Intravenous Calcium as a Pressor in a Swine Model of Hypoxic Lifeless Shock

CURRENT STATUS: UNDER REVIEW

Alexander Lindqwister
Dartmouth College Geisel School of Medicine

Joshua W. Lampe
ZOLL Medical Corp

Jeffrey R. Gould
ZOLL Medical Corp

Christopher L. Kaufman
ZOLL Medical Corp

Karen Moodie
Dartmouth College Geisel School of Medicine

Norman Paradis
Dartmouth College Geisel School of Medicine

norman.a.paradis@hitchcock.org Corresponding Author

DOI: 10.21203/rs.3.rs-22127/v1

SUBJECT AREAS
Critical Care & Emergency Medicine

KEYWORDS
pressor, calcium, PEA, pulseless electrical activity, lifeless shock
Abstract
Background: Resuscitation from hypoxic lifeless shock (LS, previously called pseudo-PEA) is often associated with hypotension refractory to catecholamine pressors. We hypothesized that this post-resuscitation state may be associated with hypocalcemia and the hypotension responsive to intravenous calcium.

Results: Using pre-existing data from our hypoxic swine LS model, we measured blood pressure, hemodynamics, and electrolytes. In 9 animals with refractory hypotension we administered 37 boluses of intravenous calcium in the dosage range of 5 -20 mg. Physiological data were analyzed on a heartbeat by heartbeat basis. The midpoint of the calcium response was defined using change of curvature feature detection. Hemodynamic parameters were shifted such that the value at the midpoint was equal to zero. Comparisons were made between the average values in the time period 40-35 seconds before the bolus and 35-40 seconds after the bolus. Of the 37 administered boluses, 34 manifested a reaction in the blood pressure, with mean aortic pressure, systolic and diastolic pressures all increasing post bolus administration.

Conclusions: Ionized hypocalcemia and hypotension may be common after resuscitation from hypoxic lifeless shock. Administration of intravenous calcium may be associated with a pressor-like response. Hypocalcemia is an etiology for hypotension, and intravenous calcium as a therapy in this setting should be further investigated.

Introduction
The post resuscitation state is frequently characterized by hypotension that is refractory to pressors.

1 The etiology of this state may be multifactorial, combining both myocardial dysfunction and vasomotor stunning. While it is likely that the myocardial and vasomotor processes are themselves complex, it has been proposed that disordered calcium homeostasis is a contributing factor. 2,3 This is a reasonable hypothesis, as ionized calcium plays an important role in both inotropy and vasomotor tone. 4

Nieman et al have previously reported that ionized hypocalcemia occurs post return of spontaneous circulation (ROSC) in their swine model of ventricular fibrillation, 2,3 and that administration of
intravenous (IV) calcium gluconate is associated with a transient improvement in hemodynamics. Historically, clinical cardiac arrest was classified into two broad categories that include ventricular fibrillation and pulseless electrical activity (PEA). The latter category was first called electromechanical dissociation (EMD), but underwent a name change when studies revealed that in a substantial fraction of patients, pulse pressures and ventricular mechanical function were detectible. The detection of pulses with advanced technology renders PEA an imprecise descriptor, as well, and we now refer to the cardiovascular state of subjective clinical cardiac arrest with detectably hemodynamics as “lifeless shock.” Specifically, lifeless shock, also called pseudo-PEA, is a global hypotensive ischemic state with retained coordinated myocardial contractile activity and an organized ECG.

Similar to ventricular fibrillation, pseudo-PEA causes a profound global ischemia and is often associated with post-resuscitation hypotension. Extending Nieman’s report of IV calcium responsive hypotension after resuscitation from fibrillation cardiac arrest, we now provide a preliminary report a calcium pressor response in hypotension after resuscitation from pseudo-PEA.

Methods
The manuscript contains a post-hoc analysis of data originally obtained in other studies using our pseudo-PEA model. These studies were conducted in accordance with the guidelines of the National Research Council of the National Academies and with the approval of the Dartmouth College Institutional Animal Care and Use Committee. The model utilized was a variation on previously described porcine asphyxial P-EMD preparation.

