A Multi-Mode System for Myocardial Functional and Physiological Assessment during Ex Situ Heart Perfusion

Thomas Duignan, BME; Alvise Guariento, MD; Ilias P. Doulamis, MD; Takashi Kido, MD; William L. Regan, CCP, LP, FPP; Mossab Saeed, MD; David M. Hoganson, MD; Sitaram M. Emani, MD; Pedro J. del Nido, MD; James D. McCully, PhD; Gregory S. Matte, CCP, LP, FPP

Department of Cardiac Surgery, Boston Children’s Hospital, Harvard Medical School, Boston, Massachusetts

Abstract: Ex situ heart perfusion (ESHP) has proven to be an important and valuable step toward better preservation of donor hearts for heart transplantation. Currently, few ESHP systems allow for a convenient functional and physiological evaluation of the heart. We sought to establish a simple system that provides functional and physiological assessment of the heart during ESHP. The ESHP circuit consists of an oxygenator, a heart–lung machine, a heater–cooler unit, an anesthesia gas blender, and a collection funnel. Female Yorkshire pig hearts (n = 10) had del Nido cardioplegia (4°C) administered, excised, and attached to the perfusion system. Hearts were perfused retrogradely into the aortic root for 2 hours before converting the system to an isovolumic mode or a working mode for further 2 hours. Blood samples were analyzed to measure metabolic parameters. During the isovolumic mode (n = 5), a balloon inserted in the left ventricular (LV) cavity was inflated so that an end-diastolic pressure of 6–8 mmHg was reached. During the working mode (n = 5), perfusion in the aortic root was redirected into left atrium (LA) using a compliance chamber which maintained an LA pressure of 6–8 mmHg. Another compliance chamber was used to provide an afterload of 40–50 mmHg. Hemodynamic and metabolic conditions remained stable and consistent for a period of 4 hours of ESHP in both isovolumic mode (LV developed pressure: 101.0 ± 3.5 vs. 99.7 ± 6.8 mmHg, p = .979, at 2 and 4 hours, respectively) and working mode (LV developed pressure: 91.0 ± 2.6 vs. 90.7 ± 2.5 mmHg, p = .942, at 2 and 4 hours, respectively). The present study proposed a novel ESHP system that enables comprehensive functional and metabolic assessment of large mammalian hearts. This system allowed for stable myocardial function for up to 4 hours of perfusion, which would offer great potential for the development of translational therapeutic protocols to improve dysfunctional donated hearts. Keywords: heart transplant, ex situ heart perfusion, myocardial function, cardioplegia. J Extra Corpor Technol. 2020;52:303–13

Ex situ heart perfusion (ESHP) is a relatively new technique that keeps the heart in a beating and oxygenated state using warm nutrient-enriched donor blood (1–3). The main aim of ESHP is to eliminate cold ischemic injury and minimize the accumulation of toxic metabolic by-products. This could ultimately lead to an increase in the donor pool by using organs that are currently considered to be high risk, such as those obtained from donation after circulatory death (DCD) or marginal donors (4). In addition to these advantages, ESHP allows for a detailed independent assessment of the donor heart’s function, regardless of the medical history and method of donation (3,5–13).

In general, current ESHP systems for use in cases of anticipated extended ischemic periods for donor hearts provide only limited organ function evaluation, primarily through biomarker levels (2,3,14–16). However, it has been recently shown that biomarkers such as lactates are not prognostic for heart function, thus making the need to determine contractile parameters of the donor heart imperative (6). Left ventricular (LV) function has been shown to be more effective than biomarkers in predicting cardiac performance after transplantation (5–8,14,16). Therefore, the establishment of ESHP systems that enable a more
comprehensive assessment of cardiac function during transportation is essential for safe expansion of the donor pool (17). Although one group has developed an ESHP system with the capability of evaluating LV function (5), replicating this system requires both mechanical and software engineering expertise as well as specific equipment that is not readily available to most of the transplant programs. In this study, we sought to establish a simple, user-friendly ESHP system with readily available equipment that goes beyond conventional biomarker analysis by providing for functional and physiological evaluation of LV function of swine hearts in 2 separate modes: isovolumic loading mode (Langendorff mode) and working heart mode. The Langendorff mode involves perfusing the heart in a retrograde manner via the aorta, whereas the working mode aims to replicate perfusion in vivo.

