Pyruvate enhancement of cardiac performance: Cellular mechanisms and clinical application

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Impact statement

This article reviews pyruvate's cardioprotective properties as an energy-yielding metabolic fuel, antioxidant and anti-inflammatory agent in mammalian myocardium. Preclinical research has shown these properties make pyruvate a powerful intervention to curb the complex injury cascade ignited by ischemia and reperfusion. In ischemically stunned isolated hearts and in large mammal models of cardiopulmonary bypass, cardiac arrest–resuscitation and hypovolemia, intracoronary pyruvate supports recovery of myocardial contractile function, intracellular Ca^{2+} homeostasis and free energy of ATP hydrolysis, and its antioxidant actions restore β-adrenergic signaling and suppress inflammation. The first clinical trials of pyruvate for cardiopulmonary bypass, fluid resuscitation and intracoronary intervention for congestive heart failure have been reported. Receiver operating characteristic analyses show remarkable concordance between pyruvate's beneficial functional and metabolic effects in isolated, perfused hearts and in patients recovering from cardiopulmonary bypass in which they received pyruvate- vs. *L*-lactate-fortified cardioplegia. This research exemplifies the translation of mechanism-oriented preclinical studies to clinical application and outcomes.

Abstract

Cardiac contractile function is adenosine-5'-triphosphate (ATP)-intensive, and the myocardium's high demand for oxygen and energy substrates leaves it acutely vulnerable to interruptions in its blood supply. The myriad cardioprotective properties of the natural intermediary metabolite pyruvate make it a potentially powerful intervention against the complex injury cascade ignited by myocardial ischemia–reperfusion. A readily oxidized metabolic substrate, pyruvate augments myocardial free energy of ATP hydrolysis to a greater extent than the physiological fuels glucose, lactate and fatty acids, particularly when it is provided at supra-physiological plasma concentrations. Pyruvate also exerts antioxidant effects by detoxifying reactive oxygen and nitrogen intermediates, and by increasing nicotinamide adenine dinucleotide phosphate reduced form (NADPH) production to maintain glutathione redox state. These enhancements of free energy and antioxidant defenses combine to augment sarcoplasmic reticular Ca^{2+} release and re-uptake central to cardiac mechanical performance and to restore β-adrenergic signaling of ischemically stunned myocardium. By minimizing Ca^{2+} mismanagement and oxidative stress, pyruvate suppresses inflammation in post-ischemic myocardium. Thus, pyruvate administration stabilized cardiac performance, augmented free energy of ATP hydrolysis and glutathione redox systems, and/or quelled inflammation in a porcine model of cardiopulmonary bypass, a canine model of cardiac arrest–resuscitation, and a caprine model of hypovolemia and hindlimb ischemia–reperfusion. Pyruvate's myriad benefits in preclinical models provide the mechanistic framework for its clinical application as metabolic support for myocardium at risk. Phase one trials have demonstrated pyruvate's safety and efficacy for intravenous resuscitation for septic shock, intracoronary infusion for heart failure and as a component of cardioplegia for cardiopulmonary bypass. The favorable outcomes of these trials, which argue for expanded, phase three investigations of pyruvate therapy, mirror findings in isolated, perfused hearts, underscoring the pivotal role of preclinical research in identifying clinical interventions for cardiovascular diseases.

Keywords: Anaplerosis, cardiopulmonary bypass, erythropoietin, Gibbs free energy, glutathione, reactive oxygen species, receiver operating characteristic, sarcoplasmic reticulum

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Introduction

Pyruvate (2-oxopropanoate) is a natural aliphatic carbohydrate and intermediary metabolite generated in cytosol by glycolysis or lactate oxidation. At serum concentrations of approximately 0.1 mM,[1,2] pyruvate is far less abundant than fatty acids, glucose and lactate, the principal blood-borne myocardial fuels, yet it is the immediate source of at least 10% of the acetyl CoA oxidized in the Krebs cycle.[2] Pyruvate is freely soluble in aqueous solution, and its circulating concentrations are readily augmented by infusion of highly concentrated pyruvate solutions. The research summarized below has established that serum pyruvate concentrations approximating 3–6 mM exert energy-yielding, antioxidant and anti-inflammatory actions that collectively augment cardiac mechanical performance and protect the myocardium from ischemic injury. Pyruvate's functional impact is especially robust in myocardium that has been reversibly injured, i.e. stunned, by ischemia and reperfusion or by oxyradical exposure.

Established cellular and molecular effects of pyruvate in mammalian myocardium

Pyruvate's enhancements of cardiac contractile performance, Gibbs free energy of ATP hydrolysis (ΔG_{ATP}), sarcoplasmic reticular (SR) Ca^{2+} cycling and antioxidant defenses have been presented in earlier reviews.[3–5] These mechanisms are summarized here because they likely contribute to pyruvate's favorable clinical effects presented in later sections.

Pyruvate enhancement of contractile function and ΔG_{ATP} in pre- and post-ischemic myocardium

Pyruvate's inotropic and lusitropic properties have been demonstrated in a variety of heart preparations. In isolated guinea-pig hearts, switching substrate supply from 16 mM glucose to 10 mM pyruvate increased left ventricular developed pressure from 97 ± 4 to 125 ± 3 mmHg while tripling phosphocreatine (PCr)/inorganic phosphate (P_i) concentration ratio, a measure of ΔG_{ATP}.[6] In isolated guinea-pig hearts consuming glucose[7] or octanoate,[8] addition of 2–20 mM pyruvate concentration dependently increased left ventricular stroke work and power, while lactate failed to increase cardiac function.[7] In *in situ* canine myocardium, intracoronary pyruvate infusion (0.15 $mmol \cdot min^{-1}$) increased systolic wall thickening by 44%.[9]

Pyruvate remained effective in hearts which were reversibly injured, i.e. stunned, by ischemia followed by abrupt reperfusion. In *in situ* canine hearts, 10 min coronary artery occlusion and 30 min reperfusion decreased systolic thickening of the post-ischemic territory by 80%, yet intracoronary pyruvate infusion restored wall thickening to 78% of the pre-ischemic value.[9] In stunned guinea-pig hearts consuming 5 mM glucose and 5 mM lactate, 5–10 mM pyruvate increased left ventricular power over 10-fold.[7]

Pyruvate augments cardiac function by several complementary mechanisms (Figure 1). A readily oxidized metabolic fuel, pyruvate increases ΔG_{ATP}, the thermodynamic driving force for the ATP-consuming processes that orchestrate cardiac mechanical function. Although mammalian myocardium consumes fatty acids, ketone acids, glucose, lactate and amino acids as energy substrates,[10–14] elevated serum pyruvate raises ΔG_{ATP} above that sustained by