Surgical Prep
Twenty-four domestic farm raised Yorkshire swine weighing approximately 30 kg were fasted over-night with free access to water and then sedated with an intramuscular injection of ketamine (30 mg/kg). After endotracheal intubation, anesthesia was initiated and maintained with isoflurane (0.5–4%) and oxygen (1–3 L/min). During preparation, ventilation was provided by a volume-controlled ventilator (GE Datex-Ohmeda Modulus SE, Madison, WI) with 100% O2 (tidal volume of 15-
20 cc/kg and ventilation rate of 8-15 breaths per minute) during initiation, reducing to 30% shortly thereafter. Ventilation rate and tidal volume were initially adjusted to maintain normocapnia (the end-expiratory partial pressure of CO₂ between 35-45 mmHg) as measured continuously by a capnometer (CO2SMO, Novametrix, Wallingford, CT placed in the airway. Arterial blood gases (I-Stat, Abbott Point of Care, Princeton, NJ) were analyzed to confirm adequate baseline ventilation. Throughout the experiment, the animals were monitored using ECG, end-tidal CO₂, and arterial blood pressure. In addition, depth of anesthesia was continuously assessed.

The animals were secured in a supine position and were given normal saline at a rate of 10 ml/kg per hour through a vein to maintain a central venous pressure of ~ 5 mm Hg. Through either ultrasound guided percutaneous cannulations or surgical cut-down, two micromanometer single pressure sensors (SPR-350, Millar Instruments, Houston, TX) and a pressure sensor with a lumen (Tru-Wave Pressure Transducer, Edwards Lifesciences, Irvine, CA) were placed into: 1) the right atrium via the femoral vein, and 2) the descending aorta through the femoral artery for pressure measurements for pressure measurement. All catheters were positioned under fluoroscopic guidance, and unfractionated Heparin (100 units/kg) was given to prevent catheter clotting. Flow probes were placed around the carotid artery (3 PS probe, Transonic, Ithaca, NY) and jugular vein (2.5 PS probe, Transonic, Ithaca, NY) via a cut down procedure.

After instrumentation, baseline measurements were obtained for all variables including blood gas analyses. Analog outputs of the physiological parameters were digitized and stored in data files on a personal computer for further analysis using a 16-channel computerized data-acquisition system at a sampling rate of 1000 Hz (Powerlab 16SP, ADInstruments, Castle Hill, Australia). Raw data channels included ECG, aortic pressure, right atrial pressure, intra-cranial pressure, capnography, carotid blood flow and jugular blood flow.

**Pseudo-PEA induction and chest compression**

Animals were converted to continuous intravenous anesthesia using ketamine (50 mcg/kg/min) and fentanyl (0.45mcg/kg/min), isoflurane was gradually discontinued. A surgical plane of anesthesia was ensured via monitoring blood pressure, heart rate, and jaw tone. The IV anesthesia protocol was
maintained 15 minutes to allow isoflurane washout and to establish a stable level of continuous IV anesthesia prior to initiation of the hypoxia protocol.

Arterial blood gases were measured at baseline and after each episode of pseudo-PEA (i-STAT, Abbott Point of Care, Abbott Park, IL). Measurements included: pH, pCO2, pO2, base excess, HCO3, TCO2, O2 percent saturation, Na (mmol/L), K (mmol/L), ionized calcium (iCa), glucose, hematocrit, and hemoglobin.

Once adequate anesthesia has been confirmed, the animals were paralyzed using Vecuronium (1.0 mg/kg) to minimize gasping. Baseline data were measured before any injury occurred. Round 1 data were measured after resuscitation from the first pseudo-PEA injury. Data were also measured after resuscitation from subsequent pseudo-PEA injuries. The number of injuries per animal are variable, but the maximum number of hypoxic episodes is 4. Animals were ventilated with a progressively hypoxic gas mixture of O2/N2. Gas concentrations were measured using an oxygen concentration analyzer (Oxygen Analyzer S-3A/II, Applied Electrochemistry, VMETEK) and the concentration of O2 was decreased until pseudo-PEA was achieved. Onset of pseudo-PEA was defined as sustained aortic systolic pressure ≤ 50 mmHg recorded by the aortic catheter in the presence of an organized cardiac rhythm.

Animals were treated with mechanical chest compressions delivered at a depth of 5 cm and at a variable rate. Compressions were delivered for a total of 6 minutes. After 6 minutes of chest compressions the FiO2 was set to 100%, and chest compressions were continued until ROSC was achieved. ROSC was defined as occurred as systolic pressure > 60 mm Hg without chest compressions. If ROSC was detected at any point during the chest compressions with ongoing hypoxia, compressions were terminated and FiO2 was set to 100%.

