Laboratory Investigation

Ex Vivo Resuscitation of Adult Pig Hearts

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One possible way to expand the human heart donor pool is to include non-heart-beating human donors. To begin validating this approach, we developed an ex vivo cardiac perfusion circuit to support large mammalian hearts in Langendorff mode and beating-ejecting mode and to assess and improve their ischemic tolerance.

In vivo hemodynamic data and heparinized blood (4.0 ± 0.5 L) were collected from 6 anesthetized pigs. Hearts were isolated and connected to a recirculating perfusion circuit primed with autologous buffered blood (pH, 7.40). After retrograde aortic perfusion in Langendorff mode, the left atrium was gravity-filled at 10–20 mmHg, and the left ventricle began to eject against a compliance chamber in series with a systemic reservoir set to a hydraulic afterload of 100–120 mmHg. Left ventricular function was restored and maintained in all 6 hearts for 30 min. Cardiac output, myocardial oxygen consumption, stroke work, aortic pressure, left atrial pressure, and heart rate were measured. The mean myocardial oxygen consumption was 4.8 ± 2.7 mL/min/100 g (95.8% of in vivo value); and mean stroke work, 5.3 ± 1.1 g · m/100 g (58.95% of in vivo value). One resuscitated heart was exposed to 30 min of normothermic ischemic arrest, then flushed with Celsior® and re-resuscitated.

The ex vivo perfusion method described herein restored left ventricular ejection function and allowed assessment of ischemic tolerance in large mammalian hearts, potentially a 1st step toward including non-heart-beating human donors in the human donor pool. ***(Tex Heart Inst J 2003;30:121-7)***

Key words: *Heart function tests; isolated heart; non-heart-beating donor; normothermic arrest; organ preservation; perfusion methods; resuscitation; solutions; swine*

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The chronic shortage of donor hearts for transplantation could be greatly alleviated by using hearts from non-heart-beating donors. The problem with this approach is that hearts from non-heart-beating donors are often harvested some time after circulatory arrest and a period of normothermic ischemia. Nevertheless, the approach is feasible.[1] Even in the case of the 1st human heart transplant in 1967, the heart was subjected to normothermic arrest before being connected to the cardiopulmonary bypass machine.[2] To validate the proposed use of hearts from non-heart-beating donors, innovative methods are needed for studying the restoration of cardiac function after prolonged periods of normothermic cardiac arrest in large mammalian animals. As a 1st step in this direction, we resuscitated isolated hearts of adult pigs on a fortified ex vivo perfusion circuit and then assessed the hearts for ischemic damage.

Materials and Methods

Animals

Six domestic adult pigs (4B Livestock; Midway, Tex), each weighing 87.77 ± 10.89 kg, were used in this study. Until cardiectomy, all pigs received humane care in compliance with the *Principles of Laboratory Animal Care* prepared by the National Society for Medical Research and the *Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources and published by the National Academy Press (1996).

Cardiectomy

Preoperative and Anesthetic Procedures. Before surgery, food was withheld from each pig for the last 24 hours and water for the last 12 hours. A physical examination was done to ascertain health. A 20-gauge angiocatheter was inserted into one of the saphenous veins to administer fluids and drugs. Atropine sulfate (0.05 mg/kg), acepromazine maleate (0.05 mg/kg), and ketamine sulfate hydrochloride (15 mg/kg) were administered intramuscularly; and thiopental sodium (10–15

mg/kg) was administered through the angiocatheter to induce anesthesia. Each anesthetized pig was placed on its stomach, orally intubated, and given isoflurane (0.5%–2.5%) to maintain general anesthesia. A rectal temperature probe was inserted. The pig was then placed on its back and its chest was shaved. A 3-lead electrocardiogram and a bovine electrocautery pad were placed on the shaved area. The chest was cleaned with 4% chlorhexidine gluconate. Pancuronium bromide (0.1 mg/kg) was given intravenously to induce muscle relaxation.

