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MAIN TEXT

Oxygenated machine perfusion at room temperature as an alternative for static cold storage in porcine donor hearts

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Abstract

Background: There is a continued interest in ex situ heart perfusion as an alternative strategy for donor heart preservation. We hypothesize that oxygenated machine perfusion of donor hearts at a temperature that avoids both normothermia and deep hypothermia offers adequate and safe preservation.

Methods: Cardioplegia-arrested porcine donor hearts were randomly assigned to six hours of preservation using cold storage (CS, $n = 5$) or machine perfusion using an oxygenated acellular perfusate at 21°C (MP, $n = 5$). Subsequently, all grafts were evaluated using the Langendorff method for 120 min. Metabolic parameters and histology were analyzed. Systolic function was assessed by contractility and elastance. Diastolic function was assessed by lusitropy and stiffness.

Results: For both groups, in vivo baseline and post-Langendorff biopsies were comparable, as were lactate difference and myocardial oxygen consumption. Injury markers gradually increased and were comparable. Significant weight gain was seen in MP ($p = 0.008$). Diastolic function was not impaired in MP, and

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lusitropy was superior from 30 min up to 90 min of reperfusion. Contractility was superior in MP during the first hour of evaluation.

Conclusion: We conclude that the initial functional outcome of MP-preserved hearts was transiently superior compared to CS, with no histological injury post-Langendorff. Our machine perfusion strategy could offer feasible and safe storage of hearts prior to transplantation. Future studies are warranted for further optimization.

KEYWORDS

evaluation, ex situ heart perfusion, machine perfusion, preservation, temperature

1 | INTRODUCTION

The demand for donor hearts exceeds the supply. As the use of extended criteria donors and donors after circulatory death (DCD) becomes increasingly popular, there is a continued interest in alternative strategies for donor heart preservation.

Since the beginning of clinical heart transplantation, static cold storage (CS) has been a cornerstone in achieving adequate ex situ heart preservation. Key elements of this preservation technique are cardioplegic arrest of the donor heart and maintenance of low myocardial temperature. Cardioplegic electrical arrest decreases the oxygen demands of myocytes by 90%.[1] Flushing the coronary arteries with a cold cardioplegic fluid and preserving the heart in a hypothermic solution enables maintenance of low metabolism rates. This is thought to mitigate ischemic injury.[2] Ischemic myocytes lose their ability to regulate ion homeostasis, leading to acidosis, necrosis, edema, and depletion of adenosine triphosphate (ATP).[3] Solely decreasing the temperature of myocardial tissue by cooling its surrounding fluid is insufficient for optimal preservation,[4] especially when pushing the boundaries for new sources of donor hearts.

Ex situ heart perfusion (ESHP) aims to prevent ischemic injury and stimulate aerobic metabolism, leading to restoration of myocardial homeostasis.[5] Regeneration of ATP is achieved by supplying the myocytes with a tailored, oxygenated perfusate during ESHP. Currently, there is no consensus on what the optimal temperature is for machine perfusion preservation. Normothermia offers the advantage of functional evaluation, though functional parameters are not available in the current clinical devices and the evaluation is based solely on metabolic parameters. When device malfunction occurs, the donor heart is immediately exposed to warm ischemia. Profound hypothermia (<10°C) during perfusion offers the same protective benefits as CS. However, lowering the temperature below 15–20°C does not offer additional significant reductions in oxygen uptake during continuous oxygenated ESHP.[1,6,7] Also, deep hypothermia itself could lead to cell death, as it results in disruption of ion regulatory mechanisms and imbalances in ATP supply and demand.[8] To date, the majority of studies describing different temperatures during oxygenated ESHP focus on profound hypothermia (<10°C) and normothermia (>35°C).

We hypothesize that oxygenated ESHP at a temperature that avoids both normothermia and deep hypothermia offers adequate and safe preservation. In this study, we compared six hours of CS porcine donor heart preservation with oxygenated acellular machine perfusion preservation at 21°C.

2 | METHODS

The study protocol was approved by the Ethics Committee of the Catholic University Leuven (ref: 108/2017). Animal care was in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health, Publication 85-23, revised 1985). Figure 1 gives an overview of the study design.

2.1 | Surgical procedure

Female-specific pathogen-free pigs (35–55 kg) were sedated using an intramuscular injection of zolazepam/tiletamine (8 mg/kg) and xylazine (2.5 mg/kg). After orotracheal intubation, anesthesia was induced with a bolus of propofol (3 mg/kg) and maintained with continuous infusion of propofol (10 mg/kg/h) and fentanyl (0.3 μg/kg/min).

The heart was exposed by median sternotomy. After intravenous administration of heparin (300 IU/kg), 400 ml of whole blood was harvested for ex situ normothermic evaluation. The ascending aorta was cross-clamped and 1 liter of NIH-2 cardioplegia (Table S1) was infused. This

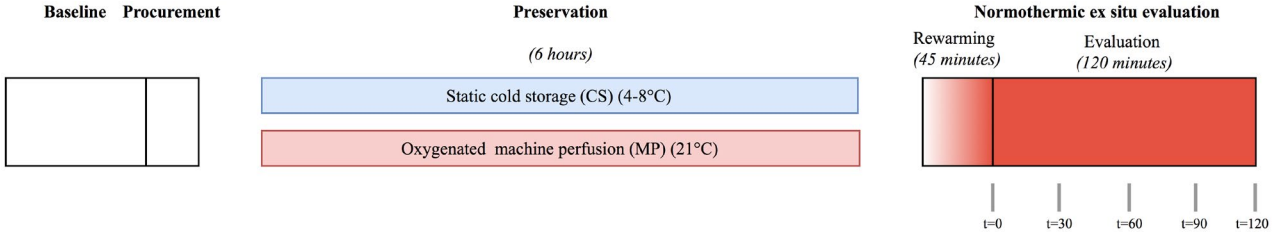

FIGURE 1 Overview of study design and timing for end points [Color figure can be viewed at wileyonlinelibrary.com]

