Published in final edited form as:

Crit Care Med. 2019 March ; 47(3): e241–e249. doi:10.1097/CCM.0000000000003620.

Hemodynamic-directed CPR Improves Neurologic Outcomes and Mitochondrial Function in Heart and Brain

Andrew J. Lautz, MD^{*,1,2}, Ryan W. Morgan, MD, MTR^{*,1}, Michael Karlsson, MD¹, Constantine D. Mavroudis, MD, MS³, Tiffany S. Ko, MS⁴, Daniel J. Licht, MD⁵, Vinay M. Nadkarni, MD, MS¹, Robert A. Berg, MD¹, Robert M. Sutton, MD, MSCE^{†,1}, and Todd J. Kilbaugh, MD^{†,1}

¹Children's Hospital of Philadelphia and University of Pennsylvania Perelman School of Medicine; Department of Anesthesiology and Critical Care Medicine

²Cincinnati Children's Hospital Medical Center; Division of Critical Care Medicine

³Children's Hospital of Philadelphia and University of Pennsylvania Perelman School of Medicine; Department of Cardiothoracic Surgery

⁴University of Pennsylvania, Department of Bioengineering

⁵Children's Hospital of Philadelphia and University of Pennsylvania Perelman School of Medicine; Department of Pediatrics, Division of Neurology

Abstract

Objective: Less than half of the thousands of children who suffer in-hospital cardiac arrests annually survive and neurologic injury is common among survivors. Hemodynamic-directed cardiopulmonary resuscitation (HD-CPR) improves short-term survival but its impact on longer term survival and mitochondrial respiration – a potential neurotherapeutic target – remains unknown. The primary objectives of this study were to compare rates of 24-hour survival with favorable neurologic outcome after cardiac arrest treated with HD-CPR versus standard depth-guided CPR (DG-CPR) and to compare brain and heart mitochondrial respiration between groups 24 hours after resuscitation.

Design: Randomized pre-clinical large animal trial.

Setting: A large animal resuscitation laboratory at a large academic children's hospital.

Subjects: Twenty-eight four-week-old female piglets (8 to 11 kg).

Interventions: Twenty-two swine underwent 7 minutes of asphyxia followed by ventricular fibrillation and randomized treatment with either HD-CPR (n=10; compression depth titrated to aortic systolic pressure of 90mmHg, vasopressors titrated to coronary perfusion pressure 20mmHg) or DG-CPR (n=12; depth 1/3 chest diameter, epinephrine every 4 minutes). Six animals (sham group) underwent anesthesia and instrumentation without cardiac arrest. The primary outcomes were favorable neurologic outcome (swine cerebral performance category 2)

Address correspondence to: Todd J. Kilbaugh, MD, Children's Hospital of Philadelphia, 3401 Civic Center Boulevard, Suite 8566, Philadelphia PA 19104, Kilbaugh@chop.edu; 267-426-2344 (P); 215-590-4327 (F).

*Co-first authors

†Co-senior authors

and mitochondrial maximal oxidative phosphorylation (OXPHOS_{CI+CII}) in the cerebral cortex and hippocampus.

Measurements and Main Results: Favorable neurologic outcome was more likely with HD-CPR (7/10) than DG-CPR (1/12; $p=0.006$). HD-CPR resulted in higher intra-arrest coronary perfusion pressure, aortic pressures, and brain tissue oxygenation. HD-CPR resulted in higher OXPHOS_{CI+CII} (pmol O₂/s*mg/citrate synthase) in the cortex (6.00 ± 0.28 vs. 3.88 ± 0.43 ; $p<0.05$) and hippocampus (6.26 ± 0.67 vs. 3.55 ± 0.65 ; $p<0.05$) and higher Complex I respiration (pmol O₂/s*mg) in the right (20.62 ± 1.06 vs. 15.88 ± 0.81 ; $p<0.05$) and left ventricles (20.14 ± 1.40 vs. 14.17 ± 1.53 ; $p<0.05$).

Conclusions: In a model of asphyxia-associated pediatric cardiac arrest, HD-CPR increases rates of 24-hour survival with favorable neurologic outcome, intra-arrest hemodynamics, and cerebral and myocardial mitochondrial respiration.

Keywords

cardiac arrest; cardiopulmonary resuscitation; mitochondria; neurologic outcomes

INTRODUCTION:

Pediatric in-hospital cardiac arrest (IHCA) occurs in approximately 6,000 children each year in the United States (1, 2). Less than half of these children will survive to hospital discharge despite nearly 80% achieving return of spontaneous circulation (ROSC) immediately after cardiopulmonary resuscitation (CPR) (3, 4). Neurologic injury and myocardial dysfunction have been implicated as primary components of the post-cardiac arrest syndrome that lead to these deaths after apparently successful resuscitation (5). As neurologic morbidity is also common among survivors of cardiac arrest (6), the development and evaluation of resuscitation techniques specific to pediatrics that promote cardiovascular and neurologic recovery is warranted.

Physiologic monitoring during CPR is recommended by consensus guidelines (7, 8), but its use is not common. Hemodynamic-directed CPR (HD-CPR) has been highlighted as a promising technique to improve survival (9-11). In pediatric models of IHCA, HD-CPR improves rates of ROSC and 4-hour survival (12), but the efficacy of this resuscitation method in improving rates of longer term 24-hour survival and clinical neurologic status among survivors remains unknown. To that end, the primary objective of this study was to determine if HD-CPR would improve 24-hour survival with favorable neurologic outcome compared with standard depth-guided CPR (DG-CPR) in a porcine model of pediatric asphyxia-associated IHCA.