MATERIALS AND METHODS

Animal Model
In this study, hearts from 10 female Yorkshire pigs (40–60 kg) were used. This investigation was conducted in compliance with the “Guide for the Care and Use of Laboratory Animals,” published by the National Institutes of Health (NIH Publication No. 85-23, revised 1985) and use was approved by the Boston Children’s Hospital’s Animal Care and Use Committee (Protocol 18-06-3717). All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals.

Surgical Procedure
General anesthesia was induced with Telazol (4–6 mg/kg, intramuscular [IM]), xylazine (1.1–2.2 mg/kg, IM), and isoflurane (for induction by the mask up to 3% and for maintenance of general anesthesia by the endotracheal tube 0.5–4%) at the beginning of the procedure. On initiation of anesthesia, the animal was intubated with a cuffed endotracheal tube and mechanically ventilated. Monitoring during the procedure included an arterial line catheter, an intravenous catheter, core temperature monitoring, a Foley urinary catheter, mechanical ventilation, and electrocardiography. A sternotomy was performed, and the pericardium was opened. Lidocaine 2% (2 mg/kg) was then administered, and the animal was fully anticoagulated with intravenous heparin (300 units/kg) to obtain an activated clotting time greater than 350 seconds. And then, 120 mL of blood was removed with a cannula placed in the femoral artery. This blood served as the blood component of the del Nido cardioplegia solution (18).

Heart Harvesting and Preparation
After acquisition of baseline measurements, a dose of 10 mL/kg of cold del Nido cardioplegia (4°C) mixed in a ratio of four parts crystalloid to one part donor patient whole blood was delivered through a cannula placed in the ascending aorta, with a cross-clamp placed distal to this cannula. Cardioplegia was vented through an incision in the right atrium (RA).

The heart was then excised, submerged in cold cardioplegia solution, and stored in a cold container for approximately 10 minutes while preparing and then connecting it to the ESHP system. Figure 1 indicates the time required to include this cold preservation time. A 3/8” straight connector was inserted into the aorta which was secured with a zip-tie (Figure 2). The RA was sutured closed so that the venous return from the coronary sinus was ejected through the pulmonary artery only, which was cannulated to monitor coronary blood flow (CBF). The entire heart was then quickly removed from the storage container, pouring out any storage solution still in the ventricles, and weighed (19). The heart was oriented in the ESHP system so that aorta was facing upward with the posterior aspect of the heart resting on a plastic sheet, which was part of our custom blood collection system. The organ procurement usually takes at least 30 minutes, whereas the time needed to collect blood to prime the system is approximately 15 minutes.

Perfusion System Setup
The primary components of the perfusion circuit consisted of either CAPIOX FX05 or CAPIOX FX15 oxygenators (Terumo Cardiovascular Group, Ann Arbor, MI), a Stockert S3 heart–lung machine (LivaNova PLC, London, England), a 3T heater–cooler Unit (HCU) (LivaNova PLC), a Computerized Diagnostics Inc. (CDI) Blood

**Figure 1.** Timeline of the study. Yorkshire pigs (40–50 kg, male, n = 10) were used for all experiments. Ten minutes after the administration of cardioplegia, hearts were harvested and were mounted on the perfusion system. Hearts were reperfused with the ex situ perfusion system for 240 minutes in total. After the initial 2 hours of unloaded perfusion, hearts were loaded either in the isovolumic (inflation of the intraventricular balloon, n = 5) or in the working mode (direction of the flow to the LA, n = 5) until the end of the study. Myocardial function assessment time points are annotated (red triangles).
Coronary sinus blood collected by the funnel flowed back to CVR via gravity drainage. We used two servo-regulated pumps on the Stockert S3 heart–lung machine; the first to perfuse the heart with regulated pressure control and the second to provide independent active ultrafiltration through the hemoconcentrator (Figure 5). The Stockert S3 heart–lung machine was also used to monitor the perfusate temperature precisely in conjunction with the 3T HCU. The perfusion circuit was equipped with a cardioplegia dump line to prevent the high-potassium cardioplegic blood returning to the perfusate in the reservoir as myocardial reperfusion was initiated.

