Myocardial Contractile Performance.** By enhancing myocardial inotropic state, supraphysiological concentrations (ca. 2–10 m*M*) of exogenous pyruvate increase cardiac output, left ventricular developed pressure and dP/dt, and external cardiac work (12–14). These inotropic effects have been ascribed to pyruvate's enhancement of cytosolic ATP phosphorylation potential and Gibbs free energy of ATP hydrolysis ($\Delta G_{ATP}$), the immediate thermodynamic energy source for contractile work. Unlike catecholamines, pyruvate does not elevate heart rate (9, 15, 16) and, therefore, does not appreciably increase cardiac internal work. Consequently, pyruvate enhancement of cardiac performance does not increase myocardial $O_2$ demand, nor does it deplete the heart's energy reserves (14).

Although increased $\Delta G_{ATP}$ would theoretically enhance all ATP-dependent cellular processes, pyruvate's effects on the sarcoplasmic reticulum (SR) have received the most attention. Cardiac function requires cyclic release and reuptake of SR $Ca^{2+}$ via the ryanodine receptor $Ca^{2+}$ channels and the SR $Ca^{2+}$ ATPase, respectively. The free energy of the SR $Ca^{2+}$ concentration gradient established by the $Ca^{2+}$ ATPase is closer to cytosolic $\Delta G_{ATP}$ than the free energy necessary for actin-myosin crossbridge cycling and sarcolemmal $Na^+$, $K^+$ ATPase, the other principal ATP-consuming processes required for contraction (17). Consequently, the SR $Ca^{2+}$ ATPase is acutely sensitive to changes in $\Delta G_{ATP}$; accordingly, enhancement of $\Delta G_{ATP}$ by pyruvate is associated with increased intra-SR $Ca^{2+}$ concentration (18) and increased turnover of the SR $Ca^{2+}$ store (13, 14). Enhanced SR $Ca^{2+}$ loading increases systolic SR $Ca^{2+}$ release and the cytosolic [$Ca^{2+}$] transient (19, 20), culminating in more forceful contractions. Indeed, 5–10 m*M* pyruvate enhances [$Ca^{2+}$] transients and cell shortening in rat ventricular myocytes (20, 21).

Studies in isolated rat ventricular cardiomyocytes by Zima *et al.* (22) have provided new insights into pyruvate's complex effects on cellular $Ca^{2+}$ homeostasis. Exposure of these cells to 10 m*M* pyruvate slowed the kinetics and increased the amplitude of the cytosolic [$Ca^{2+}$] transient and increased SR $Ca^{2+}$ content. The latter effect appeared to require mitochondrial oxidation of pyruvate and increased $\Delta G_{ATP}$. Pyruvate lowered FAD autofluorescence, indicating an increase in the reductive potential of mitochondrial flavin nucleotides and, thus, increased electron supply for the ATP-generating mitochondrial respiratory chain. Blockade of mitochondrial pyruvate uptake with α-*cyano*-4-hydroxycinnamate abrogated pyruvate's effects on mitochondrial redox state and cytosolic [$Ca^{2+}$] transients, confirming an earlier report that pyruvate enhancement of SR $Ca^{2+}$ uptake and myocardial function required its mitochondrial metabolism (14). Inhibitors of the mitochondrial respiratory chain and adenine nucleotide translocase, which blunt ATP production, also prevented pyruvate enhancement of [$Ca^{2+}$] transients.

In the study of Zima *et al.* (22), pyruvate unexpectedly suppressed SR $Ca^{2+}$ efflux by decreasing the open probability of the $Ca^{2+}$ channels. This action may have resulted from pyruvate's direct interaction with the channels, as it was observed in isolated terminal cisternal vesicles. This mechanism could be responsible for potentially arrhythmogenic induction of $Ca^{2+}$ alternans by excess pyruvate in atrial myocytes (23, 24); this proarrhythmic effect could limit the dosages of pyruvate that can be used safely in patients. Although the precise mechanism of pyruvate inhibition of $Ca^{2+}$ channels is still unknown, suppression of SR $Ca^{2+}$ release could explain the previously reported (25) temporary depression of myocardial contractile performance in the first few minutes of pyruvate administration. Eventually, pyruvate's enhancement of $\Delta G_{ATP}$ and the $Ca^{2+}$ ATPase would increase SR $Ca^{2+}$ loading and thereby augment $Ca^{2+}$ release, despite its suppression of the $Ca^{2+}$ channels.

Pyruvate also caused sustained intracellular acidification in the Zima *et al.* study (22). This $H^+$ accumulation indirectly increased resting cytosolic [$Ca^{2+}$] via sarcolemmal $Na^+/H^+$ and $Na^+/Ca^{2+}$ exchanges. Lactate, which, like pyruvate, is co-transported with a proton into cardiomyocytes (26), also lowered intracellular pH and increased resting [$Ca^{2+}$], but did not increase [$Ca^{2+}$] transients or flavin adenine dinucleotide (FAD) fluorescence. However, another report in isolated cardiomyocytes (20) did not demonstrate increased resting [$Ca^{2+}$] during pyruvate exposure.

Maier *et al.* (27) compared the inotropic effects of 5–15 m*M* pyruvate with 0.01–10 μ*M* isoproterenol in ventricular muscle strips from normal and failing human hearts. The amplitude of rapid cooling contractures provided a measure of SR $Ca^{2+}$ content. Pyruvate and isoproterenol dose-dependently increased twitch force and SR $Ca^{2+}$ content in normal myocardium. Though both actions of isoproterenol were severely impaired in failing myocardium, pyruvate's inotropic actions remained largely intact, although its enhancement of SR $Ca^{2+}$ content was blunted. Thus, pyruvate's inotropic character was not due solely to increased SR function; pyruvate was also proposed to enhance myofilament $Ca^{2+}$ responsiveness. Inorganic phosphate ($P_i$) dampens $Ca^{2+}$-activated force development by interfering with actin-myosin crossbridge cycling kinetics (28, 29). By lowering intracellular $P_i$ concentration (12–14), pyruvate could increase contractile force independent of its effects on SR $Ca^{2+}$ content. In addition, antioxidants can augment myofilament $Ca^{2+}$ responsiveness of stunned myocardium (30). Pyruvate's antioxidant properties could conceivably support its inotropic actions in failing myocardium.