Our previous work in porcine models of IHCA has demonstrated that mitochondrial dysfunction in the brain persists at least four hours after ROSC despite resuscitation with HD-CPR, indicating that neurologic injury cannot be entirely mitigated by a personalized hemodynamic approach alone (13). As mitochondrial bioenergetic alterations in the setting of ischemia-reperfusion injury play a pivotal role in ongoing secondary cellular injury in the brain and heart, measurement of mitochondrial respiration after ROSC serves not only as a

marker of cellular injury and metabolic crisis (13-16), but may represent a therapeutic target (14, 17) to potentially decrease the severity of the post-cardiac arrest syndrome. Therefore, as secondary objectives, we sought to determine if cerebral mitochondrial functional alterations persisted at 24 hours post-ROSC and if similar alterations in mitochondrial function were evident in myocardial tissue. We hypothesized that cerebral and myocardial mitochondrial bioenergetics would be more abnormal among DG-CPR survivors compared with HD-CPR survivors at 24 hours post-ROSC, but that compared to shams, both groups would still have evidence of altered respiration.

MATERIALS AND METHODS:

All procedures were approved by the Institutional Animal Care and Use Committee of the Children's Hospital of Philadelphia. Twenty-eight four-week-old female piglets (8 to 11 kg) were randomized to one of three cohorts: 1) cardiac arrest with HD-CPR (n=10); 2) cardiac arrest with DG-CPR (n=12); or 3) sham (n=6). In surviving animals, brain and heart mitochondrial assessments of the electron transport chain were conducted 24 hours post-ROSC.

Pediatric IHCA Model:

This experiment utilized an established porcine model (9-13, 18) due to the similarities in anterior-posterior chest diameter and chest compression characteristics, as well as neuroanatomy and neurodevelopment, between swine and humans (19, 20). Since 80% of pediatric intensive care unit IHCAs are preceded by respiratory insufficiency, the injury model utilized an asphyxial event prior to induction of cardiac arrest (3). Seven minutes of asphyxia was chosen based upon prior work to yield severe hypoxemia and hypercarbia (9, 10). Ventricular fibrillation (VF), which occurs in nearly one-third of all pediatric IHCAs as either the initial or a subsequent rhythm (21), was induced to ensure a uniform period of cardiac arrest during which to compare resuscitation methods. Ten minutes of CPR was provided prior to first defibrillation to assess differences in CPR between groups and because ten minutes is the median duration of CPR among survivors of pediatric cardiac arrest (22).

Cardiac Arrest Protocol:

General anesthesia was induced and maintained on a mixture of isoflurane and room air. After tracheal intubation and mechanical ventilation, femoral arterial and venous introducer sheaths were placed and micromanometer-tipped catheters were advanced to the aorta and right atrium for continuous pressure monitoring. A Licox catheter (Integra LifeSciences, Plainsboro, NJ) was inserted via a right parietal burr hole for measurement of brain tissue oxygenation (PbtO₂). The endotracheal tube was clamped to induce asphyxia, and intravenous fentanyl was continuously infused during the asphyxial period for maintenance of anesthesia. After seven minutes of asphyxia, VF was induced via transthoracic needle electrodes, the endotracheal tube was unclamped, and manual CPR commenced (Supplemental Digital Content 1).

The chest compression rate was 100 compressions per minute in all animals and was guided by metronome and a CPR quality feedback device (Zoll R Series Plus; Zoll Medical Corporation, Chelmsford, MA). Depth of chest compressions and vasopressor administration varied by group. In the HD-CPR group (Supplemental Digital Content 2), chest compression depth was continuously titrated to achieve a goal aortic systolic pressure of 90 mmHg and vasopressor bolus doses (epinephrine and vasopressin) were titrated to maintain a goal coronary perfusion pressure of 20 mmHg. Beginning two minutes into CPR, these animals received vasopressor if the CoPP was less than 20 mmHg during mid-diastole for three consecutive compressions. Epinephrine (0.02 mg/kg) was administered first, followed by a second dose of epinephrine at least one minute later if the CoPP remained below goal. Vasopressin (0.4 U/kg) was administered after an additional one minute if needed, and the cycle was repeated starting with epinephrine as needed two minutes after the dose of vasopressin. Vasopressin was used as an alternative vasopressor at this point due to epinephrine being ineffective at maintaining goal CoPP. In the DG-CPR group, a guideline-recommended (23) chest compression depth of one-third of the anterior-posterior chest diameter was targeted based on real-time feedback from the CPR quality feedback device. After two minutes of CPR, epinephrine was administered and repeated every four minutes during CPR (8).

Defibrillation (5 J/kg) was attempted after ten minutes of CPR and was repeated every two minutes if the animal remained in VF until ROSC (30 seconds of sustained aortic waveform pulsatility) or until 20 total minutes of CPR. Following ROSC, survivors received algorithmic titration of hemodynamic, ventilatory, and anesthetic support for four hours prior to extubation. Sham animals received similar total anesthetic time and identical instrumentation but did not undergo asphyxia or cardiac arrest.

Neurobehavioral outcomes were assessed utilizing the swine cerebral performance category (CPC) score, which was assessed by two independent, trained investigators at 24 hours post-ROSC (24, 25). Briefly, the swine CPC assigns a global assessment of neurologic function, ranging from normal to mild dysfunction in Categories 1 and 2, respectively, to unresponsiveness in Category 5. Categories 1 and 2 were considered favorable neurologic outcome.