**Perfusate Preparation**

The perfusion circuit was first primed with Plasma-Lyte A 7.4 (Baxter Healthcare, Deerfield, IL) heparinized to 3 international units (IU)/mL per institutional standard. Approximately 500 mL of crystalloid was added to the reservoir and recirculated. The system was then deaired, and gas flow was turned on with .75 liters per minute (LPM) of 100% oxygen flow and .05 LPM of 100% CO₂ flow. The 3T HCU was set to provide an initial perfusate temperature of 32°C.

Given known advantages over non-sanguineous perfusates, a blood-based perfusate was used (20). In our experiments, we solely used blood drained from the donor animal. Blood was obtained from the donor animal after the heart was explanted and during its preparation and connection to the ESHP system. The circuit crystalloid volume in the reservoir was reduced via a recirculating return line connected to the original Plasma-Lyte bag. Approximately 250 mL of blood (also heparinized to 3 IU/mL) was then added to the reservoir and recirculated to reach a hematocrit of ≥25%. Active zero-balance ultrafiltration of the circuit prime with Plasma-Lyte (Baxter, Deerfield, IL) was used as needed to bring the electrolyte values for calcium, potassium, and sodium within normal range. Conventional ultrafiltration, in conjunction with blood boluses to the circuit, was also used to maintain the hematocrit in the target range. The calcium was normalized to .80 mmol/L, and the pH was adjusted with NaHCO₃ as needed.

**Retrograde Perfusion: Resting Mode**

The heart was first attached to the perfusion system, and coronary perfusion was initiated in a retrograde fashion into the aortic root (Figure 2). The first 100 mL of perfusate was wasted to a separate reservoir to eliminate the high-potassium cardioplegia solution from entering the ESHP system. The heart was perfused for 2 hours before converting the system either to an isovolumic loading mode or working heart mode (Figure 1).

Root pressure was measured via a pressure transducer connected to a stopcock on the aortic root cannula. The CBF was adjusted as needed for the desired root pressure.
and adjusted per body surface area (BSA) calculated as previously described (21). The target mean arterial pressure (MAP) at the start of reperfusion was 40 mmHg with a perfusate temperature of 32°C in accordance with previous studies (1–5). The arterial pump head was servo-regulated 5 mmHg above this value to ensure maintenance of the target MAP without an excessive pressure load. The arterial pump circuit was also servo-regulated for a maximum system pressure of 300 mmHg as a safeguard against overpressurization in the ESPH circuit.

The oxygen and carbon dioxide gas flows were adjusted to maintain a partial pressure of arterial oxygen (\(\text{PaO}_2\)) between 190 and 220 mmHg and a partial pressure of arterial carbon dioxide (\(\text{PaCO}_2\)) between 40 and 45 mmHg using an in-line blood gas monitor, which was recalibrated every 30 minutes. A common \(\text{PaO}_2\) was used during cardiopulmonary bypass to ensure fully oxygenated blood while minimizing the risks which may be associated with significant hyperoxia. The temperature was increased incrementally by .5°C every 10 minutes of reperfusion until a perfusate temperature of 35°C was reached (5). A pacemaker was used to stimulate the heart at a rate of 80–100 beats per minute in case a lower spontaneous rate was present. The heart was reperfused for 30 minutes at the initial controlled reperfusion period at an MAP of 40 mmHg with a slightly hypocalcemic and moderately acidic perfusate (\(\text{Ca}^{2+} : 8 \text{ mmol/L}, \text{pH} : 7.2\)) before being allowed to rewarm to reduce myocardial edema (22).

