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TITLE: Development of a Minimal-Bulk Oxygen Delivery Product to Enhance Survival During Hemorrhagic Shock/Studies Regarding the Use of Perfluorocarbon-Derived Intravascular Microbubbles from Oxygen Transport

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14. ABSTRACT

Goal 1: Production of microbubble forming dodecafluoropentane emulsion (DDFPe); NuvOx Pharma LLC, Tucson, AZ was able to make high-quality emulsions February 2008, which have been successfully used in resuscitation of rats (sever hemodilution) and pigs (potentially lethal hemorrhagic shock) over the last 16 months. Goal 2: A shock model combining hemorrhage (~32 mL/kg total blood loss) and a fractured femur bone was developed in pentobarbital anesthetized pigs weighing approximately 25 kg. In 19 pigs not given any treatment, ~30% of the pigs survived for six hours. Goal 3: Comparison of Hextend (plasma expander) and DDFPe (blood substitute) for low volume resuscitation in the model developed during Goal 2. Eight out of ten pigs survived if Hextend was given 45 minutes after bleeding, suggesting a survival of ~80%. All six pigs given 0.6 mL/kg DDFPe (NuvOx Pharma LLC) survived with good physiological values for more than six hours. This was interpreted as 100% of the DDFPe treated pigs survived for the required time. Repeatedly observed in Hextend treated pigs was development of severe pulmonary distress with foam in the lungs, dyspnea, and gradually rising content of CO2 and falling O2 tension in arterial blood. Goal 4: Semi-conscious pigs were bled ~31.5 mL/kg over 26 min and were allowed to develop shock over the next ~35 min. Two randomly selected groups (n=6 each) were resuscitated with either 7 mL/kg Hextend or 0.6 mL/kg DDFPe over 30 min. One pig died before any treatment was given. All treated pigs survived the experiments for 11 to 14 days, then euthanized. One of the Hextend treated pigs suffered severe sequel from the shock with no weight gain and was required euthanization on the 11th day. All DDFPe treated pigs were in good condition, increasing their weight at a normal rate. Goal 5: Pigs (from model described in Goal 2) in hemorrhagic shock were successfully resuscitated with Hextend or DDFPe given over 15 min. Conclusion: The experiments performed show that DDFP emulsion is a better resuscitation fluid than Hextend.

15. SUBJECT TERMS
Hemorrhagic shock, broken bone, dodecafluoropentane emulsion, Hextend, low volume resuscitation, semiconscious pigs

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INTRODUCTION

The proposal for the project "Development of a Minimal-Bulk Oxygen Delivery Product to Enhance Survival During Hemorrhagic Shock", contract # W81XWH-07-1-0011, was submitted by Dr. Claes Lundgren MD, PhD (Director of CRESE and Distinguished Professor at Department of Physiology and Biophysics, SUNY at Buffalo, NY) and Dr. Evan Unger MD, PhD (CO of ImaRx Pharmaceuticals, Inc., Tucson, AZ) as Principal Investigators (PI).

Dr. Lundgren withdrew as PI from the project in the fall of 2007, due to retirement, and the project was continued by Dr. Ingvald Tyssebotn MD, PhD as PI. Dr. Evan Unger resigned from ImaRx Pharmaceuticals, Inc. shortly after signing the contract with the US Army. A new agreement of production of DDFP emulsion was negotiated between the University of Buffalo and NuvOx Pharma, LLC and signed in January 2008 (see Appendices).

The project was brought forward with the title: Development of a Minimal-Bulk Oxygen Delivery Product to Enhance Survival during Hemorrhagic Shock/Studies regarding the use of Perfluorocarbon derived intravascular microbubbles for oxygen transport.

The US forces have been searching for the optimal treatment of hemorrhagic shock for decades. The standard treatment for a wounded soldier in the field has recently been to stop the bleeding and give 500 mL of Hextend (6 % hetastarch in lactated electrolyte injection; Hospira Inc., Lake Forest, IL) as soon as a paramedic arrives. Assuming a standard person weighs 70 kg, the dose of the plasma expander, Hextend, will be 7 mL/kg. To imitate this scenario, we have developed a model on anesthetized pigs (**Model I**) with a broken bone and hemorrhagic shock, comparing treatment with intravascular oxygen-carrying micro bubbles of dodecafluoropentane emulsion (DDFPe), 0.6 mL/kg, and treatment with Hextend, 7 mL/kg. A survival model (**Model II**) was developed where pigs had a venous and an arterial catheter implanted during a brief Isoflurane anesthesia 2-3 days before the bleeding, and the hemorrhage performed while the pigs were tranquilized and semi- conscious. The pigs were treated by either Hextend or DDFPe, in the same doses as in **Model I**, and the pigs kept alive for the next 11 to 14 days. In **Model III** the treatment volumes were similar, but the infusion rate was doubled (15 min, compared to 30 min).

BODY OF WORK

THE AIMS OF THE STUDY

Aim 1 (*Goal 1*): To optimize, evaluate and manufacture, on a laboratory scale , a novel oxygen transporting erythrocyte replacement intravenous preparation consisting of a 2% DDFPe stabilized with PEG Telomer B, Pluronic P123 and sucrose.

Aim 2 (*Goal 2 to 5*): To demonstrate the unique properties of the preparation (produced under *Goal 1*) as low volume resuscitation fluid for treatment of hemorrhagic shock in field-realistic animal models.

Aim 1 - Development of new DDFP emulsion (*Goal 1*)

The production of 2% DDFPe terminated in September 2007 and the manufacturer ImaRx Pharmaceuticals, Inc. (Tucson, AZ) withdrew from the project in the fall of 2007. Another small company, NuvOx Pharma, LLC (Tucson, AZ) bought the production rights and negotiated a contract with State University of New York at Buffalo by UB Office of Science, Technology Transfer and Economic Outreach early 2008. The company was able to start production of the new formula in February 2008, a replica of the previously produced EchoGen, Sonus Pharmaceuticals, Inc., Bothell, WA. This emulsion is now 15 months old and the particles have been stable and so far have not shown signs to deteriorate or to grow in size. The emulsion has been stored at 25°C with 60% relative humidity and at 40°C with 75% relative humidity since production, but still not close to the planned 3 years (The EchoGen particles were mainly stable for 11 years when stored in room temperature after the production in June 1997). The new formula has been used intensively over these months in pigs and rats, and has been found to be as good as the EchoGen produced by and Abbott in 1997. The new emulsion passed our rigorous tests in a hemodiluted rat model (described by Lundgren, Bergoe and Tyssebotn, 2006) with survival of 5 rats for 2 hours at hemoglobin of less than 2.0 g/100 mL.

