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Impact of normothermic regional perfusion during DCD recovery on lung allograft function: A preclinical study

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KEYWORDS:
lung transplantation;
donation after circulatory death;
ex vivo lung perfusion;
normothermic regional perfusion

BACKGROUND: Normothermic regional perfusion (NRP) has been growing as a novel procurement strategy after circulatory death (donation after circulatory death (DCD)) in the context of heart transplantation. However, the impact of NRP on lung graft viability is largely unknown. We sought to determine lung function after thoraco-abdominal NRP (TA-NRP) in a clinically relevant porcine DCD model.

METHODS: Donor domestic pigs underwent hypoxic cardiac arrest to simulate DCD procurement and were randomly allocated to either 1-hour resuscitation on TA-NRP ($n = 4$) or direct lung procurement (direct procurement and perfusion (DPP), $n = 4$). All lungs were placed on ex-vivo lung perfusion (EVLP) and evaluated for 3 hours to assess functional outcome parameters and suitability for transplantation.

RESULTS: After 1 hour of TA-NRP, cardiopulmonary bypass was weaned, and mean systemic PaO_2/fraction of inspired oxygen was 418 ± 76 mm Hg, which was comparable to baseline (467 ± 41, $p = 0.41$). No significant differences were seen between the groups during EVLP, except for a higher pulmonary artery pressure in the TA-NRP group at 3 hours of EVLP (19.7 ± 1.5 vs 14.7 ± 2.1 mm Hg, $p = 0.02$). Perfusate inflammatory cytokines levels of IL-6 and IL-8 were higher at the first hour of EVLP in the TA-NRP group; however, these differences were not sustained as levels were similar by the last hour of EVLP. There were no differences in histology, cytokines, or metabolic profile of the TA-NRP lungs compared to DPP.

CONCLUSIONS: TA-NRP porcine lungs met functional criteria to proceed to transplantation and demonstrated no significant histological, cytokine, and metabolic differences when compared to DPP

A relevant preclinical porcine model reveals that lung allograft quality after NRP was comparable to direct procurement DCD, underscoring the potential value of considering these donor lungs to increase donor pool.

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https://doi.org/10.1016/j.jhlto.2023.100009

porcine lungs. This study highlights the value of considering TA-NRP lungs for transplant with well-established protocols.
JHLT Open 2023;2:100009

Lung transplantation is a life-saving intervention for patients with end-stage lung disease.[1] However, the growing demand for lung transplantation in this population has resulted in increasingly long waiting lists which increases the waitlist mortality to approximately 30%.[2,3] Therefore, innovative strategies are crucial to expand the donor pool to meet this rising demand. Donation after circulatory death (DCD) has presented a promising opportunity to increase the availability of organs for transplantation.[4-6] However, its utilization remains limited.[7] To address this challenge, recent advancements have shown that combining DCD with normothermic regional perfusion (NRP) offers a promising solution to decrease ischemic times and enhance organ viability, particularly in the context of heart and abdominal transplantation.[8,9]

Thoraco-abdominal NRP (TA-NRP) involves the rapid restoration of circulation after death by cannulating the aorta and right atrium and employing high perfusion rate cardiopulmonary bypass (CPB).[10] This approach allows for prompt recovery of cardiac function and minimizes total ischemic time.[11] The use of TA-NRP protocols and ex-vivo perfusion technology has allowed for an exponential growth in the use of DCD heart donors.[12] While ex-vivo lung perfusion (EVLP) has enabled the same growth in DCD lung donation,[13] the concomitant use of lungs after TA-NRP protocols is still underutilized.[14,15] Current evidence regarding the use of TA-NRP lungs is limited to feasibility reports or small series studies, and the exact impact on lung graft viability and availability remains largely unknown.[16,17]

In light of these considerations, the present study aimed to evaluate the effects of TA-NRP on lung graft quality and viability in a controlled translational pig model. By conducting an in-depth assessment of lung graft parameters during EVLP, we sought to address the existing knowledge gap regarding the impact of TA-NRP on lung allograft function.

Methods

Study groups, DCD, and thoraco-abdominal normothermic regional perfusion

Donor pigs were randomly allocated to the following 2 groups: DCD followed by direct procurement (direct procurement and perfusion (DPP) group, $n = 4$) and DCD followed by TA-NRP (TA-NRP group, $n = 4$). All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals.[18] A median sternotomy was performed to expose the heart and great vessels, and baseline hemodynamic measurements and samples were taken. Figure 1 shows study groups and detailed time points.

DCD and TA-NRP techniques were based on previously published porcine protocols.[19,20] To simulate a clinical controlled DCD scenario, hypoxic circulatory arrest was induced by withdrawing mechanical ventilation under general anesthesia. Asystole was determined as loss of pulsatility and pulse pressure on the arterial waveform. Functional warm ischemia time (f-WIT) was defined as the time since systolic blood pressure fell below 50 mm Hg to the onset of TA-NRP or lung cold flush in the DPP group. After asystole, a 5-minute stand-off period was respected before proceeding to either TA-NRP cannulation or lung cold flush. Central veno-arterial NRP was achieved through cannulation of the aorta and right atrium and supra-aortic vessels were cross clamped. Mechanical ventilation was restarted using a volume control mode set to 7 mL/kg, 5 cm H_2O of positive end-expiratory pressure (PEEP), respiratory rate of 15, and fraction of inspired oxygen (FiO_2) at 50%. A standard CPB circuit comprising of a membrane oxygenator, centrifugal pump, hardshell reservoir, and heat exchanger was used for TA-NRP. Oxygenated normothermic blood was delivered into the ascending aorta targeting a mean arterial pressure around 55 mm Hg. Vasopressor was administered as needed to maintain this target blood pressure. After 1 hour of TA-NRP, CPB was weaned, and lung function was evaluated by measuring pulmonary artery (PA) and left atrium (LA) flows, systemic oxygenation, airway pressures, and lung compliance. After functional evaluation, a Perfadex cold flush followed by lung retrieval was performed and lungs were stored at 4°C for 2 hours. Cold flush and storage happened right after the 5-minute stand-off period in the DPP group.