**Isovolumic Loading Mode**

After 2 hours of ESHP, hearts (n = 5) were transitioned to the isovolumic loading mode. A 25 x 64-mm polyethylene terephthalate balloon (Nordson Medical, Amherst, OH) was inserted in the LV cavity through the mitral valve. The balloon was sutured on a 6-Fr introducer (Terumo Interventional Systems) and subsequently stabilized in the LV by suturing the introducer on the mitral valve and closing the left atrium (LA) with a 3–0 Prolene suture (Ethicon, Bridgewater, NJ). The balloon was left deflated during the initial 2 hours of resting mode retrograde perfusion.

Following reperfusion in the resting mode, the balloon in the LV was inflated and the heart was switched to an isovolumic loading mode. Pressure in the LV balloon was monitored with a Surgivet Advisor three-parameter Vital Signs Monitor (Smiths Medical). End diastolic pressure (EDP) was adjusted by filling the balloon and was set at 6–8 mmHg. This allowed for isovolumetric contraction of the LV and assessment of myocardial function. Inotropic support of .03 micrograms (mcg)/kg/min epinephrine and 4 mcg/kg/min dobutamine was infused throughout the isovolumic loading mode.

**Working Heart Mode**

Hearts (n = 5) were transitioned to the working heart mode after 2 hours and maintained in this mode until the end of each study. Tubing with an internal diameter of 3/8” was connected from the output of the oxygenator to a cannula in the LA. During the transition of retrograde resting mode perfusion to working heart mode perfusion, the ESPH flow was partially redirected to the LA. Pressure was regulated by redirecting blood flow into the LA using a compliance chamber to maintain an LA pressure between 6 and 8 mmHg. Another compliance chamber was used to provide afterload in the aortic cannula of 40–55 mmHg.

When the LV function was stable (no sign of arrhythmia or ventricular distention), the flow was directed to the LA. Perfusion data were continuously recorded so that a comprehensive analysis of the cardiac function could be assessed. Inotropic support of .03 mcg/min epinephrine and 4 mcg/min dobutamine were infused throughout the working heart mode to maintain an acceptable blood pressure throughout the perfusion period.

**Experimental Measurements**

**Myocardial functional parameters:** Measurements on the donor heart were obtained with a 7-Fr VSL transonic
pressure/volume conductance catheter (Transonic, Ithaca, NY) inserted into the LV apex and secured with a purse-string suture. Hemodynamic parameters of the LV such as LV peak developed pressure (Pdev, mmHg), LVEDP (mmHg), and maximal change of LV pressure over time (dP/dt max, mmHg/sec) were obtained every hour after initiation of ex situ perfusion over a 4-hour reperfusion time. Data were recorded using LabChart 7 Acquisition Software (AD Instruments, Sidney, Australia).

During the isovolumic loading mode, data were obtained from the balloon inserted in the LV. This allowed for the obtaining Pdev and dP/dt max. When the ESHP system was used in a working mode, a conductance catheter was used for complete assessment of myocardial function.

**Perfusate parameters:** Samples were taken from the ESHP sampling manifold and from a catheter positioned at the coronary sinus every 30 minutes after the initiation of ex situ perfusion. Samples were then analyzed with a blood gas analyzer (Nova Biomedical, Waltham, MA) as a means to measure serum lactates, myocardial lactate metabolism, and myocardial oxygen consumption every 30 minutes (18,23).

**Statistical analysis**
All data were normally distributed as assessed by Shapiro–Wilk test and expressed as mean ± SD. Longitudinal analysis for within-group comparisons was performed using two-way repeated-measures analysis of variance (ANOVA) and by fitting mixed-effects linear regression models. Application of parametric statistical tests was opted for to provide a more powerful and robust analysis for within-group analysis throughout the experimental period. To reduce the probability of false-positive results (Type I error) due to multiple comparisons, Benjamini–Hochberg’s false discovery rate was applied to control family-wise error to α < .05. All tests reported are two-tailed. Statistical analyses were performed with GraphPad Prism version 7.00 for Mac OS X (GraphPad Software, La Jolla, CA). Between-group (Isovolumic vs. Working Mode) comparisons were deferred because such comparisons were beyond the scope of this study.

