Experimental paper

Haemodynamic-directed cardiopulmonary resuscitation promotes mitochondrial fusion and preservation of mitochondrial mass after successful resuscitation in a pediatric porcine model

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Abstract

**Objective:** Cerebral mitochondrial dysfunction is a key mediator of neurologic injury following cardiac arrest (CA) and is regulated by the balance of fusion and fission (mitochondrial dynamics). Under stress, fission can decrease mitochondrial mass and signal apoptosis, while fusion promotes oxidative phosphorylation efficiency. This study evaluates mitochondrial dynamics and content in brain tissue 24h after CA between two cardiopulmonary resuscitation (CPR) strategies.

**Interventions:** Piglets (1 month), previously randomized to three groups: (1) Std-CPR (n=5); (2) HD-CPR (n=5; goal systolic blood pressure 90mmHg, goal coronary perfusion pressure 20mmHg); (3) Shams (n=7). Std-CPR and HD-CPR groups underwent 7min of asphyxia, 10min of CPR, and standardized post-resuscitation care. Primary outcomes: (1) cerebral cortical mitochondrial protein expression for fusion (OPA1, OPA1 long to short chain ratio, MFN2) and fission (DRP1, FIS1), and (2) mitochondrial mass by citrate synthase activity. Secondary outcomes: (1) intra-arrest haemodynamics and (2) cerebral performance category (CPC) at 24h.

**Results:** HD-CPR subjects had higher total OPA1 expression compared to Std-CPR (1.52; IQR 1.02 – 1.69 vs 0.67; IQR 0.54 – 0.88, p=0.001) and higher OPA1 long to short chain ratio than both Std-CPR (0.63; IQR 0.46 – 0.92 vs 0.26; IQR 0.26 – 0.31, p=0.016) and shams. Citrate synthase activity was lower in Std-CPR than sham (11.0; IQR 10.15 – 12.29 vs 13.4; IQR 12.28 – 15.66, p=0.047), but preserved in HD-CPR. HD-CPR subjects had improved intra-arrest haemodynamics and CPC scores at 24h compared to Std-CPR.

**Conclusions:** Following asphyxia-associated CA, HD-CPR exhibits increased pro-mitochondrial fusion protein expression, preservation of mitochondrial mass, improved haemodynamics and superior neurologic scoring compared to Std-CPR.

**Institutional protocol number:** IAC 16-001023.

**Keywords:** Cardiac arrest, Cardiopulmonary resuscitation, Mitochondria, Fusion, Fission, Dynamics, Neurologic outcomes

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Introduction

Pediatric in-hospital cardiac arrests affect >15,000 children in the US each year, more than half do not survive to hospital discharge, and permanent brain injury is common among those who survive.1–3 A critical convergence point for neurologic injury following ischaemia-reperfusion injury is cerebral mitochondrial dysfunction, which mediates ongoing secondary injury and cell death.4–6 The impact of intrinsic cerebral mitochondrial quality control in response to mitochondrial dysfunction on neurologic outcomes after cardiac arrest is a novel and promising area of study.7–10

Mitochondrial function is crucial to cellular health through a variety of cellular processes, including energy production, calcium signaling,11 reactive oxygen species generation12,13 and apoptosis.14 Under basal conditions and in response to cell stressors and injury, mitochondrial quality control – ensuring appropriate numbers of normally functioning mitochondria and removing damaged ones – is an essential component of maintaining optimal mitochondrial and cellular function.15,16 The regulation of mitochondrial morphology, also known as mitochondrial dynamics, is a key element of this process and is accomplished through the expression and activity of specific proteins and their post-translational modifications that dictate a constant interplay of fusion, fission, and mitophagy.15,17 Mildly dysfunctional mitochondria can fuse with healthy mitochondria to optimize complementation, mediate oxidative stress, and improve oxidative phosphorylation; severely dysfunctional mitochondria will be sequestered and recycled through fission and mitophagy.16,18,19 Under stress, fusion may salvage bioenergetic output, redistribute mtDNA, and protect cellular components. However, with prolonged and severe stress, fission is activated, which causes mitochondrial fragmentation, increases reactive oxygen species (ROS) production, and triggers mitochondrial apoptotic pathways.19,20