Baseline characteristics, such as body weight, baseline partial pressure of oxygen (PaO_2)/FiO_2 ratio, interval between withdrawal of ventilation and asystole, f-WIT, and cold ischemic time, were similar between the DPP and TA-NRP groups (Table 1).

After 2 hours of cold static preservation, all lungs underwent 3 hours of EVLP assessment. The Toronto normothermic acellular EVLP method was used for these experiments and is well described elsewhere.[21] EVLP was used in this study as an assessment platform to validate function and suitability of lung allografts to proceed to transplantation. Judgment on the suitability of the allograft for transplantation was formulated according to the standard routine criteria utilized in clinical settings.[22,23]

Histopathological analysis

Tissue samples were taken at specific time points and formalin-fixed, paraffin-embedded, and sectioned for histologic analysis. Lung biopsies were taken at baseline, 1 hour of TA-NRP, pre-EVLP, and post-EVLP. Heart and liver biopsies were taken at the time of lung retrieval. Tissue samples were taken to assess the degree of tissue injury using standard hematoxylin and eosin staining. The International Harmonization of Nomenclature and Diagnostic's Criteria standards were used as the basis of evaluation.[24]

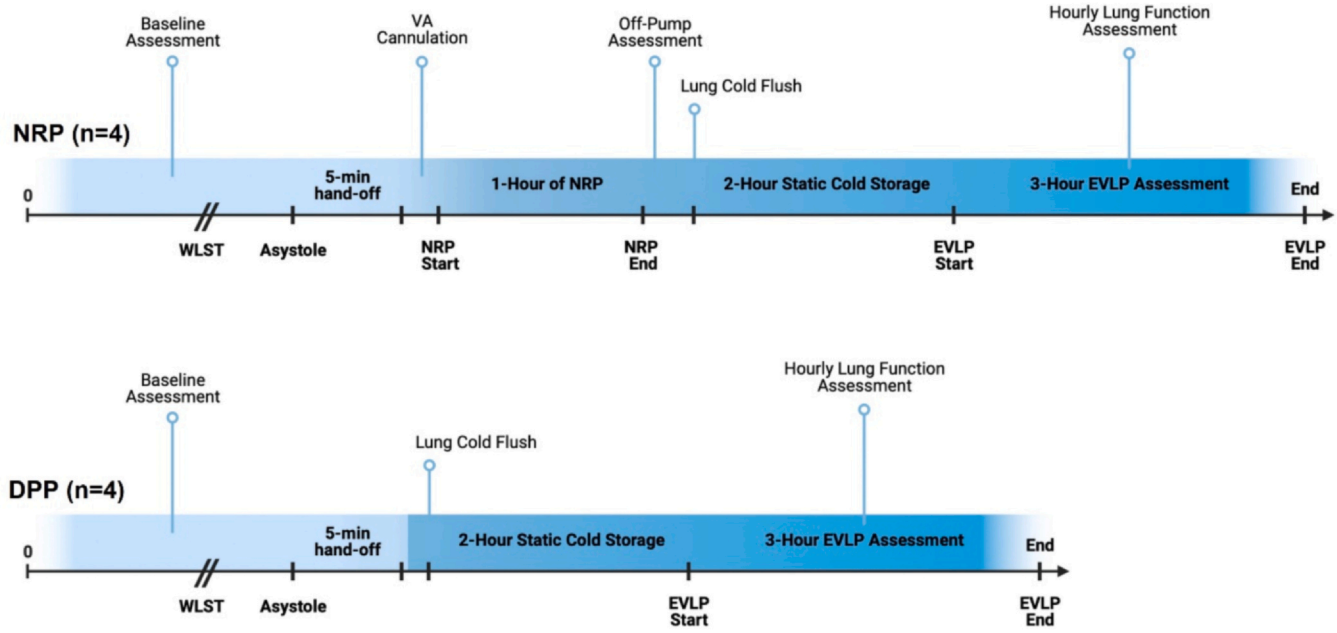

Figure 1 Experimental study design. Domestic pigs were randomly allocated to either of the following 2 groups ($n = 4$ each): (1) DCD followed by direct lung procurement (DPP) or (2) DCD followed by NRP. We used a clinically established DCD protocol by withdrawing respiratory support under deep anesthesia. Once cardiac arrest was documented, a 5-minute hand-off period was respected. For the DPP group, lung cold flush with Perfadex happened right after this 5-minute period and organs were kept at 4°C for 2 hours before normothermic ex-vivo assessment. In the NRP group, after the 5-minute hand-off period, aorta and right atrium were cannulated and normothermic perfusion was immediately started to allow for the heart and circulation to restart. After 1 hour of NRP, CPB was successfully weaned to allow for lung and hemodynamic evaluation. As in the DPP group, there was 2 hours of cold static storage followed by 3 hours of EVLP assessment. CPB, cardiopulmonary bypass; DCD, donation after circulatory death; DPP, direct procurement and perfusion; EVLP, ex-vivo lung perfusion; NRP, normothermic regional perfusion.

