

**EVALUATION OF INTRAOSSEOUS INFUSION OF
LIPOSOMAL OXYGEN IN RABBIT MODELS
OF HYPOXEMIA**

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ABSTRACT

The intravenous administration of a lipid-based oxygen suspension has been shown to raise blood oxygen saturations to survivable levels in models of asphyxia. However, emergency situations often render intravenous access impossible, so the intraosseous (IO) route is sometimes used to obtain vascular access. This study examined the pharmacokinetics of infusion of a lipid-based oxygen suspension administered through an IO line. IO infusion raised the blood oxygen content of hypoxemic animals in a manner similar to IV infusion.

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BACKGROUND

Hypoxemia

Hypoxemia is clinically defined as a condition in which a patient's blood oxygen content has fallen below a healthy level. This condition is often diagnosed using arterial oxyhemoglobin saturations (SpO₂), arterial oxygen partial pressure (PO₂) or a combination of both. In healthy individuals breathing atmospheric oxygen at sea level, normal levels are around 110 mmHg PO₂ and 99% SpO₂ (Gootman et al., 1963). Hypoxemia is recognized as a dip below 90 mmHg PO₂ or 90% SpO₂ for an extended period of time (Bowton et al., 1994).

Hypoxemia can be caused by a number of conditions. Acute respiratory distress syndrome, chronic obstructive pulmonary disorder, pulmonary edema, and atelectasis (alveolar collapse) are all known to cause hypoxemia in patients (Bone, 1980; Miller et al., 1984; Suchyta et al., 1991; Piantadosi and Schwartz, 2004). Alveolar-capillary block syndrome, a condition that thickens the alveolar walls and compromises the diffusion of oxygen, also causes arterial hypoxemia in patients (Finley et al., 1962). Pulmonary embolism causes hypoperfusion or total ischemia of the lungs, preventing adequate blood oxygenation (Wilson et al., 1971). Anatomical anomalies can also lead to low blood oxygenation. Many congenital heart defects cause right-to-left shunting, the mixing of oxygenated and deoxygenated blood (Sukumalchantra et al., 1970). A patent foramen ovale, a hole in the atrial septum, allows pre- and post-pulmonary flow to mix. Transposition of the Great Arteries, an anatomic translocation of the aorta and pulmonary

artery, causes profound hypoxemia in patients (Petit et al., 2009). There has even been a documented case of partial occlusion of the right pulmonary artery by a mediastinal tumor that led to severe hypoxemia (Takeda et al., 1999).

Dangers of Hypoxemia

The biological need for a constant supply of oxygen is rooted in the production of ATP, the basic source of energy for cells. ATP is made through three steps – glycolysis, the Krebs cycle and oxidative phosphorylation – together referred to as cellular respiration. Oxidative phosphorylation operates through the electron transport chain where oxygen acts as the final electron acceptor. The final reduction of oxygen completes the chain, and allows for the production of ATP via ATP synthase. In the absence of oxygen, however, the electron transport chain cannot function. Oxidative phosphorylation and the Krebs cycle come to a complete stop. In the absence of the electron transport chain, glycolysis is the only source of ATP. This process, called anaerobic respiration, is far less productive than full cellular respiration, producing only 2 ATP molecules per molecule of glucose, as opposed to 38 ATP in the case of aerobic respiration. In addition, the pyruvic acid that normally enters the Krebs cycle is instead reduced to lactic acid (Hole et al., 2009).

In rat models, a 50% reduction in ATP levels in the brain occurs shortly after the arterial PO_2 drops below 30 mmHg. This drop in energy corresponds with an almost immediate impairment of brain function. Neurons utilize anaerobic respiration as a source of energy, causing a rapid accumulation of lactic acid. The continuous flow of blood carrying glucose with low oxygen provides additional fuel for anaerobic respiration

and lactic acidosis. This acidotic condition triggers and accelerates further injury and insults to neuronal tissue. This condition of low oxygen delivery is known as ischemia, and is a final common pathway ending in cell death (Hass, 1983).

A higher occurrence of periventricular leukomalacia (PVL), a type of white-matter necrosis in brain tissue, has been linked to a lower average PO_2 . Newborns with D-transposition of the great arteries (d-TGA), a congenital heart defect that transposes the aorta and the pulmonary artery, are characteristically hypoxemic due to the recirculation of desaturated systemic venous blood into the systemic arteries. Petit et al. (2009) in a study of preoperative neonates with TGA, discovered a negative association between blood oxygen tension and the occurrence of PVL. Among preoperative patients, those showing signs of PVL had a significantly lower mean PO_2 than those without PVL. Additionally, the longer the patient spent in the hypoxemic state (time to palliative measures taken), the higher the probability of developing PVL (Petit et al., 2009). PVL results in significant decrement in quality of life, including motor dysfunction, in these children.

Gootman et al. (1963) conducted a study of pediatric and neonatal patients with cyanotic congenital heart defects. Researchers found a link between low blood oxygen saturation (and partial pressure) and a build-up of lactic acid. This excess lactic acid acted to bring the patients overall blood pH to acidic levels, under 7.2 (Gootman et al., 1963). Lactic acidosis often results from poor tissue oxygenation due to diminished oxygen delivery, a condition frequently present in hypoxemic patients (Mizock and Faulk, 1992).

Hypoxemia and resulting acidosis has also been linked to the development of cardiac arrhythmias. Ayres and Grace (1969) reported on nine cases involving patients admitted with symptoms indicative of acute myocardial infarction or respiratory distress. During hospitalization, the patients displayed varying degrees of hypoxemia, acidosis, and alkalosis. Analysis of the data revealed that traditional anti-arrhythmic treatments were ineffective on the patients. Rather, the arrhythmias only returned to normal sinus rhythm when the hypoxemia, acidosis or alkalosis was corrected. Arrhythmic events seen in patients often occurred when mechanical administration of oxygen was disrupted, and were corrected shortly after arterial PO₂ was raised (Ayres and Grace, 1969).

Aside from these specific anomalies, hypoxemia has been linked to survival. An in-hospital study conducted in 1994 correlate hypoxemic events with patient outcome. A group of 100 patients admitted to the hospital with varying diagnoses were followed with continuous pulse oximetry monitoring. The study stratified patients into three different groups of hypoxemia – SpO₂ <90%, <85%, and <80% – all lasting for periods of five minutes or more. APACHE (Acute Physiology and Chronic Health Evaluation) scores were calculated on all patients as a means of incorporating the severity of the diagnosis into the results. After hospital discharge, patients were contacted within four to seven months for a follow-up to determine long-term outcome (Bowton et al., 1994). Of the 100 patients studied, 26 were found to have hypoxemic episodes <90% SpO₂, 16 patients below 85% SpO₂, and 9 patients below 80% SpO₂. 74 patients experienced no prolonged periods of hypoxemia. The study revealed a striking increase in mortality among the 26 patients experiencing hypoxemia. Of the patients contacted, 32% of those with <90% SpO₂ died within the follow up period. This was significantly higher than the 7%

mortality rate among non-hypoxemic patients ($p=0.0003$). Even when adjusted for severity of illness, the hypoxemic group still exhibited a significantly higher mortality rate. Furthermore, the follow-up study revealed that mortality increased with the severity of the hypoxemic events experienced. 31% of those with $<85\%$ SpO₂, and 44% of those with $<80\%$ SpO₂, died in the months following hospital discharge. Additionally, those patients who spent longer times at lower saturations had a lower survival rate. The study ultimately revealed a correlation, though not definitively causal, between decreased arterial oxygenation and lowered survival rates. Patient mortality could not be explained by the severity of respective illnesses: those with severe injuries or illnesses were affected to a similar degree as those with non-critical diagnoses (Bowton et al., 1994).