Our group has previously shown that in pediatric porcine models of asphyxia-associated cardiac arrest, a haemodynamic-directed CPR strategy (HD-CPR) improved mitochondrial respiration and survival with favorable neurologic outcome compared to a standard, guideline-based CPR strategy (Std-CPR).21–23 To understand the effect of cardiac arrest and cardiopulmonary resuscitation (CPR) on mitochondrial dynamics in the immature brain, we performed a protein analysis of cerebral mitochondrial dynamics and measured mitochondrial content in cerebral cortex 24h after these two distinct CPR strategies. We additionally evaluated intra-arrest haemodynamics and cerebral performance category (CPC) among survivors of CA. We hypothesized that 24h after return of spontaneous circulation (ROSC), cerebral cortical tissue from animals resuscitated with HD-CPR would have protein expression consistent with mitochondrial fusion and a preservation of mitochondrial content, as well as superior intra-arrest haemodynamics and improved post-arrest clinical neurologic scoring compared to Std-CPR.

Materials and methods

Cohort description

These experiments were performed as a component of a previously reported pre-clinical trial in which animals were randomized to HD-CPR vs Std-CPR.22 In that study, 7 of 10 animals treated with HD-CPR and 5 of 12 animals treated with Std-CPR survived to 24h. The first five animals in each group that survived to 24 h were included in the present study, as were seven anesthetized shams, as described below.

Animal preparation

All animal procedures were approved by the Institutional Animal Care and Use Committee at the Children’s Hospital of Philadelphia (CHOP) and were conducted in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals. Healthy one-month-old, female swine, Sus scrofa domesticus, (8–11 kg) were anesthetized and mechanically ventilated using room air and titrated isoflurane (1.0–2.5% inspired concentration) with a tidal volume of 10–12 ml/kg and positive end-expiratory pressure of 6 cm H2O; respiratory rate was titrated to maintain end-tidal carbon dioxide (ETCO2) at 38–42 mmHg.22,24,25

Measurements

Femoral arterial and venous sheaths were placed and micromanometer-tipped pressure transducers were advanced to the aorta and right atrium for continuous measurement. Coronary perfusion pressure (CoPP) during CPR was calculated as the difference between the aortic diastolic pressure (DBP) and the right atrial pressure. The Philips Heart Start MRx defibrillator (Philips Medical Systems, Andover, MA) provided audiovisual feedback to the provider for rate, compression depth and chest wall recoil during CPR.22

Model justification

This study used an established porcine model because of similarities in neurodevelopmental and chest compression characteristic between swine and humans.26,27 The experimental model was meant to simulate an asphyxial event preceding CA, as many pediatric in-hospital cardiac arrests have a respiratory etiology.1,28,29

Experimental protocol

Briefly, anesthetized animals underwent 7min of asphyxia produced by endotracheal tube clamping followed by induction of ventricular fibrillation (VF) to ensure a standard 10-minute period of CPR that would only be terminated by defibrillation (Fig. 1). More detailed experimental protocols have been reported in previous publications.21,22,25 Compressions were provided with a target rate of 100 per minute and ventilations at 6 per minute with 100% oxygen. Animals were previously randomized to HD-CPR, standard, guideline-based resuscitation (Std-CPR), or anesthetized shams. In HD-CPR, chest compression force was adjusted to target a systolic blood pressure (SBP) of 90mmHg and vasopressors were given to maintain CoPP greater than 20mmHg. Beginning two minutes into CPR, vasopressors were administered if CoPP <20mmHg as follows: adrenaline (0.02mg/kg), adrenaline (0.02mg/kg), and vasopressin (0.4U/kg), with minimal dosing interval of 1 min following adrenaline doses, and 2min following vasopressin administration. In Std-CPR, target depth was one third of the anterior-posterior chest diameter based on real-time feedback from the CPR quality-monitoring defibrillator. After 2min of CPR, adrenaline dosing of 0.02mg/kg was given and repeated every 4min. In both treatment groups,
beginning 10 min after the start of CPR, defibrillation was attempted every two minutes as necessary until ROSC or a maximum of 20 min of CPR. After ROSC, animals received standardized post-arrest intensive care. Shams were anesthetized and instrumented with central venous and arterial catheters, underwent protocolized, similar anesthetic exposure with inhaled isoflurane (≈1.0%). Blood pressure, end-tidal carbon dioxide, oxygen saturation, temperature, and anesthesia were maintained by using the identical explicit post-ROSC protocol as for the treatment groups. Following emergence from anesthesia, all animals were extubated and monitored for 24 h, where downstream outcome metrics were performed by members of scientific team blinded to treatment status.

