

Characterizing Myocardial Metabolism During Hypothermic Storage By Carbon-13 and Proton MRS: Implications For Cardiac Transplantation

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Background: Myocardial viability for heart transplantation is limited due to depletion of energy stores. Machine perfusion preservation is one technique that has been utilized in preserving other organs [1]. This strategy can provide oxygen and metabolic substrate to sustain ischemic tissue after organ harvest and prior to reimplantation into the recipient. Myocardial energy requirements are much higher than those of other solid organs thus limiting ischemic periods to less than 4 hours before progressive donor organ dysfunction is noted [2]. Preserving hearts by continuously providing oxygen and available substrate could address the metabolic demands of stored myocardium. However, this strategy has not been fully explored. Additionally, the optimal preservation solution for myocardial preservation during conventional static storage and so-called perfusion preservation is also unclear. In the current study, we compared myocardial metabolism during storage in extracellular type preservation solution (Celsior) and intracellular type preservation solution (University of Wisconsin [UW]) utilizing both static and perfusion protective strategies.

Methods:

Experimental Design: Groups of male Sprague-Dawley rat hearts (n=10 per group) were harvested after administration of cardioplegia and stored for 200 minutes using conventional static storage and perfusion preservation. Hearts were preserved in either Celsior or UW solution. A control group consisted of hearts preserved in 25 ml UW (**UW Control Group**) or Celsior (**Celsior Control Group**). Effects of available substrate on metabolism during storage were assessed by adding 5.5mM U-¹³C glucose to the solutions (**Celsior Glucose, UW Glucose Groups**). Additional hearts were preserved by continuous Langendorff perfusion from a height of 100cm with U-¹³C glucose containing storage solution at 10°C (**Celsior Perfusion, UW Perfusion Groups**). 4 hearts in each group were used to evaluate effects of each intervention during storage as described below. The remaining 6 hearts in each group were Langendorff reperfused with Krebs-Heinselleit buffer for 120 minutes to evaluate the effects of each intervention on reperfusion cardiac function.

Preservation: In the perfusion preservation groups, coronary flow, inflow and outflow pO₂ were measured. MVO₂ was calculated from coronary flow and pO₂ data. For static storage hearts, MVO₂ was calculated by O₂ solubility at 4°C combined with pre and post storage pO₂ differences. After the preservation interval, 4 hearts in each group were freeze clamped in liquid nitrogen. Cardiac tissue was extracted with perchloric acid and lyophilized. A portion of the each extract was reconstituted in D₂O and high-resolution ¹H and proton decoupled ¹³C MR spectra were obtained on a 14.1T Varian Inova spectrometer. Glycolysis from catabolism of exogenous, labeled glucose was calculated by the enrichment of the number 3 carbon in lactate or alanine. Fractional substrate oxidation was determined by glutamate isotopomer analysis under non-steady-state conditions if sufficient enrichment of TCA cycle intermediates was present [3]. Additional extract from each heart was used to measure adenosine tri-phosphate (ATP) levels by HPLC.

Reperfusion: Heart rate, developed pressure, rate pressure product, MVO₂, coronary vascular resistance (CVR) and myocardial water content were determined. After 200 minutes reperfusion, cardiac tissue was preserved in 4% paraformaldehyde for subsequent histology. TUNEL assays using the Promega Dead End TUNEL kit were performed on tissue sections from each heart to determine apoptotic cell death. Apoptosis was reported as the number of TUNEL positive nuclei per high-power field.

Statistics: Data for all groups are reported as the mean ± SEM. Groups were compared by one-way analysis of variance. Differences between groups, when present, were determined by the Fisher LSD using SigmaStat® statistical software. A p value of less than 0.05 was considered significant.

Results: During perfusion preservation, MVO₂ was significantly greater in the Celsior Perfusion group compared to the UW Perfusion group. A corresponding increase in glycolytic and oxidative metabolism of exogenous glucose was noted (See Figure 1). None of the static storage groups had any measurable oxidative metabolism during storage. Glycolytic enrichment in the Celsior Glucose and UW Glucose groups was similar. Perfusion preservation more effectively maintained ATP levels and energy charge compared to conventional static storage. Additionally, earlier recovery of myocardial functional recovery was noted in the perfusion preservation groups compared to static storage groups (p<.05) although functional recovery as measured by the rate pressure product after 200 minutes was not different. Apoptosis was reduced 4-5 fold in perfusion preservation groups. Comparing perfusion groups, CVR and myocardial water content was reduced in the Celsior Perfusion group compared to the UW Perfusion Group (p<.05).

| Group | Storage MVO ₂ (μmol/min/g dry wt) | Storage ATP (μmol/min/g dry wt) | Apoptosis (Cells per 10 HPF) | CVR (mmHg/ml/min) | Water Content (%) |
|-------------------|---|------------------------------------|---------------------------------|----------------------|-------------------|
| Celsior Control | .01±.001 | 3.8±1 | 7.5±1 | 4.5±.2 | 84.6±.3 |
| UW Control | .01±.001 | 3.9±1 | 10±2 | 4.0±.2 | 84.8±.6 |
| Celsior Glucose | .01±.005 | 4.5±1 | 7.2±1 | 3.4±.3 | 85.1±.3 |
| UW Glucose | .01±.004 | 2.5±.4 | 8.8±1 | 3.4±.3 | 83.6±.4 |
| Celsior Perfusion | 4.1±.4*† | 8.1±1* | 1.3±.4* | 3.5±.2† | 82.8±.3† |
| UW Perfusion | 2.2±.2* | 7.0±1* | 1.4±.6* | 4.5±.3 | 84.5±.4 |

Table 1 Myocardial MVO₂, Energetics, Function, and Apoptosis

Data are mean ± SEM * - p < .05 vs all Static Groups, † - p < .05 vs UW Perfusion

Conclusion: Perfusion preservation results in greater metabolism during storage and superior cardiac function with improved myocyte survival compared to static storage. Hearts perfused with oxygenated, extracellular preservation solution have higher MVO₂ and metabolism of exogenous glucose as measured by MRS. Contrary to conventional wisdom, this increased metabolism during storage is associated with improved CVR and myocardial water content.

References:

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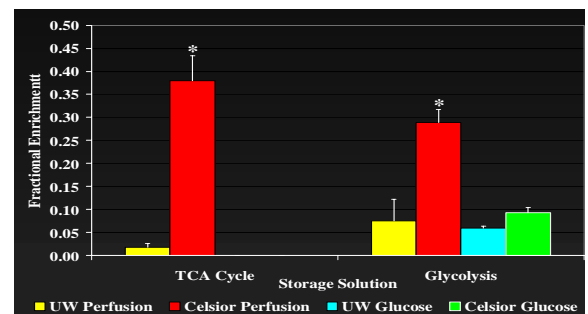


Figure 1 Glucose Utilization by MRS

* - p < .05 vs All Groups