Hasenfuss *et al.* (31) examined the impact of 1–20 m*M* pyruvate on contractile force and SR $Ca^{2+}$ content in left ventricular muscle strips isolated from failing human hearts and superfused with Krebs-Henseleit buffer. Pyruvate concentration-dependently increased systolic contractile force and $Ca^{2+}$ transients, lowered diastolic force, and enhanced the rates of systolic force development and

diastolic relaxation. Pyruvate also intensified rapid-cooling contractures, indicating increased SR $Ca^{2+}$ content. However, pharmacologic blockade of SR $Ca^{2+}$ uptake and release eliminated rapid cooling contractures but only partially attenuated pyruvate enhancement of systolic force, suggesting a contribution from an SR-independent mechanism. In contrast to Zima *et al.* (22), Hasenfuss *et al.* demonstrated modest (0.1 pH unit) intracellular alkalinization by 10 m*M* pyruvate. Protons dampen $Ca^{2+}$-activated force of cardiac myofilaments (32–34); accordingly, Hasenfuss *et al.* (31) proposed that intracellular alkalinization may have contributed to pyruvate enhancement of contractile force in the isolated left ventricular muscle. Interestingly, pyruvate enhancement of developed force occurred more rapidly than the increase in intracellular pH. Although intracellular $P_i$ was not measured in the Hasenfuss *et al.* study, a reduction in $P_i$ may have preceded the intracellular alkalinization and initiated the enhancement of developed force despite the inhibition of SR $Ca^{2+}$ transport. These results underscore the complexity of pyruvate's actions on the myocardial contractile machinery.

**Antioxidant Properties of Pyruvate.** Reactive oxygen species (ROS) generated by mono-, di-, or trivalent reduction of molecular oxygen in electron transfer reactions have been implicated in the pathogenesis of myocardial infarction and reversible postischemic contractile dysfunction (cardiac stunning) in experimental animals and patients (35–39). Superoxide ($\cdot O_2^-$), hydrogen peroxide ($H_2O_2$), and their more aggressive progeny hydroxyl radical ($\cdot OH$) and peroxynitrite ($ONOO^-$), attack and modify many cellular constituents (Fig. 1A), including membrane phospholipids, ion-transporting ATPases, contractile proteins, and metabolic enzymes (40–45). Cells are protected by a battery of antioxidant enzymes that detoxify ROS with reducing power supplied by α-tocopherol, ascorbic acid, glutathione (GSH), and other crystalloid antioxidants.

Pyruvate's antioxidant character resides in its chemical structure and the patterns of its cellular metabolism (46). Its α-keto-carboxylate structure enables pyruvate to neutralize peroxides in a direct, nonenzymatic chemical reaction (Fig. 1A), in which peroxides are reduced to their conjugate alcohols and pyruvate is decomposed to acetate and $CO_2$ (47–49). By this mechanism, co-administration of 5 m*M* pyruvate minimized contractile dysfunction and conserved energy reserves in hearts challenged by 300 μ*M* $H_2O_2$ (50). Pyruvate detoxifies peroxynitrite in a similar fashion (Fig. 1A), yielding $NO_2^-$, $CO_2$, and acetate (51).

Mitochondrial metabolism may indirectly contribute to pyruvate's antioxidant character (Fig. 1B). Malic enzyme and pyruvate carboxylase condense pyruvate and $CO_2$ to generate the Krebs cycle intermediates malate and oxaloacetate, respectively (5, 52). These anaplerotic mechanisms increase steady-state citrate content. By diverting glycolytic flux into the hexose monophosphate shunt and generating isocitrate for $NADP^+$-dependent isocitrate dehydrogenase (46), citrate increases formation of NADPH (Fig. 1B). The latter compound provides reducing equivalents to maintain the redox state of GSH, the principal intracellular antioxidant (53) and source of reducing power to detoxify peroxides and peroxynitrite. In accordance with this scenario, in perfused guinea-pig hearts, pyruvate increased citrate content and NADPH:$NADP^+$ ratio and restored GSH redox state depleted by ischemia-reperfusion (54) and $H_2O_2$ (55).

A recent study by Bassenge *et al.* (56) in isolated, Krebs-Henseleit perfused guinea-pig hearts revealed yet another antioxidant mechanism of pyruvate. Here, 0.1–10 m*M* pyruvate concentration-dependently suppressed ROS formation by the superoxide ($\cdot O_2^-$)-generating enzyme NADH oxidase. Pyruvate deprived the oxidase of its substrate by shifting the lactate dehydrogenase equilibrium toward NADH oxidation. In keeping with the NADH oxidase mechanism, pyruvate's reduced congener lactate, which increases cytosolic NADH, stimulated ROS formation (57), and pyruvate suppressed lactate's pro-oxidant effect (56).

It is unclear whether pyruvate's antioxidant mechanisms alone are sufficient to increase myocardial mechanical performance, independent of its augmentation of myocardial energy state. Both properties result from pyruvate's intermediary metabolism, so selective inhibition of only one is difficult. As an alternative approach, pyruvate's inotropic and metabolic actions in ischemically stunned (54) and $H_2O_2$-challenged (55) working guinea-pig hearts were compared with those of a membrane-permeable, pharmacologic antioxidant, *N*-acetylcysteine. Both treatments increased myocardial GSH/glutathione disulfide (GSSG); in fact, *N*-acetylcysteine was more effective than pyruvate. Nevertheless, only pyruvate increased left ventricular pressure development, cardiac output, and power, and only pyruvate enhanced cytosolic phosphorylation potential. These results indicate that pyruvate's antioxidant actions alone may be insufficient to increase cardiac performance, and that its inotropic effects also require its energy-generating capabilities as a metabolic fuel.

## Salutary Actions of Pyruvate in Metabolically Challenged Myocardium

Its considerable energy requirements make the heart exquisitely sensitive to interruption of its fuel and oxygen supply. Some of the most devastating medical conditions in western societies, including sudden cardiac death and heart failure, are the direct consequences of metabolic derangements produced by myocardial ischemia and reperfusion. This section summarizes several recent investigations that have tested pyruvate as an intervention to ameliorate myocardial ischemic injury and improve contractile performance of postischemic myocardium.

**Enhancement of β-Adrenergic Inotropism in Stunned and Failing Myocardium.** Reversibly injured, ischemically stunned myocardium (35) is remarkably

