

Eight hours of cold static storage with adenosine and lidocaine (Adenocaine) heart preservation solutions: Toward therapeutic suspended animation

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Objective: Most cardiac preservation solutions provide safe cold ischemic storage times for 4 to 5 hours. Our aim was to investigate the effects of 8 hours of cold static storage (4°C) using 2 normokalemic, polarizing adenosine-lidocaine (Adenocaine, Hibernation Therapeutics, Macon, Ga) solutions and to compare their functional recovery with hearts preserved in gold standard histidine-tryptophan-ketoglutarate and Celsior solutions.

Methods: Male Sprague–Dawley rats (350–450 g) were randomly assigned to 1 of 4 groups (n = 8): (1) adenosine-lidocaine cardioplegia with low Ca²⁺/high Mg²⁺; (2) 2× adenosine-lidocaine cardioplegia, low Ca²⁺/high Mg²⁺, melatonin, and insulin (2× adenosine, lidocaine, melatonin, and insulin); (3) histidine-tryptophan-ketoglutarate solution; or (4) Celsior. Hearts were perfused in working mode, arrested (37°C), removed, stored for 8 hours at 4°C, reattached in Langendorff mode and rewarmed for 5 minutes (37°C), and switched to working mode for 60 minutes. Myocardial oxygen consumption, effluent lactates, and troponin T levels were measured.

Results: Hearts preserved for 8 hours in adenosine-lidocaine and 2× adenosine, lidocaine, melatonin, and insulin returned 50% and 76% of aortic flow and 70% and 86% of coronary flow, respectively, at 60 minutes of reperfusion. In contrast, histidine-tryptophan-ketoglutarate and Celsior hearts returned 2% and 17% of aortic flow and 11% and 48% of coronary flow, respectively, at 60 minutes of reperfusion. Hearts preserved in adenosine-lidocaine and 2× adenosine, lidocaine, melatonin, and insulin returned 90% and 100% of developed pressures and 101% and 104% of heart rate, respectively. Hearts preserved in histidine-tryptophan-ketoglutarate failed to increase systolic pressure greater than 14 mm Hg (11% baseline) and diastolic pressure greater than 10 mm Hg (17% baseline), and recovered only 16% of heart rate. Hearts preserved in Celsior developed 70% of baseline systolic pressures and 86% recovery of heart rate. At 5 minutes of rewarming after cold storage, the myocardial oxygen consumption for hearts preserved in adenosine-lidocaine, 2× adenosine, lidocaine, melatonin, and insulin, histidine-tryptophan-ketoglutarate, and Celsior was 23.0 ± 5, 20 ± 4, 15 ± 1, and 10 ± 2 μmol O₂/min/g dry wt, respectively, with corresponding lactate outputs of 1.8 ± 0.8, 1.5 ± 0.7, 2.6 ± 0.7, and 3.2 ± 1.4 μmol lactate/min/g dry weight. Troponin T was not detected in the coronary effluent of adenosine-lidocaine or 2× adenosine, lidocaine, melatonin, and insulin hearts, whereas histidine-tryptophan-ketoglutarate and Celsior hearts had troponin T levels of 0.08 and 0.24 μg/mL, respectively.

Conclusion: We report a 78% return of cardiac output, 90% to 100% return of developed pressures, and 101% to 104% return of heart rate after 8 hours of cold static storage using normokalemic, adenosine, lidocaine, melatonin, and insulin preservation solution in the isolated rat heart compared with 55% cardiac output with polarizing adenosine-lidocaine cardioplegia alone, 4% cardiac output with histidine-tryptophan-ketoglutarate, and 25% cardiac output in Celsior preservation solutions. (*J Thorac Cardiovasc Surg* 2011; ■:1-10)

From a scientific standpoint, depolarizing potassium concentrations of 10 mEq/L and above in surgical cardioplegia or

heart preservation solutions may not afford optimal arrest and protection.¹ In 2004, we introduced a new concept of polarized arrest for surgical cardioplegia using a composition of adenosine and lidocaine (Adenocaine, Hibernation Therapeutics, Macon, Ga) in a physiologic Krebs–Henseleit solution.^{1,2} We reported that Adenocaine in a normokalemic solution arrested the heart by “clamping” the myocyte’s diastolic membrane potential at or approximately –80 mV with an accompanying decrease in myocardial oxygen consumption (MVO₂) of more than 95%.² The idea was borrowed from natural hibernating animals (or summer estivators) who do not flood their cells with high potassium and depolarize their cell membranes as they decrease their body’s

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Abbreviations and Acronyms

AF	= aortic flow
AL	= adenosine-lidocaine
ALMI	= adenosine, lidocaine, melatonin, and insulin
ANOVA	= analysis of variance
CF	= coronary flow
CO	= cardiac output
HTK	= histidine-tryptophan-ketoglutarate
MVO ₂	= myocardial oxygen consumption
RPP	= rate-pressure product

“basal” metabolic rate to pilot-light.³ The question posed was “Could the human heart in cardiac surgery be pharmacologically manipulated to operate more like a heart from a natural hibernator?” The early objective was to inhibit the voltage-dependent Na⁺ fast channels responsible for the phase O upstroke (lidocaine) and *simultaneously* decrease the action potential duration (open K⁺ channels) of atria, Purkinje fibers, and ventricles (adenosine), which would theoretically arrest the heart in a more “natural” polarized, diastolic state compared with hyperkalemic depolarized arrest.

The adenosine-lidocaine (AL) polarizing arrest and protection concept has subsequently received proof-of-concept in the canine model of cardiopulmonary bypass⁴ and is used clinically in a number of US centers as Adenocaine all-blood microplegia.⁵ In 2008, Jin and colleagues⁶ carried out a 134-patient pediatric safety trial and showed that AL crystalloid “one shot” with moderate hyperkalemia (10 mmol/L) was more protective than AL with 20 mmol/L K⁺ or 20 mmol/L K⁺ alone. In 2010, we confirmed the importance of keeping potassium within its normokalemic limits for optimal AL polarized protection at 32°C to 33°C in isolated rat hearts and showed that higher (depolarizing) or lower (hyperpolarizing) extracellular potassium arrest resulted in significantly higher coronary vascular resistances, slower times to first beat (stunning), and lower cardiac outputs (COs) with lower contractility.^{1,7}

AL cardioplegia also appears versatile as a preservation solution at both cold static storage (4°C) and warmer intermittent perfusion (28°C–30°C) compared with Celsior.⁸ In 2011, we reported that reperfusing the isolated rat heart for 5 minutes with warm, oxygenated polarizing AL arrest *after* 6 hours of cold storage in AL cardioplegia or Celsior led to significantly higher recoveries and may offer a new paradigm of polarizing protection for rewarming and implantation.⁹ The aim of the present study is to investigate the effects of 8 hours of cold static storage (4°C) using 2 modified AL (Adenocaine) preservation solutions and compare them with Custodiol histidine-tryptophan-ketoglutarate (HTK) and Celsior solutions.

