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High Oxygenation During Normothermic Regional Perfusion After Circulatory Death Is Beneficial on Donor Cardiac Function in a Porcine Model

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Background. Thoracoabdominal normothermic regional perfusion (NRP) is a new method for in situ reperfusion and reanimation of potential donor organs in donation after circulatory death by reperfusion of the thoracic and abdominal organs with oxygenated blood. We investigated effects of high oxygenation (HOX) versus low oxygenation (LOX) during NRP on donor heart function in a porcine model. **Methods.** Pigs (80 kg) underwent a 15-min anoxic cardiac arrest followed by cardiac reanimation on NRP using a heart-lung bypass machine with subsequent assessment 180 min post-NRP. The animals were randomized to HOX (FiO_2 1.0) or LOX (FiO_2 0.21 increased to 0.40 during NRP). Hemodynamic data were obtained by invasive blood pressure and biventricular pressure-volume measurements. Blood gases, biomarkers of inflammation, and oxidative stress were measured. **Results.** Eight of 9 animals in the HOX group and 7 of 10 in the LOX group were successfully weaned from NRP. Right ventricular end-systole elastance was significantly improved in the HOX group compared with the LOX group, whereas left ventricular end-systole elastance was preserved at baseline levels. Post-NRP cardiac output, mean arterial, central venous, and pulmonary capillary wedge pressure were all comparable to baseline. Creatinine kinase-MB increased more in the LOX group than the HOX group, whereas proinflammatory cytokines increased more in the HOX group than the LOX group. No difference was found in oxidative stress between groups. **Conclusions.** All hearts weaned from NRP showed acceptable hemodynamic function for transplantation. Hearts exposed to LOX showed more myocardial damage and showed poorer contractile performance than hearts reperfused with high oxygen.

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INTRODUCTION

In order to increase numbers of donor hearts, transplantation of hearts from circulatory dead donors has emerged as a potential solution.[1-5] Donor organs, however, are exposed to a period of warm ischemia from the circulatory death and may thus suffer irreversible injury. Iyer et al[6] demonstrated that cold storage directly after circulatory death is not feasible in adult heart transplantation (HTX). Oxygenated perfusion, however, has been demonstrated in and ex situ to enable successful transplantation of hearts

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All authors agree with the content of the manuscript and have made substantial contributions to the work; N.M., L.B.I., P.R., M.P., S.T., and H.E. conceived and planned the experiments, and N.M., Z.L.Z., and C.C. carried out the experiments. J.P. performed analysis of inflammatory markers. N.M., M.P., and H.E. interpreted the results, and Z.L.Z., L.B.I., P.R., M.E.E., and S.T. contributed to the surgical protocol and provided constructive feedback. N.M. wrote the first draft of the manuscript. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Supplemental Visual Abstract; http://links.lww.com/TP/C425.

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donated after circulatory death (DCD).[6-8] In addition to reperfusion, evaluating the heart for eligibility for transplantation after the ischemic insult is of utmost importance.[9]

Several preclinical studies have paved the way of thoracoabdominal normothermic regional perfusion (TANRP).[7,10,11] This method has been proven in clinical programs to allow successful transplantation of DCD hearts[3,12]; however, procedures for circulatory death and normothermic regional perfusion (NRP) differ among institutions, and no standardized protocols for TANRP have been established, including duration and oxygenation level.[13]

Oxygenation is highly debated in cardiopulmonary resuscitation, and several studies have showed higher mortality and increased morbidity with use of high oxygenation (HOX) during and after cardiopulmonary resuscitation.[14-17] The primary concern of HOX is the risk of neurological damage secondary to ischemic reperfusion injuries and oxidative stress[18]; however, other studies have demonstrated attenuation of ischemic cardiac damage using HOX during resuscitation.[19-21] Thus, the aim of the present study was to assess the effects of high and low oxygenation (LOX) level during NRP on post-NRP cardiac function in a porcine DCD model.

MATERIALS AND METHODS

The study was approved by the Danish National Committee on Animal Research Ethics (2018-15-0201-01603) and conducted in accordance with the Principles of Laboratory Animal care.[22]

Anesthesia, Monitoring, and Surgical Procedure

Female Danish landrace pigs (80 kg) were used. Premedication of Zoletil Vet 50 (Virbec, Carros, France) was administered intramuscular. The animals were orally intubated and mechanically ventilated with a tidal volume of 8 mL/kg, FiO_2 of 0.4, positive end-expiratory pressure of 5 cm H_2O, and respiratory rate of 12 to 15 per min to maintain an end-tidal CO_2 of 4.5 to 5.6 kPa. Anesthesia and analgesia were maintained with intravenous propofol (3.5 mg/kg/h) and fentanyl (15 µg/kg/h). Infusion of amiodarone (10 µg/kg/min) and a bolus of 100 mg lidocaine were administered to prevent arrhythmias. Via the right femoral artery, an aortic occlusion balloon was advanced to the abdominal aorta above the iliac bifurcation. A Swan-Ganz pulmonary arterial (PA) catheter (Edwards Lifescience, Irvine, CA) was inserted through the right external jugular vein. A median sternotomy was performed. Pressure-volume admittance catheters, 7 Fr (Transonic Systems, Ithaca, NY), were inserted in the right and left ventricle (LV) via 8 Fr sheaths in the right external jugular vein and left common carotid artery, respectively. Intravenous heparin (40 000 IU) was administered for systemic anticoagulation before cannulation of the ascending aorta and right atrium for the NRP circuit. Cannulae were inserted in the ascending aorta and PA for blood sampling and invasive blood pressure monitoring. After instrumentation, infusion of rocuronium (3 mg/kg/h) was initiated before baseline parameters were recorded and blood and tissue were sampled.