Left and right ventricular biopsies and cerebral cortex and hippocampus tissue acquisition was performed on survivors at 24 hours post-ROSC with tissue preparation according to established protocols (26-29). Euthanasia occurred under general anesthesia via the provision of intravenous potassium chloride.

Mitochondrial Respirometry:

High-resolution respirometry (Oxygraph-2k and DatLab software; Oroboros Instruments, Innsbruck, Austria) was performed to measure oxygen consumption (pmol/(s*mg)). Cortical and hippocampal specimens were analyzed as previously described using a substrate, uncoupler, inhibitor titration (SUIT) protocol (28, 30). The SUIT protocol utilized for brain tissue was adapted for myocardial tissue (Supplemental Digital Content 3).

Statistical Analysis and Outcomes:

The primary survival outcome of this study was 24-hour survival with favorable neurologic outcome, defined as a swine CPC score of 1 or 2 at 24 hours post-ROSC. The primary mitochondrial outcome was maximal oxidative phosphorylation (OXPHOS_{CI+CII}) in the cerebral cortex. Secondary outcomes included hemodynamics (specifically coronary perfusion pressure) and brain tissue oxygenation during cardiac arrest; chest compression depth (cm); and measures of mitochondrial bioenergetics in the brain and myocardium. Mitochondrial bioenergetics were compared between treatment groups and between surviving animals with favorable and unfavorable neurologic outcomes. Hemodynamic waveform data measured in PowerLab (ADInstruments, Chelmsford, MA) were averaged into 15-second data epochs by custom code (MATLAB; MathWorks, Inc., Natick, MA), and further described in Supplemental Digital Content 3.

Statistical analyses were performed using Stata Version 14 (StataCorp, College Station, TX) and PRISM Version 7 (GraphPad, La Jolla, CA). Dichotomous variables were compared with Fisher's exact test. The Skewness-Kurtosis test was utilized to determine the normality of continuous variables; normally distributed continuous variables were described as means with standard errors of the mean and compared by Student's t-test or ANOVA, while non-parametric continuous variables were described as medians with interquartile ranges and compared with the Wilcoxon rank-sum test or the Kruskal-Wallis test. Hemodynamic variables, chest compression depth, and brain tissue oxygenation during CPR were compared using generalized estimating equation regression models to account for clustering of data points within individual animals.

RESULTS:

Survival and Physiologic Data:

Animals treated with HD-CPR had higher rates of 24-hour survival with favorable neurologic outcome (7/10) compared to those treated with DG-CPR (1/12; $p = 0.006$). Rates of both ROSC and 24-hour post-ROSC survival were 7/10 for the HD-CPR group and 5/12 for the DG-CPR group ($p = 0.23$). The median duration of CPR was 10 minutes [IQR: 10, 20] in the HD-CPR group and 20 minutes [11, 20] in the DG-CPR group ($p = 0.08$). Among survivors who were included in neurologic outcome assessment and mitochondrial analyses, the median duration of CPR was 10 minutes [10, 10] in the HD-CPR group and 10 minutes [10, 12] in the DG-CPR group ($p = 0.08$). The median total number of administered vasopressors was similar between animals receiving HD-CPR (4.5; IQR 3, 9; $p = 0.32$) and DG-CPR (5; IQR 2.5, 5), but the HD-CPR group received a higher median number of vasopressors during the first ten minutes of resuscitation prior to the first defibrillation attempt (4.5; IQR 3, 6) relative to DG-CPR (2; IQR 2, 2; $p < 0.001$).

At baseline and at the end of the asphyxial period, there were no significant differences in physiologic measurements between groups (Supplemental Digital Content 4). HD-CPR resulted in significantly higher coronary perfusion pressure than DG-CPR (21.0 ± 2.8 vs. 9.6 ± 2.1 mmHg; $p < 0.001$) during minutes 2–10 of the resuscitation period (Fig. 1; Table 1). During CPR, animals treated with HD-CPR had higher aortic systolic pressure (85.5 ± 10.5

vs. 60.5 ± 7.1 mmHg; $p = 0.02$); aortic diastolic pressure (31.3 ± 4.0 vs. 16.8 ± 2.7 mmHg; $p < 0.001$); and relative brain tissue oxygenation (233.8 ± 27.9 % baseline vs. 59.2 ± 16.1 % baseline; $p < 0.001$), compared to DG-CPR. Chest compressions were shallower in the HD-CPR group (3.4 ± 0.3 cm vs. 4.4 ± 0.2 cm; $p = 0.02$). Chest compression rate, end-tidal carbon dioxide (ETCO₂), and right atrial pressures during CPR did not differ between groups (Table 1). Ten minutes post-ROSC, surviving animals treated with HD-CPR had lower heart rates (119.3 ± 2.4 bpm vs. 155.1 ± 5.8 bpm; $p < 0.001$) and higher aortic diastolic pressures (81.3 ± 3.8 mmHg vs. 65.6 ± 3.4 mmHg; $p = 0.01$) than those treated with DG-CPR. Three hours post-ROSC, there were no significant differences in heart rates or blood pressures between treatment groups (Supplemental Digital Content 4).