MATERIALS AND METHODS**Animals**

Male Sprague–Dawley rats (350–450 g, n = 40) were obtained from James Cook University’s breeding colony. Animals were fed ad libitum and housed in a 12-hour light/dark cycle. On the day of experiment, rats were anesthetized with an intraperitoneal injection of thiopentone sodium (Thiobarb; 60 mg/kg body wt), and the hearts were rapidly excised as described by Dobson and Jones.² Rats were handled in compliance with James Cook University Guidelines (ethics approval number A1084) and the “Guide for Care and use of Laboratory Animals” from the National Institutes of Health (Publication No. 85-23, revised 1985, and PHS Publication 1996). Adenosine (A9251 > 99% purity), histidine, histidine-HCl, tryptophan, alpha-ketoglutarate, mannitol, and melatonin (N-acetyl-5-methoxytryptamine) were obtained from Sigma Chemical Company (Castle Hill, NSW, Australia). Lidocaine hydrochloride (2% solution, ilium) and insulin (40 IU/mL) were obtained from Lyppard (Queensland, Australia). Celsior was purchased as a Food and Drug Administration-approved product from Clifford Hallam Healthcare (Agent for Genzyme, North Ryde, NSW, Australia).

Arrest Solutions for Normothermic Induction and Cold Static Storage**Adenosine-lidocaine solution with low Ca²⁺/high Mg²⁺.**

The AL cardioplegia contained 200 μmol/L adenosine plus 500 μmol/L lidocaine in 10 mmol/L glucose containing modified Krebs–Henseleit buffer (pH 7.7 at 37°C) with low Ca²⁺/high Mg²⁺ (0.22 mmol/L CaCl₂ and 2.6 mmol/L MgCl₂). The solution was filtered using 0.2-μm filters and maintained at 37°C. These AL concentrations have been used in previous cardioplegia and preservation studies.^{8,9} The arrest solution was not actively bubbled with 95% O₂/5% CO₂, thus the higher pH. The average Po₂ of the AL solution at the beginning of storage was 140 mm Hg and the Pco₂ was 5 to 10 mm Hg.

2× adenosine-lidocaine solution with low Ca²⁺/high Mg²⁺, melatonin, and insulin.

The composition was the same as the AL solution (above) with the following additions: twice the concentration of A and L (400 μmol/L and 1000 μmol/L), 100 μmol/L melatonin, and 0.01 IU/mL insulin. The reason for doubling AL was from previous 6-hour cold static storage studies that showed a moderate but significant 1.3-fold increase in left ventricular function (aortic flow [AF]) and improved electrical stability (n = 6, unpublished data). Higher AL concentrations may also improve protection because lidocaine is known to have a reduced ability to block sodium fast channels at lower temperatures¹⁰ (and perhaps the same exists for adenosine and adenosine receptors). The naturally occurring pineal gland hormone melatonin was chosen because it is a free radical scavenger (5 times more effective in neutralizing OH[•] radicals than glutathione) and is a powerful antioxidant with cardioprotective properties.¹¹ Insulin was chosen because of its antioxidant and cardioprotective properties (see “Discussion”).¹² Preliminary experiments showed that melatonin and insulin added to AL arrest solution, singly or in combination, improved functional recoveries after 6 hours of cold static storage. The concentrations of each drug were chosen from their cardioprotective properties in the isolated perfused heart.^{11,12}

Celsior solution. The Celsior solution contained 100 mmol/L NaOH, 15 mmol/L KCl, 13 mmol/L MgCl₂, 0.25 mmol/L CaCl₂, 20 mmol/L glutamic acid, 80 mmol/L lactobionic acid, 30 mmol/L histidine, and 3 mmol/L glutathione. The solution was used as supplied from Genzyme with no modification. The solution was filtered with a 0.2-μm filter, was not actively bubbled with 95% O₂/5% CO₂, and had an average Po₂ of 149 mm/Hg.

Histidine-tryptophan-ketoglutarate solution. The HTK solution contained 15 mmol/L NaCl, 9 mmol/L KCl, 4.0 mmol/L MgCl₂, 0.015 mmol/L CaCl₂, 1.0 mmol/L alpha-ketoglutarate, 180 mmol/L

histidine, 18 mmol/L histidine-HCl, 30 mmol/L mannitol, and 2 mmol/L tryptophan.^{13,14}

Arrest Solutions for 5 Minutes of Warm Reperfusion After Cold Static Storage

The AL and ALMI, Celsior, and HTK arrest solutions were the same as described above except they were actively bubbled with 95% O₂/5% CO₂ to achieve a Po₂ greater than 600 mm Hg, and the solutions were not recirculated.

Composition of Modified Krebs–Henseleit for Reperfusion in Working Mode

The modified Krebs–Henseleit buffer contained 10 mmol/L glucose, 117 mmol/L NaCl, 5.9 mmol/L KCl, 25 mmol/L NaHCO₃, 1.2 mmol/L NaH₂PO₄, 1.12 mmol/L CaCl₂ (free Ca²⁺ = 1.07 mmol/L), and 0.512 mmol/L MgCl₂ (free Mg²⁺ = 0.5 mmol/L), pH 7.4 at 37°C. The perfusion buffer was filtered using a 1-μmol/L membrane and then bubbled vigorously with 95% O₂/5% CO₂ to achieve a Po₂ greater than 600 mm Hg. The perfusion buffer was not recirculated.

Experimental Groups

Rats were randomly assigned to 1 of 4 groups (n = 8 each group): (1) AL cardioplegia; (2) 2 × AL cardioplegia with low Ca²⁺/high Mg²⁺, melatonin, and insulin; (3) Celsior; or (4) HTK solution.

Langendorff and working rat heart preparation. Hearts were rapidly removed from anesthetized rats and placed in ice-cold, heparinized, modified Krebs–Henseleit buffer (Figure 1). The details of heart preparation, surgical attachment, and perfusion have been described by Rudd and Dobson.^{8,9} Briefly, hearts were attached to a Langendorff apparatus and perfused at a pressure head of 90 cm H₂O (68 mm Hg). The pulmonary artery was not cannulated before arrest, and the heart was detached for 8 hours of cold static storage to prevent vessel wall damage. The heart was cannulated after cold storage for collection of coronary venous effluent and O₂ consumption measurements. For working mode operation, a small incision was made in the left atrial

appendage and a cannula was inserted and sutured, which was switched from the aorta to the left atrial cannula at a hydrostatic pressure of 10 cm H₂O (pre-load) and an afterload pressure of 100 cm H₂O (76 mm Hg). Heart rate, aortic pressure, coronary flow, and AF were measured before and after 8 hours of cold static storage (Figure 1). CO was the sum of aortic and coronary flows. During the 15-minute equilibration period, the initial criteria for exclusion of working hearts were a heart rate less than 200 beats/min, systolic pressure less than 100 mm Hg, and coronary flow less than 10 mL/min.

