Hemodynamic Differences in Recovery Following Chest Compression with Asynchronous Ventilation Using Chest Compression Rates of 90/min, 100/min, or 120/min in a Porcine Model of Neonatal Asphyxia

By

Sparsh Patel

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Abstract

Extensive neonatal resuscitation that involves chest compressions is an uncommon event impacting approximately 0.1% of term newborns, and up to 15% of preterm newborns. Despite best resuscitation efforts birth asphyxia results in approximately one million deaths annually worldwide. Neonatal resuscitation research is still considered to be a field in its infancy when it comes to optimizing extensive resuscitation efforts. This is further complicated by the infrequent occurrence of extensive neonatal resuscitation, making it difficult to conduct rigorous clinical studies. However, optimizing neonatal resuscitation is an urgent issue as current extensive resuscitation practices are associated with poor survival and morbidity. Animal models make up the primary body of scientific knowledge when it comes to optimizing chest compression techniques. To this extent, the contents of this thesis explore whether continuous chest compressions with asynchronous ventilation (CCaV) with varying rates of chest compressions reduces the time to return of spontaneous circulation (ROSC) using a porcine model of neonatal asphyxia induced cardiac arrest. We hypothesize that CCaV with 120 CC/min will have reduced time to ROSC compared to CCaV with 100 CC/min or 90 CC/min.

Thirty piglets of age 24-72h were surgically instrumented and allowed to recover to a stable baseline following surgery. Piglets in the intervention group were exposed to 30min of hypoxia followed by asphyxia until cardiac arrest, piglets in the sham-operated (n=6) group were not. Intervention piglets were randomized to receive CCaV with either 90 CC/min (n=8), 100 CC/min (n=8), or 120 CC/min (n=8) until ROSC or a maximum of 12min. Piglets that achieved ROSC were allowed to recover for 4h, after which, cerebral cortical tissues were harvested for bioassays.

Time to ROSC was similar in all intervention groups (120s, 90s, and 90s for CCaV+120, CCaV+100, and CCaV+90 respectively, p=0.93). The number of piglets achieving ROSC (p=0.83), and survival to 4h after ROSC (p=0.50) was also similar across intervention groups. However, regional hemodynamic recovery at the end of experiment was significantly worse in CCaV with 90 CC/min and 100 CC/min compared to CCaV with 120 CC/min. A similar trend was observed with pro-inflammatory markers interleukin(IL)-1β, IL-6, and lactate in the frontoparietal cortex. Where CCaV with 90 CC/min and 100 CC/min had significantly worse cortical inflammatory and injury markers compared to CCaV with 120 CC/min.

Preface

This thesis is an original work by myself (Sparsh M. Patel). I was involved in the design of the study, performed the animal surgery, observation of the piglet throughout the experiment period, and was involved in the resuscitation of the piglets. I performed data collection throughout the experimental period, performed autopsies, tissue collection and biochemical assays. I did the statistical analysis for the study in chapter 3, a version of which is a published article in Archives of Disease in Childhood - Fetal and Neonatal Edition as "Asynchronous ventilation at 120 compared with 90 or 100 compressions per minute improves hemodynamic recovery in asphyxiated newborn piglets". The manuscript of this article was drafted by all authors including myself.

The research project this thesis is based on has received ethics approval from the Animal Care and Use Committee University of Alberta (AUP00001764), and the data has been presented according to ARRIVE guidelines. The study was supported by a Grant-in-Aid from the Heart and Stroke Foundation Canada (grant #: G-15-0009284), we have no conflicts of interests to declare.

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Abbreviations

Anterior-posterior	AP
Cardiac arrest	CA
Cardiopulmonary resuscitation	CPR
Cerebral oxygen saturation	$crSO_2$
Chest compressions	CC
Chest compressions with asynchronous ventilation	CCaV
Chest compressions with sustained inflation	CC+SI
Compressions:ventilation	C:V
Coronary perfusion pressure	CPP
Endotracheal	ET
End-tidal carbon dioxide	$ETCO_2$
Heart rate	HR
Hypoxic ischemic encephalopathy	HIE
Intravenous	IV
Mean arterial pressure	MAP
Neonatal intensive care unit	NICU
Neonatal resuscitation program	NRP®
Pediatric intensive care unit	PICU
Positive pressure ventilation	PPV
Return of spontaneous circulation	ROSC
Tidal volume	V_T
Ventricular fibrillation	VF

Chapter I

Introduction

Background

The transition from intrauterine to extra-uterine life requires a complex set of adaptations which include the initiation of breathing, a switch from placental to pulmonary gas exchange, and increased vascular resistance with removal of the placenta[1–3]. Even with the complexity of transitioning to extra-uterine life 95% of term newborns transition successfully; most initiating breathing on their own, with some requiring drying and stimulation to initiate breathing[1]. Leaving approximately 5% of term newborns requiring some form of respiratory support to establish effective ventilation, which is one of the most important steps in neonatal resuscitation[1–3]. In such cases where apnea persists, positive pressure ventilation (PPV) is initiated immediately at birth until heart rate (HR) above 100 beats/min is achieved. In 0.1% of these term newborn cases, the HR continues to drop below 60 beats/min and extensive cardiopulmonary resuscitation (CPR) is initiated, and epinephrine may also be administered[1,2]. Extensive CPR refers to resuscitation interventions beyond initial ventilation through PPV, including chest compressions (CC) and medication administrations during resuscitation. The preterm newborn population is particularly vulnerable and up to 15% of this population requires extensive CPR[4]. A recent retrospective cohort study reporting survival to 1-year of age among newborns with Apgar score of 0 at 5min and 10min vs. 0 at 5 min and >1 at 10min noted 46% and 61% survival to 1-year of age, respectively[5]. Unfortunately long-term assessment of neurodevelopmental outcomes was not possible with the database used for this study, however, severe neurodevelopmental morbidity has been reported following CCs in the preterm population[5–8]. The prognosis following CCs is poor, and additional research is needed to improve upon current CPR methods and optimize the delivery of CCs to improve resuscitation outcomes of newborns.

The infrequent use of CCs for newborns has hindered conducting rigorous clinical studies in developing optimal methods of CCs in newborns. The guidelines from Pediatric Advanced Life Support and Neonatal Resuscitation Program (NRP®) recognize that most newborns experience cardiovascular collapse due to asphyxia, and the resulting recommendations emphasize the importance of ventilation in newborn resuscitation. The NRP® algorithm recommends a 3:1 Compression:Ventilation (C:V) ratio[1,2,9], while the pediatric resuscitation guidelines recommend continuous chest compressions without a pause for ventilation (continuous CC with asynchronized ventilation = CCaV) for infants with an advanced airway[10]. Due to lack of alternatives or additional algorithms, the use of NRP® has been extended beyond its original application in the delivery room into the neonatal intensive care unit (NICU), but without any research on its scientific merit or formal evaluation of its impact[11]. These recommendations rely on data extrapolated from adult or animal studies. Adult data may not fully apply to newborn resuscitation, as the primary cause of adult cardiovascular collapse is ventricular fibrillation (VF) and not asphyxia. Data from animal models also face issues with translatability because existing models use both VF and asphyxia to induce cardiovascular collapse. Therefore, studies determining the optimal method for improving hemodynamics during neonatal resuscitation are needed.

Asphyxia/Hypoxia

Asphyxia results from a lack of oxygen supply that can lead to severe hypoxemia, and hypercapnia with mixed metabolic and respiratory acidosis which leads to ischemic organ damage to newborns[12,13]. A recent study examining trends for causes of child death reported birth asphyxia as one of the leading causes for neonatal deaths, accounting for an estimated global prevalence of 10.5% of all deaths under 5 years of age, where 44% of all deaths under 5years of age were in the

neonatal period[14]. Advancing neonatal care in cases of asphyxia has the potential to significantly impact survival rates and long-term health outcomes.

Prolonged asphyxia or hypoxia in newborns can result in severe organ damage followed by death or severe life-long pathologies[12,13]. This is the result of compromised placental (perinatal) or pulmonary gas exchange (immediately postnatal) failing to deliver oxygenated blood to the vital organs causing progressive hypoxemia and hypercapnia [12,13]. Prolonged asphyxia can cause severe injury to the neonate's vital organs including the brain and ultimately leading to cardiac collapse. This requires intervention in the form of extensive CPR, which includes initiation of CCs and administration of vasoactive drugs.

The vast majority of neonatal asphyxia cases occur between labor and delivery period (intrapartum)[13]. Intrapartum events such as tight nuchal cord, cord prolapse, maternal fever, prolonged labor, and out of hospital birth can compromise fetal oxygenation[13,15]. Other relevant risk factors associated with neonatal hypoxia are maternal age, pre-eclampsia, meconium stained fluid, uterine rupture, placental abruption, diuretic drug usage, and pre-mature delivery[13,15].

Following severe perinatal hypoxia and successful initial resuscitation of the newborn, a major neurologic sequelae of hypoxia emerges: hypoxic ischemic encephalopathy (HIE)[13,16,17]. Currently, 15-20% of neonates who experienced perinatal asphyxia die, and approximately 25% of survivors experience permanent neurologic deficits[13,16]. HIE is a form of clinical encephalopathy that can result in a wide range of deficits in motor, sensory, cognitive, and behavioral development[17]. The prevalence of perinatal asphyxia and HIE can vary based on the

level of care available[14,15], and therefore developing countries typically experience higher rates of complications associated with perinatal asphyxia and related sequelae. HIE can lead to permanent brain damage, however, the severity and timing of the hypoxic period can present as a variety of neurological deficiencies. The SARNAT classification scale is typically used to help categorize the extent of neonatal HIE by assessing physical manifestations of consciousness, neuromuscular tone, reflexes, pupils, HR, and respiration to grade HIE into mild, moderate or severe[16]. Neonates with an assessment of severe HIE experienced higher mortality compared to neonates with moderate or mild HIE (84.2%, 1.4%, and 0%, respectively) at a tertiary hospital in northern Tanzania[16].

The pathophysiology of HIE occurs in three stages. First, interruption of oxygen and glucose to the brain causes immediate primary neuronal injury resulting in cell death through failure of ATP-dependent Na^+K^+ pump, cell swelling, and widespread depolarization[13]. Second, a latent period of approximately six hours during which cells are reperfused and experience some recovery. Third, late secondary neuronal injury occurs over the next two days as reperfusion spreads toxic neurotransmitters; effectively widening the area of brain injury[13]. Treatment of HIE usually includes a combination of observation, oxygen therapy, mechanical ventilation, and most commonly therapeutic hypothermia[13,16]. Therapeutic hypothermia is a time sensitive intervention most effective during the second stage, where cooling can minimize damage from cell rupture where the release of toxic neurotransmitters effectively widens the area of brain injury[13]. When therapeutic hypothermia is initiated within six hours of hypoxic injury, it can reduce mortality, severe disability while improving neonatal survival with a normal outcome (Relative Risk=1.31), and survival without neurological abnormalities (Relative Risk=1.6) in neonates with moderate to

severe HIE[13,17]. Which means therapeutic hypothermia, when utilized within six hours of hypoxic injury, will improve neonatal survival and have better neurological outcomes relative to neonates which have not received therapeutic hypothermia intervention.

Current Neonatal Resuscitation Guidelines

Current neonatal resuscitation guidelines established with international consensus in 2015 updated recommendations to emphasize the importance of effective ventilation when neonates need respiratory support to transition from intrauterine to extrauterine environment[1]. The guidelines recommend initiating ventilatory support in the form of PPV when the neonate is gasping or has HR <100bpm, where PPV helps facilitate neonatal transition. However, for those that continue declining to HR <60bpm, CCs should be initiated to improve perfusion to coronary arteries and the brain[1,2,18]. Compressions should be discontinued after achievement of HR >60bpm. The compressions should be delivered on the lower third of the sternum at a depth of 1/3 of anterior-posterior diameter of the chest using the 2-thumb technique, where the chest is allowed to recoil fully between each compression[1,2]. It is recommended that compressions and ventilations be delivered in synchrony with a ratio of 3:1 C:V at a rate of 120 events/min[1,2]. It is also recommended that CCs are initiated with 100% O_2, and oxygen is weaned as soon as the HR recovers. CCs are initiated with 100% O_2 because resuscitation efforts preceding CCs have presumably attempted resuscitating with lower concentration of oxygen with no beneficial result[1,2]. However optimal O_2 concentration delivered during resuscitation remains a topic of debate and research. Finally, the compressions and ventilations should be continued until return of spontaneous circulation (ROSC) with HR >60bpm. The primary reasoning for emphasis on ventilation stems from asphyxia being the predominant cause of cardiovascular collapse in neonates, and by providing adequate lung

aeration the fluids in lungs can be sufficiently cleared for better gas exchange[1,2,18]. However, these recommendations are based on physiological plausibility, data from adult patients, neonatal simulations, and animal models because clinical evidence is lacking[1,2,18].

Factors contributing to high-quality CCs for successful resuscitation include C:V ratio, CC rate, depth of CCs, adequate cardiac, and cerebral perfusion. These factors are all recognized to be important for high-quality successful resuscitation[19], however, extensive research is needed to determine the optimal combination of these parameters to maximize cardiac and cerebral perfusion while ensuring adequate ventilation for asphyxiated newborns.