Withdrawal From Life-sustaining Therapy and NRP

Mechanical ventilation was disconnected, resulting in asphyxiation and hypoxic circulatory arrest. Functional warm ischemic time was defined as the time from systolic arterial blood pressure below 50 mm Hg to the onset of reperfusion. Mechanical asystole was determined as pulse pressure <20 mm Hg marking the onset of circulatory arrest. An additional 15 min of warm ischemic circulatory arrest was observed after asystole to mimic the preparation time for NRP. Five minutes before reperfusion, blood and tissue samples were obtained, the arch vessels were individually snared to exclude cerebral circulation, and the abdominal aortic balloon was inflated above the iliac bifurcation to exclude lower body circulation.

After 15 min of circulatory arrest, ventilation was restarted, and NRP commenced with a FiO_2 set according to randomization; the animals were randomly assigned 1:1 to receive HOX or LOX during the NRP (**Figure S1, SDC,** http://links.lww.com/TP/C424). The FiO_2 in the HOX group was set to 1.0 during NRP and subsequently lowered to 0.6 during the first 20 min after weaning. The LOX FiO_2 was set to 0.21 at the start of NRP and gradually increased to 0.4 over the first 9 min and kept at that level for the rest of the experiment. The extracorporeal circuit, which comprised a standard extracorporeal membrane oxygenator, roller pump, and heat exchanger, was primed with 1000 mL Ringers lactate and 200 mL mannitol. A standardized NRP perfusion protocol was devised based on pilot studies (see Supplemental Information, SDC, http://links.lww.com/TP/C424). The heart was gradually volume loaded during the first 6 min of NRP to perfuse the pulmonary vasculature, and NRP flow was gradually reduced after 15 min to full weaning after 35 min. After 15 min of NRP, infusion of dobutamine (2.5 µkg/kg/min) was started. If weaning was considered unsuccessful, defined as mean arterial pressure (MAP) <60 mm Hg for 5 min posttermination of NRP, despite norepinephrine infusion (maximum of 1.0 µg/kg/min), NRP was recommenced for an additional 35 min. During NRP, arterial and mixed venous blood samples were collected and hemodynamic and biochemical parameters were corrected according to protocol during the whole study (Table 1). After successful weaning from TANRP, the animals were observed for 180 min with measurements of hemodynamics, contractility, and sampling of blood and tissue performed at 15, 30, 60, 90, 120, and 180 min.

TABLE 1.

Hemodynamic and biochemical lowest acceptable levels and corrections, if needed

Parameter	Correction
Atrial and ventricular arrhythmias	Internal defibrillation, 20 J
Mean arterial pressure <60 mm Hg	Titrate norepinephrine infusion: 0.0–0.7 µg/kg/min
pH <7.3	8.4% sodium bicarbonate, 50 mL
Ionized Ca2+ <1.20 mmol/L	Calcium chloride, 2.5 mmol
K+ <4.5 mmol/L	Potassium chloride, 5 mmol
Plasma glucose <5 mmol/L	Glucose, 2500 mg

During normothermic regional perfusion, boluses were administered through the heart-lung machine, whereas they were administered through a central venous line before and after normothermic regional perfusion.

Cardiac Function

Cardiac output (CO) measurements were obtained by thermodilution technique using the PA catheter. Admittance catheters were calibrated according to the manufacturer's specifications. Load-independent contractility of the LV and right ventricle (RV) was assessed using the end-systolic elastance (Ees) and the pressure-volume area. Diastolic function was assessed using the Tau relaxation constant. Pressure-volume data were also used to measure load-dependent hemodynamic measures: ejection fraction, stroke volume (SV), and arterial elastance (Ea) as a measure for ventricular afterload.

Biochemistry and Inflammation

Arterial and mixed venous samples were analyzed for partial pressures of oxygen, carbon dioxide, and lactate (ABL90 Flex Plus; Radiometer Medical, Copenhagen, Denmark). Creatinine kinase-MBs (CK-MBs) were measured by chemiluminescence (Advia Centaur XPT; Siemens Healthcare Diagnostics, E Walpole, MA). Plasma levels of 8-isoprostane were determined using an ELISA kit (Cayman Chemical, Ann Arbor, MI) as per the manufacturer's instructions. Cytokine plasma levels were measured at baseline, 10 min after circulatory arrest, 30 min after NRP commencement, and 60 min and 180 min after weaning from NRP. The Millipore MILLIPLEX porcine chemokine array (granulocyte macrophage colony stimulating factor, interferon-γ, IL-1 receptor antagonist, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, and tumor necrosis factor α) was analyzed with the Luminex platform, Magpix (Millipore Corp).

Statistical Analysis

Data were checked for normality by qq-plots. Continuous data were analyzed using a multivariate repeated measurement ANOVA. The model was used to allow for repeated measures, taking the study design into account, and to allow for analysis of parameters containing missing data. The model was used to compare outcomes between intervention groups with group and time (and the interaction between them) as fixed effects and subjects as random effects. No correction for multiple comparison was performed. Fisher exact test was used for comparison of categorical data. Mann-Whitney and Student *t* test were used to compare data between groups when appropriate. $P < 0.05$ was considered statistically significant. Stata (version 15.1; StataCorp, College Station, TX) and GraphPad Prism 9.0 (GraphPad Software, San Diego, CA) were used for statistical and graphical analyses.

RESULTS

Protocol Feasibility and Compliance

Nineteen animals were instrumented. Eight of 9 animals were weaned from NRP per protocol in the HOX group; 1 animal had sustained refractory ventricular fibrillation after 70 min of NRP and was terminated, whereas 6 of 10 were weaned from NRP as per protocol in the LOX group ($P = 0.30$); additionally, 1 animal in the LOX group was weaned after 60 min of NRP; 3 animals did not sustain a MAP >60 mm Hg after 70 min of NRP because of very poor contractile cardiac function ("stone heart") and were terminated. All weaned hearts across both groups supported the circulation until termination of the experiment. The Consolidated Standards of Reporting Trials flow diagram can be seen in Figure 1. No difference between the groups was observed in time to asystole, NRP time, and time to sinus rhythm (Table 2). The oxygenation levels during NRP measured as Pao_2 and Sao_2 were significantly different between groups ($P < 0.001$); see Figure 2. The HOX group required nonsignificantly more direct current shocks for defibrillation than the LOX group (median of 7.5 versus 3.5, $P = 0.68$). The average norepinephrine infusion rate from start of NRP to 180 min post-NRP did not differ significantly between the 2 groups (LOX 0.41 [0.14–0.57] versus HOX 0.11 [0.051–0.91] μg/kg, medians [interquartile range], $P = 0.53$; Table 2).