**Post resuscitation treatment**

Arterial blood gases were measured 10 minutes after ROSC in all 24 animals for all injury rounds. The first 10 animals were not treated with calcium. After determining that serum calcium decreased in these animals through the first two rounds of hypoxia, as shown in Fig. 2, calcium was added to the treatment regime. In the following 14 animals, Calcium was delivered in an ad hoc fashion to effect
during the recovery phase after successful resuscitation from pseudo-PEA when MAP was not sufficient or stable. In addition, epinephrine and sodium bicarbonate were also delivered to effect to support perfusion pressures and correct post-resuscitation blood chemistry as needed. As a result, different animals received different numbers and dosages of calcium boluses. Individual animals received as many as four periods of hypoxia induced pseudo-PEA followed by resuscitation. Time of delivery of epinephrine, bicarbonate, and calcium were annotated in the physiological data file from each experiment.

Data Selection:
To examine the effect of calcium on the hemodynamics, each physiological file was examined for notation of the delivery of a calcium bolus. Nine of the fourteen animals received calcium injections, and a total of 37 boluses of calcium were delivered. For each calcium bolus, a data subset was exported that included time before and after the bolus. Because the calcium was delivered in an un-protocolized manner, the amount of time before (or after) the bolus varied for each calcium injection. In the event that a calcium injection was preceded or followed by a different injection of calcium or injections of bicarbonate or epinephrine, the data subset started (or ended) half-way between the two injections. This was done in an attempt to isolate the effect of the injection of calcium from the effects of other injections. One critical effect of this method is that there was large variation in the amount of time before and after a calcium injection. The minimum amount of time before an injection was 40 seconds. The minimum amount of time after an injection was also 40 seconds. For this reason, our analysis focused on the 40 seconds before and after each calcium bolus.

Data Analysis:
Individual heartbeats were identified using a python script (Anaconda 1.9.6, Spyder version 3.1.2, Pandas version 0.22.0, Numpy version 1.14.0). Once the location of each heartbeat was identified, the mean arterial pressure (MAP), the systolic aortic pressure (AoS), and the diastolic aortic pressure (AoD) were all calculated using normal conventions. The effect of calcium was easily observable in most cases and the physiological effect could be approximated using a sigmoid shape. However, the heterogeneity of the mean blood pressures and their slope during recovery from pseudo-PEA would
have obscured any analysis of the raw data. To understand the size of the pressor effect of Calcium, blood pressure data were normalized such that the midpoint of observed effect occurred at a pressure of 0 mmHg and a time of 0 s. This normalization was performed by a second python script which used the zero-crossing point in the second derivative of the systolic aortic pressure, the second derivative of the coronary perfusion pressure and the second derivative of the cerebral perfusion pressure to approximate the midpoint of the sigmoid response to a calcium bolus. Once the midpoint was found, pressures were normalized by subtracting the pressure value at the midpoint and time was normalized by subtraction of the time when the midpoint occurred. Once the MAP, AoS, and AoD pressures were normalized, the mean values and standard deviations were calculated for the times – 40 s < t < 40 s.

**Statistical Methods:**
To compare physiology before and after the delivery of calcium we compared physiology from the time period – 40 < t < -37 (after) and the time period 37 < t < 40 (after). Physiology was averaged across these two 4 second periods and compared via a student’s t-test (STATA v 15.1).

**Results**
In Fig. 2, the serum ionic calcium concentrations from the first 10 animals are plotted as a function of experimental injury round. Ionic calcium decreases with injury through the first two rounds of hypoxia induced pseudo-PEA. This observation is further supported by empiric data from related experiments. Table 1 are the results of additional unpublished experiments assessing the outcomes of CPR treatment versus 100% O2 therapy alone in pseudo-PEA. The design of this experiment down to the machinery used was identical to our own, with the exception of the CPR intervention during resuscitation. None of these animals received bolus calcium. Again, there is a redemonstrated significant difference between baseline iCa and post-round 1 iCa (P = 0.029) and baseline iCa and subsequent rounds (P = 0.001). There is no significant difference in iCa between pigs who did or did not receive CPR (P = 0.89), further suggesting that the observed differences in ionized calcium from baseline are attributable to the pseudo-PEA disease state.
Table 1
Results of blood chemistries from the 14 animals that did not receive calcium treatment

|        | Baseline | Round 1 | Other Rounds |
|--------|----------|---------|--------------|
| Mean   | Std      | Mean    | Std          | Mean   | Std       |
| pH     | 7.5      | 0.015   | 7.33         | 0.12   | 7.37      | 0.096      |
| iCa [mmol/L] | 1.36    | 0.16    | 1.25*        | 0.071  | 1.24**    | 0.055      |
| Glu [mg / dL] | 84.22  | 18.23   | 182.15       | 60.47  | 136       | 49.10      |