C:V Ratios and ROSC

Current neonatal resuscitation guidelines recommend 3:1 C:V ratio, however, the most effective C:V ratio in newborns remains unknown[1,2,20]. Guidelines place emphasis on establishing effective ventilation because asphyxia causes increasing hypoxia and hypercapnia over a prolonged period, as opposed to, adult sudden cardiac arrest where the blood is still oxygenated. Therefore, the need for ventilation in neonates is more essential compared to adult resuscitation. The recommendation of 3:1 C:V ratio is based on data from adult and animal studies along with expert opinion, because there is a lack of clinical data available on neonates receiving CC interventions[1,2]. The 3:1 C:V ratio is rationalized on the basis of higher neonatal resting physiological HR and respiratory rate. However, the 3:1 C:V ratio has not been clinically examined in a rigorous scientific study. In fact, Dr. Schmölzer and colleagues are currently conducting the first randomized controlled trial examining CCs in neonates[21]. This will be the first study

comparing different CC interventions in the neonatal population, and the first time 3:1 C:V ratio is examined scientifically in the clinical setting[21].

Basic science research with neonatal piglet asphyxia models has been conducted comparing various C:V ratios, as well as, alternative techniques such as continuous compressions with either sustained inflation or asynchronous ventilation[22–29]. Solevåg *et al* conducted two separate studies comparing time to ROSC for 3:1 C:V with 9:3 C:V median (IQR) (150s (115–180)s vs. 148s (116–195)s respectively, p=ns), and 3:1 C:V with 15:2 C:V (150s (140–180)s vs. 195s (145–358)s respectively, p=ns)[26,27]. They also reported no differences in survival, mortality, oxygen delivery, hemodynamics, epinephrine administration, or cytokine concentration when 3:1 C:V was compared with either 9:3 C:V or 15:2 C:V. More recently Pasquin *et al* compared 2:1, 3:1, and 4:1 C:V ratios in a neonatal piglet model of asphyxia and found no differences in time to ROSC (127s (82–210)s, 96s (88–126)s, and 119s (83–256)s in 2:1, 3:1, and 4:1 C:V respectively, p=0.67) between groups. Additionally, they did not report differences in survival hemodynamics, proinflammatory cytokines in lung, mean arterial pressure (MAP), cerebral oxygen saturation ($crSO_2$), or carotid flow 4h post resuscitation[29]. These studies did not find any significant differences between various C:V ratios in an animal model, prompting exploration of alternative CC approaches in neonates to improve outcomes.

CCs with sustained inflation (CC+SI) and CCaV are alternative CC techniques with potential to improve neonatal resuscitation. Schmölzer *et al* first described CC+SI as an alternate CC technique in 2013 where they reported significant reduction in time to ROSC with CC+SI compared to 3:1 (38s (23–44)s vs. 143 (84–303)s respectively, p=0.0008), along with increased

survival with CC+SI (p=0.038)[28]. However, this animal model was resuscitated once HR reached 25% of baseline instead of total cardiac arrest (CA) after asphyxia. This experiment was later revisited by Mustofa *et al* using total CA following asphyxia under the context of comparing different inflation lengths using CC+SI[24]. They reported significantly improved time to ROSC, with CC+SI compared to 3:1 C:V but did not report improved survival. Schmölzer *et al* also conducted a randomized feasibility trial in neonates and concluded CC+SI to be feasible in the clinical setting[30]. They enrolled a total of nine infants receiving either CC+SI (n=5) or 3:1 C:V (n=4), and reported significantly reduced time to ROSC with CC+SI compared to 3:1 C:V mean (SD) (31s (±9)s compared to 138s (±72)s respectively, p=0.011)[30]. However, CC+SI has not been used extensively in a clinical setting.

CCaV also provides continuous CCs but they are combined with inflations without any synchronization, instead of a prolonged inflation in the case of CC+SI. Recently, CPR using 3:1 C:V was compared with CCaV with 90 CC/min and 30 inflations/min in a piglet model of neonatal asphyxia and reported a trend of faster time to ROSC during CCaV compared to 3:1 C:V (114s (88–148) vs. 143s (84–303) respectively, p=0.351). They also reported a trend towards higher survival 4h post-resuscitation in the CCaV group 6/8 vs. 3/8 in the 3:1 C:V group, however, this did not reach statistical significance[22]. The study did not report a difference in tidal volume (V_T), minute ventilation or end-tidal carbon dioxide ($ETCO_2$) between 3:1 C:V and CCaV. However, a study with newborn mannequin model reports higher minute ventilation with lower V_T compensated by higher number of ventilations in CCaV compared to 3:1 C:V[31]. The piglet study indicated a trend to lower V_T however this did not reach statistical significance[22]. Mendler *et al* similarly reported no adverse effect on minute ventilation and CO_2 removal using mechanical

ventilation with asynchronous inflations[32]. CCaV is the recommended method for resuscitation in newborns by the pediatric basic and advanced life support guidelines when an advanced airway (laryngeal mask, ET tube) is in place[10]. This is because it is easier to teach, and data comparing 3:1 C:V and CCaV was not available when the recommendations were made. The guidelines also express concern toward whether interruption of ventilation during CCaV can affect outcomes. It has since been reported that CCs interfered with 29% of total ventilations using CCaV, and 25% of total ventilations using 3:1 C:V[22]. It appears that interruptions when delivering ventilations are common in coordinated CPR also, which indicates that ventilation interruptions during CCaV may not be concerning.

Rate of Chest Compressions

The neonatal resuscitation guideline's recommendation of using 3:1 C:V results in 90 CC/min and 30 inflations/min, with a total of 120 events/min[1,2]. However, adherence to the guidelines is poor with providers failing to meet recommended rate of compression, rate of ventilation, depth, and duty cycle[33]. With recent use of CCaV and CC+SI methods, the delivery of inflations with a pause in CC is eliminated. This allows resuscitators to focus on delivering high quality CCs continuously which would otherwise be interrupted with delivering inflations. Allowing extra time to deliver more compressions than possible with the 3:1 C:V method. This prompts investigation into whether higher or lower rates of CC/min can improve outcomes for neonatal resuscitation. A mathematical model by Babbs *et al* suggests that using >120 CC/min for neonates would be the optimal CC frequency based on physical and mathematical reasoning[34]. This mathematical model used scaling rules for cardiovascular parameters related to CC frequencies. They used vascular resistance, arterial blood pressure, systemic perfusion pressure,

basal metabolic rate, cardiac index, pump filling and emptying as metrics to develop the mathematical model[34]. They reasoned that if the fraction of time in which the pump's input valve remains open is greater than the time required for pump filling, there is cardiac output to be gained by increasing the compression frequency[34]. However, it is important to note that this mathematical model does not account for the effects of increased compression frequencies on the coronary circulation which directly impacts myocardial perfusion.

Li *et al* were interested in looking at different rates of CCs and their effect on the quality of resuscitation by looking at rescuer fatigue, CC depth over time, and changes in peak pressure during CC in mannequins by comparing 3:1, CCaV with 90 CC/min (CCaV+90) and CCaV with 120 CC/min (CCaV+120). They observed a gradual decrease in peak pressure throughout their 10m resuscitation period, with significant differences from baseline at 156s, 96s, and 72s using 3:1, CCaV+90, and CCaV+120 groups, respectively[35]. They also observed a reduction in CC depth by 20%, 30%, and 50% in 3:1, CCaV+90 and CCaV+120 groups after 3min. Their findings indicate that rescuers preferred the 90 CC/min over the 120 CC/min, and rescuers were able to maintain CC quality for longer with coordinated CCs[35]. From the rescuer perspective and how it relates to the quality of resuscitation performed, rescuers clearly preferred coordinated compressions over continuous compressions and lower CC rates over higher CC rates[35,36]. In this study, rescuers were not formally trained in all of the tested resuscitation techniques which may contribute to their preference or comfort for a technique they are already familiar with. However, whether 90 CC/min or 120 CC/min is more effective from the physiological stand point is an entirely separate question.

Li *et al* later examined whether different CC rates (90 CC/min or 120 CC/min) during CC+SI reduced time to ROSC in a neonatal asphyxia piglet model[37]. They observed similar time to ROSC, survival to 4h after ROSC, respiratory, and hemodynamic parameters between CC+SI 90 and CC+SI 120 groups. Although both groups observed similar hemodynamic recovery, CC+SI 120 had higher cerebral oxygenation 4h post-resuscitation which was attributed to higher carotid blood flow[37]. Li *et al* ultimately concluded that using CC+SI 120 over CC+SI 90 did not provide any improvements in ROSC, survival, or hemodynamic recovery in their porcine model of neonatal asphyxia induced cardiac arrest. However, their animal model has already undergone the fetal to neonatal transition, the animals were ventilated using an endotracheal (ET) tube (no leak), and the resuscitation began when animal HR decreased to 25% of baseline rather than CA. With increasing rates of CC an important factor to consider is duty cycle (proportion of time spent compressing the chest), and relaxation time between CCs. Decreased relaxation time and increased duty cycle result in inadequate chest recoil, and static chest deformation[38]. Increasing duty cycle can increase coronary perfusion pressure (CPP) until the amount of time spent contracting the chest interferes with adequate chest recoil, which can reduce coronary blood flow as it primarily occurs during chest recoil/relaxation phase[38].

Optimal Depth to Optimize Ejection Fraction

The recommended CC depth in neonates is 1/3 of anterior-posterior (AP) chest diameter, which means the depth of CC varies with size of neonates. Achieving this depth consistently has been a problem for rescuers, but using the two-thumb instead of the two-finger technique can drastically improve depth consistency[39]. Meyer *et al* studied efficacy and safety of 1/4, 1/3, and 1/2 AP CC depth in neonates using computerized tomography and also estimated ejection fractions

using a mathematical model[40]. They found a positive relationship with increasing CC depth and ejection fraction (51 ±3%, 69 ±3%, and 106 ±4% with 1/4, 1/3, and 1/2 AP chest depth respectively, $p<0.001$). Under compression with 1/4 AP depth and over compression with 1/2 AP depth was also reported, while 1/3 AP depth reported neither over/under compression[40]. Using 1/3 AP depth for compressions was further reinforced with a similar study using pediatric patients[41]. These studies add vital information on appropriate CC depth since CC depth is very difficult to study in the clinical environment. These studies reinforce the neonatal and pediatric resuscitation guidelines and provide estimations on ejection fraction. Although higher ejection fraction can be achieved with deeper compressions, this has to be balanced with bone and soft tissue damage along with multiple factors encompassing overall quality of CCs.

Cardiac and Cerebral Hemodynamics

Establishing effective coronary and cerebral perfusion is key to achieving ROSC. CCs should aim to maximize these parameters in order to increase CC quality and outcomes. Complete decompression of chest wall following compression is recommended by both neonatal and pediatric guidelines, as coronary blood flow primarily occurs during the decompression phase[1,10,42]. Rescuer leaning which prevents full decompression is associated with decreasing venous return, MAP, myocardial blood flow, and CPP[43,44]. Interruptions during CCs for ventilation, HR assessment, or switching rescuers may also have a negative impact on CPP. In a large swine model interruption to CCs decreased CPP, and reported lower CPP during first few compressions when CCs were reinitiated[45]. Studies looking at uninterrupted and interrupted CCs at in-hospital and out-of-hospital settings reported better outcomes for uninterrupted CCs[46,47].

However, these studies were conducted in adults; the majority of which do not experience asphyxial CA.

Animal Models for Studying Neonatal Resuscitation

There are several animal models being used to research neonatal topics including perinatal medicine, HR monitoring, fetal growth, antenatal glucocorticoid treatments, cardiovascular physiology, neonatal resuscitation, etc.[48,49]. Animals used in studying these topics include sheep, pigs, primates, rabbits, and rats[48,49]. Each individual animal model has its strengths and weaknesses, for example, sheep have largely been used for perinatal and cardiovascular transition research because sheep have fewer offspring and it is relatively easy to conduct cesarean section under general anesthesia[49]. This has the ethical and practical advantage of having to use fewer animals for experimentation at a given time, while also providing opportunity to study fetal transition with fluid filled lungs. However, the sheep model is at a disadvantage when studying CCs during neonatal resuscitation because their chest is V-shaped as opposed to human neonates that have a cylindrical chest with a flat sternum. For studying CCs the piglet model is widely used[48]. In a 2015 systematic review Solevåg *et al* identified 24 studies using a piglet model while identifying only 1 sheep, and 2 primate models to study neonatal CCs[48]. While the primate model more closely resembles human physiology and anatomy, it is extremely expensive and inaccessible to use for research purposes.

When interpreting results of CCs using a neonatal piglet model it is important to consider how CA is induced and the degree to which it extends. Studies using the neonatal piglet model can induce CA by using asphyxia, VF, or potassium chloride[48]. The major distinction here is gradual

vs. sudden cardiac collapse. While models using VF or potassium chloride induce sudden CA, the asphyxia induced arrest is gradual and prolonged. The asphyxia model mimics the biochemical profile of neonates that need CPR in the delivery room presenting with mixed metabolic and respiratory acidosis[48,49]. Therefore, models using asphyxia to induce CA may have better translational value, while sudden CA models may be more useful in studying hemodynamics during CCs[48]. Unfortunately, the piglet model uses post-transitional animals which means the animal does not have lung fluid, high pulmonary pressures, or a patent ductus arteriosus. These are all factors which effect transitioning in neonates and somewhat limits the translational strengths of this model. However, it is important to place emphasis on utilizing results from animal models to guide translational research in the clinical setting and ultimately clinical evidence should be the driver to affect change in neonatal resuscitation guidelines.