Table 1 Baseline Characteristics and Experimental Ischemic Times

Variables	DPP ($n = 4$)	NRP ($n = 4$)	p-value
Body weight, kg	40.55 ± 2.78	38.08 ± 3.60	0.41
Baseline PaO_2/FiO_2, mm Hg	494.5 ± 68.44	467.7 ± 42.67	0.55
Time between WLS to arrest, minute	13.0 ± 2.5	14.0 ± 1.2	0.42
f-WIT, minute	14.0 ± 3.8	12.6 ± 2.7	0.70
Cold ischemic time, minute	150.5 ± 4.79	157.8 ± 14.58	0.41

Abbreviations: cold ischemic time (lung flush to EVLP start); EVLP, ex-vivo lung perfusion; f-WIT, functional warm ischemic time (systolic BP mm Hg to flush or NRP start); PaO_2/FiO_2, partial pressure of oxygen/inspired pressure of oxygen; WLS, withdrawal of life support.
Data reported as Mean ± SD.

Cytokine analysis

Blood samples were collected at baseline for both groups and at different timepoints during TA-NRP. Perfusate samples were collected at the first and last hour of EVLP. All samples were stored at −80°C and evaluated for inflammatory response using a porcine multiplex cytokine assay from MilliporeSigma. The analytes were read and analyzed with the Luminex xMAP platform (Biotechne, MN).

Metabolomic analysis

Blood and lung tissue samples were assayed for untargeted measurements of metabolites (Metabolomics Core, Mayo Clinic, MN). Samples were extracted, prepared, and analyzed following standard practice and protocols from the Metabolomics Core (described in detail in the Supplementary section). A liquid chromatography mass spectrometry platform was used to perform untargeted analysis using the METLIN database to provide putative metabolite identification.

Statistical analyses

All results are expressed as mean ± SD. Mann-Whitney U tests were performed to compare differences between groups. For longitudinal data, a 2-way analysis of variance (ANOVA) for repeated measures was performed followed by a Bonferroni correction for multiple comparisons. Statistical tests were also performed on metabolomic data after data normalization. Univariate statistical analysis, Students' unpaired t-test between the different timepoints and groups were performed with multiple testing correction to identify differentially expressed metabolites between groups with statistical significance (false discovery rate (FDR) adjusted p-value ≤0.05 and fold change ≥1.5). Figures and statistical analyses were done using GraphPad Prism 9.0 software. Statistical significance was defined at $p < 0.05$.

Results

Lung assessment after TA-NRP

After 1 hour of TA-NRP, CPB was weaned, and hemodynamic and pulmonary parameters were taken (Table S1). In the TA-NRP group, mean PaO_2/FiO_2 ratio after weaning of CPB was 418.0 ± 76.31 mm Hg, which was comparable to baseline measurement of 467.6 ± 42.67 mm Hg ($p = 0.41$).

PA pressure was measured at 30 minutes and after weaning TA-NRP. Mean PA pressure at 30 minutes of NRP was 16.5 ± 4.4 mm Hg, which after weaning went up to 19.6 ± 2.0 mm Hg. Left atrium pressure after weaning CPB was 10.5 ± 3.1 mm Hg. Airway pressure and lung compliance were similar during TA-NRP and after weaning.

Lung function during EVLP

During the 3 hours of EVLP assessment, graft oxygenation capabilities ($p = 0.21$), airway pressures ($p = 0.36$), and dynamic ($p = 0.21$), and static ($p = 0.25$) lung compliances were comparable between the study groups (Figure 2A-E). Pulmonary artery pressure was significantly higher in the TA-NRP group at the third hour of perfusion ($p = 0.01$). Lactate levels measured at the end of EVLP were 4.9 ± 1.0 mmol/liter in the DPP group and 5.6 ± 1.0 mmol/liter in the TA-NRP group ($p = 0.34$, Figure 2F). An important assessment during EVLP is edema formation, which can be evaluated both by the amount of Steen loss throughout perfusion and chest X-rays. Average Steen loss was 135 ± 53 ml in the DPP lungs and 146 ± 67 ml in the TA-NRP ones ($p = 0.97$, Figure 3A). Similarly, chest X-rays images by the end of perfusion were similar between the groups (Figure 3B). A comparison of the lung gross appearance after the perfusion period for each experimental condition is also shown in Figure 3C. Importantly, all 8 allografts were deemed transplantable by the end of EVLP assessment based on clinical criteria.

Tissue injury scores

Comprehensive histopathological analysis of lung tissue revealed findings of pleural/interlobular edema, congestion, and hemorrhage (Figure 4A). Acute lung injury scores were calculated for all samples and were similar in the 2 groups across all compared time points (baseline, pre-EVLP, and post-EVLP) (Figure 4B-D). Tissue injury scores were also calculated for samples taken at different time points in the TA-NRP group and did not reveal any differences overtime (Figure S1). Interestingly, postretrieval TA-NRP heart sections displayed higher grade and higher frequency interstitial hemorrhage,

