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Effects of periodic acceleration (pGz) on nitric oxide during resuscitation in anesthetized pigs

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EFFECTS OF PERIODIC ACCELERATION (pGz) ON NITRIC OXIDE DURING RESUSCITATION IN ANESTHETIZED PIGS

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To: Dean Ronald M. Berkman
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This thesis, written by Ingrid M. Gunnlaugsson, and entitled "Effects of Periodic Acceleration (pGz) on Nitric Oxide During Resuscitation on Anesthetized Pigs", having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

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ABSTRACT OF THE THESIS

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The purpose of this study was to compare the response of Nitric Oxide (NO) during periodic acceleration (pGz) to standard CPR in anesthetized pigs. PGz increases blood flow during resuscitation and research suggests NO may be the mediator. Twelve anesthetized pigs (9.5 to 12 kg) were stimulated into ventricular fibrillation and resuscitated via either pGz or standard CPR. Serum NO levels were drawn prior to ventricular fibrillation and at 10 and 20 minutes during resuscitation. Mean NO levels were measured using three methods: an NO Meter (ISO-NO Mark II), using both a reductase and deproteinization method, and a Greiss Reaction Assay. Data were analyzed using Friedman matched samples and Kruskal-Wallis signed ranks test. While significant differences were noted in some of the measurements, these differences were inconsistent. Variations in the NO levels in this study indicate problems with both the sampling modality and the reduction methods utilized in measurement.

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1. Introduction

Overview

Cardiac arrest, cardiopulmonary resuscitation (CPR), and low blood flow states seriously alter many physiological responses. CPR related circulatory impedance and pulmonary hypoxia result in biological alterations including: hyperkalemia, hypocalcemia, abnormal arterial blood gases, and increased nitric oxide (NO) levels (Bellamy et al., 1996; Schleien, Kuluz, & Gelman, 1998). Research studies indicate that increased serum potassium, along with decreased ionized calcium, occur during lengthy CPR events and possibly cause countershock arrhythmias and the failure of resuscitation efforts (Niemann & Cairns, 1999). Research studies also suggest that CPR induced increases in endogenous NO levels result in cerebral and cardiovascular hyperemia and injury further impeding the success of resuscitative procedures (Schleien et al, 1998). In contrast, additional evidence exists postulating that the vasodilatory properties of increased NO levels may actually be beneficial in decreasing neuronal and cardiovascular destruction and therefore possibly increase resuscitation survival rates (Adams, Mangino, Bassuk, Kurlansky, & Sackner, 2001a; Mossad, 2001; Schleien et al., 1998). Further research implies that NO may play a role in consciousness, sedation, and analgesia (Matsouka, Watanabe, Isshiki, & Quock, 1997; Tonner, Scholz, Schlamp, & Schulte, 1997).

Standard CPR, which originated in 1965, has been the focus of numerous animal and human studies (Schwenzer, Smith, & Durbin, 1993). As swine have similar metabolic and cardiovascular functions to humans, with a similar coronary anatomy, they are frequently used in modern CPR research (Idris et al., 1996). Standard CPR, as defined by

the *Utstein-Style Guidelines for Uniform Reporting of Laboratory CPR Research*, is described as "external closed chest compression and ventilation" (Idris et al., 1996, p. 529). Successful standard CPR depends on the ability of the chest compressions, to provide adequate blood flow, and the ability of ventilation mechanisms to provide and maintain sufficient oxygenation. Insufficient circulation, and resulting ischemia, can set off a cascade of hemodynamic abnormalities (Pinchak, Hancock, Hagen, & Hall, 1998).

As the goal of CPR is to provide and maintain adequate circulation and oxygenation, as well as increase patient survival rates, continued research is needed to facilitate and implement successful resuscitation methods (Jawan et al., 2000). In an ongoing study at the Harry Pearlman Research Institute at Mount Sinai in Miami Beach, Florida, intraoperative resuscitation on anesthetized pigs, using a periodic acceleration (pGz) approach, is under investigation. Resuscitation via pGz motion is implemented by moving the anesthetized animal in a foot to head direction known as a "*z-plane*" using a platform device (Adams, Mangino, Bassuk, Inman, & Sackner, 2000b). By manipulating the platform's speed and frequency, acceleration forces ventilate the anesthetized and arrested pig's lungs and stimulate cardiovascular function (Adams et al., 2000; 2001a). The pGz platform's movement also causes hemodynamic vessel shear stress that may increase the production of endogenous NO (Adams et al., 2000; 2001a). There is a possibility that pGz resuscitation may be applicable to humans as an alternate to standard CPR. To date, little research exists on pGz or possible related physiological responses such as increases in NO (Adams et al., 2000b; Adams et al., 2001a; Jawan et al., 2000).

Study Purpose

The purpose of this study was to compare the physiological responses during pGz resuscitation to standard cardiopulmonary resuscitation in anesthetized and arrested pigs.

Statement of the Research Problem

Endogenous NO has been implicated as a modulator of various physiological responses including: regulation of blood pressure and blood flow, stimulation of vascular relaxation, transmission of molecular signals, inhibition of platelet aggregation, and activation of cytokine induced inflammatory neutrophil and macrophage migration, nociception and the maintenance of consciousness (Kelly, Balligand, & Smith, 1996; Fukuda et al., 1999). Standard CPR has commonly depended on the use of vasopressors, such as epinephrine and vasopressin, in shunting circulation to the brain and cardiovascular areas in order to maintain the oxygenation of those tissues (Krismer et al., 2000). Coronary perfusion pressure has primarily been the deciding factor as to success in restarting an arrested heart (Achleitner et al., 2000). Nitric oxide, on the other hand, is a potent vasodilator and this vasodilatory response has long been viewed as detrimental to resuscitation efforts (Schleien, 1998). Nitric oxide is believed to be stimulated by receptor dependent and independent agonists, hypoxic events, and hemodynamic shear stresses (Busse, Mulsch, Fleming, & Hecker, 1993; Vanhoutte, 1999). Cardiac arrest situations cause circulatory and pulmonary impedance resulting in ischemia and hypoxia. Movement of the pGz platform device produces vasoactive modulators such as vascular shear stress and pulsatile vessel stretch (Adams et al., 2001a; Bellamy et al., 1996). Thus an increase in NO release is expected with pGz movement resuscitation. Whether or not an increase in endogenous NO actually occurs with pGz and whether such an increase is

beneficial, is not known. Therefore, the study addressed the following problem: To what extent does pGz motion affect the physiological response of nitric oxide during intraoperative resuscitation in anesthetized pigs?

Specific Aims

1. To determine the effects of intraoperative pGz on NO levels in anesthetized pigs.
2. To compare the differences in NO levels during pGz to standard CPR in anesthetized pigs.

Hypothesis

In order to achieve these aims, the following is hypothesized in arrested and anesthetized pigs:

H_1. There is a difference between NO levels during pGz resuscitation and baseline levels prior to pGz resuscitation.

H_2. There is a difference between NO levels during standard CPR and baseline levels prior to standard CPR.

H_3. The NO levels are greater during pGz resuscitation than during standard CPR.

Variables

The variables in this study are as follows:

1. Dependent variable: Nitric oxide (NO) levels.
2. Independent variables: intraoperative resuscitation (pGz and standard CPR).

Definition of Key Terms

Intraoperative resuscitation

Intraoperative resuscitation refers to the cardiovascular and pulmonary resuscitation of

anesthetized pigs, that have had induced ventricular fibrillation and subsequent cardiac arrest, via pGz or standard CPR.

pGz

The pGz motion platform is a device that moves the animal in a foot to head direction, while varying the speed and frequency of the acceleration motion, with the purpose of ventilating and stimulating cardiovascular function in arrested pigs.

Standard CPR

Standard CPR refers to external closed chest compressions and ventilation. Standard chest compressions refer to "external chest compressions applied to the area of the chest approximately the size of the heel of an adult's hand" (Idris et al., 1996, p. 529). Ventilation refers to "any movement of gas in and out of the lungs" (Idris et al., 1996, p. 529).

Nitric oxide

Nitric oxide (NO) is a colorless, odorless free radical gas and molecular intracellular messenger that diffuses easily across the cell membrane and is thought to stimulate a variety of physiological responses. Some of the physiological responses to NO include: blood pressure regulation, vascular relaxation, platelet inhibition, behavioral modification such as wakefulness and sedation, pain nociception and antinociception, and molecular signal transmission (Fukada et al., 1999; Furchgott, 1999; Kelly et al., 1996). NO is believed to be stimulated by receptor agonists, hypoxia, and hemodynamic shear stress (Furchgott, 1999).

Assumptions

1. Cardiac arrest and standard CPR, with subsequent low blood flow states and hypoxia, result in physiological alterations that include arterial blood gas abnormalities, increased potassium, decreased ionized calcium, and increased nitric oxide levels.
2. Successful standard CPR depends on the ability of chest wall compressions to provide sufficient blood flow and the ability of ventilation methods to provide and maintain sufficient oxygenation. Decompression of the chest wall, as occurs during standard CPR, may negatively alter the lung volume capacity and mechanical ventilation, with frequent high pressures, may injure lung parenchyma (Adams et al., 2000b; Pinchak et al., 1998; Voelckel, et al, 2001).
3. Receptor dependent and independent agonists, hypoxia, and hemodynamic shear stresses stimulate the production of NO (Busse et al., 1993). The pGz platform movements generate hemodynamic shear stress and pulsatile vessel stretch (Adams et al., 2001a). Hypoxia can occur during, or after, cardiac arrest and resuscitation.

Significance to Nursing

The results of this study are relevant to clinical nursing practice and may add to the knowledge base of nursing education. As staff nurses are the first to respond to patients in cardiac arrest, and the first to initiate CPR, nurses must continually expand their knowledge relating to resuscitation practices. Acute care facilities mandate that staff nurses attend basic life support courses, biannually, and nurses that work in specialty areas must also attend advanced life support courses. Critical care nurses generally

receive patients, who have arrested and been successfully resuscitated, in the critical care units and therefore must have additional knowledge of cardiac arrest, subsequent resuscitation, low blood flow states, and the expected hemodynamic abnormalities and physiological responses. Standardized treatment modalities are in place for many of these alterations (Burden & Saufl, 2001; Mancini & Kaye, 1999).

Cardiac arrest is a possible complication intraoperatively and may result from inadequate oxygen, in the anesthesia content itself, or possibly from depressed respiratory function due to the anesthesia's action (Guyton & Hall, 2000). As such, the practice of nurse anesthesia requires both competencies in cardiac arrest situations and resuscitation methods, as well as, knowledge of innovations in CPR techniques. The novel and simplistic application of pGz makes it an attractive method of cardiopulmonary support or adjunct to current CPR modalities. Resuscitation via pGz, and the subsequent production of NO, may improve CPR outcomes in human populations. Therefore, study findings will help determine if NO is increased during pGz, whether increased NO is beneficial in cardiac resuscitation, whether pGz is a viable alternative to standard CPR in decreasing mortality, and whether pGz has applicability in human populations.

The American Nurse Association's (ANA) Code for Nurses may be applied to any nursing area regarding professional standards and expectations of patient care during cardiac arrest, CPR, intraoperative resuscitation, and subsequent abnormal physiological responses (ANA, 1991). Unique and challenging situations arise involving the care of patients intraoperatively, while undergoing anesthesia and surgery, and involving the acutely ill patients in today's critical care units (Burden & Saulf, 2001). As always, nurses must maintain a competency based practice and must continue to acquire

knowledge in relevant matters (ANA, 1991). Consequently, study results may help set the stage for further research in areas such as the applicability and practicality of pGz in human cardiac arrest situations.

2. Literature Review

Introduction

Cardiac arrest is a global, ischemic assault that seriously stresses the physiological responses of both human and animal populations. Ventricular fibrillation is the most common, as well as, the most treatable cause of cardiac arrest; however, cardiac arrest occurs in many other situations (American Heart Association, AHA, 1998). Intraoperative administration of potent anesthetics, particularly succinylcholine, has been implicated in arrest situations either as an anaphylactic or toxic response (Gronert, 2001; Larach, Rosenberg, Gronert, & Allen, 1997; Wu, Rau, Lin, & Chan, 1997). Cardiac arrest is also associated with other cardiac arrhythmias; trauma events, drowning, and can accompany respiratory arrest in hypoxic situations (AHA, 1998). Cardiac resuscitation efforts date back to the mid-1800s and research, to improve resuscitation methods, continues in modern times (Woda, Dzwonczyk, Bernacki, Cannon, & Lynn, 1999). Unfortunately, the majority of cardiac resuscitation efforts fail with overall survival rates between 1 and 20% (Paiva, Kern, Hilwig, Scalabrini, & Ewy, 2000). Coronary perfusion pressure, during prolonged CPR, is often less than adequate making the possibility of retaining intact neurological function minimal (Eynon, 2000; Reid, Mullins, & Iyer, 1998). Successful resuscitation depends on maintenance of physiological factors such as: serum potassium and calcium concentrations, acid-base pH, and possibly NO levels (Niemann & Cairns, 1999; Ziglar, 2000).

Theoretical Framework

The focus of the study framework is the affect of cardiac arrest and pGz resuscitation on physiological responses, particularly nitric oxide levels. The study framework

concepts include: (1) cardiac arrest, (2) resuscitation, (3) circulation, (4) ventilation, (5) vasoactive modulators, and (6) nitric oxide. Postulates suggested by the study framework are that pGz produces hemodynamic shear stress and that this stress, along with hypoxia, increases the production of NO. The conceptual model of the study framework (Figure 2.1) is based on evidence from the research studies cited in the literature review and the study concepts are defined as follows:

(1). *Cardiac arrest* refers to the "cessation of cardiac mechanical activity" (Idris et al., 1996, p. 529). Cardiac arrest is induced in the study population via electrically produced ventricular fibrillation.

(2). *Resuscitation* refers to the use of standard CPR or pGz to restart the animals arrested heart and stimulate circulation and ventilation. Standard CPR is defined as "external chest compression and ventilation" (Idris et al., 1996, p. 529). The abbreviation pGz refers to a device that moves an animal in a foot to head direction with the purpose of stimulating circulation and ventilation (Adams et al., 2001a).

(3). *Circulation* is defined as the movement of blood through the body as measured via arterial pressures (Idris et al., 1996). Circulation is frequently less than adequate during standard CPR (Eynon, 2000; Reid et al., 1998). Research indicates that pGz provides excellent regional blood flow to the brain, heart, as well as abdominal organs (Adams, Mangino, Bassuk, Kurlansky, & Sackner, 2001b).

(4). *Ventilation* is defined as "any movement of gas in and out of the lungs" and includes such movement as accompanies standard CPR and pGz (Idris et al., 1996, p. 529). Ventilation during standard CPR frequently results in pulmonary impedance and hypoxia (Adams et al., 2000b; Bellamy et al., 1996). Noninvasive negative pressure ventilation of

anesthetized pigs has been supported during pGz in a manner similar to human respiration and appears related to the thrust of the animal's abdominal contents cephalic toward the diaphragm (Adams, Mangino, Bassuk, & Sackner, 2000a).