Treatments of Hypoxemia

The treatment of hypoxemia is limited. Many hypoxemic patients are treated with supplied oxygen, either through a nasal tube or a face mask. In more critical events, patients may be intubated and placed on mechanical ventilation (Ayres and Grace, 1969). A specific aspect of mechanical ventilation, positive end-expiratory pressure (PEEP), is used to optimize oxygenation. PEEP provides a positive pressure throughout the expiratory phase, preventing alveolar collapse (Bone, 1980). This requires an intact airway and lung unit to oxygenate the blood. A treatment of refractory hypoxemia known as extra-corporeal membrane oxygenation (ECMO) involves the *ex vivo* oxygenation of the blood. A patient's great vessels are connected to a perfusion pump which circulates the blood through a series of membranes exposed to oxygen gas which act as artificial alveoli. This method has proven to be highly effective in patients with

ARDS. However, the risks of this technique are great, including intracranial bleeding, renal injury, hemolysis and worsening of the inflammatory cascade make this a suboptimal method of emergency oxygenation. More importantly, the time, expertise and resources for the institution of this invasive therapy make it available only in a limited number of tertiary care centers. Even in centers with rapid response ECMO teams, ECMO may take more than an hour to institute during which irreversible organ injury can take place (Suchyta et al., 1991). The limitations of the modern treatments have led some physicians to seek an alternative way to increase the blood oxygen content of hypoxemic patients in an emergency setting.

Lipid-based Oxygen Suspension

Kheir et al. (2010) of Children's Hospital Boston and Harvard Medical School has recently developed a lipid-based suspension that acts as a vehicle for oxygen gas. Rather than endotracheal or extra-corporeal administration of oxygen, the suspension was initially designed to deliver oxygen gas directly into the bloodstream. In 2010, the suspension was studied *in vivo* with rabbit models of asphyxia. Rabbits were anesthetized and paralyzed, allowing researchers full control over the breathing pattern of the animal. The animals were stabilized with mechanical ventilation on 21% inspired oxygen. After stabilization, the endotracheal tube was completely occluded, resulting in no mechanical or spontaneous respiration. Experimental animals received an intravenous infusion of the lipid-based oxygen suspension at a rate matching normal oxygen consumption, and control animals received an intravenous infusion of oxygenated intravenous fluid. Asphyxia was maintained for 15 minutes. Arrested animals were

resuscitated using advanced critical life support protocol (Kheir et al., 2010). The mean time to arrest in control models was 6.7 minutes. All control animals exhibited a rapid decline in arterial PO₂ and blood oxygen saturation. Control animals also became critically hypotensive prior to arrest, adding to the oxygen delivery deficit. In contrast, 8/10 animals receiving the lipid-based oxygen suspension survived to the 15-minute endpoint with no arrest. These animals maintained arterial PO₂ and blood oxygen saturation at survivable levels. Experimental animals also initially displayed a slight increase in mean arterial blood pressure followed by a return to baseline tension (Kheir et al., 2010).

The results of this experiment reveal that an intravenous lipid-based oxygen suspension can act as a temporary supplement to inspired air. A therapy capable of supplying a patient with oxygen outside of traditional mechanical ventilation and ECMO carries profound implications in the world of critical care and emergency medicine. Given the dangers of hypoxemia and the conditions it is associated with, administration of intravenous oxygen could provide the rapid increase in arterial PO₂ and blood oxygen saturation needed for survival in critical situations.

Venous Drug Delivery

The administration of drugs in emergency situations has been done through numerous routes. Percutaneous peripheral intravenous access (PPIV), central venous catheterization (CVC), and surgical catheterizations into major veins all rely on direct access to the circulatory system (Brunette and Fisher, 1988). Because of this direct access, PPIV has been used to quickly administer therapies to patients in critical and

emergency situations. However, PPIV relies on the ability of personnel to place the catheter into the vein percutaneously. But in many cases, conditions make accurate and rapid insertion of the catheter difficult. Burns, edema, and massive limb trauma make insertion of PPIV more difficult (Rosetti et al., 1985; Blumberg et al., 2008).

Additionally, peripheral veins can be difficult to locate in obese patients and in children (Rosetti et al. 1985; Paxton et al., 2009). Dehydration, poor hemodynamics, and previous IV drug abuse can make insertion of the IV difficult and time consuming (Paxton et al., 2009). Peripheral veins will often collapse in cases of shock and cardiac arrest, rendering them useless for PPIV (Tocantins and O'Neill, 1951; Rosetti et al. 1984; Rosetti et al. 1985; Orłowski et al. 1989; Blumberg et al. 2008). Furthermore, placement of a CVC is recognized as a high-risk procedure in many patients (Rosetti et al., 1985). Surgical catheterizations are often used as a last-resort technique, involve additional trauma to the patient, and are time-consuming, making them less suited for emergency situations (Paxton et al., 2009).

Endotracheal Drug Delivery

Endotracheal drug administration is also used in some applications. This method of drug delivery can be convenient, especially if the patient is already intubated. It is, however, limited in its uses. Only a handful of drugs can be administered via the airway, thus restricting endotracheal therapies in emergency settings (Brunette and Fischer, 1988).

History of Intraosseous Infusion

Intraosseous infusion (IO) uses the medullary canal of long bones to introduce drugs into the circulatory system. IO infusion involves the placement of a rigid needle through the periosteum and into the red marrow of a patient. Drinker et al. (1922) first documented the use of IO infusion in mammals. The study showed that perfusion through a catheter inserted into the tibial red marrow of a dog was a feasible way to deliver fluids to the circulatory system. Drinker reported that the canal functions as a rigid “noncollapsible vein” with a direct connection to the rest of the circulation (Drinker et al., 1922). In 1940, L.M. Tocantins and O’Neill published the first study of IO infusion conducted on human patients. 17 infusions were conducted on 14 patients, with a success rate of 94% (Tocantins and O’Neill, 1951). Further studies pushed IO infusion into general recognition, and it was adopted by battlefield medics during World War II as a quick and efficient way of establishing vascular access. Yet with the coming of more sophisticated PPIV apparatus and techniques, IO infusion fell by the wayside in the late 1940s (Orlowski et al., 1989; Blumberg et al., 2008).

IO Infusion Physiology

The physical structure of the long bones allows intraosseous infusion of drugs and fluids. Bones, although composed of a dense and compact cellular matrix, are highly vascular. The basic unit of bone structure, the osteon, is built around an inner canal that contains arterial and venous blood flow. The Haversian canals and their vessels are interconnected by vessels running through Volkmann canals. The heads of long bones contain red marrow, which serves as generative tissue for red and white blood cells (Hole

et al., 2009). This area has a high perfusion rate – necessary to introduce new cells into circulation – and is the target for insertion of an IO line (Leidel et al., 2010). Several bones have been utilized in both experimental and clinical settings. The sternal manubrium, tibia, femur, humerus, radius and clavicle have all been successfully infused. Preferred sites for penetration in pediatric patients and adult patients are the anteromedial face of the tibia and the sternum, respectively (Rosetti et al., 1985; Blumberg et al., 2008).