Sample acquisition

At 24 h post-arrest, animals were re-anesthetized and intubated. A bilateral craniectomy was performed to expose the brain, and cortical tissue was rapidly extracted and snap frozen in liquid nitrogen. Animals were humanely euthanized while under general anesthesia. All experimental procedures were performed on previously banked tissue of animals that survived to 24 h after CA.

Laboratory analysis

Frozen tissue whole cell lysate preparation

Frozen cerebral cortex tissue was homogenized (5% wt:vol) with 4 rounds of 5 strokes in a Potter-Elvehjem teflon-glass homogenizer in RIPA buffer (#89901, Pierce, Rockford, IL, USA) with protease inhibitor (AEBSF, Aprotinin, Bestatin hydrochloride, E-64, Leupeptin, Pepstatin A) (#P8340, Sigma Aldrich, St. Louis, MO, USA) and 5 mM NaF (#201154, Sigma Aldrich, St. Louis, MO, USA) as a phosphatase inhibitor. The homogenate was centrifuged at 12,000 × g for 10 min, the supernatant whole cell lysate was preserved and the pellet discarded. Total protein was quantified using the BCA Protein Assay Kit (#23228; Pierce, Rockford, IL, USA).

Western blots

Equal amounts of lysate protein were denatured in 4 × Laemmli sample buffer with 1:10 dithiothreitol (DTT) (D0632-1G, Sigma Aldrich, St. Louis, MO, USA), separated by SDS–polyacrylamide gel electrophoresis (12% Bis-Tris, #NP0349BOX, ThermoFisher, Carlsbad, CA, USA), and transferred to nitrocellulose membranes (#88024, Thermo Scientific, Rockford, IL, USA). Membranes were blocked with Odyssey Blocking Buffer (#927-40000, Li-Cor, Lincoln, NE, USA) and incubated with primary antibody overnight for the following: DRP1 (1:15K, #32898, Santa Cruz Biotechnology, Santa Cruz, CA, USA), FIS1 (1:1K, #10956-1-AP, Proteintech, Rosemont, IL, USA), OPA1 (1:5K, #NB110-55290, Novus Biologicals, Littleton, CO, USA), MFN2 (1:1K, #PA5-42171, Invitrogen, Rockford, IL, USA) and alpha-tubulin (1:20K; #ab7291, Abcam, Cambridge, MA, USA). After washing, membranes were stained with IRDye secondary antibody (1:15K, #925-32411, Li-Cor, Lincoln, NE, USA) for 1 h, then imaged using an Odyssey Scanner (Li-Cor). Relative band densities were determined by digital densitometry using Li-Cor Odyssey Application Software v3.

Citrate synthase activity

Preserved chamber contents from previously performed high-resolution respirometry were stored at -80°C and analyzed for citrate synthase (CS) activity quantification (Citrate Synthase Assay Kit, CS0720; Sigma). CS activity (µmol/mL/min) was used as an index of mitochondrial content.5,22,30
**Porcine cerebral performance category**

Porcine Cerebral Performance Category was independently determined 24h after ROSC by two blinded, trained investigators. The scoring system is as follows: 1 is normal (no difficulty standing, walking, etc.); 2 is mild disability (can stand, but unsteady, slow to respond, drinking, not eating); 3 is severe disability (awake but not responding, cannot stand, walk, eat, drink); 4 is comatose; and 5 is dead.31,32

**Statistical outcomes and analysis**

The primary outcome of this study was mitochondrial dynamics protein expression and mitochondrial mass in cerebral cortex at 24 h after ROSC. Secondary outcomes include intra-arrest haemodynamics and cerebral performance category as a measure of neurologic function 24h after ROSC. Outcomes were compared between subjects that received HD-CPR and Std-CPR after asphyxial cardiac arrest and sham animals that received equal anesthetic time.

Statistical analysis was performed using GraphPad Prism v7 (GraphPad, La Jolla, CA) and Stata Version 14 (StataCorp, College Station, TX). Protein expression, mitochondrial content and cerebral performance category were not assumed to have normal distributions and were statistically evaluated for differences using one-way nonparametric ANOVA (Kruskal-Wallis) followed by a Dunn's multiple comparisons test. Results are expressed as medians with interquartile ranges followed by p values of Dunn's multiple comparisons test.