Myocardial Mitochondrial Respiration:

Complex I respiration (OXPHOS_{CI}) in both ventricles and maximal non-phosphorylating oxidative phosphorylation (ETC_{CI+CII}) in the right ventricle were significantly lower in both cardiac arrest groups relative to shams. In addition, HD-CPR resulted in significantly higher OXPHOS_{CI} compared to DG-CPR in both ventricles. There were no other differences in myocardial mitochondrial respiration between groups (Fig. 2; Supplemental Digital Content 5).

Brain Mitochondrial Respiration:

OXPHOS_{CI}, maximal oxidative phosphorylation (OXPHOS_{CI+CII}), and respiratory control ratio were lower in both the cerebral cortex and hippocampus in both cardiac arrest groups as compared to the sham group. Compared to shams, Complex II respiration (ETC_{CII}) was lower in the cortex and the hippocampus in animals treated with DG-CPR, but not in those treated with HD-CPR. Animals treated with HD-CPR exhibited a significantly higher OXPHOS_{CI+CII} compared to DG-CPR in both the cortex and hippocampus (Fig. 3; Supplemental Digital Content 2). This resulted in a higher respiratory control ratio for both brain regions in subjects treated with HD-CPR compared to DG-CPR (Supplemental Digital Content 2). There were no statistical differences between treatment groups for inner membrane leak respiration or complex IV respiration. There were no differences in citrate synthase activity, which was used to normalize mitochondrial respiration analyses, between the three groups (Supplemental Digital Content 2).

Mitochondrial Respiration and Neurologic Outcome:

Among survivors, those with a favorable neurologic outcome had higher cortical and hippocampal OXPHOS_{CI+CII} compared to those with a poor neurologic outcome (Supplemental Digital Content 6).

DISCUSSION:

This investigation establishes that hemodynamic-directed CPR (HD-CPR) increases the rate of 24-hour survival with favorable neurologic outcome compared to standard depth-guided CPR (DG-CPR) in a pediatric model of asphyxial cardiac arrest. During resuscitation, HD-CPR resulted in higher coronary perfusion pressures, aortic systolic and diastolic pressures, and brain tissue oxygenation. Among surviving animals, cerebral and myocardial

mitochondrial bioenergetics were markedly different in the HD-CPR and DG-CPR groups compared to shams. In addition, the HD-CPR group displayed significantly higher mitochondrial bioenergetic function in the cortex, hippocampus, and myocardium compared to DG-CPR, providing support for the hypothesis that HD-CPR improved outcomes by improving oxygen delivery and stabilizing mitochondrial bioenergetics. Among survivors, animals with favorable neurobehavioral outcome had substantially higher mitochondrial oxidative phosphorylation capacity compared to animals with unfavorable neurobehavioral outcomes, providing a critical clinical correlate of the mitochondrial findings.

This study supports previous pre-clinical findings that actively targeting predetermined coronary perfusion pressure and aortic systolic pressure goals during cardiac arrest can optimize hemodynamics and improve rates of survival (9-12, 18). The utility of HD-CPR in a pediatric model is particularly relevant because as many as 93% of pediatric IHCA occur in intensive care units where such a technique could be employed (2). Furthermore, recent multicenter clinical data supports a diastolic blood pressure threshold as a determinant of survival among patients with arterial catheters in place (31). Together, these findings support consensus guidelines that advocate for titrating therapies to CoPP or diastolic blood pressures (7) and provides a framework for how this could be accomplished. During CPR, higher aortic diastolic pressure with HD-CPR led to significantly higher coronary perfusion pressure, which is the primary determinant of myocardial blood flow (32) and is directly correlated with the likelihood of attaining ROSC (33). This was achieved despite shallower compressions with HD-CPR. Since animals resuscitated with HD-CPR received more total vasopressors in the first ten minutes of CPR, it is likely that targeted vasopressor dosing augmented systemic vascular resistance, thereby raising diastolic and systolic aortic pressures, such that goal systolic pressure was achieved with less force and depth during compressions. In the DG-CPR group, chest compression depth (4.42 ± 0.02 cm) approximated pediatric guidelines for infants and children (4 cm for infants and 5 cm for children) (18), but the HD-CPR group required a mean compression depth of only 3.37 ± 0.3 cm. This corroborates the concept that optimal chest compression depth (i.e., the depth required for optimal CPR hemodynamics) presumably depends on individual patient factors and arrest characteristics and that further work is necessary to define optimal chest compression depths in children with and without invasive hemodynamic monitoring in place. The fact that animals with shallower chest compressions were more likely to survive with favorable neurologic outcomes supports the approach of titrating CPR to physiologic response. Notably, ETCO_2 did not differ between groups during CPR – this supports previous evidence that when CPR quality is high, as it was in both groups in this study, ETCO_2 values lack specificity in their capacity to predict outcome (34).

On analysis of myocardial mitochondrial function, surviving animals in both treatment groups had evidence of Complex I dysfunction compared to sham animals that underwent anesthesia and instrumentation without cardiac arrest. This inability to use complex I substrates efficiently limits oxidative phosphorylation to generate energy and may be a component of post-cardiac arrest syndrome (35). Further, it builds upon and extends the longitudinal timeline reported in previous studies in murine models of cardiac arrest demonstrating progressive myocardial Complex I dysfunction in the acute post-arrest period (i.e., 30 and 60 minutes after ROSC) (16). Animals treated with HD-CPR had higher

Complex I-mediated respiration compared to those treated with DG-CPR, which may reflect improved myocardial perfusion. Complex II-driven respiration was no different in survivors of cardiac arrest than in shams, consistent with previous studies at earlier time-points following ROSC (16). This differential dysfunction of electron transport chain components may provide for targeted therapeutic interventions in the future. Finally, non-phosphorylating maximal respiration was more depressed in the RV than the LV. This respiratory state represents the maximal reserve capacity of mitochondrial respiration. Future investigations should delineate the role of mitochondria in post-arrest myocardial dysfunction in both the left and right ventricle following asphyxial cardiac arrest, as well as the susceptibility of the right ventricle following asphyxial arrest.