Contraindications of IO Infusion

As with all methods of drug administration, there are contraindications of IO infusion. Placing an IO line into a fractured or damaged bone results in perfusion of the drug through the fracture rather than into circulation. Bones that have previously been used for IO infusion are avoided for the same reason. Percutaneous infection in the area of insertion serves as a contraindication, as it may introduce infection to the periosteum or marrow (Blumberg et al., 2008; Paxton et al., 2009). In addition to these factors, patients also suffering from bone disorders such as osteoporosis should not receive IO infusions (Blumberg et al., 2008). In these cases, trauma inflicted to the bone during the placement of the needle could far outweigh the benefits of vascular access.

Complications of IO Infusion

Even under normal circumstances, IO infusion carries risks. Despite the trauma inflicted on the periosteum and compact bone upon penetration, risks associated with intraosseous infusion are few and for the most part benign. Main concerns lie in the

development of infection as a result of aseptic administration. Percutaneous infection or improper sterilization of the skin could introduce infectious agents to the marrow.

Subcutaneous abscesses, osteomyelitis and skin necrosis can all result from IO infusion (Rosetti et al., 1985; Christensen et al., 1991; Rosovsky et al., 1994 Paxton et al., 2009; Leidel et al., 2010). Although these conditions are possible, they are unlikely to occur in a majority of patients. In 1985, Rosetti et al. compiled data from 30 individual studies of human IO infusions conducted between 1942 and 1977. The collection profiled 4,270 patients. Of these patients, it was found that only 27, less than 0.6% of the group, developed osteomyelitis. It was also noted that in these cases, a high percentage of them occurred in patients with extended periods of infusion or previously existing infections. Subcutaneous abscesses formed in only 0.09% of the patients (Rosetti et al., 1985).

The development of fat or bone marrow emboli has been proposed as a possible risk in IO infusion. Since these emboli would be in venous circulation, they have the potential to disrupt blood flow in the pulmonary capillary beds (Rosetti et al., 1985; Orłowski et al., 1989). However, a study of 30 dogs and two pediatric patients examining the frequency and implications of emboli formation in IO infusion contradicts these concerns. Researchers found that regardless of what drug was infused, fat and bone marrow emboli were discovered in lung tissue. The study concluded that 89 to 100% of all IO infusions would lead to fat or marrow emboli. Arterial blood gas analysis revealed that despite the high prevalence of emboli, alveolar perfusion was not compromised and the emboli were not clinically relevant (Orłowski et al., 1989).

Other possible complications include compartment syndrome resulting from subcutaneous or subperiosteal infiltration, bone fracture due to the penetrating force of

the needle and epiphyseal plate disruption (Rosetti et al., 1985; Paxton et al., 2009). The latter, however, can be avoided by correct use of anatomical landmarks (Rosetti et al., 1985). As with all methods of vascular access, dislodgement of the catheter and extravasation, improper insertion and bleeding are concerns (Paxton et al., 2009; Leidel et al., 2010).

Overcoming the Downfalls of Traditional Vascular Access

Paxton et al. (2003) conducted a two-phase study comparing the efficiency of the traditional methods of intravascular access, PPIV and CVC infusion, against IO infusion. The study was conducted in the emergency department of a level I trauma center with an annual volume of 92,000 emergency patients. Phase I of the study involved 62 patients subjected to conventional PPIV access. In the event of PPIV failure, CVC was attempted. One patient received immediate CVC due to poor peripheral vein conditions, and four received CVC after failed PPIV. Phase II of the study subjected 29 patients to proximal humeral IO infusion. One patient received a subsequent infusion two weeks after the initial, resulting in an n = 30. Endpoints included time from beginning of catheter placement to proper flow and failure attempts of catheterization (Paxton et al., 2009). The data revealed a striking difference between the times required to administer PPIV or CVC versus IO lines. In phase I, the mean time to peripheral intravenous access was 3.6 minutes over a range of 1-16 minutes (SD = 3.7). On average, each successful PPIV placement required 1.5 attempts. For the five patients that received CVC, mean time to access was 15.6 minutes over a range of 11-25 minutes (SD=6.7). CVC required an average of 2.4 attempts per successful placement. In contrast, times to proximal

humeral IO insertion were much faster. Over the 29 patients (30 infusions) of phase II, the mean time to placement was 1.5 minutes over a much tighter range of 1-6 minutes (SD=1.1). IO insertion was significantly faster than PPIV ($p < 0.001$) and CVC ($p=0.0056$). In addition, IO infusion had a first attempt success rate of 80.6% versus 73.7% for PPIV and 20.0% for CVC (Paxton et al., 2009).

A similar study was conducted on strictly pediatric patients under the age of two years. 33 infants in cardiac arrest were studied. The time between arrival in the emergency department and intubation and administration of endotracheal drugs was measured. Time to the application of each kind of intravascular access was also recorded. Intravascular methods included PPIV, CVC, surgical catheterization and IO infusion. PPIV was found to have a faster mean time to administration of 3.0 +/- 2.0 minutes versus 4.7 +/- 1.49 minutes for IO infusion. However, IO infusion had a success rate of 83% and was the initial access route in 75% of patients, which were the highest of all intravascular methods. Despite PPIV having a faster time to administration, IO infusion proved to be a suitable alternative to patients with difficult access (Brunette and Fischer, 1988).

Industry Standards

IO infusion's ability to complement direct venous infusions and to nearly usurp endotracheal drug administration has prompted the medical industry to re-adopt its use in clinical situations. In the 1980s, the American Heart Association (AHA) adopted IO infusion into its pediatric advanced life support guidelines (Blumberg et al., 2008). Modern AHA pediatric guidelines recommend it for all pediatric patients. Furthermore,

the AHA also recommends that IO infusion be used in adults as the primary substitute to tradition IV access in its advanced cardiac life support guidelines (Kleinman et al., 2010; Neumar et al., 2010).

PROJECT PURPOSE

Although the intravenous administration of a lipid-based oxygen suspension has previously been shown to raise blood oxygen saturations to survivable levels in models of asphyxia, emergency situations often render intravenous access impossible, so an intraosseous (IO) route is sometimes used to ensure vascular access. The purpose of this study is to determine the effectiveness of an intraosseous infusion of the lipid-based oxygen suspension in rabbit models of hypoxemia. We hypothesized that the suspension infused through the intraosseous route would raise blood oxygen content in a manner comparable to the intravenous route.

METHODS

Rabbits

Three female New Zealand white rabbits of approximately 4.5 kg were studied. All animals were housed in the Animal Resources at Children's Hospital (ARCH) for a three-day acclimation period prior to each procedure.

Surgery

Prior to each procedure, the animal was induced and sedated using Glycopyrrolate (10 micrograms/kg) and Ketamine (10 mg/kg), both intramuscularly. 0.1 mg/kg Midazolam was given via a percutaneous IV line placed in an ear vein. The animal was then intubated and ventilated at 21% FiO₂. Upon presence of EtCO₂ (expired CO₂), the animal was sedated. A Fentanyl bolus, between 25-50 micrograms/kg titrated specifically to each animal was administered via an ear vein. The animal also received a continuous Fentanyl infusion of 50-100 micrograms/kg/hr throughout the entire procedure. The animal was then paralyzed using Pancuronium 0.1 mg/kg IV. Paralysis of the animal allowed for full control of respiration.