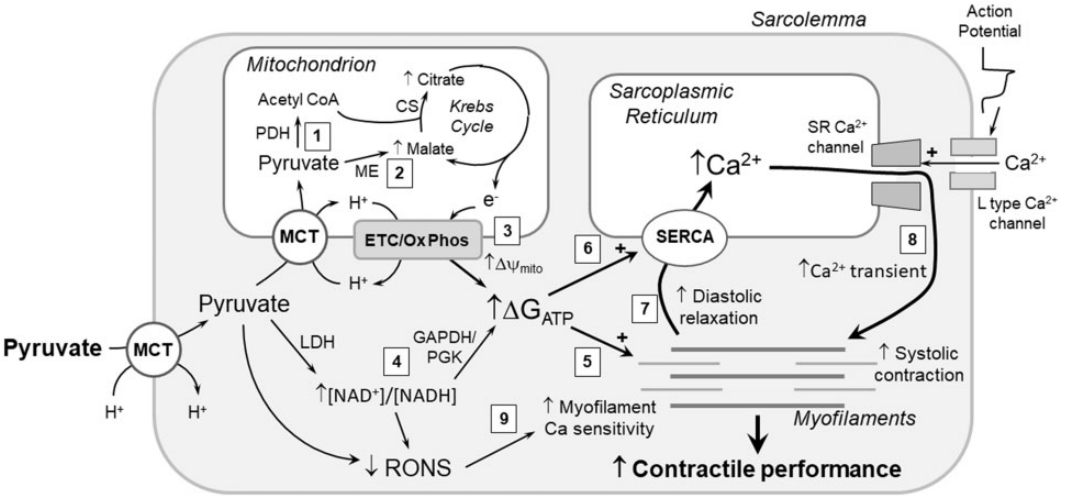

Figure 1. Mechanisms of pyruvate enhancement of myocardial contractile function. Pyruvate's energy-yielding and antioxidant actions increase cardiac performance by several mechanisms. Pyruvate metabolism increases ΔG_{ATP} by (**1**) generating Krebs cycle substrate acetyl CoA via pyruvate dehydrogenase (PDH); (**2**) increasing Krebs cycle intermediate pool sizes via anaplerotic pyruvate carboxylation by malic enzyme (ME); (**3**) augmenting mitochondrial membrane potential ($\Delta\psi_{mito}$); and (**4**) via lactate dehydrogenase (LDH), increasing cytosolic $[NAD^+]/[NADH]$ redox state, which increases ΔG_{ATP} via the near-equilibrium glyceraldehyde 3-phosphate dehydrogenase (GAPDH)/phosphoglycerate kinase (PGK) reactions. The increased ΔG_{ATP} augments contractile function by providing more energy for (**5**) myofilament crossbridge cycling and (**6**) sarcoplasmic reticular (SR) Ca^{2+} ATPase, i.e. SERCA. Increased SR Ca^{2+} loading by SERCA facilitates diastolic relaxation of the contractile machinery (**7**), and, by augmenting SR Ca^{2+} loading, increases Ca^{2+} induced Ca^{2+} release via the ryanodine-sensitive SR Ca^{2+} channels, producing a greater systolic Ca^{2+} transient (**8**) and more robust activation of the myofilaments. Pyruvate's antioxidant mechanisms, diagramed in detail in Figure 2, remove reactive O_2 and N_2 species (RONS), thereby augmenting myofilament Ca^{2+} sensitivity (**9**). Collectively, these mechanisms enhance both systolic contraction and diastolic relaxation, and thereby increase myocardial contractile performance.CS: citrate synthase; ETC/Ox Phos: electron transport chain/oxidative phosphorylation; MCT: monocarboxylate transporter. See text for details.

other fuels.[3,8] In *in situ* canine myocardium receiving its physiological complement of blood-borne fuels, pyruvate infusion to a serum concentration of 5.3 mM increased [PCr]/[ATP], a measure of ΔG_{ATP}, by 23%, and lowered the estimated cytosolic concentration of free, i.e. unbound, adenosine-5′-*bis*phosphate (ADP) by 25%.[15] Pyruvate augmentation of ΔG_{ATP} remained robust in stunned myocardium and paralleled the improved contractile recovery.[7,16] Transient (1-2 min) decreases in contractile force at the onset of pyruvate treatment followed by sustained increases in force development were reported in rabbit right ventricular trabeculae[17] and in muscle strips from failing human hearts.[18] Pyruvate enters cardiomyocytes via symport with H^+,[19–21] and the resultant cytosolic acidification may temporarily dampen the cardiac mechanical force[22,23] until surmounted by pyruvate's enhancement of ΔG_{ATP}.

Pyruvate also increased myocardial ΔG_{ATP} in large animal models of cardiopulmonary bypass (CPB)[24] and cardiopulmonary resuscitation.[25] Hearts of open-chest pigs were arrested with pyruvate- or lactate-fortified cardioplegia solution while the animal was maintained on a heart–lung machine. In comparison to the lactate cardioplegia, the pyruvate-enriched cardioplegia better preserved myocardial ATP content and ΔG_{ATP}.[24] In a canine model of cardiac arrest–resuscitation,[25] intravenous pyruvate vs. NaCl augmented the partial recovery of myocardial ΔG_{ATP} during cardiopulmonary resuscitation and increased post-arrest recovery of cardiac contractile and electrocardiographic activity and cerebral blood flow.[25]

Cytosolic and mitochondrial mechanisms of pyruvate enhancements of cardiac function and energy state

In liver mitochondria, ΔG_{ATP} and free [ATP]/[ADP] were directly correlated with inner membrane electrical potential ($\Delta\psi_{mito}$), a major component of the protonmotive driving force for oxidative phosphorylation.[26–28] In perfused rat hearts, switching fuel supply from 11 mM glucose to 5 mM pyruvate increased $\Delta\psi_{mito}$ by 25 mV[29]; thus, pyruvate, at concentrations that augment contractile function and ΔG_{ATP} in isolated and *in vivo* heart preparations,[3,7,8,15,16,24,25] increased the electrochemical driving force for mitochondrial ATP synthesis.

Pyruvate enhancement of post-ischemic cardiac function and ΔG_{ATP} required glucose as co-substrate. In glycogen-depleted guinea-pig hearts, irreversible contractile failure ensued when glucose was withdrawn despite the presence of 5 mM pyruvate.[30] Similarly, in rabbit hearts receiving 5 mM pyruvate, glycogen depletion or pharmacological inhibition of glycolysis upon reperfusion compromised recovery of left ventricular systolic and diastolic function.[31] Because pyruvate is a readily oxidized substrate, the glycolytic requirement for its enhancements of post-ischemic cardiac function and ΔG_{ATP} is surprising. The proposed coupling of cytosolic [NAD^+]/[NADH] concentration ratio to ATP phosphorylation potential ([ATP]/[ADP][Pi]) and, thus, ΔG_{ATP}, effected by the near-equilibrium glycolytic enzymes glyceraldehyde 3-phosphate dehydrogenase and phosphoglycerate kinase (Figure 1), may provide an explanation for the glycolytic requirement.[32] Pyruvate, in turn, increases cytosolic [NAD^+]/[NADH] via the near-equilibrium lactate dehydrogenase reaction. Accordingly, in isolated rabbit hearts, 10 mM pyruvate[33] increased cytosolic [ATP]/([ADP][P_i]) while raising the dihydroxyacetone phosphate/α-glycerophosphate concentration ratio, a measure of cytosolic [NAD^+]/[NADH].[34]