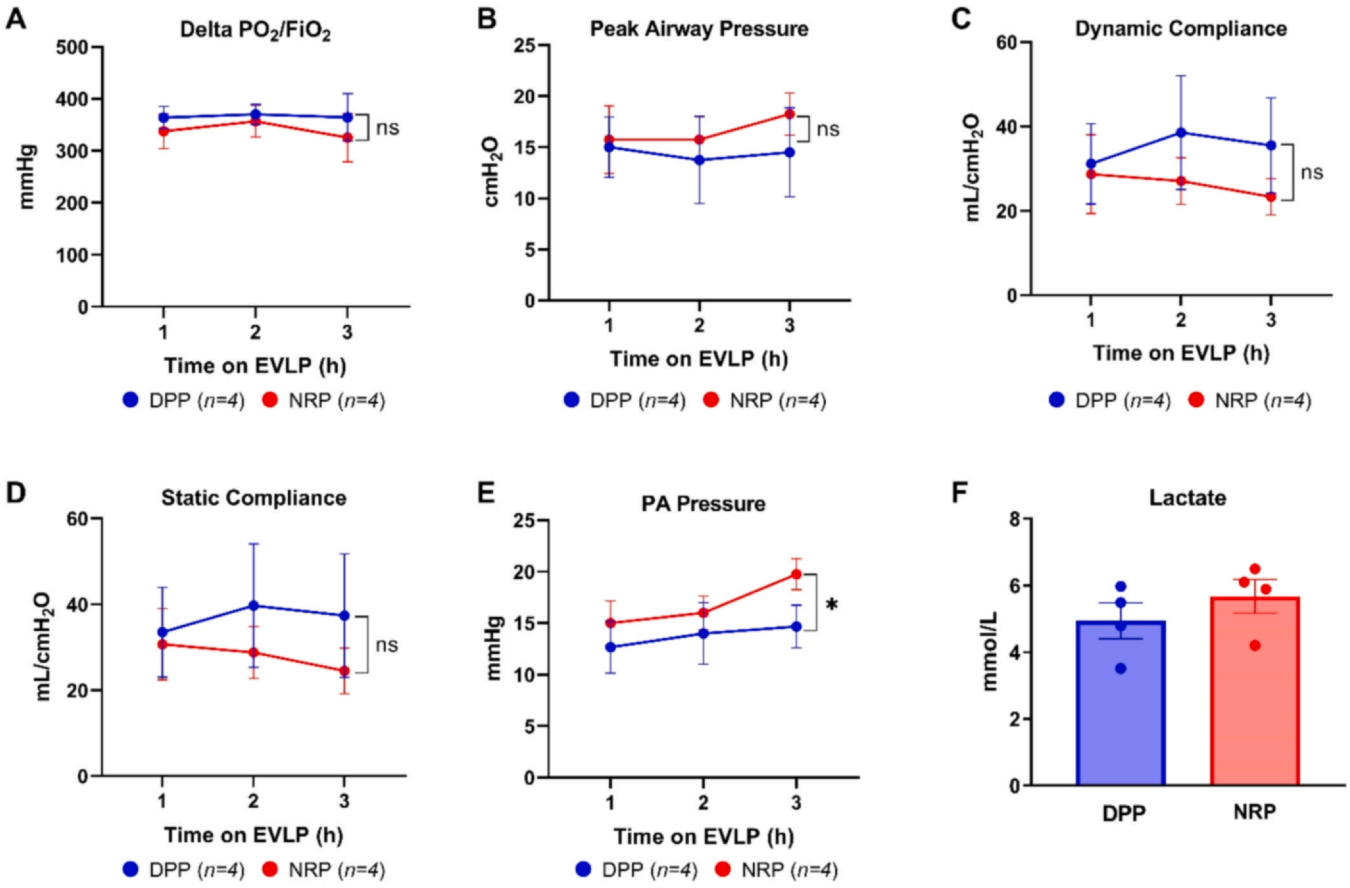

Figure 2 Physiologic parameters during the 3 hours of EVLP. Parameters were comparable between groups, except for higher PA pressure at the last hour in the NRP group. (A) Delta PaO_2/FiO_2 ratio (ΔPaO_2 = perfusate LA PaO_2 – perfusate PA PaO_2 [mm Hg]), (B) peak airway pressure, (C) dynamic and (D) static lung compliances, and (E) PA pressure. (F) shows lactate levels at the last hour of EVLP. *$p < 0.05$. DPP, direct procurement and perfusion; EVLP, ex-vivo lung perfusion; FiO_2, fraction of inspired oxygen; NRP, normothermic regional perfusion.