**RESULTS**

**In Vivo Measurements**
Animal characteristics, myocardial function indices, physiologic parameters, and electrolyte status in vivo are summarized in Table 1.

**Ex Situ Analysis**
**Myocardial function: isovolumic mode:** After 2 hours of reperfusion (beginning of loading), Pdev was 101.0 ± 3.5 mmHg and dP/dt max was 1,818.3 ± 27.4 mmHg/sec. Following 4 hours of reperfusion, no significant difference was noted neither in Pdev (99.7 ± 6.8 mmHg, p = .979) nor in dP/dt max (1,721.3 ± 112.4 mmHg/sec, p = .316) (Figure 6A and B).
LVEDP was maintained steadily at 6–8 mmHg by adjusting the volume of the intraventricular balloon (Figure 6C) to minimize confounding effects of preload among hearts. CBF was adjusted to maintain a steady MAP of 40–50 mmHg. At 2 hours of reperfusion, CBF was $127.8 \pm 25.5 \text{ mL/min/BSA}$ and was significantly increased to $178.4 \pm 58.3 \text{ mL/min/BSA}$ at the end of 4-hour reperfusion ($p = .038$) (Figure 6D).

*Myocardial function: working mode:* After 2 hours of reperfusion, $P_{\text{dev}}$ was $91.0 \pm 2.6 \text{ mmHg}$ and $dP/dt\text{ max}$ was
1,845.7 ± 42.2 mmHg/sec. Following 4 hours of reperfusion, no significant difference was noted in these indices (Pdev: 90.7 ± 2.5 mmHg, p = .942; dP/dt max: 1,737.3 ± 76.0 mmHg/sec, p = .303) (Figure 6A and B).

The height of the compliance chamber connected to the LA was adjusted to maintain a steady LVEDP pressure of 6–8 mmHg throughout the reperfusion period (Figure 6C). At 2 hours of reperfusion, CBF was 135.1 ± 4.1 mL/min/
BSA and was significantly increased at the end of 4 hours: 175.9 ± 13.7 mL/min/BSA (p = .026) (Figure 6D).

**MAP:** MAP in the isovolumic, and the working heart mode was consistent throughout reperfusion (Figure 6E).

**Pressure volume loops: working mode:** Cardiac function was assessed in the working heart mode by pressure–volume (PV) loop calculation, remaining stable after 4 hours of perfusion. Representative PV loops taken following 4 hours of perfusion are shown in Figure 6F. Pressure volume loops cannot be obtained in the isovolumic mode.

**Physiologic parameters:** Myocardial oxygen consumption and lactate metabolism Both myocardial oxygen consumption (beginning of loading: 2.3 ± 0.1 mL O₂/min/100 g; end of reperfusion: 2.6 ± 0.1 O₂/min/100 g; p = .536) and lactates (beginning of loading: 2.4 ± 0.2 mmol/L; end of reperfusion: 2.3 ± 0.2 mmol/L; p = .867) remained unchanged throughout the 2-hour loading period (Figure 7A and B).

**Perfusate components** Hemoglobin (p = .378) and glucose (p = .190) were maintained steadily throughout the 4-hour perfusion period (Figure 7C and D). This was achieved by adding fresh blood or dextrose to the perfusate.

Temperature was maintained at 35.1 ± .1°C during the 2 hours of loading (p = .782) (Figure 7E). Similarly, the perfusate was maintained at a normal range of pH = 7.4 ± .1 during the loading period (p = .998) (Figure 7F).

Figure 8A–D show that there was no significant difference in concentration of calcium, potassium, sodium or bicarbonate in the electrolyte in the perfusate across the four hours between the two modes.

**DISCUSSION**

In the present study, we propose a new design of a multimode ESHP system that allows for transition to either an isovolumic loading mode or working heart mode from the resting mode, by modifying the perfusion circuit and settings. Both the isovolumic loading mode and working heart mode allow for the evaluation of myocardial function using either a balloon or a conductance catheter inserted in the LV, respectively.