Purpose Statement

The primary purpose of this work is to examine whether varying rates of CC with CCaV can reduce time to ROSC. We further examined whether hemodynamic recovery will differ with varying CC rates.

Objectives

The primary objective of this work is to examine whether CCaV with 120 CC/min will have a reduced time to ROSC compared to CCaV with 90 CC/min or 100 CC/min. The secondary objective of this work is to observe whether CCaV with 120 CC/min confers benefits to hemodynamic recovery compared to CCaV with 90 CC/min or 100 CC/min.

Hypothesis

We hypothesized that CCaV with 120 CC/min will have a reduced time to ROSC, and better hemodynamic recovery compared to CCaV with 90 CC/min or 100 CC/min.

Chapter II

Materials and Methods

To examine the relationship between varying CC rates and ROSC we utilized a previously established neonatal porcine model of asphyxia induced cardiac arrest (CA). Following CA animals were subsequently resuscitated using intervention specific compression rates, and allowed to recover for 4h post-resuscitation. Thirty mixed breed piglets (24-72h of age, weighing 2.0kg (±0.20kg)), were acquired on experimentation days from the University Swine Research Technology Centre. Experiments were carried out according to approved guidelines from the Animal Care and Use Committee (Health Sciences) University of Alberta (AUP00001764).

Animal Model

We used a neonatal porcine model of asphyxia to induce CA, as porcine models are widely used in resuscitation research[48,49]. However, many use electrical (60 Hz current)[50,51] or chemical interventions (hyperkalemia using KCL bolus)[32,52] for inducing CA in animal models. Asphyxia induced CA models may be more robust as they introduce gradual hypoxia followed by asphyxia to CA, this stresses the animals over a prolonged duration. The introduction of prolonged hypoxia, hypercapnia, and metabolic acidosis better represents the in-utero condition of the neonate compared to sudden CA models[3,49]. The prolonged duration of hypoxia can increase pulmonary vascular resistance, peripheral vasoconstriction, MAP, cause redirection of blood to vital organs, tachycardia, and initiation of gasping reflexes[53]. In the absence of resuscitation interventions, persistent hypoxia will eventually result in peripheral vasodilation and a drop in MAP with subsequent bradycardia followed by CA[53]. Whereas sudden CA has key physiological difference that weakens the translatability of animal models using electrical or chemical methods for CA. Sudden CA lacks the mixed respiratory and metabolic acidosis present in most neonates requiring CCs. With sudden CA the blood is well oxygenated, the pH is normal, and forward flow continues

until the pressure gradient between the arterial and venous system dissapates[54]. There is a lack of initial increase in vascular resistance, carotid blood flow, and tachycardia which is concerning because the resulting inflammation and organ damage may not resemble what we see in CA from prolonged hypoxia. The preference for studying neonatal resuscitation using asphyxia models over VF models is reflected in the literature, where use of the asphyxia model has recently increased[48]. In a 2015 systematic review by Solevåg *et al* identifying 28 studies examining CCs in newborn animal models, only 8/28 studies used a sudden CA animal model[48].

Sample Size and Power Estimates

Our primary outcome measure was time of cardiopulmonary resuscitation (CPR) to achieve ROSC. Our previous studies showed a median (IQR) ROSC of 114s (88–148)s during CPR using CCaV[6]. We hypothesized that CCaV 120 CC/min during CPR would reduce time to achieve ROSC. A sample size of 24 piglets (8 per group) was sufficient to detect a clinically important (33%) reduction in time to achieve ROSC (i.e., 180s vs. 120s), with 80% power and a 2-tailed alpha error of 0.05.

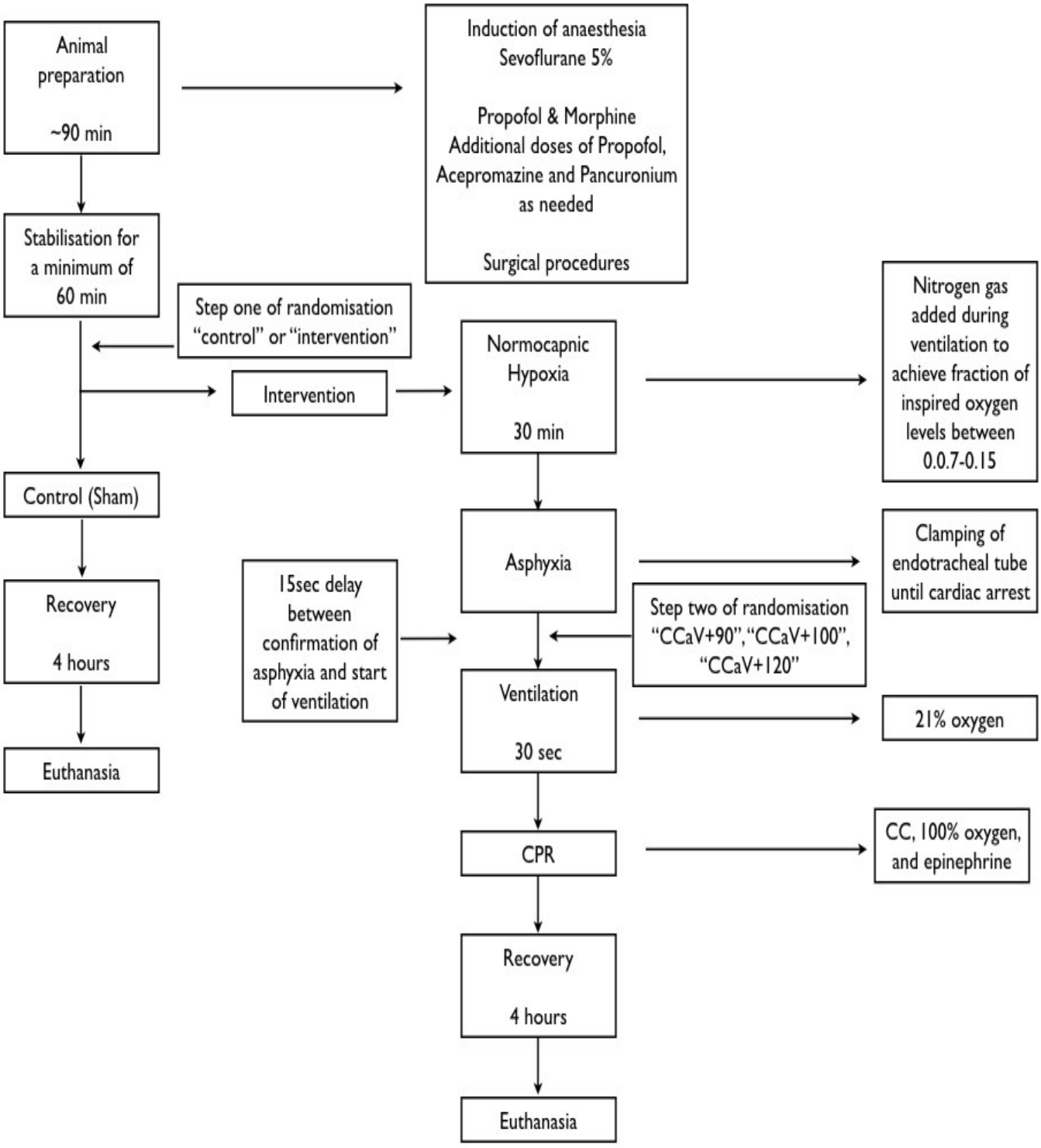
Random Allocation of Intervention

The experimental protocol has two separate random allocation phases. Allocation was block randomized with variable sized blocks (2 to 4) using a computer-generated randomization program (http://www.randomizer.org). The first is allocation to sham-operated or intervention group (Figure 2.1). Random allocation is necessary here to eliminate selection bias during surgical instrumentation and stabilization phases, ensuring similar surgical instrumentation and recovery phases between sham-operated and intervention animals by blinding the experimental team. The

experimental team opens a subsequently numbered, sealed, opaque envelope containing allocation to sham-operated or intervention group immediately before the hypoxia phase. Animals allocated to sham-operated groups do not undergo hypoxia as opposed to animals allocated to intervention group which do undergo hypoxia. The sham-operated group received the same surgical protocol, stabilization, and equivalent experimental periods without hypoxia and asphyxia.

The second subsequently numbered, sealed opaque envelope allocates intervention groups to CCaV+90, CCaV+100, or CCaV+120 (Figure 2.1). This allocation ideally occurs immediately following confirmation of asystole. However, the window before initiation of resuscitation is short, therefore, the designated operator for delivering CCs (separate from assessor of asystole) opens the second sealed opaque envelope containing intervention allocation once asphyxia is initiated. The designated operator for delivering CCs is aware of the intervention allocation once asphyxia is initiated which allows them to set the appropriate metronome frequency for CCs. This approach eliminates unplanned delays that may occur with the task of opening a sealed envelope, setting the appropriate metronome frequency, or miscommunication within the resuscitation team. A prompt announcement of intervention allocation following confirmation of asystole informs the entire resuscitation team of the intervention allocation. This ensures the assessor of asystole is blinded and unbiased in their assessment of asystole, and also allows for a smooth transition to delivery of appropriate intervention following confirmation of asystole.

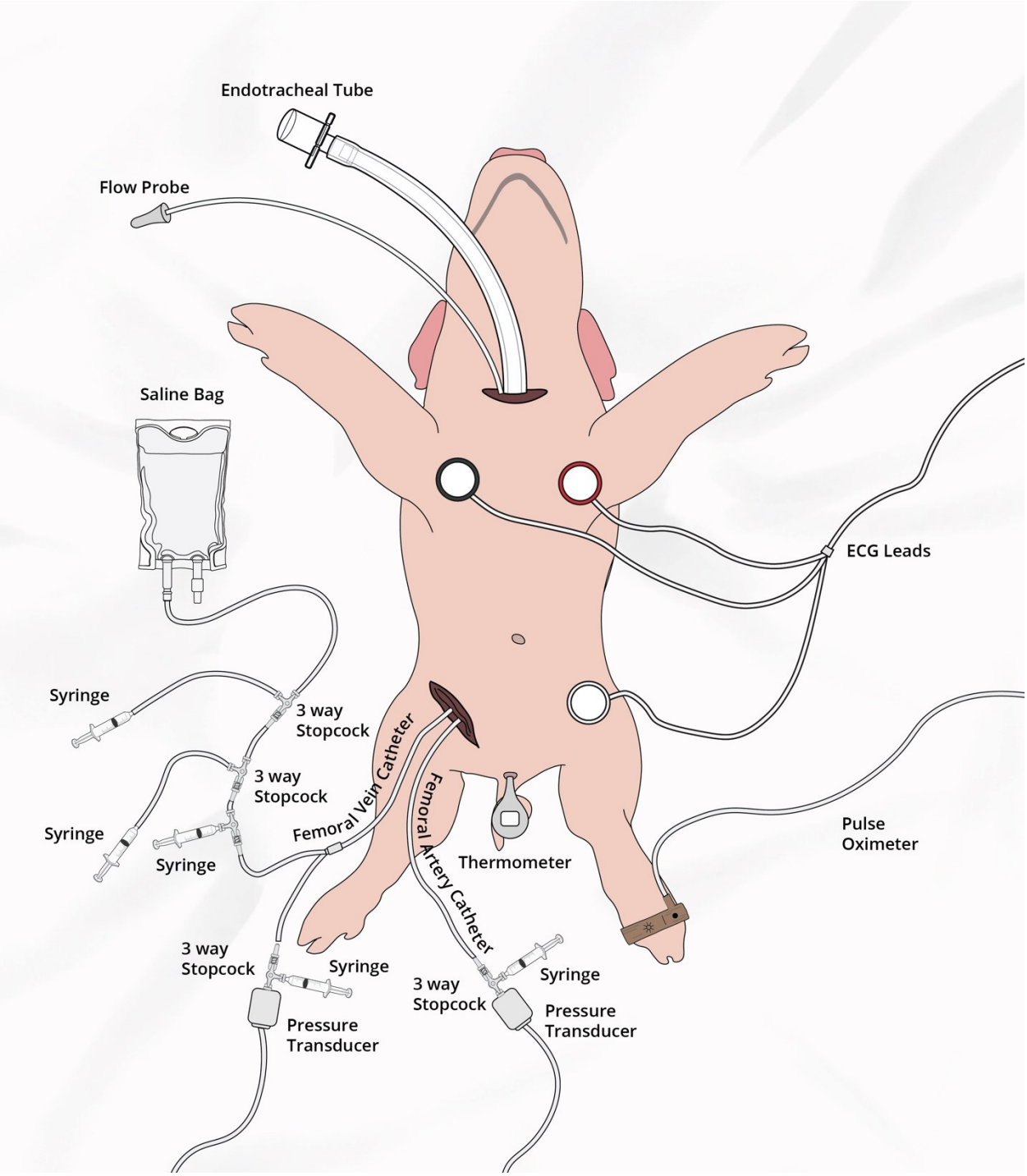
Figure 2.1: Study flow chart



Surgical Instrumentation

Piglets were instrumented as previously described with modifications[22,24,25,28,29,37,55,56]. Animals were anaesthetized with isoflurane gas (5%), sedation was confirmed by toe-pinch before any surgical procedures were initiated. Animals were intubated via tracheostomy and a 3.5 endotracheal (ET) tube was inserted in to the trachea. Mechanical ventilation was initiated immediately after insertion of ET tube. Ventilation (Acutronic Fabian HFO; Hirzel, Switzerland) was commenced at a respiratory rate of 20-25 breaths/min and pressure of 30/5 cmH_2O. Oxygen saturation was kept within 90-100%, glucose level and hydration was maintained with an Intravenous (IV) infusion of 5% dextrose at 10mL/kg/hr. Anaesthesia was maintained with IV propofol 5-10mg/kg/hr and morphine 0.1mg/kg/hr. Additional doses of propofol (1-2mg/kg), pancuronium (0.1-0.2mg/kg) and acetopromazine (0.125-0.25mg/kg) were given as needed. The piglet's body was maintained at a normal resting temperature of 38.5-39.5°C[57] using an overhead warmer and a heating pad. Lastly, no animals were lost during surgical instrumentation. A representation of the experimental setup including the surgical instrumentation is presented in Figure 2.2.