Our previous studies of HD-CPR in asphyxial cardiac arrests established higher rates of four-hour survival relative to DG-CPR (12), yet demonstrated cortical and hippocampal mitochondrial dysfunction despite treatment with HD-CPR (13). The present study expands the clinical relevance of these findings by extending survival to 24 hours, and may provide important mechanistic findings related to functional neurologic dysfunction. In both treatment groups, Complex I respiration, maximal oxidative phosphorylation, and respiratory control ratio, a biomarker of overall mitochondrial health, were each lower in both the cortex and hippocampus compared to shams. However, this mitochondrial dysfunction was partially mitigated in animals treated with HD-CPR. Specifically, HD-CPR stabilized complex II-driven respiration and improved maximum oxidative phosphorylating capacity relative to DG-CPR in both the cortex and hippocampus. We hypothesize that by optimizing coronary perfusion pressure to increase myocardial blood flow, this method of CPR also resulted in increased mean arterial pressure and oxygen delivery to the brain. Consistent with this hypothesis, the HD-CPR group had higher brain tissue oxygenation relative to baseline during CPR. Such improvements in oxygen delivery and perfusion pressure may partially alleviate ischemic injury at a cellular level. This mechanism is supported, in part, by the finding of improved maximum, coupled oxidative phosphorylation with HD-CPR relative to standard DG-CPR. However, the supranormal brain oxygen tension in the HD-CPR cohort could also lead to cell damage through reactive oxygen species generation and oxidative stress, which this study did not explicitly measure. Future research should further investigate the balance between oxygen delivery and oxidative phosphorylation and adenosine triphosphate (ATP) generation with reactive oxygen species generation and ischemia-reperfusion injury.

The finding that HD-CPR did not fully eliminate mitochondrial dysfunction in the brain or the heart underscores the need for ongoing research into mitochondrial interventions to further limit injury. Persistent Complex I depression in cortical, hippocampal, and myocardial tissue 24 hours after resuscitation from cardiac arrest may limit the ability of Complex I-linked substrates to generate sufficient ATP to meet the substantial bioenergetic needs following injury (36-38). Conversely, normal myocardial complex II-driven respiration at 24 hours and improved cortical and hippocampal complex II-driven respiration with HD-CPR suggest that mitochondrial substrates such as succinate and free fatty acids, which supply Complex II independent of Complex I, hold promise as adjunct therapies in resuscitation (17). Indeed, there is *in vitro* and *in vivo* evidence that supplementation with complex II substrates may decrease brain injury (39, 40). Future investigations could

evaluate changes in total ATP production after substrate supplementation and correlation with clinical severity of both myocardial and brain injury. Ultimately, a more personalized approach to CPR may include not only hemodynamic targets, but also targeted mitochondrial therapeutics as part of a comprehensive resuscitation strategy. Future work should focus further on mechanisms of injury related to ischemia and reperfusion in the immature brain and heart, focusing on reactive oxygen species generation, means of bypassing dysfunctional Complex I, and downstream mitochondrial dynamics and apoptotic signaling pathways.

This investigation has limitations. First, asphyxia and cardiac arrest were induced in previously healthy, anesthetized animals in a controlled laboratory setting, whereas pediatric cardiac arrest occurs among children with variable disease processes and a variety of comorbidities. Minimizing other confounding variables allowed for a focused evaluation of the resuscitation techniques being compared, but the applicability of these findings to pediatric cardiac arrests is not entirely known. Second, the experimental protocol utilized in this preclinical trial is complex in terms of the extensive measurements performed and the need to titrate therapies in real time during HD-CPR. These factors need to be considered as HD-CPR is evaluated and effectively employed in actual clinical practice. Third, the intra-arrest hemodynamics measured with DG-CPR are relatively low compared to recently published data in children with IHCA (31). This suggests a relatively severe insult and that HD-CPR is applicable to a sicker cardiac arrest population but that its relative benefit in brief or otherwise less severe cardiac arrests is unknown. Fourth, the swine CPC scale is a gross measure of neurologic function similar to scales used in humans, which has the potential for interrater variability and a lack of sensitivity for subtle neurologic dysfunction. However, assessments were standardized and performed by two trained study team members who were blinded and who previously exhibited harmonized categorization. The striking difference in maximum coupled oxidative phosphorylation capacity in the cortex and hippocampus between animals with favorable CPC scores of 1 or 2 and those with a score of 3 or greater supports the validity of comparing favorable to unfavorable neurologic outcomes using the swine CPC scale in these experiments.

CONCLUSIONS:

In this piglet model of asphyxia-associated pediatric cardiac arrest, HD-CPR resulted in a higher rate of 24-hour survival with a neurologically favorable outcome compared to standard DG-CPR. HD-CPR led to higher coronary perfusion pressure and brain tissue oxygenation during resuscitation. In surviving animals, HD-CPR resulted in greater overall maximal oxidative phosphorylation capacity in the cortex and hippocampus and complex I respiration in myocardial tissue. Persistent mitochondrial bioenergetic alterations may afford the opportunity for mitochondrial-targeted therapeutics to ameliorate cerebral and myocardial injury when combined with HD-CPR.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS:

The authors wish to thank Melissa Gabello, William P. Landis, George Bratinov, Yuxi Lin, Sejin Jeong, Ting-Chang Hseih, Wesley Shoap, Francis X. McGowan, Heather Wolfe, Thomas Conlon, Maryam Naim, and Heather Griffiths.