After the animal had been properly sedated and securely intubated, the abdomen, neck, and groin area were fully shaved. A pulse oximetry probe was fixed to the ear, paw or tongue. An esophageal probe was inserted to measure heart rate, body temperature and EKG. Following this, placement of arterial and venous lines via surgical catheterizations began. A 22-gauge catheter was placed in both the left and right femoral arteries. The left femoral artery catheter was connected to an extension and a three-way

stopcock, both primed with normal saline. A saline flush was administered to ensure proper flow. This line was used specifically for the drawing of arterial blood gas samples. The right femoral artery catheter was connected to a three-way stopcock, held permanently in the open position. Through this stopcock, and into the femoral artery, an Oxford Optronix fiber optic PO₂ probe was fed for continuous oxygen tension measurement. The auxiliary port of the three-way stopcock was used to transduce systolic and diastolic blood pressure. A 4 French Cordis sheath was inserted into a femoral vein for intravenous administration of the lipid-based oxygen suspension. Using a 15 gauge Life/form® intraosseous (IO) infusion needle, an intraosseous line was placed in the animal's humerus. Because of the large gauge of the needle and small size of the bone, the needle was inserted into the proximal epiphysis of the humerus, longitudinally, running parallel with the medullary canal. An extension set and stopcock were attached to the IO needle to prevent movement of the needle. A 10 ml saline flush was administered to ensure proper placement and flow.

Following line placements, the FiO₂ was decreased from 21% to 11%. This caused alveolar hypoxia and reduced the overall blood oxygen tension and saturation of the animal. The animal was allowed to reach a stable blood oxygen tension and saturation, and an arterial blood gas was drawn.

After the animal had stabilized at 11% FiO₂, preparation for the infusion began. A 140ml syringe of the lipid-based oxygen suspension was fixed to the 4-French Cordis sheath in the right femoral vein. With the stopcock closed to the vein, the line was primed with the lipid-based oxygen suspension. At this time, a baseline arterial blood gas was drawn to determine starting-point measurements. A blood gas was drawn every 30

seconds throughout the infusion. The stopcock attached to the 4-French Cordis sheath was opened to venous flow, and the suspension was hand infused. The infusion continued for 1:45 minutes. Following a 1:15 minute observation period, the FiO_2 was increased to 21% for a 10-minute recovery period.

After the 10-minute recovery period, the FiO_2 was again reduced to 11% as described earlier. Another baseline arterial blood gas was drawn. One 140ml syringe of the lipid-based oxygen suspension was attached to the stopcock leading to the IO line in the humerus of the animal. The lipid-based oxygen suspension was hand-infused for a period of 1:45 minutes. At the end of the infusion, the animal was given a recovery period as described above. Infusion alternated between IV and IO, with a recovery period following each one, so that two of each type of infusion was recorded. Following the end of the final infusion, the animal was terminated using FatalPlus (pentobarbital sodium).

Autopsy

After death, the animal underwent a sternectomy to reveal the heart, lungs and great vessels. The heart and lungs were excised. The heart was dissected along the transverse plane to expose the atria and ventricles. The heart and samples of both lungs were placed into formalin for preservation. Tissue samples were also taken from the liver. The humerus of the rabbit was then excised and longitudinally bisected to reveal the bone marrow. This entire procedure was documented with photographs.

Endpoints included systolic, diastolic and mean arterial blood pressure, temperature, heart rate and pulse oximetry, as recorded on a SurgiVet Monitor.

Continuous arterial blood oxygen tension was measured via the fiber optic probe in the right femoral artery. Lung mechanics were also recorded. Arterial blood gas measurements were used to corroborate blood oxygen tension and saturation. All data was recorded on a one-second time resolution, and blood gasses were drawn every minute throughout the infusions.

RESULTS

This project investigated the effectiveness of infusing a lipid-based oxygen suspension intraosseously compared to intravenous administration.

Three rabbits underwent infusions during this study. Animal #1 was successfully prepped for surgery and intravenous infusion began. No increase in arterial oxygen saturation was seen, even as the infusion surpassed 1:30 minutes. Suspensions manufactured in a different manner than usual were utilized for this experiment, and were more viscous than usual. The infusion was continued beyond the 1:45 minute endpoint with no increase in oxygen tension. The animal then became hypotensive and increasingly hypoxemic. The infusion was stopped, and the rabbit underwent a failed resuscitation. Autopsy revealed that the lipid-based oxygen suspension had backed up in the inferior vena cava, forming a massive embolus and preventing blood flow. The death of this animal was attributed to a different manufacturing of the suspension.

The second animal subject also experienced difficulties during the operation. Researchers experienced difficulty placing the venous and femoral catheters resulting in a significant loss of blood volume prior to the beginning of any infusions. Both the left and right femoral veins were perforated, and catheterization of these vessels was deemed impossible. An attempt to catheterize the right jugular resulted in perforation of the jugular vein. It was decided that further attempts at venous catheterization would jeopardize the immediate survival of the animal, and, as is often the case in hemorrhaging patients, that intraosseous line insertion was the only remaining solution. An intraosseous line was placed in the proximal humerus running parallel to the diaphysis.

A total of 40 ml of normal saline was flushed through this line, both to ensure proper placement and to restore volume lost due to line placement.

A total of three intraosseous infusions were administered to animal #2, each lasting 1:45 minutes. The animal survived the first two infusions with no hemodynamic perturbations. A 0.1 mg bolus of epinephrine was given after each infusion to treat hypotension in the animal, possibly due to the acidosis found on blood gases. During the final infusion, the animal again showed signs of hypotension. End tidal CO₂ levels dropped to zero, indicating that the animal had become entirely anaerobic despite the administration of oxygen. FiO₂ was increased to 21% with little effect, and the animal was terminated 10 minutes after the final infusion.

Upon necropsy of the animal, upwards of 20 ml of the suspension was found to have infiltrated the fascia surrounding the triceps (**Fig.1**). Examination of the thoracic cavity revealed no embolus to the lungs. A transverse dissection of the heart revealed evidence of subendocardial ischemia in the left ventricle (**Fig.2**).

Data collected from animal #2 was recorded on a 1-second time resolution from the SurgiVet monitor and the Oxford Optronix PO₂ probe. Ventilation data from the DATEX, however, was recorded on a 10-second time resolution. Accordingly, plots of SurgiVet and PO₂ probe data are far smoother than plots of DATEX data.

The third animal did not experience any noteworthy problems during preparation. The first intravenous infusion was successful, and the animal was recovered on 21% FiO₂. The second infusion was administered intraosseously through a line placed in the right proximal humerus. Towards the end of the infusion, suspension was seen emerging from the puncture site of the intraosseous line. After another period of stabilization, the

rabbit was given another intravenous infusion. This infusion was also successful, however the animal became severely hypotensive and end-tidal CO₂ levels began to drop shortly after. 0.4 mg epinephrine was administered, and CPR was performed for 5 minutes before the animal was declared dead.

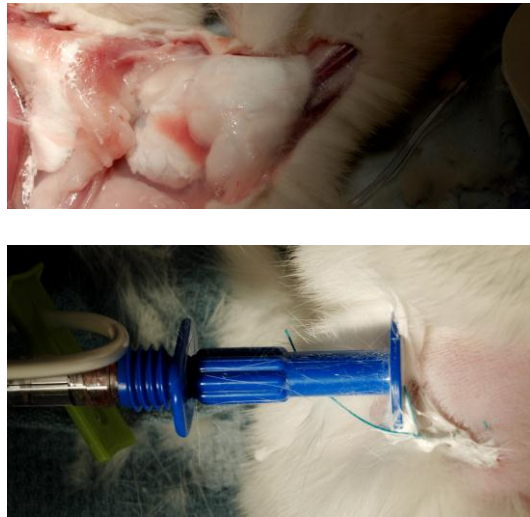


Fig.1 Images of infiltration (top) and extravasation (bottom) of the lipid-based oxygen suspension from the intraosseous lines placed in the proximal humerus of animals #2 and #3, respectively.