Figure 2.2 Experimental setup with surgical instrumentation.



With permission from RETAIN Labs Medical Inc.

Hemodynamic Parameters

A 5-French Argyle® (Klein-Baker Medical Inc. San Antonio, TX) double-lumen catheter was inserted via the right femoral vein for administration of fluids and medications. A 5-French Argyle® single-lumen catheter was inserted above the right renal artery via the femoral artery for continuous arterial blood pressure monitoring in addition to arterial blood gas measurements. The right common carotid artery was also exposed and encircled with a real-time ultrasonic flow probe (2mm; Transonic Systems Inc., Ithica, NY) to measure cerebral blood flow. Piglets were placed in the supine position and allowed to recover from surgical instrumentation until baseline hemodynamic measurements were stable (approximately one hour). Ventilation rate was adjusted to keep the partial arterial CO_2 between 35-45mmHg as determined by periodic arterial blood gas analysis. Mean systemic arterial pressure, HR, and percutaneous oxygen saturation were continuously measured and recorded throughout the experiment with a Hewlett Packard 78833B monitor (Hewlett Packard Co., Palo Alto, CA).

Respiratory Parameters

A respiratory function monitor (NM3, Respironics, Philips, Andover, MA) was used to continuously measure tidal volume (V_T), airway pressures, gas flow, and end-tidal CO_2 ($ETCO_2$). The combined gas flow and $ETCO_2$ sensor was placed between the ET tube and the ventilation device. V_T was calculated by integrating the flow signal[58]. $ETCO_2$ was measured using non-dispersive infrared absorption technique[59]. The accuracy for gas flow is ±0.125 L/min, $ETCO_2$ ±2 mmHg.

Cerebral and Renal Perfusion

Cerebral and renal oxygenation levels were measured using the Invos^{TM} Cerebral/Somatic Oximeter Monitor (Invos 5100, Somanetics Corp., Troy, MI). The near-infrared spectroscopy (NIRS) sensors were placed on the forehead and right kidney of the piglet and secured with tape. The Invos^{TM} Cerebral/Somatic Oximeter Monitor calculates $crSO_2$, which is expressed as the percentage of oxygenated haemoglobin (oxygenated haemoglobin/total haemoglobin). Values of regional oxygen saturation are stored every second with a sample rate of 0.13Hz[60,61].

Animal Monitoring

Animals are initially anaesthetized using isoflurane (5%). Following femoral vessel catheter placement, animals were transitioned to IV propofol (5-10 mg/kg/hr) and morphine (0.1 mg/kg/hr). As excessive sedation can interfere with compensatory mechanisms in response to hypoxia, IV drug administration was halved with the initiation of hypoxia. Sedation was monitored throughout the remainder of experiment where drug administration was enough to keep the piglet from feeling pain (confirmed via toe-pinch), however, we used as little as possible to accomplish this goal for prevention of drug interference with animal recovery following ROSC. The toe-pinch was performed at intervals of 10min before intervention protocols, and every 15min following ROSC until end of experiment.

Hydration and glucose levels were maintained with an IV infusion of 5% dextrose (10 mL/kg/hr). However, we often encountered animals with increasing hemoglobin readings when collecting metabolic data. This was used as an indirect indicator for hydration level of the animal and infusion rate was increased (1-2 mL/kg/hr) where appropriate.

Mechanical respiration was initiated at 20-25 breaths/min with a pressure of 30/5 cmH_2O following ET tube placement. Respiration was closely monitored as over-ventilation or under-ventilation can increase or decrease blood pH, respectively. We had $ETCO_2$ data available from our respiratory monitor (NM3) which we used to fine tune respiratory rates throughout the experiment; excluding the hypoxia and asphyxia phase in which the objective was to stress the animal. Chalak *et al* reported baseline $ETCO_2$ data for piglets weighing 2.2 ±0.6 kg to be 40 ±4 mmHg[62], which was similar to what we observed at baseline readings while monitoring the piglets. When $ETCO_2$ increased we often observed animals attempting rapid spontaneous breathing, which was compensated by increasing the mechanical respiratory rate to expel the excess $ETCO_2$. Conversely, over-ventilation is accompanied by low $ETCO_2$ compensated by decreasing the mechanical respiratory rate.

Experimental Protocol

The experimental protocol consists of surgical instrumentation, recovery period (1h), hypoxia/asphyxia period, and post-resuscitation recovery period (4h). Surgical instrumentation allows us to monitor and record both hemodynamic, and respiratory parameters. Following surgery, the piglet was allowed to rest and recover to a stable baseline. Hypoxia was introduced with the addition of nitrogen diluting O_2 (FiO_2: 0.10), ventilation is gradually reduced to a minimum 2 breaths/min over 30 min of hypoxia. However, close monitoring of both hemodynamic and respiratory parameters was required to maintain balance ensuring animals are well stressed during hypoxia while maintaining adequate cardiac function. Following 30min of hypoxia, the ET tube was clamped to prevent air exchange to and from the lungs; until asystole. Following asystole animals were randomized into three intervention groups: CCaV+90, CCaV+100, CCaV+120.

Resuscitation is initiated with specific protocol according to intervention allocation, with compressions using the two-thumb encircling technique at a depth of 1/3 anterior-posterior diameter of the chest. Once the piglet achieves ROSC, they were observed for a 4h recovery period before euthanization with an IV overdose of phenobarbital (100mg/kg).

Asystole was confirmed by the assessor using auscultation, ECG, and carotid blood flow in conjunction and subsequently the intervention allocation was called out to the entire experimental team. An audible metronome with the correct rate of CCs was played. Fifteen seconds following confirmation of asystole positive pressure ventilation (PPV) was initiated for 30s at a rate of 60 inflations/min using a Neopuff T-Piece (Fisher & Paykel, Auckland, New Zealand). The delay in PPV was intended to simulate clinical delays in initiation of appropriate resuscitation protocols. Following PPV, CCs were initiated at the appropriate compression rate until ROSC was achieved or a maximum of 12min. Inflations were delivered at a rate of 30 inflations/min during CCs, with peak inflation and peak end expiratory pressures of 30/5 cmH_2O, with a gas flow of 8L/min. 100% oxygen was commenced 30s after start of CC, this is a deviation from clinical protocols and is a limitation of this study. A maximum of 4 epinephrine doses (0.02mg/kg per dose) were administered during CCs, beginning 2min after initiation of PPV at an interval of 3min thereafter. The recommended epinephrine dosing for neonatal resuscitation by NRP® is 0.01-0.03mg/kg, we chose a 0.02mg/kg dose based on our own experience in the lab that indicated a dose of 0.01mg/kg was not adequate[24,37]. During CCs ECG and carotid flow were continuously monitored for signs of cardiac activity, and CCs were paused briefly to assess for ROSC; defined as heart rate (HR) $\geq$100 bpm for at least 15s. Compressions were paused every 60s briefly to assess for HR as per NRP® recommendations[1]. In assessing HR we utilized both ECG

and carotid flow, this is important to note in the context of recent concerns regarding pulseless electrical activity[63]. Assessing HR using ECG and carotid flow allows us to confirm restoration of cardiac electrical activity, as well as, pulse.

Interventions were highly coordinated, and each team member was assigned a specific task. The assessor auscultates and confirmed asystole, the CC operator confirmed intervention allocation, sets the appropriate metronome frequency, and performed quality CCs. The intervention lead directed the intervention protocol making sure PPV and CCs were initiated at the appropriate time and epinephrine doses were given at specified times according to the intervention protocol; while another team member was in charge of taking blood samples before and after CPR and also in charge of administering epinephrine.

Data Collection and Analysis

Demographics of study piglets were recorded. Transonic flow probes, HR and pressure transducer outputs were digitized and recorded with LabChart® data recording software continuously (ADInstruments, Houston, TX). Airway pressures, gas flow, V_T, and $ETCO_2$ were measured and analysed using Flow Tool Physiologic Waveform Viewer (Philips Healthcare, Wallingford, CT, USA). Post-mortem, the brain was removed from surviving piglets 4h after ROSC (sham-operated n=6; CCaV+90 n=4; CCaV+100 n=4, and CCaV+120 n=6) and placed in ice-cold 2-methylbutane for 10 min before storing at –80ºC. Frontoparietal cortex was isolated from the whole brain and was homogenized in a 50mM phosphate buffer containing 1mM EDTA (pH 7.0). The supernatants were retained after centrifugation at 3,000x*g* for 10min at 4°C and protein concentration was quantified using the Bradford method. Evidence of brain injury was

determined by quantification of the concentrations of the pro-inflammatory cytokines interleukin (IL)-1β, -6, -8, and tumor necrosis factor (TNF)-α in tissue homogenates by using commercially available ELISA kits (PLB00B, P6000B, P8000, PTA00, R&D Systems, Minneapolis, USA). Cytokine concentrations were expressed relative to protein concentration.

The data are presented as mean (standard deviation (SD)) for normally distributed continuous variables and median (interquartile range - IQR) when the distribution was skewed. The data was tested for normality and compared using one-way ANOVA for comparisons of continuous variables, and χ^2 for categorical variables. *P*-values are 2-sided and $p<0.05$ was considered statistically significant. Statistical analyses were performed with SigmaPlot (Systat Software Inc, San Jose, USA).

Metabolic & Hemodynamic data

Following surgical instrumentation and femoral artery access via catheter, blood samples were taken at the end of surgery, beginning of hypoxia, every 10min until end of hypoxia (0, 10, 20, 30min), immediately before initiation of resuscitation (at asystole), immediately after CPR (ROSC), 10min following ROSC, and every hour following ROSC until end of experiment (60, 120, 180, 240min).

Hemodynamic data was recorded at every time point mentioned above, as recordings were continuously available throughout the experiment. However, metabolic data was collected only throughout hypoxia, before and after CPR, 1h following ROSC, and at the end of experiment before euthanization. Metabolic data was collected at these time points only, because cartages

required for blood sample analysis are one time use only and economically straining. Therefore, we identified reasonable time points in our experiment that would warrant examination of the metabolic state of the animal. Animals were monitored vigilantly throughout the hypoxia period using both metabolic and hemodynamic data available to ensure all animals were under significant stress during the hypoxia period. Hypoxia causes mixed respiratory and metabolic acidosis while providing an additional layer of accuracy in the form of metabolic stress experienced by the piglet. Metabolic data was also obtained during the recovery phase following ROSC, for this we chose to collect metabolic data 1h and 4h following ROSC. We collected 1mL of blood sample preserved in EDTA immediately before hypoxia, immediately before and after CPR, 1 h, and 4 h following ROSC. However, we did not utilize these samples for further biochemical analysis at the end of the study.

Bioassays

We quantified pro-inflammatory cytokines (tumor necrosis factor-α (TNF-α), interleukin(IL)-1β, IL-6, and IL-8) in the frontoparietal cortex of the animals that survived to end of experiment (4h following ROSC) using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Appendix A). ELISA uses the quantitative sandwich enzyme immunoassay technique where the first monoclonal antibody specifically binds a single substrate which is later flanked by an enzyme-linked monoclonal antibody that yields color. The intensity of the color is measured and is proportional to the amount of bound substrate. Cytokine concentrations were expressed relative to protein concentration, so we could compare relative concentration between samples as opposed to absolute concentrations which can vary based on size of the initial sample. (detailed protocols can be found here: https://www.rndsystems.com/products/elisas)

We also quantified lactic acid concentration in the frontoparietal cortex of the animals that survived to end of experiment using a L-Lactic Acid Assay (Appendix B). This assay ultimately converts L-Lactate to NADH, the light absorbance of NADH is measured and lactate concentration is calculated using the following equation:

$$c = \frac{V * MW}{\varepsilon * d * v * 1000} * \Delta A$$

V = final volume (mL)

v = sample volume (mL)

MW = molecular weight of the substance to be assayed (g/mol)

d = light path (cm)

ε = extinction coefficient of NADH ($L*mmol^{-1}*cm^{-1}$)

ΔA = Absorbance of sample – Absorbance of blank

(A detailed protocol can be found here: https://food.r-biopharm.com/products/l-lactic-acid/)

Additional methodological details are available in the published paper presented in chapter 3 of this thesis.