In 23 experiments, the pig did not receive CPR. In 29 experiments, the pig did receive CPR. There is no significant difference in iCa between pigs who did or did not receive CPR (P = .89)

* Significant difference from Baseline vs Round 1 for iCa (P = .029)

** Significant difference from Baseline vs Other Rounds for iCa (P = .001)

Animals resuscitated from hypoxia induced pseudo-PEA are often unstable. The observation that ionic calcium decreased with pseudo-PEA round led to the addition of Ca Gluconate to the treatment regimen post-resuscitation. A bolus of Ca Gluconate, a bolus of calcium would be delivered intravenously if the aortic blood pressure was decreasing after resuscitation. This meant that Calcium might be given to treat blood pressures before the blood chemistries were drawn, resulting in some missing data. The mean values and standard deviations for the blood chemistry of the animals that received IV calcium are shown In Table 2 as a function of experimental round. Because of the calcium treatments, measured iCa values were not different between the rounds. Base excess (BE), Bicarbonate (HCO3) and total carbon dioxide (TCO2) were lower after PEA than at baseline but were not affected by subsequent pseudo-PEA injuries.

Table 2
Results of blood chemistries from 14 animals that received calcium treatment

|        | Baseline | Round 1 | Other Rounds |
|--------|----------|---------|--------------|
| Mean   | Std      | Mean    | Std          | Mean   | Std       |
| pH     | 7.45     | 0.024   | 7.22         | 0.14   | 7.31      | 0.077      |
| PCO2 [mmHg] | 44.42  | 3.30    | 52.48        | 7.04   | 47.23     | 10.62      |
| pO2 [mmHg] | 238.67 | 57.67   | 329.78       | 154.50 | 365.4     | 119.59     |
| BEecf [mmol/L] | 7       | 1.73    | -16          | 31.40  | -2.67     | 2.23       |
| HCO3 [mmol/L] | 31.06  | 1.62    | 21.57        | 4.34   | 23.54     | 1.87       |
| TCO2 [mmol/L] | 32.22  | 1.64    | 23.33        | 4.21   | 24.87     | 1.92       |
| sO2%   | 99.88    | 0.33    | 96.89        | 8.96   | 99.93     | 0.26       |
| Na [mmol/L] | 137.55 | 1.88    | 140.22       | 9.90   | 139.87    | 2.26       |
| K [mmol/L] | 4.2     | 0.23    | 3.79         | 0.43   | 4.12      | 0.62       |
| iCa [mmol/L] | 1.3867 | 0.05    | 1.22         | 0.14   | 1.33      | 0.15       |
| Glu [mg / dL] | 83.22  | 23.75   | 213.11       | 127.61 | 152       | 85.83      |
| HCT [% PCV] | 23.22  | 3.27    | 32.44        | 3.81   | 30.13     | 4.31       |
| HB [g/dl] | 7.911   | 1.13    | 11.03        | 1.31   | 10.25     | 1.47       |

* Improved base excess, pCO2, and pH between Round 1 and other rounds are attributable to bicarbonate infusions during stabilization to prevent lethal acidemia.

Calcium doses were delivered in the range of 5 mg – 20 mg with the majority of the doses being 10.

In total 37 boluses of calcium were delivered and annotated in the experimental record. The normalized aortic blood pressures from all 37 boluses are shown in Fig. 3. The data in this figure have
been smoothed to reduce the ventilation artifact so that the individual responses are easier to discern. The different responses visible in Fig. 3 demonstrate the hyperdynamic nature of the hemodynamics after resuscitation from pseudo-PEA.

The effect of the calcium bolus is observed before time 0 because time 0 was set to the midpoint of the pressor effect. Because the amount of data exported for each delivered calcium bolus depended on the time interval between the calcium bolus of interest and the injection of other medications such as epinephrine, bicarbonate, and other doses of calcium, the different lengths of the data segments also demonstrate the variability in the amount of hemodynamic support the animals needed after resuscitation.

