

Mitochondrial ATP Synthase Tetramer Disassembly following Blood-Based or del Nido Cardioplegia during Neonatal Cardiac Surgery

Bartholomew V. Simon, MD;* Gisela Beutner, PhD;† Michael F. Swartz, PhD;* Ron Angona, CCP;* Karen Smith, CCP;* George A. Porter, Jr., MD, PhD;† George M. Alfieris, MD*

*Department of Surgery, University of Rochester Medical Center, Rochester, New York; and †Department of Pediatrics University of Rochester Medical Center, Rochester, New York

Abstract: Conservation of mitochondrial adenosine triphosphate (ATP) synthase proteins during ischemia is critical to preserve ATP supply and ventricular function. Following myocardial ischemia in adults, higher order ATP synthase tetramer proteins disassemble into simpler monomer units, reducing the efficiency of ATP production. However, it is unknown if myocardial ischemia following the use of cardioplegia results in tetramer disassembly in neonates, and whether it can be mitigated by cardioplegia if it does occur. We investigated myocardial ATP synthase tetramer disassembly in both a neonatal lamb cardiac surgery model and in neonatal children requiring cardiac surgery for the repair of congenital heart disease. Neonatal lambs (*Ovis aries*) were placed on cardiopulmonary bypass (CPB) and underwent cardioplegic arrest using a single dose of 30 mL/kg antegrade blood-based potassium cardioplegia ($n = 4$) or a single dose of 30 mL/kg antegrade del Nido cardioplegia ($n = 6$). Right ventricular biopsies were taken at baseline on CPB ($n = 10$) and after approximately 60 minutes of cardioplegic arrest

before the cross clamp was released ($n = 10$). Human right ventricular biopsies ($n = 3$) were taken following 40.0 ± 23.1 minutes of ischemia after a single dose of antegrade blood-based cardioplegia. Protein complexes were separated on clear native gels and the tetramer to monomer ratio quantified. From the neonatal lamb model regardless of the cardioplegia strategy, the tetramer:monomer ratio decreased significantly during ischemia from baseline measurements ($.6 \pm .2$ vs. $.5 \pm .1$; $p = .03$). The del Nido solution better preserved the tetramer:monomer ratio when compared to the blood-based cardioplegia (Blood $.4 \pm .1$ vs. del Nido $.5 \pm .1$; $p = .05$). The tetramer:monomer ratio following the use of blood-based cardioplegia in humans aligned with the lamb data (tetramer:monomer $.5 \pm .2$). These initial results suggest that despite cardioprotection, ischemia during neonatal cardiac surgery results in tetramer disassembly which may be limited when using the del Nido solution. **Keywords:** Cardioplegia, Mitochondria, Myocardial Ischemia. *J Extra Corpor Technol. 2022;54:203–11*

Adenosine triphosphate (ATP) synthase is a mitochondrial enzyme that uses electrons generated by the electron transport chain to synthesize ATP. Monomers of ATP synthase can form tetramers and oligomers (synthasomes) that alter the mitochondrial shape and increase the efficiency of ATP production (1,2). Ischemia can result in ATP synthase tetramer and synthasome disassembly into simpler monomer units, reducing

ATP supply and ventricular function (2–4). Strategies to protect the mitochondria and prevent ATP synthase disassembly during ischemia have focused on maintaining the closure of the permeability transition pore (PTP). The PTP is an inner mitochondrial membrane channel that regulates mitochondrial calcium, pH, and volume (2,5). Within adult cardiomyocytes the PTP closes during ischemia, limiting the disassembly of higher order ATP synthase proteins, release of reactive oxygen species, and apoptosis (6).

Unlike the adult myocardium, neonatal cardiomyocytes and mitochondria resemble a fetal and immature state, and the mitochondrial PTP remains open (7–11). Although the mitochondria eventually mature and the PTP closes, ischemia during the first several weeks of

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Address correspondence to: George M. Alfieris, MD, Cardiac Surgery Resident, Department of Surgery University of Rochester Medical Center, 601 Elmwood Ave., Box Pediatric Cardiac Surgery, Rochester, NY 14642. E-mail: george_alfieris@urmc.rochester.edu
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life could leave the mitochondria unprotected with the resultant disassembly of higher order ATP synthase proteins (tetramers and synthasomes) into monomers, decreasing the efficiency of ATP production (9,10).