Financial support for this study: Support provided through Russell Raphaely Endowed Chair funds at The Children's Hospital of Philadelphia. TJK received NIH funding (NS-103826). RWM, RAB, and RMS received NIH funding (HD-089132). TSK received NIH funding (HD-085731). DJL received funding from the NIH (NS-072338 and NS-60653) and the June and Steve Wolfson Family foundation.

Copyright form disclosure: Dr. Morgan's institution received funding from National Institutes of Health (NIH) National Institute of Child Health and Human Development (NICHD). Drs. Morgan, Licht, Nadkarni, Berg, and Kilbaugh received support for article research from the NIH. Dr. Karlsson received funding from Neurovive Pharmaceutical AB. Dr. Licht's institution received funding from the NIH. Dr. Berg's institution received funding from NICHD laboratory CPR grant. Dr. Sutton's institution received funding from National Heart, Lung, and Blood Institute R01; he received funding from Zoll Medical (Speaking Honoraria); and he disclosed he is a member of American Heart Association's Get with the Guidelines-Resuscitation Pediatric Research Task Force. The remaining authors have disclosed that they do not have any potential conflicts of interest.

REFERENCES:

1. Knudson JD, Neish SR, Cabrera AG, et al. Prevalence and outcomes of pediatric in-hospital cardiopulmonary resuscitation in the United States: an analysis of the Kids' Inpatient Database*. Crit Care Med 2012;40(11):2940–2944. [PubMed: 22932398]
2. Berg RA, Sutton RM, Holubkov R, et al. Ratio of PICU versus ward cardiopulmonary resuscitation events is increasing. Crit Care Med 2013;41(10):2292–2297. [PubMed: 23921270]
3. Berg RA, Nadkarni VM, Clark AE, et al. Incidence and Outcomes of Cardiopulmonary Resuscitation in PICUs. Crit Care Med 2016;44(4):798–808. [PubMed: 26646466]
4. Girotra S, Spertus JA, Li Y, et al. Survival trends in pediatric in-hospital cardiac arrests: an analysis from Get With the Guidelines-Resuscitation. Circ Cardiovasc Qual Outcomes 2013;6(1):42–49. [PubMed: 23250980]
5. Nolan JP, Neumar RW, Adrie C, et al. Post-cardiac arrest syndrome: epidemiology, pathophysiology, treatment, and prognostication. A Scientific Statement from the International Liaison Committee on Resuscitation; the American Heart Association Emergency Cardiovascular Care Committee; the Council on Cardiovascular Surgery and Anesthesia; the Council on Cardiopulmonary, Perioperative, and Critical Care; the Council on Clinical Cardiology; the Council on Stroke. Resuscitation 2008;79(3):350–379. [PubMed: 18963350]
6. Slomine BS, Silverstein FS, Christensen JR, et al. Neurobehavioral Outcomes in Children After Out-of-Hospital Cardiac Arrest. Pediatrics 2016;137(4).
7. Meaney PA, Bobrow BJ, Mancini ME, et al. Cardiopulmonary resuscitation quality: [corrected] improving cardiac resuscitation outcomes both inside and outside the hospital: a consensus statement from the American Heart Association. Circulation 2013;128(4):417–435. [PubMed: 23801105]
8. de Caen AR, Berg MD, Chameides L, et al. Part 12: Pediatric Advanced Life Support: 2015 American Heart Association Guidelines Update for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. Circulation 2015;132(18 Suppl 2):S526–542. [PubMed: 26473000]
9. Sutton RM, Friess SH, Bhalala U, et al. Hemodynamic directed CPR improves short-term survival from asphyxia-associated cardiac arrest. Resuscitation 2013;84(5):696–701. [PubMed: 23142199]
10. Sutton RM, Friess SH, Naim MY, et al. Patient-centric blood pressure-targeted cardiopulmonary resuscitation improves survival from cardiac arrest. Am J Respir Crit Care Med 2014;190(11):1255–1262. [PubMed: 25321490]
11. Naim MY, Sutton RM, Friess SH, et al. Blood Pressure- and Coronary Perfusion Pressure-Targeted Cardiopulmonary Resuscitation Improves 24-Hour Survival From Ventricular Fibrillation Cardiac Arrest. Crit Care Med 2016;44(11):e1111–e1117. [PubMed: 27414479]
12. Morgan RW, Kilbaugh TJ, Shoap W, et al. A hemodynamic-directed approach to pediatric cardiopulmonary resuscitation (HD-CPR) improves survival. Resuscitation 2017;111:41–47. [PubMed: 27923692]