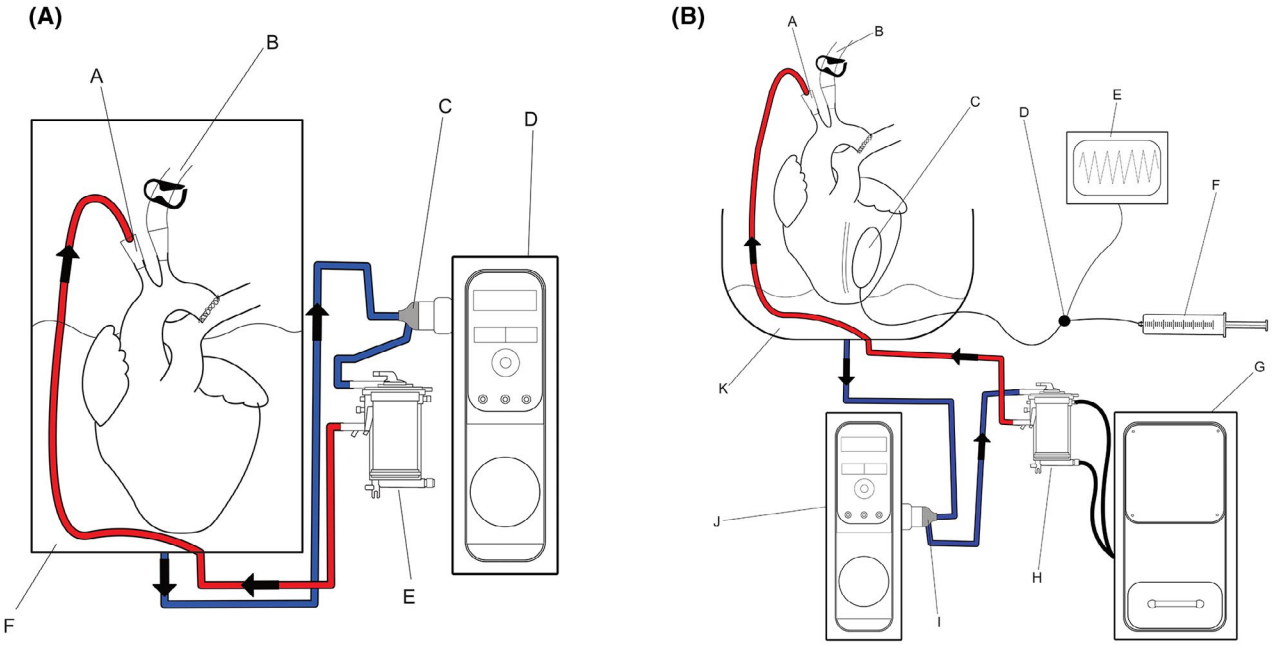

FIGURE 2 (A) Overview of preservation machine and cannulation sites. A: inflow cannula; B, de-airing cannula with pinch clamp; C, centrifugal pump; D, pump unit; E, oxygenator; F, reservoir. (B) Overview of normothermic evaluation machine and cannulation sites. A, inflow cannula; B, de-airing cannula with pinch clamp; C, balloon in left ventricular, with modifiable volume; D, three-way stopcock; E, pressure monitor for intraventricular balloon; F, syringe with saline; G, thermo unit; H, oxygenator; I, centrifugal pump; J, pump unit; K, reservoir [Color figure can be viewed at wileyonlinelibrary.com]

cardioplegic solution is used in the clinical setting of our transplant program. The heart was topically cooled. Cardiectomy was performed, leaving the supra-aortic vessels attached to the graft to facilitate cannulation. A perfusion cannula was placed in the brachiocephalic truncus, a de-airing cannula was placed in the left subclavian artery, and the distal aorta was sutured closed.

2.2 | Preservation technique

By drawing lots, hearts were randomly assigned to one of two preservation techniques: (CS, $n = 5$) or machine perfusion (MP, $n = 5$). All hearts were preserved for 6 h. CS hearts were statically stored at 4–8°C in 1 L of cardioplegia without continuous monitoring of the temperature of the solution or myocardium. MP hearts were placed in a heart holder and attached to the perfusion set via the perfusion cannula (Figure 2A). The disposable perfusion set (Organ Assist, Groningen, The Netherlands) consisted of a reservoir, an oxygenator (Medos Hilite 1000, XENIOS, Heilbronn, Germany), and a centrifugal pump (Medos DP2, XENIOS, Heilbronn, Germany). Antegrade coronary perfusion was pressure-controlled (max. 40 mm Hg, based on pilot experiments, to avoid insufficient coronary flow in lower pressures) and pulsatile (60/min, 20% above and below the set perfusion pressure; as the heart was arrested, synchronization was not necessary). This ensured a continuously closed aortic valve. A pulsatile flow was chosen as this has been proven to keep the capillary bed open and improve perfusion in the setting of extracorporeal perfusion.[9] For MP hearts, a vent was inserted through the mitral valve to ensure unloading of the left ventricle (LV) during preservation in the event of undetected aortic valve insufficiency. The perfusion solution (1000 ml) was based on the solution described by Steen et al.,[10] containing

hormones, catecholamines, and cocaine, with a decreased potassium concentration (10 mmol/L), and without erythrocytes. The temperature was maintained and monitored at 21°C. The temperature of the myocardium was not monitored. During perfusion of the arrested heart, target values for blood gas and electrolyte concentrations were: pO_2 100–200 mm Hg, pH 7.3–7.4, pCO_2 35–45 mm Hg, HCO_3^- 25 mmol/L, K^+ 10 mmol/L, and Ca^{2+} 1.3 mmol/L.

2.3 | Normothermic evaluation

After preservation, hearts were connected to a Langendorff-type normothermic evaluation machine.[11] The Heart Assist (Organ Assist, Groningen, The Netherlands) consisted of a heater-cooler and a pump unit. The disposable perfusion set consisted of a reservoir, an oxygenator (Medos Hilite 2800, XENIOS, Heilbronn, Germany), and a centrifugal pump (Medos DP2, XENIOS, Heilbronn, Germany) (Figure 2B). The perfusion solution was based on the preservation solution (600 ml) supplemented with whole blood (400 ml) and 10.000 IU of heparin. Perfusate targets were: pO_2 100–200 mm Hg, pH 7.3–7.4, pCO_2 35–45 mm Hg, HCO_3^- 25 mmol/L, Ca^{2+} 1.3 mmol/L, K^+ 3.0–5.5 mmol/L, and hemoglobin (Hb) 4.0–4.5 g/L. The perfusion of the heart was pressure controlled with continuous flow. The perfusion pressure was initiated at 40 mm Hg and 20°C. The temperature was increased with steps of 3°C every 5 min, in order to reach normothermia (38°C) after 30 min. Once the temperature of the perfusate in the reservoir had reached 34°C, dobutamine was infused (5 μg/min).[12] Once the temperature of the perfusate had reached 38°C, the perfusion pressure was increased to 60 mm Hg.[13] Another 15 min of perfusion were observed to ensure equal myocardial temperatures for both groups. All hearts were paced at 100 beats/min.

2.4 | Endpoints

2.4.1 | Metabolic parameters

Veno-arterial lactate differences (ΔVA-lactate) were analyzed and myocardial oxygen consumption (MVO_2) was calculated using the formula:

$$\text{Oxygen consumption (ml } O_2\text{/100 g/min)} = ((((C_aO_2 - C_vO_2)/100) * \text{CBF})/\text{heart weight}) * 100$$

with C_aO_2 and C_vO_2 as arterial and venous oxygen content, CBF as coronary blood flow (ml/min), and heart weight in grams. Arterial and venous oxygen content was calculated using the following formulas:

$$C_aO_2 = (1.34 * \text{Hb}*SO_{2\,\text{arterial}}) * (K * pO_{2\,\text{arterial}}) \text{ and}$$
$$C_vO_2 = (1.34 * \text{Hb}*SO_{2\,\text{venous}}) * (K * pO_{2\,\text{venous}})$$

where 1.34 (ml O_2/Hb (g)) is multiplied with Hb (g/dl), SO_2 is the oxygen saturation, pO_2 is the arterial or venous oxygen pressure (mm Hg), and K represents the solubility coefficient for oxygen in human plasma. For the 21°C preservation perfusion, K was assumed to be 0.0036 (ml O_2/mm Hg/100 ml solvent), and 0.0028 (ml O_2/mm Hg/100 ml solvent) at normothermia.[14]

2.4.2 | Blood and perfusate samples

During preservation, perfusate samples were analyzed after 30 min and at 360 min of perfusion in MP. During normothermic ex situ evaluation, perfusate samples were analyzed prior to the connection of the heart (baseline), after a gradual rewarming period of 45 min ($t = 0$), 30 min after the rewarming period ($t = 30$), and at the end of reperfusion ($t = 120$) (Figure 1). All samples were analyzed for creatine kinase (CK), lactate dehydrogenase (LDH), and troponin T (TnT).