The screening of the DDFPe formulation produced by ImaRx Pharmaceuticals, Inc., was reported on March 2008.

All work and results from here on are discussed in relation to the microbubble-producing DDFPe (NVP-108) manufactured by NuvOx Pharma, LLC, Tucson, AZ.

Aim 2 - DDFP emulsion and Hextend as resuscitation fluids

To show the unique properties of the DDFPe that creates microbubbles when heated above 29ºC in a warm-blooded body, the bubbles were used for treatment of hemorrhagic shock in several animal models. During the first passage through the lungs, the bubbles (diameter: 2-3 µm) take up oxygen from the alveoli similar to red blood cells. The bubbles are transported with the blood to the tissues where oxygen is unloaded and carbon dioxide absorbed for transportation back to the lungs (Burkard and Van Liew, 1994). The size of the microbubbles is determined by the size of the emulsion particles (~200 nm in diamenter, NuvOx Pharma, LLC).

The studies in aim 2 were divided into four different goals using three different animal models all with pathogen free Yorkshire pigs.

<u>*Goal 2:*</u> Develop an animal model that after hemorrhagic shock combined with a broken femur, soft tissue injuries and laparotomy with no resuscitation will give a survival rate of ~30% (**Model I**)

<u>*Goal 3:*</u> Comparison of DDFPe treatment and Hextend treatment in **Model I** (reported March 2008).

<u>*Goal 4:*</u> Study the long term consequences of hemorrhagic shock in slightly sedated pigs (surviving 14 days) (**Model II**). Comparison of resuscitation with DDFPe treatment and Hextend treatment in **Model II**.

Goal 5: Evaluation of increased infusion rates on the outcome of DDFPe and Hextend treatments using the experimental setup as in Model I (**Model III**)

METHODS

TESTING OF DDFP EMULSIONS ON RATS

In this project, the two NuvOx Pharma, LLC DDFP emulsions produced mid-February, 2008, were tested on eight Wistar rats. All rats were tested for survival with the aforementioned hemodilution model (Lundgren, Bergoe and Tyssebotn 2006). They all survived surgery. After hemodilution to less than 2 g hemoglobin/100 mL blood while given 0.7 mL DDFPe/kg body weight, the rats lived for 2 hours, which was our preset requirement of a high-quality emulsion.

MODEL I: HEMORRHAGE IN ANAESTHETIZED PIGS WITH BROKEN FEMUR AND LAPAROTOMY

Most results from **Model I** pigs were reported in the written report on March 31, 2008 and verbally given at the ATACCC Conference, St. Pete's Beach, FL, August 2008.

Since March 2008, more pigs have been added to each study group. The results confirm and strengthen the conclusions from last year. Non-treated pigs had a survival rate of ~30% after suffering a fractured femur, soft tissue damage and severe blood loss. When Hextend was used as treatment in this model, the survival rate increased to 80% (8 out of 10). When the new DDFP emulsion from NuvOx Pharma was applied, the survival rate was 100% (6 out of 6) in healthy pigs.

MODEL II: HEMORRHAGE IN SEDATED PIGS - COMPARISON OF DDFPe TREATMENT AND HEXTEND TREATMENT

A model for hemorrhagic shock and bleeding in sedated, awake pigs was developed over the spring of 2008. After 3 months of negotiations with the IACUC committee at the university, we were granted approval for the protocol to conduct the experiments under semiconscious conditions. Two members of the IACUC committee supervised the bleeding procedure during the first two experiments to ensure the pigs did not suffer in any way.

The timeline for sedated pig experiments

Habituation: Habituation of the pigs lasted for 4-5 weeks to adapt them to be relaxed in a supporting sling when sedated for many hours during the hemorrhage experiment.

Day 1 (surgery): Pigs were anesthetized and surgically prepared with a limited number of arterial and venous catheters.

Day 2 or 3 (hemorrhage): Hemorrhagic shock experiments were performed.

Day 16 (necropsy): The pigs were anesthetized and physiological measurements performed, followed by euthanization, and harvesting of tissue samples.

The training/habituation program

Sixteen pathogen free neutered male pigs weighing 8-10 kg were purchased from Michal Fanning Farm, IN, and grew to ~23 kg by the time of experiment.

The pigs were trained daily by a limited number of people starting with touching and grooming. They were gradually exposed to the restraining sling and the equipment used in the experiments. For motivation and as a reward during and after each training session the pigs were given treats, such as apple pieces, until they were calm and relaxed in the sling. Whenever the pigs were in their pen, they had free access to regular food and water. After approximately 4 weeks of training the pigs were willing to stay in the sling for the duration of the experiments.

Surgical preparation - Day 1

The pigs were pre-medicated with Telazol, 1 mL /25 kg, and anesthetized for approximately one hour with Isoflurane gas and mechanically ventilated with 4 L of O_2/min. The entire surgery was performed aseptically and in a sterile surgical suite at the Laboratory Animal Facility of University at Buffalo (SUNY). Each pig was intubated with an endo-tracheal tube, size 5.5 to 7 mm. A small skin incision on the right side of the neck was made and the right carotid artery was located and cannulated with a catheter to facilitate bleeding during the experimental procedure. The right jugular vein was cannulated and the catheter tip located close to the right atrium to be used for infusions of sedative drugs and fluids. The catheters were tunneled under the skin and the ends taken out on the pig's back. Arterial blood was collected anaerobically for measurements of the arterial acid-base chemistry, blood gases, sodium, potassium, base excess, lactate and glucose on an ABL725 Blood Gas Analyzer (Radiometer, Copenhagen, Denmark). Blood pressures and heart rate (HR) were measured for approximately 1 hour and at least 2 arterial blood samples were taken during the procedure. The pigs were given an infusion of lactated Ringer's solution in the amount of 1 mL/min for the duration of the surgery. At the end of the surgical procedure, the catheters were closed and put into a small pocket sewn onto a fitting jacket worn by the pig to ensure that the pigs did not reach them. The catheters were flushed with heparinized fluids daily to reduce the risk of coagulation.

Hemorrhagic shock experimental procedure - Day 2 or 3

Post surgery and until time of euthanization, the pigs were checked daily by a veterinarian. The pigs were removed from their pen after given a small dose of either of two drugs, Midazolam or Diazepam i.v. and placed in a supporting sling that allowed them to keep their feet on the ground. Of the 16 pigs that had catheters implanted, 4 pigs had to be excluded from the study. One had severe lung infection (verified with bacteriology), two had clogged catheter lines (dedicated as shams for histology), and the last did not survive the hemorrhage.