Cold Static Storage (4°C) and Early Reperfusion

The method of cold static storage for rat hearts has been described by Rudd and Dobson.^{8,9} Briefly, hearts were arrested at a constant pressure head of 90 cm H₂O (68 mm Hg) using AL cardioplegia or Celsior solution for 5 minutes (50–100 mL) at 37°C for their respective groups. Hearts were gently removed from the apparatus and placed in a 50-mL tube containing their respective air-equilibrated preservation solutions, and the sealed tube was immersed in the water bath at 4°C for 8 hours (Figure 1). After 8 hours of storage, the hearts were immediately reattached to the perfusion apparatus, and fresh, warm oxygenated arrest solutions (AL, 2 × ALMI, HTK, or Celsior) were used to reperfuse the heart in non-working Langendorff mode at a pressure head of 90 cm H₂O (68 mm Hg) for 5 minutes (Figure 1). The heart's surface temperature was 28°C to 32°C.⁹ Temperature was measured using a Cole-Palmer thermistor-thermometer (8402-20), which was tucked under the left auricle. Hearts were then switched to working mode and reperused for 60 minutes at 37°C using oxygenated glucose containing Krebs–Henseleit pH 7.4. Hearts were allowed to spontaneously return to function during reperfusion, and they were not electrically assisted if function did not return. Functional data (aortic and coronary flow, heart rate, and systolic and diastolic pressures) were measured at 5, 10, 15, 30, 45, and 60 minutes during the 60-minute reperfusion. These data were compared with the baseline (pre-arrest) data for each group. Myocardial oxygen consumption (μmole O₂/min/g dry wt heart) was calculated using equations and methods described by Dobson and Jones² and Rudd and Dobson.^{8,9} Coronary venous effluent was measured in milliliters/minute, and heart weight was expressed as

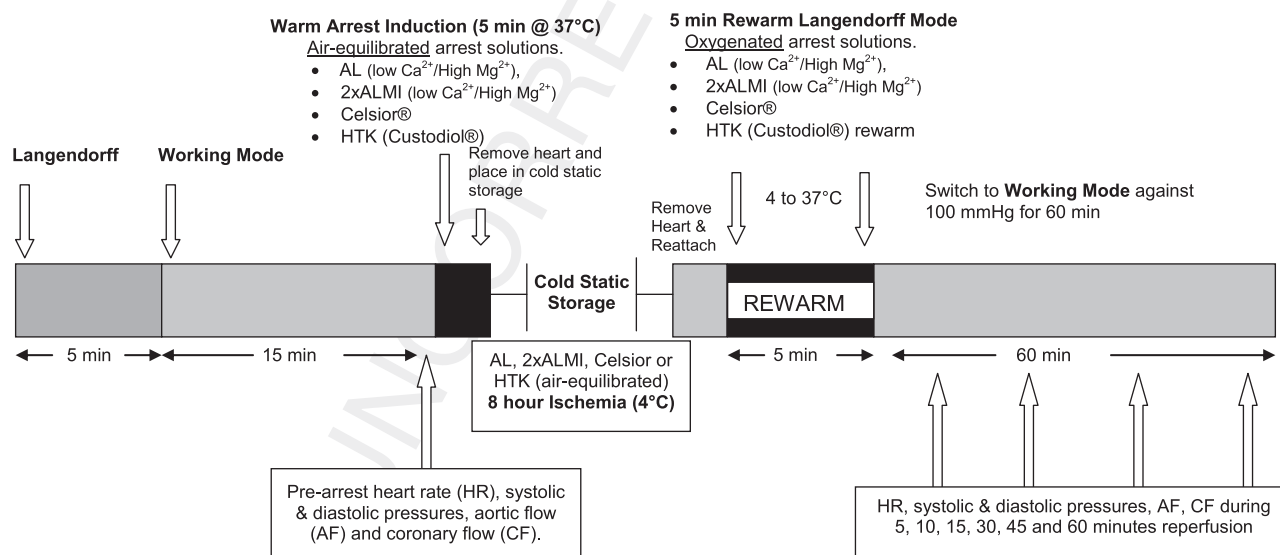


FIGURE 1. Experimental protocol. Isolated rat hearts were placed in cold static storage for 8 hours in (1) AL polarizing cardioplegia with low Ca²⁺/high Mg²⁺ (0.22 mmol/L/2.6 mmol/L); (2) AL doubled in concentration with melatonin (100 μmol/L) and insulin (0.01 IU/mL) (2 × ALMI); (3) HTK (Custodiol); and (4) Celsior depolarizing preservation solutions. Each group was rewarmed in Langendorff mode for 5 minutes with their respective oxygenated arrest solution (see “Materials and Methods” for details). AL, Adenosine-lidocaine; ALMI, adenosine, lidocaine, melatonin, and insulin; HTK, histidine-tryptophan-ketoglutarate.

grams of dry weight. Total tissue water (%) was determined by the difference in wet weight and dry weight divided by wet weight and multiplied by 100.

Lactate Output and Troponin T Release at 5 Minutes Warm Reperfusion

Coronary effluent (1.0–1.5 mL) was collected in small 1.5-m Eppendorf tubes during the last minute of the rewarming period and stored at -20°C until analysis (~ 2 weeks). Perfusate lactates were analyzed on Cobas Integra 800 using a colorimetric kit purchased from Roche Diagnostics, Australia. Output results were in millimoles/liter and converted to micromoles/minute/gram dry weight using coronary flow (CF) and heart weight as in equation (1):

$$\text{Lactate } (\mu\text{mol}/\text{min}/\text{g dry wt}) = \text{Lactate } (\mu\text{mol}/\text{mL}) \times \text{CF}(\text{mL}/\text{min}) \times 1/\text{heart weight } (\text{g dry wt})^{-1} \quad (1)$$

Troponin T was measured on fresh effluent using the enzyme immunoassay method of the Roche Cardiac T Quantitative test on the Roche Cardiac Reader System (Roche Diagnostics). The method has a lower limit of detection of approximately $0.03 \mu\text{g}/\text{L}$. Broad clinical prognostic values for troponin T reference range are as follows: no myocardial damage, $0.0\text{--}0.05 \mu\text{g}/\text{L}$; myocardial damage possible, $0.05\text{--}0.1 \mu\text{g}/\text{L}$; myocardial damage detected, greater than $0.1 \mu\text{g}/\text{L}$.¹⁵

Statistical Analysis

All results are expressed as mean \pm standard error of the mean. One-way analysis of variance (ANOVA) was used to compare rat weights, arrest times, and time to first beat and AF. Two-way ANOVA with repeated

measures was used to compare recovery variables (eg, AF, systolic and diastolic pressures, heart rate, CO) over multiple time points for the different treatment groups. One-way ANOVA was used to compare recovery of variables at specific times points (5, 10, 30, 45, and 60 minutes) during reperfusion in working mode. Significance was then assessed using Bonferroni and Dunnett 2-way post hoc tests.

RESULTS

Heart functional properties before and after cold static storage in the 4 different preservation solutions are shown in Table 1 and Figures 2 and 3. During pre-arrest, there were no significant differences among the groups in AF, CF, CO, heart rate, developed pressures, or rate-pressure product (RPP) (Table 1).