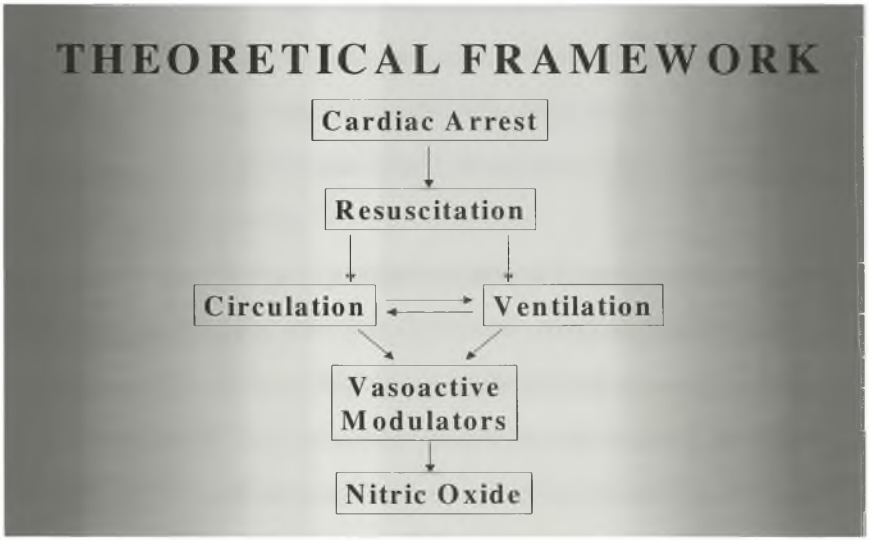
(5). *Vasoactive modulators* refer to such factors as hemodynamic shear stress, pulsatile vessel stretch, and hypoxic events. Cardiac arrest and resuscitation cause circulatory and pulmonary impedance and result in hypoxia and inadequate tissue perfusion and oxygenation (Bellamy et al., 1996; Paiva et al., 2000; Reid et al., 1998). The movement of pGz is thought to produce vascular hemodynamic shear stress and pulsatile vessel stretch (Adams et al., 2001a).

(6). *Nitric oxide* refers to a reactive diffusible free radical gas that is produced in the vascular endothelium, and various other biological tissues, in response to such stimulants as hypoxia, hemodynamic shear stress, pulsatile vessel stretch, and receptor agonists (Busse et al., 1993; Miller et al., 1986).

The relationships postulated by the variables in the study framework are based on previous research findings and linked via the following statements: Cardiac arrest and resuscitation impede circulation and ventilation (Bellamy et al., 1996). Resuscitation, via standard CPR and pGz, attempts to correct this impedance by increasing circulation and ventilation with the goal of adequately oxygenating cardiac and cerebral tissue (Schwenzer et al., 1993). Standard CPR is thought to produce various negative physiological responses such as: hyperkalemia, hypocalcemia, and abnormal arterial blood gases (Bellamy et al., 1996; Neimann & Cairns, 1998). Research has also indicated that increased NO levels occur during standard CPR (Furchgott, 1999; Mossad, 2001; Schleien et al., 1998). Additional research suggests that the production of NO is

stimulated by hypoxic events, hemodynamic shear stress, and pulsatile vessel stretch (Busse et al., 1993; De Keulenaer et al., 1998; Inoue et al., 1996; Miller et al., 1886; Utematsu et al., 1995). The movement that occurs during pGz is thought to produce hemodynamic shear stress, and pulsatile vessel stretch, and therefore stimulate the release of NO (Adams et al., 2001a). Nitric oxide is a potent vasodilator and plays a role in numerous physiological responses such as: blood pressure regulation, cerebral function, skeletal muscle activity, and neuroendocrine mediation (Christopherson & Bredt, 1997; Kelly et al., 1996). Whether or not an increase in NO production during pGz is beneficial to resuscitation outcomes is, as yet, unknown.

Figure 2-1. Theoretical Framework.



Standard CPR

Early CPR consisted of a chest-pressure arm lift technique without accompanying ventilation (Woda et al., 1999). Modern or standard CPR, composed of closed external chest compressions and ventilation, originated in 1965 (Schwenzer et al., 1993). Standard CPR commonly consists of ventilation by intermittent positive pressure via bag-valve or endotracheal tube and/or mechanical device (Pinchak et al., 1988). Research indicates that decompression of the chest wall, as occurs during standard CPR, may negatively alter lung volume capacity, displacing the abdominal contents upward, and thus decreasing the ventilatory ability (Voelckel et al., 2001). Furthermore, mechanical ventilation, with its frequent need for high-pressure levels to provide adequate oxygenation, may injure the lung parenchyma (Adams et al., 2000b, Idris et al., 1996). The need for both ventilation and chest compression, in bystander CPR, is currently under debate (Berg, Hilwig, Kern, & Ewy, 1999: Idris, 1995). However, CPR, utilizing both ventilation and chest compression, remains the treatment of choice (Idris, Wenzel, Becker, Banner, & Orban, 1995).

Cardiac arrest and CPR result in circulatory impedance and pulmonary hypoxia which deplete energy and adenosine triphosphate (ATP) levels causing movement of sodium (Na^+), calcium (Ca^{++}), and water (H_2O) from extracellular spaces to intracellular spaces, leakage of potassium (K^+) from intracellular spaces to extracellular spaces, and overall failure of the Na^+/K^+ membrane pump (Bellamy et al., 1996). Hypoxia leads to oxygen starvation and cells revert to anaerobic metabolism resulting in lactic acid as a byproduct (Bellamy et al., 1996). One of the primary causes of cerebral injury is the production of

lactic acid in brain tissue (Karkela, Pasanen, Kaukinen, Morsky, & Harmoinen, 1992). Lactic acid binds with calcium causing further depletion in calcium levels. Research has indicated that postcountershock rhythm disturbances occur, during CPR, in hyperkalemic and hypocalcemic states and that these disturbances decrease chances for successful resuscitation (Neiman & Cairns, 1998).

pGz Resuscitation

The pGz motion platform is a new noninvasive approach to cardiopulmonary resuscitation currently under investigation at the Harry Pearlman Research Center in Miami Beach, Florida. The pGz method is based on a horizontal foot to head direction or "z-plane" fluctuation with alternating and periodic acceleration-deceleration platform movement as occurs when using swine for study (Adams et al., 2000b). Variations are made in the speed, frequency (0.5-10 Hz), and intensity (0.1-1.5 G.) of the platform's motion (Adams et al., 2000a). The pGz platform moves the animal's entire body, which produces inertial energy and stimulates both cardiovascular and pulmonary function (Adams et al., 2000a; Adams et al., 2000b). The force of the acceleration-deceleration motion drives the abdominal contents toward the diaphragm and results in negative pleural pressure ventilation similar to human respiration (Adams et al, 2000b). These periodic movements also produce vascular flow and pulsatile shear stress, to arterial and capillary walls, and possibly stimulate the production of NO (Adams et al., 2000a; Adams et al., 2000b).

Adams and associates (Adams et al, 2000a; 2000b; 2001a) have performed several swine studies using the pGz platform device. Study findings suggest that by manipulating the acceleration and frequency of inertial forces, noninvasive ventilation of an animal can

be supported at continuous positive airway pressures as opposed to intermittent positive pressure commonly associated with standard CPR (Adams et al., 2000a). Additional experiments were performed to investigate the effects of the pGz motion on meconium aspiration in pigs (Adams et al., 2000b). Results indicate that this form of ventilation attenuates the pulmonary hypertension and systemic vascular resistance that frequently accompany meconium aspiration conditions (Adams et al., 2000b). Once again, this attenuation of pulmonary and vascular resistance may be due to the hemodynamic vessel shear stress, and subsequent increase in NO production, as possibly induced by pGz motion (Adams et al., 2000b; 2001a). Finally, in an experiment on pGz as a form of CPR, evidence supports the hypothesis that pGz alone produces sufficient capillary circulation and oxygenation, to both myocardial and cerebral tissue, to maintain a fibrillating animal for eighteen minutes with post resuscitation arterial blood gases similar to baseline values (Adams et al., 2001a).

Nitric Oxide

Nitric oxide (NO) is a colorless, odorless, free radical gas and reactive molecular messenger that diffuses easily across the cell membrane and has a short half-life (2-30 seconds) (Berkels, Purol-Schnabel, & Roesen, 1999; Mossad, 2001). Formerly known as endothelial relaxing factor (EDRF), NO was identified in the 1980s as an important biological mediator with multiple actions (Furchgott, 1993). Nitric oxide is produced in the vascular smooth muscle endothelium, as well as other biological tissues, and the synthesis of NO is activated by a group of enzymes known as NO synthases (NOS) (Liao, 1999). These enzymes catalyze the conversion of the amino acid L-arginine to L-citrulline and NO in the presence of oxygen (O_2) and require reduced nicotinamide-

adenine-dinucleotide phosphate (NADPH) for optimal function (Vanhoutte, 1999). A product of the urea cycle, L-arginine circulates in the blood until actively transported into the cell (Harrison, 1997). Once produced, NO activates guanylate cyclase in the endothelium and increases cyclic 3'-5'-guanosine monophosphate concentrations (cAMP) which mediates relaxation in vascular smooth muscle (Lushcer, Boulanger, Yang, Noll, & Dohi, 1993). The formation of cAMP also inhibits endothelin, a vasoactive contracting factor produced in the endothelium (Luscher et al., 1993). Therefore, NO is a potent endogenous vasodilator (Furchgott, 1999). In addition, large amounts of NO are found in the central and peripheral nervous systems and skeletal muscle (Christopherson & Bredt, 1997). In these areas, NO is thought to modulate neuronal activity, gastrointestinal motility, regional blood flow, neuroendocrine actions, behavioral and memory function, as well as, muscle metabolism and contractility (Christopherson & Bredt, 1997).

Three isoforms of the enzyme NOS have been identified and include: (1) neuronal tissue synthase (nNOS or NOS 1), (2) cytokine inducible synthase (iNOS or NOS2) and, (3) large vessel endothelial synthase (eNOS or NOS3) (Kelly et al., 1996). The action of all three isoforms depends on the binding of calmodulin, an intracellular protein that combines with calcium to stimulate various physiological responses (Kelly et al., 1996; Thomas, 1993). Neuronal (nNOS) and endothelial (eNOS) are constitutive and calcium sensitive, with calmodulin binding regulated by intracellular concentrations of calcium ions (Kelly et al, 1996). Conversely, the inflammatory mediated cytokine inducible NOS is not dependent on calcium as its calmodulin remains tightly bound without regard to calcium concentration (Kelly et al, 1996). Additionally, the level of NO, by itself, is

thought to play a role in the regulation of NOS activity (Kelly et al, 1996). Due to the complexity of the subject matter, this paper will deal primarily with endothelial NOS and the possible increase in NO production during resuscitation.

The vascular endothelium produces two types of constitutive NOS, basal and stimulated. Basal production of NO is continuous; however, various agonists such as: receptor dependent (acetylcholine (ACh), adenosine triphosphate (ATP), and bradykinin) and receptor independent (Ca^{++} ionophore and Ca^{++} ATPase inhibitor) can stimulate the production of NO (Busse et al., 1993). Increased NO production can also be stimulated by hypoxic events (decreased arterial PO_2), as well as, physical stimulation (fluid shear stress or pulsatile stretching of the vessel) (Busse et al., 1993). Hemodynamic shear stress, as produced by increased vessel blood flow, has been implicated in the increased production of EDRF/NO and subsequent vascular relaxation (Miller, Aarhaus, & Vanhoute, 1986). In addition, the short half-life of NO is thought to impact numerous biological processes and results from NO's rapid destruction via three major reactions: (1) reaction with oxyhemaglobin or oxymethaglobin to produce nitrite/nitrate, (2) binding with ferrous heme iron of guanylate cyclase or other proteins, and (3) the scavenging by superoxide anion (SO) to form peroxynitrite anion ($ONOO^-$) during oxidation (Beckman, 1996). Peroxynitrite is a potent oxidant and may be responsible for neural injury in cerebral ischemia and reperfusion (Liao, 1999). However, endothelial cells also produce an enzyme, identified as superoxide dismutase (SOD), as a defense against SO's rapid destruction of NO and the consequent cell damage (Mugge, Elwell, Peterson, & Harrison 1991). Research has indicated that fluid shear stress may increase the production of cytosolic copper/zinc SOD (Cu/Zn SOD), and endothelial NOS, thus increasing the half-

life and protective properties of NO (Inoue, Ramasamy, Fukai, Nerem, and Harrison, 1996).

Furchgott and Zawadzki (1980) first reported the discovery of EDRF after performing an experiment on rabbit thoracic aorta endothelial cells which suggested "the obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine" (Furchgott, 1993). Furchgott and associates (1986) further experimented with rabbit aorta, theorizing that EDRF might be the same substrate as NO. Thereafter, EDRF and NO were compared via perfusion bioassay procedures in numerous studies such as the experiments by Ignarro et al., (1987) using bovine pulmonary vessels and by Palmer, Ferrige, and Moncada (1987) using cultured porcine aortic endothelial cells. These studies also indicated that the chemical characteristics of NO and EDRF were similar, if not the same (as cited by Furchgott, 1999).

Mugge, et al., (1991) conducted a study on the activation of EDRF/NO by the oxygen derived free radical superoxide dismutase. Experiments were performed to determine the existence of endothelial cell defense mechanisms, such as SOD's scavenging of superoxide anions, by impairing antioxidant defenses in normal vasculature tissue using rabbit aorta in vitro and cultured bovine aortic endothelial cells (BAEC). Endothelium dependent and independent relaxation was measured in the rabbit aorta both before and after inhibition of Cu/Zn SOD with diethyldithiocarbomate (DETC). SOD activity was visualized by Beauchamps and Fredovich (1971) assay with enzyme activity indicated by the appearance of achromatic bands. Results revealed that aortic preparations incubated with DETC diminished the Cu-Zn SOD activity, as well as, the vasodilator activity of BAEC effluent, and the NO recovered from that effluent, under basal conditions when

measured by bioassay. Experiments were also performed using stimulation by various agonists. Decreased vasodilatation, in the DETC model, was evident after bradykinin stimulation. Furthermore, results suggest that inhibition of Cu-Zn SOD, with DETC, diminished both endothelial dependent and independent relaxation and indicate that Cu-Zn SOD is a necessary component in the production of EDRF/NO (Mugge et al., 1991).

In a study by Inoue, Ramasamy, Fukai, Nerem, and Harrison (1996) the effect of laminar shear stress on the expression of Cu-Zn SOD, in cultured human aortic endothelial cells and vascular smooth muscle cells, was examined. These cells were grown to confluence and placed in a flow chamber with two reservoirs. The temperature and pH of the fluids was kept constant at a respective 37 °C and 7.4 pH with shear flow rate regulated by the height of the reservoirs. Western analysis was performed with a sheep antibody against human Cu-Zn SOD and enzyme activity was assayed. Data was obtained from the shear and control groups with comparisons made by paired t-tests and ANOVA with Fischer's least significant difference post hoc test. Results indicated that steady fluid shear stress at 15 dynes/cm^2, for either 24 or 48 hours, showed an increase in Cu-Zn SOD mRNA transcription, protein, and enzyme activity in human aortic endothelial cells but no increase was detected in human vascular smooth muscle cells. These results suggest that Cu-Zn SOD increase may be endothelial specific and that enhanced endothelial dependent relaxation in vessels denuded by shear stress may be induced by SOD. Results also suggest that SOD may synergistically stimulate the vasodilatory properties of endothelial NO (Inoue et al., 1996).