Although several neonatal cardioplegia strategies have focused on preserving overall ATP content (12–14), preservation of ATP synthase activity has been left unexplored. Blood-based depolarizing solutions have demonstrated good efficacy in preserving myocardial metabolism (15). However, modified solutions containing magnesium and lidocaine such as del Nido cardioplegia act to close the PTP, which may prevent ATP synthase disassembly (16). We investigated myocardial ATP synthase disassembly in both a neonatal lamb cardiac surgery model and in neonatal children requiring cardiac surgery for the repair of congenital heart disease.

MATERIALS AND METHODS

Animal Procedures

All procedures described were approved by the University of Rochester (University Committee on Animal Resources), protocol #101910. All animals used in the study received humane care in compliance with the “Guide for the Care and Use of Laboratory Animals,” published by the National Institutes of Health (NIH Publication No. 85–23, revised 1985). Neonatal lambs (*Ovis aries*) 10–14 days old were used given their similar cardiac anatomy to humans and prior use in pediatric cardiac surgery experiments (17). This age range was chosen to ensure closure of the ductus arteriosus. Baseline echocardiograms, as well as electrolytes, were examined preoperatively to ensure that the animals were healthy and had normal ventricular function.

Operative Methods

Anesthesia was induced with intravenous ketamine (2.0–7.5 mg/kg) and maintained with inhaled isoflurane (1–3%) and propofol (.25 mg/kg/min). Endotracheal intubation and pressure-controlled ventilation was performed to maintain arterial $PO_2 > 70$ mmHg and $PCO_2 < 50$ mmHg. Central venous and arterial access were achieved and a three-lead electrocardiogram was monitored. Hemodynamics were maintained within normal limits.

The animal was placed in the left lateral decubitus position. A cervical incision exposed the right external jugular vein and carotid artery, and a right thoracotomy exposed the mediastinal contents. Following the administration of heparin (200 IU/kg) to achieve an Activated Clotting Time (ACT) of >480 seconds, the carotid artery was cannulated using an 8-Fr Medtronic Biomedicus arterial cannula (Medtronic, Minneapolis, MN). The external jugular vein was cannulated with a 12Fr Medtronic Biomedicus Pediatric Jugular

Venous cannula (Medtronic). A Medtronic infant circuit with a Medtronic Affinity Pixie Oxygenator (Medtronic) and sanguineous prime (details within data supplement) were used to achieve full cardiopulmonary bypass (flow rate 150 mL/kg/min).

A single dose of either 30 mL/kg del Nido cardioplegia or 30 mL/kg of blood-based depolarizing cardioplegia was given after the ascending aorta was cross clamped (Table 1). Following approximately 60 minutes of ischemia, the aortic cross clamp was removed, the animal was separated from cardiopulmonary bypass, and the venous reservoir contents were transfused. The venous and arterial cannulae were removed and the cervical vessels ligated. Intercostal nerve blocks were performed with .5% bupivacaine and the incisions were closed in layers. Animals were transitioned to sternal recumbency in a custom-made Panepinto Sling (Lomir Biomedical, Malone, NY) immediately following chest closure to promote lung expansion. Warming blankets and a space heater were used to prevent hypothermia. Animals were returned to the vivarium living quarters once they were off oxygen and standing without support, and were allowed to recover for a maximum of 72 hours.

Following either 72 hours of recovery or a witnessed hemodynamic deterioration, the previous right thoracotomy was opened. A right atrial cannula was placed and used to exsanguinate the animal under deep anesthesia, and biopsies of the left and right ventricle were obtained.

Biopsies

Baseline right ventricular biopsies were taken after the initiation of cardiopulmonary bypass before cardioplegic arrest, and again following approximately 60 minutes of cardioplegic arrest before the cross clamp was removed. If the animal either recovered or had a witnessed deterioration, left and right ventricular biopsies were obtained at the 72-hour mark or at the time the animal decompensated. All biopsies were immediately flash frozen and stored at -80°C until needed for further study.

Human Samples

Following parental permission and institutional review board (IRB) approval (URMC IRB #73408 “Electron

Table 1. Comparison of blood and del Nido cardioplegia.

	Blood (500 mL)	del Nido (1,000 mL)
Base solution (500 mL)	PlasmaLYTE-A	PlasmaLYTE-A
Blood:crystalloid ratio	4:1	1:4
KCl (mEq/mL)	1	2
NaHCO ₃ ⁻ (mEq/mL)	–	1
20% Mannitol (mL)	–	16.3
1% Lidocaine (mL)	–	13
50% Magnesium sulfate (mL)	–	4

Transport Assembly following Infant Cardioplegic Arrest” approved 10/11/18), infants requiring a right ventriculotomy during cardiac surgery where right ventricular muscle tissue would be discarded were recruited. Arterial cannulation was achieved using either direct aortic cannulation or after a 3.5 mm PTFE graft (Gore Medical, Flagstaff, AZ) was sewn to the innominate artery. In all cases, venous drainage was achieved with bicaval cannulation. The heart was arrested using 50 mL/kg of antegrade blood-based depolarizing cardioplegia per standard institutional practice. Excised right ventricular tissue was frozen at -80°C .