Recovery of Aortic Flow, Coronary Flow, and Cardiac Output

Baseline values of AF ranged from 54 to 63 mL/min⁻¹. Five minutes after switching to working mode, the 2 \times ALMI heart group was the only one that generated AF (2 mL/min) (Table 1, Figure 2, A). At 10 minutes, AL and ALMI hearts had spontaneously generated approximately 20% of their respective baseline AF, whereas HTK hearts had zero flow (0%) and Celsior hearts had 1 mL/min ($\sim 2\%$ of pre-arrest). At 30 minutes, AL and ALMI hearts generated 47% and 71% of their pre-arrest AF and

TABLE 1. Functional parameters of isolated working rat hearts after 8-hour cold static ischemic storage (4°C) and 5-minute oxygenated rewarming in polarizing adenosine-lidocaine and 2 \times adenosine, lidocaine, melatonin, and insulin solutions or depolarizing Custodiol-HTK and Celsior solutions

Time	Cold arrest + rewarm treatment	n	Aortic flow (mL/min)		Coronary flow (mL/min)		Cardiac output (mL/min)		Heart rate (beats/min)		Systolic pressure (mm Hg)	Diastolic pressure (mm Hg)	Rate-pressure product (beats/mm Hg/min)
			Value (% PA)	Value (% PA)	Value (% PA)	Value (% PA)	Value (% PA)	Value (% PA)					
15 min pre-arrest ⁸	AL	8	60 \pm 2		20 \pm 1		80 \pm 2		280 \pm 7		124 \pm 2	60 \pm 0	34,561 \pm 584
	2 \times ALMI	8	62 \pm 1		22 \pm 1		85 \pm 2		303 \pm 7		128 \pm 1	60 \pm 0	38,836 \pm 957
	HTK	8	54 \pm 2		19 \pm 1		73 \pm 2		275 \pm 8		125 \pm 1	60 \pm 0	34,404 \pm 943
	Celsior	8	63 \pm 4		21 \pm 2		85 \pm 5		286 \pm 12		126 \pm 2	60 \pm 0	36,041 \pm 1268
5-min recovery	AL	8	0 \pm 0	0	1 \pm 1	1	1 \pm 1	1	4 \pm 4	1	3 \pm 3	1 \pm 1	80 \pm 80
	2 \times ALMI	8	3 \pm 3	1	2 \pm 2	9	5 \pm 5	6	25 \pm 25	8	16 \pm 16	8 \pm 8	3250 \pm 3250
	HTK	8	0 \pm 0	0	1 \pm 1	5	1 \pm 1	1	22 \pm 7	8	14 \pm 5	9 \pm 3	446 \pm 201
	Celsior	8	0 \pm 0	0	3 \pm 2	14	3 \pm 2	4	48 \pm 13	17	31 \pm 9	19 \pm 3	1998 \pm 935
10-min recovery	AL	8	11 \pm 6	18	11 \pm 3	55	22 \pm 9	28	139 \pm 44	50	71 \pm 17	40 \pm 10	14,673 \pm 5320
	2 \times ALMI	8	14 \pm 7	22	8 \pm 4	36	22 \pm 11	26	118 \pm 50	39	51 \pm 20	25 \pm 10	13,078 \pm 6316
	HTK	8	0 \pm 0	0	2 \pm 1	11	2 \pm 1	3	39 \pm 21	14	15 \pm 9	10 \pm 5	1795 \pm 1377
	Celsior	8	1 \pm 1	2	8 \pm 2	38	9 \pm 3	11	176 \pm 39	62	66 \pm 10	48 \pm 8	13,944 \pm 3680
30-min recovery	AL	8	28 \pm 5 [†]	47	15 \pm 2 [†]	75	43 \pm 1	54	293 \pm 22	105	110 \pm 3	63 \pm 2	32,155 \pm 2515
	2 \times ALMI	8	44 \pm 2 [*]	71	18 \pm 1 ^{‡§}	82	62 \pm 3 ^{‡§}	73	294 \pm 16 [§]	97	121 \pm 3 ^{‡§}	60 \pm 0 [§]	35,294 \pm 1496 ^{‡§}
	HTK	8	0 \pm 0	0	2 \pm 1	11	2 \pm 1	3	41 \pm 27	15	13 \pm 11	10 \pm 9	2411 \pm 2221
	Celsior	8	8 \pm 4	13	10 \pm 2	48	18 \pm 6	21	213 \pm 32	75	88 \pm 8	59 \pm 5	19,998 \pm 3834
60-min recovery	AL	8	30 \pm 5 [†]	50	14 \pm 2 [†]	70	44 \pm 6 [†]	55	283 \pm 19	101	111 \pm 3	63 \pm 2	31,518 \pm 2346
	2 \times ALMI	8	47 \pm 3 [*]	76	19 \pm 1 ^{‡§}	86	66 \pm 3 ^{‡§}	78	314 \pm 12 [§]	104	117 \pm 3 [§]	60 \pm 0 [§]	36,800 \pm 1021 ^{‡§}
	HTK	8	1 \pm 1	2	2 \pm 1	11	3 \pm 2	4	44 \pm 30	16	14 \pm 12	10 \pm 9	3396 \pm 2781
	Celsior	8	11 \pm 5	17	10 \pm 2	48	21 \pm 7	25	247 \pm 28	86	89 \pm 6	62 \pm 4	21,473 \pm 3642

See "Materials and Methods" for details. No significant differences between pre-arrest groups. *ALMI significantly different from all other groups, $P < .05$. †AL significantly different from HTK or Celsior or both, $P < .05$. ‡ALMI significantly different from Celsior only, $P < .05$. §ALMI significantly different from HTK only, $P < .05$. ||Celsior significantly different from HTK, $P < .05$.

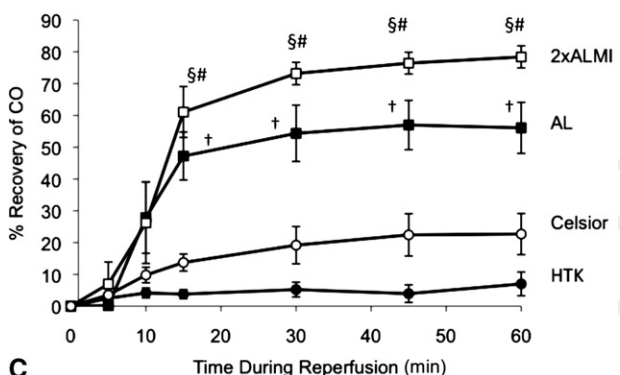
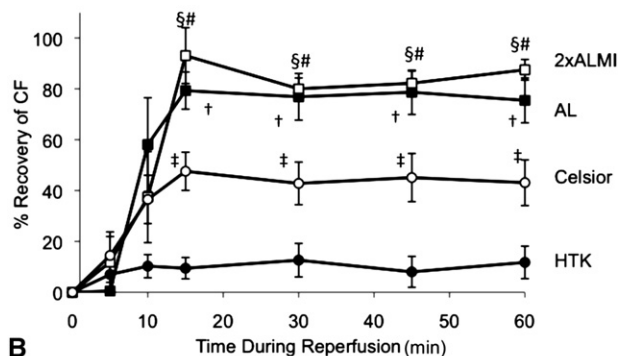
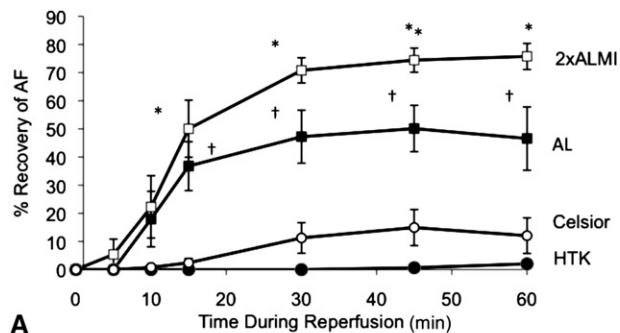