When the pigs had relaxed for 15 min in the supporting sling, the arterial pressure line was connected to a Y-adaptor where one branch was connected to a pressure transducer and the other to a bleeding line (Fig 1). The central venous line was connected to another Y-adaptor with one branch connected

to a pressure (CVP) transducer and the other branch to a pump to be used for infusion of sedative drugs, plasma expander fluids and DDFPe (Figure 1).

Prior to bleeding, baseline measurements were recorded for 30 min and 3 arterial blood samples and 1 venous sample were taken and assessed. The pigs were then bled ~31.5 mL/kg over 2 6 min, reducing the blood pressure below 50 mmHg. The pigs remained calm during the entire bleeding procedure. From 30 min prior to the onset of bleeding, arterial and venous pressures, respiratory frequency (RF) and heart rate (HR) were measured continuously. The start of hemorrhage was set as time zero. For measurements of O_2 and CO_2 tensions, base excess, hemoglobin, potassium, sodium, chloride, glucose and lactate concentrations and pH, arterial blood was sampled every 5 min (~1 mL each) for the first 45 min and every 15 min thereafter, while venous blood (~1 mL) was sampled every 60 min for the entire experiment. In addition, 5 mL arterial blood was sampled every 60 min throughout the experiment for analysis of urea, creatinine and DDFP concentrations. As a final result, blood sampling ended at roughly 10 mL/hr for the duration of the experiment.

Hemorrhagic shock was allowed to develop from the onset of bleeding until ~63 min mark, where after treatment with either DDFPe (0.6 mL/kg) or Hextend (7 mL/kg) was infused over the next 30 min. After the DDFPe infusion, 7 mL of venous blood was sampled to clean the DDFPe out of the venous line. An identical volume was sampled from the Hextend pigs.

After a period of 3 hours, 500 mL of Hespan (6% hetastarch in a 0.9% NaCl solution; H.Braun Medical Inc., Irvine, CA) was infused over 60 min in all pigs and the wounds closed using local anesthetics, after which the pigs were returned to their pen.

Physiological measurements and euthanasia - Day 11 or 14

A Hextend pig that the veterinarian required to be euthanized on day 11 had an extremely poor condition without any detectable reason. It was unable to feed or stand up. All the other pigs were apparently healthy, eating and drinking and lived until Day 14, when they were euthanized as planned.

All pigs were anesthetized with Isoflurane after a standard premedication of Telazol, 1 mL/25 kg. All pigs had new catheters implanted in the right femoral artery and the left jugular vein for arterial and venous pressure measurements. A Foley catheter was implanted into the urinary bladder through a small incision in the lower abdominal wall.

After a stabilization period of 45 min, at least 3 parallel arterial and venous blood samples as well as urine samples were taken in order to assess kidney function by calculation of urea and creatinine clearance, as well as estimation of renal blood flow.

From the arterial and venous blood samples, O_2 and CO_2 tensions, base excess, hemoglobin, potassium, sodium, chloride, glucose, urea, creatinine, lactate concentrations and pH were determined. The measurements were performed over 1 hour after which a light hemodilution, reducing hemoglobin concentration by 30%, was performed before the pigs were euthanized with a lethal dose of pentobarbital (Fatal Plus, Vortech Pharmaceutical, Dearborn, MI). Tissue samples were harvested from the following organs: heart, lungs, cerebrum, cerebellum, brain stem and spinal cord,

spleen, liver, stomach, small and large intestines, kidneys, and adrenals. The tissue samples were stored in 4% buffered formaldehyde before processed for light microscopy. So far, only histopathology of pig kidney has been provided by Professor Peter Nickerson, PhD, SUNY at Buffalo. The following groups of pigs were examined: Control (sham operated), Hextend and DDFPe. Following the fixation with buffered formaldehyde, kidneys were processed by conventional techniques for light microscopy; 4 µm sections were cut and stained with hematoxylin and eosin.

MODEL III - RAPID INFUSIONS OF DDFPe AND HEXTEND

Six pigs, weighing 20-25 kg, anesthetized with pentobarbital sodium (25 mg/kg), were tested for their tolerance of increased infusion rate of DDFPe or Hextend treatments. The pigs were instrumented as was done for the broken bone/hemorrhagic model (**Model I**) (Figure 11) reported in March 2008. The leg was broken 5 min before the bleeding was started. A bleeding of ~ 28 mL/kg was performed over 18 min in all pigs. The six pigs were randomly divided into two groups treated with an infusion of either Hextend (7 mL/kg) or DDFPe (0.6 mL/kg) over a 15 min time period.

Oxygen consumption and CO_2 production were measured by an airflow meter, and mass spectrometer measurements of O_2 and CO_2 in expired gas, were also taken. Results were converted to mL/kg (STDPD). Cardiac output was measured by a Millar blood flow catheter (Millar Instruments, Houston, TX) placed in the left ventricle. Tissue electrodes from Radiometer (Copenhagen, Denmark) were used for tissue O_2 and CO_2 measurements.

STATISTICS AND CALCULATIONS

All values are given as means ± SEM (standard error of the mean). P-values of 0.05 or less were considered significant. Creatinine and urea clearances were calculated from plasma and urine concentrations and urine flow on the final day of the experiments. Clearance of creatinine and glomerular filtration rate (GFR) are believed to be equal. Assuming the filtration fraction to be 20%, the renal plasma flow and blood flow were calculated from the measured creatinine clearance (GFR) and adjusted by the hematocrit. Blood volume was calculated from the fall in hemoglobin after infusion of known amounts of Hextend (Model II and III) and Hespan (Model II). Shock index was calculated as heart rate divided by mean arterial pressure and cardiac work index as heart rate times systolic arterial pressure.

RESULTS

MODEL II - SEDATED PIGS GIVEN DDFPe OR HEXTEND TREATMENTS

The training/ habituation

All sixteen pigs were successfully trained to stand relaxed in the supporting sling for up to two hours.

Day 1 - Physiological measurements during anesthesia

Arterial pressure, CVP, HR, RF and arterial blood parameters were similar and within normal range in all sixteen pigs post surgery (Figure 2 to 10). They all recovered from the Isoflurane anesthesia within 15 min after surgery was completed and started eating immediately after being returned to the pen.