Objectives

The primary objective was to understand the degree of ATP synthase tetramer disassembly during ischemia in a neonatal lamb model of cardiac surgery. Secondary objectives were to 1) identify if the human ATP synthase tetramer levels following the use of cardioplegia mirrored the lamb model, 2) identify if the del Nido cardioplegia solution would better limit ATP synthase disassembly vs. blood cardioplegia within the lamb model of cardiac surgery, 3) understand if ATP synthase tetramer levels return to baseline 72 hours after ischemia within the lamb model of cardiac surgery.

Details regarding troponin measurements, echocardiography, native electrophoresis, and statistical methods have been placed within the data supplement.

RESULTS

Ten lambs underwent myocardial arrest using either blood ($n = 4$) or del Nido cardioplegia ($n = 6$). In all cases, myocardial arrest was achieved after approximately 5 mL/kg of cardioplegia. One animal within the blood-based group developed continuous myocardial activity after approximately 45 minutes. The cross clamp was removed at that point and the animal was included in the analysis.

Table 2. Perioperative demographics—neonatal lamb model.

	Blood ($n = 4$)	del Nido ($n = 6$)	p Value
Age (days)	12.8 ± 1.5	12.3 ± 1.5	.7
Male gender	2 (50%)	5 (83.3%)	.5
Weight (kg)	5.3 ± 1.3	4.7 ± 1.2	.5
CPB time (min)	164.8 ± 41.9	182.3 ± 26.2	.4
Aortic cross clamp time (min)	52.8 ± 15.1	61.5 ± 2.5	.3
Postoperative			
Ventricular arrhythmia	1 (25%)	2 (33%)	.52
Max vasoactive inotrope score	3.3 ± 2.5	0	.08
Echocardiogram LVEF (%)			
Baseline	82.1 ± 8.1	82.8 ± 6.4	.9
Post-CPB	79.9 ± 3.5	72.1 ± 10.2	.2

CPB, cardiopulmonary bypass; LVEF, left ventricular ejection fraction.

Perioperative demographics (Table 2) demonstrate no significant differences in age, gender, cardiopulmonary bypass or cross clamp time, need for inotropic therapy, or change in left ventricular ejection fraction between cardioplegia groups. Two animals in the del Nido group achieved recovery at 72 hours. Three animals had a witnessed clinical deterioration (Blood-2, del Nido-1). Five animals deteriorated during the late postoperative period (8–24 hours) and final biopsies could not be obtained. Four of these five animals developed respiratory failure (Blood-2, del Nido-2), and one died suddenly after doing well for approximately 24 hours postoperative.

ATP Synthase Assembly during Ischemia

Both monomer and tetramers of ATP synthase were identified in all baseline and ischemic right ventricular samples. Higher order synthasomes were observed in only two animals, both of which received del Nido cardioplegia and both of which survived to 72 hours. Representative immunoblots in Figure 1A demonstrate the monomer, tetramer, and synthasome bands. From the entire cohort ($n = 10$), ischemia significantly lowered the tetramer:monomer ratio from baseline ($.6 \pm .2$ vs. $.5 \pm .1$; $p = .03$) (Figure 1B) by 18.4%. In both animals where ATP synthase synthasomes were present, ischemia decreased the synthasome:monomer ratio ($.9 \pm .3$ vs. $.8 \pm .2$; $p = .3$). Biopsy data analyzed from animals during recovery at the 72-hour mark and during clinical deterioration are provided within the data supplement.