The proposed [NAD^+]/[NADH]:[ATP]/([ADP][P_i]) coupling[32] was challenged by the finding that pharmacological inhibition of mitochondrial pyruvate uptake, which did not disrupt pyruvate's enhancement of cytosolic [NAD^+]/[NADH], prevented pyruvate-increased left ventricular power and [ATP]/([ADP][P_i]), despite provision of medium chain fatty acid octanoate as alternative fuel.[8] Thus, mitochondrial metabolism of pyruvate was required for its enhancements of ΔG_{ATP} and contractile performance. Similarly, pyruvate augmentation of cell shortening and systolic [Ca^{2+}] transient in isolated rat ventricular cardiomyocytes was blunted by pharmacological inhibition of mitochondrial pyruvate uptake.[35] Unlike fatty acids, pyruvate can undergo reductive carboxylation in the mitochondrial matrix generating Krebs cycle intermediates,[36–38] which augments oxidative capacity and initiates formation of cardioprotective antioxidants (Figure 2). It thus appears that pyruvate's cytosolic and mitochondrial metabolism may act synergistically to bolster ΔG_{ATP} and antioxidant systems.

Pyruvate-enhanced SR Ca^{2+} transport

The myocardial contractile machinery is activated by Ca^{2+}, most of which is mobilized from the SR. Mechanisms that alter SR Ca^{2+} uptake and release modulate the heart's contractile performance. Diastolic relaxation requires Ca^{2+} reuptake by SR Ca^{2+} ATPase, a major ATP consumer in myocardium. The amount of Ca^{2+} released via the ryanodine-sensitive SR Ca^{2+} channels varies in proportion to SR Ca^{2+} loading,[39] which in turn is augmented by increased ΔG_{ATP}.[40] Approximately 80–85% of the free energy from ATP hydrolysis is conserved in the SR Ca^{2+} gradient,[41,42] so even modest changes in ΔG_{ATP} will modulate SR Ca^{2+} uptake. In buffer-perfused rabbit hearts, 1 mM pyruvate increased left ventricular developed pressure, ΔG_{ATP}, and the free energy of the SR Ca^{2+} gradient,[42] and in isolated guinea-pig hearts, pyruvate increased SR Ca^{2+} turnover, in parallel with its augmentation of contractile performance.[8,43] These effects were abrogated by pharmacological blockade of mitochondrial pyruvate uptake, despite provision of an alternative fatty acid substrate, demonstrating that pyruvate enhancements of SR Ca^{2+} transport, and thus, cardiac function require its mitochondrial oxidation.[8]

In rat ventricular cardiomyocytes, 10 mM pyruvate increased the amplitude but slowed the kinetics of the systolic Ca^{2+} transient and increased SR Ca^{2+} content.[44] Inhibition of mitochondrial monocarboxylate transport, adenine nucleotide translocase or the mitochondrial respiratory chain, blunted pyruvate amplification of [Ca^{2+}] transients, implicating mitochondrial pyruvate metabolism and ATP production in pyruvate's SR effects. Interestingly, pyruvate decreased the frequency of Ca^{2+} sparks and lowered the open probability of SR Ca^{2+} channels by

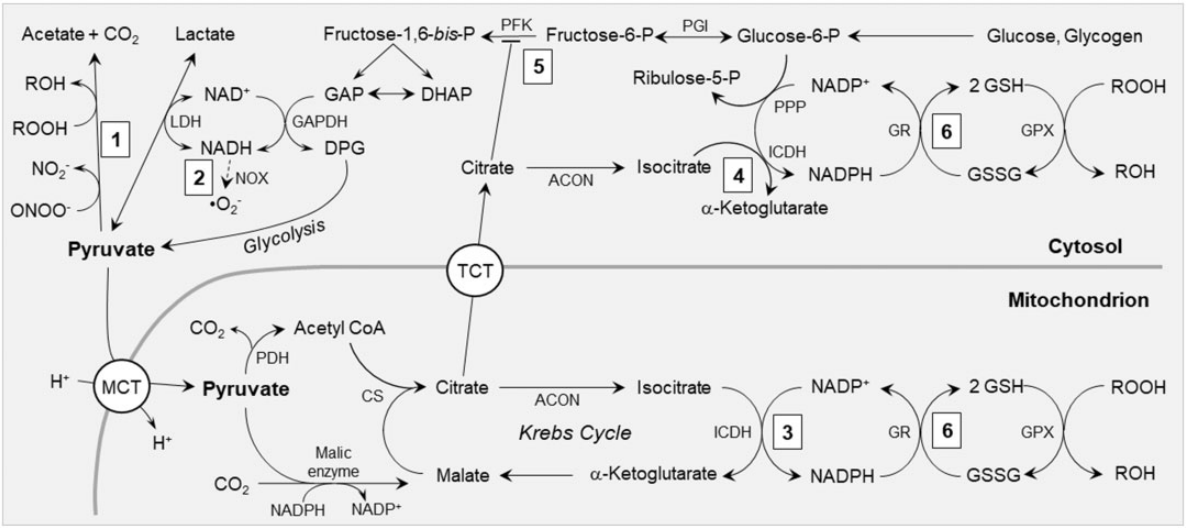

Figure 2. Antioxidant mechanisms of pyruvate. (**1**) Pyruvate non-enzymatically detoxifies peroxynitrite ($ONOO^-$) to nitrite and peroxides (ROOH) to their conjugate alcohols (ROH). (**2**) Via the lactate dehydrogenase (LDH) equilibrium, pyruvate oxidizes cytosolic NADH, regenerating NAD^+ for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and decreasing superoxide ($\bullet O_2^-$) formation by NAD(P)H oxidase (NOX). (**3**) Pyruvate carboxylation by malic enzyme increases mitochondrial concentrations of Krebs cycle intermediates, including citrate and isocitrate, thereby supporting NADPH production by mitochondrial $NADP^+$-dependent isocitrate dehydrogenase (ICDH). (**4**) Citrate effluxes to the cytosol via tricarboxylate transporters (TCT), where it is converted by aconitase (ACON) to isocitrate, providing substrate for NADPH formation by cytosolic ICDH. (**5**) Citrate inhibits phosphofructokinase (PFK), thereby diverting glycolytic flux into the pentose phosphate pathway (PPP). (**6**) NADPH generated by mechanisms 3, 4 and 5 provides reducing power to reduce glutathione disulfide (GSSG) to glutathione (GSH), thereby maintaining GSH redox state for the glutathione peroxidases (GPX) and other GSH-dependent antioxidant systems. CS: citrate synthase; DAP: dihydroxyacetone phosphate; DPG: 1,3-*bis*-phosphoglycerate; GAP: glyceraldehyde 3-phosphate; GR: glutathione reductase; MCT: monocarboxylate transporter; PDH: pyruvate dehydrogenase; PGI: phosphoglucose isomerase. See text for details.

approximately 60%. Thus, pyruvate-augmented ATP production and ΔG_{ATP} increased SR Ca^{2+} content, while pyruvate's interaction with SR Ca^{2+} channels suppressed Ca^{2+} release.