The proposed isovolumic setting represents an innovative method to analyze myocardial function in experiments where a high volume (> .5 L) of donor blood is not available. In this mode, approximately 400 mL of blood is needed to perfuse the heart across a 4-hour period. In

![Figure 7. Physiologic parameters during reperfusion in the isovolumic mode and working heart mode.](image-url)
addition, a low volume of perfusate can also be primed and electrolyte-adjusted more easily and efficiently. CBF is easily regulated from the pump so that the desired root pressure can be maintained. Balloon inflation in the left ventricle is very easy and intuitive, and useful images of myocardial contraction can be obtained using an epicardial echocardiogram to calibrate the position. Similarly, very insightful results can be obtained from LV-developed pressure and maximal change of LV pressure over time.

However, the isovolumic mode fails to provide accurate physiological measurements of cardiac function. In particular, the inflated balloon does not simulate the structure and contraction that the LV can generate ex vivo. Although there was no rise in lactates after 2 hours of isovolumic mode, the heart starts to undergo a fair deal of myocardial injury likely because of the LV contracting around the rigid LV balloon. Furthermore, the isovolumic mode causes permanent damage to the mitral valve as it is over-sewn to keep the balloon in place. As a result of the mitral valve damage, hearts examined in the isovolumic mode are not suitable for further studies including actual transplantation into another animal. Although the use of an inflated balloon to measure myocardial contraction does not provide a comprehensive assessment of the myocardial function because of different physiology compared with in vivo conditions, it is a well-established method to investigate the impact of pharmaceutical interventions on cardiac performance.

Perfusing hearts in the working mode provides a more accurate physiological analysis of cardiac function than hearts perfused in an isovolumic mode. Unlike the isovolumic mode, the cardiac structure remains fully intact in this setting. The LV in each heart can generate a full contraction, and blood is ejected through the aortic valve. As a result, hearts are found to have less myocardial injury than hearts perfused in the isovolumic mode.

It is important to note that although the working mode provides a better physiological analysis of cardiac function, it requires a higher level of skill to operate. The working mode requires approximately 2.5–3.5 L of blood to accommodate the additional compliance chamber and tubing, as well as the extra blood required to fill the left side of the heart as compared with .4 L needed in the isovolumic mode.

A comparison between the isovolumic loading mode and working heart mode on the same heart was not assessed in this study. Switching from the isovolumic loading mode to working heart mode or working heart mode to isovolumic loading mode was both found to be unsuitable for assessment. When the sutures from the suture closed, the mitral valve in the isovolumic loading mode was removed and perfusion was switched to working heart mode, and mitral valve injury led to mitral regurgitation and heart dysfunction. In the case of switching from the working heart mode to isovolumic loading mode, the technical skill required to insert the LV balloon into a beating heart was deemed too challenging to replicate, even during the resting mode. During insertion of the LV balloon, contraction of the LV was found to destabilize the LV balloon so that the mitral valve could not be fully sutured. Accounting for these
challenges, a single heart comparison of both methods was deemed beyond the scope of this study.

In the isovolumic mode, the LV balloon was secured by suturing closed the mitral valve before reperfusion on the circuit began. In the working heart mode, a 3/8” line tubing was inserted into the LA and secured with zip-ties. Manipulation of the LA during reperfusion was found to negatively impact the heart, often leading to cardiac fibrillation. For this reason, it was not feasible to compare the functional assessment of both methods on the same heart.

A relatively high sweep flow (up to .75 L/min) of 100% oxygen was needed in this ESHP system to prime the perfusate. The overall sweep flow rate was governed in part because of the limitation in CO₂ flow rates. Carbon dioxide was required in the sweep gas because the neonatal oxygenator in use was much more efficient than required for only removing myocardial CO₂ production. Even if a very low sweep gas rate was used, PaCO₂ would have been too low without the additional of CO₂. The lowest flow from the CO₂ flow meter was used, and this obligated us to use a higher overall sweep to compensate for the CO₂ flow meter limitation.