Day 2 - Hemorrhagic shock development and treatments during light sedation

The arterial pressures, CVP, HR and RF values prior to bleeding were similar between the two groups of animals (Figure 2 to 5). Blood samples taken before the hemorrhage also had identical compositions, as shown in Figure 7 to 10. The differences in values from 2 days before were most likely caused by mechanical ventilation, O_2 breathing, and Isoflurane anesthesia.

The arterial pressures fell similarly in both groups during bleeding (Figure 2). While hemorrhage increased the HR during bleeding of pentobarbital anesthetized pigs as previously demonstrated (annual report March 2008), HR fell during bleeding of the semiconscious pigs (Figure 3). Both groups of pigs were bled identically (31.9 ± 0.5 mL/kg in 26 ± 0 min), the shock developed similarly until the treatment began 63 min after the onset of bleeding (Figures 2 to 6), and the blood composition developed equally (Figures 7 to 10).

<u>HEXTEND TREATED PIGS</u>

A few minutes after the Hextend infusion was initiated, the arterial pressures started to increase gradually as demonstrated in Figure 2, upper panel, and stabilized around 100 mmHg during the rest of the period before the plasma expander (Hespan) was initiated. A smaller increase was observed during the plasma expander infusion. The HR fell after the Hextend infusion, but increased during the infusion of plasma expander (Figure 2, upper panel). The respiratory frequency increased during bleeding, then started to fall transiently before a significant and a marked increase (~40%) occurred during Hextend infusion. The rate again fell at the end of the infusion, followed by another increase after start of plasma expander infusion (Figure 4, upper panel).

<u>DDFPe TREATED PIGS</u>

The arterial pressure showed a rapid, transient increase during the first 10 min of DDFPe infusion. After the treatment, arterial pressure continued to increase gradually during the whole period before Hespan infusion. The HR was unchanged during bleeding and before start of DDFPe treatment, increased slightly during treatment, gradually fell during the period before Hespan treatment, and increased gradually afterwards (Figure 3, lower panel). The RF remained mostly unchanged during bleeding and treatment and the period before Hespan was given, but fell abruptly during this infusion. At the end of Hespan infusion, the RF increased steeply (Figure 4, lower panel).

<u>COMPARISON BETWEEN THE TWO GROUPS OF ANIMALS</u>

The shock index (Figure 5) and cardiac work index (Figure 6) described similar patterns and were not statistically different in the two groups. The dilution effect of Hextend on arterial blood is shown in Figure 7, resulting in an arterial oxygen content significantly higher ($P<0.01$) in the DDFP group than the Hextend treated group (Figure 8). Since the O_2 content is calculated from oxyhemoglobine

concentration and oxygen tension measured by the ABL 725, the O_2 content values in the DDFPe group (during and after treatment) is most likely a minimum value since the machine might not be measuring the O_2 in the microbubbles. Concentrations of lactate, glucose, potassium and base-access were similar during the experiment in the two groups of animals (Figure 10).

When returned to the pen after the bleeding experiment, all DDFPe treated pigs immediately started to eat and drink, while the Hextend treated pigs primarily slept.

Day 14 – Physiological measurements, euthanasia, and necropsy

By the day of necropsy, most of the pigs had developed small, local, superficial infections in the wound surfaces on the neck. As mentioned previously, one of the Hextend pigs required to be sacrificed the 11th day after hemorrhage, as it had not gained any weight, was inactive, and was in very poor condition. There was no indication of infection or temperature increase. All other pigs in both groups had gained weight, 0.3 ± 0.1 kg/day (Hextend) and 0.4 ± 0.1 kg/day (DDFPe), were in good condition, and lived for the 14 days as required. On the day of necropsy, the arterial and central venous pressures as well as blood compositions were similar to the values during identical anesthesia on Day 1. The pressures and blood composition values were similar in both treatment groups and similar to the values measured in anesthesia prior to the bleeding experiment. The creatinine clearance was similar in both groups (182 ± 13 and 184 ± 11 mL/min, DDFPe and Hextend group, respectively), while the protein clearance was significantly higher in the Hextend group than the DDFPe group (0.58 ±0.28 and 0.05 ± 0.01 mL/min, $P<0.05$, respectively) indicating significantly more proteins were excreted, possibly due to glomerular membrane leakage. Renal blood flows calculated from creatinine clearance were 1253 ± 88 mL/min for the DDFPe group and 1257 ± 81 mL/min for the Hextend group. Only one animal from the DDFPe group had detectable concentration of DDFP in the lung. No animals had DDFP detected in the fat.

During the necropsy, pale, wedge- shaped areas within the cortex and the outer medulla of the kidneys were regularly found in the Hextend treated pigs, but not in the DDFPe treated pigs.

By light microscopy, the histopathology of kidneys, including the glomeruli in the cortex from the DDFPe group, was virtually identical to the control group (sham-operated). Glomeruli in the Hextend group showed a small amount of vacuolation that corresponds to prominent capillaries. Tubules throughout the cortex and medulla were similar among all three groups of animals.

MODEL III - INREASED INFUSION RATE DURING DDFPe OR HEXTEND TREATMENT

These pigs were anesthetized with pentobarbital that reduced the arterial O_2 and increased the CO_2 tensions compared to the sedated pigs in the previously described series (Model II). Otherwise the results are comparable between sedated and anesthetized pigs. Only 1 pig from each treatment group survived for the required 6 hours (Figure 12). Two Hextend treated pigs died at a mean of 130 min after start of bleeding due to pulmonary distress, reduced arterial O_2 and increased CO_2 tensions. The two pigs that died after DDFPe infusion survived both for a marked longer time (mean: 233 min) than the Hextend pigs that died. One of the DDFPe treated pigs that died had an anomaly with a considerable channel in the septum between the two atriums of the heart mixing the oxygenated and venous blood (verified by high O_2 tensions in venous blood samples). This circulatory pattern could

possibly reduce the loading of O_2 into the microbubbles of DDFP. Results from the measurements are shown in Figures 12 to 19.

During deep anesthesia, it was remarkable that when the femur was fractured, the O_2 consumption and CO_2 production immediately fell in all animals by 25-30 % before the bleeding started 5 min later (Figure 14 and 15). Similarly, minute ventilation fell after the bone break and started to increase during bleeding. During treatment, only Hextend increased the minute ventilation by 42% while DDFPe treatment increased the minute ventilation by only 4 %. The work of breathing/min (calculated as: (tidal volume)x(pressure changes during the breathing cycle) x(breathing frequency)) increased by 67% during Hextend treatment while it only increased by 9% during the DDFP treatment.