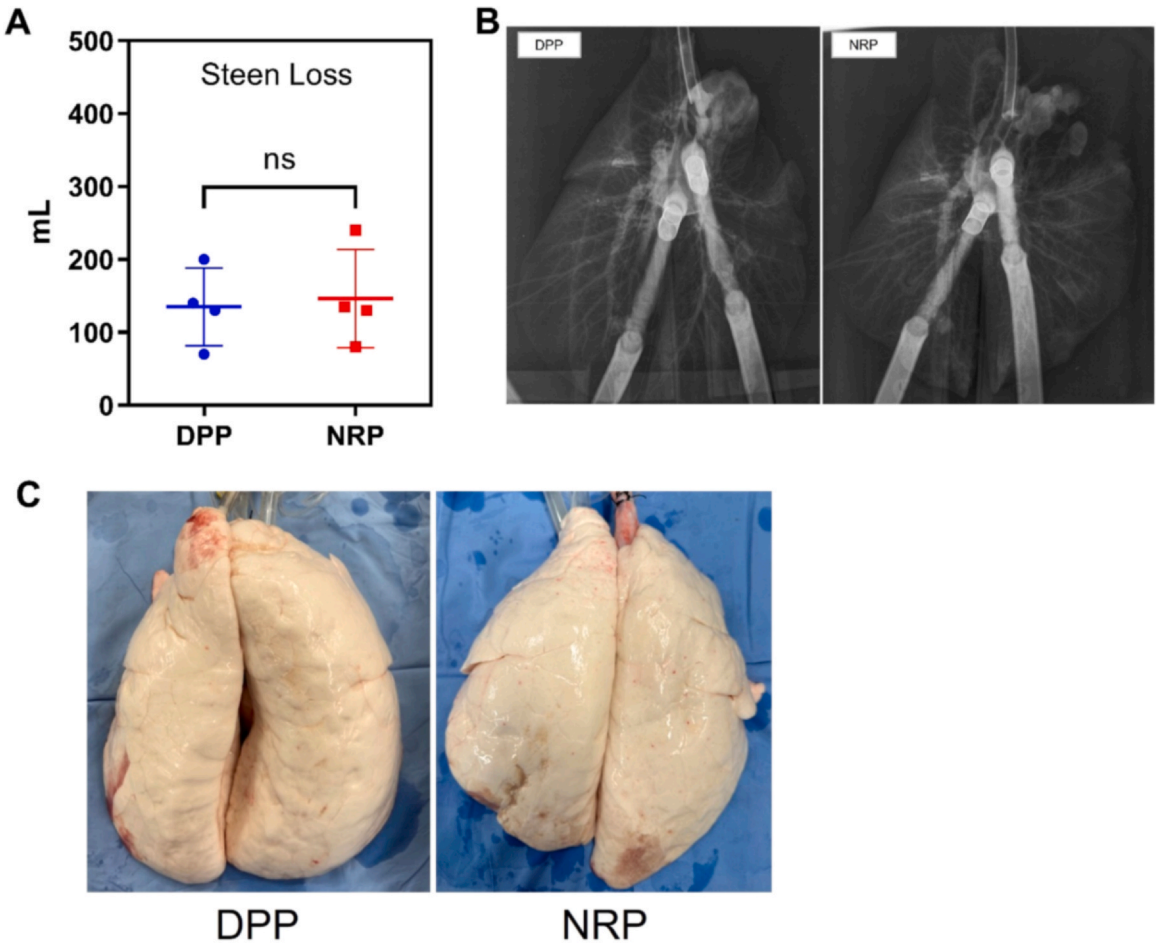

Figure 3 Pulmonary edema assessment. (A) Steen loss during the 3 hours of EVLP were recorded and were low and similar in both groups. (B) Additional assessment of edema formation was done by getting a chest X-ray at the end of EVLP and were also comparable between groups. (C) Representative pictures of macroscopic appearance after EVLP. DPP, direct procurement and perfusion; EVLP, ex-vivo lung perfusion; NRP, normothermic regional perfusion; ns, nonsignificant.

edema, and contraction band necrosis compared to DPP hearts, which is explained as a result of the 1 hour of reperfusion in the TA-NRP group and no reperfusion period in the DPP group (Figure S2). No changes were identified in postretrieval liver sections.

Inflammatory response

Plasma cytokines levels of tumor necrosis factor alpha (TNF-α), interleukine-2 (IL-2), interleukine-8 (IL-8), and interleukine-1-beta (IL1-β) were below the detection limit for all measured timepoints during TA-NRP. Table 2 shows the levels of interferon-gamma (IFN-γ), interleukine-4 (IL-4), interleukine-6 (IL-6), interleukine-10 (IL-10), and interleukine-12 (IL-12) at baseline, 5, 30, and 60 minutes of TA-NRP. There were no significant increases over time across all inflammatory cytokines.

Il-2, IL1-β, and IL-4 levels were undetectable in EVLP perfusate samples measured at the first and last hour of perfusion in DPP and TA-NRP lungs. A similar increase during EVLP was noted in TNF-α, IFN-γ, IL-6, IL-8, IL-10, and IL-12 levels in both study groups. Interestingly, the TA-NRP lungs demonstrated higher levels of IL-6 and IL-8 and lower levels of IL-10 at the first hour of EVLP compared to the DPP lungs. However, by the third hour of perfusion, the levels of these cytokines were back to similar levels in both groups (Table 3).

Metabolic profile of TA-NRP allografts is similar to DPP despite metabolic changes during TA-NRP

To understand whether there are metabolic changes occurring during TA-NRP, plasma was collected before DCD induction (baseline) and at different timepoints during CPB (30 and 60 minutes). Plasma concentrations of 75 metabolites were significantly different between baseline and 30 minutes, whereas only 31 were different between baseline and 60 minutes (Tables S2 and S3, respectively, $p < 0.05$, fold change > 1.5). Principal component analysis (PCA) plot visualization showed that samples from each timepoint form distinct and separate clusters compared to baseline, suggesting a clear shift in metabolic profile after initiation of TA-NRP (Figure 5A, B). Interestingly, the PCA plots also show that the metabolic processes dynamically evolve over time during TA-NRP and become less distinct toward the end of TA-NRP, suggesting that the duration of TA-NRP might have an impact on the metabolic state of the samples.