2.4.3 | Heart weight and biopsies

Hearts were weighed prior to preservation, after preservation, and at the end of normothermic ex situ evaluation. Biopsies from standardized locations on the apex of the LV were taken and stored in formaldehyde: at baseline in vivo, prior to preservation, after preservation, and after normothermic ex situ evaluation. Five-micrometer-thick cross-sections were prepared, embedded in paraffin, and stained with Sirius Red. A senior cardiac pathologist (EKV) was blinded and scored the samples 0 (none) to 3 (severe) for edema, contraction bands, cytoplasmic coagulation necrosis, congestion, and the influx of neutrophils.

2.4.4 | Functional endpoints

A conic-shaped balloon (length 90 mm, diameter 70 mm, and potential volume 100 ml) was inserted into the LV through the mitral valve annulus for functional evaluation. The balloon was connected to a pressure transducer for continuous pressure recording. Intraventricular balloon diastolic pressure was kept at 0–10 mm Hg, in order to avoid diminished endocardial flow due to high intraventricular pressure. The first functional evaluation was performed after 45 min of rewarming ($t = 0$), and every half hour thereafter, for a total of 120 min ($t = 120$). Volume was gradually added to the balloon (maximum of 40 ml). Systolic function was represented by contractility,

defined as the maximum rate of intraventricular pressure rise (dP/dt_{max}), and elastance. Diastolic function was represented by lusitropy, defined as the maximum rate of intraventricular pressure fall (dP/dt_{min}), and stiffness.

2.5 | Statistical analysis

The non-normal-distributed continuous variables were tested with a Mann–Whitney *U* test, expressed as the median [interquartile range]. A Wilcoxon-signed rank test was performed for both paired ordinal and paired non-normal-distributed continuous variables. The chi-square test was used for non-paired ordinal and nominal variables. dP/dt_{max} and dP/dt_{min} were assessed at a statistically comparable intraventricular balloon volume. For both stiffness and elastance, a linear curve was fit through the diastolic and systolic pressures, respectively, for every successive volume increase. At least two successive data points were needed in order to fit the curve. The slope of the linear curve was compared. A *p*-value <0.05 was considered statistically significant. SPSS version 24 (IBM Corp, Armonk, USA) was used for the statistical analyses. Prism 8 (GraphPad, San Diego, USA) was used for creating the graphs.

3 | RESULTS

3.1 | Baseline

In vivo, CK was 1166 U/L [921–1446] in CS and 1336 U/L [1051–2648] in MP ($p = 0.548$). LDH was 535 U/L [491–732] in CS and 510 U/L [494–560] in MP ($p = 0.389$). TnT was 0.122 μg/L [0.037–0.169] in CS and 0.159 μg/L [0.106–0.513] in MP ($p = 0.310$). There was no difference in histological scores between both groups for in vivo biopsies or biopsies prior to preservation (Figure 3A,B). In the MP group, one biopsy was not taken prior to preservation; therefore, only four out of five were available for histological analysis. CS hearts weighed 219 g [202–243], which was comparable to the MP group which weighed 231 g [213–252] ($p = 0.69$). Time from incision to preservation was comparable for both groups ($p = 0.151$). In the MP group, the time from initiation of cardioplegia to the start of machine perfusion ranged from 17 to 19 min ($n = 3/5$).

3.2 | Preservation period

For MP hearts, the median perfusion pressure was 39 mm Hg [32–39], resulting in a median flow of 198 ml/min [181–213] throughout the preservation. The median measured perfusion temperature was 21°C [20–22]. MP heart weight increased significantly (54% [43–79]) compared to CS after preservation ($p = 0.008$). Histological analysis was comparable between CS and MP after preservation in terms of edema, cytoplasmic coagulation necrosis, congestion, or influx of neutrophils (Figure 3C). In the MP group, contraction bands were more severe (0.5 vs. 0 in CS, $p = 0.008$) after preservation. After 60 min of machine preservation, MVO_2 was 0.30 ml/100 g/min [0.27–0.37], which changed to 0.13 ml/100 g/min [0.12–0.20] at 6 h of preservation ($p = 0.063$) (Figure 4A). The ΔVA-lactate was stable over time ($p = 0.438$) (Figure 4B). Levels of myocardial injury markers are shown in Table 1.

3.3 | Normothermic ex situ evaluation

The median coronary flow during evaluation ranged from 248 to 432 ml/min and 370–468 ml/min in the CS and MP groups, respectively (*p*-value between 0.548 and 0.841 at all evaluation time points). MP graft weight decreased by 10% [4–12] compared to weight after preservation, resulting in an overall increase of 43% [35–56] compared to baseline. This was comparable to the 34% [27–29] increase of graft weight from baseline to end of the evaluation seen in CS ($p = 0.151$). At the end of normothermic ex situ evaluation, CS and MP hearts had comparable histological scores (Figure 3D). No significant differences were seen for MVO_2 or ΔVA-lactate (Figure 5A,B). For both groups, at 60 min, ΔVA-lactate indicated lactate consumption. This continued until the end of the evaluation. Prior to the onset of evaluation, baseline perfusate CK levels were significantly higher in the MP group (753 U/L [497–1158]) compared to the CS group (437 U/L [262–488], $p = 0.032$). At baseline, LDH and TnT levels were comparable. After 120 min of evaluation, the injury markers in the MP group did not differ significantly from the CS group (Figure 6A–C). For LDH and TnT at 120 min of evaluation, one sample of the CS group was not available for analysis due to insufficient volume.

3.3.1 | Functional outcome

MP hearts showed superior lusitropy at 30, 60, and 90 min of evaluation (Figure 7). At the end of the evaluation, the lusitropy of CS and MP hearts were comparable ($p = 0.151$). MP hearts also showed significantly less stiffness at 30 and 60 min of evaluation (Figure 8B,C). This was reflected by the smaller slope: 0.46 mm Hg/ml (vs. 1.73 mm Hg/ml in CS at 30 min, $p = 0.032$) and 0.57 mm Hg/ml (vs. 1.81 mm Hg/ml in CS at 100 min, $p = 0.008$). In the MP group, contractility was superior at 0, 30, and 60 min of evaluation (Figure 7). Elastance was comparable between the groups throughout the entire evaluation phase ($p \geq 0.05$, Figure 9A–E). As we