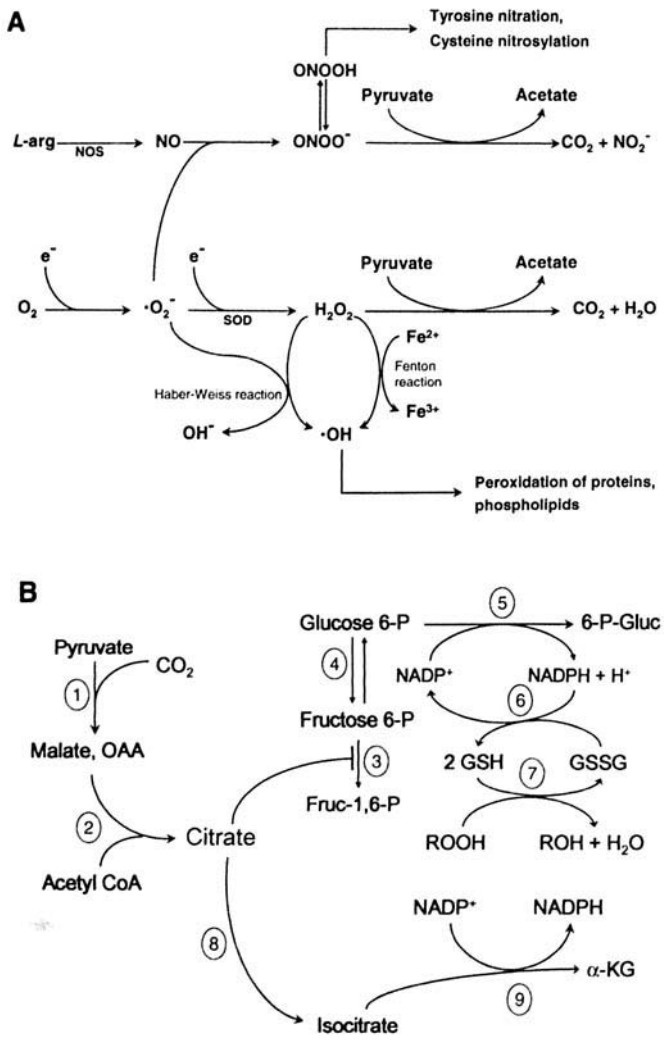


**Figure 1.** Antioxidant mechanisms of pyruvate. (A) Pyruvate detoxifies peroxynitrite ($ONOO^-$) and $H_2O_2$ in direct, nonenzymatic reactions, in which pyruvate decarboxylation yields acetate as a by-product. NOS, nitric oxide synthase; SOD, superoxide dismutase; ONOOH, peroxynitrous acid. (B) Metabolic antioxidant mechanisms. Here, pyruvate metabolism increases formation of NADPH, providing reducing power for reduction of glutathione disulfide (GSSG) to glutathione (GSH). Numbered ovals indicate enzymatic reactions: 1, malic enzyme/pyruvate carboxylase; 2, citrate synthase; 3, phosphofructokinase; 4, phosphoglucose isomerase; 5, glucose 6-phosphate dehydrogenase; 6, glutathione reductase; 7, glutathione peroxidase; 8, aconitase; 9, $NADP^+$-dependent isocitrate dehydrogenase. OAA, oxaloacetate; α-KG, α-ketoglutarate.

responsive to metabolic intervention with pyruvate (15, 58). Studies in this laboratory demonstrated a novel interaction of pyruvate and pharmacologic inotropes in ischemically stunned guinea-pig myocardium (54, 59). Contractile responses to the β-adrenergic agonist isoproterenol were severely impaired in stunned versus nonischemic control hearts, but pyruvate treatment largely restored β-adrenergic sensitivity (59) and potentiated isoproterenol stimulation of cardiac performance. Pyruvate also prevented β-adrenergic depletion of myocardial energy reserves (59). This β-adrenergic potentiation appeared to be a product of pyruvate's antioxidant actions. Pyruvate increased GSH redox state in these hearts, and *N*-acetylcysteine, a sulfhydryl antioxidant and GSH precursor but not a metabolic fuel, also potentiated isoproterenol and increased GSH redox state (54). Moreover, acetoacetate, a natural myocardial fuel when its plasma concentrations are elevated, did not increase phosphorylation potential in stunned myocardium, but it did augment GSH redox state, and, like pyruvate, it restored inotropic responses to isoproterenol (46, 60).

Hermann *et al.* (61) examined pyruvate:catecholamine interactions in isolated ventricular muscle preparations from failing human hearts. Here, 10 m*M* pyruvate shifted in an upward direction the relationship between isoproterenol concentration and developed force. Thus, pyruvate increased developed force from 9 to 21 $mN \cdot mm^{-2}$ in the absence of isoproterenol and from 31 to 47 $mN \cdot mm^{-2}$ during maximal stimulation with 1 μ*M* isoproterenol. These promising *in vitro* results and those from our laboratory indicate that co-administration of pyruvate could augment the inotropic actions of catecholamines and, thus, lower the catecholamine dosage required to increase cardiac function, while minimizing β-adrenergic depletion of myocardial energy reserves.

Pyruvate has also been found to improve cardiac function in patients with congestive heart failure, even without β-adrenergic support. In an extension of their earlier clinical study (62), Hermann *et al.* (63) infused pyruvate to produce estimated intracoronary concentrations of 1.5, 3, and 6 m*M* in NYHA Class II–III heart failure patients. Pyruvate concentration-dependently improved both systolic and diastolic left ventricular function. The highest dosage increased left ventricular $dP/dt_{max}$ and $dP/dt_{min}$ by 40% and 23%, respectively, lowered left ventricular end-diastolic pressure from 17 ± 2 to 12 ± 2 mm Hg, and increased left ventricular ejection fraction from 30% ± 4% to 39% ± 4%. Importantly, no arrhythmias or other undesirable side effects of the pyruvate treatment were reported.

**Pyruvate Protection Against Myocardial Infarction.** Depletion of cellular energy reserves during myocardial ischemia, and the massive burst of ROS formation and intracellular $Ca^{2+}$ overload following reperfusion, inflict lethal injury culminating in myocardial infarction. By increasing energy reserves, neutralizing ROS, and improving cellular $Ca^{2+}$ homeostasis, pyruvate could mitigate ischemic injury, yet early reports of pyruvate's impact on infarction are sparse and contradictory (64, 65).

A recent investigation (66) in Lasley's laboratory tested pyruvate's ability to limit infarction in *in situ* pig hearts. The left anterior descending coronary artery was occluded for 60 min, followed by 3 hrs of reperfusion. Pyruvate was continuously infused (10 $mg \cdot kg^{-1} \cdot min^{-1}$) into the left atrium. Pyruvate administration from 30 mins before occlusion until reperfusion reduced infarct volume from 66% ± 1% to 49% ± 3% of the ischemic territory. When pyruvate treatment was extended until 60 mins reperfusion, infarctions were further limited to 30% ± 2% of the ischemic region.

Stanley *et al.* (67) tested a novel pyruvate derivative, dipyruvyl-acetyl-glycerol (DPAG), in pigs subjected to 60-min occlusion of the left anterior descending coronary artery and 2-hr reperfusion. DPAG was continuously infused into the femoral vein throughout reperfusion. Cleavage of DPAG by circulating esterases released pyruvate, raising its plasma concentration from <0.1 to approximately 0.8 m*M*. Although this increase in plasma pyruvate was rather modest, DPAG treatment reduced the infarction from 30.8% ± 4.6% to 20.1% ± 4.2% of the ischemic myocardium.

In an earlier study by Gutterman *et al.* (65) in dogs subjected to 3-hr occlusion of the left circumflex coronary artery and 90-min reperfusion, intracoronary pyruvate infusions (0.4 mmol · $min^{-1}$) failed to decrease infarct volume when given during the first 60-min reperfusion alone or from 15 mins preocclusion until 60 mins reperfusion. Aside from differences in species and route and rate of pyruvate administration, comparison of the report of Gutterman *et al.* (65) with those of Kristo *et al.* (66) and Stanley *et al.* (67) indicates that pyruvate can mitigate myocardial damage inflicted by 1-hr coronary occlusions, but is less effective against more prolonged ischemic insults.