DISCUSSION

The fundamental goal of this study is to elucidate the most effective treatment of hemorrhagic shock in a field-applicable setting. There is no obvious difference in the outcome of the two treatment modalities, except that one of the sedated Hextend treated pigs in Model II required early euthanization and other pigs during Hextend infusion developed respiratory distress (Model I under Goal 3, Model II and Model III). Treatments with the microbubbles created from DDFP emulsion was, in our view, the best of the two options since blood pressure steadily increased during and after the infusion, then remained stable for several hours (Model I under Goal 3 and Model II). In addition, the animals seemed healthier during the recovery after the experiment, eating better, no death occurred over 14 days, and no renal changes were observed microscopically (Model II). Perhaps a combination of DDFP microbubbles and a small amount of Hextend could give a better outcome, although this was not evaluated in the present study. Additional positive observations are that DDFP emulsion does not need refrigeration during storage, the shelf life is long (16 months, so far, for the emulsion produced by NuvOx LLD in February 2008; 10 years for the product made by Sonus Pharmaceuticals, Inc., Bothell, WA), the difficulties of matching donor/recipient blood type is prevented, and the volume is so small that an individual soldier could bring his/her own sterile bottle of DDFPe solution into the field. Indeed, Hextend used as a resuscitation fluid is 12 times as voluminous as DDFPe, putting an extra burden to the paramedic in the field (500 mL given to a soldier). Additional disadvantages of Hextend discovered in this study include lower weight gain than normal, and occasional deaths, as only 84% of the animals survived for 14 days (Model II).

One of the aims in this study was to increase the infusion rate of a full dose of Hextend and DDFPe to be given in 15 min (Model III). This was possible in both types of treatment, but only one out of three pigs in each group survived for 6 hours which was our requirement for the study. The two Hextend treated pigs died after an average of 130 min, whereas the two DDFPe treated pigs died after about 240 min, even with one of the latter pigs having a significant hole in the cardiac septum, possibly influencing the survival time. The two Hextend treated pigs died with pulmonary distress, reduced arterial O_2, and increased CO_2 tensions suggest that the increased infusion rate caused a detrimental overload of fluid.

KEY RESEARCH ACCOMPLISHMENTS

- New DDFP emulsion (NVP-108, NuvOx Pharma, LLC) is produced in laboratory scale.
- The emulsion appears to be stable after 16 months.
- The emulsion functions well when tested in both oxygen depleted rats and pigs.
- Three hemorrhagic shock models in pig have been successfully developed and tested.
- Treatment with 0.6 mL/kg DDFP emulsion demonstrates excellent outcome in pigs after hemorrhagic shock.
- Hextend infusion of 7 mL/kg showed some dangerous side effects and demonstrated lesser survival in hemorrhagic shock than infusion of DDFPe.
- The oxygen content in arterial blood was kept higher in DDFPe treated pigs than in Hextend treated pigs providing more O_2 to the tissue if blood flow was similarly maintained.
- Kidney physiology and histology was normal in the DDFPe treated and sham-operated pigs.
- The Hextend treated surviving pigs lost proteins in urine on necropsy day, most likely caused by membrane destruction in the glomeruli.
- Histology of the kidneys in Hextend treated pigs showed vacuolization in the glomeruli.
- Increased rate of Hextend infusion provoked symptoms of pulmonary distress.
- The higher infusion rate gave longer survival time in DDFPe treated pigs compared to Hextend treated pigs.
- Treatment with DDFP emulsion remains the most promising treatment module for Hemorrhagic shock.

REPORTABLE OUTCOME

Tyssebotn, I, GW Bergoe: Treatment of Hemorrhagic Shock: Comparison between 0.5 mL/kg DDFP emulsion (microbubbles) and 7 mL/kg Hextend. ATACCC Conference, St. Pete Beach, Florida, 11th 13th August 2008.

Tyssebotn, I, GW Bergoe; Treatment of Hemorrhagic Shock: Comparison between 0.6 mL/kg DDFP emulsion (microbubbles) and 7 mL/kg Hextend in semiconscious pigs. ATACCC Conference, St. Pete Beach, Florida, to be held 10th-13thAugust, 2009.

CONCLUSIONS

1. A new and stable DDFP emulsion is available.
2. Pigs in hemorrhagic shock can be successfully resuscitated with an extremely small dose of DDFP emulsion (15 mL/25 kg).
3. The volume of Hextend used is twelve times the volume of DDFPe.
4. DDFPe as resuscitation fluid seems more effective than Hextend

5. Low volume resuscitation can be accomplished with DDFP emulsion.
6. If pertinent, a wounded soldier in the field can be treated with about 45 mL of DDFPe emulsion.

SO WHAT

Since the pigs survived after one dose of DDFPe or Hextend, and the time frame for experiments was too short a second dose was not attempted.

The older DDFP emulsion (Echogen) produced by Sonus Pharmaceuticals Inc. has been tested in roughly 2500 humans, volunteers and patients, and approved for ultrasound imaging in humans in Europe. Sonus was not able to get Echogen approved by FDA. NuvOx Pharma, LLC (Tucson, AZ) has been able to make a replica of Echogen that has been used in the present study. This replica is working equally well as the predecessor. NuvOx Pharma, LLC is a small company with limited resources and will not, without funding, be able to get the product approved by FDA and clinically tested.

Treatment with DDFP emulsion is still the most promising resuscitation fluid for hemorrhagic shock, but more research has to be performed to evaluate the safety of the DDFP emulsion.

PROBLEMS MET AND SOLVED DURING THE PROJECT

The work planned under Aim 1 in the original proposal, would have brought the preparation to maturity in terms of fulfilling the sponsor's requirements under Product Considerations (page 3, Supplement 1) as well as with regards to toxicology and GMP. This was not accomplished since ImaRx Pharmaceuticals, Inc. met financial problems. They closed down the production and later abandoned the project. Therefore, at the end of the grant period (i.e. end of year 2), the product from the new company NuvOx Pharma, LLC was still not ready for an FDA IND permit, even as a successful outcome of work under Aim 2 (in the original proposal) has been demonstrated.

Change of PI created some delays in the middle of the grant period.

One of the key researchers became seriously ill and was on sick leave for three months.

Negotiations between NuvOx Pharma, LLC and the University of Buffalo took several months, but discrepancies were solved in January 2008 (see Appendices).