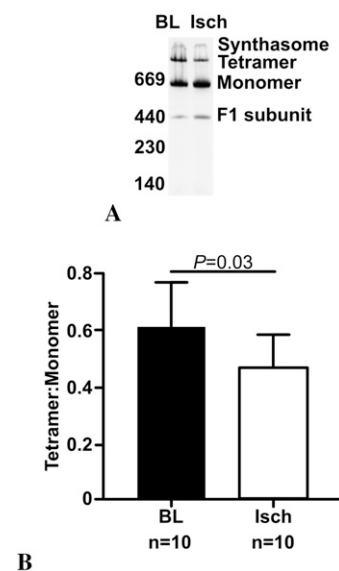
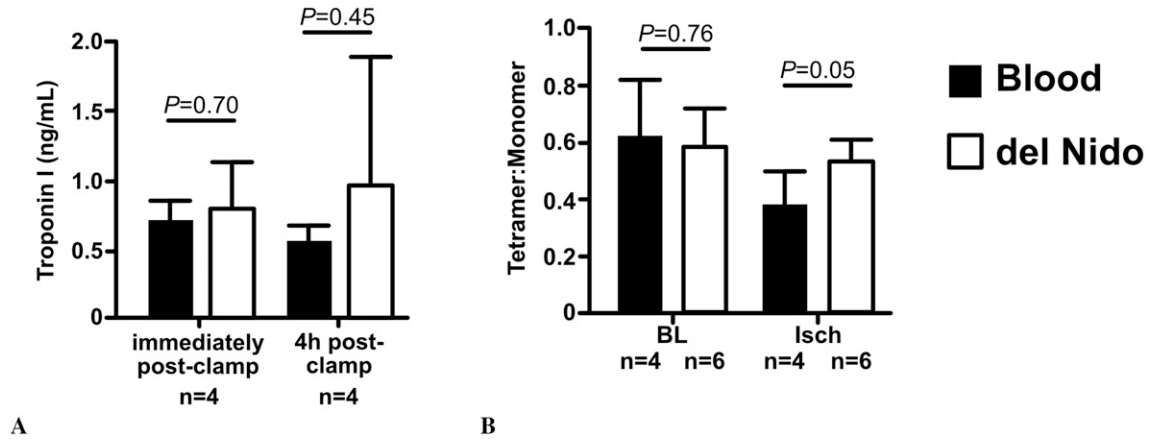


Figure 1. (A) Representative immunoblot of monomer, tetramer, and synthasome bands from neonatal lamb right ventricular muscle samples taken at baseline and following ischemia—also visible at 440 kD is the F1 subunit of adenosine triphosphate (ATP) synthase. (B) Bar graph displaying cumulative tetramer:monomer ratio at baseline (black) and following ischemia (white) from 10 neonatal lambs. BL, baseline; Isch, ischemia.

Figure 2. (A) Bar graph displaying troponin levels immediately following reperfusion and after 4 hours of reperfusion between animals given blood-based (black) or del Nido (white) cardioplegia. (B) Bar graph displaying tetramer:monomer ratio at baseline and ischemia when using either blood-based or del Nido cardioplegia. BL, baseline; Isch, ischemia.



Blood vs. del Nido Comparison

Both serum troponin I (Figure 2A) and ventricular systolic function (Table 2) were not statistically different between groups, with ejection fraction being numerically but not statistically lower in the del Nido group. During ischemia, the right ventricular tetramer:monomer ratios were lower than baseline measurements in all animals that received blood-based cardioplegia. However, the administration of del Nido cardioplegia preserved the tetramer:monomer ratio in 66% ($n = 4$) of animals. As a result, the tetramer:monomer ratio during ischemia was significantly greater within the del Nido group compared to the blood group ($.5 \pm .1$ vs. $.4 \pm .1$; $p = .05$) (Figure 2B).

ATP Synthase Assembly during Ischemia in Humans

Right ventricular muscle was obtained from three neonates requiring repair of congenital heart disease (Table 3). Two samples were obtained from the anterior portion of the right ventricle, and one from several right ventricular bands that required division to patch a ventricular septal defect. A representative immunoblot demonstrates human monomer and tetramer bands of identical weights and similar ratios to the ovine samples (Figure 3A and 3B). ATP synthase synthasomes were not observed in these human samples.

DISCUSSION

The results from this study demonstrate: 1) despite cardio-protection with cardioplegia, during ischemia in an ovine model of pediatric cardiac surgery there is a significant reduction in right ventricular ATP synthase tetramer:monomer ratio; 2) del Nido cardioplegia better preserves the tetramer:monomer ratio during ischemia,

but without significantly impacting serum markers of myocardial injury or left ventricular function; 3) during cardiac surgery after the use of blood-based cardioplegia, human right ventricular tetramer:monomer ratios during ischemia are similar to the ovine model.