To compare the hemodynamics before and after the calcium bolus we chose the minimum timespan $[-40 \text{ s} < t < 40 \text{ s}]$ for which data are present for all injections. Mean aortic pressures (MAP), systolic aortic pressure (AoS), and diastolic aortic pressure (AoD) were averaged as a function of time. The mean aortic pressure responses are represented by the blue line in Fig. 4. The shaded region above and below the blue line represents the standard deviation of those values. The mean pressures and the standard deviation of the pressures approaches zero as the time approaches zero due to the normalization procedure discussed in the methods. Comparing blood pressures from the timespan $[-40 \text{ s} < t < -36 \text{ s}]$ with blood pressures from the timespan $[36 \text{ s} < t < 40 \text{ s}]$ we find that the MAP increased by 11.03 mmHg ($p < 0.05$), the AoS increased by 15.91 mmHg ($p < 0.05$) and the AoD increased by 7.79 mmHg ($p < 0.05$).

The physiological response to the calcium boluses manifested in a variety of MAP responses, as shown in Fig. 5. In the top tracing, no increase in MAP is observed. In the middle tracing a physiological response is observed, but the MAP resumes its decline after about 20 seconds of improvement. In the bottom tracing, the MAP improves due to the calcium bolus and then continues to improve and stabilize as a function of time.

Discussion

Our preliminary results indicate that, similar to the post resuscitation study after ventricular fibrillation, ionized hypocalcemia may be common after resuscitation from pseudo-PEA, and the post-
disease state refractory hypotension may be responsive to administration of IV calcium gluconate. Specifically, administration of IV calcium gluconate in this setting is often associated temporally with a relatively rapid pressor response. Calcium administration was frequently associated with a stabilization of blood pressure that ended a progressive overall downward trend, and this stabilization often is maintained until the end of that cycle in the experiment. In instances where stabilization was not achieved, bolus calcium either resulted in either a temporary pressure increase followed by a return to the previously demonstrated decline or no measurable effect.

The relationship between ionized calcium and critical cardiovascular states is not completely understood. Prior studies describe hypocalcemia in other related cardiac arrest states. Of the metabolic abnormalities in these other hypotensive states, it was noted that there was consistently a relationship between ionized calcium and pH. Proposed explanations of this phenomena includes intracellular calcium influx from ion compartment shifting, changes from impaired transmembrane pump activity, and extracellular protein complexing. Under ischemic conditions, it has been demonstrated that cells increase their uptake of calcium as a result of altered membrane transport; moreover, intracellular and mitochondrial calcium sequestration is an essential apoptosis initiation.

It is likely that a combination of these factors that result in depleted ionic calcium stores in pseudo-PEA, as tissues are repeatedly exposed to hypoxic stress. Ischemia and the resultant loss of normal cellular energy state may underlie these processes.

It appears likely that hypocalcemia likely contributes to the hypotension that is common the recovery period of lifeless shock. Both cardiac and vascular smooth muscle are dependent on calcium for function. In cardiac myocytes, calcium-dependent calcium release is a critical component initiating robust ventricular contraction; hypocalcemia is a known cause of negative inotropy and dyrythmia.

For vascular smooth muscle, calcium is needed to activate calmodulin to allow for fiber crosslinking. Effective calcium starvation impairs vasomotor tone and results in a decrease in peripheral resistance. For this, it is unsurprising that resultant hypotension is refractory catechol pressors, as functional deficits in both the myocardium and vascular smooth muscle likely overcome the enhanced
sympathetic stimulation. Diffuse hypoxic insult with associated metabolic derangement affects different cell lines at different rates and to different degrees, which could explain continued hypocalcemia even after ROSC is achieved.

Hypotension after resuscitation from profound global hypoxia and ischemia, as in our porcine model of pseudo-PEA, is a particularly challenging cardiovascular state for clinicians. Leaving it untreated may worsen outcome through secondary injury, as in a “two-hit” model. As noted before, it is often refractory to catecholamine pressers\textsuperscript{1}, which may themselves be associated with a worse outcome. It was relatively common during the 1960s and 1970s to treat this condition with boluses of IV calcium but without supporting clinical trials.\textsuperscript{13} This has become much less common in the era of evidence-based ACpseudo-PEA.

pseudo-PEA is common clinically - often representing half of all clinical cardiac arrests - and outcomes are often poor.\textsuperscript{14} It has not been studied pre-to the same extent as ventricular fibrillation. The pseudo-PEA cardiovascular state can be created by hypoxia, asphyxia, cardio tonic drug overdose, and post-defibrillation.\textsuperscript{15} Unfortunately, it has been difficult to create stable models using these insults. Our model is relatively stable and reproducible. However, the hypoxic insult in juvenile pigs may be most representative clinically of pediatric partial asphyxiation such as is seen in asthma. The applicability of our model and its results to adult patients suffering ischemic cardiac arrest may be limited.