Haemodynamic data were recorded at a high sampling rate during CPR using PowerLab (ADInstruments, Colorado Springs, CO) and the mean of each 15-second epoch was then calculated. The haemodynamic data from the first ten minutes of CPR (excluding the first minute to account for the typical period of chest wall molding) were compared between the two CPR groups with a generalized estimating equation regression model accounting for animal-level clustering of data points.

**Results**

**Intra-arrest haemodynamic measurements**

During CPR, mean arterial pressure was higher with HD-CPR than Std-CPR (point estimate +8.1 [CI95: 1.4, 14.8]mmHg; p=0.018), as were diastolic blood pressure (+6.1 [CI95: 0.01, 12.1]; p=0.049) and coronary perfusion pressure (+6.2 [CI95: 0.2, 12.2]mmHg; p=0.042) (Fig. 2). There were no significant differences in systolic blood pressure or right atrial pressure between groups.

![Fig. 2](image-url)  
**Fig. 2**—Increased intra-arrest haemodynamic measurements in HD-CPR versus Std-CPR in animals that survived to 24h after CA.

(A) Invasive mean arterial pressure monitoring during CPR period. Time zero represents initiation of chest compressions. Error bars represent SEM. MAP was higher with HD-CPR compared to Std-CPR (point estimate +8.1 [CI95: 1.4, 14.8]mmHg; p=0.018).

(B) Calculated coronary perfusion pressure as DBP minus RAP. Time zero represents initiation of chest compressions. Error bars represent SEM. CoPP was higher with HD-CPR compared to Std-CPR (point estimate +6.2 [CI95: 0.2, 12.2]mmHg; p=0.042).

(C) Invasive systolic blood pressure monitoring during CPR period. Time zero represents initiation of chest compressions. Error bars represent SEM. No statistical difference was found between HD-CPR and Std-CPR. (point estimate +8.43 [CI95: -11.9, 28.8]mmHg; p=0.416).

(D) Invasive diastolic blood pressure monitoring during CPR period. Time zero represents initiation of chest compressions. Error bars represent SEM. DBP was higher with HD-CPR compared to Std-CPR (point estimate +6.1 [CI95: 0.01, 12.1]mmHg; p=0.049).
**Expression of fusion and fission proteins**

Animals treated with HD-CPR had a near three-fold median increase in total OPA1 expression (1.52; IQR 1.02–1.69 vs 0.67; IQR 0.54–0.88, p=0.001) compared to those treated with Std-CPR (Fig. 3). OPA1 cleavage analysis showed higher OPA1 long to short chain ratio in animals treated with HD CPR compared to both animals treated with Std-CPR (0.63; IQR 0.46–0.92 vs 0.26; IQR 0.26–0.31, p=0.016) and shams (0.63; IQR 0.46–0.92 vs 0.28; IQR 0.25–0.36, p<0.025). Additionally, OPA1 cleavage analysis showed higher L-OXA1 (0.43; IQR 0.18–0.54 vs 0.06; IQR 0.05–0.08, p=0.002) and S-OXA1 (0.56; IQR 0.40–0.64 vs 0.22; IQR 0.13–0.31, p=0.003) in the HD-CPR arm compared to sham animals (Fig. 4). No significant effect was seen for MFN2 protein expression. There was also no significant effect on expression of total DRP1 or FIS1 between intervention cohorts (Fig. 3).

**Mitochondrial mass**

There was a decrease in mitochondrial mass in animals that were treated with Std-CPR compared to sham animals (11.0; IQR 10.15–12.29 vs 13.4; IQR 12.28–15.66, p=0.047) and a preservation of mitochondrial mass in animals treated with HD-CPR compared to sham animals (Fig. 5). There was also a trend towards increased mitochondrial mass in the HD-CPR compared to Std-CPR (14.16; IQR 11.97–15.29 vs 11.02; IQR 10.15–12.29, p=0.085).

**Porcine cerebral performance category**

At 24 h after cardiac arrest, survivors of HD-CPR had normalization of CPC to shams, while Std-CPR animals had significantly worse neurologic scores than both animals that were treated with HD-CPR (3; IQR 2–3 vs 1; IQR 1–2, p=0.014) and shams (3; IQR 2–3 vs 1; IQR 1–1, p=0.0094) (Fig. 6).