Hemodynamics

Neither systemic nor PA pressures were different between the groups (Figure 3). Mean PA pressure increased immediately after withdrawal of life-sustaining therapies (WLST) and returned to baseline levels during the post-NRP period, whereas MAP was titrated to >60 mm Hg post-NRP. CO did not differ between groups and showed

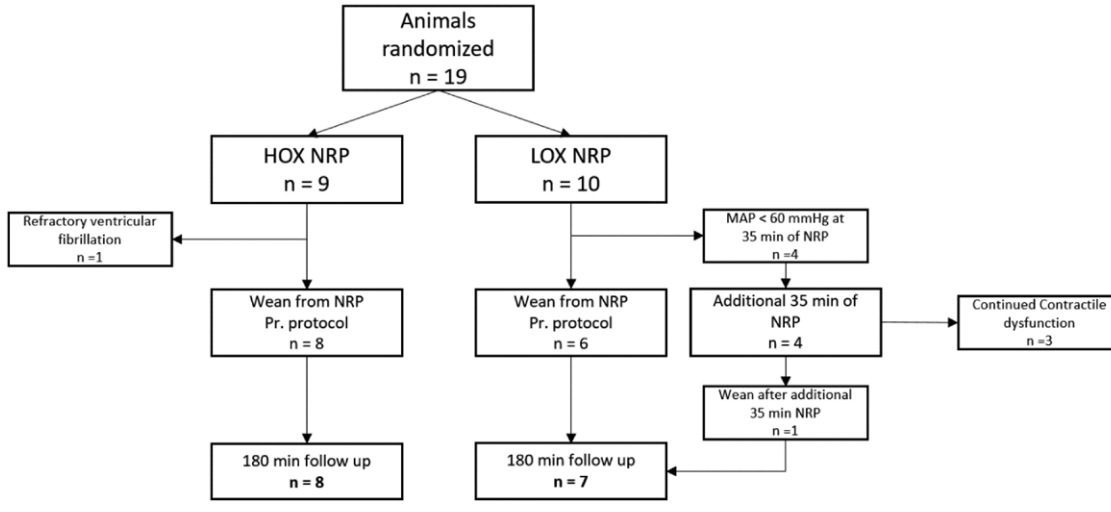

FIGURE 1. Consolidated Standards of Reporting Trials diagram. HOX, high oxygenation; LOX, low oxygenation; MAP, mean arterial pressure; NRP, normothermic regional perfusion.

TABLE 2.

Important time points, defibrillations, and infused norepinephrine

	HOX (n = 8)	LOX (n = 7)	*P*
WLST to asystole, min	8.3 ± 1.6	8.4 ± 1.8	0.84[a]
FWIT (SBP <50 mm Hg), min	18.3 ± 0.6	15.8 ± 0.7	0.0223[a]
NRP, min	36.0 (34.3–37.0)	36.0 (35.0–37.0)	0.78[b]
Reperfusion to electrical activity, min	1.0 (0.75–2.0)	1.0 (1.0–1.8)	0.87[b]
Reperfusion to sinus rhythm, min	6.0 ± 4.5	5.6 ± 3.5	0.85[a]
Number of internal defibrillations	7.5 (1.0–10.5)	3.5 (2.0–6.5)	0.68[b]
Infused NE from NRP start to 180 min post-NRP, µg/kg	0.11 (0.05–0.91)	0.41 (0.14–0.57)	0.53[b]

Data are displayed as median (interquartile range) or mean ± standard error of the mean.
[a]Student *t* test.
[b]Mann-Whitney *U* test.
FWIT, functional warm ischemic time (sat <70% to reperfusion); HOX, high oxygenation; LOX, low oxygenation; NE, norepinephrine; NRP, normothermic regional perfusion; SBP, systolic blood pressure; WLST, withdrawal from life-sustaining therapies.

a similar increase from baseline immediately post-NRP with decreasing CO toward baseline at 60 min post-NRP. Central venous pressure remained unchanged at baseline levels post-NRP in both groups. Pulmonary capillary wedge pressure and pulmonary vascular resistance showed no significant change over time with no difference between groups (**Figure S2, SDC,** http://links.lww.com/TP/C424). SvO_2 was slightly higher in the HOX group than in the LOX group ($P = 0.051$) post-NRP and showed an increase from baseline to 15 min post-NRP but declined thereafter toward baseline in both groups ($P < 0.001$).

Pressure-volume Recordings

Left ventricular SV remained stable in both groups between baseline and the post-NRP period (Figure 4), whereas RV SV was higher in the LOX group ($P = 0.011$) than in the HOX group. Stroke work remained unchanged and similar between both groups. LV and RV ejection fractions were similar between groups but showed a non-significant decrease over time post-NRP. Ea increased non-significantly in the LV but remained at baseline levels in the RV ($P = 0.51$). No difference was found between groups in LV Ea, whereas the HOX group showed significantly higher Ea than the LOX group at all time points ($P = 0.016$). The ventricular relaxation constant, Tau, remained unchanged over time for both ventricles. No difference in Tau was found between treatment groups. Left ventricular end-systole elastance was preserved after weaning from NRP compared with baseline ($P = 0.48$), and no difference was found between the HOX and LOX group. RV Ees, however, increased significantly from baseline to the post-NRP period in the HOX group compared with the LOX group ($P = 0.013$).