Pyruvate-enhanced Ca^{2+} sensitivity of the contractile machinery

Pyruvate produced a powerful Ca^{2+}-sensitizing effect, i.e. increased Ca^{2+}-activated contractile force, in rabbit right ventricular trabeculae.[17] Pyruvate of 10 mM doubled peak cytosolic [Ca^{2+}] and peak contractile force, arguing that pyruvate's inotropic actions were due to increased [Ca^{2+}] transients, yet when SR Ca^{2+} cycling was pharmacologically inhibited, pyruvate increased contractile force *without* increasing systolic [Ca^{2+}]. Instead, pyruvate produced a striking increase in myofilament Ca^{2+} sensitivity, manifest by a leftward shift in the force:[Ca^{2+}] relationship and an increased Hill coefficient, which would augment contractile force in response to a given Ca^{2+} transient. When the trabeculae were chemically skinned to disrupt the sarcolemma and mitochondria, pyruvate increased neither myofilament Ca^{2+} sensitivity nor the Hill coefficient. It was proposed[17] that pyruvate enhanced Ca^{2+} sensitivity by lowering P_i concentration, an effect that requires oxidative phosphorylation and, thus, intact mitochondria. This indirect myofilament Ca^{2+}-sensitizing mechanism may complement pyruvate's enhancement of SR Ca^{2+} transport to potentiate systolic contractile force. Accordingly, 20 mM pyruvate increased developed force in failing human heart muscle even after pharmacological inhibition of SR Ca^{2+} storage, indicating that enhanced SR Ca^{2+} turnover was not solely responsible for pyruvate's inotropic effects.[18]

Oxido-nitrosative stress and cardiac injury

Myocardial ischemia–reperfusion provokes overproduction of toxic reactive oxygen and nitrogen species, i.e. RONS, including superoxide, H_2O_2 and lipid peroxides, hydroxyl radicals, singlet oxygen, hypochlorous acid and peroxynitrite. These aggressive compounds attack cellular biomolecules, compromising electrolyte homeostasis, membrane integrity, ATP production and, ultimately, cardiomyocyte survival and cardiac function. The myriad targets of RONS include polyunsaturated acyl moieties in membrane phospholipids,[45] enzymes catalyzing intermediary metabolism and energy production,[46–48] membrane ion transporters and channels,[49,50] contractile proteins,[51,52] and nuclear[53,54] and mitochondrial[55,56] DNA. Many of the enzymes that orchestrate fuel metabolism are potential RONS targets, including glyceraldehyde 3-phosphate dehydrogenase[47] of the glycolytic pathway, aconitase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase of the Krebs cycle,[46,57,58] mitochondrial respiratory complexes I and III,[48,59,60] and creatine kinase,[61,62] which shuttles ATP from mitochondria to myofilaments and ion pumps.[63,64] Inactivation of these enzymes compromises ATP supply essential for cardiac function.

The actions of RONS on myofilaments can depress Ca^{2+} sensitivity of the contractile machinery.[65] *S*-nitrosylation of cysteinyl residues in sarcomeric proteins can dampen Ca^{2+}-activation of myofibrillar ATPase and contractile force.[66] Peroxynitrite has been found to depress left ventricular force development, myofilament Ca^{2+} sensitivity,[67] and the energetic efficiency of cardiac performance, i.e. the contractile work produced at a given rate of ATP expenditure.[68,69] The electrolyte transport mechanisms that

orchestrate excitation–contraction coupling and diastolic relaxation also are RONS targets, including the Na^+, K^+ ATPase, which maintains sarcolemmal Na^+ and K^+ electrochemical gradients and, thus, resting membrane potential,[49,70] the ryanodine-sensitive SR Ca^{2+} channels which release massive amounts of Ca^{2+} to trigger systolic contraction,[71–73] the sarcolemmal Na^+/Ca^{2+} exchangers which effect Ca^{2+} efflux to the extracellular space,[71,74,75] and the SR Ca^{2+} ATPase,[76,77] which sequesters Ca^{2+}, affording diastolic relaxation and re-loading the SR for the next systole. Impairment of these processes by RONS can profoundly impact cardiac function, and the ensuing Ca^{2+} overload may initiate mitochondrial permeability transition and cardiomyocyte injury or death.[78,79]

Pyruvate's cardioprotective antioxidant mechanisms

In addition to its ATP-yielding properties as a metabolic fuel, pyruvate also is a powerful antioxidant.[80] Pyruvate exerts its antioxidant effects via several complementary mechanisms, summarized in Figure 2.[4,5] Aliphatic α-keto-carboxylates, including pyruvate, undergo oxidative decarboxylation, yielding electrons to reduce and detoxify peroxides, hydroxyl radicals and peroxynitrite in direct, non-enzymatic reactions.[81,82] In this fashion, pyruvate (a) reduces lipid peroxides to their conjugate alcohols, thereby interrupting propagation of membrane lipid peroxidation;[24,83] (b) reduces hydrogen peroxide (H_2O_2) and hydroxyl radicals (·OH) to water[81] and (c) converts peroxynitrite to a benign product, nitrite.[82] Pyruvate decarboxylation also yields an oxidizable fuel, acetate.

The principal natural antioxidant in cells, the tripeptide glutathione (GSH), contains a sulfhydryl moiety capable of neutralizing peroxides, peroxynitrite and hydroxyl radicals.[84,85] Massive amounts of these toxic metabolites are generated during ischemia and particularly during efforts to restore perfusion, e.g. revascularization and fluid resuscitation.[86,87] This oxyradical burden depletes GSH, compromising cellular GSH-dependent antioxidant systems. Pyruvate maintains GSH in two ways (Figure 2).[4,5] By directly detoxifying RONS, pyruvate unburdens GSH-dependent antioxidant enzymes, thereby preserving cellular GSH content. Secondly, pyruvate carboxylation by malic enzyme[37,38,88] increases the contents of Krebs cycle intermediates, including citrate, a physiological inhibitor of the glycolytic enzyme phosphofructokinase.[89,90] Thus, pyruvate administration sharply increased citrate content in isolated hearts[8,91,92] and in myocardium *in vivo*.[24,93] After its efflux into the cytosol via tricarboxylate transporters in the inner mitochondrial membrane[89] (Figure 2), citrate inhibits phosphofructokinase, causing buildup of glucose 6-phosphate,[91] the substrate for the hexose monophosphate pathway, a major source of NADPH which is utilized to maintain GSH via glutathione reductase.