Autopsy of the animal revealed bruising of the lungs due to the trauma of CPR. No other cardiopulmonary abnormalities were seen. A transverse dissection of the heart again revealed signs of subendocardial ischemia (**Fig. 2**). Both the left and right humerus were excised from the animal and bisected longitudinally. No signs of marrow disruption, gas emboli or lipid deposits were seen in either the infused or uninfused humeri (**Fig. 3**).

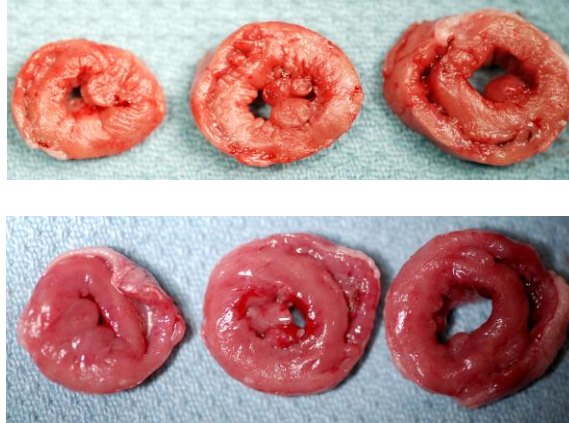


Fig. 2 Transverse dissections of the hearts collected during autopsy of animals #2 (top) and #3 (bottom). Both exhibit signs of subendocardial ischemia inside the left ventricle. They appear as darker areas, similar to bruising, on the inner walls of the ventricles.



Fig. 3 The left humerus (top, unfused) and right humerus (bottom, infused) bisected longitudinally. No gas emboli or lipid deposits can be seen. The white areas seen in the marrow of the bottom humerus is glare from surgical lamps.

The data collected from animal #3 was collected on a 1 second interval on the SurgiVet monitor and a 5-second interval on the probe and DATEX. Arterial blood pressure measurements were disrupted by an occlusion in the catheter that prevented transduction. This occlusion was later found to be due to the PO₂ probe being forced too far into the catheter. Instead of transducing from the same stopcock as the probe, the

blood pressure measurements were taken from the stopcock used for arterial blood gas sample collection. Because the flow to the transducers was disrupted for drawing samples, reliable blood pressure data from animal #3 was not measured. On both animals #2 and #3, pulse oximetry was never reliably obtained.

In total, animal #2 received 3 intraosseous infusions, and animal #3 received 2 intravenous and one intraosseous infusions. Thus, 4 successful intraosseous infusions and 2 successful intravenous infusions were completed for the study.

The intravenous infusions served as comparators for this experiment as the gold-standard for drug adsorption. Both intravenous infusions on animal #2 began when the animal was desaturated to a mean PO_2 of 31.76 mmHg. An increase in arterial tension was seen beginning at 00:30 seconds into the infusion. The oxygen tension increased at a steady rate throughout the entire infusing period, ending at a mean tension of 57.74 mmHg. The arterial tension continued to rise for approximately 00:45 seconds post infusion. Over the infusion, the ventilator was set at an FiO_2 of 12%, however measured inspiratory oxygen fraction fluctuated between 9.92 – 11.73%. FeO_2 , the fraction of oxygen exhaled by the animal, increased over the period of the infusion, even surpassing the inspired oxygen fraction for a short period of time, a finding present only when pulmonary arterial blood (normally the most desaturated blood in the body) is at a higher partial pressure of oxygen than inspired air (**Fig. 4**).

Compiled Data of Intravenous Infusions

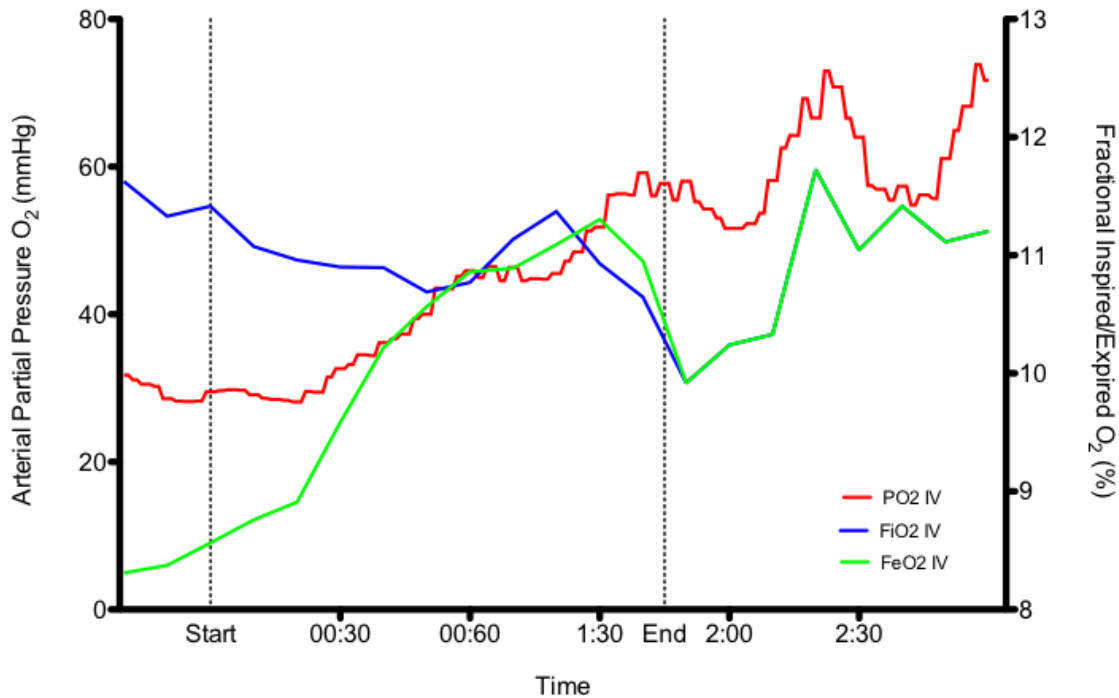


Fig. 4 The arterial oxygen tension measured using the invasive fiberoptic probe placed in the femoral artery is plotted over the inspired and expired oxygen percentage for the intravenous infusions (n=2). PO₂ is plotted on the left y-axis and Fe/FiO₂ is plotted on the right y-axis. Vertical dashed lines denote the start and end of the 1:45 minute infusion.

The intraosseous infusions began at a mean PO₂ of 37.23 mmHG. PO₂ increased at a gradual rate, and peaked in the middle of the infusion period. The infusion ended with mean arterial oxygen tension of 44.49 mmHG. The arterial oxygen tension began to decrease approximately 00:20 seconds before the end of the infusion, and continued to decrease after the end of infusion. An increase in arterial oxygen tension occurred shortly after infusion. Measured FiO₂ levels fluctuated between 11.44 – 11.89%. The FeO₂ of the animal increased steadily throughout the infusion, and peaked just after the endpoint. The FeO₂ values did not surpass FiO₂ values at any point (**Fig. 5**).