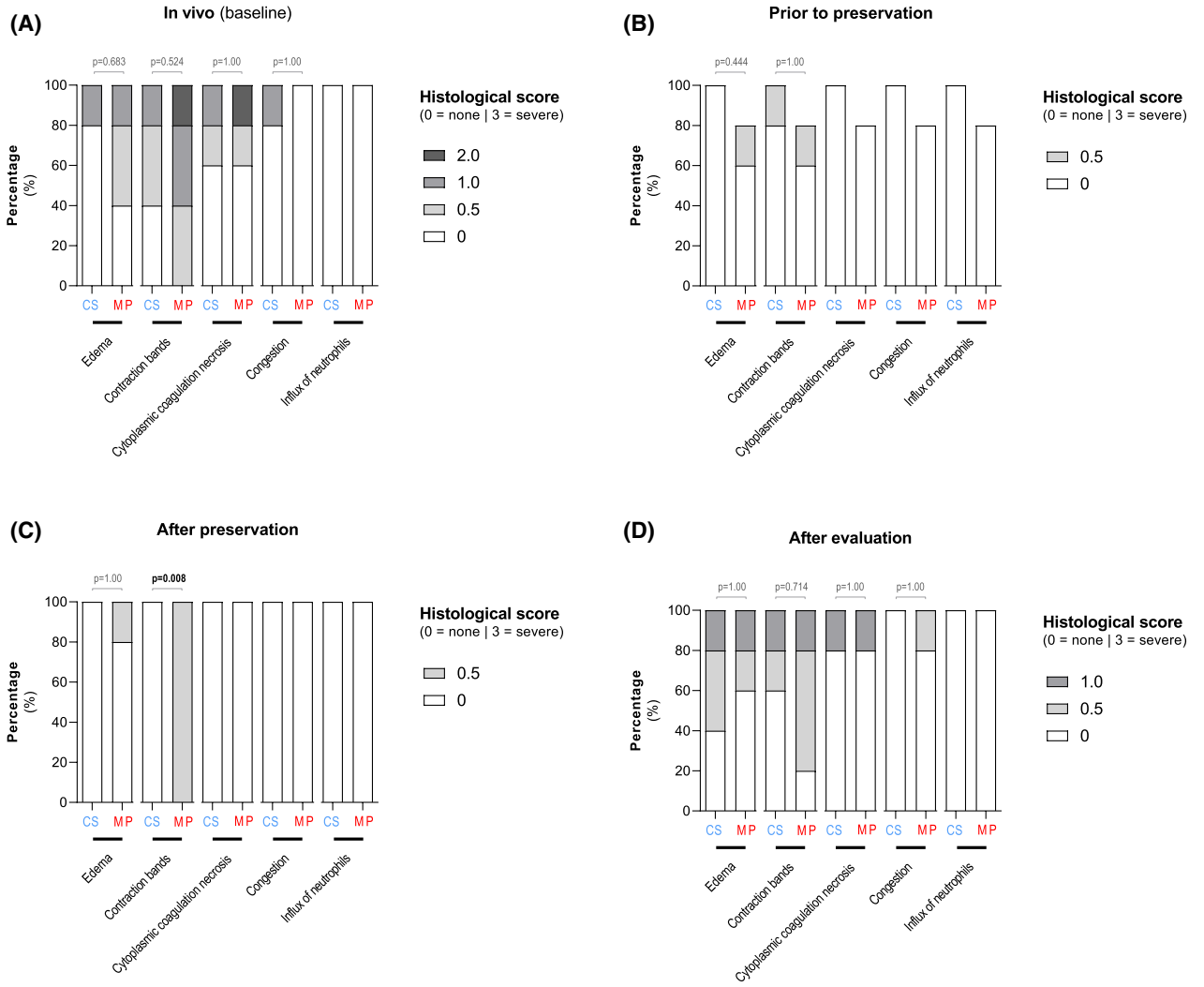

FIGURE 3 Histological analyses of biopsies. (A) in vivo, baseline; (B) prior to preservation; (C) after preservation; (D) after normothermic evaluation. The cumulative percentage of hearts with a specific histological score is depicted on the *y*-axis. The different aspects within the histological analyses are shown on the *x*-axis. Histological end points on Sirius Red stained samples were scored (0 = none, 3 = severe injury) on severity by a blinded pathologist. CS, cold storage group; MP, machine perfusion group [Color figure can be viewed at wileyonlinelibrary.com]

adhered to the predefined maximum balloon volume of 40 ml and maintained the intraventricular balloon diastolic pressure at 0–10 mm Hg at the start of functional evaluation time points, the slopes of both stiffness and elastance were based on a limited number of available data points per heart when reaching 90 and 120 min of evaluation. In MP hearts, in 3 out of 5 experiments ($t = 90$) and in 2 out of 5 experiments ($t = 120$) it resulted in the inability to fit a curve. In CS hearts, it resulted in 1 out of 5 experiments ($t = 120$) in which no curve was fitted.

4 | DISCUSSION

ESHP has become clinically available as an alternative for traditional CS donor heart preservation, allowing for both extended donor heart and DCD heart transplantation.[15,16] In this emerging field, there is no consensus on what the optimal temperature is for oxygenated machine perfusion preservation, but machine perfusion could have significant benefits over CS. We have shown that a 21°C oxygenated acellular ESHP strategy results in the functional outcomes which are comparable to CS, especially in terms of contractility, relaxation, and stiffness. At certain time points, functional outcome was superior.

Though a cold ischemic time <4 h is preferred, 40% of all clinical heart transplantations are performed with grafts that were subjected to >4 h of ischemic time in Europe.[17] Six hours of CS is considered the absolute upper limit of what is acceptable in clinical practice.[18] By preserving porcine hearts for 6 h, we compared the

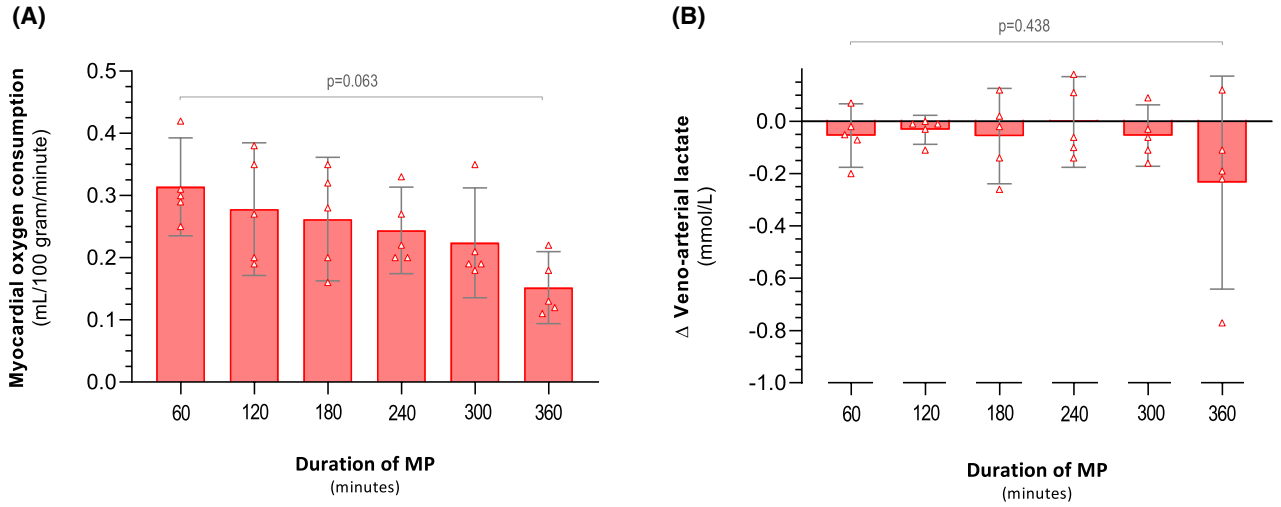

FIGURE 4 Metabolic parameters during 21°C machine preservation. (A) myocardial oxygen consumption. (B) veno-arterial lactate differences. Bars represent the median and red triangles represent the individual data points. Interquartile ranges are shown in gray. ΔVA-lactate, veno-arterial lactate difference; MP, machine preservation [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Levels of myocardial injury markers during 21°C machine preservation

	CK (U/L)		LDH (U/L)		Troponin (μg/L)	
MP	**30 min**	**360 min**	**30 min**	**360 min**	**30 min**	**360 min**
Median [IQR]	342 [335–485]	10 098 [5902–13 804]	86 [75–111]	866 [640–1498]	0.345 [0.3055–0.5225]	4.76 [3.195–7.565]
Heart 1	607	6808	125	866	0.621	3.93
Heart 2	363	4997	97	803	0.424	4.76
Heart 3	342	13 804	86	1768	0.307	9.51
Heart 4	303	2525	70	477	0.345	2.46
Heart 5	328	10 098	79	1227	0.304	5.62

Abbreviations: CK, creatine kinase; IQR, interquartile range, LDH, lactate dehydrogenase; MP, machine perfusion; TnT, troponin T.