Earlier research on the mechanism of shear stress as involved in the regulation of endothelial cell NOS mRNA expression was performed by Uematsu et al. (1995). The

study utilized both bovine aortic endothelial cells (BAEC) and human aortic endothelial cells (HAEC) and investigated the effects of shear stress on eNOS mRNA, protein expression, and endothelial cell NO production. NOS activity was measured by examination of the production of nitrite, a byproduct of NO, in the sheared versus the control groups. Cells were grown to confluence and exposed to shear forces as delivered via a flow chamber similar to the Inoue study (1996). However, in this study fluid temperature was maintained at 37 °C with a pH of 7.2. Similarly, shear stress was set at 15 dynes/cm^2 and measured from 1 to 24 hours. Significant results were obtained at 3 hours. Nitrite was converted to NO gas and measured via a chemiluminescence analyzer, which was appropriately calibrated. Results indicated that increases in eNOS mRNA occurred at 3 hours in both BAEC and HAEC. Results also suggested an increase in shear-induced production of NO approximately two-fold in BAEC in both basal conditions and with administration of the agonist, calcium ionophore. Additional study data indicated that these increases might be initiated by the opening of K^+ channels. Study findings conflict with earlier research studies that described eNOS as a constitutive or static enzyme (Uematsu et al., 1995).

In an experiment by De Keulenaer et al., (1998) on the redox state of cultured human umbilical vein endothelial cells, after either low pulsating oscillatory flow shear stress or steady laminar flow shear stress, was investigated. Once again, flow chambers were used to produce shear stress with modifications of lower flow and intermittent pulsatile action in the oscillatory model. Appropriate fluid temperature and pH were maintained. Changes in Cu-ZN SOD, presumed adaptive in oxidative stress, were measured via Northern Blot analysis after endothelial cell exposure to oscillatory shear flow (5 dynes/cm^2, 1 Hz) at

intervals of 1, 5, and 24 hours. In this experiment, no change was evident in the level of Cu-Zn SOD. Conversely, when endothelial cells were exposed to steady laminar shear flow (5 dynes/cm^2) for 24 hours, Cu-Zn SOD mRNA and protein levels were increased. Oxidative stress was also investigated in measurements of NADH oxidase, intracellular O_2 levels, and heme-oxygenase-1 (HO-1). Intracellular oxygen (O_2) was measured after both oscillatory and laminar shear stress and O_2 levels were found to be three times higher in the oscillatory model. Study findings are consistent with previous research indicating that increases in Cu-Zn SOD, and accompanying protective antioxidant properties, may be stimulated by shear stress. However, these findings suggest that increases in Cu-Zn SOD may be laminar shear specific (De Keulenaer et al., 1998).

In a study by Schleien et al., (1998) experiments were done to examine whether the increases in NO activity associated with cardiac arrest, CPR, and reperfusion might be detrimental in relation to successful resuscitation. This study examined the inhibition of NO activity by N-nitro-L-arginine methyl ester (L-name; 3 mg/kg) and tested the hypothesis that such inhibition would (1) lessen cerebral and cardiovascular hyperemia, (2) benefit vascular effects on other organs allowing for successful resuscitation and reperfusion, and (3) maintain cerebral oxygen and glucose rates without altering lactate production by the lessened blood flow after resuscitation. The study measured vascular pressures, regional blood flow, and cerebral metabolism before, during, and after CPR on anesthetized pigs via aortic, right atrial, pulmonary artery, and sagittal sinus pressures, arterial blood gases, and plasma glucose and lactate concentrations. Study findings indicated that (1) L-name did not decrease cerebral or myocardial flow or adversely affect the rate of successful CPR, (2) L-name lessened cerebral hyperemia, and (3) L-

name decreased cardiac output before and after arrest. L-name was believed to have a negative inotropic affect on cardiac function. Study results also suggested that possible adverse affects in cerebral and cardiac function, as well as decreased circulation to the kidneys and other organs, might result from the attenuation of cerebral and cardiovascular hyperemia (Schleien et al., 1998).

NO and Anesthesia

Although controversial, research studies indicate that the NO/cGMP pathway plays an important role in maintaining consciousness and facilitating nocioception, whereas the inhibition of NOS maybe responsible for sedation and analgesia (Fukada et al., 1999; Tonner et al., 1999). Moreover, the action of certain anesthetics on NO production and inhibition may account for the variability found among numerous studies investigating the properties and production of NO (Galley, Cras, Logan, & Webster, 2000). Evidence exists suggesting that NO has a dual action of either nociception or antinociception dependent on the tissue site where it's found (Lauretti, Oliveira, Juliao, Reis, & Pereira, 2000; Lauretti, Oliveira, Reis, Mattos, & Pereira, 1999). In fact, NO appears to inhibit pain perception in the descending spinal pain pathway and may act directly on *mu* opiod receptors enhancing their effect (Lauretti et al., 1999; 2000). The NO pathway is the foundation for the pharmacodynamics of certain nitrate medications such as Nitroglycerin, Sodium Nitroprusside, and Hydrazaline (Morgan, Mikhail, & Murray, 2002). Nitroglycerine patches have been shown to have a possible synergistic effect when administered with intrathecal neostigmine in gynecological surgeries, with intrathecal sufentenil in orthopedic surgeries, and with morphine administered either by intrathecal or intravenous methods (Lauretti, 1999; 2000). This synergistic effect helps delay the

need for post-operative rescue pain medications for up to 14 hours (Lauretti et al., 2000). Additionally, the neurotransmitter and intracellular messenger properties of NO may influence some of the systemic effects of local anesthetics such as: cardiac depression, cardiac arrhythmias, and grand mal seizures (Heavner, Shi, & Pitkanen, 1999). The cardiotoxicity of lidocaine, a commonly used antiarrythmic, and anesthetics such as: tetracaine and cocaine appear attenuated with NO inhibition (Heavner, Shi, & Pitkanen, 1999; Roig et al., 2000). Research indicates that adverse alcohol withdrawal symptoms are also decreased with the inhibition of NO and this result may have important treatment ramifications (Adams & Cicero, 1998). Some studies suggest that Halothane, formerly one of the most frequently used volatile anesthetics, alters the NO pathway via inhibition of calcium release from both the sarcoplasmic reticulum and the calcium release induced by bradykinin (Galley et al., 2001; Fukada et al., 1999; Loeb, Raj, & Longnecker, 1998; Tsuchida, Tanaka, Seki, Inoue, & Namiki, 1999). Both types of calcium release are necessary for NO production as eNOS and nNOS are calcium dependent (Tsuchida et al., 1999). Halothane and isoflurane, when administered with a NO inhibiting agent such as L-NAME, may decrease the threshold for minimum alveolar anesthetic concentration (MAC) by blocking the NO pathway at the level of cGMP (Fukada et al., 1999; Galley et al., 2001). Research indicates that inhaled NO is also a potent selective bronchodilator and is commonly administered by anesthesia machines to treat pulmonary hypertension and hypoxia during surgery (Ceccarelli et al., 2000). Obviously, understanding the stimulation and inhibition of NO production may have important implications regarding the dosage and route of many anesthetics, pain treatment modalities, and research study modifications.

The review of the literature on nitric oxide research reveals evidence that NO and EDRF have the same chemical characteristics. Study findings indicate that NO and SOD are both products of endothelial and laminar flow sheer stress. Research also proposes that Cu-Zn SOD is an important component in the biological synthesis of NO and in scavenging super oxide anions that limit NO's effect. Study results indicate that the release of SOD, and its stimulation of NO, may be laminar sheer flow specific. Although, research (De Keulenaer et al., 1998) proposes a difference between steady laminar and low flow oscillatory shear stress and the resulting stimulation of Cu-Zn SOD or NO production, the magnitude of the flow force produced by pGz motion is thought to produce hemodynamic shear stress and pulsatile vessel stretch during pGz resuscitation (Adams et al., 2001a). Additionally, research opposes the previous held belief that eNOS is a constitutive or static enzyme. Research findings on the inhibition of NOS show this effect to reduce hyperemia, in cerebral and cardiovascular tissue, but whether this attenuation is beneficial during cardiac arrest is questionable. Variability among NO studies may be explained by the inhibition or stimulation of NO by certain anesthetic agents. Finally, a great deal of evidence exists suggesting that NO release may be induced by fluid and pulsatile shear stress. Furthermore, the strong evidence reported here supports the researcher's proposal to study the effect of pGz platform motion on NO levels and to determine whether a related increase in NO levels occurs during resuscitation in anesthetized pigs.

3. Methodology

Introduction

Research studies investigating resuscitation using standard CPR methods suggest that standard CPR produces a variety of physiological responses, many of which are detrimental to successful resuscitation outcomes. The pGz model is a new form of resuscitation procedure devised with the hope of possibly decreasing resuscitation mortality rates. The free radical NO is thought produced during hypoxia, ischemia, and hemodynamic shear stress events and therefore a possible byproduct of resuscitation procedures. This study was designed to investigate the effects of pGz motion platform resuscitation on the production of NO in anesthetized pigs. The production of NO was measured at baseline and at 10-minute intervals during resuscitation using the pGz model. Nitric oxide measurements were compared between the two models, pGz and standard CPR.

The foundation for the proposed hypotheses to be studied was based on a theoretical framework created from previous research findings as cited from studies examining standard CPR and pGz methods and the induced production of NO. The study attempted to determine if (H_1) there is a difference between NO levels during pGz resuscitation and baseline levels prior to pGz resuscitation, if (H_2) there is a difference between NO levels during standard CPR and baseline levels prior to standard CPR, and if (H_3) the NO levels are greater during pGz resuscitation than during standard CPR. Basic to the study framework was the theory that NO production is stimulated by various vasomodulators, namely hemodynamic shear stress and pulsatile vessel stretch. The movement of the pGz platform was assumed to produce both of these vasomodulators during the resuscitation

of anesthetized pigs and consequently an increase in NO production is expected to follow.

Design

The study used an experimental repeated measures design to determine the possible increase in NO levels in anesthetized pigs during pGz and standard CPR resuscitation. A total of twelve pigs was used in the study and divided into two groups of 6 pigs each. In order to test the study hypothesis, two experiments were performed: (1) measurement of NO production during pGz resuscitation, and (2) the measurement of NO production during standard CPR resuscitation. Comparisons were be made between the two levels.

Variables and Measurements

Independent Variables

pGz. Periodic acceleration (pGz) refers to a novel method of resuscitation, which uses a motion platform device, to move an animal in a footward to headward direction, with the purpose of ventilating and stimulating cardiovascular circulation in anesthetized and arrested pigs. The frequency of acceleration and deceleration movements ranges from 0.5 – 10 Hz at a force of 0.1 – 1.5 G. Approximation of heartbeat per minute (bpm) to corresponding frequency of motion is 1 Hz = 60 bpm (Adams et al., 2001a). Ventilations occur via the force of acceleration movement, which thrusts the animal's abdominal contents toward the diaphragm and produces negative pleural pressure respiration (Adams et al., 2000b).

Platform design. The motion platform design (Adams et al., 2000a) is as follows:

> The motion platform was constructed around a linear displacement current motor (Model 400, 12 volt; APS Dynamics, Carlsbad, CA). The motor is powered by

dual-mode power amplifier (model 144, APS Dynamics) connected to a sine-wave controller (model 140-072; NIMS, Miami Beach, FL). The controller allows for the control of the frequency of the table oscillation, the amplitude of the voltage reaching the motor and subsequent acceleration of the stroke, and the duty cycle of the motor. The motor is secured in the bottom and center of a frame constructed from treated pine lumber. The table platfrom is directly driven by the underlying motor and articulates across the frame on stainless steel tracks and nylon wheels. The unit has a maximum weight capacity of ~30 kg and operates at a frequency of 0.5 – 10 Hz at a force of 0.1-1.5 G (Adams et al., 2000b, p. 2448).

Standard CPR. Standard CPR refers to external closed chest compressions and ventilation. Standard chest compressions refer to "external closed chest compressions applied to an area of the chest approximately the size of the heel of an adult's hand" (Idris et al., 1996, p. 529). Chest compressions were done via the *Thumper*, ". . . a mechanical deice for compressing the chest in a uniform, repetitive fashion with constant rate and force" (Pinchak et al., 1988). Standard CPR is commonly performed at a rate of 15 compressions to 2 ventilations manually (approximately 80-100 bpm).

Ventilation. Ventilation refers to "any movement of gas in and out of the lungs" (Idris et al., 1996, p. 529). Ventilation includes intermittent positive pressure via bag valve, endotracheal tube, mechanical ventilation, and gas movement resulting from chest compression or pGz platform movement.

Dependent Variable.

Nitric Oxide (NO). NO is a colorless, odorless, free radical gas that is produced in the vascular endothelium, and in other biological tissue sites, in response to such stimulants

as hypoxia, ischemia, hemodynamic shear stress, pulsatile vessel stretch, and receptor dependent and independent agonists (Busse et al., 1993; Miller et al., 1986). Normal or basal levels for NO were not found in the literature; therefore, NO will be measured with baseline levels used as control. NO levels are dynamic with baseline levels specific to each individual pig. NO measurements were made using *the Isolated Nitric Oxide Meter and Sensors* (ISO-NO Mark II; World Precision Instruments Inc., Sarasota, FL, 2000).

ISO-NO Mark II. The ISO-NO analyzer (WPI, 2000) is a highly sensitive instrument for the measurement of NO in biological tissues. NO is measured via the reduction of nitrate/nitrite, the byproducts of NO, to NO using an amperometric sensor (2.0 mm) (Berkels et al., 2001). The ISO-NO analyzer has the ability to measure basal and agonists stimulated NO production in both aqueous solutions and gas mixtures. First, nitrate is converted to nitrite via an enzyme (nitrate reductase) and then nitrite is reduced to NO using an acidic solution (Berkels et al., 2001). The detection limit of nitrite is as low as 1 nM when using the ISN-NO sensor electrode (WPI, 2000). The electrode has a membrane cover which NO diffuses through producing an electrical current proportionate to the NO concentration of the specimen (WPI, 2000). Accuracy of measurement is based on correct calibration of the instrument using a titration method (nitrite in the presence of H_2SO_4 and KI) (WPI, 2000). Accurate calibration requires construction of a standard curve to determine sensitivity of the sensor (slope of the standard curve) precisely as noted by pA/nM NO (WPI, 2000). The ISO-NO 2.0 sensor electrode can be used to measure NO reduced from nitrate/nitrite and NO concentration in cell cultures and sensitivity is 2 pA/nM (WPI, 2000). Numerous publications citing the validity, reliability,

and sensitivity of the ISO-NO Mark II instrument, relating to measurement of NO production during experimental studies, are available (WPI, 2000).

The principle investigator received training, along with other pGz research investigators, in NO measurement using the ISO-NO Mark II instrument. Training included a session with the WPI ISO-NO representative and took place at the Mount Sinai Research facility. Training required approximately a one-month period of 2-3 days a week.

Control Variables

Arrest. Arrest refers to cardiac arrest or "the cessation of cardiac mechanical activity" as indicated by the "sudden loss of arterial pulsations on intravascular pressure monitors and a systolic aortic blood pressure of < 25 mmHg" (Idris et al., 1996, p. 529). Cardiac arrest will be induced via the stimulation of ventricular fibrillation using a bipolar wire placed at the apex of the heart and delivering 30 V of A.C. current at 60 Hz (Adams et al., 2000, 2001a). All animals under study had induced ventricular fibrillation.