**Discussion**

Prior studies by our group comparing HD-CPR to Std-CPR in this model of pediatric asphyxia-associated cardiac arrest have demonstrated that HD-CPR results in improved mitochondrial respiration and higher rates of both ROSC and 24-h survival with favorable neurologic outcome.\(^1\)\(^2\) The present study builds on this line of work by demonstrating that HD-CPR subjects had protein expression consistent with mitochondrial fusion in the cerebral cortex 24 h after cardiac arrest when compared to sham animals. This conclusion is supported by an upregulation of total OPA1 expression, increased OPA1 long to short chain ratio, and a preservation of mitochondrial mass. In contrast, compared to HD-CPR, Std-CPR resulted in decreased mitochondrial mass 24 h post-arrest, as measured by citrate synthase activity. This study also demonstrates improved intra-arrest haemodynamics and post-arrest neurologic scoring in subjects that received HD-CPR. Cumulatively, these data suggest that in subjects receiving HD-CPR, mitochondrial fusion may be a driven by superior intra-arrest haemodynamics and represent an adaptive response that optimizes clinical neurologic recovery after asphyxial cardiac arrest.

Mitochondrial fusion is regulated by GTPase proteins optic atrophy 1 (OPA1) and mitofusins 1 and 2 (MFN1, MFN2). OPA1 exists in multiple isoforms; long isoform (L-OPA1) is embedded in the inner mitochondrial membrane and promotes mitochondrial fusion. A low

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**Fig. 3 – OPA1 protein expression is increased at 24 h after CA in animals receiving HD-CPR.**

(A) Representative Western blot analysis of expression of fusion/fission proteins in homogenized cerebral cortex at 24 h in sham (n=7), Std-CPR (n=5) and HD-CPR (n=5).

(B) Relative densitometry of OPA1 and MFN2 protein expression in relation to alpha tubulin loading control expressed as box plots. HD-CPR animals have increased OPA1 expression compared to sham (mean rank difference 10.4, p =0.001).

(C) Relative densitometry of DRP1 and FIS1 protein expression in relation to alpha tubulin loading control expressed as box plots. No significant differences between groups.
ATP state or loss of mitochondrial potential can stimulate excessive OPA1 cleavage to short isform (S-OPA1), which stimulates fission, increases mitochondrial membrane permeability, and promotes cell death. Mitochondrial fission is influenced by total expression and post-translational modifications of dynamin related protein (DRP1), a cytosolic GTPase that translocates to the mitochondrial outer membrane and binds to mitochondrial fission protein (FIS1). \cite{22,34} DRP1 inhibition with small molecule mdivi-1 is associated with improved survival and neurologic scores after ROSC in a murine model of cardiac arrest. \cite{9}

Our findings are the first to document protein expression changes pertaining to mitochondrial dynamics in cerebral cortex following a cardiac arrest in a large animal model. To our knowledge, the only study of cerebral mitochondrial dynamics following ischaemia reperfusion injury was performed by Li et al. in an adult rodent asphyxial cardiac arrest model. \cite{10} Their study reported an upregulation of fusion proteins MFN1 and MFN2 at 4h post-ROSC, followed by a rise in fission proteins DRP1 and FIS1 at 12–24h. We found our most significant changes in OPA1 expression and OPA1 cleavage following cardiac arrest treated with HD-CPR, and did not detect differential expression of fission proteins DRP1 and FIS1. The differences in these findings may be attributed to the degree of injury (e.g., differences in injury model or resuscitation techniques), different protein expression in a higher order mammal that is more directly related to human response, or a difference in response to insult in the developing cortex versus an adult animal model. \cite{22,35,36}

Additionally, in an adult rodent diffuse axonal injury traumatic brain injury (TBI) model, Di Petro et al. showed that moderate TBI yielded elevated OPA1 expression 48h after injury, but an increased L-OPA1 to S-OPA1 ratio as early as 6h after injury. \cite{15} Furthermore, rodents subjected to moderate TBI had preserved citrate synthase expression, also consistent with fusion. After severe TBI, differential cleavage yielded greater S-OPA1 versus L-OPA1 and an increase in DRP1 expression, consistent with mitochondrial fission. \cite{16} Their findings support an emerging paradigm of differential mitochondrial dynamics responses dictated by the severity of injury. During moderate stress or injury, mitochondrial fusion allows for maximal metabolic efficiency and redistribution of mitochondrial DNA, which is thought to be protective. However, after severe injury, a sustained energy deficit leads to fission, which precedes mitophagy and can lead to ROS generation and cell death. \cite{15,16,19,37}