Although collected retrospectively, our preliminary data is relatively convincing that ionized hypocalcemia may be present after resuscitation from pseudo-PEA and that bolus IV calcium is associated with a sustained pressor response. Intriguingly, this pressor effect often stabilized blood pressure. These finding should be investigated prospectively. If confirmed, they may justify a clinical trial of intravenous calcium in the setting of pseudo-PEA or post-pseudo-PEA hypotension.

However, it should be emphasized that hypotension per se is a clinical outcome. It is possible that administration of intravenous calcium temporarily improves blood pressure, but then is associated with an overall worse clinical outcome because it enhances vital organ injury by priming of
It is important that clinicians not respond to this preliminary post-hoc data set by empiric administration of IV calcium. A clinical trial with a primary endpoint of improved neurologically intact long-term survival would need to be completed preliminary to a change in clinical practice.

Limitations – our report has a number of limitations: 1) this is an animal model of hypoxic pseudo-PEA, 2) the porcine model is juvenile and without senescent comorbidities, 3) intravenous calcium levels and the blood pressure response to calcium administration were not the primary processes under study, these are retrospectively observed results, 4) improved MAP is not a clinical outcome.

Conclusion
Our preliminary results indicate that the hypotension after hypoxia induced pseudo-PEA is frequently associated with decreased serum ionized calcium levels and a hypotension that is responsive to administration of IV Calcium. These results should be confirmed prospectively before consideration is given to clinical trials.

Abbreviations
**ACLS:** Advanced cardiac life support, **AoD:** Diastolic aortic pressure, **AoS:** Systolic aortic pressure, **BE:** Base Excess, **CPR:** Cardiopulmonary resuscitation, **EMD:** Electro mechanical dissociation, **ETCO2:** End-tidal carbon dioxide, **FiO2:** Inspired fraction of oxygen, **HCO3:** Bicarbonate, **iCA:** Ionic calcium, **IV:** Intra-venous, **pseudo-PEA:** Lifeless shock, **MAP:** Mean arterial pressure, **N2:** nitrogen, **O2:** oxygen, **pCO2:** partial pressure of carbon dioxide, **PEA:** Pulseless electrical activity, **P-EMD:** Pseudo-electromechanical dissociation, **pO2:** partial pressure of oxygen, **ROSC:** Return of spontaneous circulation, **TCO2:** Total carbon dioxide

Declarations
**Ethics approval and consent to participate:** These studies were conducted in accordance with the guidelines of the American Physiological Society for the Care and Use of Laboratory Animals and with the approval of the Dartmouth College Institutional Animal Care and Use Committee.

**Consent for Publication:** Not applicable

**Availability of data and materials:** The authors declare that all data supporting the findings of this study are available within the article.
**Funding:** The authors gratefully acknowledge funding from ZOLL Medical Corporation.

**Competing Interests:** The authors declare that they do not have any competing interests.

**Authors Contributions:** ALL conducted experiments and wrote the manuscript. JWL analyzed the data and was a major contributor in writing the manuscript. JRG and CLK designed the study and conducted the experiments. KM conducted the experiments. NAP designed the study, conducted the experiments and was a major contributor in writing the manuscript. All authors consent to the publication of the manuscript.

**Acknowledgements:** The authors gratefully acknowledge funding from ZOLL Medical Corporation. The opinions expressed in the manuscript are held by the authors and do not represent the opinion of the sponsor.