**Pyruvate Cardioprotection During Cardiopulmonary Resuscitation.** By interrupting nutritive blood flow, cardiac arrest imposes severe, global ischemia on the heart, brain, and other organs. The prognosis for victims of cardiac arrest remains grim, despite substantial improvements in recent decades in delivery of emergency medical care. Only a small minority of victims survives to hospital discharge (68, 69), and a host of devastating morbidities confront those who survive the initial arrest. Of particular concern in the early recovery period is the postresuscitation syndrome (70, 71), wherein the injured heart fails to adequately perfuse its own tissue or that of peripheral organs, including brain (72), raising the specter of multiple organ failure. Postresuscitation cardiac insufficiency exhibits the hallmarks of cardiac stunning (73) and likely has a similar pathogenesis initiated by energy depletion and mediated by ROS.

An energy-yielding fuel and antioxidant, pyruvate could be a powerful intervention to mitigate cardiac injury and facilitate postarrest recovery. This proposal was tested (7) in open-chest, anesthetized dogs subjected to 5-min cardiopulmonary arrest and 5-min open chest cardiac compression (OCCC) + mechanical ventilation, then defibrillated with epicardial countershocks and monitored for another 3 hrs. Pyruvate was continuously infused into a femoral vein during OCCC and the first 25 mins of recovery, achieving a steady-state concentration of 3.6 ± 0.2 m*M* in the systemic arterial plasma. Control experiments received NaCl infusions. Myocardial phosphorylation potential collapsed and GSH redox state fell sharply by 5 mins into arrest, indicating severe depletion of energy reserves and antioxidant defenses. Despite partial recovery of energy and GSH redox states during OCCC and more complete recovery following defibrillation, the control hearts suffered postarrest electromechanical impairment: persistent ST segment displacement was evident in standard limb lead II electrocardiogram, and left ventricular dP/dt and carotid blood flow, a measure of craniocephalic perfusion, fell sharply after 2 hrs of recovery.

Pyruvate hastened recovery of phosphorylation potential during OCCC and GSH redox state following defibrillation (7). Although these metabolic improvements subsided after pyruvate infusion was discontinued, its enhancement of cardiac electromechanical recovery did not wane: ST segment displacement completely resolved by 2 hrs postdefibrillation, and dP/dt and carotid blood flow stabilized at or near prearrest baselines throughout the recovery period. Thus, temporary pyruvate treatment supported appreciable improvements in myocardial metabolism and function during the first few hours of recovery. Whether pyruvate enhancement of postarrest myocardial function persists beyond the initial recovery period remains to be determined.

**Pyruvate-Enhanced Cardioplegia: Protecting the Myocardium During Cardiopulmonary Bypass Surgery.** Coronary artery bypass surgery requires interruption of coronary blood flow to permit anastomoses of the grafts to the target vessels. Moreover, because coronary revascularization and other delicate cardiac surgical procedures require a motionless surgical field, the heart is cardioplegically arrested, which interrupts blood flow to the entire organ. Although cardiac arrest lowers myocardial energy demand, these measures nevertheless impose ischemic stress, which can injure the myocardium, deplete its energy reserves, and compromise postsurgical recovery of cardiac mechanical function. However, myocardial ischemic injury is a malleable process, potentially responsive to cardioprotective interventions administered via the coronary vasculature.

Pyruvate's dual energy-yielding and antioxidant capabilities could provide powerful cardioprotection during cardiac surgery, but it had never been tested as a cardioplegia component in the clinical arena. Accordingly, Olivencia-Yurvati *et al.* (74) conducted a prospective, randomized, semiblinded trial of pyruvate-fortified cardioplegia in adult patients undergoing elective coronary artery bypass grafting. Cardiac arrest was induced with 4:1 blood to crystalloid cardioplegia. In 15 patients the crystalloid component contained 24 m*M* lactate, and in another 15 patients it contained 10 m*M* pyruvate. The two groups were well-matched for gender, age, preoperative left ventricular ejection fraction, cardiopulmonary bypass and aortic cross-clamp times, and administered cardioplegia volume. Surgical arrest was induced by administering 500 ml cardioplegia antegradely via the aortic root, then retrogradely via the coronary sinus. Supplemental cardioplegia was administered periodically during crossclamp to maintain arrest.

In the lactate cardioplegia group, the left ventricular

stroke work index fell markedly during the first 4 hrs postbypass, then recovered gradually; this pattern typifies postbypass left ventricular function (75–78). In striking contrast, left ventricular function of the pyruvate group returned to baseline by 4 hrs into recovery and remained at the higher level. The cardioplegia solutions were cleared from the heart by reperfusion with the patient's blood, so the persistent functional enhancements postbypass must have resulted from pyruvate's salutary effects during the antecedent period of surgical arrest and possibly the first few minutes of reperfusion. Pyruvate also lowered cardiac release of the cardiac troponin I isoform and creatine kinase MB by 67% and 53%, respectively, versus lactate cardioplegia ($P < 0.05$), indicating that pyruvate ameliorated myocardial injury during cardioplegic arrest. Ten lactate cardioplegia patients required β-adrenergic inotropic support postbypass, but only four pyruvate-treated patients required β-adrenergic support ($P = 0.067$). Thus, pyruvate-fortified cardioplegia mitigated myocardial injury during coronary bypass surgery and supported robust, sustained postsurgical recovery of cardiac performance. Consequently, use of pyruvate- versus lactate-fortified cardioplegia shortened postoperative hospitalization from 6.3 ± 0.3 to 5.2 ± 0.1 days ($P < 0.002$).

Studies have been undertaken in pigs to delineate pyruvate's salutary mechanisms in cardioplegically arrested myocardium. In these experiments, addition of 24 m*M* pyruvate to the glucose-fortified crystalloid component of blood cardioplegia dampened the burst of 8-isoprostane release, a measure of lipid peroxidation (79, 80), in the first few minutes of reperfusion (Fig. 2). Pyruvate cardioplegia also enhanced recovery of phosphorylation potential following reperfusion of left ventricular myocardium (81).