Sample Selection. The use of a randomly selected homogenous breed of animals in this study was used as a sample control. The swine model is commonly used in CPR research due to the similarity of metabolic processes, cardiovascular functions, and coronary anatomies between swine and human populations (Idris et al., 1996). In addition, juvenile pigs are noted as a homogenous breed due to similarity in size and structure at corresponding ages and weights (Idris et al., 1996).

Baseline: Baseline measurements of NO concentrations were used as control in the within group experiments. Baseline refers to "conditions attained before induction of cardiac arrest, usually in an anesthetized, intubated, ventilated, and instrumented animal.

They do not represent the normal physiological state of an animal subject" (Idris et al., 1996, p. 528).

pGz manipulation. The frequency and force of the pGz motion platform movement were used as a control with frequency set at 2 Hz (approximately 120 bpm) and the force set at 0.6 G for all experiments (Adams et al., 2000b; 2001a).

Data Collection

The first experiment (n=6) measured the production of NO, as obtained from serum specimens, at baseline and at 10-minute intervals (x 2) during pGz resuscitation. The second experiment (n=6) measured the production of NO levels, as obtained from serum specimens, at baseline and at 10-minute intervals (x 2) during standard CPR resuscitation. Comparisons were made between the pGz and standard CPR groups measured serum levels of NO concentration (Figure 3-1). The serum specimens obtained consisted of whole blood, collected at the previously stated times, which was quickly centrifuged and frozen for later analysis. Serum levels of NO were measured, via the presence of nitrate concentrations, using three modalities: (1) reductase method using the ISO-NO Mark II instrument, (2) deproteinization method using the ISO-NO Mark II method, and (3) assay quantification via the Greiss reaction using a reductase specimen.

Measurement Techniques

The measurement of serum NO levels via the ISO-NO Mark II Analyzer was performed using two different measurement methods. The first method using the reductase preparation was performed after first preparing nitrate standards to ensure internal control in a graduated fashion from 0 μM to 500 μM. Biological NO is rapidly oxidized to nitrite and nitrate. As nitrite concentration is the preferred serum

STUDY PROTOCOL

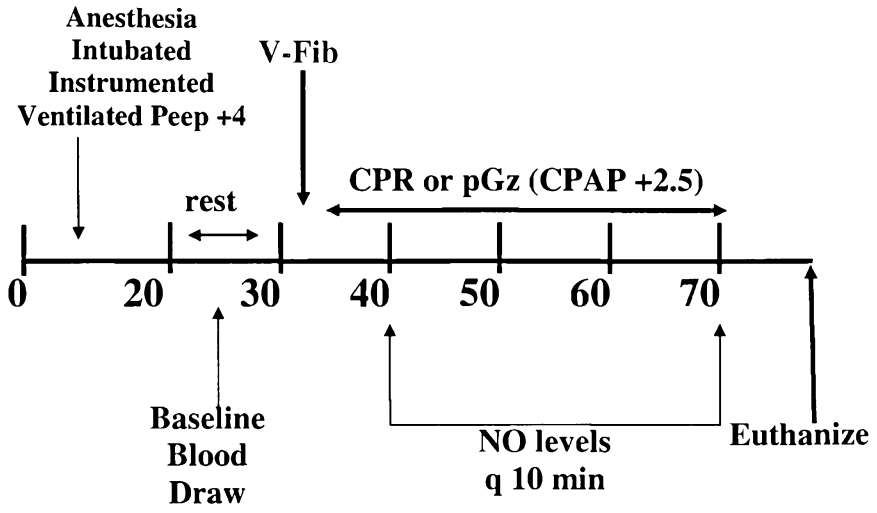


Figure 3-1. Study Protocol

measurement, thawed room temperature serum NO samples were reduced from nitrate to nitrite by treating the samples with cooperized cadmium pellets (Cd-Cu) that were washed with both cleaning and 1X buffer solutions. These solutions were removed via a pipetting technique. The NO serum sample and a 3X buffer were added to the specimen tubes containing the remaining Cd-Cu Reducer pellets, shaken for 25 minutes and centrifuged for an additional 5 minutes. The second ISO-NO Mark II Analyzer method, deproteinization, used the above reductase preparation after two additional solutions were

added to remove protein. The same procedure for the preparation of internal control standards was used as in the reductase preparation. Serum NO was also quantified using a Greiss reaction on reductase samples, prepared as described earlier, and the Greiss assay carried out in a 96-well microtiter plate format. The Greiss reaction assay was then read in a microtiter plate scanner at 540nm, with water as the reference, and had a sensitivity of 10.6 μM. Internal control was also established using known amounts of sodium nitrite in a graduated fashion and via a standard curve.

Sample

Twelve randomly selected juvenile piglets (9.5 $\pm$ 12.09 kg) were used for this study and were provided by a grant from Miami Heart Institute and housed at the Harry Pearlman Research Institute at Mount Sinai Medical Center. The swine model used in the study was presumed free of any pathology.

Estimation of Sample Size

A sample of two groups of 6 piglets each was used in the study. This sample size was based on previous studies using the pGz model, Utstein guidelines, provisions of Miami Heart Institute, and was appropriate for a pilot study for feasibility. The pGz experiments, on the production of NO in anesthetized pigs, is part of an ongoing study on various biological responses during pGz resuscitation currently in progress at the Mount Sinai Research facility.

Ethical Considerations

The principle investigator completed the animal certification course as required by the Institute for Animal Care and Use Committee (IACUC) and approved by Mount Sinai Medical Center-Miami Heart Institute and Florida International University. The research

protocol was performed and reported per compliance with the *Utstein-Style Guidelines for Uniform Reporting of Laboratory CPR Research*, the Animal Welfare Act, and any other public laws and regulations as are appropriate. The use of a swine model allowed for necessary invasive procedures, stimulation of ventricular fibrillation, and performance of subsequent resuscitation processes as were needed to complete the experiment. The animals were housed and cared for at the Mount Sinai Research facility in the appropriate manner. The experimental procedures utilized during the study endeavored to minimize both animal use and frequency of invasive procedures. At the completion of the experiments, all animals that survived were euthanized by a lethal barbiturate dose as per IACUC requirements.

Animal Preparation

The preparation of the study animals was performed using the protocol of previous pGz experiments (Adams et al., 2000a; 2000b; 2001a). Animals under study were anesthetized with Ketamine (10mg.kg, I.M.) prior to entering the research laboratory procedure site. Additional Ketamine was administered prior to invasive procedures and the animals were held in a surgical plane of anesthesia with an intravenous Diprivan and Ketamine combination. Paralysis was induced via Pancurium Bromide at 0.1 mg/kg with continued supplementation as needed. The depth of anesthesia was determined by loss of the corneal and spinal reflex with accompanying animal heartbeat of 150 bpm (Adams et al., 2000a; 2000b; 2001a).

Prior to pGz movement or standard CPR procedures, the animals were intubated via an endotracheal tube and the femoral artery was cannulated to facilitate the procurement of arterial blood specimens, arterial blood gases, and arterial blood pressure

measurements. An electrocardiogram continuously monitored the animals' heart rhythm and the animals received 100% O_2 with 5cmHg CPAP in order to reduce hypoxic consequences. Additionally, a right atrial catheter was placed in the internal jugular vein and used for administering fluids and medications and measuring right atrial pressure. All invasive catheters were sized according to animal anatomical requirements. A bipolar fibrillating wire was placed at the apex of the heart and this was used to induce ventricular fibrillation in the study animals. Stimulation of ventricular fibrillation was initiated via delivery of 30 volts of A.C. current at 60 Hz (Adams et al., 2000a; 2000b; 2001a).

The animals were placed on the motion platform with extremities bound in order to maintain close approximation with the platform device. The platform moved in a cephalic foot to head direction with periodic accelerations and decelerations. This motion allowed for manipulation of the speed and frequency of oscillations (Adams et al., 2000a; 2000b; 2001a).

Method of Data Analysis

The repeated measures non-parametric experimental design for the within group experiments (H_1), and the between group comparison (H_2), was performed using various statistical methods. Originally, a repeated measures two-way ANOVA was the statistical analysis chosen for the study as this design is a robust parametric method that allows multiple values to be obtained from the same animal, using the animal as its own control, and ensuring a high amount of equivalence among the study subjects (Polit & Hungler, 1999). Parametric statistical methods are used to analyze experimental studies for examining causality but must have three essential elements: (1) random sampling, (2)

manipulation of the independent variable, and (3) control of the experiment (Burns & Grove, 1993). The decision to use a non-parametric statistical method was reached once it became evident that the criteria for parametric testing was not met. Parametric testing infers the following assumptions: (1) a normally distributed population in which the variance can be calculated, (2) an interval or ordinal level of study data available, and (3) the available data appropriate for use as random samples (Burns & Grove, 1993). The appropriateness of parametric testing for this study was evaluated via the following analysis. First, a Mauchly's test of sphericity was performed with the results indicating the assumption of symmetry met and suggesting a univariate approach appropriate. The Greenhouse-Geiser and Huynh-Felt tests, used for correction of compound symmetry for repeated measures ANOVA, were also performed but here the criteria were not met. At this point, the repeated measures ANOVA parametric design was deemed inappropriate for use in this study. Next, the Kolmogorov-Smirnov test for normality was performed showing an abnormal distribution, based on the sample size, and indicating the need for nonparametric testing. The nonparametric test chosen for the study was the Friedman matched samples, which parallels repeated measures ANOVA in populations lacking normality. In the samples where Friedman showed significance, the non-parametric Wilcoxon signed ranks test was chosen to evaluate changes in pretest (baseline)/ posttest (10 minute intervals). Both the magnitude and direction of the change can be measured by the Wicoxon design and the signed-ranking is particularly useful in studies where negative values (decreases in NO levels) are possible. The Kruskal-Wallis test was used to compare the two groups (pGz and standard CPR) and is the most powerful nonparametric one-way ANOVA to detect differences between groups while testing the

null hypothesis that all samples are from the same population (Burns & Grove, 1993). For the non-parametric tests, Kruskal-Wallis signed ranks and Friedman matched samples, scores were converted into ranks with the analysis comparing the mean ranks in each group.

4. Results

This study examined the effects of intraoperative resuscitation, via pGz or standard CPR, on the production of NO. Twelve randomly selected juvenile pigs, ranging in weight from 9.5 to12.0 kg were used in both experiment sets of this study. Data were analyzed using nonparametric tests using a univariate approach. Based on the results of the Mauchly's test of sphericity, the assumption of compound symmetry was met; therefore, the univariate approach was used. All data analyzed failed the test for normality and the homogeneity of variance requirement was not met. Analysis of residuals indicated that the assumptions underlying the ANOVA were not satisfied; therefore, the nonparametric test, Kruskal-Wallis, was used. In addition, overall resuscitation method, time, and interaction effect were not significant using the Greenhouse-Geiser and Huynh-Feldt correction for lack of compound symmetry, an assumption underlying Repeated Measures ANOVA. Therefore, the nonparametric Friedman matched samples test was used. To reduce the possibility of making a Type I error, rejecting the null hypothesis when it is true, alpha was set at 0.05 for all hypothesis tests. To reduce the probability of rejecting the null hypothesis when it is false (Type II error), power was set at 0.80. A sample size of 12 (6 per group) was used for this study based on prior studies using the pGz model, Utstein guidelines, provisions of Miami Heart Institute, and as is appropriate in a pilot study for feasibility. Data were analyzed using the SPSS statistical software for Windows 7.0 on a PC compatible computer.

Results of Specific Hypotheses

Hypotheses proposed for this study dealt with (a) levels of NO during pGz resuscitation (H_1), (b) levels of NO during standard CPR resuscitation (H_2), (c) levels of

NO during pGz and standard CPR resuscitation (H_3). Results are reported in means and standard deviations (SD) unless otherwise noted.

Effects of pGz on NO Levels

Hypothesis 1: There is a difference between NO levels during pGz resuscitation and baseline levels prior to pGz resuscitation.

Mean scores of NO were compared between baseline levels of NO and NO levels at 10 and 20 minutes (Table 4-1) (Figure 4-1). Using the reductase method, after pGz was initiated, NO levels rose to 2.63 (18.80) at 10 minutes, which was not significant from baseline and at 20 minutes NO levels decreased to -5.13 (11.15), which also was not significant. Using the deproteinizing method, after pGz was initiated, NO levels decreased to -4.83 (14.41) at 10 minutes, which was not significant from baseline and at 20 minutes NO levels decreased to -8.97 (13.23), which also was not significant. However, when the Greiss method was utilized, the decreases in NO levels were significant at both 10 minutes and 20 minutes. At 10 minutes NO levels were -4.57 (3.68), which was significant at $p = 0.046$ and at 20 minutes mean NO levels were -6.70 (5.14), which was significant at $p = 0.028$. There was no significance between 10 and 20 minutes. Therefore, the hypothesis was supported only when the Greiss method of NO measurement was used.

Hypothesis 2: There is a difference between NO levels during standard CPR and baseline levels prior to standard CPR.

Mean scores of NO were compared between baseline levels of NO and NO levels at 10 and 20 minutes (Table 4-1) (Figure 4-2). Using the reductase method, after CPR was initiated, NO levels decreased to -13.97 (36.07) at 10 minutes, which was not

Table 4-1. Mean Delta No levels, pGz vs CPR (n = 6)

Time	Resuscitation Method	Reductase	Deproteinizing	Greiss
Time 0	pGz	Baseline	Baseline	Baseline
10 min	pGz	2.63 (18.80)	-4.83 (14.41)	-4.57* (3.68)
20 min	pGz	-5.13 (11.15)	-8.97 (13.23)	-6.70* (5.14)
Time 0	CPR	Baseline	Baseline	Baseline
10 min	CPR	-13.97 (36.07)	4.07 (12.22)	5.34* (7.10)
20 min	CPR	-40.45*± (37.53)	-2.46 (17.08)	1.93 (7.95)

This table summarizes the comparisons of the different methods of NO measurement during pGz and CPR at baseline, 10 minutes and 20 minutes.

* $p < 0.05$, significantly different from baseline; ± $p < 0.05$, significantly different from 10 min

Figure 4-1. Delta NO Means-pGz

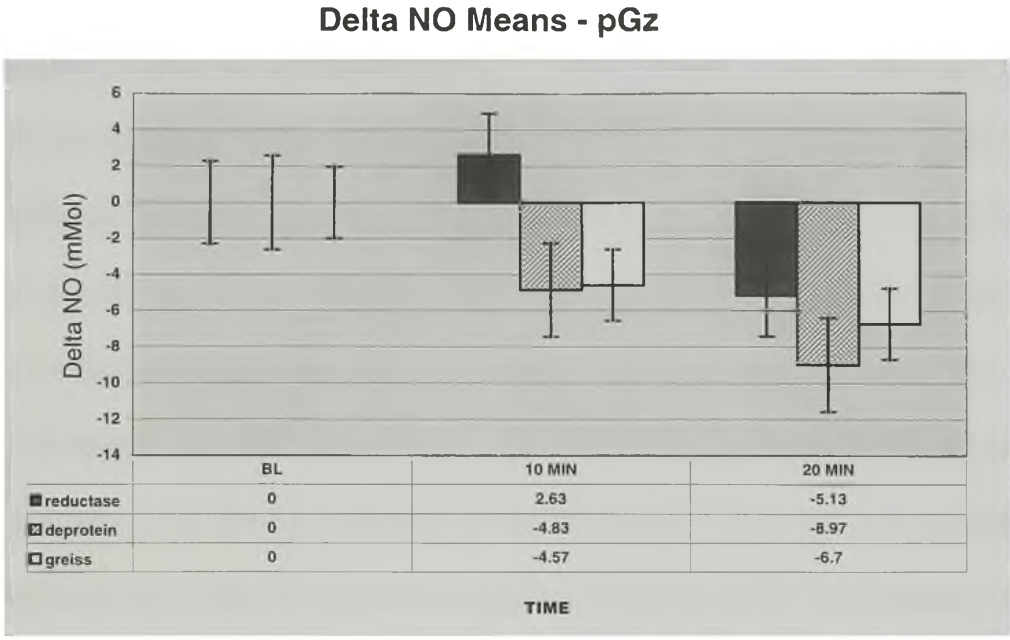


Figure 4.1. Within group comparison of NO levels during pGz at baseline (BL), 10 minutes and 20 minutes.