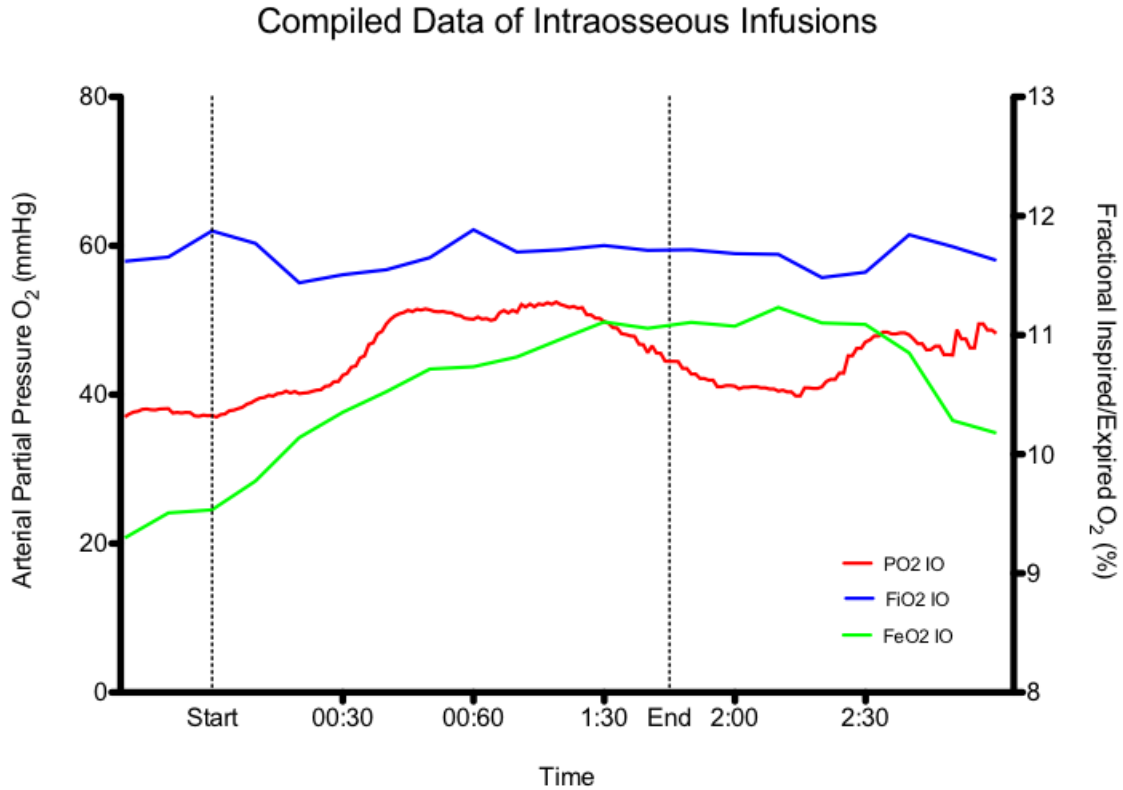


Fig. 5 Arterial oxygen tension measured using the femoral fiber optic probe plotted with the inspired and expired oxygen percentage measured during the intraosseous infusions (n=4). PO₂ is plotted on the left y-axis and Fe/FiO₂ is plotted on the right y-axis. Vertical dashed lines denote the start and end of the 1:45 minute infusion.

When directly compared to one another, the mean PO₂ curves of the intravenous and intraosseous infusions both show an increase in arterial oxygen tension. Linear regression analysis revealed that the rate of increase in arterial oxygen tension in the intravenous group was almost three times greater than that of the intraosseous group (0.3034 ± 0.009502 vs. 0.1198 ± 0.01612 , $p < 0.0001$) over the course of the infusion (**Fig. 6**).

Arterial Oxygen Tension

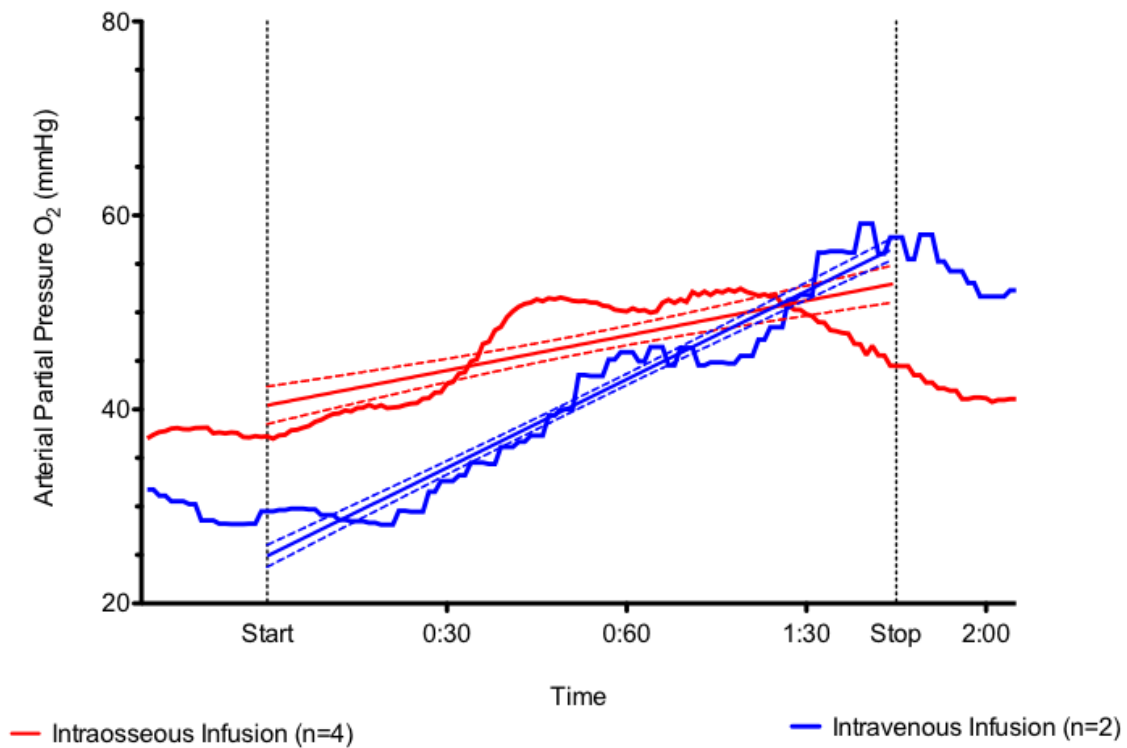


Fig. 6 The arterial oxygen tensions of both the intravenous and intraosseous infusions plotted on the same field along with corresponding regression lines. Dashed lines represent 95% confidence values of the regression line.

As a supplement to the constant arterial oxygen tension measurements taken by the fiberoptic probe, arterial oxyhemoglobin saturation was also measured. These measurements were taken from arterial blood samples drawn at infusion start and every 30 seconds during the infusion and the immediate post infusion period.

The SaO₂ values of the intravenous and intraosseous infusions show an increase over the infusion period. Linear regression analysis reveals that there is no statistical significance between the two lines ($p=0.253$), though the slopes of both lines were significantly non-zero. These results do, however, corroborate the rises in arterial tension seen in the animals (**Fig. 7**).