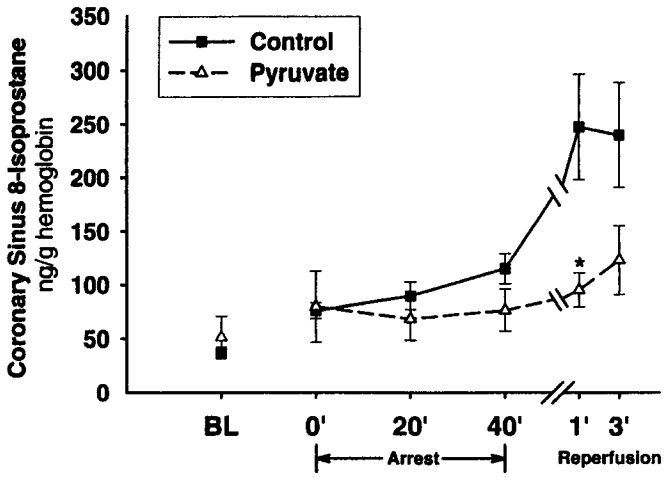


**Figure 2.** Coronary sinus 8-isoprostane content in pigs undergoing cardioplegic arrest and reperfusion. Pigs received hypothermic (4°C) 4:1 blood:crystalloid cardioplegia for 60 mins, then the heart was reperfused with cardioplegia-free whole blood. The crystalloid component contained 188 m*M* glucose alone (control group) or glucose + 24 m*M* pyruvate (pyruvate group) as energy substrates. Values are means ± SEM from eight experiments per group. $^*P < 0.05$ versus control.

These initial findings support the working hypothesis that pyruvate protects the cardioplegically arrested myocardium both by improving energy supply and by intervening against oxidant attack.

## Electroneutral Pyruvate Derivatives

An organic anion at physiologic pH, pyruvate is supplied as a sodium salt in most commercial formulations. Intravenous administration of sodium pyruvate to effective circulating concentrations imposes a significant sodium burden, which could compromise control of extracellular volume and blood pressure, especially in patients with renal insufficiency or congestive heart failure. Furthermore, in neutral or alkaline aqueous solutions, pyruvate undergoes an irreversible, aldol-like condensation to form a nonmetabolizable dimer, γ-methyl-γ-hydroxy-α-ketoglutarate (i.e., para-pyruvate) (82–84). This compound inhibits the key Krebs cycle enzyme α-ketoglutarate dehydrogenase (82, 85), an effect that would impede mitochondrial ATP production. These limitations have prompted development of electroneutral, chemically stable pyruvate derivatives.

Ethyl pyruvate, an ester formed by condensation of pyruvate and ethanol, has been proposed as an alternative to authentic pyruvate to protect tissues threatened by ischemia or oxidative stress. Ethyl pyruvate preserved GSH/GSSG and $Na^+$, $K^+$ ATPase activity in cultured lens epithelium challenged by the prooxidant menadione (86). More recently, studies by Fink *et al.* in rats (87) and mice (88) demonstrated that intravenous infusions of ethyl pyruvate–fortified crystalloid solutions preserved intestinal mucosal integrity following occlusion and reperfusion of the superior mesenteric artery. Recently, ethyl pyruvate's cardioprotective capabilities were tested in *in situ* rat hearts subjected to 30 mins of coronary artery occlusion and 30 mins of reperfusion (89). Relative to control Ringer's solution, intravenous bolus administration of ethyl pyruvate–fortified Ringer's solution slowed ATP depletion during ischemia, minimized postischemic lipid peroxidation, increased postischemic recovery of left ventricular developed pressure, dP/dt, and cardiac output, and decreased myocardial infarct size by 25%. The weaker water solubility of ethyl pyruvate relative to authentic pyruvate, and the potentially detrimental effects of ethanol released by cleavage of the ester, may limit the concentrations that can be administered by continuous intravenous infusions.

We compared ethyl pyruvate's ability to detoxify hydrogen peroxide in aqueous solution with that of its parent compound. As expected, 1 and 0.5 m*M* pyruvate quickly consumed $H_2O_2$ (Fig. 3). Ethyl pyruvate also detoxified the oxidant, albeit at a somewhat slower rate. Its ester bond stabilizes ethyl pyruvate until it is cleaved by esterases to yield pyruvate and ethanol. The protein-free buffer lacked esterases, so the disappearance of $H_2O_2$ must have resulted from direct reaction of ethyl pyruvate with the oxidant. Incubation of pyruvate and ethyl pyruvate with 0.5

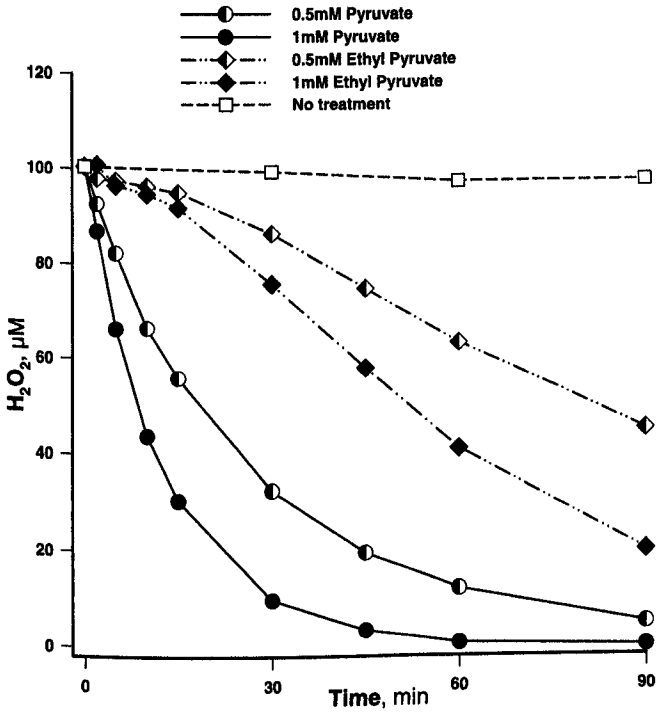


**Figure 3.** Direct detoxification of hydrogen peroxide by pyruvate versus ethyl pyruvate. Aqueous solutions of pyruvate and ethyl pyruvate (0.5, 1 m*M*) were incubated with 0.1 m*M* $H_2O_2$ at 37°C. $H_2O_2$ concentrations were measured at selected intervals by spectrophotometric assay using peroxidase and 2,2′-azino-di-[3-ethyl-benzothiazolidine-(6)-sulphonic acid], as previously described (90).

m*M* $H_2O_2$ yielded 0.387 and 0.395 m*M* acetate, respectively, after 2 hrs. Thus, ethyl pyruvate, like its parent compound, can neutralize $H_2O_2$ in a direct, nonenzymatic reaction centered on the covalent bond between the carbonyl and carboxyl carbons of its pyruvate moiety.

Recently, Stanley *et al.* (67) developed another pyruvate derivative, DPAG, and tested its cardioprotective capabilities in *in situ* pig hearts subjected to ischemia-reperfusion. This novel triglyceride was infused intravenously for 2 hrs, beginning immediately after release of a 60-min occlusion of the left anterior descending coronary artery. Cleavage of the compound by plasma esterases increased plasma pyruvate concentration from approximately 0.1 to 0.82 m*M*. Even at these elevated but suboptimal pyruvate concentrations, the treatment decreased infarct volume by 35%.