FIGURE 2. A, Percentage recovery of AF (%) during 60-minute reperfusion in working mode immediately after 5 minutes of oxygenated reperfusion in Langendorff mode. The 4 groups were (1) AL low Ca^{2+} /high Mg^{2+} (■), (2) 2× ALMI (□), (3) HTK (●), and (4) Celsior (○). Significance ($P < .05$) was as follows: *2× ALMI different from all groups. †AL different from Celsior and HTK. B, Percentage recovery of coronary flow (%) during 60-minute reperfusion (see A). §ALMI significantly different from Celsior. #ALMI significantly different from HTK only. †AL significantly different from HTK or Celsior. ‡Celsior significantly different from HTK. C, Percentage recovery of CO (%) during 60-minute reperfusion (see A). §ALMI significantly different from Celsior. #ALMI significantly different from HTK only. †AL significantly different from HTK or Celsior. CO, Cardiac output; CF, coronary flow; AF, aortic flow; ALMI, adenosine, lidocaine, melatonin, and insulin; AL, adenosine-lidocaine; HTK, histidine-tryptophan-ketoglutarate.

increased to 50% and 76% at 60 minutes reperfusion, respectively. In contrast, hearts preserved in HTK generated 0% and 2% AF after 30 and 60 minutes, respectively,

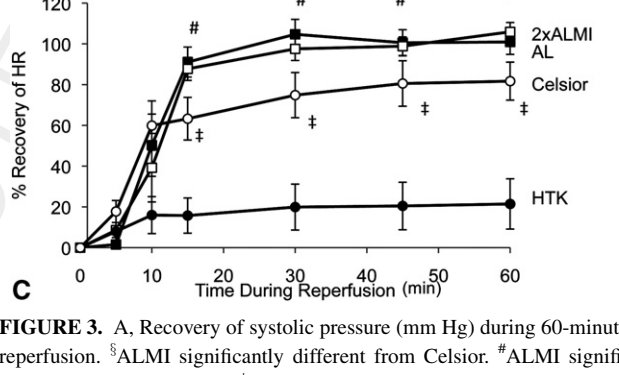
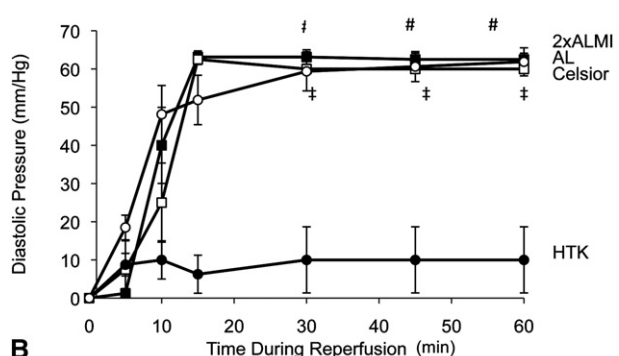
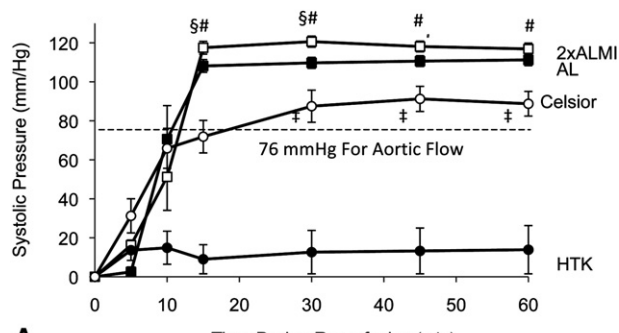


FIGURE 3. A, Recovery of systolic pressure (mm Hg) during 60-minute reperfusion. §ALMI significantly different from Celsior. #ALMI significantly different from HTK. †Celsior significantly different from HTK. B, Recovery of diastolic pressure (mm Hg) during 60-minute reperfusion. #Celsior significantly different from HTK. †Celsior significantly different from HTK. C, Percentage recovery of heart rate (%) during 60-minute reperfusion in working mode immediately after 5 minutes of oxygenated reperfusion in Langendorff mode. The 4 groups were (1) AL low Ca^{2+} /high Mg^{2+} (■), (2) 2× ALMI (□), (3) HTK (●), and (4) Celsior (○). Significance ($P < .05$) was as follows: #ALMI significantly different from HTK. †Celsior significantly different from HTK. ALMI, Adenosine, lidocaine, melatonin, and insulin; AL, adenosine-lidocaine; HTK, histidine-tryptophan-ketoglutarate; HR, heart rate.

and Celsior hearts generated 13% and 17% pre-arrest AF after 30 and 60 minutes, respectively (Table 1, Figure 2, A).

The spontaneous return of CF at 5 minutes for AL, 2× ALMI hearts, HTK, and Celsior was 1%, 9%, 5%, and 14% of baseline values, respectively, and at 10 minutes CF increased to 55%, 36%, 11%, and 38%, respectively (Table 1, Figure 2, B). At 30 minutes reperfusion, the CF

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increased to 75% and 48% for the AL and Celsior hearts, respectively, whereas the 2× ALMI hearts increased 2.3-fold (82% pre-arrest). At 60 minutes, the return of CF for AL, 2× ALMI, and Celsior hearts was 70%, 86%, and 48%, respectively. HTK hearts did not increase CF from 10 to 60 minutes and remained at 11% of pre-arrest value (2 mL/min⁻¹) (Table 1, Figure 2, B).

Recovery of CO at 5 minutes for all groups in working mode ranged from 1% to 6% (Table 1, Figure 2, C). At 10 minutes, the CO for AL, 2× ALMI hearts, HTK, and Celsior was 28%, 26%, 3%, and 11%, respectively. At 30 minutes, the AL and 2× ALMI hearts doubled and tripled their outputs, respectively, the Celsior hearts doubled their output, and the HTK hearts failed to increase CO above 3% of pre-arrest value (Table 1, Figure 2, C). At 60 minutes, the AL hearts stabilized CO at 55% pre-arrest output, 2× ALMI hearts increased to 78%, and Celsior increased to 25% pre-arrest output. HTK failed to increase CO over the 60-minute period (Table 1, Figure 2, C). Stroke volume was calculated by dividing CO by heart rate and ranged from 0.27 to 0.30 mL beat⁻¹ for the 4 groups at baseline (Table 1). At 60 minutes reperfusion, the AL and 2× ALMI hearts had recovered 55% and 75%, respectively. In direct contrast, the HTK and Celsior hearts recovered 5% and 23% of baseline, respectively.