Figure 4-2. Delta NO Means – Thumper

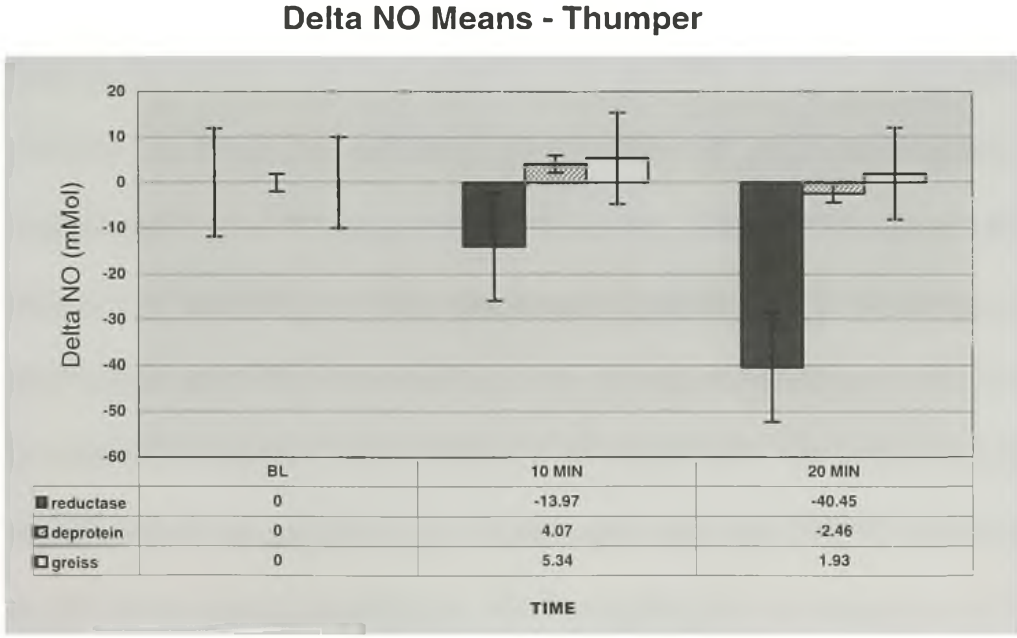


Figure 4.2. Within group comparison of NO levels during thumper at baseline (BL), 10 minutes and 20 minutes.

significant from baseline (p = 0.249) and at 20 minutes NO levels decreased to -40.45 (37.53), which was significant ($p = 0.028$). In addition, NO levels were significantly different between 10 and 20 minutes (p = 0.028). Using the deproteinizing method, after CPR was initiated, NO levels increased to 4.07 (12.22) at 10 minutes, which was not significant from baseline and at 20 minutes NO levels decreased to -2.46 (17.08), which also was not significant. However, when the Greiss method was utilized, the increases in NO levels were significant only at 10 minutes with NO levels of 5.34 (7.10), $p = 0.028$. At 20 minutes mean NO levels were 1.93 (7.95), which was not significant at $p = 0.600$. There was no significance between 10 and 20 minutes. Therefore, the hypothesis was supported only when the reductase and Greiss methods of NO measurement was used.

Hypothesis 3: The NO levels are greater during pGz resuscitation than during standard CPR.

Mean rank scores of NO levels were compared between pGz and CPR at 10 and 20 minutes (Table 4-2) (Figure 4-3). Comparing pGz with CPR using the reductase method, at 10 minutes pGz was ranked at 7.33 and CPR was 5.67, which was not significant. At 20 minutes, pGz was 8.42 and CPR 4.58, which also was not significant. Comparing pGz with CPR using the deproteinizing method, at 10 minutes pGz was ranked at 5.50 and CPR was 7.50, which was not significant. At 20 minutes, pGz was ranked at 6.00 and CPR 7.00, which also was not significant (Figure 4-4). Comparing pGz with CPR using the Greiss method, at 10 minutes pGz was ranked at 3.50 and CPR was 9.50, which was significant ($p = 0.004$), indicating that CPR NO levels are higher than pGz levels during resuscitation. At 20 minutes, pGz was ranked at 4.67 and CPR

Table 4-2. Mean Ranks (n = 6)

Time	Resuscitation Method	Reductase	Deproteinizing	Greiss
Baseline	pGz	6.50	6.50	6.50
Baseline	CPR	6.50	6.50	6.50
10 min	pGz	7.33	5.50	3.50
10 min	CPR	5.67	7.50	9.50*
20 min	pGz	8.42	6.00	4.67
20 min	CPR	4.58	7.00	8.33

This table summarizes the ranked comparisons of NO levels between pGz and CPR at baseline, 10 minutes and 20 minutes.

* p = 0.004, significantly different from baseline

Figure 4-3. Delta NO Means – Reductase. Thumper vs. pGz.

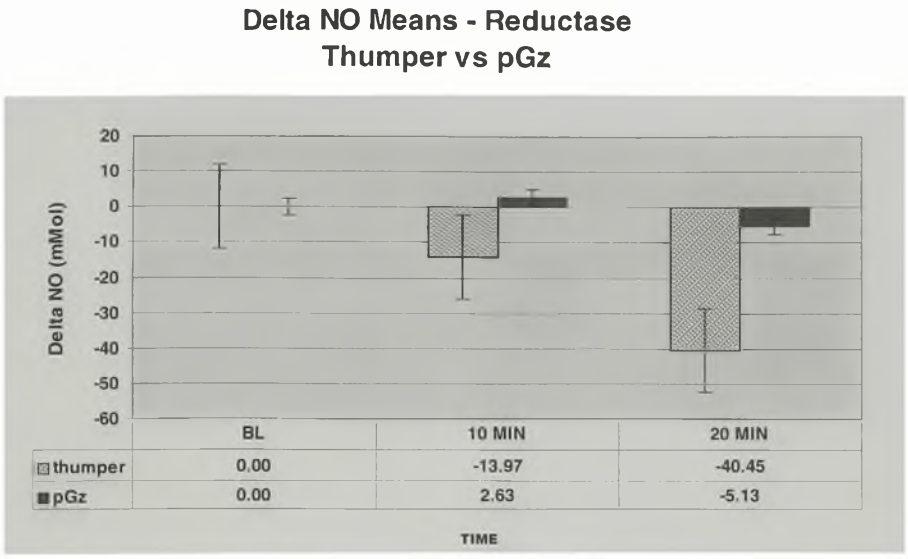


Figure 4.3. Between group comparison of NO levels using reductase measurement.

Figure 4-4. Delta NO Means – Deproteinization. Thumper vs. pGz.

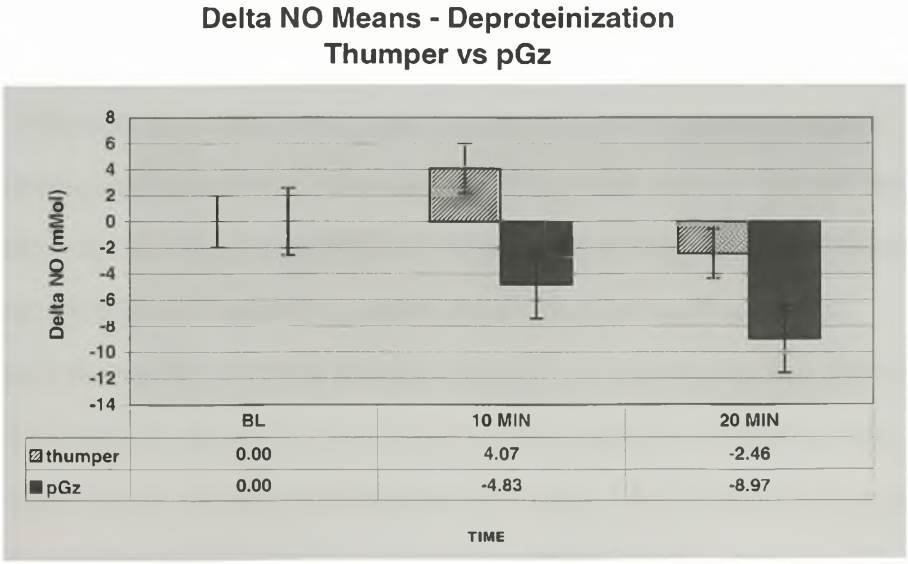


Figure 4.4. Between group comparison of NO levels using deproteinization measurement.

Figure 4-5. Delta NO Means – Greiss Assay. Thumper vs. pGz.

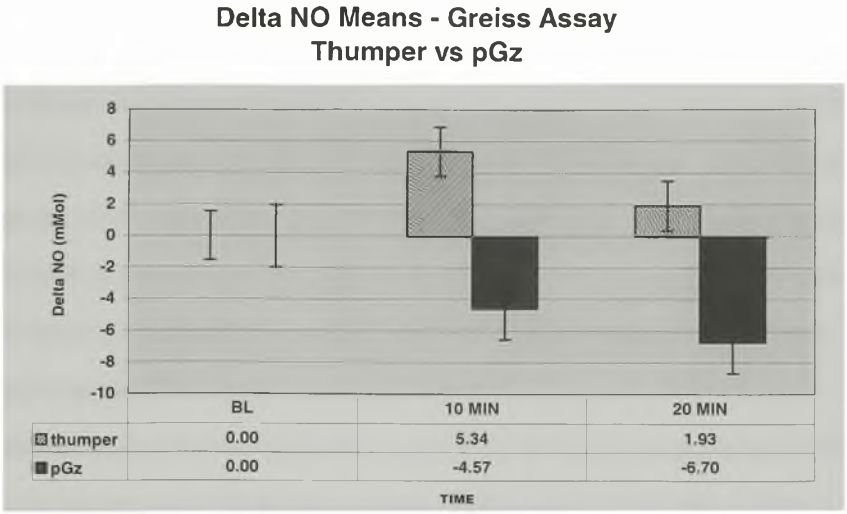


Figure 4.5. Between group comparison of NO levels using Greiss Assay measurement.

8.33, which was not significant (Figure 4-5). Therefore, the third hypothesis was not supported.

Major Findings

Differences in serum NO levels from baseline to 10 and 20 minutes, in animals undergoing pGz resuscitation, were noted as follows: (1) the reductase ISO-NO Mark II analysis showed a rise in mean NO levels from baseline at 10 minutes and a decrease in mean NO levels at 20 minutes but neither change was found significant, (2) the deproteinization ISO-NO Mark II analysis showed a decrease from baseline in NO levels at both 10 and 20 minutes but these were also without significance. However, when using a Greiss reaction, with pGz resuscitation serum samples, a significant decrease in mean NO levels from baseline was noted at both 10 and 20 minutes. Since it was hypothesized

that there would be a change in serum NO levels from baseline, in animals undergoing pGz resuscitation, the first hypothesis was only supported in the NO levels measured via the Greiss reaction. Differences in NO serum levels from baseline to 10 and 20 minutes, in animals undergoing standard CPR, were noted as follows: (1) the reductase ISO-NO Mark-II analysis showed a decrease in mean NO levels from baseline at both 10 and 20 minutes, (2) the deproteinization ISO-NO Mark II analysis showed an increase in mean NO levels from baseline at 10 minutes and a decrease at 20 minutes. Here, the increase in NO levels at 10 minutes from baseline using the reductase measurement, in animals under standard CPR, was not significant; however, the decrease in NO levels at 20 minutes from baseline was significant. A significant difference was also noted in NO levels between 10 and 20 minutes using the reductase method. The deproteinization method of NO measurement, in animals undergoing standard CPR, did not show significance in either the 10 minute increase in NO from baseline or the 20 minute decrease in NO from baseline. When using the Greiss reaction to measure NO levels from baseline, in animals undergoing standard CPR, a significant increase was noted at 10 minutes. Although an increase in mean NO levels from baseline at 20 minutes was also noted, it was without significance and there was no significance between the 10 and 20 minute NO levels using the Greiss method. The second hypothesis stated that there would be a difference between NO levels, during standard CPR, and baseline levels prior to standard CPR, which was, only supported in NO levels using the reductase method at 20 minutes and between 10 and 20 minutes under ISO-NO Mark II analysis, as well as, NO levels at 10 minutes using the Greiss reaction. When testing the third hypothesis, which stated that the NO levels would be greater during pGz resuscitation than during

standard CPR, the following was noted: (1) when using the reductase measurement the mean pGz NO levels were greater than CPR at both 10 and 20 minutes but without significance, (2) when using the deproteinization measurement the pGz NO levels were less than CPR at both 10 and 20 minutes also without significance, and (3) when using the Greiss reaction measurement the mean NO levels under pGz resuscitation were less than CPR at both 10 and 20 minutes with only the 10 minute measurement significant. Therefore, the third hypothesis was not supported.

Although, significant differences in NO levels from baseline were noted in some of the reductase, deproteinization, and Greiss reaction methods of measurement, under both pGz resuscitation and standard CPR, these differences were inconsistent and none of the hypotheses well supported. These inconsistencies may be due to various factors, such as flaws with the measurement tools, unknown pathology in the animal, and small size of the study sample.

5. Discussion and Conclusion

The basis of this study was a theoretical framework created from the literature review of research supporting the postulate that the production of NO in pigs can be induced by shear stress and pulsatile vessel flow as believed to occur during pGz motion platform resuscitation. In order to support the study hypotheses, animals were studied under two types of resuscitation, pGz and standard CPR, to determine changes in serum NO levels from baseline versus NO levels at 10-minute intervals during resuscitation. The changes in serum NO levels were also compared between the two forms of resuscitation. Increased production of NO was expected under pGz resuscitation due to the movement of the motion platform. As pGz resuscitation is a novel approach, not much literature exits on its effect on physiological responses. Therefore, this study was performed to determine whether an increase in endogenous NO is actually a physiological response to pGz resuscitation and whether such an increase in NO is greater in magnitude during pGz resuscitation than the NO found during standard CPR.

Three hypotheses were tested in this study on the effect of manipulating two types of intraoperative resuscitation, pGz and standard CPR, on physiological responses such as endogenous NO levels in anesthetized pigs. In order to test the hypotheses, three forms of serum NO measurement techniques were used: reductase and deproteinization using the ISO-NO Analyzer and the Greiss reaction assay using the microtiter plate scanner. Although each measurement technique showed some changes in NO levels from baseline, the results were not consistent and none of the hypotheses well supported. In fact, significant increases in serum NO levels were found during standard CPR contrary to the expected increase during pGz. The difficulty in accurately measuring a local mediator

such as NO, found in such small basal quantities with a half-life of 3-5 seconds and that rapidly oxides to nitrite and nitrate, has been written of in numerous research studies over the past 20 years (Beckman, 1996; Berkels et al., 1999; Palmer, Ferrige, & Moncada, 1987). Therefore, to have found any difference in NO levels from baseline in this study is believed to be extremely promising. Possible explanations for the variations in the study results will be addressed in the following discussion.