Biochemistry

Arterial lactate increased dramatically from baseline to 10 min after circulatory arrest. Lactate increased significantly more in the LOX group during circulatory arrest than in the HOX group, before start of intervention, despite a significantly longer HOX functional warm ischemic time of 2.5 min. Lactate increased again after onset of NRP ($P < 0.001$). Overall, lactate in the HOX group tended to be lower but not significant and cleared faster than the LOX group lactate by 120 min post-NRP (Figure 5). At 60 min post-NRP, the lactate levels started decreasing in both groups from the peak at onset of NRP. In **Figure S3, SDC,** http://links.lww.com/TP/C424) the absolute lactate concentrations are shown. CK-MB increased significantly from baseline to post-NRP in both groups ($P < 0.001$). CK-MB increased more in the LOX group than in the HOX group (Figure 5). The biomarker 8-isoprostane is a free-radical-catalyzed metabolite of arachidonic acid used as a reliable biomarker of oxidative stress. No overall difference was observed between the groups, nor any significant development over time ($P = 0.093$; Figure 5). All measured inflammatory markers increased from baseline to post-NRP and kept rising during the period (Figure 6). A tendency to a larger increase

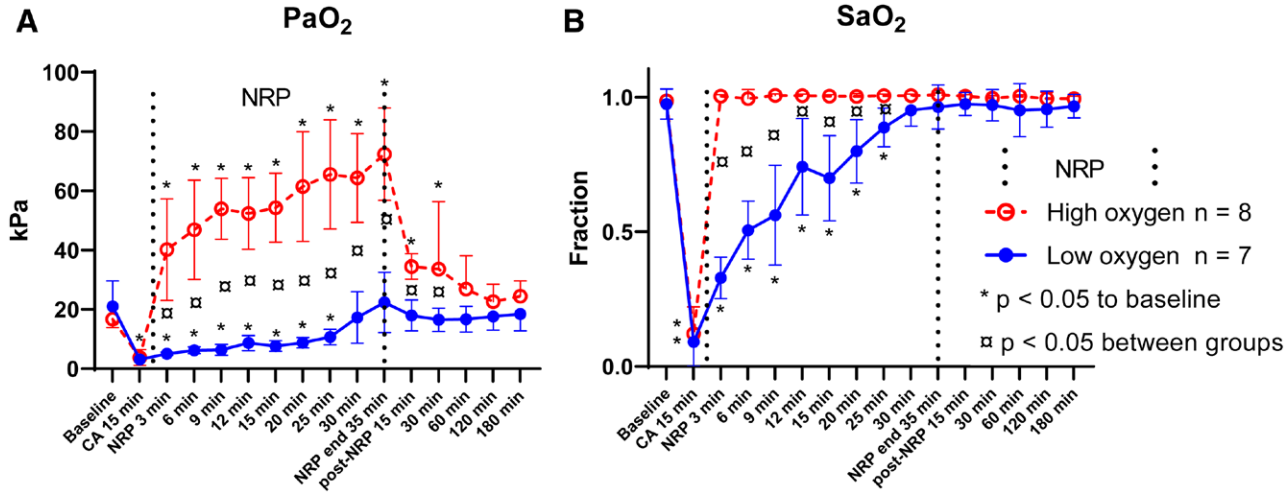

FIGURE 2. Systemic arterial Pao_2 and saturation during and after NRP. Blood oxygen content was significantly higher in the high oxygenation than the low oxygenation group during NRP (A) and (B), whereas Sao_2 and Pao_2 group differences diminished post-NRP (A) and (B). Data are expressed as mean ± SD. Multivariate repeated measurement ANOVA was used for analysis. CA, circulatory arrest; NRP, normothermic regional perfusion; Pao_2, partial pressure O_2; Sao_2, arterial saturation.