**References**

1. Trzeciak S, Jones AE, Kilgannon JH et al (2009) Significance of arterial hypotension after resuscitation from cardiac arrest. Crit Care Med 37:2895–2903

2. Cairns CB, Niemann JT, Pelikan PC, Sharma J (1991) Ionized hypocalcemia during prolonged cardiac arrest and closed- chest CPR in a canine model. Ann Emerg Med 20:1178–1182

3. Niemann JT, Adomian GE, Garner D, Rosborough JP (1985) Endocardial and transcutaneous cardiac pacing, calcium chloride, and epinephrine in postcountershock asystole and bradycardias. Crit Care Med 13:699–704

4. Erdmann E, Reuschel-Janetschek E (1991) Calcium for resuscitation? Br J Anaesth 67:178–184

5. Paradis NA, Martin GB, Goetting MG, Rivers EP, Feingold M, Nowak RM (1992) Aortic pressure during human cardiac arrest. Identification of pseudo-electromechanical dissociation. Chest 101:123–128

6. Paradis NA, Halperin HR, Zviman M, Barash D, Quan W, Freeman G (2012) Coronary perfusion pressure during external chest compression in pseudo-EMD, comparison of
systolic versus diastolic synchronization. Resuscitation 83:1287-1291

7. Jentzer JC, Chonde MD, Dezfulian C (2015) Myocardial Dysfunction and Shock after Cardiac Arrest. Biomed Res Int 2015:314796

8. Paradis NA, Crockett SC, Moodie K, Gould JR (2017) Relationship Between Right Atrial Pressure With Jugular Flow in an Asphyxial Model of Pseudo-Pulseless Electrical Activity in Swine. Circulation 136:A15994

9. Paradis NA, Crockett SC, Gould JR, Kaufman C, Moodie K (2018) Development of a Hypoxic Asphyxial Model of Pseudo-Pulseless Electrical Activity. Circulation 136:A15914

10. Larabee TM, Paradis NA, Bartsch J, Cheng L, Little C (2008) A swine model of pseudo-pulseless electrical activity induced by partial asphyxiation. Resuscitation 78:196-199

11. Urban P, Scheidegger D, Buchmann B, Barth D. Cardiac arrest and blood ionized calcium levels. Annals of Internal Medicine 1988 Jul 15; 109(2):110-3

12. Youngquist ST, Heyming T, Rosborough JP, Niemann JT (2010) Hypocalcemia following resuscitation from cardiac arrest revisited. Resuscitation 81(1):117-122. doi:

13. Donovan PJ, Propp DA (1985) Calcium and its role in cardiac arrest: understanding the controversy. J Emerg Med 3:105-116

14. Andrew E, Nehme Z, Lijovic M, Bernard S, Smith K (2014) Outcomes following out-of-hospital cardiac arrest with an initial cardiac rhythm of asystole or pulseless electrical activity in Victoria, Australia. Resuscitation 85:1633-1639

15. Rabjohns J, Quan T, Boniface K, Pourmand A. “Pseudo-Pulseless Electrical Activity in the Emergency Department, an Evidence Based Approach.” The American Journal of Emergency Medicine, October 2019, S0735675719306527

16. Carraro M, Bernardi P (2016) Calcium and reactive oxygen species in regulation of
the mitochondrial permeability transition and of programmed cell death in yeast. Cell Calcium 60:102-107

Figures

Experiment Design. Each experiment was started by a stabilization period to ensure constant hemodynamic parameters before initiating hypoxic insult. There was an intentional ~1 minute delay post paralytic administration to demonstrate stability. Following resuscitation and recovery, the animal entered a stabilization phase, starting the next experiment. There were at maximum 4 experiments per pig.
Serum calcium concentrations measured via arterial blood gases from the first 10 experiments. Blood gases were drawn 10 minutes after successful resuscitation. Serum calcium decreases with experimental round through the first two rounds of pseudo-PEA.
Normalized mean arterial pressure tracings are shown for all 37 boluses of calcium. Displayed pressures have been smoothed using a 15-sample windowed average to reduce the ventilation artifact. There are a variety of physiological responses to a bolus of calcium.
In each subplot, mean normalized arterial pressures are shown with a blue line, and the gray shaded area represents the standard deviation of the same pressures. The top subplot shows the effect of calcium on the mean aortic pressure. The middle subplot shows the effect of calcium of the systolic aortic pressure. The bottom subplot shows the effect of calcium on the diastolic aortic pressure. A calcium bolus results in a sigmoidal increase in arterial pressures. Time 0 is the midpoint of the pressor effect as determined by computer script. The pressures have been normalized by the subtraction of the pressure measured at time 0.
There are a variety of individual responses to a calcium bolus. The top subplot shows a calcium injection that has no observable effect on the mean arterial pressure. The middle subplot shows a calcium injection that has a modest effect on the mean arterial pressure. The bottom subplot shows a calcium injection that has a large effect on arterial pressure.

These tracings were chosen to represent the spectrum of observed responses.