Discussion

Hypothesis 1 postulated that there would be a difference between NO levels during pGz resuscitation and baseline levels prior to pGz resuscitation. The hypothesis was not supported. Results from the present study indicate that mean NO levels were decreased at both 10 and 20 minutes when measured via the Greiss reaction but no significance was found utilizing the other two measurement techniques (reductase and deproteinization) when using the ISO-NO Analyzer. As previously mentioned, measuring NO is extremely difficult and the inconsistency in the results may result from factors such as the extremely short half-life of NO in biological systems (3-5 seconds). Another possibility may be related to the use of serum samples rather than cell cultures as a medium. All the research cited in the literature review was performed using cell cultures and studies that measured NO utilizing only serum samples were not found. However, Adams et al (2001) found increased serum NO levels during their studies on regional blood flow during pGz resuscitation. In Adams experiment, NO was measured via the Greiss reaction but utilized a different reduction (nitrate to nitrite) modality than was used in the current study. The quantification of NO via reductase or deproteinization methods using the ISO-NO Analyzer or measurement of NO via the Greiss method requires the reduction of

nitrate to nitrite. This reduction is accomplished by treating the samples with metals, such as cadmium or zinc, or by using reductase enzymes (Beckman, 2001). The current study utilized cadmium (Cd-Cu Reducer), which depends on factors such as time, surface area, sample volume ratio, temperature, and pH for conversion ability (WPI, 2001). Alterations or inconsistencies in any one of these factors may interfere with accuracy. Although a deproteinization method was used to avoid the deposition of proteins and/or particulate substances on the reducer surface, this may be another possible reason for inaccuracy in the NO results. Preparation of the Cd-Cu Reducer also requires a buffering agent, that may possibly become contaminated with transition metals, iron or copper and which may bind and deactivate NO. Contamination with O2 has also been cited as problematic when transferring NO samples in syringes (Beckman, 1996). In the study by Adams, the reduction method employed was diazotization with 1% sulfanilamide in 30% acetic acid and Azo coupling performed combining the diazo product with an equal volume of 0.1% N-(l-napthyl) ethyldiamine hydrochloride in 60% acetic acid (Adams et al, 2001). This may be a better method of NO reduction when utilizing a Greiss method of measurement in future studies.

Another possible explanation for the decrease in NO during pGz resuscitation is the effect of reperfusion injury as occurs during cellular hypoxia resulting from low flow states, circulatory shock, and/or cardiopulmonary resuscitation. Research indicates hat ischemia alters or primes cells making them more vulnerable to reperfusion injury related to an oxygen burst of toxic reactive oxygen intermediates (ROIs) (Waxman, 1996). Endothelial cells in particular may be directly injured by reperfusion and the inflammation process which follows. Such injury includes damage to cell membranes,

proteins, and chromosomes resulting in an increased cell permeability and production of procoagulant substances (Waxman, 1996). These effects are generally considered opposite to the effects of NO production and may be due to altered production by the damaged endothelial cell. Endothelial production of NO during normal conditions, as occurs during laminar shear stress or pulsatile vessel flow, is thought to have anticoagulant and antioxidant properties that are protective against reperfusion injury (Maier & Bulger, 1996).

Endothelial cell injury has been suggested as a result of the secondary inflammatory process more than a result of the original ischemia or reperfusion (Maier & Bulger, 1996; Waxman, 1996). Inflammation has been shown as responsible for alterations in coagulation, regulation of vascular tone, vascular permeability, and regulation of leukocyte adherence (Maier & Bulger, 1996; Waxman, 1996). Reperfusion stimulates pro-inflammatory cytokines and macrophages to simultaneously produce both superoxide and NO (Beckman, 1996). Research has shown that superoxide rapidly reacts with NO converting the majority of NO to form peroxynitrite (ONOO-), a toxic reactive oxygen intermediary. In *Nitric Oxide Principles and Actions*, Joseph Beckman (1999) states, "For each 10-fold increase in the concentration of nitric oxide and superoxide, the rate of peroxynitrite formation will increase by 100-fold" (p. 40). Peroxynitrite has been implicated in DNA alterations, cell damage, and cell death (Szabo, 1996). Research also suggests that it is the ratio of NO to superoxide that is responsible for the production of peroxynitrite and that enhanced production of NO may actually limit the oxidant reactivity of peroxynitrite (Szabo, 1996). Whether or not reperfusion injury and endothelial cell damage accounts for decreases in NO during pGz resuscitation in this

study is questionable; however, the measurement of arterial blood gases (ABG's) and superoxide levels would be helpful in evaluating possible cellular hypoxia and NO/superoxide ratios.

Another possible factor in the NO measurement inconsistencies is whether or not pGz produced laminar or pulsatile flow and vessel stretch during the resuscitation studies. Adams et al., studied regional blood flow and novel CPR during pGz resuscitation and indicated that the platform motion produces pulsatile shear stress and vessel wall stretch resulting in vasodilation (2001a; 2001b). Numerous studies have suggested that laminar flow related shear stress plays a role in the production of NO (Inoue et al., 1996; Uematsu et al., 1995; Zafari et al., 1999). Conversely, research suggests that oscillatory shear stress may reduce the steady-state level of NO and is implicated in the development of atherosclerosis in areas of turbulent flow (Zafari et al., 1999).

Laminar flow is defined as flow that is streamlined and smooth, moving in layers or lamina, in areas of high viscosity (Davis, Parbrook, & Kenny, 2001). Viscosity is associated with shearing forces, the amount and type of which has importance in cell orientation and blood vessel integrity (Davis et al., 2001; Traub & Beck, 1998.) Laminar and pulsatile flow occur in linear areas and produce a positive shear stress that is associated with the beat-to-beat changes of the cardiac cycle (Traub & Beck, 1998). Research suggests that while the laminar flow produced during various research studies may yield similar effects as the physiologic beat-to-beat positive shear stress or pulsatile flow, the flow produced during experimentation is a steady shear stress and may have quantitative and qualitative differences related to cell orientation (Traub & Beck, 1998). However, research suggests that the flow produced by the pGz motion platform more

closely mirrors the cardiac related beat-to-beat pulsatile flow than other experimental modalities (Adams et al., 2001a, 2001b). In areas of blood vessels that have curvature or angling, turbulent flow is produced with periods of flow reversal or oscillation (Traub & Beck, 1998). This oscillatory flow is associated with the fatty streaks and plaque that produce atherosclerosis and numerous other pathologic conditions (Traub & Beck, 1998). The production of NO has been suggested as the primary factor responsible for the athero-protective mechanisms of laminar and pulsatile positive shear stress (Traub & Beck, 1998).

Hypothesis 2 postulated that there would be a difference between NO levels during standard CPR and baseline levels prior standard CPR. This hypothesis was not supported. Significance was found in decreased NO levels at 10 minutes and between 10 and 20 minutes using reductase and the ISO-NO Analyzer. Once again, these inconsistencies are probably related to the measurement tools, use of serum versus cell culture sample, and possible reperfusion effects on endothelial cells as explained above. Interestingly, when the Greiss method was used to measure NO levels during standard CPR, NO levels were significantly increased at 10 minutes. Standard CPR was performed using the *Thumper* resuscitation tool, which vigorously applied 100 compressions per minute to the pig's chest wall. On autopsy, it was noted that all six pigs, resuscitated via the *Thumper* tool, had fractured ribs. There is a possibility that the increase in NO, noted at 10 minutes, may be due to cytokine inducible NO (iNOS). Cytokine inducible NO is stimulated by a wide variety of cells, including macrophages, as may accompany the inflammatory process associated with bone fracture (Kelley et al., 1996). It may be helpful to note animal pathology related to NO results in future studies.

Hypothesis 3 postulated that the NO levels would be greater during pGz resuscitation than during standard CPR. This hypothesis was also not supported. Comparing pGz to standard CPR using the Greiss method indicated that NO levels were significantly higher than pGz levels at 10 minutes. Once again, these results are most likely due to the various possible problems with the measurement tools. Additionally, there is a possibility that the increase in NO during CPR is related to an increase in iNOS associated with the animal's pathological rib fractures.

Recent research has suggested that the action of certain anesthetic agents, on the production or inhibition of NO pathways, may account for the variability of results found in numerous research studies evaluating the properties of NO. Galley et al., (2001) found that ketamine and xenon anesthesia increased cGMP in the brain and spinal cord. The formation of cGMP is the primary route in which NO acts as an intracellular messenger in neural pathways (Galley et al., 2001). Conversely, diazepam and pentobarbital had no effect on cGMP in the same study (Galley et al., 2001). Whether or not ketamine played a role in the inconsistency of the current study's results is impossible to determine; however, ketamine was used as a sedative and anesthetic agent in all 12 animals. It may be beneficial to use a neutral agent such as pentobarb in future studies.

Conclusion

In conclusion, NO was significantly increased at 10 minutes during standard CPR and using the Greiss method of measurement. Decreases in NO were found during pGz under Greiss assay at both 10 and 20 minutes. The increase in NO was greater at 10 minutes during standard CPR than during pGz. Although some changes in NO levels were

significant, none of the hypotheses were supported. It is unclear from this study whether or not pGz resuscitation has an effect on the production of NO.

The inconsistencies in the serum NO level results indicate possible problems with both the use of serum as a sampling modality and the reduction methods utilized in measurement via both the ISO-NO Analyzer and the Greiss assay tools. Other possible factors associated with the inconsistent results may be unknown animal pathology, unknown effects of anesthetic agents, and unknown flow produced during resuscitation. The nature of NO in itself, with such a short half-life, is also problematic.

Limitations

The design of this study used an experimental repeated measures format to determine the possibility of increased NO levels in anesthetized pigs during pGz and standard CPR. Animal preparation, maintenance, stimulation of ventricular fibrillation, and motion platform variables utilized the protocol employed in previous pGz studies. Serum was drawn from an arterial line at 10-minute intervals, frozen, and samples prepared and measured via the ISO-NO Analyzer and Greiss reaction method at a later date. Serum samples were kept frozen in an -80° freezer until the time of use. Serum specimens required preparation via a reduction method described previously which recommended the use of room temperature serum. A limitation of this study is that the temperature of the thawed samples was not ascertained prior to sample preparation. As stated previously, alterations in variables such as temperature can interfere with the reduction of nitrate to nitrite and hence accurate measurements.

Prior to the induction of the pGz platform motion, animals were paralyzed, intubated, and the femoral artery cannulated for blood draws. Although, the animals were

maintained with only 100% CPAP during pGz, they were placed on mechanical ventilation prior to the onset of ventricular fibrillation. During the rest period before the pGz platform motion was initiated, arterial blood gases were drawn at 5-minute intervals and adjustments made to the ventilator parameters. A limitation of this study is that no record was kept regarding these ventilator changes, nor were the arterial blood gases included in this study. The lack of recorded ventilatory changes and associated arterial blood gases may have impacted the knowledge of animal status prior to experimentation.

Additionally, autopsy of the animals post *Thumper* resuscitation revealed that all six of the study animals had fractured ribs. The fact that autopsy results were not recorded on any of the animals under study is another possible limitation of this study.

Generalization of the study findings is not possible because the study used only male pigs as study specimens. It is not known whether a difference exists in the production of NO between male and female pigs. Therefore, this is a limitation of the study. Although swine are known to have a similar cardiovascular system to humans, it is also not possible to generalize study findings as representative of human populations and this is another limitation of the study.

The greatest limitation of this study is associated with the small sample size (n=12). Although, this sample size was appropriate in a pilot study for feasibility, results were inconsistent. Small sample sizes result in abnormal distributions of measurement numbers, which makes it necessary to use non-parametric statistics for analysis. Parametric statistical methods are generally deemed appropriate for analyzing true experimental results as they have rigid requirements, one of which is a larger sample size. However, as a rule of thumb in research is to obtain data from as large a sample as is

practical and at a feasible cost, the sample size used in this study was appropriate (Polit & Hungler, 1995). Unfortunately, the small sample size increases the probability of inconsistent results and errors in measurement.

The results inferred from this study must be seen as groundwork for feasibility of future studies on the production of NO during pGz resuscitation. Measurements of NO were taken from serum samples of unknown temperature and as such may not be accurate. Generalization of study findings is not possible due to the use of only male animals, nor is generalization between animal and human populations possible although they share a similar cardiovascular anatomy. Ventilator assisted respiratory parameters were adjusted prior to the onset of pGz, based on arterial blood gases, but these were not recorded. Autopsy results on the animals post-experimentation were also not recorded and may have impacted the study. The smallness of sample size skewed the distribution abnormally and necessitated the use of non-parametric statistical analysis, which may not be appropriate for analysis of the study experiments. Although, numerous limitations and inconsistencies exist in this study, the ability to obtain measurement values for a short biological mediator such as NO, is extremely encouraging.

Recommendations for Future Research

This study revealed issues and weaknesses in certain areas, which may be corrected in future studies. A fundamental question arising from the study is to find a better method of reducing nitrate to nitrite for measurement. Repeating the study utilizing the method outlined by Adams et al., (2001b), as described previously, might ensure more accurate results using the Greiss assay method. Ascertaining consistent room temperature specimens, with associated and appropriate pH, may ensure greater accuracy in the

reduction preparation of the serum samples for measurement. The use of cell culture confluent rather than serum, for measurement of NO via the ISO-NO analyzer, may also yield more accurate results.

Repeating the study with a larger sample size, and a mixture of male and female animals, is probably the most important change to make in the study design and would possibly produce more normally distributed data and increase the ability to generalize study results. The larger sample size would also allow the use of parametric statistical analysis possibly providing greater accuracy and consistency in results.

Another recommendation for future studies is to use a different standard CPR technique than the *Thumper* to compare NO levels between pGz and standard resuscitation. Although the *Thumper* provided consistent and vigorous chest compressions, its effectiveness was negated by the concomitant production of rib fractures in all the animals under study utilizing that form of standard CPR. Numerous other modalities are available such as: vest CPR, active compression-decompression (ACD) CPR, an inspiratory impedence threshold valve, and phased thoracic-abdominal compression and decompression (PTACD) (Lurie, 1997) An additional alternative to these resuscitation techniques is manual CPR performed by the investigators; however, the use of manual CPR has produced variability related to the effectiveness of chest compressions between performers (Pinchak et al., 1988).

Future studies would benefit from recording all arterial blood gases, and ventilator changes, both before and after the induction of resuscitation. Knowledge of these values might reveal pathological conditions in the study animals. The measurement of

superoxide and the superoxide to NO ratio might unveil the presence of reperfusion injury and subsequent endothelial damage, which could alter the production of NO. Repeating the study with the addition of calcium and potassium levels during pGz resuscitation might also be beneficial. All three isoforms of NOS, and therefore the synthesis of NO, are dependent on the binding of calmodulin, an intracellular protein that binds with calcium to stimulate various biological responses (Kelley et al., 1996). In addition, research has indicated that increased potassium and decreased ionized calcium occur during prolonged CPR and may be responsible for failure of resuscitation efforts (Neiman & Cairns, 1999). As such, alterations in these electrolytes could influence the production of NO. The use of a different anesthetic agent than ketamine may decrease the possibility of interference in the NO pathway, altering the production of NO, which has been suggested with ketamine use (Galley et al., 2001).