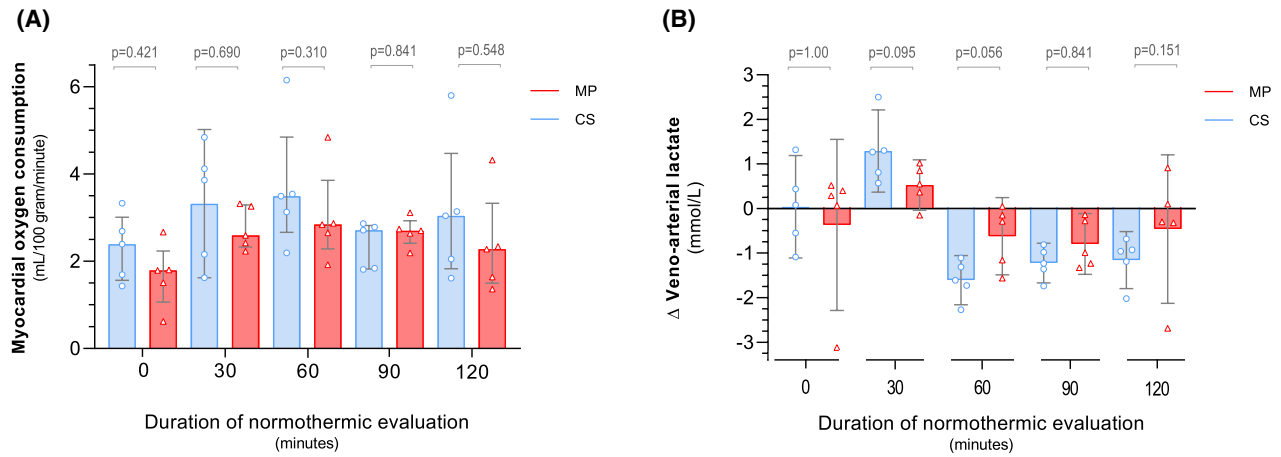

FIGURE 5 Metabolic parameters during normothermic evaluation. (A) myocardial oxygen consumption. (B) veno-arterial lactate differences. Red bars represent the median and red triangles represent the individual data points in the machine perfusion group. Blue bars represent the median and blue circles represent the individual data points in the cold storage group. Interquartile ranges are shown in gray. CS, cold storage group; ΔVA-lactate, veno-arterial lactate difference; MP, machine perfusion group [Color figure can be viewed at wileyonlinelibrary.com]

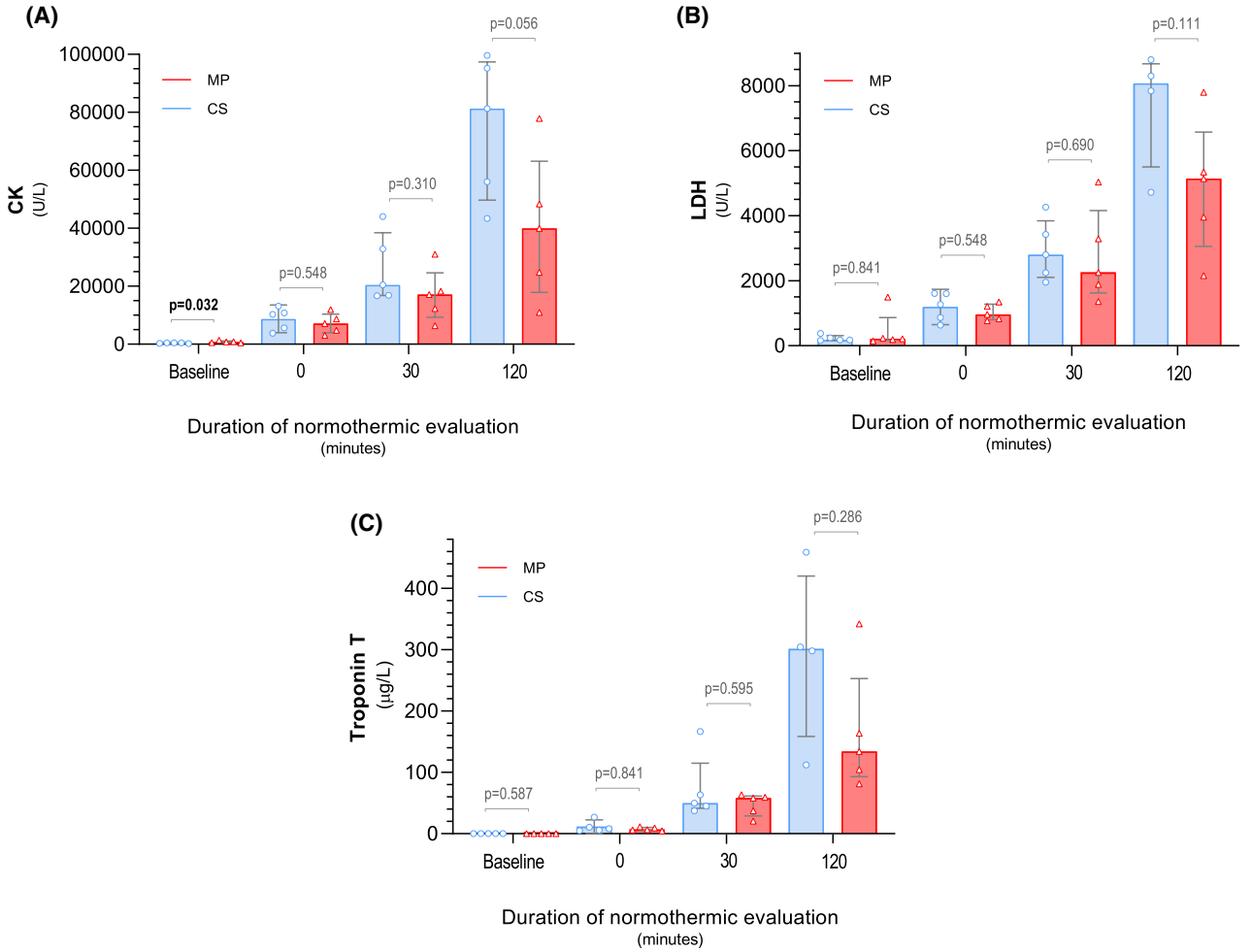

FIGURE 6 Myocardial injury markers during normothermic evaluation. (A) creatine kinase levels. (B) Lactate dehydrogenase levels. (C) Troponin T levels. Red bars represent the median and red triangles represent the individual data points in the machine perfusion group. Blue bars represent the median and blue circles represent the individual data points in the cold storage group. Interquartile ranges are shown in gray. Values at "baseline" represent the values prior to the connection of the heart. CK, creatine kinase; CS, cold storage group; LDH, lactate dehydrogenase; MP, machine perfusion group [Color figure can be viewed at wileyonlinelibrary.com]