Chapter III

Results

Baseline Parameters

Thirty piglets were randomly assigned to sham-operated (n=6), CCaV+90 (n=8), CCaV+100 (n=8), and CCaV+120 (n=8). Baseline demographics are presented in Table 3.1. Baseline characteristics of sham-operated, CCaV+90, CCaV+100, or CCaV+120 were statistically similar between all study groups with respect to age, weight, hemodynamic, and metabolic parameters. It is important for baseline parameters to be similar between groups, because differences in baseline parameters may influence resuscitation outcomes which can become a severe limitation in the interpretation of study results. It's also important to note that baseline parameters were collected after an hour of stabilization following surgical instrumentation. This may have influenced the resting physiological levels of these parameters, however, of importance is the fact that all animals in our study experienced similar physiological conditions following initial surgical instrumentation.

Resuscitation Parameters

Our primary outcome was time to ROSC, time to ROSC for CCaV+90 (90s (90-243)s), CCaV+100 (90s (60-473)s), and CCaV+120 (120s (75-192)s, p=0.93) was similar between intervention groups (Table 3.2). Hence, our hypothesis of CCaV+120 reducing time to ROSC when compared to CCaV+90 or CCaV+100 was not confirmed in our porcine model of neonatal asphyxia induced cardiac arrest (CA). Studies examining various compression techniques, and frequencies report similar findings[26,27,29,37].

Parameters associated with ROSC, such as, the number of piglets which achieved ROSC (p=0.83), total number of epinephrine doses (p=0.84), number of piglets that received 100% O_2

(p=0.32), and survival to end of experiment (p=0.50) were similar across intervention groups (Table 3.2).

The time to CA following clamping of ET tube was similar between intervention groups (CCaV+90 (229s (172-473)s), CCaV+100 (375s (215-564)s), and CCaV+120 (260s (83-408)s, p=0.36). Severity of asphyxiation which was indicated by metabolic parameters like pH (p=0.91), $paCO_2$ (p=0.97), lactate (p=0.89), and base excess (p=0.46) were also similar across intervention groups (Table 3.2); indicating a similar level of metabolic stress following the hypoxia period. Animals that did not achieve ROSC were not included in the calculation of time to ROSC.

Changes in Hemodynamic Parameters

Changes in hemodynamic parameters throughout the study period are summarized in Table 3.3. As expected, there were no differences observed in baseline hemodynamic parameters between sham-operated and intervention groups.

HR was significantly higher in intervention groups compared to sham-operated at the end of hypoxia and 10min following ROSC (Table 3.3). Which is an expected finding as HR increases in response to hypoxia and rebound hypertension is associated with epinephrine administration following resuscitation[64]. However, HR returned baseline levels at the end of experiment in all intervention groups and was comparable to HR of sham-operated group at the end of experiment (Table 3.3).

MAP was similar across experimental groups at the end of hypoxia and 10min following ROSC (Table 3.3). While the MAP for CCaV+120 and CCaV+90 group returned to baseline, MAP in CCaV+100 group piglets was significantly decreased compared to baseline and sham-operated at the end of experiment (Table 3.3).

At 10min after ROSC, the carotid flow was similar between all groups (Table 3.3). While carotid blood flow returned to baseline at the end of experiment in CCaV+120, both CCaV+90 and CCaV+100 groups observed significant decrease in carotid flow compared to their own baselines and sham-operated piglets (Table 3.3). Indicating that cerebral blood flow continued to decrease following ROSC, which may have contributed to increased levels of pro-inflammatory markers and lactate in the brain of animals resuscitated with CCaV+90 and CCaV+100 (Figure 3.2, 3.3).

Plasma lactate levels were significantly higher across intervention groups following end of hypoxia when compared to their own baselines (Table 3.3). However, they were similar across intervention groups at the end of hypoxia. Unfortunately, arterial blood gas analysis was not performed 10min after ROSC. Most interestingly, at the end of experiment plasma lactate levels of CCaV+90 and CCaV+100 were significantly higher when compared to sham-operated animals and their own baselines (Table 3.3). In contrast, CCaV+120 plasma lactate levels returned to a comparable baseline and was similar to sham-operated animals at the end of experiment (Table 3.3). Additional statistical analysis revealed CCaV+120 plasma lactate levels were significantly lower at the end of experiment when compared to CCaV+90 and CCaV+100 ($p=0.03$).

Both cerebral and renal oxygenations were severely reduced compared to baseline and sham-operated animals following end of hypoxia (Table 3.3). However, they were similar across intervention groups at the end of hypoxia. The cerebral oxygenation was similar in all three intervention groups at 10min after ROSC (Table 3.3). The brain and renal oxygenation levels returned to baseline in the CCaV+120 group. While in the CCaV+100 and CCaV+90 groups the cerebral oxygenation levels were significantly reduced compared to sham-operated piglets and their own baselines, the renal oxygenation was only significantly different from sham-operated piglets (Table 3.3). Significantly higher oxygen concentrations were required to maintain oxygen saturation in the CCaV+90 group compared to sham-operated (43(8)% vs. 31(2)%, respectively, p=0.03) by the end of the 4h recovery period. Overall, we observed worse hemodynamic recovery in CCaV+90 and CCaV+100 compared to CCaV+120.

Respiratory Parameters

There was no difference in respiratory markers-such as tidal volume, peak inflation pressure, positive end expiratory pressure, peak inflation flow, and peak expiratory flow-between CCaV+90, CCaV+100, or CCaV+120 during resuscitation (Table 3.4). This is an expected finding because inflations were delivered using a ventilator with identical respiratory setting throughout the intervention groups at a rate of 30 inflations/min. We did not observe significant differences in the $ETCO_2$ during resuscitation either (Table 3.4), $ETCO_2$ monitoring is said to predict adequacy of CCs and useful in determining ROSC[65].

Brain injury Markers

There were no significant differences in brain injury markers TNF-α and IL-8 in forntoparietal cortex tissue between sham-operated and intervention groups (Figures 3.1A and 3.1D). The concentration of pro-inflammatory marker IL-1β was significantly higher in CCaV+90 and CCaV+100 intervention groups when compared to sham-operated piglets (Figure 3.1B). Whereas, IL-1β concentration in CCaV+120 group was not statistically higher compared to sham-operated piglets or CCaV+90 and CCaV+100. IL-6 concentration was similar between CCaV+90, CCaV+120, and sham-operated groups and significantly higher in CCaV+100 (Figure 3.1C). Interestingly, IL-6 concentration in CCaV+120 was significantly lower when compared to CCaV+100 (Figure 3.1C).

Cortical lactate levels in the frontoparietal cortex were significantly higher in CCaV+90 and CCaV+100 groups compared to sham-operated piglets, while CCaV+120 group had similar levels of lactate compared to sham-operated and significantly lower lactate levels compared to CCaV+100 (Figure 3.2). This corresponded with plasma lactate levels (7.6(2.9), 10.4(4.4) and 4.2(3.1)mmol/L for CCaV+90, CCaV+100, and CCaV+120 respectively, p=0.03) at the end of experiment.

Figure 3.1: Concentrations of (A) tumor necrosis factor-α (TNF-α), (B) interleukin(IL)-1β, (C) IL-6, and (D) IL-8 in frontoparietal cortex homogenates, expressed relative to protein concentration, in sham-operated piglets (n=6), and in piglets resuscitated using 90 chest compressions per minute (CCaV+90, n=4), 100 chest compressions per minute (CCaV+100, n=4), or 120 chest compressions per minute (CCaV+120, n=6). *Significantly different from sham-operated. $p<0.05$; # Significantly different from CCaV+100 group, $p<0.05$.

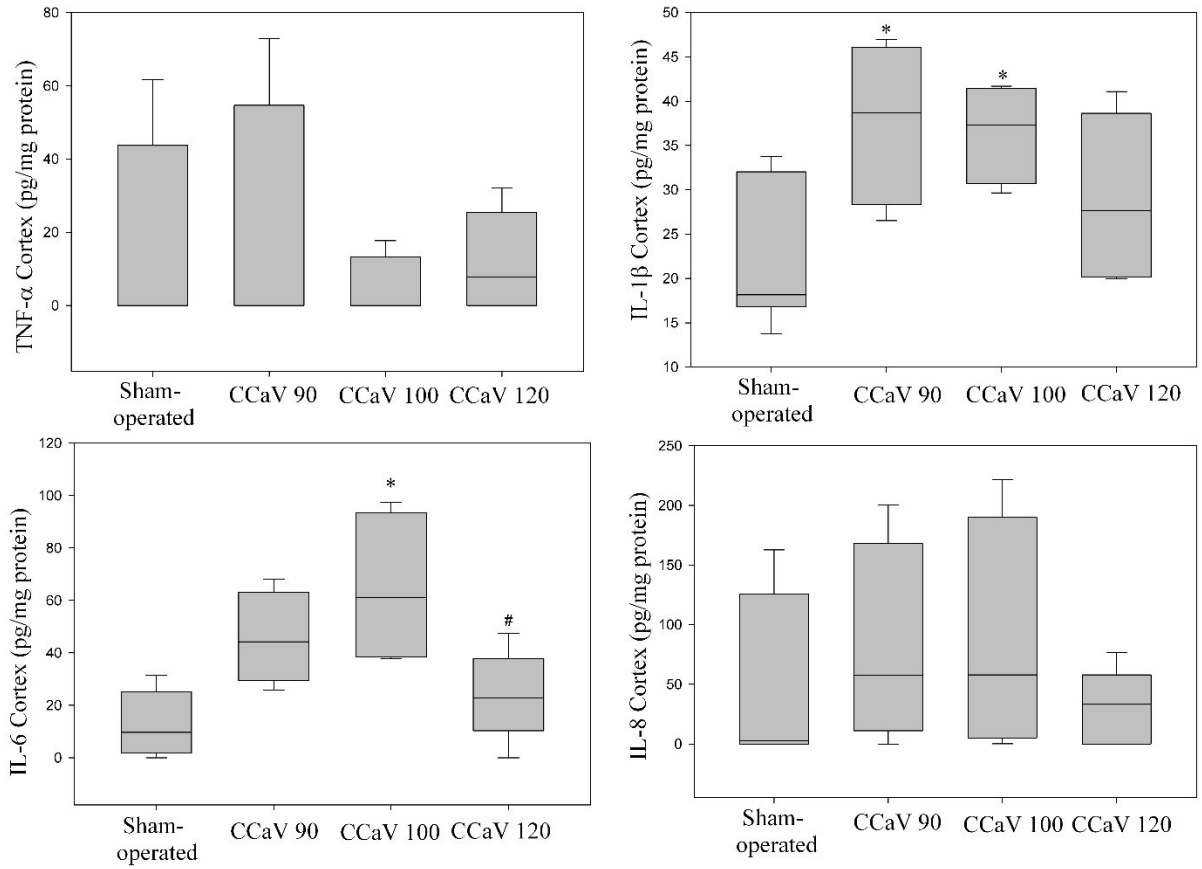


Figure 3.2: Concentrations of lactate in frontoparietal cortex homogenates, expressed relative to protein concentration, in sham-operated piglets (n=6), and in piglets resuscitated using 90 chest compressions per minute (CCaV+90, n=4), 100 chest compressions per minute (CCaV+100, n=4), or 120 chest compressions per minute (CCaV+120, n=6). *Significantly different from sham-operated. P<0.05; # Significantly different from both CCaV+90 and 100 groups, p<0.05.