Recovery of Systolic and Diastolic Developed Pressures

The systolic and diastolic pressures are shown in Table 1 and Figure 3, A and B. Baseline values ranged from 124/60 to 128/60 mm Hg and were not significantly different. At 5 minutes, the AL and 2× ALMI hearts generated 2% and 13% of baseline pressures, respectively, which rapidly increased to 57% to 67% systolic and 40% to 42% diastolic at 10 minutes, respectively (Table 1, Figure 3, A, B). At 30 and 60 minutes of reperfusion, the AL and 2× ALMI hearts had recovered 89% to 94% of systolic pressures and 105% to 100% of diastolic pressures, respectively. Celsior hearts showed a faster recovery at 5 minutes (25%–32% baseline) and at 10 minutes had recovered 52% systolic and 80% diastolic pressures. At 30 and 60 minutes, Celsior hearts increased systolic pressure to 70% baseline, but this pressure was not high enough to generate sufficient AF, and the diastolic pressure was 98% to 100%. HTK failed to increase systolic pressure greater than 14 mm Hg (11% baseline), and diastolic pressure was not greater than 10 mm Hg (17% baseline) over the 60-minute reperfusion period.

Recovery of Heart Rate and Rate-Pressure Product

Spontaneous return of heart rate is shown in Table 1 and Figure 3, C. Baseline rates ranged from 275 to 303 beats/min⁻¹. At 5 minutes, the AL, 2× ALMI, and HTK hearts had a rate of 4 to 25 beats/min⁻¹; however, the Celsior hearts

had a rate of 48 beats/min⁻¹. At 10 minutes, the AL and 2× ALMI hearts increased their rates to 50% and 39% baseline, respectively, and Celsior hearts were beating at 62% baseline. The HTK hearts increased their heart rate from 22 to 39 beats/min⁻¹ to 44 beats/min⁻¹ (14%–16% baseline) at 10 minutes and did not change during the 60-minute reperfusion. At 30 minutes, AL and 2× ALMI hearts returned 105% and 97% of their baseline heart rates, respectively, and Celsior hearts had 75% of the baseline rate. At 60 minutes, full recovery of heart rate was found in the AL and 2× ALMI hearts, and 86% recovery was found in the Celsior hearts.

Baseline RPP ranged from 34,404 to 38,836 beats/mm Hg/min⁻¹ for the 4 preservation groups (Table 1). At 5 minutes, return of RPP was 0.2% to 8% baseline in all groups. At 10 minutes, the AL and 2× ALMI hearts generated 42% and 34% of baseline RPP, respectively, and the Celsior hearts generated 39% (Table 1). The HTK hearts generated 5% to 10% baseline RPP during the entire 60-minute reperfusion period. At 30 minutes, the AL and 2× ALMI hearts nearly had full recovery of RPP (91%–93% return) and 2× ALMI hearts had 95% at 60 minutes. The Celsior hearts returned 55% and 59% of their RPP at 30 and 60 minutes, respectively.

Maintenance Myocardial Oxygen Consumption in the Last Minute of the 5-Minute Rewarming Phase Before Switching to Working Mode

At the end of the rewarming phase, the MVO₂ for AL, 2× ALMI, HTK, and Celsior hearts in Langendorff mode perfused with their oxygenated arrest solutions was 23.0 ± 5, 20 ± 4, 15 ± 1, and 10 ± 2 μmol O₂/min/g dry wt, respectively (Figure 4, A). AL and 2× ALMI hearts were significantly higher than Celsior. The coronary effluent flows in the final minute were 13.7 ± 0.9 and 14.2 ± 2.3 mL for AL and 2× ALMI hearts, respectively, and 11.1 ± 0.9 and 10.8 ± 1.4 mL for HTK and Celsior hearts, respectively.

Lactate and Troponin T Output in the Last Minute of 5-Minute Rewarming

The lactate outputs for AL, 2× ALMI, HTK, and Celsior hearts perfused with their oxygenated arrest solutions were 1.8 ± 0.8, 1.5 ± 0.7, 2.6 ± 0.7, and 3.2 ± 1.4 μmol lactate/min/g dry wt, respectively (Figure 4, B). No troponin T was detected in the coronary effluent of AL or 2× ALMI hearts after rewarming (Figure 4, C). In contrast, the troponin T levels for HTK and Celsior hearts were 0.08 and 0.24 μg/mL effluent, respectively.

DISCUSSION

Over the past 3 decades, the design of organ preservation solutions has been based on 3 fundamental principles: (1) hyperkalemic arrest, (2) a suitable cold, milieu to maintain cell viability during global ischemia, and (3) protection

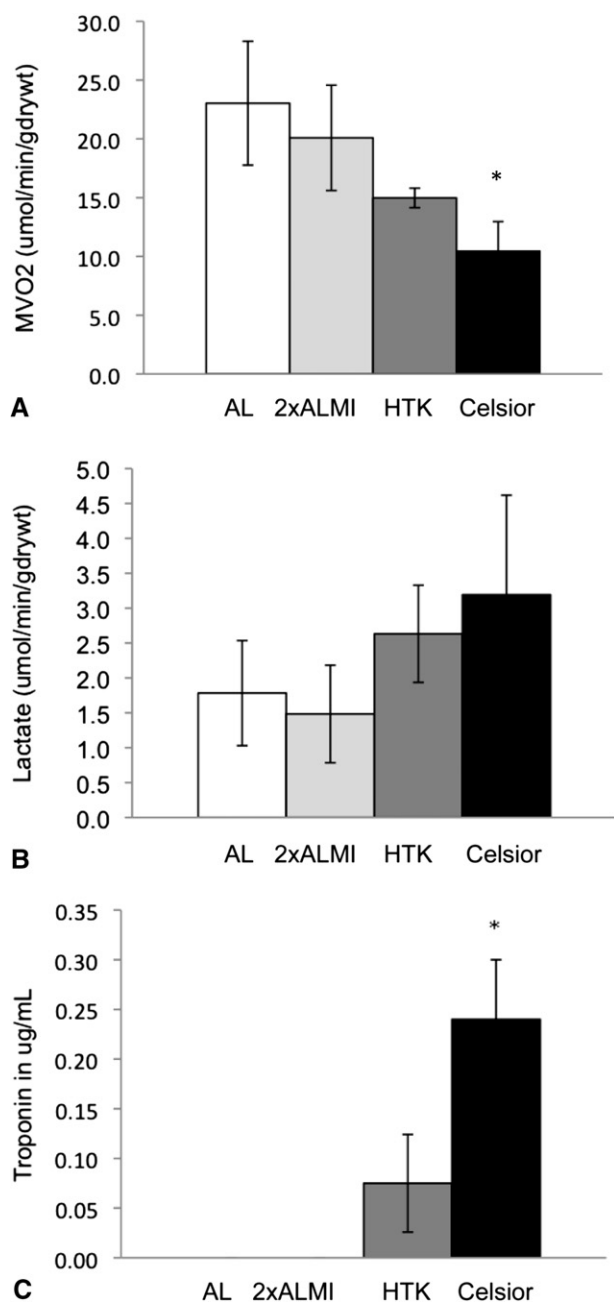


FIGURE 4. A, MVO₂ for the 5 different groups during 5-minute rewarming period in Langendorff mode and at 10 and 60 minutes reperfusion in working mode. Celsior was significantly different from AL and ALMI ($P < .05$). B, Lactate output ($\mu\text{mol}/\text{min}/\text{g}$ dry wt heart) measured in the last minute of the 5-minute rewarming period. Differences were not significant ($P < .05$). C, Troponin T measured in the last minute of the 5-minute rewarming period. Celsior was significantly different from AL and ALMI ($P < .05$). MVO₂, Myocardial oxygen consumption; AL, adenosine-lidocaine; ALMI, adenosine, lidocaine, melatonin, and insulin; HTK, histidine-tryptophan-ketoglutarate.