In the spring of 2008, 3-4 months of negotiations and modifications of the research protocol were invested before the IACUC committee at the University was willing to approve the protocol for bleeding of habituated, sedated pigs.

Shortage of a technician in our lab lasted for more than 6 months and therefore delayed processing of data.

The histology of tissue specimens harvested during necropsy has not yet been fully processed due to shortage of personnel in the histology lab of Dr. Peter Nickerson. The kidney specimens were

however processed a week ago and the results includ ed in this report. The remaining histology mentioned is still pending.

REFERENCES

Burkard ME, Van Liew, HD. Oxygen transport to tissue by persistent bubbles: Theory and simulations. J. Appl. Physiol. **77**, 2874-2878, 1994.

Lundgren C, Bergoe G, T yssebotn I. Intrava scular fluor ocarbon-stabilized micr obubbles protect against fatal anemia in rats. Artificial Cells, Bl ood Substitutes, and Biotechnology, and Internationa l Journal. **34**, 473-486, 2006.

APPENDICES

1 - FIGURES WITH LEGENDS

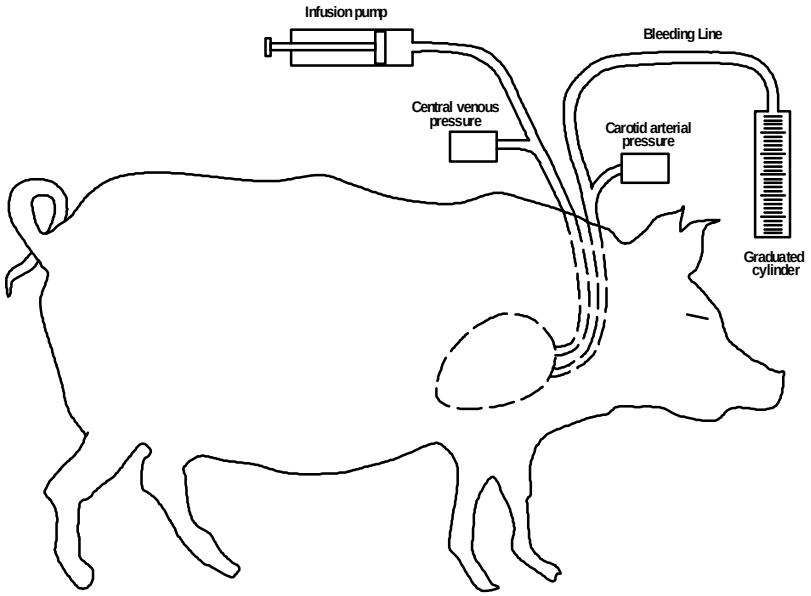


Figure 1: A schematic drawing of a sedated pig positioned in a sling prepared for experimental hemorrhagic shock (Model II). Catheters for bleeding and arterial pressure recording from aorta, and infusion of sedative and treatment in a vein (vena jugularis) are shown. The pigs were bled 31.9 ± 0.5 mL/kg body weight during 26 ± 0 min. When all the blood samples were added at the end of the experimental day, 344 ± 0 min after start of bleeding, the animals had lost 35.3 ± 0.8 mL/kg. All values are means ± SE. The experiments were performed under heavy scrutiny of the veterinarian staff.