The $NADP^+$-dependent isocitrate dehydrogenase is another source of NADPH. Cardiomyocytes harbor distinct $NADP^+$-isocitrate dehydrogenase isoenzymes in the cytosol and mitochondrial matrix.[94,95] Because aconitase, the Krebs cycle enzyme that converts citrate to isocitrate, is near-equilibrium, pyruvate-induced citrate accumulation will, in turn, generate isocitrate, the substrate for isocitrate dehydrogenase. Thus, pyruvate carboxylation could support production of NADPH, the source of electrons to maintain GSH in its reduced, antioxidant form. It should be noted that NADPH provides electrons for reductive pyruvate carboxylation to malate by malic enzyme,[36] so conversion of this malate to isocitrate, followed by isocitrate oxidation by $NADP^+$-isocitrate dehydrogenase, does not afford net NADPH production. Instead, the sequential conversion of pyruvate to malate and then citrate, citrate efflux from the mitochondria and its conversion to isocitrate and isocitrate oxidation by cytosolic $NADP^+$-isocitrate dehydrogenase effectively shuttles NADPH across the mitochondrial membrane (Figure 2).

In isolated working guinea-pig hearts, low-flow ischemia and reperfusion sharply lowered myocardial GSH redox state, i.e. the concentration ratio of GSH to its oxidized form, glutathione disulfide (GSSG). Post-ischemic pyruvate treatment restored GSH/GSSG and increased $NADPH/NADP^+$ redox state and citrate content.[91] Moreover, pyruvate prevented inactivation of oxyradical-sensitive enzymes in H_2O_2 exposed hearts,[92] *in situ* porcine myocardium subjected to cardioplegia-induced arrest,[96] and in canine myocardium subjected to cardiac arrest and cardiopulmonary resuscitation.[97]

Bassenge *et al*.[98] demonstrated that shifts in cytosolic $[NAD^+]/[NADH]$ driven by pyruvate vs. lactate modulate RONS production by NADH oxidase. In cardiac homogenates, pyruvate concentration dependently suppressed NADH oxidase activity, with 55% inhibition by 5 mM pyruvate. The xanthine oxidase inhibitor oxypurinol did not alter lactate-stimulated ROS formation, and neither pyruvate nor lactate affected RONS production by an *in vitro* xanthine/xanthine oxidase system, excluding xanthine oxidase as the pyruvate-suppressed RONS source. In isolated guinea-pig hearts, an intense, 60 s burst of RONS production ensued upon reperfusion after 5 min severe ischemia. Pyruvate concentration dependently dampened this RONS burst, with *c.* 75% suppression by 5 mM pyruvate. In contrast, lactate increased post-ischemic RONS production. Notably, the monocarboxylate transport inhibitor α-cyano-3-hydroxycinnamate, at a concentration (0.5 mM) which selectively inhibits mitochondrial but not sarcolemmal pyruvate uptake,[8,20] did not impede pyruvate's suppression of RONS. Thus, pyruvate's antioxidant actions in this model are mainly cytosolic, unlike its energy-generating and inotropic effects which require its mitochondrial metabolism.[8]

Pyruvate's antioxidant capabilities likely contributed to its potentiation of β-adrenergic mechanisms in stunned myocardium.[16,91] In isolated guinea-pig hearts, ischemia–reperfusion blunted, yet pyruvate treatment largely restored, β-adrenergic stimulation of contractile performance.[16] Ischemia–reperfusion shifted the relationship between cardiac power and isoproterenol concentration, such that the EC_{50} increased from 0.3 ± 0.06 nM in pre-ischemic to 5.2 ± 1.9 nM in stunned hearts, yet 5 mM pyruvate, initiated at 15 min reperfusion, lowered the EC_{50} to 1.1 ± 0.3 nM isoproterenol. Consequently, 2 nM isoproterenol, which produced a robust inotropic effect in

pre-ischemic hearts, was ineffective in stunned myocardium, yet 5 mM pyruvate largely restored the inotropic response to isoproterenol.[16] Although pyruvate alone increased ΔG_{ATP}, during 2 nM isoproterenol stimulation, ΔG_{ATP} was similar in the absence vs. presence of pyruvate; thus, increased ΔG_{ATP} could not explain the fivefold greater power of the hearts receiving 2 nM isoproterenol + pyruvate vs. 2 nM isoproterenol alone. On the other hand, pyruvate sharply increased antioxidant GSH/GSSG and NADPH/$NADP^+$ ratios, and the sulfhydryl antioxidant *N*-acetylcysteine potentiated isoproterenol-induced power and augmented GSH/GSSG to similar extents as pyruvate, without increasing ΔG_{ATP}.[91] Thus, pyruvate's antioxidant mechanisms potentiated β-adrenergic inotropism in stunned myocardium.

Recently identified cellular and molecular effects of pyruvate

Investigations conducted since the most recent comprehensive pyruvate review[5] have documented several additional cardioprotective actions of this pleiotropic compound.

Anti-inflammatory actions of pyruvate in CPB and hypovolemia

Although essential for many cardiothoracic surgeries, CPB can ignite an intense systemic inflammatory response that complicates post-surgical recovery.[99,100] RONS, generated in myocardium by intermittent cardioplegia infusion and abrupt reperfusion upon release of the aortic cross-clamp,[101,102] ignite myocardial inflammation by provoking neutrophil infiltration[103] and activating metalloproteinases that degrade the extracellular matrix.[104] The authors tested the hypothesis that physiological antioxidant pyruvate could quell post-bypass inflammation in pigs undergoing CPB with pyruvate-enriched vs. pyruvate-free cardioplegia.[105] By 4 h reperfusion after cardiac arrest with control cardioplegia, coronary sinus [GSH]/[GSSG], a measure of [GSH]/[GSSG] within the cardiomyocytes,[106] fell by 70% vs. non-arrested pigs, but in pigs receiving pyruvate-enriched cardioplegia, coronary sinus [GSH]/[GSSG] was increased nearly 10-fold vs. control cardioplegia, indicating a robust antioxidant effect still present 4 h post-CPB. Pyruvate cardioplegia also suppressed matrix metalloproteinase-9 and myeloperoxidase activation, C-reactive protein accumulation, and neutrophil infiltration in left ventricular myocardium.[105] Thus, pyruvate-enriched cardioplegia exerted antioxidant and anti-inflammatory effects that persisted at least 4 h after treatment.