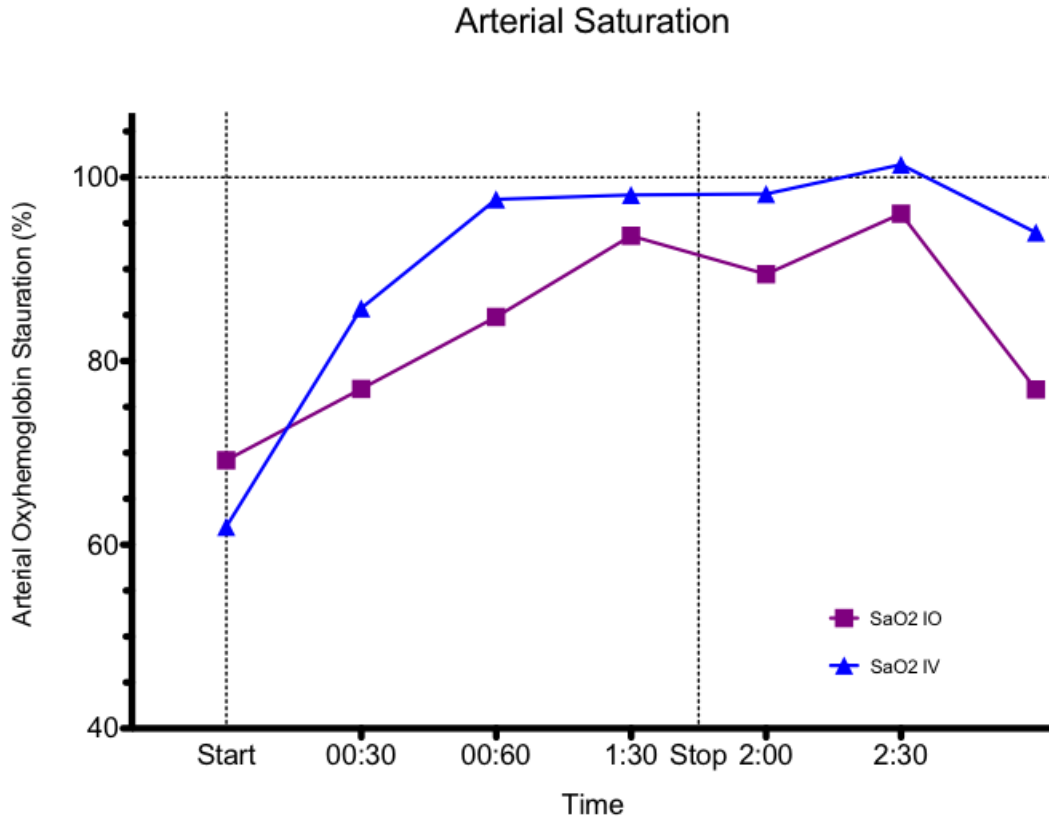


Fig. 7 Arterial oxyhemoglobin saturations obtained from arterial blood gas analysis. The dashed line at 100 represents the theoretical maximum value attainable.

The plots of the compiled intravenous and intraosseous infusion data included plots of the total mean values at each second, but did not show any data regarding the individual infusions. Each infusion reached a different maximum value at a different time in the infusion period. For the intravenous group, the mean maximum PO_2 of the infusions was 60.98 ± 0.84 . It took the infusions a mean time of 98.5 ± 9.19 seconds to reach this time. The intraosseous group had a mean maximum PO_2 value of 57.87 ± 12.07 and took a mean time of 79.75 ± 26.17 seconds after the start of the infusion to reach that point. Overall change in PO_2 values were calculated by determining the difference between the maximum and minimum values over the infusion period. The

intravenous group displayed a higher mean change in PO₂ of 32.95 ± 1.80 than that of the intraosseous group (23.12 ± 11.70) (**Table 1**).

	Mean Maximum PO ₂	Mean Change in PO ₂	Mean Time to Max
IV	60.98 ± 0.84	32.95 ± 1.80	98.5 ± 9.19
IO	57.87 ± 12.07	23.12 ± 11.70	79.75 ± 26.17

Table 1 Calculated mean values of the intravenous and intraosseous infusions with standard deviation included. Standard deviation values are considerably higher in the intraosseous group.

The mean plots of the Fe/FiO₂ values collected from intravenous and intraosseous infusions, although plotted separately in Figures 4 & 5, are easier to compare to one another when shown on the same field. The mean FeO₂ of the intravenous infusions increases to surpass the FiO₂. Although the FeO₂ of the intraosseous infusions rises over the infusion, it never surpasses the values of the FiO₂. Over the period of the infusion, the FeO₂ lines were not found to be significantly different ($p= 0.1736$) (**Fig. 8**).

Expired vs Inspired Oxygen Difference

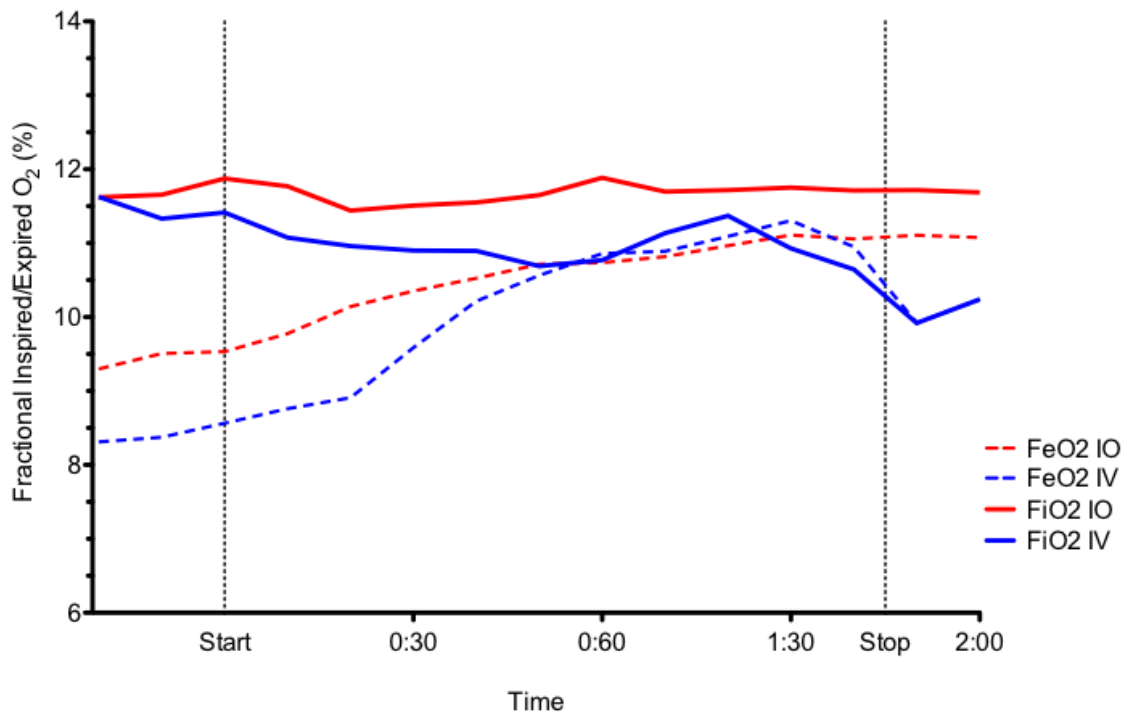


Fig. 8 The fractional inspired and expired oxygen measured during the intravenous and intraosseous infusions. The expired oxygen measure in the intravenous infusions surpasses the inspired oxygen. In contrast, the inspired and expired oxygen levels during the intraosseous infusions begin to converge, but never meet.

Mean arterial blood pressure was only collected on the first three intraosseous infusions because of difficulties transducing blood pressure on animal #3. The mean arterial pressure data of the three intraosseous infusions performed on animal #2 shows a slow rise over the infusion. Linear regression analysis revealed a best-fit line with a slope significantly non-zero (**Fig. 9**).