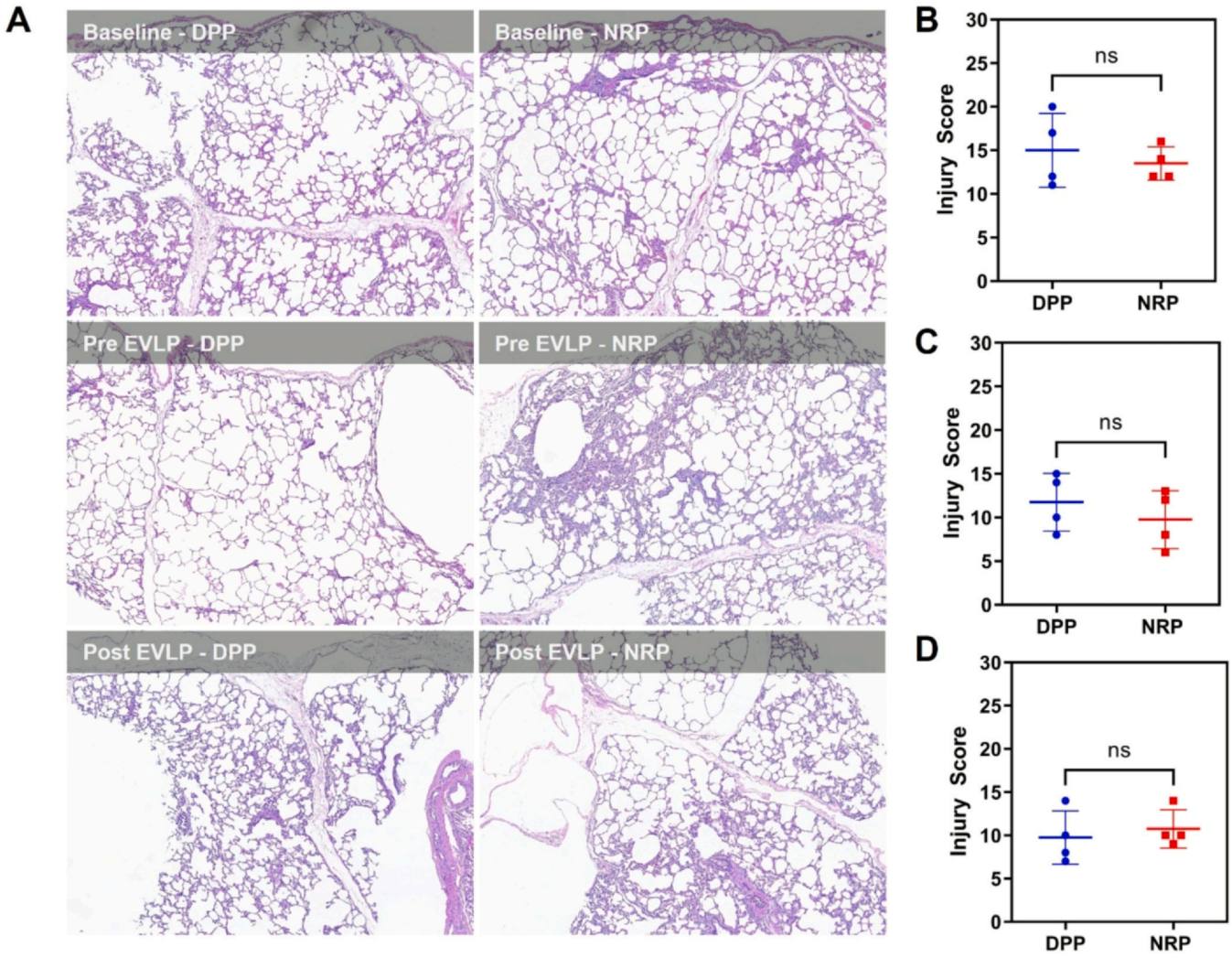

Figure 4 Histopathological analysis of lung tissue. (A) Representative image of hematoxylin-eosin-stained specimen at different timepoints of the experiment. Pleural/interlobular edema, congestion, and hemorrhage were noted in all samples with the same frequency. Semiquantitative lung injury scores were low and similar when comparing DCD and NRP lungs at baseline (A), pre-EVLP (B), and post-EVLP (C). DPP, direct procurement and perfusion; EVLP, ex-vivo lung perfusion; NRP, normothermic regional perfusion; ns, non-significant.

In contrast, metabolic analysis of lung tissue samples collected pre-EVLP and post-EVLP in both TA-NRP and DPP groups did not show any significantly differences in the metabolites. Likewise, PCA plot visualization showed that samples clustered together when comparing metabolic profile of TA-NRP and DPP lung allografts, suggesting similar metabolic state of both type of allografts despite metabolic differences seen in the plasma during TA-NRP (Figure 5C, D).

Discussion

By comparing DCD-NRP lungs with direct procurement DCD lungs, we sought to elucidate the effects of TA-NRP on lung graft quality and viability using a controlled porcine preclinical model. While studies investigating the tolerable duration of warm ischemia in DCD lung transplantation settings have been published,[19,25] we established our TA-NRP porcine model to assess the impact of this reperfusion

Table 2 Levels of Inflammatory Cytokines in Plasma During NRP

Cytokine levels in plasma (pg/ml)	Baseline	5 minutes	30 minutes	60 minutes	*p*-value
IFN-g	2140 ± 2020	2358 ± 1973	1084 ± 464	2904 ± 1925	0.76
IL-4	1372 ± 1056	614 ± 497	596 ± 474	456 ± 329	0.89
IL-6	542 ± 507	54 ± 31	60 ± 25	61 ± 23	0.84
IL-10	402 ± 191	746 ± 469	740 ± 441	612 ± 356	0.90
IL-12	678 ± 193	476 ± 69	562 ± 64	556 ± 57	0.72

Table 3 Levels of Inflammatory Cytokines in Perfusate During EVLP

Cytokine levels in EVLP perfusate	DPP ($n = 4$)	NRP ($n = 4$)	p-value
EVLP 1 hour (pg/ml)			
TNF-α	625 ± 232	282 ± 213	0.31
IFN-γ	570 ± 90	646 ± 95	0.55
IL-6	20 ± 0.0	104 ± 11	0.007
IL-8	22 ± 6.3	94 ± 30	0.03
IL-10	42.5 ± 8.5	20 ± 0.0	0.04
IL-12	72.4 ± 34.2	48 ± 12.4	0.88
EVLP 3 hour (pg/ml)			
TNF-α	4785 ± 2294	3272 ± 1782	0.55
IFN-γ	647 ± 316	2306 ± 985	0.06
IL-6	1177 ± 434	1894 ± 359	0.16
IL-8	1912 ± 755	5915 ± 3188	0.91
IL-10	510 ± 151	474 ± 181	0.96
IL-12	285 ± 101	192 ± 47	0.73

strategy on lung allografts. The comprehensive assessment of lung graft quality during TA-NRP and EVLP allowed us to evaluate critical parameters, such as oxygenation capacity, tissue integrity, inflammatory status, and metabolic profiles, to address the knowledge gap surrounding the impact of TA-NRP on lung allograft function. All TA-NRP lungs demonstrated effective oxygenation and preserved lung function after weaning from CPB. Lung function, gas exchange, dynamic and static compliance, metabolic profile, and edema formation were comparable between the TA-NRP and DPP groups during EVLP assessment. Macroscopic and microscopic analysis also showed similar findings, and objective tissue injury score evaluation was overall low and not different between and within the groups. Interestingly, TA-NRP did not induce a significant systemic inflammatory response, although initial differences in cytokine levels were observed during EVLP, which became attenuated over time. Despite metabolic changes during TA-NRP, this did not translate to changes in the lung allografts of both groups which demonstrated similar metabolic profiles. Importantly, in this experimental model of TA-NRP after EVLP assessment, all the allografts were deemed suitable for transplantation.