Inflammation is a major comorbidity and cause of death in patients suffering from hypovolemic and septic shock.[107–109] Pyruvate's antioxidant actions and its enhancement of SR Ca^{2+} transport suggested pyruvate-enriched resuscitation for hypovolemia may exert anti-inflammatory effects in a manner superior to lactate resuscitation. To test this hypothesis, domestic goats were exsanguinated to lower arterial pressure to a critical point approaching decompensation. A tourniquet was applied to impose hindlimb ischemia and released 90 min later during resuscitation with pyruvate- vs. lactate-enriched Ringer's solution. Relative to non-hemorrhaged shams, in the lactate-resuscitated gastrocnemius, NADPH oxidase, a source of oxyradicals, and the pro-apoptotic enzyme poly (ADP-ribose) polymerase were activated, peroxynitrite formation and lipid peroxidation were exacerbated and the oxyradical-sensitive enzymes creatine kinase and aconitase were inactivated.[93,110] In contrast, pyruvate-enriched fluid resuscitation prevented all of these untoward effects in the post-ischemic muscle.[110,111] Pyruvate Ringer's resuscitation also proved superior to lactated Ringer's for stabilizing cardiac function. Compared to lactated Ringer's, pyruvate Ringer's afforded increased recovery of systemic arterial pressure, prevented pro-arrhythmic changes in the electrocardiographic QT interval, and blunted myocardial accumulation of the peroxynitrite derivative nitrotyrosine.[93,112]

Pyruvate induction of myocardial erythropoietin production and cytoprotective signaling

Erythropoietin is known classically as an activator of erythrocyte maturation in response to hypoxia.[113] More recently, a second function of the hormone has emerged: protecting cells in heart, brain and other organs from ischemic injury and inflammation.[114–116] Indeed, erythropoietin and its analogs are being developed as treatments for myocardial infarction and ischemic stroke.[117–119] Furthermore, it is now becoming evident that heart[120] and brain[121,122] are capable of synthesizing erythropoietin as an endogenous cardio- and neuroprotectant. Treatments that induce endogenous erythropoietin hold great promise for treating ischemic syndromes of heart and brain, provided they can gain access to the target tissues.

In left ventricular myocardium of pigs subjected to cardioplegic arrest on CPB, pyruvate-enriched cardioplegia induced a nearly 1000-fold increase in the abundance of messenger RNA encoding erythropoietin, and doubled erythropoietin protein content, 4 h after pyruvate treatment.[120] Pyruvate-treated myocardium secreted erythropoietin into the coronary effluent for at least 4 h post-pyruvate; thus, pyruvate-enriched cardioplegia stimulated myocardial production of the cytoprotective hormone. Phosphorylation and activation of extracellular signal-regulated kinase-1/2 (Erk-1/2), an erythropoietin-responsive signaling kinase which has been implicated in cardioprotection,[123,124] and stabilization of cardiac mitochondria[125] were sharply increased 4 h after pyruvate treatment. To our knowledge, this study was the first to show erythropoietin expression in myocardium, and it was pyruvate that produced this novel effect. In a subsequent study in a rat model of ischemic stroke, intravenous pyruvate infusion, for the last 60 min of 2 h middle cerebral artery occlusion and the first 30 min reperfusion, decreased lesion volume by 84% and DNA fragmentation by 77%, in a manner paralleled by increased cerebrocortical erythropoietin content.[122]

Clinical application of pyruvate

Although pyruvate has been repeatedly found beneficial in preclinical models of cardiovascular disease, only a limited number of clinical trials have examined pyruvate therapy for cardiovascular syndromes. Pioneering studies by Hermann *et al*.[126] evaluated intracoronary pyruvate in patients with dilated cardiomyopathy and New York Heart Association class III heart failure. Pyruvate was infused into the left main coronary artery at 0.925 and 1.85 $mmol \cdot min^{-1}$ for 15 min at each dose. Both doses similarly increased stroke volume index and cardiac index, while lowering pulmonary capillary wedge pressure and heart rate. These variables returned to their respective pretreatment values within 15 min after pyruvate infusion was discontinued. In patients in cardiogenic shock after acute myocardial infarct receiving primary percutaneous coronary intervention and intra-aortic balloon pump, intracoronary pyruvate infusion (*c*. 0.016–0.04 $mmol \cdot kg^{-1} \cdot min^{-1}$) increased cardiac index, stroke volume index and mean arterial pressure.[127] These effects plateaued within 30 min and again subsided by 10 min post-infusion.

In a recent clinical trial, Dong *et al*.[128] compared 28 mM pyruvate-enriched Ringer's to NaCl solution for intravenous resuscitation of patients in septic shock. Relative to NaCl, pyruvate Ringer's decreased circulating activities of the pro-inflammatory cytokines tumor necrosis factor α and interleukin 6, and increased central venous O_2 saturation, a measure of pulmonary function, and urine output. Most importantly, while 9 of the 45 control patients died, only 2 of the 45 pyruvate-treated patients died ($P = 0.01$). Thus, intravascular pyruvate treatment afforded significant clinical benefit without producing any untoward effects.

Pyruvate-fortified cardioplegia for CPB

During open-heart surgeries, CPB mechanically circulates and oxygenates blood for the body while bypassing the heart and lungs.[129] CPB uses a heart–lung machine to maintain organ perfusion while the surgeon works in a bloodless surgical field. Meanwhile, the heart is arrested by intracoronary administration of a depolarizing cardioplegia solution, affording a quiescent surgical field. Afterward, the aortic cross-clamp is released, the heart is reperfused and, upon recovery of sufficient cardiac function, the patient is separated from the heart–lung machine. Although CPB permits lifesaving cardiac surgery, it depletes myocardial energy and antioxidant reserves, provokes myocardial oxyradical formation, and ignites an intense systemic inflammatory response triggered by the collective effects of anesthesia, surgical trauma, hypothermia, and the repeated passage of leukocytes and other blood components through an artificial, extracorporeal circuit.[99,100]

Although cardioplegic arrest interrupts coronary flow, imposing ischemia on the myocardium, it also affords opportunities for intervention with pyruvate. As an energy substrate, antioxidant and anti-inflammatory agent, pyruvate may be uniquely capable of simultaneously addressing myocardial energy depletion, oxidative stress and inflammation associated with CPB. Accordingly, the authors conducted the first clinical trial of pyruvate-enriched cardioplegia and compared it with conventional, lactate-fortified cardioplegia.[130] Patients undergoing primary coronary artery grafting on CPB were randomized to receive cardioplegia containing glucose, insulin and either lactate or pyruvate. The two groups of 15 patients were well matched for age, gender and other demographic factors, and the duration of aortic crossclamp and bypass time were virtually identical in the two groups. Upon separation from bypass, both groups showed the typical post-CPB cardiodepression, a manifestation of cardiac stunning,[102,131] yet within 4 h, those patients who had received the pyruvate cardioplegia experienced robust, complete recovery of left ventricular function, while those receiving the lactate cardioplegia recovered more gradually, as is typically seen following CPB.[132] Relative to lactate cardioplegia, pyruvate cardioplegia also sharply lowered cardiac release of the injury marker proteins cardiac troponin I and creatine kinase MB.[130] The pyruvate group required substantially less dobutamine inotropic support, and those patients met the criteria for hospital discharge on average 27 h earlier than the lactate cardioplegia cohort, at a savings of several thousand dollars per patient. Importantly, there were no adverse events associated with pyruvate treatment.

The results of this clinical trial demonstrate pyruvate's effectiveness to improve post-cardioplegic arrest recovery of patients at risk of ischemia–reperfusion and its sequelae. Moreover, the clinical trial validates preclinical studies in a variety of animal models which demonstrated pyruvate's multiple mechanisms that make it a potentially powerful cardioprotective intervention for ischemic and pro-inflammatory syndromes.