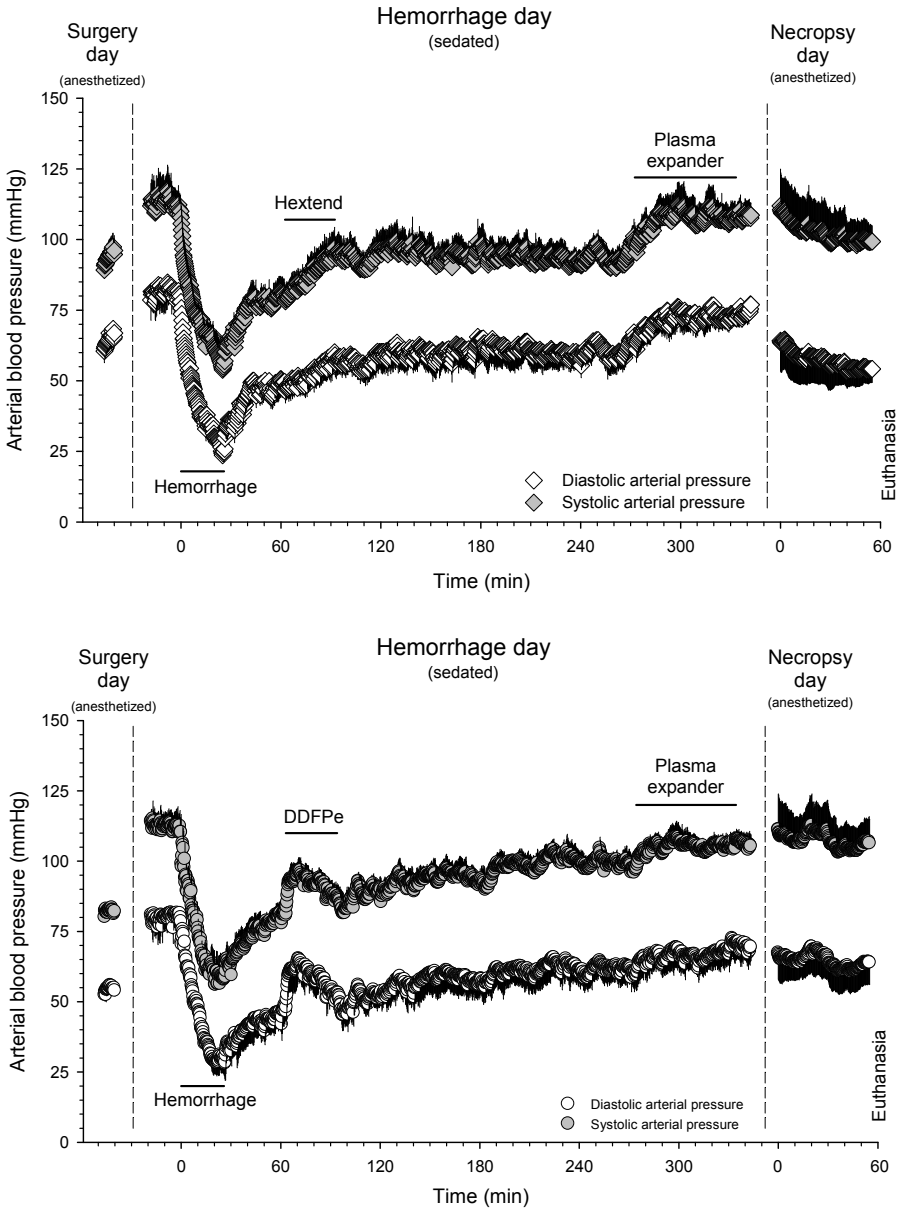


Figure 2: The figure shows the timeline and events during hemorrhagic shock experiments in sedated pigs. This includes the surgery day 2 to 3 days before the hemorrhage day, and the necropsy day 11 to 14 days after the severe blood loss (31.9 ± 0.5 mL/kg). Systolic and diastolic arterial pressure are shown in two groups of pigs (n=6 in each). About 35 min after the hemorrhage the pigs were treated over 30 min with either 7 mL/kg of Hextend (6% hetastarch in lactated electrolyte injection) (upper panel) or 0.6 mL/kg of DDFPe (dodecafluoropentane emulsion) (lower panel). Which of the treatments to be applied was chosen by coin toss 10 min before the infusion was to start. Three hours after the treatment had finished, a second infusion of plasma expander (500 mL of Hespan (6% hetastarch in 0.9% sodium chloride injection) during 60 min) was given to all animals. The catheters were closed and the wounds sutured in local anesthesia and heavy sedation about 10 min after the end of the last infusion.

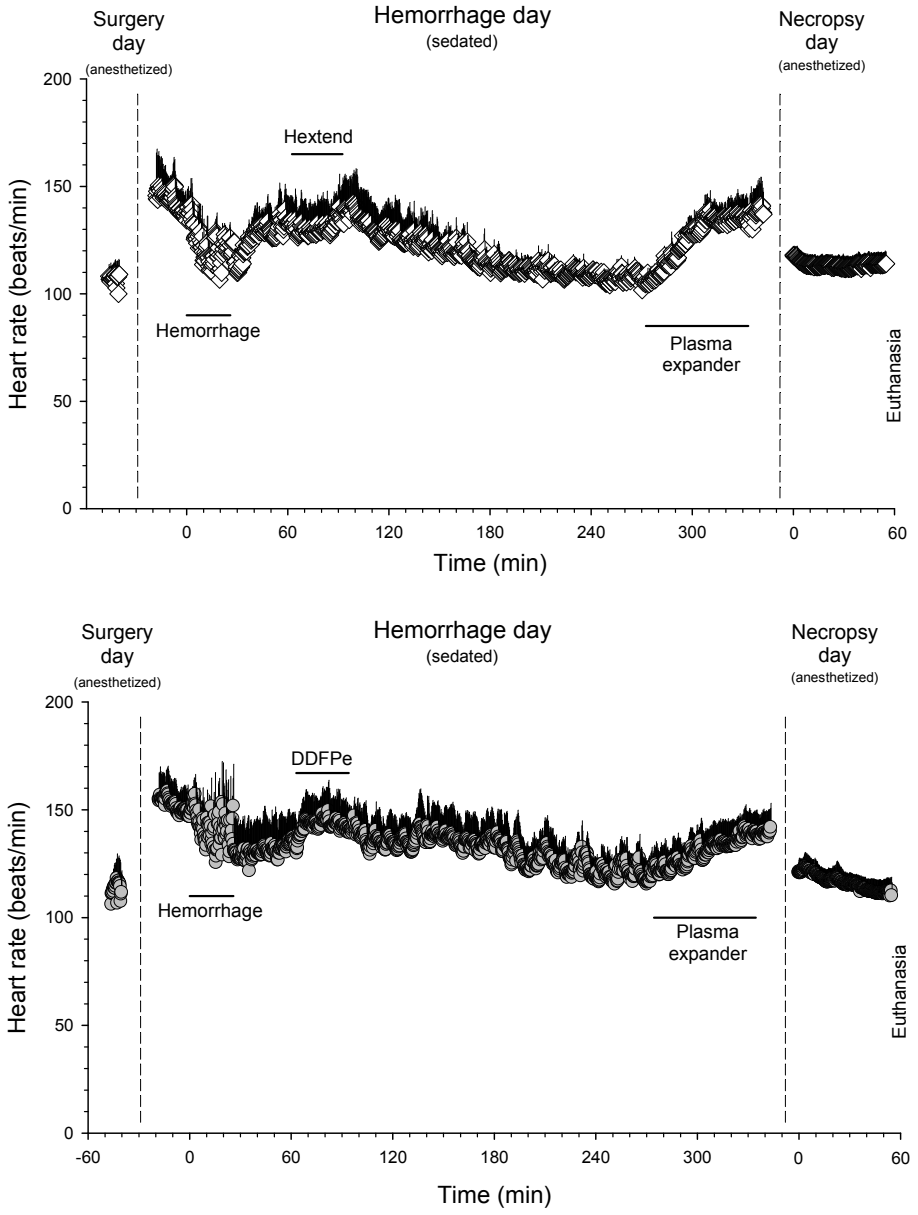


Figure 3: The effects on heart rate of hemorrhage and treatments thereof are demonstrated in two groups of sedated pigs. Hextend (n=6) treated pigs are shown in upper panel and DDFPe (n=6) treated ones in lower panel. For other details see Figure 1 and 2.