The increase in energy production from the organization of ATP synthase into tetramers and synthasomes has been only recently elucidated (2,18). Particularly during the neonatal period, disassembly of ATP synthase limits the ATP available for myocyte differentiation and lowers systolic ventricular function (11). In adults, disruption of the ATP synthase during ischemia reduces ATP availability by nearly 50% (19,20). Similarly, ATP synthase activity is decreased during chronic cardiomyopathy (21) and congestive heart failure (22), two conditions associated with reduced cardiac energetics. Similarly, we observed a

Table 3. Demographics of neonates with congenital heart disease.

Preoperative Demographics	N = 3
Age (days)	9.6 ± 5.1
Male gender	66.7% (2)
Weight (kg)	3.6 ± .2
Fundamental diagnosis	
Truncus arteriosus	33.3% (1)
TOF/PA	33.3% (1)
CoA/VSD	33.3% (1)
Operative data	
CPB time (Minutes)	142 ± 40.8
Ao X-Clamp time (minutes)	60.3 ± 27.4
RV sample timing (mins of ischemia)	40.0 ± 23.1
Ventricular arrhythmia	0
Max vasoactive inotrope score	11.7 ± 2.8

Ao X-Clamp, aortic cross clamp; CoA, coarctation; CPB, cardiopulmonary bypass; PA, pulmonary atresia; RV, right ventricle; TOF, tetralogy of Fallot; VSD, ventricular septal defect.

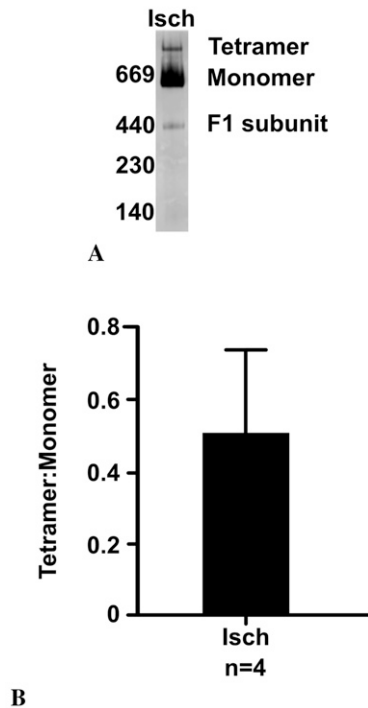


Figure 3. (A) Representative immunoblot of monomer and tetramer bands from human right ventricular muscle samples following the use of a blood-based cardioplegia- also visible at 440kD is the F1 subunit of adenosine triphosphate (ATP) synthase. (B) Bar graph displaying cumulative tetramer:monomer ratio following the use of a blood-based cardioplegia in three human neonates requiring repair for congenital heart disease. Isch, ischemia.

20% reduction in the ATP synthase tetramer:monomer ratio from the lamb experiments.

Previous data on myocardial ATP synthase have primarily been obtained from mitochondria (23), cultured myocytes (24), or small animal Langendorff experiments (25). The critical next step in ascertaining the importance of ATP synthase preservation requires a large animal model. Although large animal models increase heterogeneity compared to cellular level or Langendorff experiments, they allow the mirroring of physiologic conditions observed in human patients. The bypass and aortic cross clamp times in this manuscript are similar to those seen during surgery for congenital heart disease (26). Unfortunately, recovery to 72 hours was not achievable for the majority of animals and an understanding of the ATP synthase tetramer:monomer ratio during recovery remains unknown.

Although controversial, recent data strongly suggest a close relationship between the ATP synthase and the PTP. The PTP has long been a target for the protection of both myocytes and mitochondria during ischemia and reperfusion (27). Opening of the PTP results in mitochondrial calcium overload, release of reactive oxygen

species, and apoptosis (28,29) in both adults and children. The use of pharmacology to prevent PTP opening has not significantly impacted clinical outcomes in adult cardiac ischemia (30). However, neonates represent a unique population where the PTP is more likely to open than in adults (11). Previous experiments from our group have demonstrated that closure of the PTP in neonates maintains higher order ATP synthase organization, initiates mitochondrial maturation, and increases myocyte differentiation (7,11). Myocytes and mitochondria that are further differentiated may be more likely to survive the limited ischemia associated with cardioplegia and cardiac surgery. This may partially explain the higher incidence of early cardiac dysfunction and death following cardiac surgery during the early neonatal period.

It is interesting that synthasome formation was observed in only two animals. Previous mouse and myocyte data from our group have consistently demonstrated synthasome formation in a variety of physiologic conditions (2,11). Emerging data suggest that ATP synthase may assemble into higher order structures or disassemble depending upon the myocytes' current energetic needs (2,31). Therefore, although the baseline tetramer:monomer ratios in the two animals that recovered were similar to those that eventually developed cardiac failure, the presence of synthasome activity at baseline may have afforded these animals with increased ATP availability before surgery and a survival advantage.