Early clinical reports on the experience of lung graft viability after TA-NRP protocols have been controversial and the lack of studies investigating the effect of TA-NRP on the quality of lungs have limited the use of these type of donors. In fact, a recent study from our group looking into national trends of DCD donor lung utilization in the United States highlighted a lower utilization rate of donor lungs after TA-NRP.[15] The study also reported that concomitant lung retrieval rate during DCD heart procurements was as low as 19% when compared to 38% in donation after brain death. Survival and post-transplant outcomes are favorable after TA-NRP in lung transplantation,[15,26-28] which align with the results of our present study. Malas et al[29] analyzed the UNOS database and found that of the 422 reported lung allografts from TA-NRP perfused donors, only 14.9% of lungs were transplanted. Despite this low utilization rate, outcomes after transplantation, such as rates of extracorporeal membrane oxygenation requirement, mechanical ventilation, and inhaled nitric oxide, were numerically lower in the TA-NRP group compared to direct procurement DCD.[29] Six-month survival was 85.7% and comparable to the direct procurement group. Longer-term follow-up is required, but these findings provide additional evidence for the use of TA-NRP as a viable strategy during concomitant heart DCD procurement.

One of the concerns associated with the use of TA-NRP is the potential inflammatory and metabolic changes triggered by high flows during CPB.[30] Interestingly, we collected blood at different timepoint during the 1-hour TA-NRP run and no significant increase in plasma cytokine levels overtime was found. Regarding the levels of tissue lung inflammation as measured by cytokines in the EVLP perfusate samples, there was a similar increase in TNF-α, IFN-γ, IL-6, IL-8, IL-10, and IL-12 levels during EVLP in both the DPP and TA-NRP lungs. While the finding of a relatively mild plasma inflammatory response partially contrasts with the known notion of a strong systemic response elicited by any form of extracorporeal circulation, it could be related to the duration of CPB. Importantly, during the 3 hours of EVLP, the inflammatory cytokines levels in the perfusate increased significantly. It should also be noted that, in our study, blood transfusions were not performed during TA-NRP, which contrasts with certain clinical practices,[31] which could potentially contribute to additional inflammation. Of note, the TA-NRP lungs demonstrated higher levels of IL-6 and IL-8 and lower levels of IL-10 at the first hour of EVLP compared to the DPP lungs. However, these differences were not sustained, as the cytokine levels became similar between the groups by the third hour of perfusion. These findings suggest that TA-NRP may induce an initial inflammatory response at the beginning of EVLP due to the prior period of in situ reperfusion, but the response becomes attenuated over time.

Interestingly, there was a clear shift in metabolic profile in plasma samples collected during the 1-hour of TA-NRP; however, this does not seem to affect the metabolic profile of TA-NRP lungs, which demonstrate similar metabolic profile to DPP lungs. This further corroborates the phenotypic similarity between DPP and TA-NRP allografts. We did observe that the duration of TA-NRP might have an impact in the metabolic changes seen in the plasma. Importantly, clinical TA-NRP protocols try to wean CPB as soon as possible, with reports ranging from less than 25 to up to 45 minutes of TA-NRP duration.[29,32] Our study pushed the CPB duration to 60 minutes which could explain the metabolic and inflammatory changes we observed. Therefore, future studies focusing on shorter and even prolonged durations of TA-NRP would be interesting to understand the impact on lung allografts of CPB duration in the context of TA-NRP.

An important observation in our study was the higher PA pressure noted in the TA-NRP lungs during EVLP. It is important to note that we did not observe increased vascular leakage in the lungs and the histological analysis did not