Receiver operating characteristic analyses of pyruvate effects in *in vitro* perfused hearts vs. clinical cardioplegia for CPB

A receiver operating characteristic (ROC) diagram plots the true-positive rate, i.e. sensitivity of a test or procedure outcome, on the ordinate against the false-positive rate, i.e. 100-specificity, on the abscissa, yielding an ROC curve. The area under the ROC curve, ROCAUC, is a measure of the accuracy of the test or procedure, i.e. its ability to distinguish outcomes without false negatives or false positives. At the maximum sensitivity and specificity of 100%, ROCAUC equals 1.0, and there is no overlap of the test results from, for example, the groups receiving control vs. experimental treatments. The most useful ROC information from a clinical study may come from pooling the results of several studies examining the same or comparable clinical interventions or drug tests in different situations, generating "averaged" specificity, sensitivity and ROC statistics which provide an optimum understanding of the intervention's utility.

The ROC curve provides important information about a clinical intervention's performance: the closer the apex of the curve approaches the upper left corner, indicating a high true-positive rate and a low false-positive rate, the greater the test's reliability to discriminate an experimental condition from an appropriate control. Outcome or

treatments are expressed as numeric categories labeled as 1 (outcome positive, diseased, treatment applied) or 0 (outcome negative, healthy, treatment not applied). Thus, positive in this context means a successful outcome, a treatment provided, an expected drug effect present, or a positive clinical test relative to control. Additionally, the ROCAUC as well as the estimates of false-positive rates (100-specificity) and false-negative rates (100-sensitivity) are of great interest to the clinician,[133,134] because an ROCAUC value is an estimate of the accuracy of the results from a diagnostic test, procedure or drug test. An ROCAUC value of 1.0 indicates a perfect test/outcome where there are no overlapping data from the control and interventional states, and, therefore, no false negatives (100% sensitivity) or false positives (100% specificity). The opposite is true for an ROCAUC value of 0.5 which shows the test/procedure is no better than random chance, and therefore has no diagnostic or prognostic value. ROCAUC values between 0.9 and 1.0 are considered excellent, those between 0.8 and 0.9 are good, and those between 0.7 and 0.8 are considered fair, requiring more cases even if they are statistically significant. ROCAUC between 0.6 and 0.7 are considered to reflect poor outcome and therefore are usually clinically unacceptable; ROCAUC <0.6 should likely be considered failures.[135] Another precaution is that ROCAUCs from small sample sizes may be interpreted as inherently "noisy" due to their low statistical power and should be interpreted with extreme caution[136] even if the ROCAUC is high.

On the other hand, the ROC analysis offers the unique possibility for the clinician to determine, through the lens of clinical experience, the so-called cut-off value for optimizing diagnostic strategies. In other words, the clinician, evaluating the ROCAUC, must decide which cut-off value will be associated with acceptable odds ratios, likelihood ratios or confidence intervals for minimizing misclassification (e.g. diseased/healthy, test success/failure, etc.) using a sensitivity/specificity pair that promises to be maximally error-free. Thus, ROC analysis, like other statistical modeling techniques (e.g. multivariate analysis, logistic regression or non-linear regression), has specific limitations[134,136,137] and is therefore probably best used as an adjunct or supplemental analysis instrument available to the practicing clinician.

The beneficial effects of pyruvate demonstrated in clinical settings are foreshadowed by results of basic cardiovascular research on pyruvate by the authors and other investigators.[5] As summarized above, these preclinical efforts demonstrated the beneficial efficacy of pyruvate administration in a variety of experimental models including *in situ* and *ex vivo* heart preparations and cultured cell lines. We focused on data obtained in isolated, working and non-working guinea-pig hearts perfused for 90–120 min, including measured variables related to the myocardial energy state, particularly ΔG_{ATP}, cytosolic [ATP]/([ADP][P_i]), intracellular P_i concentration and the PCr/creatine concentration ratio, hydraulic work output of the left ventricle, and $\Delta\psi_{mito}$. The latter variable was estimated from the relationship between $\Delta\psi_{mito}$ and ΔG_{ATP} in non-respiring liver mitochondria,[26] where $\Delta\psi_{mito} = -233 +$ (ΔG_{ATP}, kJ/mol)[1.47]. Indeed, according to this equation, the addition of 5 mM pyruvate increased $\Delta\psi_{mito}$ by 22 mV in perfused guinea-pig hearts receiving 5 mM glucose, in excellent agreement with the 25 mV increase reported by Wan *et al*.[29] in isolated rat hearts. The guinea-pig hearts metabolized 5–10 mM glucose plus either 0.2–20 mM pyruvate or 5–20 mM *L*-lactate as the main fuels.[3,7,30,43,138,139]

To enable ROC analysis of the isolated heart data, the variables were first analyzed in a logistic regression model with the treatment category as the dependent variable, labeled as 1 if pyruvate present ($n = 20$ experimental protocols) or 0 if absent ($n = 22$ experimental protocols) and the myocardial parameters (measures of intracellular energy state or mechanical work output) as the independent variables. This multivariant logistic model yielded coefficients $\pm$ standard errors for each independent variable with P values between 0.043 and 0.11 and Wald statistics between 5.18 and 2.55; the model, based on 20 "positive" and 22 "negative" protocols, was statistically highly significant at $P = 0.0004$, $n = 42$ protocols (5–7 experiments per protocol; *c.* 210 individual experiments). The Shapiro–Wilk statistic for normal distribution of the residuals approached 1.0 at $W = 0.97$, with a P value of 0.35, confirming normality of the residuals. The predicted pyruvate treatment categories from these logistic regressions were then subjected to standard ROC analyses (MedCalc Software v. 17.9, Ostend, Belgium) in which the actual pyruvate categories were compared with the logistically predicted categories. These ROC estimates from the pyruvate- and lactate-perfused hearts (Figure 3(a)) yielded a good to excellent ROCAUC of 0.89 ± 0.051, $n = 42$, $P < 0.0001$, with a sensitivity of 90% and specificity of 82%. This result strongly suggests that our protocols clearly distinguished the glucose plus pyruvate treated hearts from those receiving glucose plus *L*-lactate, examined under diverse but relatively physiological conditions. This differentiation is also evident from the classification table generated by the logistic regression, which showed an 83.3% overall correct prediction, with 19 of 22 cases (86.4%) correctly predicted in the negative (glucose $\pm$ lactate) series and 16 of 20 cases (80%) correctly predicted in the positive (pyruvate + glucose) series. Thus, there was a relatively high degree of certainty that pyruvate treatment of perfused guinea-pig hearts bolstered contractile performance and myocardial energy state in these preclinical experiments.