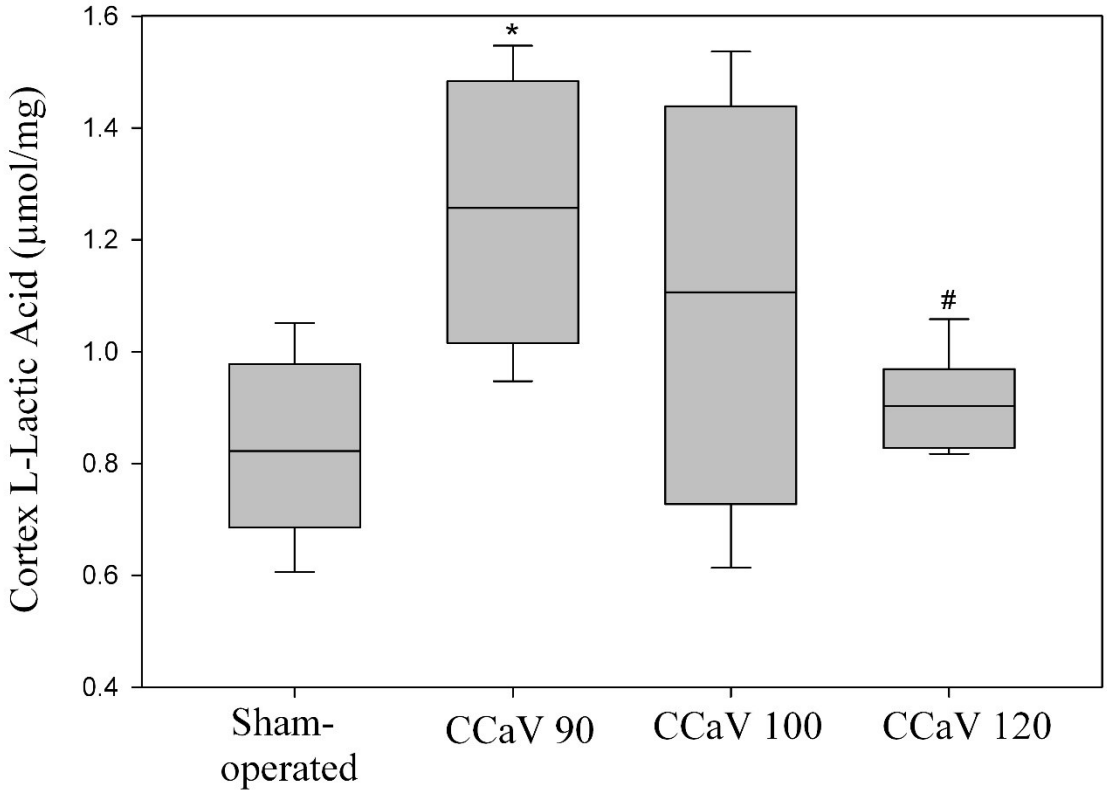


Table 3.1: Baseline characteristics

	Sham-operated (n=6)	CCaV+90 (n=8)	CCaV+100 (n=8)	CCaV+120 (n=8)	p-value
Age (hours) †	48 (13)	54 (11)	48 (18)	48 (21)	0.84
Weight (kg)	1.97 (0.225)	1.99 (0.18)	1.95 (0.23)	2.09 (0.20)	0.56
Heart rate (bpm) †	184 (29)	193 (26)	201 (37)	210 (36)	0.71
Carotid flow (mL/min/kg)	48.2 (11.53)	43.9 (14.0)	39.1 (4.09)	43.8 (9.79)	0.47
Cerebral oxygenation (%)	49.0 (10.32)	48.8 (6.65)	42.5 (6.70)	43.5 (7.13)	0.24
pH†	7.49 (7.42-7.53)	7.47 (7.42-7.51)	7.48 (7.47-7.58)	7.50 (7.42-7.51)	0.90
Base excess (mmol/L)	1.00 (2.37)	0.88 (4.12)	3.13 (4.19)	2.75 (2.52)	0.47
$paCO_2$ (torr)	33.2 (3.91)	33.4 (3.57)	33.1(3.46)	34.9 (2.25)	0.68
SpO_2 (%)†	97.0 (96.8-98.3)	97.0 (97.0-97.8)	96.5 (96.0-98.0)	97.5 (96.0-98.0)	0.88
Lactate (mmol/L)	3.22 (1.11)	4.32 (1.48)	3.80 (0.99)	4.10 (1.30)	0.42
Arterial hemoglobin (g/L)	7.92 (1.29)	7.96 (1.65)	7.78 (1.19)	7.58 (0.84)	0.93

Data are presented as mean (SD), unless indicated †median (IQR)

Table 3.2: Characteristics of asphyxia, resuscitation and survival of asphyxiated piglets (n=8 in each group)

		CCaV+90	CCaV+100	CCaV+120	p-value
Asphyxia time (s) †		229 (172-473)	375 (215-564)	260 (83-408)	0.36
Immediately before resuscitation	pH#	6.71 (0.4)	6.96 (0.07)	6.70 (0.12)	0.91
	$paCO_2$ (torr) #	87.3 (24.4)	86.7 (18.8)	84.8 (21.3)	0.97
	Lactate (mmol/L) #	15.5 (2.4)	15.0 (3)	15.4 (1.7)	0.89
	Base excess (mEq/L)†	-27 (-29~-24)	-25 (-27~-16)	-26 (-28~-24)	0.46
Resuscitation	Received 100% oxygen (n(%))	8 (100)	6 (75)	7 (88)	0.32
	Total number of epinephrine doses†	1.0 (0-4)	2.5 (0-4)	1.0 (0-3.3)	0.84
Achieving ROSC (n (%))		5 (63)	5 (63)	6 (75)	0.83
ROSC time (s) †		90 (60-243)	90 (60-473)	120 (75-192)	0.93
Survival 4h after ROSC (n (%))		4 (50)	4 (50)	6 (75)	0.50

Data are presented as n (%), unless indicated †median (IQR), and #mean (SD); ROSC – return of spontaneous circulation, "Immediately before resuscitation" refers to the time of cardiac arrest immediately before PPV was initiated.

Table 3.3: Hemodynamic changes before and after resuscitation

	Sham-operated	CCaV+90	CCaV+100	CCaV+120
Heart rate (bmp)				
Baseline	184(29)	193(26)	201(37)	210(36)
End of hypoxia	177(26)	221(38)*	228(26)*	249(30)*
10min after ROSC	177(41)	233(26)*	256(35)*	261(35)*
End of experiment	228(54)	248(41)	224(44)	237(37)
Mean Arterial Pressure (mmHg)				
Baseline	58(12)	60(6)	63(7)	66(8)
End of hypoxia	60(10)	47(13)	53(18)	54(17)
10min after ROSC	58(9)	74(14)	62(14)	73(11)
End of experiment	52(11)	40(16)	27(9)*†	54(16)
Carotid flow (mL/min/kg)				
Baseline	48(12)	43(14)	39(4)	44(10)
End of hypoxia	42(12)	46(36)	51(11)	40(32)
10min after ROSC	37(11)	34(10)	28(5)	42(8)
End of experiment	30(15)	8(13)*†	9(12)*†	18(5)
Plasma Lactate (mmol/L)				
Baseline	3.2(1.1)	4.3(1.5)	3.8(1.0)	4.1(1.3)
End of hypoxia	3.8(1.0)	15.5(2.4)*†	15.0(3.0)*†	15.4(1.7)*†
10min after ROSC	Not collected	Not collected	Not collected	Not collected
End of experiment	3.7(1.6)	7.6(2.9)*†	10.4(4.4)*†	4.2(3.1)
Cerebral oxygenation (%)				
Baseline	49(10)	49(7)	43(7)	44(7)
End of hypoxia	49(10)	28(16)*†	19(6)*†	22(14)*†
10min after ROSC	48(9)	51(6)	51(2)	59(3)
End of experiment	48(9)	25(19)*†	22(10)*†	47(13)

Renal oxygenation (%)				
Baseline	57(6)	61(6)	53(16)	61(11)
End of hypoxia	59(4)	16(2)*†	16(2)*†	18(5)*†
10min after intervention	59(2)	36(16)	30(15)*	39(16)
End of experiment	48(6)	37(16)†	29(21)†	54(13)

Data are presented as mean(SD) *Significantly different from sham-operated group, $p<0.05$; †Significantly different from baseline, $p<0.05$.

Table 3.4: Respiratory parameters during resuscitation

	CCaV+90 (n=8)	CCaV+100 (n=8)	CCaV+120 (n=8)	p-value
Tidal volume (mL/kg)	12.7 (4.2)	14.3 (4.4)	12 (4)	1.00
End-tidal CO_2 (mmHg)	14.1 (5)	14.6 (6.3)	17 (10)	1.00
Peak Inflation Pressure (cmH_2O)	29.5 (0.7)	29.8 (0.4)	29 (3.5)	1.00
Positive End Expiratory Pressure (cmH_2O)	4.9 (0.5)	5.3 (0.4)	5.3 (0.9)	1.00
Peak Inflation Flow (L/min)	8.2 (1)	8.9 (1.3)	8.7 (1.8)	1.00
Peak Expiratory Flow (L/min)	-6 (-2)	-6.4 (-1.4)	-5.2 (-1.9)	1.00

Data are presented as mean (SD)

Chapter IV

Discussion

A key difference between neonatal and pediatric resuscitation guidelines besides the use of synchronous vs. asynchronous resuscitation techniques is the rate of compressions. The neonatal resuscitation guidelines recommend the use of 3:1 C:V with 90 CC/min and pediatric resuscitation guidelines recommend the use of CCaV with 100-120 CC/min[1,2,10]. In addition, most newborns admitted to a pediatric intensive care unit (PICU) or an emergency room any time after the delivery room will be resuscitated using the pediatric resuscitation algorithm[10]. This is in contrast to newborns admitted to the neonatal intensive care unit (NICU), who will receive resuscitation according to neonatal resuscitation guidelines until discharge[1,2,9]. While mathematical modeling suggests a rate of >120 CC/min may be optimal for neonates[34], no study has examined the optimal CC rate during CCaV in newborn infants. We aimed to examine whether CCaV using 120 CC/min would achieve ROSC faster than CCaV using 90 CC/min or 100 CC/min in a porcine model of neonatal asphyxia induced cardiac arrest. We hypothesized that CCaV using 120 CC/min would have a faster time to ROSC compared to CCaV using 90 CC/min or 100 CC/min during neonatal CCs. The widespread use of CCaV in the clinical setting warrants investigation into its optimization as a resuscitation technique.

ROSC and Survival

Our primary outcome was time to ROSC, which was similar across intervention groups (p=0.93). Therefore, our hypothesis that CCaV+120 would reduce the time to ROSC was not supported in our findings using the porcine neonatal asphyxia induced cardiac arrest (CA) model. Studies examining various compression techniques, and frequencies reported similar findings[26,27,29,37].

Studies comparing 9:3 C:V with 3:1 C:V (148s (116-195)s and 150s (115-180)s respectively, p=ns), 15:2 C:V with 3:1 C:V (195s (145-358)s and 150s (140-180)s respectively, p=ns), and 2:1, 3:1, 4:1 C:V (127s (82-210)s, 96s (88-126)s, and 119s (83-256)s respectively, p=ns) reported similar time to ROSC, and all other characteristics associated with ROSC[26,27,29]. CC+SI has been an exception to this when compared to 3:1 C:V, CC+SI significantly reduces time to achieve ROSC [24,28,30,66]. CC+SI is an interesting alternative CC technique which superimposes a sustained inflation alongside continuous CCs, allowing passive ventilation where every compression expels air out of the lungs and inflates the lungs with passive chest recoil[28]. The first study reporting reduced time to ROSC used 90 CC/min for 3:1 C:V and 120 CC/min for CC+SI (143s (84-303)s and 38s (23-44)s respectively, p=0.0008). Later, Li *et al* studied 90 CC/min vs 120 CC/min with CC+SI[37]. They reported similar time to ROSC between CC+SI 90 and CC+SI 120 (34s (28-156)s, and 99s (31-255)s respectively, p=0.29). Overall, studies comparing various rates, and ratios have observed similar time to achieve ROSC and other ROSC related parameters[26,27,29,37,67]. Also, considering that all of these studies are conducted in animal models, further clinical research is needed to elucidate the true differences between these approaches to resuscitation.

ROSC was used as the primary outcome for this study based on several factors. According to the American Heart Association ROSC is frequently used for immediate assessment of CA therapies in adults, the definition of ROSC includes mechanical activity/pulse of the heart and it is a cost effective method for conducting CA research[68]. They recommend for early studies that are likely to have fewer patient enrollment to use immediate endpoints for primary outcomes, such as, ROSC, hospital mortality, and hemodynamic parameters[68]. However, they also recognize the

fact that simply restoration of cardiac activity as a primary outcome often fails to improve long-term outcomes, such as, multiple organ injury and neurological recovery[68]. However, studying these endpoints often involves long term follow-up of patients which can present a cost effectiveness barrier for early interventional studies. Ideally, CA therapies should aim to restore pre-arrest function. However, this requires large randomized controlled trials with long-term endpoints. The American Heart Association recommended 90 days follow-up for assessing neurological recovery, quality of life, and cardiac function[68].

We observed a 50% survival rate in both CCaV+90 and CCaV+100 and a 75% survival rate in CCaV+120, however, this did not reach statistical significance ($p=0.50$). A 25% difference in overall survival rate between interventions is an exciting finding, and warrants further detailed investigation. The major hurdle in assessing differences in survival between interventions for CA research is the sample size required. For example, if we designed an experiment based on the above findings to compare only two intervention groups for a clinically important difference of 25% in survival rate with a power of 80% and $\alpha=0.05$; we would need a sample size of 116 animals (58 in each intervention group). This is with a study design that would involve only two independent study groups and a dichotomous outcome (survived: yes/no). A large sample size is one of the primary hurdles in using survival as a short term outcome for animal studies, however, it is an important measure for larger trials which may have the sample size to detect these clinically important differences[68].

Baseline Parameters

Thirty piglets were randomly assigned to sham-operated (n=6), CCaV+90 (n=8), CCaV+100 (n=8), and CCaV+120 (n=8). Piglets receiving intervention did not differ from sham-operated piglets at baseline values, as presented in Table 3.1. The baseline parameters were recorded following surgical instrumentation and stabilization phase, which may explain the elevated lactate levels in our baseline readings. We speculate that this is a result of the stress surgical instrumentation has on the animal, based on the fact that we saw elevated baseline lactate levels in intervention and sham-operated animals. There were also no significant differences measured in characteristics of asphyxia at CA prior to resuscitation in the intervention groups (Table 3.2).