from reperfusion injury.¹⁶ In 2009, we introduced a new cold and warm normokalemic, polarizing concept to organ preservation,^{1,8} and in 2011 we reported that AL

cardioplegia with 1 mmol/L Ca²⁺/0.5 mmol/L Mg²⁺ returned approximately 68% CO, 101% heart rate, 90% to 105% developed pressures, and no detectable troponin T ($<0.03 \mu\text{g}/\text{mL}$) after 6 hours of cold static storage. The present study showed that further protection is afforded using higher AL levels in the presence of melatonin (100 $\mu\text{mol}/\text{L}$) and insulin (0.01 IU/mL) added to the AL preservation solution (2 \times ALMI); the return of CO increased to 78% with higher functional recoveries. In contrast and under identical conditions, HTK hearts failed to function with 4% CO, 16% heart rate, 11% to 17% developed pressures, and troponin T of 0.13 $\mu\text{g}/\text{mL}$. Celsior hearts, despite an 86% return of heart rate, also failed to return sufficient left ventricular pump function with 25% CO and troponin T of 0.24 $\mu\text{g}/\text{mL}$ after rewarming for 8 hours and 5 minutes.

Rewarming (5 Minutes) After Cold Static Storage: Higher Aerobic Maintenance Metabolism and Lower Lactate Efflux in Polarized Adenosine-Lidocaine Hearts

An interesting outcome from the present study was that the higher functional recoveries in AL hearts were associated with higher (1.5–2-fold) rates of MVO₂ in oxygenated, normokalemic, polarized arrested hearts (AL, 2 \times ALMI) before switching to working mode compared with hyperkalemic HTK and Celsior arrested hearts (Figure 3, A). Previously, we also reported a higher aerobic maintenance metabolism for AL hearts during 5 minutes of rewarming compared with Celsior hearts after 6 hours of cold static storage (arrested in normal AL with 1.0 mmol/L Ca²⁺/0.5 mmol/L Mg²⁺).⁹ The higher MVO₂ values in the present study were associated with equivalent decreases in effluent lactate (Figure 3, B), indicating an increased reliance on mitochondrial oxidative phosphorylation to replenish ATP compared with hyperkalemic HTK and Celsior groups. These differences in MVO₂ were not due to differences in coronary effluent outflows, which ranged from 11 to 14 mL/min. The higher troponin T levels in the hyperkalemic HTK and Celsior groups indicate some myocardial cell damage during 5 minutes of rewarming and reperfusion. Troponin T was not detected in the effluent of the AL or 2 \times ALMI hearts (Figure 3, C). A higher MVO₂ in AL-arrested hearts may provide a new polarizing protection paradigm for rewarming and implanting donor hearts after cold static storage.⁹

Higher Functional Recoveries in Adenosine-Lidocaine Hearts After 8 Hours of Cold Static Storage

Adenosine-lidocaine preservation solutions. A standout result of the present study was the spontaneous return of 55% to 78% CO after 8 hours of cold ischemic storage with approximately 100% heart rate and 90% to 105%

developed pressures for AL and 2× ALMI hearts. AL preservation encompassed approximately 10 hours of protection from harvest, arrest induction, cold static storage (4°C), 5 minutes of rewarming, to 60 minutes of reperfusion in working mode (38°C). We previously reported a return of 66% CO in rat hearts stored in AL cardioplegia (1.0 mmol/L Ca²⁺ and 0.5 mmol/L Mg²⁺) after 5 minutes of rewarming after 6 hours of cold static storage.⁹ Improved recovery in the 2× ALMI hearts may be due to (1) the higher AL concentration exerting a stabilizing effect on membrane polarity,¹⁰ and thereby reducing damage from Na⁺ and Ca²⁺ loading during cold storage or rewarming; (2) insulin's effect to possibly facilitate myocardial glucose uptake,¹⁷ stimulation of nitric oxide production via ischemia-induced myocyte protein kinase C-dependent phosphatidylinositol 3'-kinase-Akt-dependent signaling, and inhibition of superoxide anion (O₂⁻)^{12,18}; and (3) melatonin's cardioprotective properties. Melatonin is a powerful antioxidant and scavenges superoxide radical (O₂⁻), hydroxyl radical (OH⁻), and the lipid peroxy radical. Melatonin prevents the mitochondrial permeability transition pore from opening and collapsing the membrane potential; helps to maintain cardiolipin levels, which constitute approximately 20% of the total lipid composition of the inner mitochondrial membrane; and has a stabilizing effect on components of the electron transport chain, including preventing the loss of cytochrome c.¹¹ The present study did not investigate the relative contributions of 2× AL, melatonin, or insulin on improved pump function, and further studies are required to tease apart their mechanisms of action in AL preservation and recovery.

In both AL preservation groups, the higher return of left ventricular pump function compared with the HTK and Celsior hearts may also have been associated with improved maintenance of cell membrane polarity during all phases of the preservation protocol.^{1,9} The basic electrophysiology behind polarized versus depolarized diastolic arrest is that at "resting" diastolic membrane potentials there are fewer "open" membrane channels, pores, and exchangers compared with depolarized states that can lead to Na⁺ and Ca²⁺ loading, mitochondrial dysfunction, cell injury, and possibly death.^{2,7,19} The diastolic membrane potential of AL hearts is approximately -80 mV.^{2,7} By using the Nernstian relation between membrane potential (E_m) and extracellular potassium, where E_m (mV) = 26.23 ln [K⁺ (mmol/L)] - 123.44,⁷ we predict a membrane potential for HTK hearts of -63 mV for an extracellular potassium level of 10 mmol/L K⁺. Our Nernstian estimate agrees with the direct microelectrode membrane measurement of -60 ± 3.6 mV reported by Krohn and colleagues²⁰ on sheep cardiac Purkinje fibers bathed in Custodiol-HTK solution. We predict the voltage of the Celsior hearts to be approximately -50 mV (15 mmol/L K⁺).