We then applied ROC analysis (without prior logistic regression estimates) to left and right ventricular stroke work indices from the CPB clinical trial[130] to assess whether outcomes with pyruvate cardioplegia were statistically differentiated from the standard lactate cardioplegia. Specifically, ROC analyses were applied to identify whether pyruvate cardioplegia was superior to lactate cardioplegia for clinical CPB in terms of post-CPB recovery of left or right ventricular function, in this cohort of 30 patients. The categorical independent parameter was pyruvate cardioplegia = 1 vs. lactate cardioplegia = 0. ROC analysis of left ventricular function index 4, 6 and 12 h after CBP (Figure 3(b)) yielded a high ROCAUC value: 0.91 ± 0.029, $n = 90$ data points, $P < 0.0001$. The 95% confidence interval

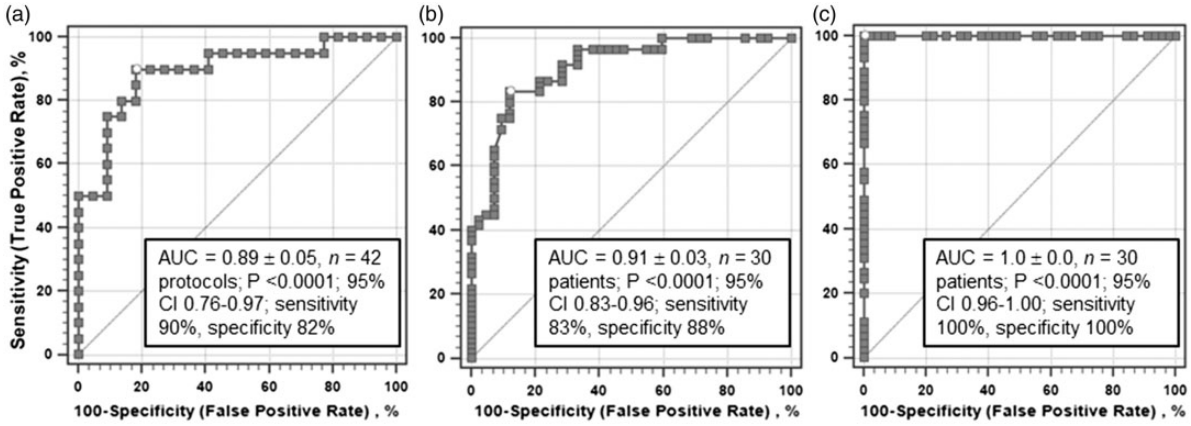

Figure 3. Receiver operating characteristic (ROC) analyses of isolated, perfused guinea-pig hearts and patients recovering from coronary revascularization surgery on cardiopulmonary bypass. (a) Isolated guinea-pig hearts: Analyses of mean values of contractile function and myocardial energy state from 42 separate series[3,7,30,43,138,139] comprising approximately 210 isolated hearts metabolizing glucose alone, glucose + lactate or glucose + pyruvate. Analysis of left ventricular (b: LVSWI post-bypass) and right ventricular (c: RVSWI post-bypass) stroke work indices in 30 patients (15 patients per group) at 4, 6 and 12 h recovery following cardiopulmonary bypass with lactate- vs. pyruvate-enriched cardioplegia (total 90 data points).[130]AUC: area under the ROC curve; CI: 95% confidence interval; LVSWI: left ventricular stroke work index; RVSWI: right ventricular stroke work index.

of the entire ROC curve ranged from 0.83 to 0.96, implying good to excellent precision and showing a clear statistical separation of the two patient groups. However, the estimated rate of false-negative classification (1-sensitivity) was approximately 17%, while the estimated false-positive rate (1-specificity) was approximately 12%, suggesting that this first CPB trial of pyruvate-enriched cardioplegia likely requires independent confirmation. Interestingly, ROC analysis of the right ventricular stroke work index 4–12 h post-CPB produced an estimated ROCAUC = 1.0, strongly suggesting a perfect test for that variable in these patients (Figure 3(c)).

Collectively, these first CBP trial results predicted with relatively high confidence that the pyruvate cardioplegia could have significant clinical utility, at least in the setting of cardiothoracic surgery on CPB. Schillinger *et al.*[127] reached a similar conclusion regarding the use of intracoronary pyruvate treatment of cardiogenic shock. It is also interesting to note that pyruvate intervention was found to be effective for resuscitation for patients with septic shock,[128] in which pyruvate-enriched Ringer solution decreased systemic pro-inflammatory cytokines and simultaneously improved survival rate from 80% to 95% without any signs of toxicity.

The ROCAUC estimates from the isolated guinea-pig hearts (Figure 3(a)) and the left ventricle of patients 4–12 h post-CPB (Figure 3(b)) were nearly identical: AUC 0.89 $\pm$ 0.058 and 0.91 $\pm$ 0.029, respectively; $z = 0.34$, $P = 0.733$. Thus, direct comparison of these ROCAUC estimates demonstrates remarkable concordance of the preclinical and clinical ROC estimates. The preclinical data from isolated, perfused guinea-pig hearts revealed considerable predictive power, at least with respect to the clinical post-CPB recovery trial detailed above. This robust agreement underscores the value of basic research as an essential precursor to clinical trials, both for proof of concept and for defining mechanisms responsible for therapeutic benefits.

Factors limiting the clinical application of pyruvate

Several practical considerations must be taken into account before pyruvate-enriched formulations can be introduced into general clinical practice. For instance, the shelf lives of such formulations have not been firmly established, but may be shorter than conventional Ringer's lactate and possibly more expensive. Currently, pyruvate-enriched solutions must be prepared at or near the time and location of use and must be sterilized, e.g. by micropore filtration, as was done in the cardioplegia clinical trial.[130] The source pyruvate and intravenous solutions must be tested to ensure they are endotoxin-free. These requirements may hamper clinical use of pyruvate, especially in emergency and critical care medicine where pyruvate may be particularly beneficial, yet treatments are given urgently. Another consideration is the profitability of pyruvate formulations for the pharmaceutical industry; if such formulations are unprofitable, there may be little motivation for developing and marketing them, despite their clinical efficacy.

Conclusions

The research summarized above has defined pyruvate's unique combination of energy-yielding, antioxidant and anti-inflammatory properties, which makes it a powerful cardioprotectant against the effects of myocardial ischemia–reperfusion and oxidant stress. Thus, pyruvate, particularly at 3–6 mM, augmented post-ischemic contractile performance and myocardial ΔG_{ATP}, the energy source for myofilament crossbridge cycling and SR Ca^{2+} uptake. Pyruvate also increased cytosolic $NAD^+/NADH$, thereby alleviating NADH suppression of energy-yielding glycolytic flux at glyceraldehyde 3-phosphate dehydrogenase and dampening superoxide production by NADH oxidase. By augmenting antioxidant GSH/GSSG and $NADPH/NADP^+$ redox states, pyruvate protected

RONS-vulnerable SR Ca^{2+} transport and myofilament Ca^{2+} sensitivity, and blunted myocardial inflammation. The first clinical trials of pyruvate intervention for heart failure, septic shock and CPB have demonstrated pyruvate's safety and therapeutic efficacy. Expanded, phase three clinical trials are essential to confirm and extend the favorable outcomes of pyruvate-based interventions.

Authors' contributions: RM and RB evaluated the literature and drafted the manuscript. AY conducted the clinical trial of cardiopulmonary bypass. RB conducted receiver operating characteristic analyses of preclinical and cardiopulmonary bypass data. RM and RB prepared the figures. RM, AY and RB edited the manuscript.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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