The time to CA following clamping of ET tube (p=0.36), pH (p=0.91), $paCO_2$ (p=0.97), lactate (p=0.89), and base excess (p=0.46) were also similar across intervention groups immediately following confirmation of CA; indicating a similar level of metabolic stress between intervention groups following the hypoxia and asphyxia period. If animals are not metabolically stressed to the same degree during hypoxia and the subsequent asphyxia period, resuscitation outcomes can be affected. Severely stressed animals would experience worse resuscitation outcomes compared to relatively healthier animals. Which can be a major confounding factor, therefore, we measured the metabolic conditions throughout the hypoxia period and adjusted our monitoring accordingly. We were able to ensure there were no statistically significant differences in metabolic stress between intervention groups because of our continued animal monitoring informed with regular collection metabolic data in conjunction with our two-step randomization.

This helped eliminate selection bias and ensured uniform monitoring of animals throughout hypoxia period.

Hemodynamic Outcomes

Changes in hemodynamic parameters throughout the study period are summarized in Table 3.3. As expected, there were no differences observed in baseline hemodynamic parameters between sham-operated and intervention groups. We did observe significantly worse hemodynamic recovery in CCaV+90 and CCaV+100 compared to CCaV+120 at the end of experiment. CCaV+120 was comparable to sham-operated piglets at the end of experiment for every hemodynamic parameter reported (Table 3.3). In contrasting CCaV+90 and CCaV+100 were significantly worse with respect to sham-operated animals for carotid flow, lactate, and cerebral oxygenation. Li *et al* reported a trend toward worse carotid flow and cerebral oxygenation with 90 CC/min compared to 120 CC/min using CC+SI[37]. When comparing resuscitation using 3:1 C:V with 90 CC/min and using CC+SI with 120 CC/min Schmölzer *et al* reported worse cardiac output using 3:1 C:V with 90 CC/min compared to CC+SI with 120 CC/min, while observing no differences in other hemodynamic parameters reported[28]. Solevåg *et al* reported no differences in hemodynamic parameters when comparing 3:1 with 15:2 C:V, however, they only reached mean (SD) CC rate of 58 (7) and 75 (5) respectively. It is difficult to ascertain the effects of varying rates of CC on neonatal resuscitation and hemodynamic recovery. The evidence present in the neonatal literature regarding CC rate often accompanies different approaches to resuscitation techniques, which makes interpretation of these results difficult. We cannot tease apart CC rate outcomes from the underlying resuscitation techniques used, therefore, experiments specifically designed to observe hemodynamic differences with varying rates need to be developed. ROSC

focused resuscitations have the potential to neglect long-term cardio vascular function, which directly influences hemodynamics. We have not directly assessed long-term cardiovascular function in this study. It would be interesting to determine whether resuscitations using higher compression rates have a significant impact on cardiovascular function and recovery in the long term; assessed by cardiac output, ejection fraction, filling pressure, mixed or central venous oxygenation, and lactate clearance[68].

Coronary Perfusion Pressure

Coronary perfusion pressure (CPP) is an important determinant in achieving ROSC. Quality compressions should aim to maximize CPP in order to re-oxygenate myocardium, as myocardial ischemia can prevent ROSC[54]. CPP is the pressure gradient between the aorta and right atrium at aortic diastole and coronary blood flow occurs primarily during the decompression (chest recoil) phase of CCs which is effectively the diastole phase of CCs[42,54,69]. Brief retrograde coronary blood flow has also been reported in animal studies during compression phase[54,69]. CPP is predictive of ROSC in adults as low initial CPP and maximal CPP were associated with no ROSC, 1.6 (8.5)mmHg and 8.4 (10)mmHg, respectively[70]. ROSC was positively associated with patients that had a higher initial and maximal CPP, 13.4 (8.5)mmHg and 25.6 (7.7)mmHg, respectively[70]. Paradis *et al* also noted that only patients with maximal CPP above 15mmHg achieved ROSC, however, this did not guarantee ROSC. Leaning during the decompression phase of CCs is a detrimental factor to CPP, leaning impedes venous return and CPP[43,44]. Coronary perfusion is reportedly limited at rates greater than 120 CC/min as an increase in duty cycle compromises diastolic perfusion time[38,69].

Prolonged resuscitation can also cause chest deformation, compromise proper chest relaxation and thoracic filling of blood, having an overall negative impact on perfusion[41]. Therefore, active decompression of chest wall during CCs may be worth investigating in neonatal resuscitation. Active decompression is being explored as an experimental practice within adult resuscitation, where an adhesive attachment on the chest helps active relaxation following compression. Interestingly, fraction of CCs with complete release were significantly better with 120 CC/min compared to 100 CC/min, therefore, an increase in recommended CC/min may help reduce leaning related decrease in CPP[71].

The association between CC interruptions and CPP, to my knowledge, has not been studied in the neonatal population. However, Interruptions in CCs during resuscitation have been studied using large swine models, which indicate that interruptions can have a negative impact on hemodynamics and CPP[45,72]. Berg *et al* observed a decrease in CPP when CCs were paused for two rescue breaths (4s) in a large swine model of VF induced CA[45]. They reported significantly lower CPP in compressions following a pause for rescue breathing compared to CPP before pause for rescue breathing (14 (1) mmHg versus 21 (2)mm Hg respectively, $p<0.001$). Kern *et al* in a similar follow-up study reported significantly increased normal neurological survival 24 hours following resuscitation with continuous CCs, as opposed to CCs with interruptions in a large swine model ($p=0.0001$)[72]. Sanders *et al* also reported significantly better neurologic function 24hrs following resuscitation with fewer interruptions in a large swine model ($p=0.007$)[73]. Recently, a Cochrane review of out of hospital CA reported an increased proportion of survival with compression only CPR from 11.6% to 14% in both adults and children[47]. Although, this review only included non-asphyxia related causes of CA. However, when taken in context with earlier

studies in large swine, CPP may have been a contributing factor in the increased proportion of survival with compression only CPR. Lastly, Morgan et al reported similar diastolic and systolic blood pressures following interruptions (>1s) in pediatric in-hospital cardiac arrest when comparing the average of 20 compressions pre and post interruptions[74]. This study did not compare CCs without interruptions as it was an observational study utilizing data from patients with invasive BP monitoring in place at the time of cardiac arrest, data for this study was collected post resuscitation. They speculated that the absence of a hemodynamic difference pre and post interruption was multifactorial and included short duration of interruptions (2.4s (1.4-7.0s)). Epinephrine administration, changes in rescuers, and most importantly their experimental design's inability of blinding rescuers to arterial blood pressure monitoring which may have helped rescuers titrate CCs to hemodynamics are major limitations of this study[74]. However, these findings bring a clinical relevance to interruptions during CCs which have been absent in previous, highly controlled animal experiments. The authors argue that the clinically significant relationship between CC interruptions may be counterintuitive as pauses in CCs may be opportunities for providing arrest specific interventions which may help improve outcomes[74].

Cerebral Injury Markers

TNF-α, IL-1β, IL-6, and IL-8 are pro-inflammatory cytokines, which are signaling molecules involved in the inflammatory response. Increased levels of cytokines in the brain ultimately result in neuronal injury via leukocyte migration, blood brain barrier disruption, and glial cell activation[75–78]. Elevated levels of these pro-inflammatory cytokines is associated with neuroinflammation and eventual neuronal injury[75,77,79]. These inflammatory markers play a crucial role in cerebral injury following ischemia, which is typically associated poor neurological

outcomes. Following ischemic injury pro-inflammatory cytokine activation of microglia and astrocytes is detrimental, and increased levels of IL-1β have been shown to positively correlate with hypoxic ischemic encephalopathy (HIE) severity[80]. These cytokines can activate astrocytes to produce more pro-inflammatory cytokines, worsening HIE severity with increased activation[80]. Studies have established increased serum concentrations of both IL-1β and IL-6 are associated with adverse outcomes and severity[81,82]. Here, we observed a trend toward lower concentration of pro-inflammatory markers IL-1β and IL-6 in the CCaV+120 group. This trend corresponds with our hemodynamic recovery results, where we observed worse hemodynamic recovery in CCaV+90, and CCaV+100 groups compared to CCaV+120 and sham-operated piglets at the end of experiment.

Comparatively, previous studies experimenting with various C:V ratios (9:3, 15:2, 2:1, 3:1, and 4:1 C:V) have not shown differences in pro-inflammatory cytokine levels between intervention groups[17,18,19]. However, TNF-α mRNA is produced within 1h of ischemic insult yet reaches a peak about 6-12h after the ischemic insult[75]. Our experiment and the others discussed earlier allow recovery for approximately 4-5h after ischemic insult in their animal models, which means cytokine concentrations may not have reached their peak. This introduces a temporal hurdle toward ascertaining an accurate picture of pro-inflammatory cytokine concentrations and cerebral injury in our study. Therefore, the relationship between CC rate, hemodynamic recovery, and cerebral inflammation remains unclear and requires longer post-resuscitation recovery periods to ascertain clearly.

Lactate concentration exhibit a similar pattern to IL-6. CCaV+120 had significantly lower levels of cortical lactate compared to CCaV+100, this was also observed in plasma lactate levels at the end of experiment ($p=0.03$). Lactate is found to have a neuroprotective effect following transient focal cerebral ischemia in rat models[83,84]. While recent evidence in neonatal HIE suggests cortical lactate concentration is increased in areas of severe injury, the authors also found a correlation between serum and cerebral lactate concentrations measured with moderate to severe brain injury[85]. These results may provide insight into the pattern of lactate concentrations we observed in our interventions groups. CCaV+90 and CCaV+100 both exhibit elevated levels of lactate which combined with pro-inflammatory markers suggests increased brain injury in these interventions groups compared to CCaV+120. Overall, our analysis of cerebral injury markers indicates increased cerebral injury with CCaV+90 and CCaV+100 interventions, as opposed to, resuscitations using CCaV+120.

Chest Compression Rate in Adults

Several studies examining the relationship between CC rate and ROSC have been conducted in the adult population[86–88]. These studies have indicated better ROSC and resuscitation related outcomes with compression rates of 120 CC/min compared to lower compression rates. Idris *et al* analyzed CC rates in 3,098 cases of out of hospital resuscitations and reported peak ROSC rates at compression rate of 125 CC/min[86]. Kern *et al* compared the effectiveness of 80 CC/min vs. 120 CC/min on $ETCO_2$. $ETCO_2$ is used as a surrogate marker for blood flow, hence, quality of CCs. They reported significantly higher $ETCO_2$ with 120 CC/min compared to 80 CC/min and noted higher $ETCO_2$ when compressions were guided with an audible metronome[87]. Lastly, Kilgannon *et al* studied CC rates and clinical outcomes for in-hospital CA and reported

highest rate of ROSC (64%) for patients receiving 121-140 CC/min. However, they did not observe a significant difference in good neurological function at hospital discharge for patients receiving 100-120 CC/min compared to121-140 CC/min (5%, and 11% respectively)[88]. The fact that their study groups encompass a large range of compression rates may have weakened the association between CC rate and neurological outcomes they reported. This may be the case because CC quality differs significantly at compression rates of 100 CC/min, 120 CC/min, and 140 CC/min[71]. The fraction of CCs with complete release, sufficient depth, and correct hand positioning were significantly better with 120 CC/min compared to 100 CC/min in a simulated 1-person CCs only CPR study on adult sized mannequins[71]. These factors are key in performing quality compressions as increased CCs with complete release reduces rescuer leaning and the sufficient depth achieved improves ejection fractions. Overall, the evidence in the adult population points to better CC quality and ROSC outcomes at compression rates close to 120 CC/min[86–88]. However, it remains to be seen if these recommendations can translate to neonatal CCs. Key physiological and anatomical differences, as well as, CC force, hand positioning, and compression depth in adults may be factors that influence the efficacy of higher CC rates and their translatability to neonatal resuscitation. Since evidence examining the optimal CC rate in neonates is lacking, clinical studies are needed to address this knowledge gap.

Resuscitation Practices in ICUs

Most pediatric CA is a result of progressive respiratory failure that causes hypoxia, acidosis, and bradycardia with eventual CA[89–92]. Although studies have reported etiology of CA from respiratory causes in the PICU between 13%-17%[90,92], inconsistencies in accurate reporting of age and clear classification of CA by underlying causes makes it difficult to determine

accurately. These studies also report on the full spectrum of the pediatric population ranging from 1day-18years, both of which may contribute to underestimation of the reported statistics for neonates (age: 1day-1month). These neonates admitted to PICU are resuscitated using the pediatric resuscitation algorithm[10], as opposed to neonates admitted to the NICU which are resuscitated using the neonatal resuscitation algorithm[1,2]. This exposes a knowledge gap with regards to which resuscitation approach is best in this neonatal population regardless of ICU type.