Surgical Procedure. A midsternal thoracotomy was performed. A Swan-Ganz catheter was inserted in the pulmonary artery through the jugular vein to measure cardiac output and pulmonary wedge pressure (from which left atrial pressure was derived), and a Millar® catheter (Millar Instruments, Inc.; Houston, Tex) was inserted in the left ventricle retrograde through the aorta to measure left ventricular pressure. The inferior vena cava was isolated with large silk-free ties, heparin (1 mg/kg) was administered, and 6 to 8 units of autologous blood were drawn from the inferior vena cava. The root of the aorta was cross-clamped, and 1,000 mL of cold (4 °C) hyperkalemic cardioplegic solution (Central Admixture Pharmacy Services; Houston, Tex) was delivered antegrade to stop the heart. Small venting incisions were made in the left inferior pulmonary vein and right auricle. The heart was cooled externally with 4 °C normal saline solution. Once cardioplegia was achieved, the heart was flushed with 1,000 mL of 4 °C normal saline solution to remove potassium. Finally, the heart was excised, weighed, wrapped in a towel to avoid direct contact of heart tissue with ice, and placed in an insulated container filled with ice-cold saline solution. The cadaver of the pig was then discarded, and all further experiments were performed on the isolated heart.

Preparation of Isolated Hearts

Each pig heart was placed in an insulated container filled with ice-cold saline solution to reduce damage to the heart tissue due to ischemia. The aorta and the left atrium were cannulated using ¾-inch cannulas to connect the left side of the heart to an ex vivo circuit (see below). The pulmonary artery was cannulated to measure coronary sinus outflow. Epicardial electrodes (Ethicon, Inc.; Somerville, NJ) were placed on the aorta, left atrial surface, and left ventricular surface to monitor electrical activity, to allow electrographic recording, and to pace the heart (if necessary).

Connection of Isolated Hearts to the Circuit

The perfusion circuit (Fig. 1) was assembled and primed with a normothermic (37 °C) 1:1 mixture of Krebs-Henseleit buffer (4.0 ± 0.5 L) (Sigma-Aldrich,

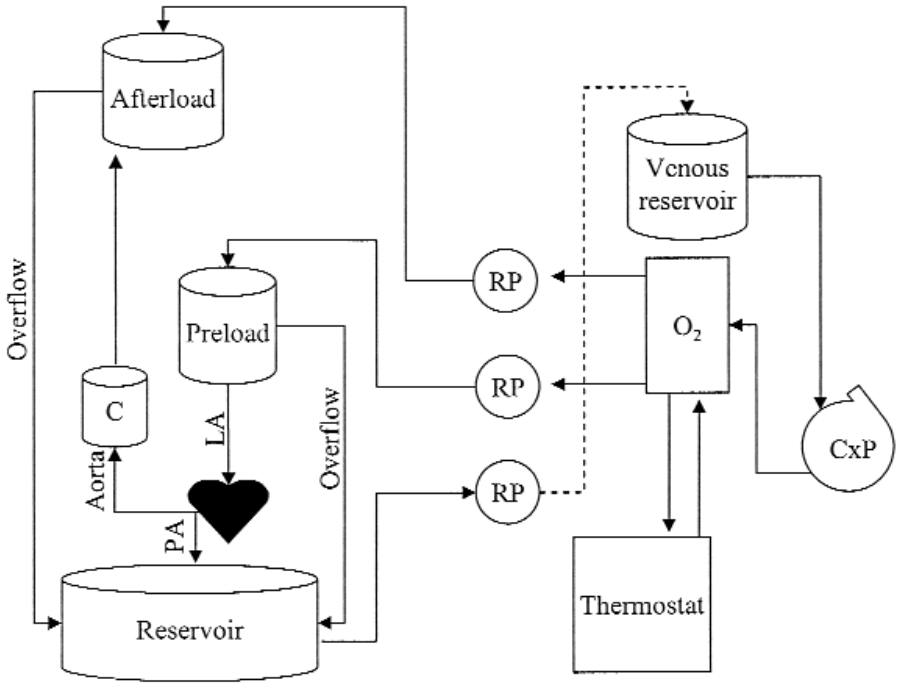


Fig. 1 *Schematic diagram of the ex vivo perfusion circuit used in the present study. The center of the circuit is the heart. The circuit consists of a preload reservoir (Preload) that feeds into the left atrium (LA), a compliance chamber (C) that is fed by the aorta, an afterload reservoir (Afterload), a collection reservoir, a venous reservoir, a centrifugal pump (CxP), an oxygenator (O_2), a thermostat, and 3 roller pumps (RP).*

PA = pulmonary artery

TABLE I. Composition of Krebs-Henseleit Buffer*

Component	Amount (g/L)
D-Glucose	2
Magnesium sulfate (anhydrous)	0.141
Potassium phosphate (monobasic)	0.16
Potassium chloride	0.35
Sodium chloride	6.9
Calcium chloride dihydrate	0.373
Sodium bicarbonate	2.1

*The pH of the perfusate was adjusted to 7.40 using 1 N bicarbonate or 1 N HCl.