## Conclusions

Research conducted in the last 5 years has yielded important insights on the complex mechanisms linking pyruvate's energetic and antioxidant properties to its salutary actions in metabolically challenged myocardium. The first reports of pyruvate application in the clinical arena have appeared. These studies have demonstrated $\leq$10 m*M* pyruvate to be safe and efficacious for improving contractile performance of failing hearts and protecting cardioplegically arrested myocardium during cardiopulmonary bypass. Higher concentrations of pyruvate should be used with extreme caution as a result of the risk of arrhythmias, sodium loading, and possibly other, heretofore unidentified toxic effects.

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1. Mallet RT. Pyruvate: metabolic protector of cardiac performance. Proc Soc Exp Biol Med 223:136–148, 2000.
2. Panchal AR, Comte B, Huang H, Kerwin T, Darvish A, Des Rosiers C, Brunengraber H, Stanley WC. Partitioning of pyruvate between oxidation and anaplerosis in swine hearts. Am J Physiol Heart Circ Physiol 279:H2390–H2398, 2000.
3. Panchal AR, Comte B, Huang H, Dudar B, Roth B, Chandler M, Des Rosiers C, Brunengraber H, Stanley WC. Acute hibernation decreases myocardial pyruvate carboxylation and citrate release. Am J Physiol Heart Circ Physiol 281:H1613–H1620, 2001.
4. Lloyd S, Brocks C, Chatham JC. Differential modulation of glucose, lactate, and pyruvate oxidation by insulin and dichloroacetate in the rat heart. Am J Physiol Heart Circ Physiol 285:H163–H172, 2003.
5. Khairallah M, Labarthe F, Bouchard B, Danialou G, Petrof BJ, Des Rosiers C. Profiling substrate fluxes in the isolated working mouse heart using $^{13}$C-labeled substrates: focusing on the origin and fate of pyruvate and citrate carbons. Am J Physiol Heart Circ Physiol 286:H1461–H1470, 2004.
6. Lloyd SG, Wang P, Zeng H, Chatham JC. Impact of low-flow ischemia on substrate oxidation and glycolysis in the isolated perfused rat heart. Am J Physiol Heart Circ Physiol 287:H351–H362, 2004.
7. Sharma AB, Knott EM, Bi J, Martinez RR, Sun J, Mallet RT. Pyruvate improves cardiac electromechanical and metabolic recovery from cardiopulmonary arrest and resuscitation. Resuscitation (in press), 2005.
8. Mongan PD, Fontana JL, Chen R, Bünger R. Intravenous pyruvate prolongs survival during hemorrhagic shock in swine. Am J Physiol Heart Circ Physiol 277:H2253–H2263, 1999.
9. Mongan PD, Cappachione J, Fontana JL, West S, Bünger R. Pyruvate improves cerebral metabolism during hemorrhagic shock. Am J Physiol Heart Circ Physiol 281:H854–H864, 2001.
10. Owen OE, Mozzoli MA, Boden G, Patel MS, Reichard GA Jr, Trapp V, Shuman CR, Felig P. Substrate, hormone, and temperature responses in males and females to a common breakfast. Metabolism 29:511–523, 1980.
11. Meierhenrich R, Jedicke H, Voigt A, Lange H. The effect of erythropoietin on lactate, pyruvate, and excess lactate under physical exercise in dialysis patients. Clin Nephrol 45:90–96, 1996.
12. Zweier JL, Jacobus WE. Substrate-induced alterations of high energy phosphate metabolism and contractile function in the perfused heart. J Biol Chem 262:8015–8021, 1987.
13. Mallet RT, Bünger R. Energetic modulation of cardiac inotropism and sarcoplasmic reticular $Ca^{2+}$ uptake. Biochim Biophys Acta 1224:22–32, 1994.
14. Mallet RT, Sun J. Mitochondrial metabolism of pyruvate is required for its enhancement of cardiac function and energetics. Cardiovasc Res 42:149–161, 1999.
15. Bünger R, Mallet RT, Hartman DA. Pyruvate-enhanced phosphorylation potential and inotropism in normoxic and postischemic isolated working heart: near-complete prevention of reperfusion contractile failure. Eur J Biochem 180:221–233, 1989.