13. Kilbaugh TJ, Sutton RM, Karlsson M, et al. Persistently Altered Brain Mitochondrial Bioenergetics After Apparently Successful Resuscitation From Cardiac Arrest. *J Am Heart Assoc* 2015;4(9).
14. Ayoub IM, Radhakrishnan J, Gazmuri RJ. Targeting mitochondria for resuscitation from cardiac arrest. *Crit Care Med* 2008;36(11 Suppl):S440–446. [PubMed: 20449908]
15. Chouchani ET, Pell VR, Gaude E, et al. Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. *Nature* 2014;515(7527):431–435. [PubMed: 25383517]
16. Han F, Da T, Riobo NA, et al. Early mitochondrial dysfunction in electron transfer activity and reactive oxygen species generation after cardiac arrest. *Crit Care Med* 2008;36(11 Suppl):S447–453. [PubMed: 20449909]
17. Ehinger JK, Piel S, Ford R, et al. Cell-permeable succinate prodrugs bypass mitochondrial complex I deficiency. *Nat Commun* 2016;7:12317. [PubMed: 27502960]
18. Friess SH, Sutton RM, Bhalala U, et al. Hemodynamic directed cardiopulmonary resuscitation improves short-term survival from ventricular fibrillation cardiac arrest. *Crit Care Med* 2013;41(12):2698–2704. [PubMed: 23887237]
19. Neurauther A, Nysaether J, Kramer-Johansen J, et al. Comparison of mechanical characteristics of the human and porcine chest during cardiopulmonary resuscitation. *Resuscitation* 2009;80(4):463–469. [PubMed: 19195761]
20. Duhaime AC. Large animal models of traumatic injury to the immature brain. *Dev Neurosci* 2006;28(4–5):380–387. [PubMed: 16943661]
21. Samson RA, Nadkarni VM, Meaney PA, et al. Outcomes of in-hospital ventricular fibrillation in children. *N Engl J Med* 2006;354(22):2328–2339. [PubMed: 16738269]
22. Matos RI, Watson RS, Nadkarni VM, et al. Duration of cardiopulmonary resuscitation and illness category impact survival and neurologic outcomes for in-hospital pediatric cardiac arrests. *Circulation* 2013;127(4):442–451. [PubMed: 23339874]
23. Atkins DL, Berger S, Duff JP, et al. Part 11: Pediatric Basic Life Support and Cardiopulmonary Resuscitation Quality: 2015 American Heart Association Guidelines Update for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. *Circulation* 2015;132(18 Suppl 2):S519–525. [PubMed: 26472999]
24. Berg RA, Chapman FW, Berg MD, et al. Attenuated adult biphasic shocks compared with weight-based monophasic shocks in a swine model of prolonged pediatric ventricular fibrillation. *Resuscitation* 2004;61(2):189–197. [PubMed: 15135196]
25. Berg RA, Sanders AB, Kern KB, et al. Adverse Hemodynamic Effects of Interrupting Chest Compressions for Rescue Breathing During Cardiopulmonary Resuscitation for Ventricular Fibrillation Cardiac Arrest. *Circulation* 2001;104(20):2465–2470. [PubMed: 11705826]
26. Letellier T, Malgat M, Coquet M, et al. Mitochondrial myopathy studies on permeabilized muscle fibers. *Pediatr Res* 1992;32(1):17–22. [PubMed: 1635840]
27. Fontana-Ayoub M, Fasching M, Gnaiger E. Selected media and chemicals for respirometry with mitochondrial preparations. *Mitochondr Physiol Network* 2016;03.02(18):1–10.
28. Kilbaugh TJ, Sutton RM, Karlsson M, et al. Persistently Altered Brain Mitochondrial Bioenergetics After Apparently Successful Resuscitation From Cardiac Arrest. *J Am Heart Assoc* 2015;4(9):e002232. [PubMed: 26370446]
29. Pesta D, Gnaiger E. High-resolution respirometry: OXPHOS protocols for human cells and permeabilized fibers from small biopsies of human muscle. *Methods Mol Biol* 2012;810:25–58. [PubMed: 22057559]
30. Kilbaugh TJ, Karlsson M, Byro M, et al. Mitochondrial bioenergetic alterations after focal traumatic brain injury in the immature brain. *Exp Neurol* 2015;271:136–144. [PubMed: 26028309]
31. Berg RA, Sutton RM, Reeder RW, et al. Association Between Diastolic Blood Pressure During Pediatric In-Hospital Cardiopulmonary Resuscitation and Survival. *Circulation* 2018;137(17):1784–1795. [PubMed: 29279413]
32. Kern KB, Ewy GA, Voorhees WD, et al. Myocardial perfusion pressure: a predictor of 24-hour survival during prolonged cardiac arrest in dogs. *Resuscitation* 1988;16(4):241–250. [PubMed: 2849790]