Mean Arterial Blood Pressure of IO Infusions

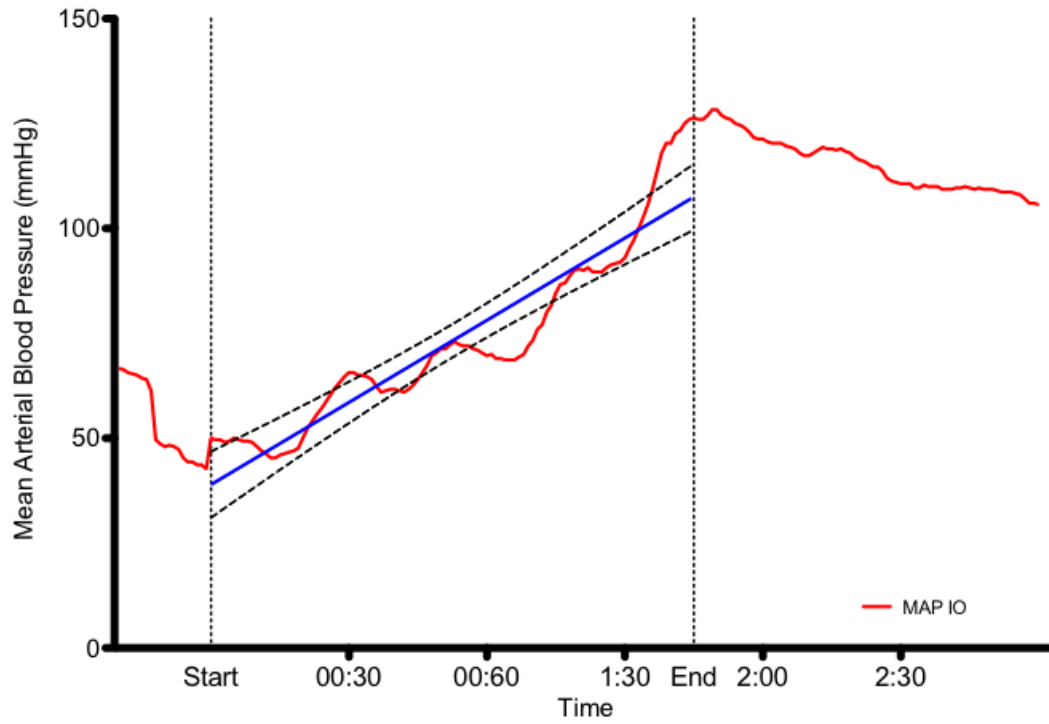


Fig. 9 Mean arterial pressure measured from the first three intraosseous infusions. Included is a linear regression analysis of the mean values, plotted in blue, with 95% CI (black dashed-line).

DISCUSSION

This study has many limitations, the most important of which is the low sample number and variations imposed upon manufacturing of the microparticle suspensions. The study suggests that infusion of a lipid-based oxygen suspension through the intraosseous route can increase the oxygen content of the blood of a hypoxemic patient. The compiled data collected from intraosseous infusions depicted a steady rise of arterial oxygen tension while the animal was held at a constant FiO_2 . The only possible source of the increase in tension over that time frame was the lipid-based oxygen suspension delivered via the IO line. The rise in oxyhemoglobin saturations measured throughout the IO infusions verifies that the animals were receiving supplemental oxygen over the period of infusion, and signifies an important rise in circulating oxygen content. Further confirmation of oxygen delivery can be found in the increase in FeO_2 seen over the infusion. A rapid increase in expired oxygen under constant inspired oxygen is a physiologic anomaly; mammals do not produce oxygen as waste. Therefore, this increase in FeO_2 indicates the rapid oxygenation of venous blood after the start of infusion. The blood passing through the pulmonary circulation had a higher oxygen tension than alveolar gas, causing back-diffusion of oxygen into the alveoli.

The linear regression analysis performed on oxygen tension during the intravenous and intraosseous infusions revealed that intravenous infusion increases blood oxygen tension at a higher rate than intraosseous infusion (**Fig. 6**). The slope of the intravenous line was almost three times greater than that of the intraosseous line, and the difference in the slope values was found to be significant. However, because the

infusions were all hand injections, these differences may represent differences in infusion rates alone, as intraosseous infusions required subjectively more force to infuse than did intravenous injections. Further studies which include quantification of injection rates are needed to accurately comment on the kinetics of absorption.

The oxyhemoglobin saturations collected from both the intravenous and intraosseous studies were not significantly different, suggesting that intraosseous infusion acts to resaturate hemoglobin in a manner similar to the intravenous route established by Kheir et al. in 2010 (**Fig. 7**). This, in fact, is a more relevant variable for oxygen delivery, as it indicates oxygen content absorbed and carried rather than the surrounding oxygen tension. Calculated mean peak PO₂ values of the individual infusions (**Table 1**) suggest that both the infusion routes achieve a similar level of oxygen saturation (IV: 60.98 ± 0.84 , IO: 57.87 ± 12.07). A large gap between the overall change in PO₂ would suggest that intravenous infusion carries the most impact (IV: 32.95 ± 1.80 , IO: 23.12 ± 11.70), however the large standard deviation of the intraosseous value brings this conclusion into question as well. Linear regression analysis of the FeO₂ values from both infusion groups suggested that the increases seen in expired oxygen content were not significantly different, consistent with our hypothesis that there is not a substantial difference in oxygen delivery between these two groups (**Fig. 8**). The mean arterial pressure shows a steady rise over the period of infusion (**Fig. 9**). Unfortunately, mean arterial pressure was only reliably transduced on animal#2. Therefore, with only one data group, no legitimate conclusions can be drawn.

As addressed by Paxton et al. (2009), dislodgement of the intraosseous needle from the bone was a problem encountered in both animals #2 and #3. One instance led to infiltration of approximately 20 ml of the suspension into surrounding tissue, and the other involved percutaneous leakage from the puncture site (**Fig. 2**). In both cases, the complications were likely due to a pressure buildup within the bone. The high rate of infusion needed to compensate for the animals' oxygen intakes could feasibly create pressure that would push the needle out of the marrow. A different, more secure intraosseous line could provide the needed stability throughout infusion.

The subendocardial ischemia seen in the hearts of both animals #2 and #3 could potentially be related to hypotension, however there is no way to determine which specific instance caused the damage (**Fig. 3**). Additional studies could potentially reveal whether this ischemia is present in all infused patients or is an iatrogenic insult incurred due to the infusion. The humeri that were bisected showed no signs of lipid deposits from the suspension (**Fig.1**). Furthermore, no oxygen emboli or visible disruption of the integrity of the marrow was seen.

Future experiments are needed to draw sound conclusions regarding the hemodynamics of intraosseous infusion of the suspension. There are not enough tests subjects and not enough data points to be confident in any accessory conclusions. To ensure proper data collection, future experiments should correctly align the time resolutions of all recording devices. Also, all means of recording data, such as the pulse oximeter and blood pressure transducers, should be functioning soundly. Proper data collection of future experiments will allow for more reliable conclusions to be drawn.

To better profile the hemodynamics of intraosseous infusion of the suspension, a radioopaque suspension could be infused into an animal. Fluoroscopic images taken at a set time interval could reveal the pathway of the suspension from the humerus to the heart and the approximate time it takes to reach arterial circulation. Histological analysis of tissue samples taken from the necropsy could reveal any underlying damages to the heart, brain, lungs or bone marrow that may be overlooked or invisible to the human eye.

Although many of the results were clouded by a low number of data points, the experiment still fulfilled its overall purpose; it has provided evidence that intraosseous infusion of a lipid-based oxygen suspension increases the oxygen content of the blood. This serves as a pilot study carrying proof of concept, allowing for funding of future experiments of intraosseous infusion.

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