The addition of ultrafiltration through the hemconcentrator was proven to be highly beneficial to the overall design. Marginal donors may also donate blood to the perfusion system. This fact, coupled with the concern for residual cardioplegia getting into the perfusate, compelled us to incorporate a simple active hemofiltration circuit for electrolyte management. Prebypass ultrafiltration with a balanced electrolyte solution as the additive allowed for efficient control of the potassium and lactate levels in the donor blood before ESHP. Ultrafiltration on bypass was also used as needed to maintain the hematocrit. The ability to ultrafiltrate the perfusion circuit will be of paramount importance with marginal donors.

To allow for translation to a human model, hearts were perfused for a total of 4 hours, as this is a translatable time frame for transport and transplantations in clinical practice. Our results demonstrate that myocardial function showed no significant difference between 2 and 4 hours after the initiation of ex situ perfusion in either the isovolumic or the working mode.

At present, lactate concentration in the perfusate and across the coronary vascular region is one parameter used to determine myocardial viability (3,10). However, recent clinical trials have raised concerns about the validity of lactate measurements as a metabolic marker (3). Messer et al. (24) performed an assessment of cardiac function during normothermic regional perfusion in 13 adult DCD donor hearts before organ care system (OCS) placement. The cardiac index of the DCD donor heart showed no significant correlation with the initial arterial lactate level on the OCS. This group concluded that many good-quality DCD donor hearts would have been unnecessarily abandoned without a functional assessment. Although the measurement of the contractility of the heart is limited during the non-working mode (retrograde resting mode) (25,26), the ESHP described herein allows for a real-time analysis of functional parameters while maintaining the desired aortic and left atrial pressure, and the associated flow rates.

Despite the importance of ESHP in providing simple and reproducible metabolic and functional assessments of viable hearts with objective results, it is worth noting that a prolonged perfusion time still leads to a decline in cardiac function (6,23). It is, therefore, possible that our ESHP system in the working mode over an extended period may lead to a decline in cardiac function. However, further studies would be required to indicate the best method of metabolic support for a heart during ESHP. It is important to qualify that working mode perfusion during ESHP requires well-trained personnel, despite the simplicity shown in this system.

Taking these discrepancies into account, our ESHP system nevertheless demonstrated an efficacious stability across a 4-hour perfusion period and good functionality by two different modes, an isovolumic loading mode and a working heart mode. This was best shown by the possibility of an independent control of left ventricle pressure to facilitate LV loading. In both cases, a reduced lactate level was observed across the perfusion period, thus reflecting a predominance of the aerobic metabolism. The increase in CBF was required after the 2-hour perfusion period to maintain good cardiac function because of the increased workload of the left ventricle.

This study has several limitations. First, our experimental groups have a relatively small sample size. A larger sample size would be needed for a more comprehensive assessment of each mode; however, this was deemed beyond the scope of this exploratory validation study. Second, the level of edema was neither minimized with mannitol nor with albumin because the primary focus was the assessment of myocardial function. For this reason, hearts were not weighed after ESHP. In a clinical scenario, optimal drug support would be applied to combat the systemic inflammatory and stress response during prolonged ESHP. Nonetheless, these results provide a basis for future studies that can evaluate the clinical applicability of ex situ continuously loaded hearts. It is important to note that the scope of this project was limited to research purposes, and considerable modifications and optimization would be needed for a clinical translation. The system described is a univentricular ex situ perfusion model. Nonetheless, this system represents a simple and relatively inexpensive platform for the assessment of ex situ heart hemodynamics, and it has been an improvement over our institution’s previous Langendorff models.

The present study proposes a novel ESHP system that enables comprehensive functional and metabolic assessment of large mammalian hearts. This system allowed for stable
myocardial function for up to 4 hours of perfusion, which would offer great potential for the development of translational therapeutic protocols to improve dysfunctional/suboptimal donated hearts.

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