16. Ochiai K, Zhang J, Gong G, Zhang Y, Liu J, Ye Y, Wu X, Liu H, Murakami Y, Bache RJ, Ugurbil K, From AHL. Effects of augmented delivery of pyruvate on myocardial high-energy phosphate metabolism at high workstate. Am J Physiol Heart Circ Physiol 286:H2237–H2242, 2004.
17. Kammermeier H. High energy phosphate of the myocardium: concentration versus free energy change. Basic Res Cardiol 82:31–36, 1987.
18. Chen W, London R, Murphy E, Steenbergen C. Regulation of the $Ca^{2+}$ gradient across the sarcoplasmic reticulum in perfused rabbit heart: a $^{19}F$ nuclear magnetic resonance study. Circ Res 83:898–907, 1998.
19. Fabiato A. Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. Am J Physiol Cell Physiol 245:C1–C14, 1983.
20. Martin BJ, Valdivia HH, Bünger R, Lasley RD, Mentzer RM Jr. Pyruvate augments calcium transients and cell shortening in rat ventricular myocytes. Am J Physiol Heart Circ Physiol 274:H8–H17, 1998.
21. Mellors LJ, Kotsanas G, Wendt IR. Effects of pyruvate on intracellular $Ca^{2+}$ regulation in cardiac myocytes from normal and diabetic rats. Clin Exp Pharmacol Physiol 26:889–897, 1999.
22. Zima A, Kockskämper J, Mejia-Alvarez R, Blatter LA. Pyruvate modulates cardiac sarcoplasmic reticulum $Ca^{2+}$ release in rats via mitochondria-dependent and -independent mechanisms. J Physiol 550:765–783, 2003.
23. Hüser J, Wang YG, Sheehan KA, Cifuentes F, Lipsius SL, Blatter LA. Functional coupling between glycolysis and excitation-contraction coupling underlies alternans in cat heart cells. J Physiol 524:795–806, 2000.
24. Kockskämper J, Blatter LA. Subcellular $Ca^{2+}$ alternans represents a novel mechanism for the generation of arrhythmogenic $Ca^{2+}$ waves in cat atrial myocytes. J Physiol 545:65–79, 2002.
25. Hermann H-P, Zeitz O, Keweloh B, Hasenfuss G, Janssen PML. Pyruvate potentiates inotropic effects of isoproterenol and $Ca^{2+}$ in rabbit cardiac muscle preparations. Am J Physiol Heart Circ Physiol 279:H702–H708, 2000.
26. Halestrap AP, Price NT. The proton-linked monocarboxylate transporter (MCT) family: structure, function and regulation. Biochem J 343:281–299, 1999.
27. Maier LS, Braunhälter J, Horn W, Weichert S, Pieske B. The role of SR $Ca^{2+}$-content in blunted inotropic responsiveness of failing human myocardium. J Mol Cell Cardiol 34:455–467, 2002.
28. Kentish JC. The effects of inorganic phosphate and creatine phosphate on force production in skinned muscles from rat ventricle. J Physiol 370:585–604, 1986.
29. Kawai M, Güth K, Winnikes K, Haist C, Rüegg JC. The effect of inorganic phosphate on the ATP hydrolysis rate and the tension transients in chemically skinned rabbit psoas fibers. Pflügers Arch 408:1–9, 1987.
30. Perez NG, Gao WD, Marbán E. Novel myofilament $Ca^{2+}$-sensitizing property of xanthine oxidase inhibitors. Circ Res 83:423–430, 1998.
31. Hasenfuss G, Maier LS, Hermann H-P, Lüers C, Hünlich M, Zeitz O, Janssen PML, Pieske B. Influence of pyruvate on contractile performance and $Ca^{2+}$ cycling in isolated failing human myocardium. Circulation 105:194–199, 2002.
32. Gulati J, Babu A. Effect of acidosis on $Ca^{2+}$ sensitivity of skinned cardiac muscle with troponin C exchange. Implications for myocardial ischemia. FEBS Lett 245:279–282, 1989.
33. Ball KL, Johnson MD, Solaro RJ. Isoform specific interactions of troponin I and troponin C determine pH sensitivity of myofibrillar $Ca^{2+}$ activation. Biochemistry 33:8464–8471, 1994.
34. Watanabe A, Tomoike H, Endoh M. $Ca^{2+}$-sensitizer Org-30029 reverses acidosis- and BDM-induced contractile depression in canine myocardium. Am J Physiol Heart Circ Physiol 271:H1829–H1839, 1996.
35. Bolli R, Marbán E. Molecular and cellular mechanisms of myocardial stunning. Physiol Rev 79:609–634, 1999.
36. Ambrosio G, Tritto I. Reperfusion injury: experimental evidence and clinical implications. Am Heart J 138:S69–S75, 1999.
37. Gross GJ, Kersten JR, Warltier DC. Mechanisms of postischemic contractile dysfunction. Ann Thorac Surg 68:1898–1904, 1999.
38. Lefer DJ, Granger DN. Oxidative stress and cardiac disease. Am J Med 109:315–323, 2000.
39. Kaminski KA, Bonda TA, Korecki J, Musial WJ. Oxidative stress and neutrophil activation—the two keystones of ischemia/reperfusion injury. Int J Cardiol 86:41–59, 2002.
40. Chatham JC, Gilbert HF, Radda GK. The metabolic consequences of hydroperoxide perfusion on the isolated rat heart. Eur J Biochem 184:657–662.
41. Janero DR, Hreniuk D, Sharif HM. Hydroperoxide-induced oxidative stress impairs heart muscle cell carbohydrate metabolism. Am J Physiol Cell Physiol 266:C179–C188, 1994.
42. Slater AFG, Nobel SI, Orrenius S. The role of intracellular oxidants in apoptosis. Biochim Biophys Acta 1271:59–62, 1995.
43. Vaage J, Antonelli M, Bufi M, Irtun O, DeBlasi RA, Corbucci GG, Gasparetto A, Semb AG. Exogenous reactive oxygen species deplete the isolated rat heart of antioxidants. Free Radic Biol Med 22:85–92, 1997.
44. Tretter L, Adam-Vizi V. Inhibition of Krebs cycle enzymes by hydrogen peroxide: a key role of α-ketoglutarate dehydrogenase in limiting NADH production under oxidative stress. J Neurosci 20:8972–8979, 2000.
45. Eaton P, Hearse DJ, Shattock MJ. Lipid hydroperoxide modification of proteins during myocardial ischemia. Cardiovasc Res 51:294–303.
46. Mallet RT, Sun J. Antioxidant properties of myocardial fuels. Mol Cell Biochem 253:103–111, 2003.
47. Constantopoulos G, Barranger JA. Nonenzymatic decarboxylation of pyruvate. Anal Biochem 139:353–358, 1984.
48. DeBoer LWV, Bekx PA, Han L, Steinke L. Pyruvate enhances recovery of rat hearts after ischemia and reperfusion by preventing free radical generation. Am J Physiol Heart Circ Physiol 265:H1571–H1576, 1993.
49. Crestanello JA, Kamelgard J, Whitman GJR. The cumulative nature of pyruvate's dual mechanism for myocardial protection. J Surg Res 59:198–204, 1995.
50. Leon H, Atkinson LL, Sawicka J, Strynadka K, Lopaschuk GD, Schulz R. Pyruvate prevents cardiac dysfunction and AMP-activated protein kinase activation by hydrogen peroxide in isolated rat hearts. Can J Physiol Pharmacol 82:409–416, 2004.
51. Vásquez-Vivar J, Denicola A, Radi R, Augusto O. Peroxynitrite-mediated decarboxylation of pyruvate to both carbon dioxide and carbon dioxide radical anion. Chem Res Toxicol 10:786–794, 1997.
52. Comte B, Vincent G, Bouchard B, Jetté M, Cordeau S, Des Rosiers C. A $^{13}C$ mass isotopomer study of anaplerotic pyruvate carboxylation in perfused rat hearts. J Biol Chem 272:26125–26131, 1997.
53. Schafer FQ, Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic Biol Med 30:1191–1212, 2001.
54. Tejero-Taldo MI, Caffrey JL, Sun J, Mallet RT. Antioxidant properties of pyruvate mediate its potentiation of β-adrenergic inotropism in stunned myocardium. J Mol Cell Cardiol 31:1863–1872, 1999.
55. Mallet RT, Squires JE, Bhatia S, Sun J. Pyruvate restores contractile function and antioxidant defenses of hydrogen peroxide–challenged myocardium. J Mol Cell Cardiol 34:1173–1184, 2002.
56. Bassenge E, Sommer O, Schwemmer M, Bünger R. Antioxidant pyruvate inhibits cardiac formation of reactive oxygen species through changes in redox state. Am J Physiol Heart Circ Physiol 279:H2431–H2438, 2000.
57. Mohazzab-H. KM, Kaminski PM, Wolin MS. Lactate and $Po_2$