Inc.; St. Louis, Mo) and heparinized autologous blood (4.0 ± 0.5 L) (final hematocrit, 15% ± 1%; pH, 7.40 ± 0.05) supplemented with insulin (0.32 units/L) (Table I). In room air, each pig heart was suspended in an elastic sleeve, and the left side of the heart was connected to the circuit as follows. The aortic cannula was connected to the circuit to induce modified Langendorff mode.[3] The heart was perfused retrograde from the aorta into the coronary arteries. Coronary perfusion pressure was kept at 70 mmHg. After approximately 5 min with the heart in modified Langendorff mode, regular myocardial contractile activity was reestablished, and the left atrial cannula was connected to the circuit to induce beating-ejecting mode. The heart was perfused anterograde from the left ventricle into the coronary arteries through the aorta. The heights of the preload and afterload reservoirs were adjusted to create a preload of 10 to 20 mmHg and an afterload of 100 to 120 mmHg.

Perfusion of Isolated Hearts on the Ex Vivo Circuit

In beating-ejecting mode, the left ventricle ejected the perfusate into the aortic cannula, where aortic pressure (AoP) was measured. The compliance chamber, placed in series between the aorta and the afterload reservoir, partially corrected cyclic fluctuations in pump pressure. Through an overflow tube in the afterload reservoir, the perfusate was collected into a reservoir and pumped by the 1st roller pump into the venous reservoir, where it then passed through a filter. A centrifugal pump routed the perfusate into the oxygenator, which contained 95% oxygen and 5% carbon dioxide. The oxygenator was connected to the heater-cooler, which kept the perfusate at 37 °C by recirculating heated water through the oxygenator. The heated and oxygenated perfusate was then pumped by the 2nd roller pump back into the preload reservoir, where its temperature was measured. From the preload reservoir, the left atrium was supplied with oxygenated perfusate. Coronary venous perfusate was returned to the circuit through the cannula leading from the pulmonary artery to the reservoir. The 3rd roller pump was used to fill the afterload reservoir initially for modified Langendorff-mode perfusion. The preload reservoir could be emptied into the reservoir through an overflow tube. Left atrial pressure (LAP) was measured with a pressure transducer connected to the left atrial cannula.

Data Collection

After a hemodynamic steady state was reached in beating-ejecting mode, data about AoP, LAP, electrocardiography, and heart rate (HR) were collected using a data acquisition system (Model ES1000 8-channel physiologic monitor/recorder (Gould Instrument Systems; Cleveland, Ohio). Coronary flow (Q_c) and aortic flow (Q_a) were measured by emptying the contents of the pulmonary cannula and the overflow tube of the afterload reservoir into a measuring cylinder for 1 minute. Cardiac output, stroke work, oxygen content, and myocardial oxygen consumption were calculated. Coronary sinus and arterial blood samples were taken from the circuit to assess the partial pressure of oxygen (pO_2), partial pressure of carbon dioxide (pCO_2), oxygen saturation (O_2 sat), and hemoglobin levels.

Cardiac output (CO) was calculated (in mL/min) as follows:

$$CO = Q_a + Q_c$$

Stroke work (SW) was calculated (in g · m/100 g heart weight) as follows:

$$SW = \frac{(AoP_{mean} - LAP_{mean}) \cdot CO}{HR \cdot (W/100)} \cdot 0.0136$$

where *0.0136* is a conversion constant for mmHg and *W* is the weight of the heart.

Oxygen content (CtO_2) was calculated (in mL O_2/dL blood) for venous and arterial blood samples as follows:

$$CtO_2 = O_2\ Sat \cdot Hemoglobin \cdot 1.34 + pO_2 \cdot 0.01275$$

where *0.01275* is calculated from the solubility coefficients of Krebs-Henseleit buffer[4] and blood.

Myocardial oxygen consumption (MVO_2) was calculated (in mL O_2/min/100 g heart weight) as follows:

$$MVO_2 = (Arterial\ CtO_2 - Venous\ CtO_2) \cdot Q_c$$

After 30 min in beating-ejecting mode, each heart was disconnected from the circuit to terminate the experiment.