Several research studies have indicated evidence of a relationship between the NO pathway and pain nociception and antinociception (Fukada et al., 1999; Lauretti et al., 1999). It has been postulated that NO may play opposing roles in areas such as peripheral, spinal, and supraspinal pain sites. While NO is thought to enhance pain perception in the periphery, NO appears to inhibit pain perception in the descending spinal pathway and may even act directly on *mu* opiod receptors enhancement (Lauretti, 1999; 2000). Synergistic effects have been elicited by combining the NO pathway derivative nitroglycerine, via transdermal patch, with intrathecal neostigmine, sufentanil and both intrathecal and intravenous morphine for post-operative pain control. This synergistic effect has delayed the need for post-op rescue pain medication up to 14 hours

(Laurettti et al., 1999; 2000). Future research exploring the synergistic use of induced NO production and analgesia in chronic pain control might also be advantageous.

Clinical Implications

Although this study did not support evidence of an increase in NO during pGz, several studies exist that describe the vasodilatory properties of pGz resuscitation and point to a vasoactive mediator such as NO (Adams et al., 2000a; 2000b; 2001a; 2001b). Additionally, elevated NO levels during pGz resuscitation were obtained by Adams et al., during regional blood flow studies (2001b). In spite of the existence of several limitations in this study and the obvious need for repeating the experiments, the possibility of enhanced NO production during pGz remains likely. Previous studies utilizing pGz resuscitation have shown that pGz both ventilates the lungs, and perfuses the organs, providing and maintaining oxygenation and circulation which is the goal of CPR (Adams et al., 2000a; 2000b; 2001a; 2001b). Adequate circulation and ventilation has been maintained in animals under study in cardiac arrest for 20 minutes with pGz platform movement. These animals were successfully resuscitated, found to have insignificant reperfusion injuries, and little changes in ABG's (Adams et al., 2001a, 2001b). As such, pGz appears to possess clinical usefulness as a modality in situations of prolonged cardiac resuscitation and possibly the ability to increase survival rates in humans.

Evidence indicates that standard CPR provides only 15-20% of the normal cardiac perfusion and 25-30% of cerebral perfusion (Lurie, 1997). Not only does pGz provide excellent regional blood flow to the heart and brain during cardiac arrest, it also provides almost 50% of flow to the intestinal mucosa via the sphlanchnic artery, were occlusion is responsible for much of the ischemic sequelae and subsequent mortality after standard

resuscitation (Adams 2001b). The ability of pGz to provide increased blood flow is related to vasodilation of the vasculature, which allows driving forces of the platform motion to perfuse target organs. Once again, this vasodilation is thought related to the enhanced production of NO and the associated decreased vascular resistance. The ability to produce increased perfusion to the brain, heart, and other organs, under low-pressure situations, may be clinically significant in human populations experiencing any low flow related pathology. Therefore, pGz and increased production of NO, may be clinically helpful as a treatment modality in decreasing the development of artherosclerosis, preventing cardiovascular changes associated with diabetes, preventing re-stenosis of cardiac vessels post surgical innovation, maintaining circulation in quadraplegic or paraplegic patients, and providing improved circulation during multiple organ failure, congestive heart failure, and sepsis. Additionally, the enhanced production of NO during pGz motion may be of clinical value in maintaining cadaveric or non-beating heart organ donors or brain dead donors for organ transplantation. With the ever-increasing shortage of organs for transplantation, particularly those from living donors, the ability to maintain perfusion and decrease ischemic damage to these organs may also have clinical significance.

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APPENDIX

IACUC APPLICATION AND APPROVAL

FLORIDA INTERNATIONAL UNIVERSITY
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

ANIMAL CARE AND USE FORM

IACUC Approval # _____

I. GENERAL INFORMATION

A. Project Title: EFFECTS OF PERIODIC ACCELERATION (pGz) ON NITRIC OXIDE DURING RESUSCITATION IN ANESTHETIZED PIGS

B. Principal Investigator: Ingrid Gunnlaugsson, BSN, RN SS#: _____

Position: ☐ Faculty ☒ Graduate Student* ☐ Undergraduate Student* ☐ Other (specify): _____

Animal Care Training/Certification: ☐ No ☒ Yes, please indicate date 06 - 06 - 2001

***FACULTY SUPERVISOR** (if PI is a student): John P. McDonough, CRNA, Ed.D., ARNP

Department / Campus Address: Nursing/ ACII 240C Zip: _____

Phone #: _____ Fax #: _____ Email Address: _____

C. Co-Investigators

	Faculty	Grad	Undergrad	Other	Animal Care Training/Certification? If yes indicate date		
Charlotte A. Richmond, PhD, RN	☒	☐	☐	☐	☐ No	☒ Yes	1996
Jose A. Adams, MD	☐	☐	☐	☒	☐ No	☒ Yes	2001
Jorge Bussak, MD	☐	☐	☐	☒	☐ No	☒ Yes	2002
Dongmei Wu, MD, OhD	☐	☐	☐	☒	☐ No	☒ Yes	2002

II. PROJECT INFORMATION

A. Status Of Project Review:

☒ New Project ☐ New Teaching ☐ Revision of previously approved Project ☐ Continuation of approved project

Source Of Funding: Miami Heart Institute ☒ Not Seeking Funding

Start Date: 2001 End Date: 2003

B. Project Summary: In the space below, summarize in lay terms the objectives, animals used and major procedures of the project.

In an ongoing study at the Harry Pearlman Research Institute at Mt. Sinai, intraoperative resuscitation on anesthetized pigs, using a pGz approach (motion platform), is under investigation. Resuscitation via pGz motion is implemented by moving the anesthetized and arrested animal in a headword–to-footward direction using a platform device. By manipulating the platform's speed and frequency, acceleration forces ventilate the pig's lungs and stimulate cardiovascular function. The pGz platform's movement also produces vasoactive modulators such as hemodynamic shear stress and pulsatile vessel stretch that may increase the production of endogenous NO. As such, an increase in endogenous NO is expected with pGz motion resuscitation. Whether an increase in NO actually occurs with pGz and whether such an increase is beneficial, is not known. To date, little research exists on the possible physiological responses to pGz. Furthermore, there is a possibility that with continued research, pGz resuscitation may become applicable to humans in the future as an alternate to standard CPR.

The study will address the following problem in Yorkshire pigs: To what extent does periodic acceleration (pGz) motion affect the physiological response of nitric oxide (NO) during intraoperative resuscitation in anesthetized pigs? The purpose of this study is to compare the physiological responses during pGz resuscitation to standard cardiopulmonary resuscitation in anesthetized and arrested pigs. Two groups of randomly selected male juvenile Yorkshire pigs (n=6 each) weighing (9.5+1.2 kg) will be studied in an experimental repeated measures design. In order to test the study hypothesis, two experiments will be performed: (1) measurement of NO production during pGz resuscitation, and (2) measurement of NO production during standard CPR resuscitation. Comparisons will be made between the two levels. Nitric Oxide levels will be measured at baseline and at 10-minute intervals (X 3) during resuscitation. In order to monitor the animal's cardiovascular function, heart rate (HR) and arterial pressure will be measured via electrocardiogram and cannulation of the femoral artery, respectively. The arterial line will also allow for procurement of blood gases and blood specimen draws. The animals will also undergo endotracheal intubation and will receive 100% O2 with 5 cmHg CPAP in order to reduce hypoxic consequences. Additionally, a right venous catheter will be placed in the internal jugular vein and used for administering fluids and medications and measuring right atrial pressure. All invasive catheters will be sized according to animal anatomical requirements. A bipolar fibrillating wire will be placed subcutaneously, across the apex of the heart, and used to induce ventricular fibrillation in the study animal via delivery of 30 volts of A.C. current at 60 Hz. The animals will be placed on the motion platform with extremities bound in order to maintain a close approximation with the platform device. The platform will move in a cephalic head to foot direction with periodic accelerations and decelerations. This motion will allow for manipulation of the speed and frequency of oscillations. The frequency of acceleration and deceleration movements range from O.5-10 Hz at a force of 0.1-1.5 G. Approximation of beats per minute (bpm) to corresponding frequency of motion is 1 Hz = 60 bpm.

III. PROCEDURE CHECKLIST:

			Affirmative answer requires completion of:
A. Tissue only?	☐Yes	☒No	Section VI

B. Live vertebrates involved?	☒Yes	☐No	Section VI
C. Immunization or bleeding	☒Yes	☐No	Section VII
D. Ascites production and harvesting?	☐Yes	☒No	Section VII
E. Drug administration(other than anesthetics or analgesics)	☐Yes	☒No	Section VIII
F. Surgical procedure?			
1. Terminal?	☒Yes	☐No	Section VI
2. Survival?	☐Yes	☒No	Section IX
3. Multiple survival?	☐Yes	☒No	Section IX
G. Special circumstances:			
1. Exposure of live vertebrates to hazardous agents? (infections, radioactive or carcinogenic agents)	☐Yes	☒No	Section X(A)
2. Restraint of animal's freedom of movement?	☒Yes	☐No	Section X(B)
3. Special diets, fasting or housing conditions	☐Yes	☒No	Section X(C)
4. Pain or distress experienced by subjects	☐Yes	☒No	Section X(D)
5. Production of blindness or paralysis	☐Yes	☒No	Section X(D)
6. Death as an endpoint in any procedure	☒Yes	☐No	Section X(E)
7. Will animals be fasted	☒Yes	☐No	Section X(F)
8. Special assistance from facility staff	☐Yes	☒No	Section X(G)
9. Special circumstances requiring additional documentation	☐Yes	☒No	Section X(H)

IV. ANIMALS

A. What species and how many animals/species will be used?
Yorkshire pigs. In total we propose the use of 12 pigs. Due to arrythmia and other possible complications, we expect to utilize a total of 16 animals.

B. Justify the necessity to use this (these) species. Indicate why non-animal alternatives cannot be used by describing the methods and sources which helped you determine that alternatives are not available.
This is a part of a feasilbilty study of non-invasive motion platform for CPR currently being conducted at Mt. Sinai Medical Center. This project requires invasive monitoring of systemic and cerebral hemodynamics. This animal

model has been used extensively in CPR research and neonatal research due to its human similarities of both cerebral and systemic hemodynamics.

C. On what do you base your assurance that this study is not unnecessarily duplicative?
This motion platform device is a new device. Other than previous work done at Mt. Sinai, there is no prior experience using this platform for CPR studies.

D. Justify the number of animals to be used. Please note the statistical methods used in arriving at these numbers.
The power to detect 20% change in hemodynamic parameters would require a minimum of 10 animals per study. Data collection for this proposed protocol will be collected during the time these animals are participating in the on-going study by Dr. Jose A. Adams (Mt. Sinai) addressing the feasibility of this non-invasive motion platform for CPR.

E. Indicate the names and qualifications/training of the personnel having contact with the animals.
If you have a need for personnel training, please indicate ☐Yes ☒No
Ingrid Gunnlaugsson, BSN, RN - Graduate nursing student who has an extensive background in monitoring critically ill patients, including pulmonary & hemodynamic measurement. She has been trained to use the Isolated Nitric Oxide Meter and Sensors
Dr. Charlotte Richmond, Ph.D., RN - Nurse physiologist experienced in pulmonary & hemodynamic measurement.
Jose A. Admas, MD - Experienced in surgical procedure for this animal species as well as all pulmonary and hemodynamic measurement.
Jorge Bassuk, MD - Anesthesiologist - Experienced in surgical procedure for this animal species as well as all pulmonary and hemodynamic measurements assessment of anesthesia.
Dongmei Wu, MD, PhD - Experienced in surgical procedure for this animal species as well as all pulmonary and hemodynamic measurements.

F. How many animals (each species) are anticipated to be housed at any one time?
3 - 4 at the Harry Perlamn Reseach Institute at Mt. Sinai Medical Center

G. List the building and room number where project efforts will take place. Indicate the time period animals will be outside the housing facility.
The procedures will take place in room # 249 in the Harry Pearlman Research Institute at Mt. Sinai Medical Center.

H. Fully describe the method of euthanasia, indicating the agent, dose and route of administration and how dead animals will be disposed of.
Concentrated Pentobarbital (Euthanol) dose of 5cc IV. The animals will be red-bagged and incinerated per Mt. Sinai's policies & procedures.

The following items must be completed *only for items marked yes* on Page 2.

V. Immunization and bleeding:

A. Frequency of bleeding:
baseline and every 10 minutes X 3

B. Volume of blood withdrawn:
0.2 - 0.3cc per draw

C. Method of collection:
Indwelling arterial catheters

D. Hybridoma or ascites:

Agents used:

Volume injected:

Site of injection:

Frequency of injection:

Frequency of fluid withdrawn:

Volume of fluid withdrawn:

Maximum number of collections:

Frequency of animal observation:

F. Incomplete Freund's adjuvant used? ☐Yes ☐No Number of times? _____

G. Name(s) and frequency of other adjuvant's used:

VI. Administration of drugs other than anesthetic and analgesics:
Indicate the drug(s) to be utilized, the amounts(s), routes(s) of administration.

VII. Surgical procedure:

Note: Asceptic techniques are required for all warm blooded animals. Surgery must be performed in a surgical area approved by IACUC.

A. Describe what happens to the animal during the surgical procedure (if multiple surgeries, justify):

The animals will be anesthetized and undergo endotrAcheal intubation and will receive 100% O2 with 5 cmHg CPAP in order to reduce hypoxic consequences. Additionally, arterial and venous catheters will be placed in the femoral artery and right atrium and used for administering fluids and medications and measuring arterial pressure and right atrial pressure. All invasive catheters will be sized according to animal anatomical requirements. A bipolar fibrillating wire will be placed subcutaneously, across the apex of the heart, and used to induce ventricular fibrillation in the study animal via delivery of 30 volts of A.C. current at 60 Hz. The animals will be placed on the motion platform with extremities bound in order to maintain a close approximation with the platform device. The platform will move in a cephalic head to foot direction with periodic accelerations and decelerations. This motion will allow for manipulation of the speed and frequency of oscillations. The frequency of acceleration and deceleration movements range from O.5-10 Hz at a force of 0.1-1.5 G. Approximation of beats per minute (bpm) to corresponding frequency of motion is 1 Hz = 60 bpm.

Buildin Mt Room 249

B. Where will the surgery be performed? g Sinai

C. List the names and training of those who will perform the surgery.

Drs. Adams and Bussak have been performing these surgical procedures for over 5 years. Dr Wu has been trained by Drs. Adams and Bussak and has been performing these procedures for 6 months.

D. Identify the anesthetic agent(s), dosage(s) and route(s) of administration. Describe the method used to monitor level of anesthesia.

All anesthetic agents will be adminsitered by Dr. Bussak, who is an anesthesiologist. The preparation of the study animals will be performed using the protocol of previous pGz experiments. Animals under study will be anesthetized with Ketamine (10mg. Kg, I.M.) prior to entering the research laboratory procedure site. Additional Ketamine will be administered prior to invasive procedures and the animals will be held in a surgical plane of anesthesia with an intravenous propofol and succinylcholine combination. Paralysis will be induced via Pancurium Bromide at 0.1 mg/kg with continued supplementation as needed. The depth of anesthesia will be determined by the loss of the corneal and spinal reflex with accompanying animal heartbeat of 150 bpm as is considered normal in swine.