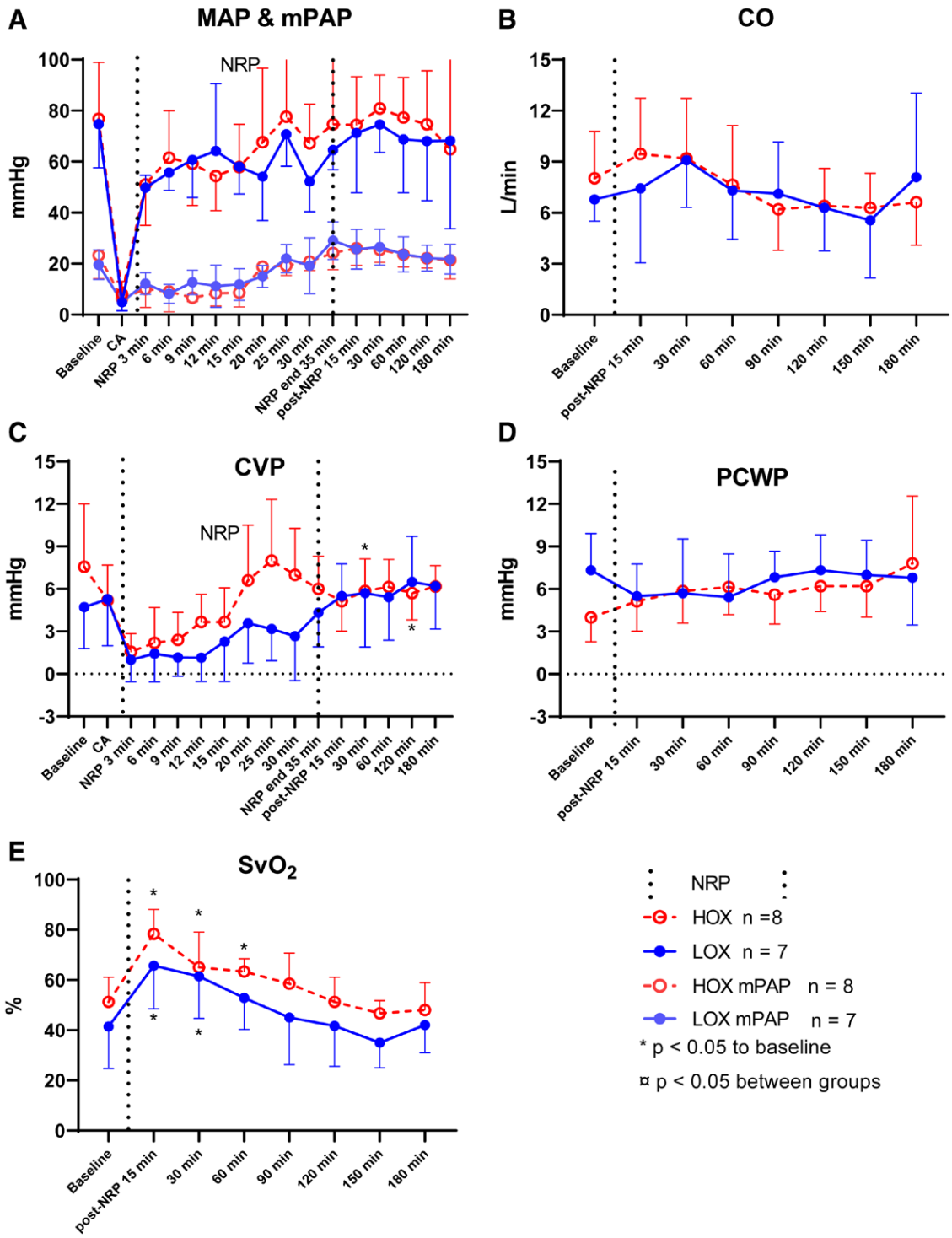

FIGURE 3. Hemodynamic parameters measured by a Swan-Ganz catheter and arterial line. MAP was titrated >60 mm Hg by norepinephrine infusion (A). CO measured by thermodilution was preserved at baseline levels and showed no difference between groups (B). Only baseline and post-NRP measurements were included in the analysis, in which no group difference was found. At 15 and 120 min post-NRP, HOX CVP was significantly lower than baseline (C). PCWP showed no differences between groups and remained on baseline levels (D). SvO_2 showed a trend to significant overall group difference (P = 0.061) and showed significantly improved oxygenation 30 min post-NRP in both groups and 60 min post-NRP in the HOX group (E). Data are expressed as mean ± SD. Multivariate repeated measurement ANOVA was used for analysis. CA, circulatory arrest; CO, cardiac output; CVP, central venous pressure; HOX, high oxygenation; LOX, low oxygenation; MAP, mean arterial blood pressure; mPAP, mean pulmonary arterial blood pressure; NRP, normothermic regional perfusion; PCWP, pulmonary capillary wedge pressure; SvO_2, central venous O_2 saturation.

in the HOX group than in the LOX group was noted in several proinflammatory cytokines, such as IL-1α, IL-1β, IL-6, IL-8, and tumor necrosis factor α. Anti-inflammatory cytokines IL-1 receptor antagonist, IL-2, and IL-10 also showed a significant increase from baseline to the post-NRP period with no differences between the groups. Absolute plasma levels are shown in the **Figure S3, SDC,** http://links.lww.com/TP/C424.