33. Paradis NA, Martin GB, Rivers EP, et al. Coronary perfusion pressure and the return of spontaneous circulation in human cardiopulmonary resuscitation. *JAMA* 1990;263(8):1106–1113. [PubMed: 2386557]
34. Morgan RW, French B, Kilbaugh TJ, et al. A quantitative comparison of physiologic indicators of cardiopulmonary resuscitation quality: Diastolic blood pressure versus end-tidal carbon dioxide. *Resuscitation* 2016;104:6–11. [PubMed: 27107688]
35. Neumar RW, Nolan JP, Adrie C, et al. Post-cardiac arrest syndrome: epidemiology, pathophysiology, treatment, and prognostication. A consensus statement from the International Liaison Committee on Resuscitation (American Heart Association, Australian and New Zealand Council on Resuscitation, European Resuscitation Council, Heart and Stroke Foundation of Canada, InterAmerican Heart Foundation, Resuscitation Council of Asia, and the Resuscitation Council of Southern Africa); the American Heart Association Emergency Cardiovascular Care Committee; the Council on Cardiovascular Surgery and Anesthesia; the Council on Cardiopulmonary, Perioperative, and Critical Care; the Council on Clinical Cardiology; and the Stroke Council. *Circulation* 2008;118(23):2452–2483. [PubMed: 18948368]
36. Vanasco V, Magnani ND, Cimolai MC, et al. Endotoxemia impairs heart mitochondrial function by decreasing electron transfer, ATP synthesis and ATP content without affecting membrane potential. *J Bioenerg Biomembr* 2012;44(2):243–252. [PubMed: 22426814]
37. Distelmaier F, Koopman WJ, van den Heuvel LP, et al. Mitochondrial complex I deficiency: from organelle dysfunction to clinical disease. *Brain* 2009;132(Pt 4):833–842. [PubMed: 19336460]
38. Esteitie N, Hinttala R, Wibom R, et al. Secondary metabolic effects in complex I deficiency. *Ann Neurol* 2005;58(4):544–552. [PubMed: 16044424]
39. Sakamoto M, Takeshige K, Yasui H, et al. Cardioprotective effect of succinate against ischemia/reperfusion injury. *Surg Today* 1998;28(5):522–528. [PubMed: 9607905]
40. Laplante A, Vincent G, Poirier M, et al. Effects and metabolism of fumarate in the perfused rat heart. A ¹³C mass isotopomer study. *Am J Physiol* 1997;272(1 Pt 1):E74–82. [PubMed: 9038854]

Figure 1. Coronary Perfusion Pressure during Cardiopulmonary Resuscitation.

Coronary perfusion pressure during ten minutes of cardiopulmonary resuscitation in depth-guided cardiopulmonary resuscitation (DG-CPR; dashed gray line) vs. hemodynamic-guided cardiopulmonary resuscitation (HD-CPR; solid black line). Error bars represent SEM.

Coronary perfusion pressures differed between groups using generalized estimating equation regression model ($p < 0.001$).

Right Ventricle

Left Ventricle

Figure 2. Myocardial Mitochondrial Respiration.

Comparisons of measures of mitochondrial respiration in the right and left ventricular myocardium 24 hours after return of spontaneous circulation (treatment groups) or 24 hours post-anesthesia (sham). (a) Right and (d) left ventricle complex I respiration (OXPHOS_{CI}) with complex I substrates malate, pyruvate, and glutamate. (b) Right and (e) left ventricle complex II respiration (ETC_{CII}) with the addition of succinate and inhibition of complex I respiration with rotenone. (c) Right and (f) left ventricle maximal non-phosphorylating oxidative phosphorylation (ETC_{CI+CII}) with the titration of the protonophore, FCCP, in the presence of complex I and complex II substrates. ANOVA with multiple comparisons of treatment groups to sham (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). ANOVA with multiple comparisons between treatment groups (#, $p < 0.05$; ##, $p < 0.01$; ###, $p < 0.001$). *Definition of abbreviations:* DG-CPR = depth-guided CPR; HD-CPR = hemodynamic-directed CPR.

Cortex

Figure 3. Cerebral Mitochondrial Respiration.

Comparisons of measures of mitochondrial respiration in the cerebral cortex (a-c) and hippocampus (d-f) 24 hours after return of spontaneous circulation (treatment groups) or 24 hours post-anesthesia (sham). (a) Cortical and (d) hippocampal complex I respiration (OXPHOS_{CI}) with complex I substrates malate and pyruvate. (b) Cortical and (e) hippocampal complex II respiration (ETC_{CII}) with the addition of succinate and inhibition of complex I respiration with rotenone. (c) Cortical and (f) hippocampal maximal oxidative phosphorylation convergence respiration (OXPHOS_{CI+CII}) with both complex I and complex II substrates. Statistical analyses performed with ANOVA with multiple comparisons of treatment groups to sham (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$) and ANOVA with multiple comparisons between treatment groups (#, $p < 0.05$; ##, $p < 0.01$; ###, $p < 0.001$). *Definition of abbreviations:* DG-CPR = depth-guided CPR; HD-CPR = hemodynamic-directed CPR.

Table 1.

Physiologic Measurements during Cardiopulmonary Resuscitation.

Physiologic Measurements	DG-CPR	HD-CPR	<i>p</i>
Aortic Systolic Pressure (mmHg)	60.5 (7.1)	85.5 (10.5)	0.02
Aortic Diastolic Pressure (mmHg)	16.8 (2.7)	31.3 (4.0)	<0.001
Right Atrial Diastolic Pressure (mmHg)	9.5 (0.6)	10.8 (0.9)	0.16
Coronary Perfusion Pressure (mmHg)	9.6 (2.1)	21.0	<0.001
End-tidal Carbon Dioxide (mmHg)	39.7 (3.1)	35.9 (4.5)	0.39
Brain Tissue Oxygenation (% baseline)	59.2 (16.1)	233.8 (27.9)	<0.001
Chest Compression Rate (min ⁻¹)	100.1 (0.1)	100.0 (0.1)	0.40
Chest Compression Depth (cm)	4.4 (0.2)	3.4 (0.3)	0.02
Chest Compression Release Velocity (mm/sec)	276.8 (7.3)	257.4 (9.7)	0.12

Comparisons between treatment groups from minutes 2-10 of resuscitation period, compared using generalized estimating equation (GEE) regression model accounting for clustering of data points within individual animals. Values are means from entire 8-minute period, with standard errors of the mean in parentheses. *Definition of abbreviations:* DG-CPR = depth-guided cardiopulmonary resuscitation; HD-CPR = hemodynamic-guided cardiopulmonary resuscitation.