limits of CS to the new perfusion strategy. Longer exposure of myocytes to ischemia results in loss of ability to regulate ion homeostasis, leading to acidosis, necrosis, edema, and depletion of ATP.[3] To slow this detrimental process and to lower the high metabolic rates encountered at normothermia,[6,19] the heart is kept at profound hypothermic temperatures during CS. The reduction of MVO_2 is most outspoken in the arrested heart during the initial cooling, with a 47% reduction in MVO_2 when cooling from 37°C to 30°C, versus a 37% reduction when cooling from 22°C to 15°C.[19] However, hypothermia itself also results in ion regulatory mechanism disruptions and an imbalance in ATP supply and demand, with a significant increase in the influx of calcium and sodium.[8] These mechanisms can lead to cell death.[8] Classic CS has the disadvantage that the temperature is not monitored and thus freezing may occur,[20] leading to injury such as protein denaturation. In this study, the temperature of the CS hearts was not monitored. Using MP, continuous temperature monitoring is an added value. Downregulation of metabolism in the present study was achieved by electrically arresting the heart[6] and cooling it to 21°C. This temperature was chosen as a point between both extremes of preservation temperature (0–8°C and normothermia). MVO_2 at the start of perfusion was similar to the values reported by other research groups of preservation at temperatures ±20°C.[6,19] At the end of perfusion, the MVO_2 was more similar to values seen in preservation with profound hypothermic temperatures.[19] The difference in MVO_2 between start and end was not statistically significant ($p = 0.063$). By choosing a moderate temperature, temperature changes in the heart during the entire cooling-rewarming process of procurement and transplantation are less drastic.

In the current clinical practice, normothermic perfusion using the Organ Care System (OCS, TransMedics, Andover,

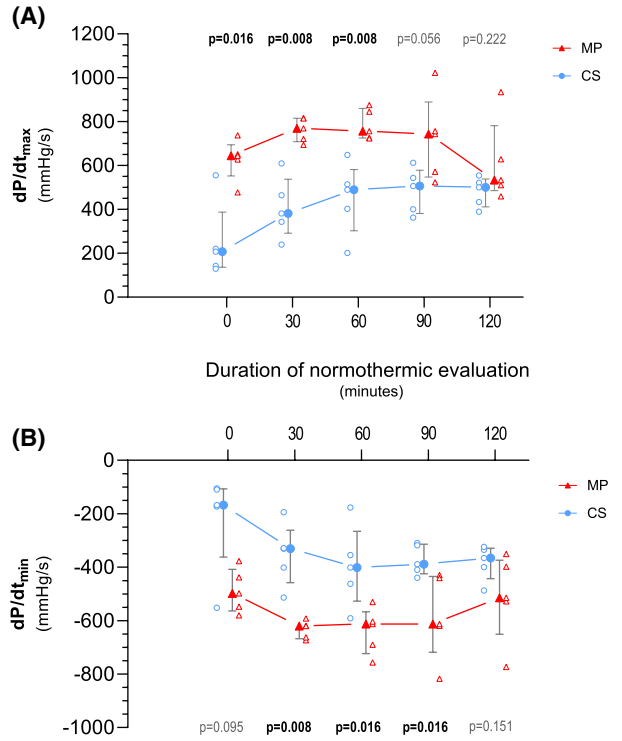

FIGURE 7 Contractility and lusitropy. (A) (contractility). Maximum rate of intraventricular pressure rise (dP/dt_{max}). (B) (lusitropy). The maximum rate of intraventricular pressure fall (dP/dt_{min}). Red solid triangles represent the median and small red triangles represent the individual data points in the machine perfusion group. Blue solid circles represent the median and small blue circles represent the individual data points in the cold storage group. Interquartile ranges are shown in gray. CS, cold storage group; MP, machine perfusion group [Color figure can be viewed at wileyonlinelibrary.com]

USA) is the only commercially available method,[21] and clinical trials have been started using 8°C perfusion (XVIVO, Gothenburg, Sweden).[22] There is an abundance of experimental data focusing on refinements on normothermic perfusion strategies and the development of <10°C perfusion. ESHP at 20–25°C has not frequently been described in the literature. Jones and colleagues successfully preserved rabbit hearts for 8 h at 20°C, leading to a comparable cardiac function compared to CS for 4 h.[23] Bovine PEG-Hb was added to the preservation perfusate, but normothermic ex situ evaluation was performed using a crystalloid perfusate. Zhang and colleagues compared 26°C perfusion oxygenated blood cardioplegia for 8 to 4 h of CS and presented superior outcomes in their perfusion group.[24] In this study, pressure-controlled perfusion was used. Recently, Kobayashi and colleagues reported that flow-controlled perfusion leads to superior functional recovery.[25] The model used by Kobayashi and colleagues differs from our animal model: A DCD model was used in juvenile pigs (11 kg, vs. 35–55 kg in our study). Both of these elements influence the demands of the heart, which is indicated by the low physiological mean arterial pressure in juvenile pigs (40 mm Hg)[25] and the potentially higher metabolic needs in DCD hearts.[26] The impact of flow-controlled perfusion on adolescent or adult porcine models is unknown.

The perfusate in our study was based on the perfusate used by Steen and colleagues,[10] which was designed to meet the specific demands of the DBD donor heart. We lowered potassium levels and omitted the erythrocytes. The latter poses the advantage of simplified logistics and cost reduction, obviating the extraction of blood during the donor procedure. Moreover, whole donor blood often contains high levels of catecholamines[27] and cytokines.[28] If banked blood is used, impaired microcirculation may result from increased cell membrane rigidity, due to a decrease in temperature.[29] Without an oxygen carrier, oxygen delivery (DO_2) of an acellular perfusion solution is solely dependent on the solubility of oxygen. Our perfusate theoretically contained 0.0036 ml O_2/mm Hg/100 ml of perfusate.[14] We supplied the heart with a median pO_2 of 197 mm Hg (Table S2) and perfused the heart at a median flow of 198 ml/min. The calculated median DO_2 was, therefore, 1.40 ml/min (0.0036 * 197 * 1.98). The highest MVO_2 (at 60 min in the MP group) was 0.30 ml/100 g/min. The median heart weight in the MP group was 231 g, resulting in an MVO_2 of 0.69 ml/min, which is considerably lower than the DO_2. Though the MVO_2 decreased during preservation, suggesting inadequate oxygen delivery, DO_2 to the heart was sufficient in this study. The target pO_2 level (100–200 mm Hg) was similar to the values reported by Stowe and colleagues (26°C, acellular, pO_2: 168 mm Hg)[30] and Toledo-Pereyra and colleagues (plasma, pO_2: 200 mm Hg).[31] Increased pO_2 may be necessary, especially in injury models.[26] Lastly, when increasing the temperature of machine preservation, the oxygen-carrying capacity of the perfusate should be reviewed and the addition of an oxygen carrier could be reconsidered.