Normothermic Ischemic Arrest and Re-Resuscitation of a Selected Pig Heart

To simulate the condition of the heart in a human non-heart-beating donor (such as a trauma victim suffering from heavy blood loss, cardiogenic shock, and respiratory arrest), one of the excised pig hearts was randomly selected and subjected to normothermic arrest after 30 min in beating-ejecting mode. In brief, the rate of perfusion was gradually reduced over 5 min to 10 mL/min/100 g by clamping the left atrial cannula to simulate blood loss due to trauma. Then oxygen flow was stopped to simulate respiratory arrest secondary to cardiogenic shock. After the mechanical and electrical activities of the myocardium ceased, the heart was kept in a state of normothermic ischemia for 30 min. The aorta was then cross-clamped, and 1,000 mL of cold (4 °C) Celsior® solution (SangStat Medical Corporation; Menlo Park, Calif) was administered via a luer lock on the aortic cannula to flush the coronary arterial and venous systems of the heart. The coronary eluate was discarded. Next, the heart was re-resuscitated as described above, kept in beating-ejecting mode for another 30 min, and monitored hemodynamically (for AoP, left ventricular volume, and left ventricular pressure) and electrocardiographically. Tissue was collected by needle biopsy from the left ventricle before and after the 30-min period of normothermic ischemic arrest and was evaluated histologically.

Results

In Vivo and Ex Vivo Hemodynamic Data

In vivo, the mean MVO_2 was 5.0 ± 2.8 mL/min/100 g heart weight, and the mean stroke work was 8.9 ± 3.9 g · m/100 g heart weight.

After cardiectomy and perfusion on the fortified ex vivo perfusion circuit, left ventricular function was spontaneously restored in all hearts. The mean MVO_2 was 4.8 ± 2.7 mL/min/100 g heart weight. The calculated mean stroke work in beating-ejecting mode reached 5.3 ± 1.1 g · m/100 g heart weight and was successfully maintained at that level for 30 min (Table II). On average, ex vivo MVO_2 reached 95.8% and ex vivo stroke work reached 58.95% of the original in vivo values.

Data from the Re-Resuscitated Pig Heart

In the randomly selected and re-resuscitated pig heart, stroke work recovered to 75% of its original ex vivo value. Hemodynamic and electrocardiographic data are shown in Figure 2. Histologic examination of tissue obtained by needle biopsy showed multifocal areas of contraction band changes and interstitial edema.

Discussion

In a 1st step toward the long-term goal of increasing the human donor heart pool, we have shown that it is possible to resuscitate large mammalian (pig) hearts and to restore their left ventricular ejection function using a fortified ex vivo perfusion circuit of our de-

TABLE II. Characteristics of Pig Hearts In Vivo and Ex Vivo in Beating-Ejecting Mode

	Heart Weight (g)	Heart Rate (beats/min)	CO (mL/min)	Mean AoP (mmHg)	Mean LAP (mmHg)	SW* (g · m/100 g)	MVO_2* (mL/min/100 g)
In vivo (n=2)							
Mean (± SD)	358 ± 31	99 ± 14	4,350 ± 173	49 ± 12	8.0 ± 0.1	8.9 ± 3.9	5.0 ± 2.8
Ex vivo (n=6)							
1	382	150	2,268	100	19	4.4	4.1
2	382	140	2,040	87	12	3.9	3.6
3	368	124	2,328	93	16	5.4	3.3
4	340	114	2,634	93	16	6.5	9.2
5	384	93	2,370	93	16	7	4.6
6	277	115	1,944	72	18	4.5	3.8
Mean (± SD)	355 ± 47	123 ± 13	2,264 ± 284	90 ± 11	16 ± 1.3	5.3 ± 1.1	4.8 ± 2.7

*Per 100 g heart weight

AoP = aortic pressure; CO = cardiac output; LAP = left atrial pressure; MVO_2 = myocardial oxygen consumption; SW= stroke work

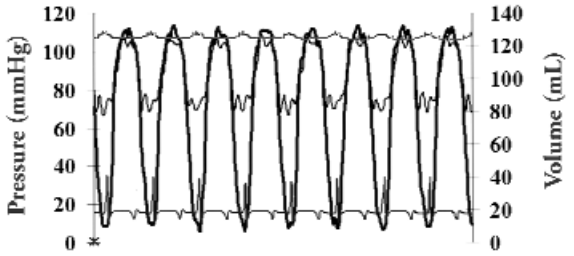


Fig. 2 *Physiologic tracings of a randomly selected and re-resuscitated pig heart in beating-ejecting mode in the ex vivo perfusion circuit. Left ventricular volume (mL) is represented by the tracing at top; electrocardiogram, by the tracing at bottom; left ventricular pressure (mmHg), by the thickest tracing; and aortic pressure (mmHg), by the remaining tracing.*

sign. In this regard, our technique is an improvement over other methods of perfusion.