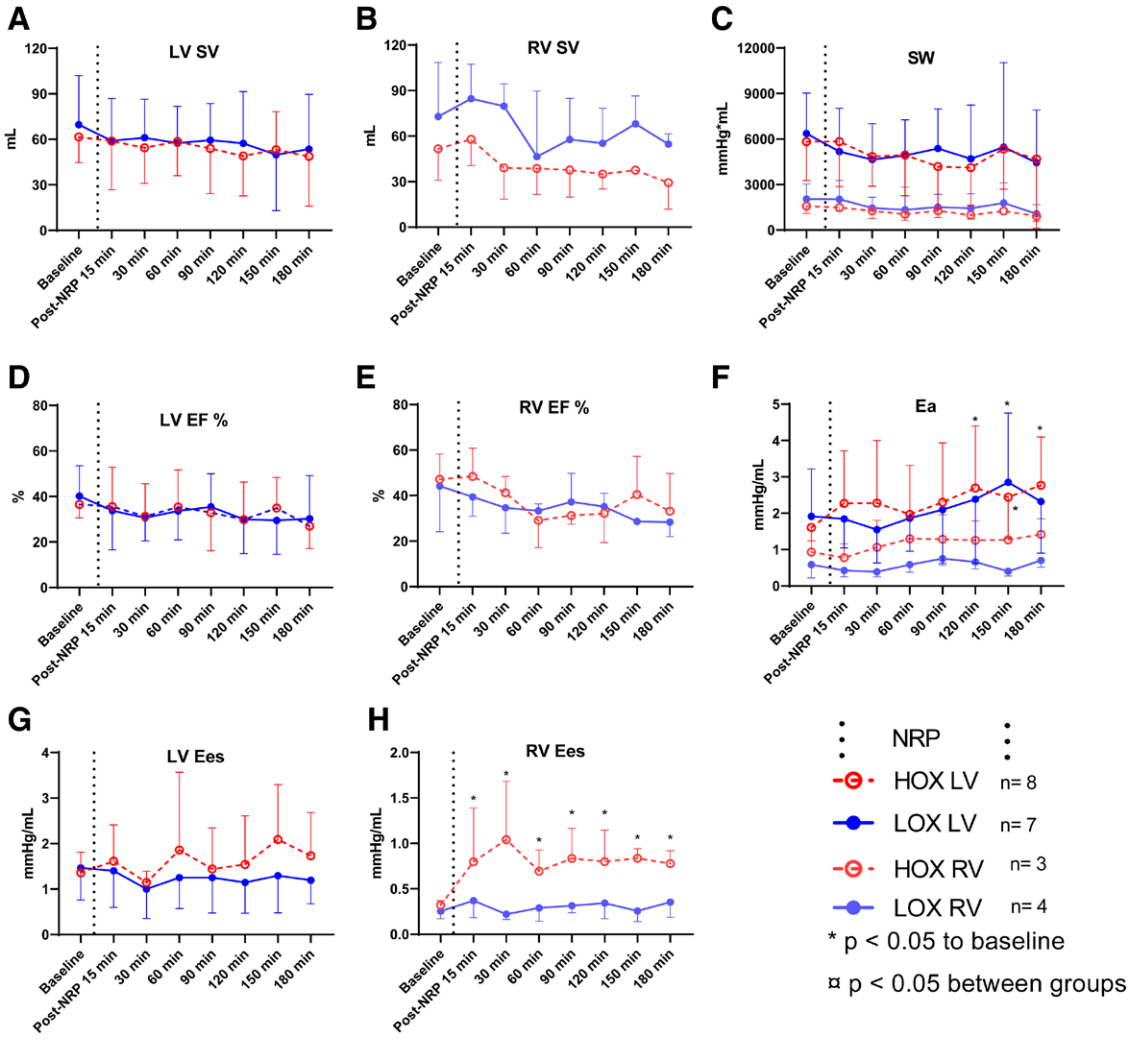

FIGURE 4. Pressure-volume measurements. LV and RV SV remained at baseline levels post-NRP (A) and (B), whereas LOX RV SV was overall significantly higher than HOX (B). LV and RV SW remained at baseline levels post-NRP (C). LV EF% and RV EF% were preserved at baseline levels post-NRP (D) and (E). LV Ea increased significantly 120 min post-NRP in both groups, indicating increasing ventricular afterload, whereas RV Ea remained at baseline levels, with RV HOX Ea being significantly higher than LOX ($P < 0.001$; F). LV Ees was similar between groups and was preserved at baseline levels in both groups (G), and HOX RV Ees was significantly increased post-NRP compared with LOX ($P < 0.001$; H). Data are presented as mean ± SD. Multivariate repeated measurement ANOVA was used for the analysis. Ea, arterial elastance; Ees, end-systole elastance; EF, ejection fraction; HOX, high oxygenation; LOX, low oxygenation; LV, left ventricular; NRP, normothermic regional perfusion; RV, right ventricular; SV, stroke volume; SW, stroke work.

DISCUSSION

In this study, we described a novel porcine model with a standardized NRP protocol for in situ reperfusion of thoracic and abdominal organs in DCD donors. The model proved relatively reliable for the reanimation of donor hearts with 14 of 19 animals successfully weaned from NRP. All animals that were weaned off NRP displayed acceptable cardiac function with preserved Ees in both ventricles. There was increased RV contractility in the HOX group. Both groups showed similar CO values >4 L/min. CK-MB was lower in the HOX group.

The resuscitation rate is comparable to similar DCD NRP porcine studies, where Ribeiro et al reported resuscitation in 10 of 12 animals where no interventions during the NRP period were performed.[7,10] In our study, we found a tendency to higher NRP failure rate, which was driven by the LOX group, suggesting that LOX during NRP can lead to sustained contractile dysfunction; however, the LOX animals that successfully weaned off NRP had acceptably cardiac function and performed to a similar level as the HOX hearts. These findings support Yeh et al, demonstrating higher CPR success rate with HOX.[19,21]

CO was similar between study groups and was preserved in the immediate post-NRP period with a maximum at 30 min post-NRP in both groups. For the rest of the post-NRP period, CO declined despite biochemical corrections and inotropic support. Mixed venous oxygen saturation displayed a similar declining pattern. These observations suggest that a prolonged period before explantation after weaning from NRP might be harmful to the reanimated donor hearts. Both CVP and pulmonary capillary wedge pressure remained at baseline levels post-NRP. Both of these filling pressures were well below the established acceptability criteria of 12 mm Hg.[12] Left ventricular contractility was preserved in both groups with a tendency to increased LV contractility in the HOX group. We found a significant increase in RV Ees, a measure of preload independent contractility, in the HOX group, whereas it stayed