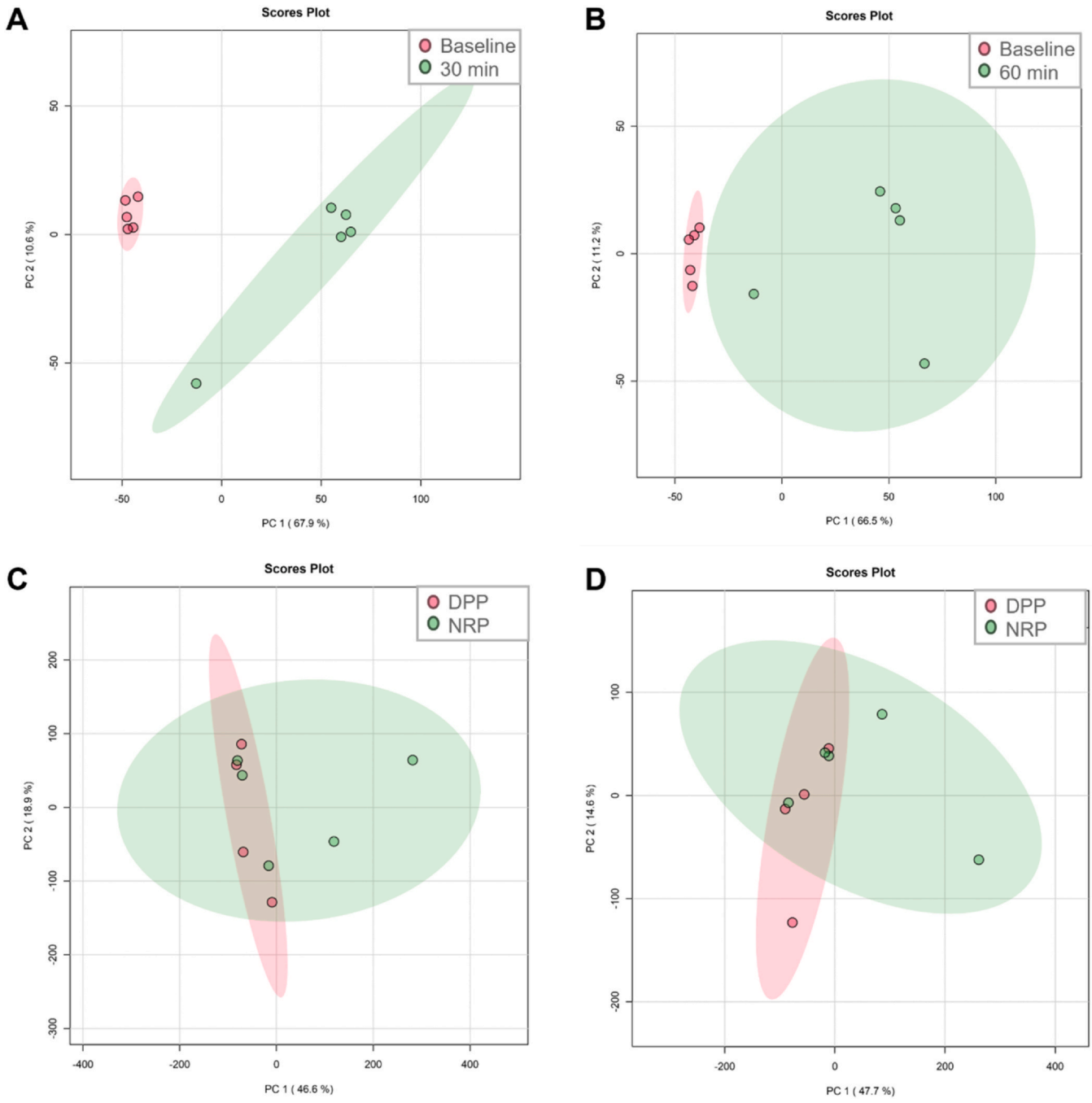

Figure 5 Principal component analysis of global metabolome. (A) Plasma at baseline compared to 30 minutes of NRP and (B) plasma at baseline compared to 60 minutes. Lung tissue samples comparing DPP and NRP allografts pre-EVLP (C) and post-EVLP (D). PC, principal component; DPP, direct procurement and perfusion; NRP: normothermic regional perfusion.

show signs of significant structural damage. Edema formation, assessed by Steen loss, and chest X-ray images at the end of perfusion were normal and comparable to the DPP lungs. Lung grafts procured after TA-NRP may therefore undergo transient vascular reactivity, resulting in increased pulmonary artery pressures. Whether this effect persists after EVLP and impact a potential further implant of the allograft in the host could not be determined. The 1-hour timing of reperfusion during TA-NRP might have been a contributing factor to this observation and warrant additional exploration to identify the optimal TA-NRP duration for DCD lung transplantation.

The limitations of our study should be acknowledged. First, we did not perform a lung transplant model; however, it is worth noting that existing literature supports the correlation between EVLP findings and post-transplant outcomes.[19,33] Our study focused on acute tissue injury and immediate graft function, and further investigations are required to assess longer term outcomes. Despite not finding any statistically significant differences in the EVLP parameters during 3 hours of EVLP, TA-NRP lungs did demonstrate a trend of under performing when compared to the DPP ones. This could be due to the small number of animals in each group. However, all the TA-NRP lungs met criteria to proceed to transplantation. Future studies

should include a transplant model and perhaps investigate different TA-NRP protocols and extend the duration (both in situ and ex vivo) to evaluate if there's any impact by prolonging the CPB run. Third, continuous left atrium pressure was not measured during TA-NRP, which could have provided valuable information. However, we did measure left atrial pressure during TA-NRP and after weaning CPB, and levels were within normal limits. In conclusion, our study provides valuable insights into the impact of TA-NRP on lung allograft function and viability. The comparable lung function, oxygenation capabilities, and absence of excessive edema formation observed in our study support the feasibility of TA-NRP as a strategy without detrimental effects on lung allografts. In fact, this is consistent with our clinically findings, as we have transplanted lungs following TA-NRP.[33] Additional studies are needed to expand the knowledge regarding longer term outcomes after TA-NRP; however, our findings have the potential to influence clinical practice and, importantly, increase the donor pool available by increasing the use of DCD lungs during concomitant heart procurement.

CRediT authorship contribution statement

Conceptualization: R.V.P.R., F.A.R., M.C., S.A.S.; Methodology: R.V.P.R., F.A.R., C.S., C.C., A.R., S.A.S.; Investigation: R.V.P.R., F.A.R., T.L.S., C.S., C.C., A.R., J.L.B., D.G.E., S.A., B.T.D., M.C., S.A.S.; Writing—original draft: R.V.P.R., S.A.S.; Writing—review and editing: R.V.P.R., F.A.R., T.L.S., C.S., C.C., A.R., J.L.B., D.G.E., S.A., B.T.D., M.C., S.A.S.

Data availability

All data generated or analyzed during this study are included in this published article and its additional file. The data collected and analyzed for this study are available from the corresponding author upon reasonable request.

Disclosure statement

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Sahar Saddoughi reports financial support was provided by Mayo Clinic Minnesota. No conflict of interest to disclose. M.C. is founder of Traferox Technologies Inc. (Traferox devices were not used for the performance of this study) and consultant for Lung Bioengineering.

We thank Joanne Pedersen, Lisa Yngsdal, Andrew Albertsen, and Shari Sutor for surgical and technical support.

This work was supported by the CAO/Lung Transplant Award, Mayo Clinic internal funding to S.A.S.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhlto.2023.100009.

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