Unlike earlier techniques, ours allows the gathering of data regarding left ventricular function for use in calculating stroke work and MVO_2 ex vivo. Earlier studies used autoperfusing heart–lung preparations to study postischemic cardiac recovery but did not attempt to measure ventricular function.[5] We previously reported our experience with resuscitation of the human recipient heart for prolonged periods;[6] however, that experience, like other attempts with animal hearts,[7] was limited to a Langendorff perfusion system in which an intraventricular balloon was used to measure left ventricular developed pressure and to adjust preload in the empty beating heart.[6,7] However, since such a system does not allow for afterloading, and since it is the afterload in particular that increases MVO_2,[8] MVO_2 was clearly unsuitable as a marker of cardiac work.

In contrast, the left heart in our system was filled with perfusate that was then ejected against an afterload. Since this allowed a more physiologic approach involving adjustable preloads and afterloads, the stroke work and MVO_2 values that we observed ex vivo were closer to those observed normally in vivo. In addition, our system enabled the heart itself (rather than an artificial pump) to adjust the coronary blood flow rate.

Compared with data from other ex vivo studies,[9,10] our ex vivo MVO_2 values were high. Compared with data from in vivo studies,[11] they were low. This disparity was most likely due to a combination of low left ventricular output and negligibly low work of the right heart, and perhaps even ischemia- or perfusion-induced injury of the myocardium. In addition, the coronary flow values that we observed ex vivo were considerably higher than the 5% of cardiac output seen in vivo. This may have been due to the lower oxygen-bearing capacity of the perfusate in comparison with blood and was an attempt at autoregulation in order to maintain the heart's oxygen supply.[12]

One important deviation from in vivo physiology was evident in the observed average cardiac output of the ex vivo working hearts, which was roughly half that of control hearts in vivo (2,264 mL/min ex vivo vs 4,350 mL/min in vivo) (Table II). It is important to point out, in this regard, that the circuit was intentionally designed to keep the cardiac output low by limiting left atrial inflow. We considered it an advantage to control the perfusion pressure rather than the blood flow rate, thus enabling intracardiac physiologic and neural mechanisms to autoregulate the vascular tone and to determine the instantaneous blood flow rates. In contrast, forcing a fixed volume of pressurized blood through the circuit without any consideration for myocardial oxygen demand or coronary vascular resistance would have caused either coronary hypoperfusion leading to myocardial ischemia or coronary hyperperfusion resulting in intramyocardial hemorrhage. Therefore, in designing our circuit, we chose passive filling of the coronaries (during Langendorff mode) and of the left atrium (during working-ejecting mode) by gravity rather than by pressure filling from a roller or centrifugal pump.

In addition, we used ¼-inch-diameter tubing for venous inflow (from the reservoir to the left atrium) and for arterial ejection (from the left ventricle into the compliance chamber and the aorta). Under a fixed hydraulic pressure head through a given cross-sectional area, the governing hydrodynamic equations (Bernoulli and continuity) can yield a reasonable estimate of the maximum-allowed flow rates, which, in our case, were 3.9 L/min for atrial filling in working-ejecting mode and 9 L/min for retrograde coronary perfusion in Langendorff mode. Thus, the maximum cardiac output allowed by our experimental system in the working-ejecting mode could not exceed 3.9 L/min. In addition, we used screw-type partial tubing clamps in the inflow and outflow circuitry to manipulate "pulmonary" and "peripheral" vascular resistance, respectively, further limiting the cardiac output. Although we could have used tubing of a larger diameter to carry blood from the pulmonary reservoir to the left atrium and so allow a higher flow rate (that is, higher cardiac output), this would have substantially increased the volume required to prime the circuit. (In part, it is this increased volume requirement that has previously made it impractical to perfuse large animal hearts in working-ejecting mode.)

Approximately one third of the cardiac output in our system was collected from the coronary sinus, from which we could extrapolate an average coronary perfusion rate of 240 cc/min/100 g of myocardium for the average heart weighing 355 g. Since this is almost twice the minimum rate required for normal

coronary perfusion, we could have lowered the height of the fluid column by a factor of 1.42 (square root of 2) and still have provided sufficient coronary blood flow. However, we chose not to do so, because we wanted to keep the afterload within the normal physiologic range.