We analyzed CK, LDH, and TnT as clinical myocardial injury markers. In comparison to the known reference values in vivo, the levels of all markers were high during both MP and normothermic ex situ evaluation. First, a surgical cardiac procedure induces myocardial injury, as is known from general postoperative course in cardiac surgery. Second, it is conceivable that perfusion in an ex situ circuit could contribute to injury due to an inherent lack of physiological circumstances and blood contact with foreign surfaces. Additionally, there is an accumulation of all substances not metabolized by the myocardium. It remains unclear what the clinical relevance is of these injury markers as marker of perfusion quality and predictor of outcome. Although we did not perform such analyses, there might be some degree of correlation between a trend of injury marker accumulation and poorer outcome.

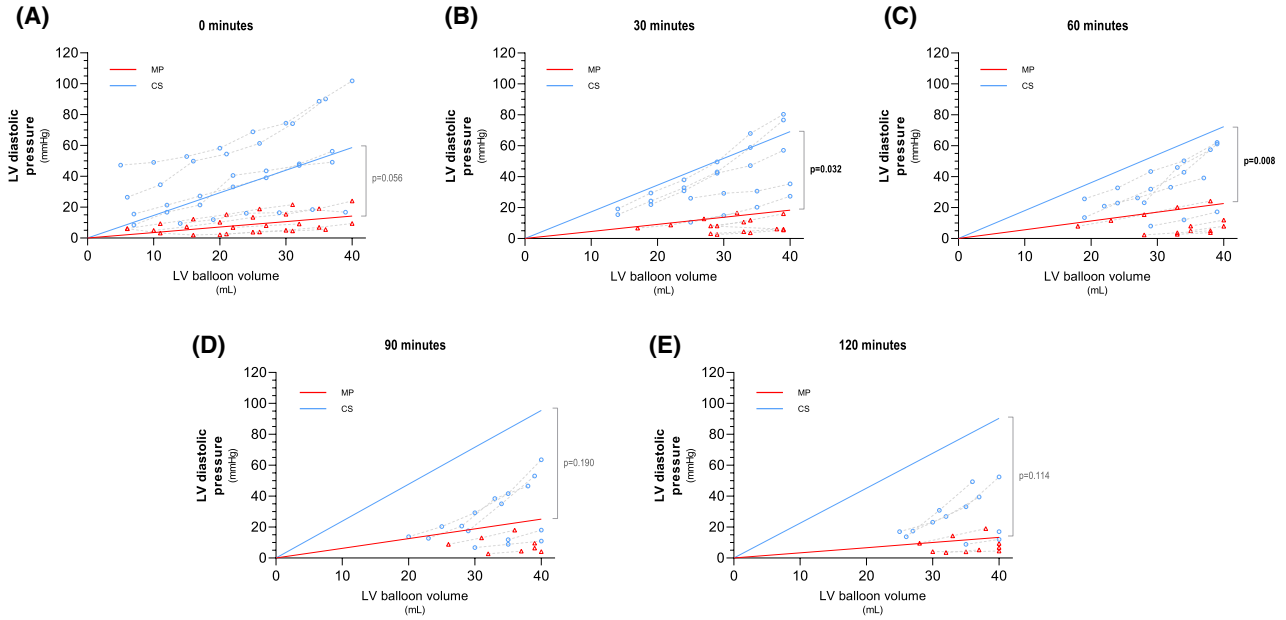

FIGURE 8 Stiffness (passive diastolic filling) of the left ventricle during normothermic evaluation. (A–E) Stiffness at the different time points during evaluation. Solid lines (blue, cold storage group; red, machine perfusion group) represent the median slope. Red triangles represent the individual data points in the machine perfusion group. Blue circles represent the individual data points in the cold storage group. Both median slopes were made to initiate from $y = 0$. CS, cold storage group; LV, left ventricle; MP, machine perfusion group [Color figure can be viewed at wileyonlinelibrary.com]

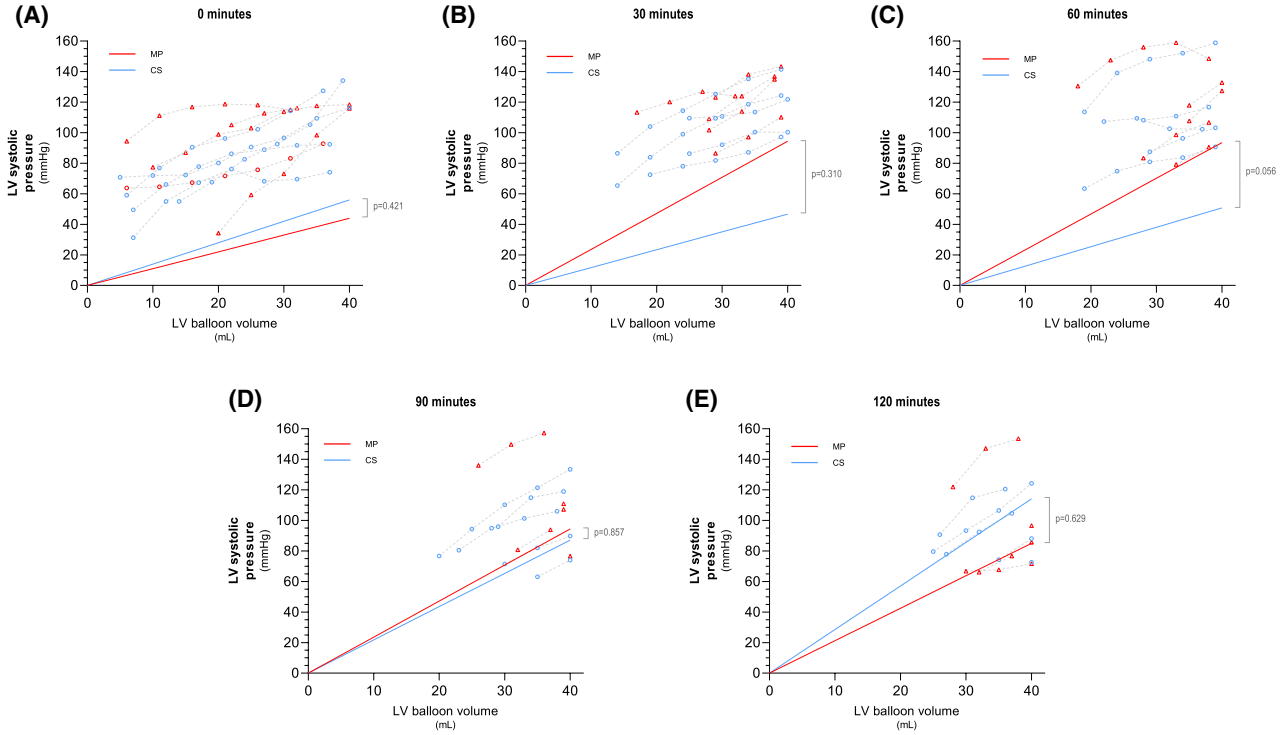

FIGURE 9 Elastance of the left ventricle during normothermic evaluation. (A–E) Elastance at the different time points during evaluation. Solid lines (blue, cold storage group; red, machine perfusion group) represent the median slope. Red triangles represent the individual data points in the machine perfusion group. Blue circles represent the individual data points in the cold storage group. Both median slopes were made to initiate from $y = 0$ [Color figure can be viewed at wileyonlinelibrary.com]

In contrast to the injury markers, no major histological injury was seen. However, the contraction band score was higher in MP hearts. These bands may have occurred as a result of intracellular calcium influx during reperfusion.[12,32] Although a healthy model without injury was used, all hearts were subjected to some degree of cold ischemia inherent to benching prior to initiation of machine perfusion. This could explain the small but significant difference in contraction bands. Moreover, the hearts of the CS group were not yet reperfused at that time point. Importantly, histological scoring after ex situ evaluation was comparable to the histology at baseline in vivo, implying that both preservation strategies demonstrated preserved histological integrity and absence of significant myocardial injury.