E. If a gas anesthetic is used, is a scavenging system in place? ☐Yes ☒No

F. Will paralytic drugs be used? ☒Yes ☐No

If yes, indicate the agent(s), dosage(s) and frequency of administration and the method of monitoring anesthesia.

Paralysis will be induced via Pancurium Bromide at 0.1 mg/kg with continued supplementation as needed. The depth of anesthesia will be determined by the loss of the corneal and spinal reflex with accompanying animal heartbeat of 150 bpm as is considered normal in swine.

G. Will post-operative antibiotics be used? ☐ Yes ☒No

If yes, name the antibiotic and how it will be administered. If no, explain/justify.

H. What post-operative measures will be taken to minimize discomfort? If none, explain/justify.
Pigs will be humanely euthanized with concentrated Pentobarbital (Euthanol). immediately after the study.

I. If analgesia is used post-operatively, indicate:

Type (name):

Dose:

Frequency and route of administration:

J. Name the individual(s) responsible for post-operative care and indicate where post-operative records will be maintained. ****Note.** A medical history chart must be maintained with no less than daily entries.

VIII. Special conditions:

A. Identify the isotope or hazardous material:

1. Exposure route:

2. Exposure dose:

3. Exposure duration:

4. Will the agent be excreted? ☐Yes ☐No

If yes ☐ in urine ☐ in feces ☐ exhaled

5. Indicate where animal(s) will be housed during and after exposure.

6. Indicate the method of disposal for the material/animal.

B. How will animal activity be restricted (as a result of surgical procedures or by chairs, tethers, stanchions, metabolism cages, etc?)

1. Indicate duration of restriction: During surgery and during CPR, while anesthetized, pig will be

2. Duration: 1-3 hours

3. Frequency: continuosly during anesthesia, surgey & resuscitation

4. How frequently will the animal be observed during restraint? continuously

C. List any special housing requirements required for the project (lighting, feed, caging, etc.).

none

D. Describe measures which will be taken to alleviate pain or distress. If no measures are taken, you must justify this decision.

Animals will be humanely euthanized at end of resuscitation study

E. Justify why death as an endpoint is necessary to this study.

This protocol being presented is a small piece of a large project that includes adminsitration of microspheres and microscopic examination of brain slices post resuscitation.

F. How long and how often will a single animal be fasted.

5 hours prior to surgical procedure

G. Describe the special assistance required from the animal facility supervisor or staff.
none

H. Indicate any procedure or occurrence which might be questioned upon review of your study and provide additional explanation or justification that will be helpful in reviewing this procedure. If describing behavioral conditioning, indicate the purpose of the conditioning, reinforcement techniques and criteria used to monitor the animal's condition.
Investigators at the Harry Pearlman Institute have been using this Mt. Sinai approved protocol for over 5 years.In order to compare data collected over the last 5 years with data being collected in the current year, no changes will be made to the present protocol This student's protocol is a small piece of a larger project. The required amount of blood, an additional 0.2 to 0.3cc drawn at baseline and repeated X3, will be taken from the blood already being obtained for the main protocol.

XI. Assurance:

The policies and procedures of Florida International University and the USDA apply to all activities involving live vertebrate animals performed by the personnel at this institution. No activities involving the use of these animals are to be initiated without prior written approval of the FIU Institutional Animal Care and Use Committee (IACUC).

The undersigned is familiar with and agrees to adhere to the PHS Policy on Humane Care and Use of Laboratory Animals, the regulations and policies of the USDA, the NIH Guide to the Care and Use of Laboratory Animals and FIU policies. I assure that any change in the care and use of animals involved in this protocol will be promptly forwarded to the IACUC for review and approval prior to implementation of the change. I also assure that only properly trained individuals will participate in this project.

Animals will not be transferred between investigators without prior written approval of the IACUC.

Signature of Principal Investigator

1/15/2002
Date

FLORIDA INTERNATIONAL UNIVERSITY
Miami's public research university

DEPARTMENT OF BIOLOGICAL SCIENCES

TO: Ingrid Gunnlaugsson, BSN, RN

FROM: Keith Condon, Ph.D.
Acting Chair, IACUC

DATE: 2/26/02

RE: FIU IACUC Protocol: Effects of Anesthesia and Periodic Acceleration (pGz) on Thermoregulatory Responses During Resuscitation in Pigs

The majority of the committee has approved your protocol pending modification (see below). However, a minority has requested a meeting with the PI and adviser to address concerns about the statistical analysis. Since an assembling of the committee takes a good deal of time (two members are off campus), I would like to propose that the PI respond to the issues raised below, paying particular attention to the statistical analysis (Section IV.D.). If the submitted modifications, including the explanation of the statistical analysis, are acceptable to the committee, then an approval with modifications will be issued. If the submitted modifications are unacceptable, then I will arrange a meeting with the PI.

If this arrangement is not acceptable to you, please notify me and I'll arrange a meeting as soon as possible.

In regards to power analyses to determine sample sizes, the statement should include an estimate of the differences in the parameters being measured between groups, the statistical test(s) being employed, an effect size and an alpha level.

On the following pages are a synopsis of the points that must be addressed in the modification

If I can assist you in the re-submission, please don't hesitate to contact me at x2604 or by e-mail at condon@fiu.edu.

II.B. Please define (not just describe) pGz; that is, to what does each of these letters refer?

How long will the animals be in fibrillation and/or subject to resuscitations? You are monitoring every 10 minutes suggesting a rather prolonged period. How long will the resuscitations last?

Also, please confirm in this section that the animals will be euthanized at the end of the experiment surgery without regaining consciousness.

Curare wipes out muscle tone – does this affect blood pressure or other physiological variables that would in turn affect NO production?

III.G.3. Should be yes; complete section X(C)

This is, since the animals are not being house at FIU, please describe the housing conditions at the study site.

III.G.4. Should be yes; complete section X(D)

IV.A. The species is *Sus scrofa*.

Please describe more fully "other possible complications" which may require the use of additional animals

IV.D. Is the 10 animals "per study" suggested by your analysis the number of animals total or the number of animals in each of your two groups. If the latter, then your effective sample size is likely too small (N = 12) to detect a significant difference. Explain.

The section on statistical power made no sense [to me.] What statistical tests are used to detect changes in NO level within subjects and to compare groups? What is the predicted variance in NO levels across comparison groups. What is the alpha level?

VII.B. The methods of standard CPR resuscitation that will be used for one of the groups are not described.

VII.H. Again, please clarify if the pigs are euthanized at the end of the surgery without gaining consciousness.

To: The Department of Biological Sciences

Attention: Keith Condon, Ph.D. and FIU IACUC Committee

From: Ingrid Gunnlaugsson, BSN, RN, CCRN and Principle Investigator

Date 3/17/02

Re: FIU IACUC Protocol: Effects of Periodic Acceleration (pGz) on Nitric Oxide During Resuscitation in Anesthetized pigs.

The following statements are in response to the letter from Dr. Condon dated 2/26/02 and hope to clarify any questions regarding the protocol used during pGz and Nitric Oxide (NO) experiments.

Clarification of Specific Questions

11B. Please define (not just describe) pGz; that is, to what does each of these letters refer?

Periodic Gz acceleration (pGz) refers to a novel cardiopulmonary resuscitation method (periodic Gz acceleration) and is the application of head to foot oscillations, which occur on a motion platform device, and cause periodic sinusoidal inertial forces (G's) in the spinal axis of anesthetized pigs. The motion platform device moves in a cephalic head to foot direction with periodic acceleration and deceleration. The speed and frequency of the pGz motion can be manipulated. The frequency and force of pGz platform movements are used as control with frequency set at 2 HZ with 1 HZ equaling approximately 60 heat beats per minute. The inertial force is set at 0.6 G's for all experiments. Movements of the platform both ventilate and stimulate cardiovascular circulation in anesthetized and arrested pigs. Ventilations occur via the force of acceleration movement, which thrusts the animal's abdominal contents toward the diaphragm, producing negative pleural pressure respiration similar to human breathing.

The protocol in use for the NO experiments is the same protocol used for previous pGz experiments by Dr. Tony Adams and associates. Dr. Adams (Director of Neonatology at Mount Sinai Medical Center) has been investigating the effects of pGz motion on numerous biological modalities for over 5 years and his research is funded by a grant from Miami Heart Institute and is approved by the Mt. Sinai IACUC Committee of which he is a member. The Principle Investigator's (PI) measurement of NO in this model is only one of many studies under current investigation by Dr. Adams and his associates, Dr.'s Wu and Bassuk. Dr. Adams et al. have several publications regarding pGz and its effects in the Journals of Applied Physiology, Circulation, and Resuscitation.

How long will the animals be in fibrillation and/or subject to resuscitation? You are monitoring every 10 minutes suggesting a rather prolonged period. How long will the resuscitations last?

The animals will be in fibrillation for up to 40 minutes. First baseline specimens are drawn; thereafter ventricular fibrillation is induced with the platform set into motion after a three-minute period. Blood draws are at 10-minute intervals (X3). The animals are sedated in the holding pen, prior to arrival in the research lab, and held in a surgical plane of anesthesia throughout the experiments without regaining consciousness. If the animals survive the experiment (ventricular fibrillation for up to 40 minutes) they are immediately euthanized with a lethal barbiturate dose (euthasol/ pentobarbital concentration of 5cc IV push) as per IACUC requirements. This protocol has been used by Dr. Adams in prior pGz experiments and with IACUC approval.

Curare wipes out muscle tone – does this affect blood pressure or other physiological variables that would in turn affect NO production?

Curare is not used in any pGz experiments. Neuromuscular blockade is induced with Pancurium Bromide at 0.1mg/kg with continued supplementation as necessary. The depth of anesthesia is determined by loss of corneal and spinal reflex with accompanying heart-beat of 150 per minute as is the norm in young pigs. The PI has reviewed several pharmacology and anesthesia references and has not found any report of Pancurium Bromide affecting the production of NO. However, side effects of Pancurium Bromide are listed as hypertension and tachycardia. As NO is a potent vasodilator, the possibility of these side effects occurring, resulting from NO's production or inhibition, will be addressed during the discussion and limitations portion of the thesis document and defense.

The animals are premedicated with ketamine (10 mg/kg, intramuscularly), in the holding pen prior to entering the lab, followed by anesthesia with propofol given at 6 mg/kg for induction IV and 0.5mg/kg/min for maintenance. Possible side effects of these medications will also be discussed in the final thesis document.

111. G. 3. Special housing.
Yes, the animals undergo special housing as they are housed at the Harry Pearlman Research Center which is the Animal Research Laboratory at Mt. Sinai Medical Center and is accredited by the American Association for Accreditation of Laboratory Animal Care and adheres to all requirements and provisions of the Animal Welfare Act, PHS policies on Animal Care and Use, and USDA regulations. Three to five pigs are housed at one time. Staff for feeding, cleaning, and routine veterinary care is provided by the Research Department at Mt. Sinai. The staff veterinarian is Julia Zarias, D.M.V.

111. G. 4. Section X (D)
Pain or distress experienced by subjects.

The answer should be "no" as the animals are anesthetized, prior to entering the lab, and continuously supplemented with anesthesia throughout experimentation (as described in section **IIB)** and therefore experience no pain or distress. This information is confirmed by the Mt. Sinai Research Laboratory Veterinarian Julia Zarias, D.M.V.
X(D). Describe the measures which will be taken to alleviate pain or distress. If no measures are taken, you must justify this decision.

The measures taken to alleviate pain or distress are fully described in section **IIB.**

1V.A. The Yorkshire species is *Sus Scrofa*.

Please describe more fully "other possible complications" which may require the use of additional animals.

Other possible complications requiring use of additional animals, for the pGz experiments, include death from ventricular arrhythmia, unknown existing pathology in the animal, and complications from intubation, vessel cannulation, and anesthesia. However, as an anesthesiologist (Dr. Bassuk) is present during all experiments and performs all invasive procedures and anesthesia administration, the animals are closely monitored.

1V. D. Is the 10 animals "per study" suggested by your analysis the number of animals total or the number of animals in each of your two groups? If the latter, then your effective sample size is likely too small (N=12) to detect a significant difference. Explain.

The study uses 12 animals total, 6 in each experiment and is based on previous pGz studies, recommendations by Dr. Adams, Utstein guidelines, provisions of Miami Heart Institute, and as is appropriate for a pilot study for feasibility. Should the study support trends or significant differences between types of resuscitation (pGz or Standard CPR), Dr. Adams plans to repeat the study, after conducting a power analysis using the results of this pilot study, with at least 10 animals in each group.

The section on statistical power made no sense (to me). What statistical tests are used to detect changes in NO level within subjects and to compare groups? What is the predicted variance in NO levels across comparison groups? What is the alpha level?

A repeated measures experimental design will be used for the within group experiments, differences in NO levels during pGz and standard CPR, and performed using a one-way analysis of variance (ANOVA) consisting of one independent variable (resuscitation) and one dependent variable (NO). The advantage of the repeated measures design is that it

allows for multiple values to be obtained from the same animal using the animal as its own control and ensuring a high amount of equivalence among the study subjects. Comparison between groups, greater NO levels during pGz resuscitation versus standard CPR, will be made using a two- way ANOVA consisting of two independent variables (pGz and standard CPR) and one dependent variable (NO). The dependent variable remains continuous as per protocol for ANOVA testing. Frequency distribution of the data will be determined and an F-ratio obtained. If the F-ratio is deemed significant, the source of significance will be determined using the appropriate post hoc test to compare group means and decrease possibility of a type-1 error. Data will be analyzed using an Instat software program and expressed as means $\pm$ SD, with statistical significance (alpha) set at $p<0.05$.

As this study represents pilot work, and as NO has never been measured in the pGz model before, it is not possible to estimate or predict variance in NO levels across comparison groups.

V11.B. The methods of standard CPR resuscitation that will be used for one of the groups are not described.

The method of standard CPR used for comparison in these experiments is via the *Thumper* device. The *Thumper* is a mechanical device for compressing the chest in a uniform, repetitive fashion with constant rate and force and simulates manual chest compressions. The *Thumper* has also been utilized for resuscitation in humans, specifically by paramedics in the field.

V11. H. Again, please clarify if the pigs are euthanized at the end of surgery without gaining consciousness.

At the completion of the experiments, all animals will be euthanized by a lethal barbiturate (as stated in **11.B**.) dose as per IACUC requirements without regaining consciousness.

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FLORIDA INTERNATIONAL UNIVERSITY
Miami's public research university

Institutional Animal Care and Use Committee

MEMORANDUM

To: Ingrid Gunnlaugsson, Principal Investigator
Dr. McDonough

From: Yvette Peterson, IACUC Coordinator

Date: January 29, 2003

Re: Approval of Protocol

The Institutional Animal Care and Use Committee at FIU has approved the following protocol:

Protocol Title: Effects of Periodic Acceleration on Nitric Oxide During Resuscitation in Anesthetized Pigs

Protocol Approval Number: **02-019**

Approval Date: **10/22/02** **Renewal Date:** **10/22/03**

Modifications:
None

FIU Animal Welfare Assurance Number: #A3096-01
Please address questions or concerns about this protocol to:
Yvette Peterson, IACUC Coordinator
Phone: 305-348-2494
Fax: 305-348-4117
Email: irbiacuc@fiu.edu