Animals that were treated with HD-CPR had greater systemic blood pressure and cerebral perfusion pressure during cardiac arrest than those treated with Std-CPR. Presumably, this resulted in a less severe ischaemia reperfusion injury. Consistent with the paradigm that injury severity is an important determinant of mitochondrial dynamics, it appears that the less severe brain injury that occurred in HD-CPR was associated with a more pro-fusion state compared to Std-CPR. This result suggests that cerebral mitochondrial dynamics can be altered in cardiac arrest and potentially other forms of ischaemia reperfusion injury by optimizing brain perfusion and fuels speculation that pharmacologically targeting fusion could be beneficial.
Our findings supporting an environment of mitochondrial fusion in the cerebral cortex 24h after HD-CPR in conjunction with our findings of improvements in haemodynamics and neurologic scoring, as well as previously reported improvements in bioenergetics and survivorship are consistent with evidence that mitochondrial fusion may be protective. In addition to indirect promotion of fusion by optimizing cerebral perfusion during cardiac arrest, directed therapies that manipulate mitochondrial dynamics will be an exciting future direction. Our findings in this study suggest that OPA1 expression and cleavage may be important in cerebral cortex after such an injury in the developing brain, and that further study into neurotherapeutics that promote fusion by targeting OPA1 expression and post-translational modification are justified. Mdiv1, an inhibitor of DRP1 translocation, has been shown in a rodent model to improve neurologic outcomes after cardiac arrest and warrants further study in a higher level animal model of cardiac arrest.

**Limitations**

Alterations in mitochondrial dynamics found in this study are only in survivors at 24h after cardiac arrest. Because of the significantly increased survival in subjects who received haemodynamic-directed CPR after cardiac arrest compared to guideline based CPR, the magnitude of difference in protein expression and mitochondrial mass may be less than what we would have found had all Std-CPR animals (presumably with greater injury) survived. Similarly, mitochondrial dynamics described after injury in other models have an evolving time course, which we were not able to assess in these experiments. Although DRP1 translocates to the mitochondrial membrane during fission, we did not study DRP1 expression in isolated mitochondria due to concern for a non-representative mitochondrial fraction. We also were not able to corroborate our protein expression findings with mitochondrial imaging (e.g., confocal or electron microscopy) or RNA expression because of experimental constraints. Future studies of the time course of proteomic, transcriptomic and histopathologic measures of mitochondrial dynamics with this highly relevant model will allow us to better define the injury and the optimal timing of therapeutic interventions. Finally, our findings were limited by a small sample size per group of piglets undergoing cardiac arrest and cardiopulmonary resuscitation.

**Conclusion**

We have demonstrated increased expression of proteins that promote mitochondrial fusion and preservation of mitochondrial mass in animals who received HD-CPR as compared to Std-CPR at 24h after CA. This result is associated with higher intra-arrest haemodynamics parameters and superior post-arrest neurologic scoring, as well as previously described mitochondrial bioenergetics and survival. Further studies are warranted to define the time course and mechanisms of brain mitochondrial dynamics following cardiac arrest,
and to interrogate therapeutics that target mitochondrial dynamics to confer neuroprotection and promote neurologic recovery following cardiac arrest.

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**Conflict of interest statement**

The authors reported no other conflicts of interest specific to this manuscript.

**CRediT authorship contribution statement**

Kumaran Senthil: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Visualization. Ryan W. Morgan: Conceptualization, Methodology, Formal analysis, Investigation, Writing - review & editing, Supervision, Project administration. Marco M. Hefti: Formal analysis, Writing - review & editing. Michael Karlsson: Conceptualization, Methodology, Investigation, Writing - review & editing. Andrew J. Lautz: Conceptualization, Methodology, Investigation. Constantine D. Mavroudis: Methodology, Investigation. Tiffany Ko: Conceptualization, Methodology, Investigation, Funding acquisition. Vinay M. Nadkarni: Conceptualization, Methodology, Investigation, Resources, Writing - review & editing, Supervision. Johannes Ehinger: Conceptualization, Methodology, Writing - review & editing. Robert A. Berg: Conceptualization, Methodology, Investigation, Resources, Writing - review & editing, Supervision, Project administration. Robert M. Sutton: Conceptualization, Methodology, Investigation, Resources, Writing - review & editing, Supervision, Project administration. Francis X. McGowan: Conceptualization, Methodology, Formal analysis, Writing - review & editing, Supervision. Todd J. Kilbaugh: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

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