There are vast physiologic differences between the beating heart ex vivo and the beating heart in situ. Under ex vivo conditions, extracardiac neurologic regulatory mechanisms and humoral (hormonal) feedback are completely unavailable, as are renal and corticorenal mechanisms, which not only control the peripheral vascular tone (that is, afterload and preload) but also regulate the electrolyte and chemical balances of the lactate-producing cardiomyocytes. We attribute the lower performance of the hearts in our ex vivo system (that is, the reduced stroke work index and the impossibility of maintaining our setup for very long periods due to progressive deterioration in cardiac function) to the absence of these and other natural regulatory pathways. In the future, we plan to add a serial dialysis unit to the circuit and improve the buffering capacity and nutrient level of our perfusate in order to prolong the duration of our experiments.

These limitations aside, ours is a promising model from which to learn more about minimizing perfusion injury in non-beating donor hearts. For instance, our totally ex vivo model might be used to 1) evaluate ischemic tolerance by comparing left ventricular function before and after ischemia, 2) expose the heart to prolonged normothermic ischemic arrest and then resuscitate it, 3) compare ex vivo data gathered before ischemic arrest with ex vivo data gathered after reperfusion, and 4) investigate the potentially protective effect of flushing solutions. The flushing solution we used (Celsior) prevents early graft loss by slowing tissue degradation, reducing cellular edema, and minimizing ischemia secondary to perfusion injury.[13]

One possible clinical use of our ex vivo perfusion circuit would be to collect preimplantation data on the ventricular function of non-beating donor hearts after prolonged normothermic ischemic arrest. This could be especially useful when donors were brought into the hospital either dead or while being resuscitated.[14] However, this application may be limited by the deterioration in myocardial function that can occur during temporary resuscitation before final in vivo resuscitation. On the other hand, if the beneficial effect of any flushing solution or additives can be demonstrated, then it might be advisable to treat the ischemic donor heart with this solution before transplanting it into the recipient.

Our perfusion circuit also allows for the incorporation of a conductance catheter to create pressure-volume loops. In future studies, such a system might be used to assess contractility by recording such loops at varying preloads and afterloads. In the present study, however, we did not systematically change the loading condition of the heart, because stroke work (which is sensitive to changes in preload or afterload) was a parameter of interest, and contractility (which is independent of preload or afterload) was not.

Future studies with our perfusion circuit could proceed in several directions. Some studies might be aimed at improving the associated perfusion technique, while others might attempt to evaluate the effect of additives to the perfusion and flushing solutions in decreasing ischemia- or reperfusion-induced injury after periods of normothermic ischemia. Candidate additives are metabolic enhancers or oxygen-free radical scavengers. Dichloroacetate, a metabolic agent that reduces lactic acidemia through activation of pyruvate dehydrogenase, has been shown to enhance myocardial metabolism and functional recovery after global ischemia.[15] Scavengers such as allopurinol, deferoxamine, and coenzyme Q_{10} are postulated to delay the onset and diminish the progression of ischemia- or reperfusion-induced injury to the myocardium. Allopurinol, a xanthine oxidase inhibitor used clinically to lower blood uric acid levels, has been shown to improve functional recovery of the stunned myocardium.[16] During global ischemia, iron levels can increase, which catalyzes the decomposition of hydrogen peroxide into hydroxyl radicals.[17] Deferoxamine chelates iron, thereby reducing the production of hydroxyl radicals.[18] Coenzyme Q_{10} has been shown to have an antioxidant effect and to preserve endothelium-dependent vasorelaxation.[19] Therefore, the circuit and the resuscitation method presented here could be used to test the additive effect of any or all of the above substances in improving left ventricular ejection function after prolonged normothermic ischemic arrest. Other studies could investigate the use of the circuit for resuscitating human cadaver hearts and for evaluating the effectiveness of different perfusion patterns.

In conclusion, we describe an ex vivo perfusion technique that can restore left ventricular ejection function in resuscitated large mammalian (pig) hearts and allow assessment of ischemic tolerance. The technique will likely be useful for investigating, ex vivo, the functional viability of hearts that have suffered extended in vivo periods of hypoxia, ischemia, or both. This study constitutes a 1st step toward the long-term goal of expanding the human donor heart pool to include hearts from non-heart-beating donors.

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