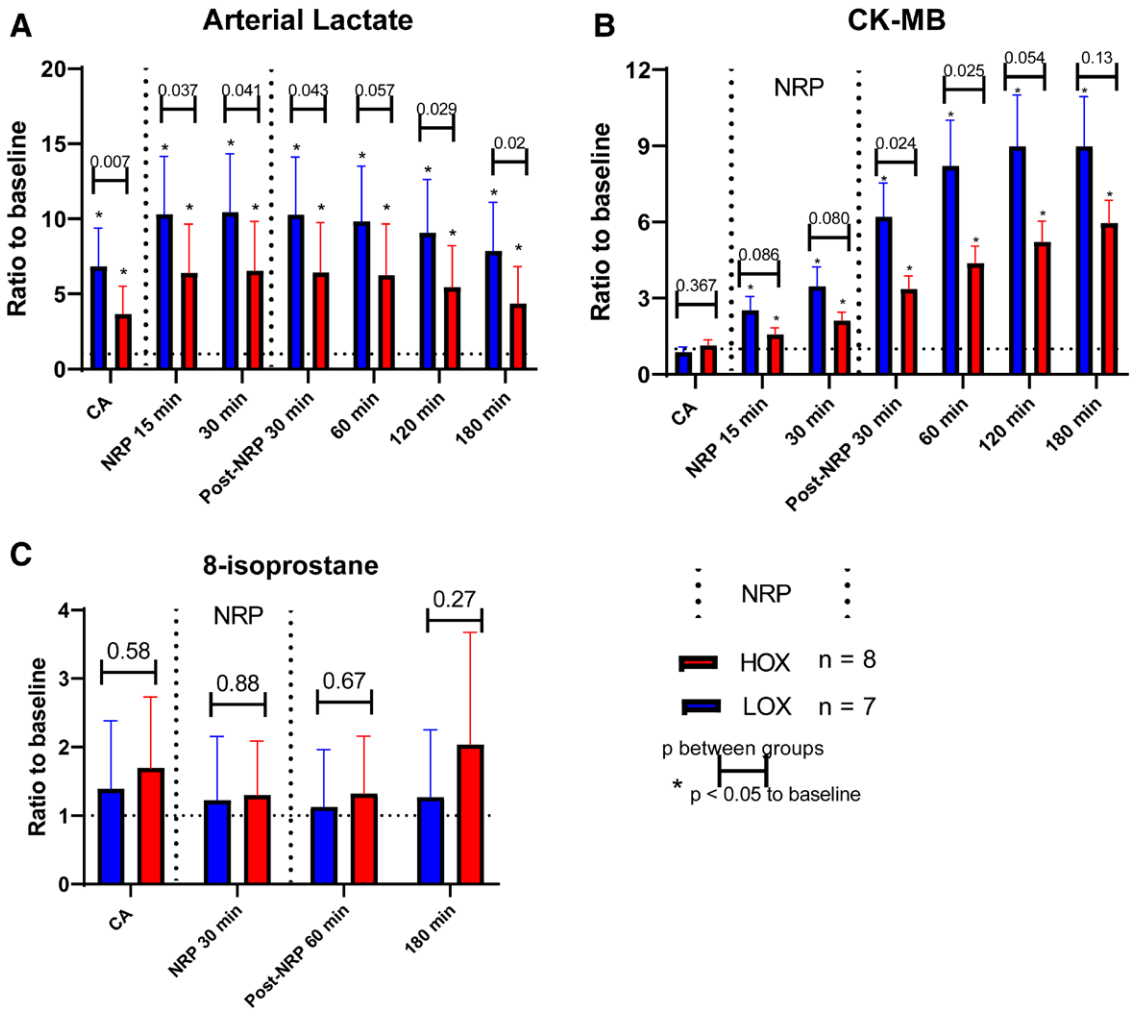

FIGURE 5. Biomarkers. Arterial lactate increased significantly from baseline ($P < 0.001$) during the circulatory arrest and during NRP in both groups, whereas LOX lactate was significantly higher than HOX at all time points. By 120 min post-NRP, lactate started clearing in both groups (A). CK-MB increased significantly more in the LOX group than in HOX (interaction: $P = 0.019$; B), and venous 8-isoprostane remained at baseline levels in both groups (C). Data are expressed as mean ± SD as a ratio to baseline. Multivariate repeated measurement ANOVA was used for the analysis. CA, cardiac arrest; CK-MB, creatinine kinase-MB; HOX, high oxygenation; LOX, low oxygenation; NRP, normothermic regional perfusion.

at baseline levels in the LOX group. This finding suggests that HOX during NRP might be beneficial to the RV function by possibly reducing PVR by pulmonary vasodilatation. It is known that RV is prone to distension during the hypoxic phase of a DCD.[23,24] Therefore, the RV might be vulnerable to hypoxic conditions after weaning from NRP. Distension increases wall stress accompanied by reduced myocardial perfusion and risk of exacerbating myocardial cell death that may lead to RV dysfunction after transplantation. Right ventricular dysfunction is a feared and common complication in the immediate phase after HTX, and any attempts, including optimal oxygenation, to avoid such condition should be pursued.

LOX lactate was significantly higher at CA before the start of the intervention. The same difference was seen during and post-NRP, which may be attributed to the initial higher lactate at CA in the LOX group; however, by 120 min of the post-NRP period, the HOX group showed significantly improved lactate clearance compared with the LOX group. Lactate clearance is an important prognostic marker for survival in patients with sepsis. Whether lactate clearance during NRP is of clinical and prognostic relevance in DCD HTX remains unknown. The increase in CK-MB from baseline was significantly greater in the LOX group than in the HOX group, which may serve as a marker of more severe myocardial ischemic damage due to the lower arterial oxygen content during NRP. HOX has been shown to have deleterious effect by inducing increased oxidative stress in several animal models[15,17]; however, the present results showed that HOX did not induce exaggerated oxidative stress. Inflammatory cytokines increased in both groups after circulatory death and NRP. Several proinflammatory cytokines, including IL-1β, IL-6, and interferon γ, tended to increase more in the HOX group than in the LOX group. In the context of advanced resuscitation after cardiac arrest, HOX has been associated with poor neurological outcome and death.[17] We believe that HOX during NRP could increase the risk of inflammatory damage to the donor organs; however, in our study, this causality was not found in the short term, as both groups showed acceptable cardiac function after NRP. As the donor hearts were not transplanted and followed, we cannot say if the relative higher increase of inflammatory cytokines is a risk factor for later development of graft complications like

