Pyruvate is Superior to Glucose in Supporting Metabolism of Machine Perfused Donor Hearts for Transplantation

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Introduction

Heart transplantation is limited by the relatively brief ischemic tolerance of preserved myocardium that restricts optimal organ allocation. Experimental data suggest machine perfusion preservation of hearts for transplantation may be an effective strategy to extend the donor ischemic interval and utilize extended donor organs [1]. We have previously demonstrated that machine perfusion preserves graft oxidative metabolism and reduces myocardial injury as measured by CKMB release and apoptotic cell death even after a standard ischemic interval [2,3]. The preserved myocardium contains limited quantities of endogenous substrates and providing an exogenous fuel may be important to avoid myocardial substrate depletion. Conventional preservation solutions utilize glucose as a potential energy source but the value of glucose in supporting the energy demands of the machine perfused heart remain unclear. Other studies from our laboratory in small and large animal models using ^{13}C and ^{1}H magnetic resonance spectroscopy (MRS) demonstrated that glucose is a minor substrate of the machine perfused donor heart and that the lactate/alanine ratio is a discriminator of graft aerobic and anaerobic metabolism [3]. Additional data suggest that pyruvate becomes an increasingly important myocardial substrate during hypothermia [4]. In the current study we applied increasing concentrations of ^{13}C glucose and pyruvate to the preservation solution of machine perfused rat hearts and measured incorporation of exogenous substrate into metabolic intermediates by ^{1}H and ^{13}C magnetic resonance spectroscopy. We hypothesized that pyruvate is a more effective substrate for supporting metabolism of the machine perfused donor heart thus preserving endogenous energy stores.

Methods

In this study, we perfused 8 groups of isolated rat hearts (n=4/group) with University of Wisconsin Machine Perfusion Solution containing increasing concentrations of uniformly Carbon 13 (U-^{13}C) labeled glucose (2.5mM, 5mM, 10mM, 20mM) or 3-^{13}C labeled pyruvate (5mM, 10mM, 20mM, 40mM) at 0.5 mL/min at 8°C for 6 hours. Myocardial oxygen consumption (MVO_2) and myocardial water content were measured. At end-perfusion, tissue was rapidly freeze clamped with liquid nitrogen and stored at -80ºC. ^{13}C and ^{1}H magnetic resonance spectroscopy was performed on ventricular extracts to determine the lactate/alanine ratio and the relative contribution of exogenous, labeled substrate to (1) glycolysis (measured by lactate enrichment) (2) oxidative pathways (measured by alanine enrichment) and (3) tricarboxylic acid cycle (TCA) intermediates. Groups were compared by ANOVA. A p-value < .05 was considered significant.

Table 1 – Exogenous Substrate Contribution to Metabolic Pathways

Group	Lactate/Alanine	Glycolysis (% Lactate Enrichment)	Oxidative (% Alanine Enrichment)
2.5mM Glucose	1.0±.4	44±10	16±2
5mM Glucose	.67±.2	37±14	11±4
10mM Glucose	1.3±2	57±.1	18±5
20mM Glucose	1.3±.3	62±5	30±7
5mM Pyruvate	.48±.1	70±6†	73±3*
10mM Pyruvate	.50±.2	70±6†	73±2*
20mM Pyruvate	.75±.2	77±5†	77±3*
40mM Pyruvate	.28±.1	82±2†	87±2*

* - p <.05 versus all glucose groups
† - p < .05 versus 2.5mM and 5mM glucose groups

Results

MVO_2 and myocardial water content did not differ between groups. Lactate/alanine ratios were low consistent with aerobic myocardial metabolism in both groups. Significant differences in substrate contributions to metabolic pathways were noted. Exogenous glucose was metabolized mainly by glycolysis and was a minor contributor to oxidative metabolism, suggesting primarily endogenous substrate oxidation. Pyruvate contributed equally to the lactate dehydrogenase reaction and presumed oxidative pathways and was the major substrate contributing to oxidative metabolism. See **Table 1**. Examples of ^{13}C enrichment patterns of lactate and alanine are shown in **Figures 1** and **2**. Tricarboxylic acid (TCA) cycle enrichment was greater in all pyruvate groups compared to the glucose containing groups ($p<.05$). Isotopomer analysis of TCA cycle intermediates suggested that in the pyruvate groups, most substrate oxidized by the TCA cycle was derived from exogenous pyruvate. The glucose contribution to oxidative metabolism was less than 30% for all groups.

Figure 1 - Pyruvate

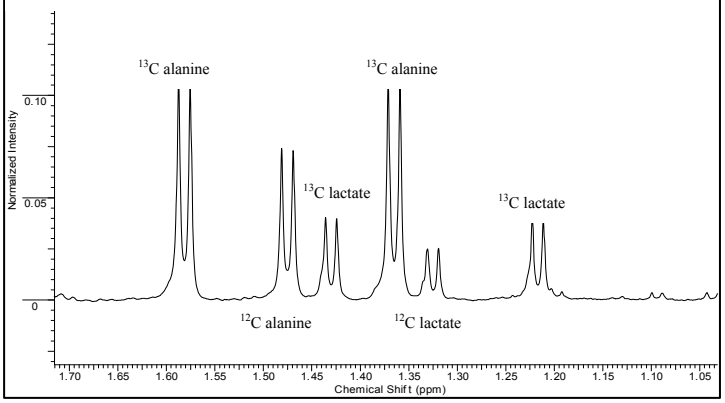
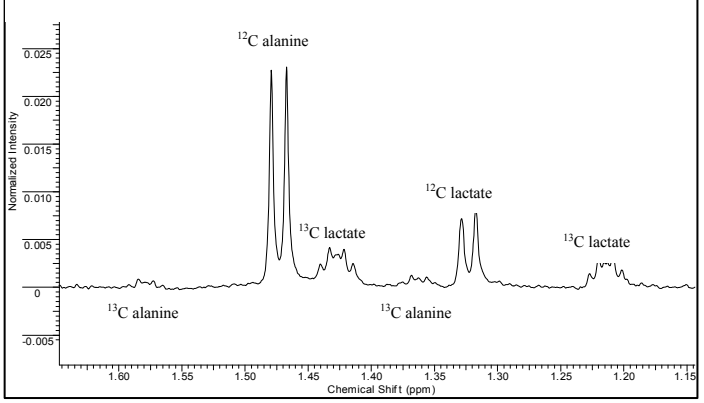


Figure 2 - Glucose



Conclusions

In conclusion, the preservation solution substrate composition influences myocardial substrate selection of cold perfused hearts. Exogenous glucose is a minor substrate in machine perfused hearts. Glucose is primarily metabolized by glycolysis and does not contribute greatly to oxidative metabolism. Pyruvate appears more effective in supporting myocardial metabolism and may limit depletion of endogenous stores. Further experiments with other myocardial substrates and to determine influences of substrate modifications on reperfusion function are warranted.

References

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