Celsior Versus Histidine-Tryptophan-Ketoglutarate Preservation Solution

Our finding that Celsior hearts performed better than HTK hearts has been reported in a number of experimental and clinical investigations for solid organ transplantation in heart,²¹⁻²⁵ lung,²⁶ and liver.²⁷ Our results are consistent with the rat heart study of Gao and colleagues,²⁸ who reported CO recoveries in Celsior stored hearts of 20% at 30 minutes after 6 hours and less than 5% at 30 minutes after 10 hours of cold static storage (2°C-3°C). In our study, only 10% of left ventricular function (AF) and 25% CO were spontaneously returned after 8 hours of cold storage (Figure 2, A-C). Celsior has also been reported to be clinically superior to University of Wisconsin (Viaspan).^{22,25,29} Superior preservation in Celsior is believed to be due to the lower potassium levels (15 mmol/L), low Ca²⁺/high Mg²⁺, impermeant lactobionate, and potent antioxidant properties of reduced glutathione.^{21,23,30-33} Celsior seems to have been originally designed as a single-solution platform for use during most of the successive steps of the preservation procedure. However, it was not designed as a normokalemic, normothermic reperfusion solution where the heart may be challenged during cold-to-rewarm transitions, as we recently showed in the rat heart after 6 hours of cold static storage.⁹

We were also surprised with the low ventricular outputs from HTK hearts with less than a few percent recovery (Figure 2, A), because they, like Celsior hearts, appeared healthy and soft to touch with no visual signs of ischemia after 8 hours of cold static storage. As with Celsior hearts, the HTK hearts could not generate sufficient left ventricular output against a fixed pressure head, that is, they could not perform adequate physical work (force × distance) against the 76 mm Hg afterload. In 1993, Reichenspurner and colleagues³⁴ evaluated HTK for myocardial preservation in cooperation with Eurotransplant and 5 heart transplant centers, and it was concluded that HTK provided good results as long as the ischemic time did not exceed 4 hours. The 5 main features of the HTK solution claimed to be useful for heart preservation are (1) its lower depolarizing extracellular K⁺, low Na⁺, low Ca²⁺ concentrations; (2) the presence of energy substrate alpha-ketoglutarate; (3) the presence of antioxidant tryptophan; (4) the presence of mannitol to reduced cell swelling; and (5) a high histidine (198 mmol/L) buffering to counter tissue acidosis during cold global ischemia.^{20,34} In 2010, Lee and colleagues,³⁵ using a rat transplant model, reported superiority of HTK over Celsior on the basis of lower serum creatine kinase levels and less macroscopic deterioration of the graft after 6 or 18 hours of cold storage. However, no pump function was reported, and, as the authors acknowledged, these hearts were not loaded and cannot be extrapolated to clinical conditions.³⁵

The overall functional recoveries of hearts after cold static storage in HTK solution reported in the literature vary widely. In 2000, Saitoh and colleagues³⁶ reported a return of 79.9% CO after 8 hours of cold static storage (4°C) compared with 4% in our study (which was predominately coronary flow) (Figure 2, A–C). Saitoh and colleagues' study had the following differences: (1) male Wistar rats versus male Sprague Dawley rats, (2) lower working afterload (60 mm Hg) compared with 76 mm Hg in our study, (3) unspecified Ca²⁺ level in their HTK solution (Table 1 in Saitoh and colleagues), (3) 15 minutes of reperfusion in Langendorff mode at unspecified temperatures compared with 5 minutes and 37°C in our study, and (4) working mode function for 25 minutes at unspecified temperatures (Figure 1 in Saitoh and colleagues) compared with 60 minutes and 37°C in our study. Also in their methods, Saitoh and colleagues state that hearts were reperfused for 20 minutes in Langendorff and 40 minutes in working mode after cold storage.³⁶ These omissions and discrepancies in their methodology make functional comparisons difficult. In 1992, Galinanes and colleagues³⁷ reported a return of 22% CO in isolated rat hearts stored in HTK for 8 hours (4°C). In these latter experiments, hearts were subjected to 15 minutes reperfusion followed by 20 minutes working mode.³⁷

In our study, the low COs in HTK (and Celsior) hearts after 8 hours may have been due to the shorter 5-minute re-warming flush period (Figure 1). Warm reperfusion in an oxygenated depolarized state may have promoted unnatural heterogeneous membrane voltage, ionic and metabolic imbalances, and possibly altered lipid membrane organization, such as temperature-dependent phase transitions influencing fluidity, which may have led to suboptimal function after switching to working mode. Nonetheless, we showed that normokalemic, polarizing AL preservation was versatile at both profoundly hypothermic states and during 5-minute cold-to-warm transitions immediately after 8 hours of cold static storage. Functional deficits of HTK hearts may also have arisen from the lack of broad-spectrum antioxidant protection. Schröder and colleagues¹⁴ recently reported cold-induced mediated oxidant injury in isolated cells after HTK storage, and they have proposed that the addition of iron chelators (or other broad-spectrum antioxidants) to the conventional Custodiol-HTK solution may bolster protection. Custodiol-N base solution (modified HTK-solution) is now being developed to address some of the concerns with the traditional Custodiol-HTK solution.³⁸

Possible Clinical Significance and Future Directions

The clinical significance of AL preservation solutions exceeding the performance of 2 leading Food and Drug Administration-approved heart preservation solutions HTK and Celsior in isolated rat hearts after 8 hours offers new opportunities. Despite decades of laboratory studies

with the reporting of some extraordinary functional recoveries after 6, 8, 12, and 24 hours of heart preservation in rat, rabbit, porcine, canine, and sheep models using a myriad of preservation solutions, the clinical reality is that 4 to 5 hours remains the safe period for the human donor heart. Storage time continues to be a critical factor determining the viability of stored solid organs, and extending these times may usher in a new era of organ donation. Longer times may expand the use of organs from marginal beating and non-beating heart donors, and donors may be sought from larger geographic areas, including rural and remote regions. Longer-term 15- to 24-hour preservation times are currently being investigated with AL solutions, as well as constant perfusion studies using isolated pig hearts and kidneys. The possibility also exists that the new polarized arrest and reperfusion paradigm may find utility in preserving other solid organs from a wide patient base, such as lung, liver, and pancreas, or isolated cells, such as stem cells or islet cells.

CONCLUSIONS

We report that AL polarized arrest solution with low Ca²⁺/high Mg²⁺ (0.22 mmol/L/2.6 mmol/L) returned 55% CO, 101% heart rate, and 90% to 105% developed pressures after 8 hours of cold static storage. When AL was doubled in concentration in the presence of melatonin (100 μmol/L) and insulin (0.01 IU/mL), the return of CO increased to 78% with no detectable troponin T (<0.03 μg/mL). In contrast, HTK hearts returned 4% CO, 16% heart rate, and 11% to 17% developed pressures, and troponin T was 0.13 μg/mL in effluent 5 minutes after the rewarm. Celsior hearts, despite an 86% return heart rate, also failed to return sufficient left ventricular function (~10%) with a return of 25% CO and a troponin T level of 0.24 μg/mL.

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1225 **000 Eight hours of cold static storage with adenosine and lidocaine (Adenocaine)** 1286
 1226 **heart preservation solutions: Toward therapeutic suspended animation** 1287
 1227 1288
 1228 *Donna M. Rudd, MSc, and Geoffrey P. Dobson, PhD, Queensland, Australia* 1289

1229 We report a 78% return of cardiac output after 8 hours of cold static storage (4°C) using 1290
 1230 normokalemic, polarizing adenosine, lidocaine, melatonin, and insulin solution in the isolated rat 1291
 1231 heart compared with 55% cardiac output with AL cardioplegia alone, 4% cardiac output with HTK 1292
 1232 preservation solution, and 25% cardiac output with Celsior preservation solution. 1293
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