The neonatal guidelines do not recommend using CCaV in any scenario, while the pediatric guidelines recommend using CCaV when an advanced airway is in place[1,10]. However, a national survey based in USA found PICU directors favored using pediatric guidelines to resuscitate neonates[93]. In contrast, NICU directors endorsed use of neonatal guidelines throughout initial hospitalization of neonates. Resuscitations were found to follow neonatal or pediatric guidelines not based on infant age or etiology of arrest, but rather the type of intensive care unit[93]. This is concerning because there is no clear consensus on which guidelines to follow based on patient characteristics which provide a specific framework to apply the most appropriate resuscitation techniques. While infants as old as one year are resuscitated using neonatal guidelines, infants are also resuscitated using pediatric guidelines any time after the immediate newborn period[93]. Along with varying opinions on which resuscitation guidelines to use, there is often failure to comply with resuscitation guidelines and CCaV is used instead[33]. It is often simpler to use CCaV which is uncoordinated over coordinated techniques like 3:1 C:V. If an advanced airway is present, CCaV can be performed with a single rescuer by making use of mechanical ventilation which also delivers precise ventilation during CCs. The ease of learning involved with CCaV can also be a

factor in its use with neonates outside the NICU, where rescuers would otherwise need to obtain and maintain multiple certifications.

Strengths and Limitations

We utilized a neonatal porcine model of asphyxia induced CA, which was chosen based on several factors. The neonatal piglets resemble neonatal humans in size, shape and birth weight; while providing the closest analog to the cylindrical chest shape of a human neonate[49]. The induction of CA via gradual hypoxia followed by asphyxia is also a major strength of this model. The primary cause of cardiac collapse in human neonates is prolonged hypoxia, and this animal model allows us to manifest the physiological symptoms and stressors a human neonate experiences in a hypoxic physiological state.

Our allocation of piglets into study groups was block randomized using an online randomization program (http://www.randomizer.org), with a two-step randomization process to reduce selection bias. We used a block randomized model to control as best we can the variability introduced with piglets from separate litters. We also used sequentially numbered, sealed, opaque envelopes allocating piglets to "intervention" or "SHAM" groups (step-one). The intervention group was allocated to specific interventions once asystole was confirmed, by a second sealed and opaque envelope allocating piglets to "CCaV+90", CCaV+100", or "CCaV+120" (step-two). This two-step randomization process is a strength of this study, eliminating selection bias in assessment of asystole during asphyxia. However, random allocation can be difficult to achieve in the clinical setting because allocation during resuscitations may delay intervention and can be a potential source of confusion/miscommunication between the resuscitators. Resuscitation research typically

has poor reporting of randomization method or allocation concealment, and timing of intervention allocation also interferes with randomization in clinical resuscitation research[48,94,95].

Limitations to using this neonatal piglet model of asphyxia induced CA should be considered when interpreting results from these studies. Piglets in these studies have already undergone fetal to neonatal transition limiting their translatability to delivery room resuscitation. Piglets are intubated and a tight seal prevents leak, however, mask leak is often present in clinical settings. Close adherence to pediatric or neonatal resuscitation guideline was not observed in terms of oxygen delivery, and epinephrine administration. However, there was no significant difference in number of epinephrine doses received. The initiation and delivery of 100% oxygen was delayed in our protocol to begin following 30s of chest compressions compared to NRP® guidelines where CCs and 100% oxygen are initiated simultaneously, which also may have influenced our results. Rescuer fatigue was discussed as a detrimental factor to quality of compressions, however, we opted to utilize a single experienced rescuer to provide CCs for the entirety of the resuscitation which extended to a maximum of 12min. We chose to utilize a single rescuer to provide CCs to avoid variability introduced in the quality of compressions when switching rescuers frequently. This neonatal piglet model is currently the closest anatomical and physiological analog for studying neonatal CCs. Improvements in the model can be made to introduce mask leak, observe closer adherence to neonatal or pediatric resuscitation algorithms, and ultimately conduct resuscitations on transitioning animals.

Chapter V

Future Directions and Conclusions

Neonatal CC research is still in its infancy with the first clinical trial currently underway. Animal research is presently the primary source on neonatal research related to CCs. In our porcine model of neonatal asphyxia induced cardiac arrest, we did not observe a reduction in time to ROSC using CCaV with 120 CC/min compared to CCaV with 90 CC/min or 100 CC/min. However, we observed worse hemodynamic recovery in both CCaV with 90 CC/min and 100 CC/min compared to sham-operated piglets and CCaV with 120 CC/min. The worse hemodynamic recovery was accompanied by increased brain injury markers in CCaV with 90 CC/min and 100 CC/min groups compared to sham-operated piglets and CCaV with 120 CC/min. I speculate that the comparatively better hemodynamic outcomes with CCaV+120 may be attributed to better CPP which may also optimize duty cycle and relaxation time, and that quality of CCs may be better with a compression rate of 120 CC/min.

Upcoming clinical trials will clearly move neonatal resuscitation research forward to elucidate how translatable the animal research will be in the clinical environment. In the meantime, animal models can still offer a better understanding of physiological phenomenon associated with resuscitation. The development of models using mechanical compression devices to standardize compression force, depth, duty and relaxation cycles have the potential to clarify the impact of each individual factor in a highly controlled and repeatable environment. Animal models with additional data of cardiac hemodynamics using Millar catheters during resuscitation can further our understanding of how factors such as CC rate, duty cycle, and effective chest relaxation impact cardiac hemodynamics during resuscitation, as well as, cardiovascular function in the hours following ROSC. The compression relaxation cycle during CCs may also play a crucial role in providing quality CCs. To this extent, studies further examining optimal duty cycle and relaxation

time with varying compression rates may improve CC quality. Active relaxation by pulling the chest in-between CCs, which is experimentally used in adult CPR, may be worth exploring to examine if CPP can be improved. However, reduced time to ROSC and survival don't necessarily go hand in hand. Instead, studies focusing resuscitation efforts on improving long term survival alongside ROSC may also help reduce the severe morbidity associated with neonatal resuscitation. Longer post-resuscitation recovery periods may help study long term hemodynamic and neurological outcomes. Ultimately, optimizing cardiac and cerebral hemodynamics during CCs may help improve ROSC and overall neonatal resuscitation outcomes in the future.

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Appendix A: Enzyme-Linked Immunosorbent Assay (ELISA) General Protocol

The ELISA protocols were used to quantify pro-inflammatory cytokine levels in the frontoparietal cortex of animals that survived until the end of experiment. ELISA kits are commercially available for specific substrates hence the specific contents of each kit vary. However, the overarching principles of the ELISA protocol are similar between substrates and therefore the following is a general ELISA protocol. Specific ELISA protocol based on substrates can be found here: https://www.rndsystems.com/products/elisas

Assay:

1. Bring all reagents to room temperature (porcine [substrate] control, wash buffer, substrate solution, and porcine substrate standard)
2. Reconstitute the porcine (substrate) control with 1.0mL of distilled water
3. Add 20mL of wash buffer concentrate with distilled water to prepare 500mL of wash buffer
4. Reconstitute the porcine (substrate) standard with calibrator diluent and dilute this standard specifically to substrate protocol
5. Add 50µL of assay diluent to each well
6. Add 50µL of standard, control or sample per well (duplicate)
7. Cover with adhesive strips and incubate for 2h at room temp. on a microplate shaker
8. Aspirate each well and wash by filling each well with wash buffer for a total of five washes
9. Invert plate and blot against clean paper towel to ensure complete removal of liquid
10. Add 100µL of porcine (substrate) conjugate to each well, cover with adhesive strips and incubate for 2h on a microplate shaker
11. Repeat step 8-9

12. Add 100µL of substrate solution to each well and incubate for 30min at room temp. (protect from light)
13. Add 100µL of stop solution to each well and mix thoroughly
14. Determine optical density of each well with a microplate reader set to 450nm

Appendix B: Lactic Acid Assay

1. Dilute 15µL of frontoparietal cortex tissue with 45µL of ddH_2O for a 4x dilution
2. Make the glycylglycine cocktail by combining glycylglycine buffer, ddH_2O, NAD^+, and GPT (store on ice until ready to use)
3. Pipette 50µL of ddH_2O in the first row to be used as a blank
4. Pipette 50µL of the samples in duplicate into a microplate
5. Pipette 172µL of the glycylglycine cocktail to each well
6. Read the plate at 340nm every 5min until 3 consecutive readings do not change by more than 0.05 of an absorbance unit (this is the baseline)
7. Pipette 2µL of LDH to each well
8. Repeat step 6
9. Subtract baseline absorbance from absorbance acquired from step 8
10. Subtract blank absorbance from sample absorbance (ΔA)
11. Calculate lactic acid concentration: $c = (V * MW)/(\varepsilon * d * v * 1000) * \Delta A$

Appendix C: ARRIVE Guidelines Checklist

The ARRIVE Guidelines Checklist

Animal Research: Reporting In Vivo Experiments

Carol Kilkenny[1], William J Browne[2], Innes C Cuthill[3], Michael Emerson[4] and Douglas G Altman[5]

[1]The National Centre for the Replacement, Refinement and Reduction of Animals in Research, London, UK, [2]School of Veterinary Science, University of Bristol, Bristol, UK, [3]School of Biological Sciences, University of Bristol, Bristol, UK, [4]National Heart and Lung Institute, Imperial College London, UK, [5]Centre for Statistics in Medicine, University of Oxford, Oxford, UK.

	ITEM	RECOMMENDATION	Page
Title	1	Provide as accurate and concise a description of the content of the article as possible.	First page, not numbered
Abstract	2	Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study.	ii
INTRODUCTION			
Background	3	a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale. b. Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study's relevance to human biology.	2-15
Objectives	4	Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.	15
METHODS			
Ethical statement	5	Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal [Scientific Procedures] Act 1986), and national or institutional guidelines for the care and use of animals, that cover the research.	iv
Study design	6	For each experiment, give brief details of the study design including: a. The number of experimental and control groups. b. Any steps taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. if done, describe who was blinded and when). c. The experimental unit (e.g. a single animal, group or cage of animals). A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out.	18-26

Experimental procedures	7	For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example: a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s). b. When (e.g. time of day). c. Where (e.g. home cage, laboratory, water maze). d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used).	26-28
Experimental animals	8	a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range). b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc.	18
Housing and husbandry	9	Provide details of: a. Housing (type of facility e.g. specific pathogen free [SPF]; type of cage or housing; bedding material; number of cage companions; tank shape and material etc. for fish). b. Husbandry conditions (e.g. breeding programme, light/dark cycle, temperature, quality of water etc for fish, type of food, access to food and water, environmental enrichment). c. Welfare-related assessments and interventions that were carried out prior to, during, or after the experiment.	N/A
Sample size	10	a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group. b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used. c. Indicate the number of independent replications of each experiment, if relevant.	19
Allocating animals to experimental groups	11	a. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done. b. Describe the order in which the animals in the different experimental groups were treated and assessed.	19,20
Experimental outcomes	12	Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes).	19
Statistical methods	13	a. Provide details of the statistical methods used for each analysis. b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron). c. Describe any methods used to assess whether the data met the assumptions of the statistical approach.	28,29
RESULTS			
Baseline data	14	For each experimental group, report relevant characteristics and health status of animals (e.g. weight, microbiological status, and drug or test naïve) prior to treatment or testing. (This information can often be tabulated).	33, 40
Numbers analysed	15	a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g. 10/20, not 50%[2]). b. If any animals or data were not included in the analysis, explain why.	33

Outcomes and estimation	16	Report the results for each analysis carried out, with a measure of precision (e.g. standard error or confidence interval).	33-37
Adverse events	17	a. Give details of all important adverse events in each experimental group. b. Describe any modifications to the experimental protocols made to reduce adverse events.	N/A
DISCUSSION			
Interpretation/ scientific implications	18	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including any potential sources of bias, any limitations of the animal model, and the imprecision associated with the results[2]. c. Describe any implications of your experimental methods or findings for the replacement, refinement or reduction (the 3Rs) of the use of animals in research.	46-48, 59, 61,62
Generalisability/ translation	19	Comment on whether, and how, the findings of this study are likely to translate to other species or systems, including any relevance to human biology.	18
Funding	20	List all funding sources (including grant number) and the role of the funder(s) in the study.	iv

References:
1. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. *PLoS Biol* 8(6): e1000412. doi:10.1371/journal.pbio.1000412
2. Schulz KF, Altman DG, Moher D, the CONSORT Group (2010) CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *BMJ* 340:c332.

Appendix D: CCaV Study Data Sheet (Hypoxia-Resuscitation)

Piglet#_____ Weight_____Kg Age_____hours Sex ♂/♀ Date_____
Intervention Group_____ Asphyxia Time_____ ROSC Time _____

Time of Day	PHASE	FiO_2	HR	OSM3/ Pulse-ox	MAP	CVP	CA	Brain	Kidney	TEMP	hB	pH	$PaCO_2$	PaO_2	Base Excess	HCO_3	sO_2%	Lactate
	End of Surgery																	
	0'*																	
	10'																	
	20'																	
	30'																	
	Before CPR																	
	After ROSC																	
	10'																	
	60*																	
	120'																	
	180'																	
	240*																	

Table 2.1 Hemodynamic and Metabolic data recording sheet. (') indicates blood sample taken at this time was 0.2mL. (*) indicates blood sample taken at this time was 1mL.