modulate superoxide anion production in bovine cardiac myocytes: potential role of NADH oxidase. Circulation 96:614–620, 1997.
58. Zhou Z, Lasley RD, Hegge O, Bünger R, Mentzer RM. Myocardial stunning: a therapeutic conundrum. J Thorac Cardiovasc Surg 110:1391–1401, 1995.
59. Tejero-Taldo MI, Sun J, Caffrey JL, Mallet RT. Pyruvate potentiates β-adrenergic inotropism of stunned guinea-pig myocardium. J Mol Cell Cardiol 30:2327–2339, 1998.
60. Squires JE, Sun J, Caffrey JL, Yoshishige D, Mallet RT. Acetoacetate augments β-adrenergic inotropism of stunned myocardium by an antioxidant mechanism. Am J Physiol Heart Circ Physiol 284:H1340–H1347, 2003.
61. Hermann H-P, Zeitz O, Lehnart SE, Keweloh B, Datz N, Hasenfuss G, Janssen PML. Potentiation of beta-adrenergic inotropic response by pyruvate in failing human myocardium. Cardiovasc Res 53:116–123, 2002.
62. Hermann H-P, Pieske B, Schwarzmüller E, Keul J, Just H, Hasenfuss G. Haemodynamic effects of intracoronary pyruvate in patients with congestive heart failure: an open study. Lancet 353:1321–1323, 1999.
63. Hermann H-P, Arp J, Pieske B, Kögler H, Baron S, Janssen PML, Hasenfuss G. Improved systolic and diastolic myocardial function with intracoronary pyruvate in patients with congestive heart failure. Eur J Heart Fail 6:213–218, 2004.
64. Regitz V, Azumi T, Stephan H, Naujocks S, Schaper W. Biochemical mechanism of infarct size reduction by pyruvate. Cardiovasc Res 15:652–658, 1981.
65. Gutterman DD, Chilian WM, Eastham CL, Inou T, White CW, Marcus ML. Failure of pyruvate to salvage myocardium after prolonged ischemia. Am J Physiol Heart Circ Physiol 250:H114–H120, 1986.
66. Kristo G, Yoshimura Y, Niu J, Keith BJ, Mentzer RM Jr, Lasley RD. The intermediary metabolite pyruvate attenuates stunning and reduces infarct size in in vivo porcine myocardium. Am J Physiol Heart Circ Physiol 286:H517–H524, 2004.
67. Stanley WC, Kivilo KM, Panchal AR, Hallowell PH, Bomont C, Kasumov T, Brunengraber H. Post-ischemic treatment with dipyruvyl-acetyl-glycerol decreases myocardial infarct size in the pig. Cardiovasc Drugs Ther 17:209–216, 2003.
68. Thel MC, O'Connor CM. Cardiopulmonary resuscitation: historical perspective to recent investigations. Am Heart J 137:39–48, 1999.
69. Eisenberg MS, Mengert TJ. Cardiac resuscitation. New Engl J Med 344:1304–1313, 2001.
70. Cerchiari EL, Safar P, Klein E, Cantadore R, Pinsky M. Cardiovascular function and neurologic outcome after cardiac arrest in dogs: the cardiovascular post-resuscitation syndrome. Resuscitation 25:9–33, 1993.
71. Kern KB. Postresuscitation myocardial dysfunction. Cardiol Clin 20:89–101, 2002.
72. Safar P, Xiao F, Radovsky A, Tanigawa K, Ebmeyer U, Bircher N, Alexander H, Stezoski SW. Improved cerebral resuscitation from cardiac arrest in dogs with mild hypothermia plus blood flow promotion. Stroke 27:105–113, 2002.
73. Kern KB, Hilwig RW, Rhee KH, Berg RA. Myocardial dysfunction after resuscitation from cardiac arrest: an example of global myocardial stunning. J Am Coll Cardiol 28:232–240, 1996.
74. Olivencia-Yurvati AH, Blair JL, Baig M, Mallet RT. Pyruvate-enhanced cardioprotection during surgery with cardiopulmonary bypass. J Cardiothorac Vasc Anesth 17:715–720, 2003.
75. Mangano DT. Biventricular function after myocardial revascularization in humans: deterioration and recovery patterns during the first 24 hours. Anesthesiology 62:571–577, 1985.
76. Breisblatt WM, Stein KL, Wolfe CJ, Follansbee WP, Capozzi J, Armitage JM, Hardesty RL. Acute myocardial dysfunction and recovery: a common occurrence after coronary bypass surgery. J Am Coll Cardiol 15:1261–1269, 1990.
77. Royster RL. Myocardial dysfunction following cardiopulmonary bypass: recovery patterns, predictors of inotropic need, theoretical concepts of inotropic administration. J Cardiothorac Vasc Anesth 7:19–25, 1993.
78. Savaris N, Polanczyk C, Clausell N. Cytokines and troponin-I in cardiac dysfunction after coronary artery grafting with cardiopulmonary bypass. Arq Bras Cardiol 77:114–119, 2001.
79. Sakamoto H, Corcoran TB, Laffey JG, Shorten GD. Isoprostanes—markers of ischaemia reperfusion injury. Eur J Anaesthesiol 19:550–559, 2002.
80. Mehlhorn U, Krahwinkel A, Geissler HJ, LaRosee K, Fischer UM, Klass O, Suedkamp M, Hekmat K, Tossios P, Bloch W. Nitrotyrosine and 8-isoprostane formation indicate free radical-mediated injury in hearts of patients subjected to cardioplegia. J Thorac Cardiovasc Surg 125:178–183, 2003.
81. Knott EM, Ryou M-G, Sun J, Heymann A, Martinez RR, Sharma AB, Mallet RT, O-Yurvati AH. Pyruvate cardioplegia suppresses oxidative stress and preserves phosphorylation potential of arrested myocardium (abstract). FASEB J 19:A 690, 2005.
82. Montgomery CM, Fairhurst AS, Webb JL. Metabolic studies on heart mitochondria. III. The action of parapyruvate on α-ketoglutaric oxidase. J Biol Chem 221:369–376, 1956.
83. Von Korff RW. Pyruvate-$C^{14}$, purity and stability. Anal Biochem 8:171–178, 1964.
84. Margolis SA, Coxon B. Identification and quantitation of the impurities in sodium pyruvate. Anal Biochem 58:2504–2510, 1986.
85. Montgomery CM, Webb JL. Metabolic studies on heart mitochondria. II. The inhibitory action of parapyruvate on the tricarboxylic acid cycle. J Biol Chem 221:359–368, 1956.
86. Varma SD, Devamanoharan PS, Ali AH. Prevention of intracellular oxidative stress to lens by pyruvate and its ester. Free Radic Res 28:131–135, 1998.
87. Sims CA, Wattanasirichaigoon S, Menconi MJ, Ajami AM, Fink MP. Ringer's ethyl pyruvate solution ameliorates ischemia/reperfusion-induced intestinal mucosal injury in rats. Crit Care Med 29:1513–1518, 2001.
88. Uchiyama T, Delude RL, Fink MP. Dose-dependent effects of ethyl pyruvate in mice subjected to mesenteric ischemia and reperfusion. Intensive Care Med 29:2050–2058, 2003.
89. Woo YJ, Taylor MD, Cohen JE, Jayasankar V, Bish LT, Burdick J, Pirolli TJ, Berry MF, Hsu V, Grand T. Ethyl pyruvate preserves cardiac function and attenuates oxidative injury after prolonged myocardial ischemia. J Thorac Cardiovasc Surg 127:1262–1269, 2004.
90. Pütter J, Becker R. Peroxidases. In: Bergmeyer HU, Ed. Methods of Enzymatic Analysis (3rd ed.). New York: Academic Press, Vol 3: pp286–293, 1983.