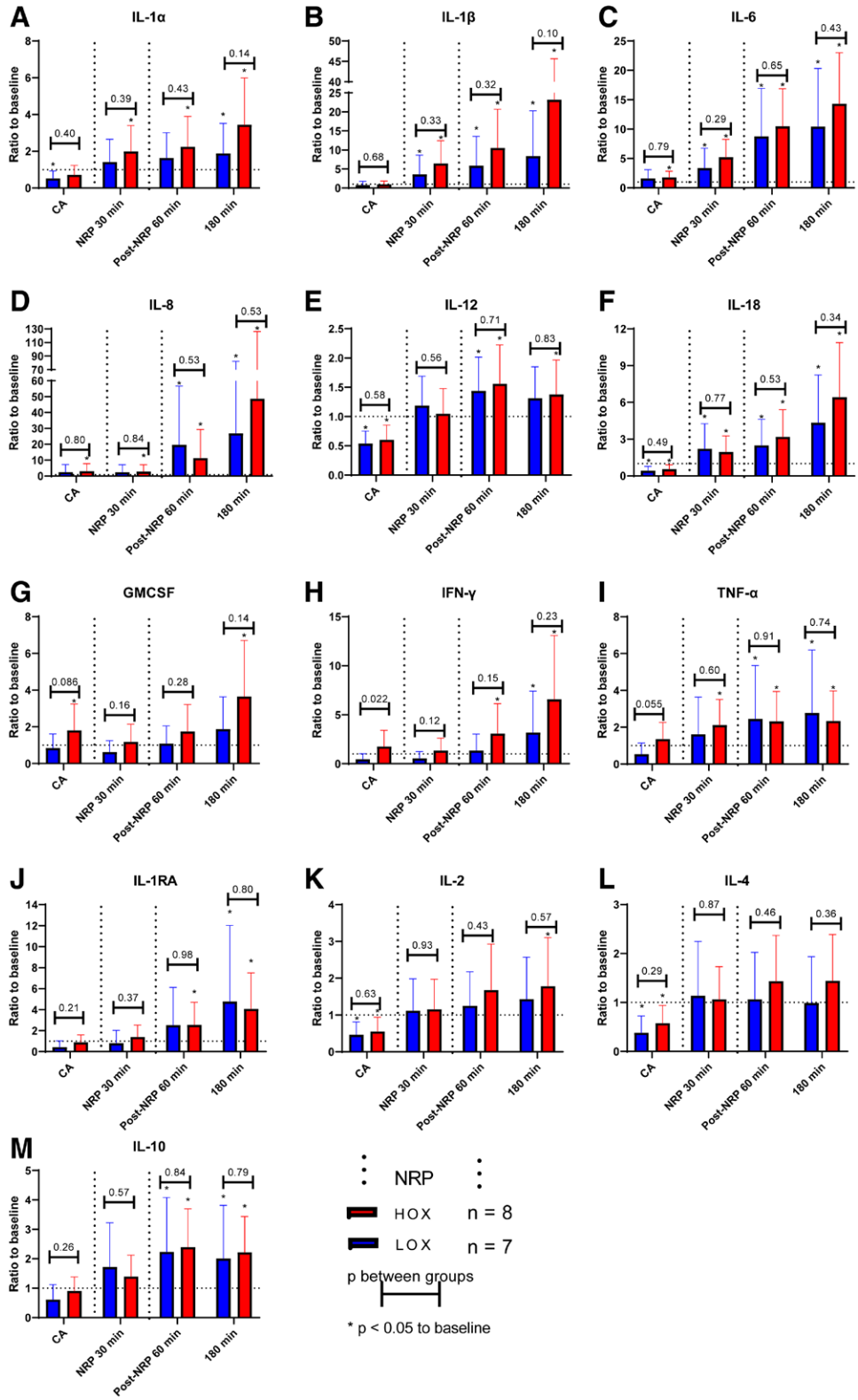

FIGURE 6. Relative change in cytokines from baseline. Cytokines are grouped as proinflammatory (A–I) and anti-inflammatory (J–M). By 180 min post-NRP, nonsignificant trends toward higher pro-inflammatory cytokines (IL-1α, IL-1β, and GMCSF) were found in the HOX group compared with the LOX group. There was no difference in anti-inflammatory cytokines (J–M). Data are expressed as mean ± SD as a ratio to baseline. Multivariate repeated measurement ANOVA was used for the analysis. CA, circulatory arrest; GMCSF, granulocyte macrophage colony stimulating factor; HOX, high oxygen; IFN, interferon; IL, interleukin; LOX, low oxygen; NRP, normothermic regional perfusion; TNF, tumor necrosis factor.

primary graft failure or cardiac allograft vasculopathy. The success of cardiac transplantation is determined by the damage from ischemic and inflammatory injuries sustained in the donor during preservation and from reperfusion in the recipient, as is the possibility to sustain damage in the whole organ donation and transplantation process and as

is the possibility to attenuate the damage by pharmaceutical means. Several compounds have been investigated in mitigating the inflammatory damage from cardiac arrest in both animal models and clinical trials.[25-27] These compounds may play an important role in the future for DCD HTX. Simple means to combat inflammatory damage in HTX has been investigated in porcine models comprising of hypertonic saline infusion to the donor and recipient[28,29] which showed promising results in attenuation inflammatory and ischemic damage from organ donation and transplantation; the method has, however, not been investigated in a DCD setting.

Today, only few animal studies on TANRP after circulatory death have been reported, including a rat study[11] and 2 porcine studies.[7,10] Meanwhile, clinical programs have already emerged in the United Kingdom,[30] Belgium,[1] Spain,[31] and the United States. The rat and porcine studies by Ali et al[7,11] demonstrated the feasibility of reanimating hearts after circulatory death and subsequent transplantation. Ribeiro et al[10] demonstrated the feasibility of prolonged static cold storage after TANRP and tested a novel heart preservation fluid. In both animal and clinical studies, no specific alterations to the conditions used during NRP were investigated, and our study is first to investigate donor interventions and the significance of oxygenation strategy during TANRP.

Limitations

We did not transplant the reanimated hearts, and thus, we cannot conclude how our management strategy may impact the success of HTX; however, important parameters like CO, MAP, and contractility did not change significantly compared with baseline, and these physiological values were comparable to those previously reported from porcine studies.[7,10] We used an open chest and precannulation model during circulatory death to standardize warm ischemic time, which is different from the clinical situation and may have allowed the heart to distort during the hypoxic circulatory arrest; however, we do not believe that the open chest affected the hemodynamics compared with variance in warm ischemic time. Because of ethical concerns, the animals were heavily anaesthetized during WLST, which may have expedited hypoxic circulatory arrest compared with clinically WLST. We are aware of the impact of the low number of animals and interpret the results with caution. Because of the small number of included animals, several trends were found, but they did not reach statistical significance. Only female pigs were used for the study because it is easier to insert a urinary catheter and they are more easily available. Theoretically, it may have limited the applicability of the findings to males; however, the present study found post-NRP hemodynamic function similar to what has been reported in male pigs (Ribeiro et al).[8]

Conclusions

We succeeded in creating a standardized TANRP flow-protocol that enabled the reanimation of circulatory dead donor hearts for evaluation for up to 180 min post-NRP. Reanimated hearts maintained with lower oxygenation during NRP showed greater myocardial damage and a tendency to reduced contractile performance compared with hearts reanimated with higher oxygenation during NRP. The biventricular function of the DCD donor heart was optimal in the immediate phase after weaning, supporting early retrieval of the DCD heart after successful weaning from NRP. This study is first to investigate specific reperfusion conditions during TANRP, and further studies that explore other parameters during NRP are warranted.

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Supplemental Information

Additional supporting information may be found online in the Supplemental Information section.

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