The tetramer:monomer ratio from the human data during ischemia correlated with the lamb data, providing some validation of the ovine model for studying ATP synthase tetramer assembly. Additionally, the linear relationship between the left and right ventricular ATP synthase assembly supports the serial right ventricular sampling used in this study. Although the failure of either ventricle can occur following cardiac surgery (32,33), sampling of left ventricular tissue is often more technically difficult. In contrast, the right ventricle and particularly the right ventricular outflow tract is anterior, thinner, has relatively few epicardial coronary branches, and is more likely to be sampled during human congenital heart surgery.

The del Nido solution better maintained the ATP synthase tetramer:monomer ratio during ischemia. In children, the del Nido solution has demonstrated superiority in preserving ventricular function and limiting myocardial injury in prior basic science (34), retrospective (35), and prospective studies (36). Unlike previous investigators (36), we did not observe a significant difference in ventricular function or myocardial injury, which may be related to our limited sample size as well as obtaining serial ventricular biopsies, a source of myocyte injury and troponin release. Better preservation of the ATP synthase tetramer:monomer ratio using the del Nido solution may be associated with the prevention of calcium

influx. We have previously demonstrated that the addition of calcium to mitochondria opens the PTP and reduces ATP synthase synthasome levels by nearly 20% (2). The del Nido solution contains lidocaine, a sodium channel blocker, which indirectly prevents calcium influx as well as magnesium, a natural calcium channel blocker, which also blocks the PTP. Prevention of calcium influx into the mitochondria in the animal model may have limited PTP opening, which would be a potential explanation for the decreased ATP synthase disassembly we observed with the del Nido solution (Supplemental Figure S1).

LIMITATIONS

There are several limitations to this work. Although our cardiac surgery animal model was a physiologic experiment, not all animals survived to the end of the 72-hour recovery period. Cardiac output data routinely obtained from a Langendorff model could not be collected due to the short ovine ascending aorta which was needed for cross clamping and cardioplegia administration. In addition, we were unable to obtain biopsy data during reperfusion. With two prior biopsy sites, there was limited opportunity for a third biopsy without compromising the right ventricle. Similarly, in trying to recover the animals for three days we did not do an analysis of tetramer disassembly under ischemia without cardioplegia which may have aided the comparison of blood-based and del Nido cardioplegia. Animals in the ovine model had normal oxygenation while some of the human samples were from cyanotic patients, which may make them less comparable due to the tendency of mitochondria to adapt under cyanotic conditions. Finally, other aspects of the mitochondrial energetics and electron transport such as Complexes I, III, and IV, overall ATP content, and PTP activity during and after ischemia were not measured given the limited biopsies taken in this survival model.

CONCLUSIONS

In an ovine model of neonatal cardiac surgery, cardioplegic arrest using cardioplegia results in the disassembly of ATP synthase tetramer proteins. Human data from infants during cardiac surgery demonstrated similar levels of ATP synthase tetramer activity. Finally, del Nido cardioplegia better preserved mitochondrial ATP synthase tetramer assembly following ischemia compared to blood-based cardioplegia. Collectively, these preliminary findings warrant further investigation to better characterize the impact of ATP synthase preservation.

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APPENDIX

DATA SUPPLEMENT FOR MITOCHONDRIAL ATP SYNTHASE TETRAMER DISASSEMBLY FOLLOWING THE USE OF BLOOD-BASED OR DEL NIDO CARDIOPLEGIA DURING NEONATAL CARDIAC SURGERY

MATERIALS AND METHODS

Troponin

Serum samples were taken after just cross clamp release and 4 hours following cardiopulmonary bypass, frozen, and analyzed in duplicate for troponin I following the manufacturer's (AbClonal, Woburn, MA) recommendations at an optical density of 450 nm on a SpectraMax Plus 384 spectrophotometer (BioTek Instruments, Winooski, VT).

Echocardiography

Transthoracic echocardiograms were performed preoperatively and after chest closure. Left ventricular end-diastolic diameter and left ventricular end-systolic diameter were measured in the parasternal short axis view. Left ventricular volumes and ejection fraction were calculated using the Teichholz method (1).

Native Electrophoresis

Protein complexes from cardiac muscle or mitochondria were separated on a 3–8% clear native (CN) gel (2).