The significant weight gain recorded in hearts at the end of MP stands in contrast with the absence of edema histologically. The development of myocardial edema is a well-known consequence of ESHP, but it is not clear to what degree it is clinically relevant.[12,33] It is not clear whether a lower perfusion pressure would have led to less edema. In a clinical case report of 8 h of normothermic ESHP, visually observed edema resulted in primary graft dysfunction.[34] Van Caenegem and colleagues, however, reported no significant influence of edema on diastolic function in pre-clinical studies, despite considerable edema.[33] It has been reported that (normothermic) ESHP leads to an inflammatory response,[35] which in turn leads to myocardial edema.[36] The inflammatory response could be one of the explanations for the edema in our study. Only minor levels of steroids (420 pmol/L)[10] were added to the perfusate, in comparison to 500 mg/L in the OCS perfusate. Sandha and colleagues reported that although the supplementation of corticosteroids in normothermic perfusion significantly reduced edema in porcine donor hearts, it did not affect functional outcome.[36] It raises the question of whether edema in itself is a significant finding. In general, the effect of edema is expected to be observed as a transient increase in stiffness.[37] Edema has been described to diminish as time after heart transplantation increases, and, therefore, the transience might be more relevant than the occurrence itself.[38] This hypothesis is supported by the reduction of weight by 10% for the MP hearts between the onset of and end of normothermic ex situ evaluation.

Despite substantially more edema at the initiation of rewarming in the MP group, it did not result in diastolic inferiority in the MP group during evaluation. In MP, both functional markers for diastolic function—stiffness and lusitropy—were either comparable or superior, depending on the time point of evaluation. It is unsure why the superiority of functional outcomes disappeared at the end of the evaluation. It is unlikely that a difference in myocardial temperature at the time of normothermic evaluation was the explanation for worse diastolic functional outcome in CS compared to MP. Myocardial hypothermia can influence the diastolic function, resulting in lower dP/dt_{min} and higher stiffness,[39] and might have been expected in the CS group. Before initiation of evaluation, rewarming to 38°C lasted 45 min, in order to prevent discrepancies in myocardial temperature between both groups. The significant difference in diastolic function (both dP/dt_{min} and stiffness) was seen after 30 min (which was 75 min of the onset of active rewarming), not upon initiation of rewarming. Second, after 120 min of evaluation, the function of MP hearts lost superiority and became comparable to the CS group. It was the result of a deterioration of function in the MP group rather than an improvement of the CS group. Prolonged normothermic MP has been reported to reduce myocardial function.[40]

In vivo, the LV end-diastolic pressure-volume relationship (stiffness) defines the passive physical properties of the LV. It is generally graphed using an exponential curve.[41] As we adhered strictly to a maximal LV balloon volume of 40 ml and maintained the intraventricular balloon diastolic pressure at 0–10 mm Hg at the start of functional evaluation time points, we obtained limited successive data points. We, therefore, chose linear curves to represent the stiffness in our experiments. A greater amount of volume could have been infused into the balloon, in order to get sufficient data points.

A mild decrease in myocardial temperature (up to 31°C) is known to have positive inotropic effects.[39] If the myocardium of the CS group had been colder during evaluation due to insufficient rewarming, systolic function would have been expected to be superior, especially in the early phases of evaluation. However, for MP, dP/dt_{max} was superior for all time points within the first hour. A plateau in dP/dt_{max} was already reached at 60 min of evaluation in the CS group. Lastly, systolic function tended to deteriorate in the MP group over time, as was the case for diastolic function. Interestingly, elastance did not reflect differences in systolic function between CS and MP at any points of evaluation.

To date, evaluation of the machine-perfused heart remains experimental. The importance of metabolic measurements is controversial.[27] We believe that functional evaluation is key, though there is no consensus on what parameter has a clear correlation with post-transplant outcome. There is a need for standardized experimental settings and parameters of evaluation to improve (comparability of) future research.

5 | LIMITATIONS

As we used healthy pigs euthanized by clamping of the aorta and administration of cardioplegia, these hearts were superior to hearts donated after brain death or after circulatory death. Future studies should incorporate an injury model. Post-preservation function was evaluated using isovolumetric recordings of the LV during continuous myocardial perfusion. ECG-triggered pulsatile

flow may have improved the normothermic perfusion. Moreover, the authors acknowledge that isovolumetric evaluation is not as clinically representative as working-mode evaluation with variable preload and volume, or a transplantation model. However, a validated working-mode or transplantation model was not present when these experiments were performed. The maximal balloon volume used was low from a physiological point of view but was chosen to prevent endo-myocardial injury due to high wall balloon pressures. The choice of 21°C was not compared to other temperatures using the same ESHP strategy, thus, limiting our ability to compare the effect of this particular temperature to others. A head-to-head comparison in the future will be crucial in order to draw conclusions on the superiority of a specific temperature for ESHP. In that, uniform and standardized experimental settings and outcomes are imperative to help this area of research forward.

6 | CONCLUSIONS

Machine perfusion at 21°C may be a viable option for oxygenated ESHP, offering theoretical physiological advantages compared to normothermia or deep hypothermia. The initial superior functional outcome of the porcine LV after 6 h of preservation, despite the relatively high injury markers in the perfusate, suggests that our ESHP strategy could offer adequate and safe preservation of hearts for transplantation. Whether the transient trend of superiority in diastolic and systolic function would be more pronounced and sustained in other normothermic models should be investigated. Second, future research is necessary to determine the optimal temperature, composition of the perfusate, and its oxygen delivery capacity for optimal cardiac preservation using machine perfusion.

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CONFLICT OF INTEREST

V. van Suylen and M.E. Erasmus received disposables and perfusion machines from Organ Assist b.v., Groningen. F. Nijhuis and A. van der Plaats are employed by Organ Assist b.v., Groningen, The Netherlands. They developed the technology and device, and participated in defining the protocol, provided technical assistance during the experiments, and reviewed the manuscript on technical subjects. They did not participate in data analysis. The other authors of this manuscript have no conflict of interest to disclose.

AUTHOR CONTRIBUTIONS

Concept and design: Vincent van Suylen, Katrien Vandendriessche, Arne Neyrinck, Foppe Nijhuis, Arjan van der Plaats, Filip Rega, Michiel E. Erasmus. Data collection: Vincent van Suylen, Katrien Vandendriessche. Data analysis/interpretation: Vincent van Suylen, Katrien Vandendriessche, Arne Neyrinck, Erik K. Verbeken, Pieter Vermeersch, Bart Meyns, Massimo A. Mariani, Filip Rega, Michiel E. Erasmus. Drafting article: Vincent van Suylen, Katrien Vandendriessche. Critical revision of the manuscript: all authors. Approval of article: all authors.

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SUPPORTING INFORMATION

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