Samples were solubilized on ice with 2 μ g lauryl-maltoside/ μ g protein in extraction buffer (50 mM NaCl, 50 mM imidazole/HCl, 2 mM 6-aminohexanoic acid, 1mM EDTA, pH 7.0). After separation at 200 V for 2 hours on ice, the protein complexes were wet-transferred onto nitrocellulose membranes (25 V, 16 hours). Membranes were stained with Ponceau red after transfer and photographed. ATP synthase monomers, tetramers, and when possible synthasomes were identified by immunoblotting against ATP5A (ThermoFisher # 439,800), and the ratio of tetramer:monomer or synthasome:monomer was calculated densitometrically using Image J. These ratios were chosen because ATP synthase exists in a dynamic continuum with tetramers and synthasomes being assembled and disassembled from monomers depending upon the myocyte's energy needs (3,4). The use of the ratio rather than signal intensity alone also excluded any differences in protein loading between lanes.

CARDIOPULMONARY BYPASS CIRCUIT BLOOD PRIME PROTOCOL

Approximately 300 mL of Plasmalyte was used to prime the circuit and cardioplegia line. Following the mixing of 250 mL of ewe blood with 5,000 units of heparin, the blood was washed with 700 mL Plasmalyte, which was removed from the circuit by ultrafiltration. Once washed, 2.2 mL of 10% calcium chloride and 3 mL of 8.4% sodium bicarbonate were added and a blood sample was obtained for pH and ionized Ca^{2+} .

Titration of pH and ionized calcium were performed as follows:

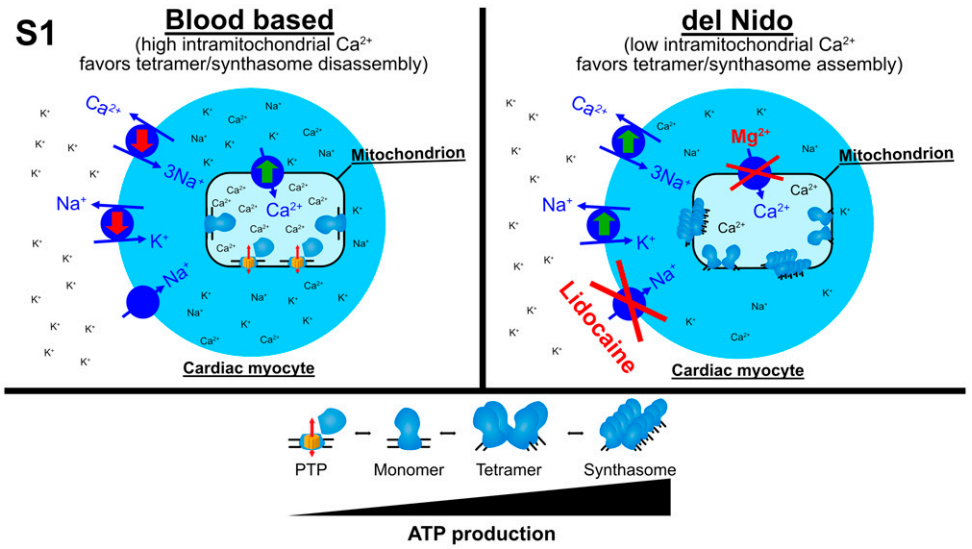
- $\text{iCa}^{2+} \leq 1.35$ —administer additional 2 mL 10% calcium chloride
- $\text{pH} \leq 7.35$ —administer 1 mL 8.4% sodium bicarbonate
- $\text{pH} \leq 7.25$ —administer 2mL 8.4% sodium bicarbonate
- $\text{pH} \leq 7.15$ —administer 3mL 8.4% sodium bicarbonate

Following the addition of sodium bicarbonate and after adding the blood to the pump prime, the sweep was increased to 50 mL/min for 1 minute. Additional blood gases were obtained to achieve an ionized $\text{Ca}^{2+} > 1.35$ and $\text{pH} > 7.35$.

Statistical Analysis

All data are presented as mean \pm SD for normally distributed continuous variables, median with interquartile range for data not-normally distributed continuous variables, or as a frequency and percentage for non-continuous variables. After ensuring the equality of variables using a Shapiro–Wilks test, univariate analysis was performed using a two-tailed *t* test. Fisher's exact test or χ^2 analysis was used for the comparison of qualitative variables. Linear regression was used to identify a correlation between two variables and the Pearson coefficient was calculated. Two-way analysis of variance (ANOVA) compared the baseline to postischemic differences between blood-based and del Nido samples. All statistics were completed using GraphPad Prism version 5.0b (GraphPad Software, San Diego, CA) or SPSS 25.0 (IBM Corporation,

Figure S1. Proposed mechanism of the differential effects of blood and del Nido cardioplegia on cardiac myocyte ion transport and intramitochondrial calcium levels in this study. Blood-based cardioplegia results in intracellular potassium accumulation. This in turn leads to sodium and calcium accumulation once the sodium–potassium and sodium–calcium exchangers lack favorable gradients to move sodium and calcium out of the cell. Calcium then accumulates in mitochondria favoring tetramer and synthasome disassembly into monomers and permeability transition pore (PTP) opening. Less adenosine triphosphate (ATP) production results. del Nido cardioplegia contains lidocaine that blocks the voltage gated sodium channel and magnesium, which blocks mitochondrial calcium channels. The net effect is less intramitochondrial calcium accumulation, more tetramer and synthasome assembly, and more ATP production.



Armonk, NY) whereby a *p* value of .05 or less was considered statistically significant.

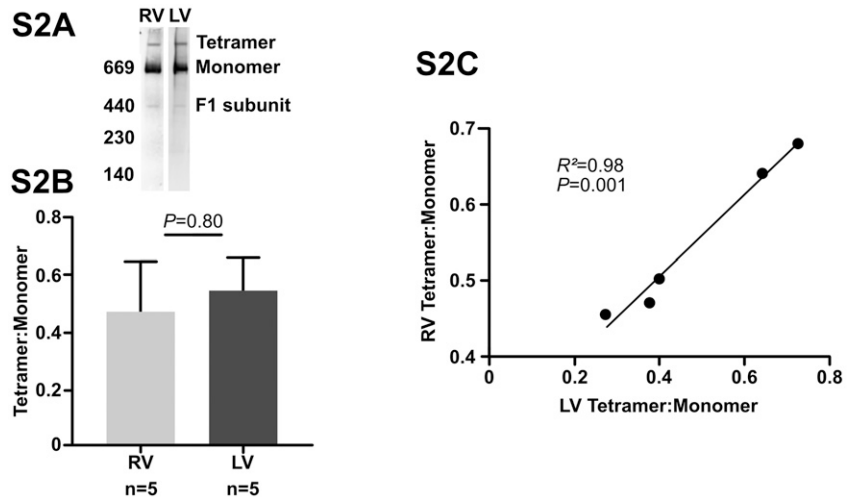
RESULTS

Final Biopsies

Final left and right ventricular muscle samples were obtained in five animals, three during clinical deterioration (blood based:2, del Nido:1) and two after 72 hours of recovery (del

Nido:2). A representative immunoblot of right and left ventricular samples demonstrates similar signal intensity of the tetramer bands in both left and right ventricular samples (Figure S2A). The final samples demonstrated a similar cumulative tetramer:monomer ratio between the right and left ventricular tissues (Figure S2B). Additionally, there was a strong linear correlation when comparing the tetramer:monomer ratios of left and right ventricular tissue (Figure S2C). Synthasomes were not observed in any of the final samples. The tetramer:

Figure S2. (A) Representative immunoblot of lamb right and left ventricular monomer and tetramer bands as well as the F1 subunit of adenosine triphosphate (ATP) synthase. (B) Bar graph displaying cumulative (*n* = 5) right and left ventricular tetramer:monomer ratio from final lamb samples. (C) Linear relationship between the left and right ventricular tetramer:monomer ratio in final lamb samples. RV, right ventricle; LV, left ventricle.



S3

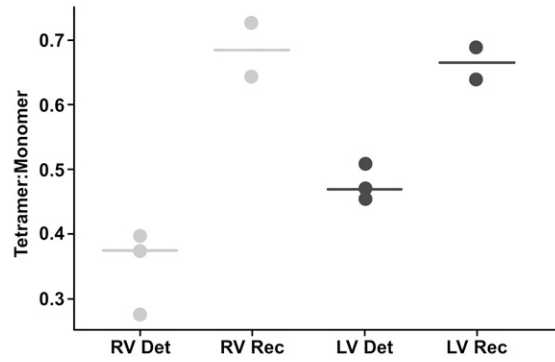


Figure S3. Column scatter plot of tetramer:monomer ratios from right and left ventricular tissue of animals during deterioration (Det) and after 72 hours of recovery (Rec). RV, right ventricle; LV, left ventricle.

monomer ratios from both right and left ventricular samples during deterioration were lower than at recovery (Figure S3).

APPENDIX REFERENCES

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