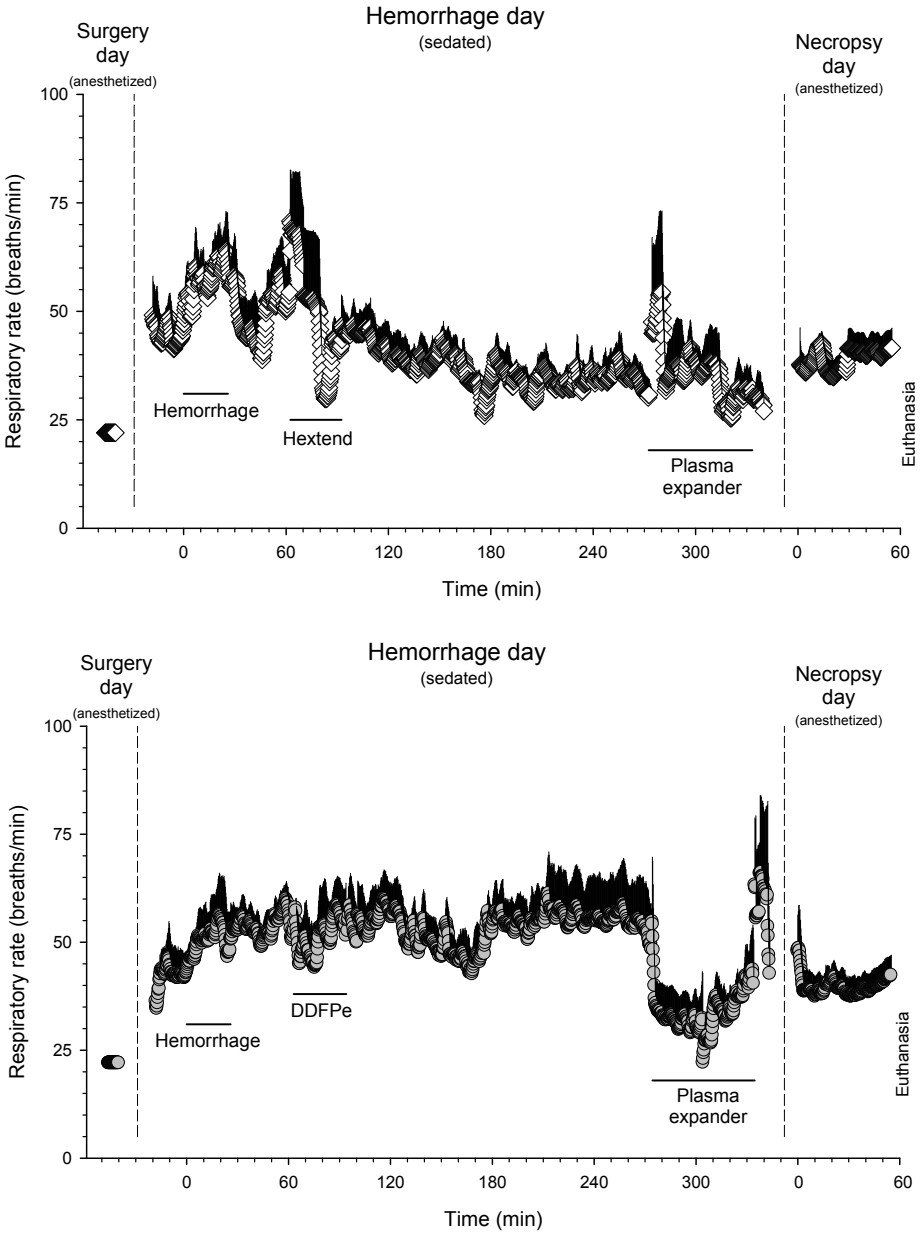


Figure 4: Respiratory rates are shown in two groups of sedated pigs during hemorrhage and treatments thereof. Hextend (n=6) treated pigs are shown in upper panel and DDFPe (n=6) treated ones in lower panel. For other details see Figure 1 and 2.

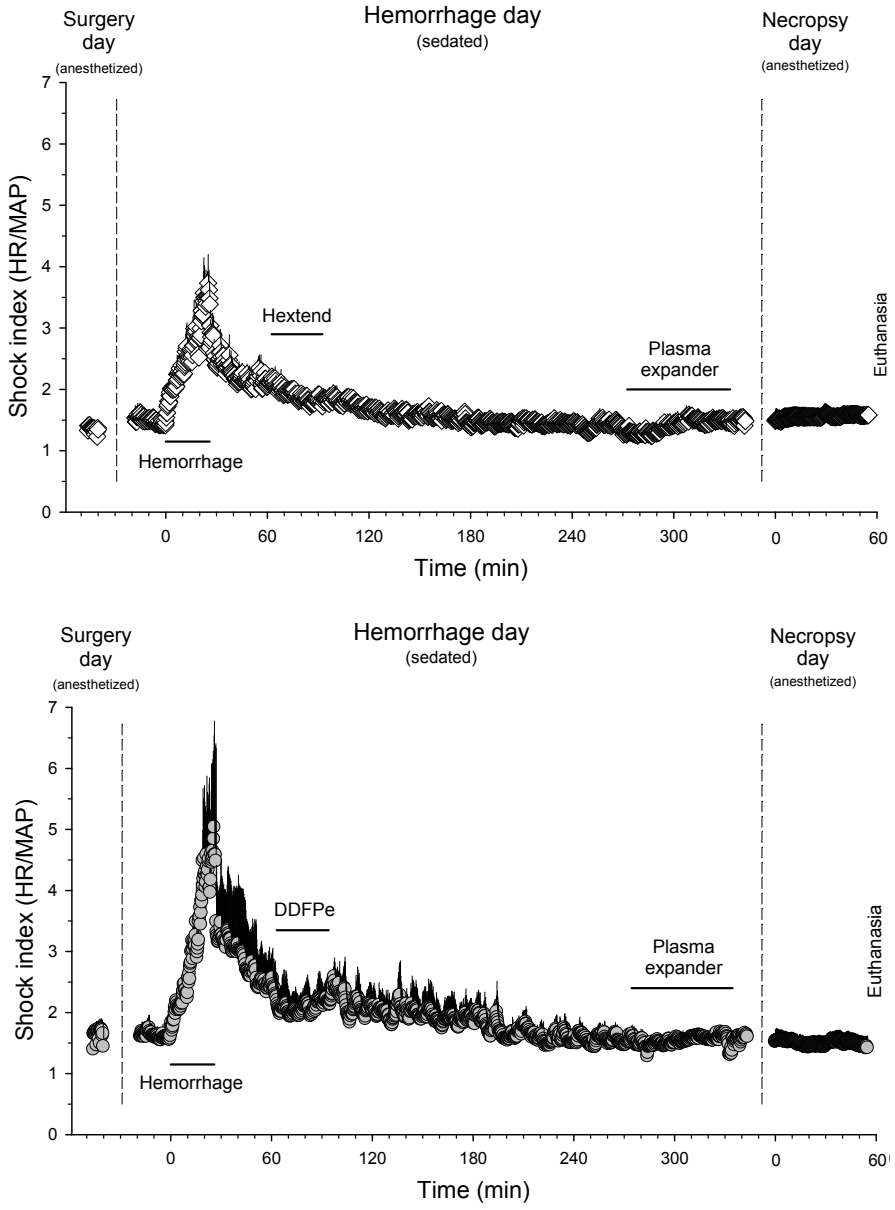


Figure 5: The effect of hemorrha ge on shock indexes, calculated a s heart rate divided by mean arterial pressure, are demonstrated in two grou ps of sedated pigs. Hextend (n=6) treated pigs are shown in up per panel a nd DDFPe (n=6) treated ones in lower panel. F or other det ails see F igure 1 and 2.

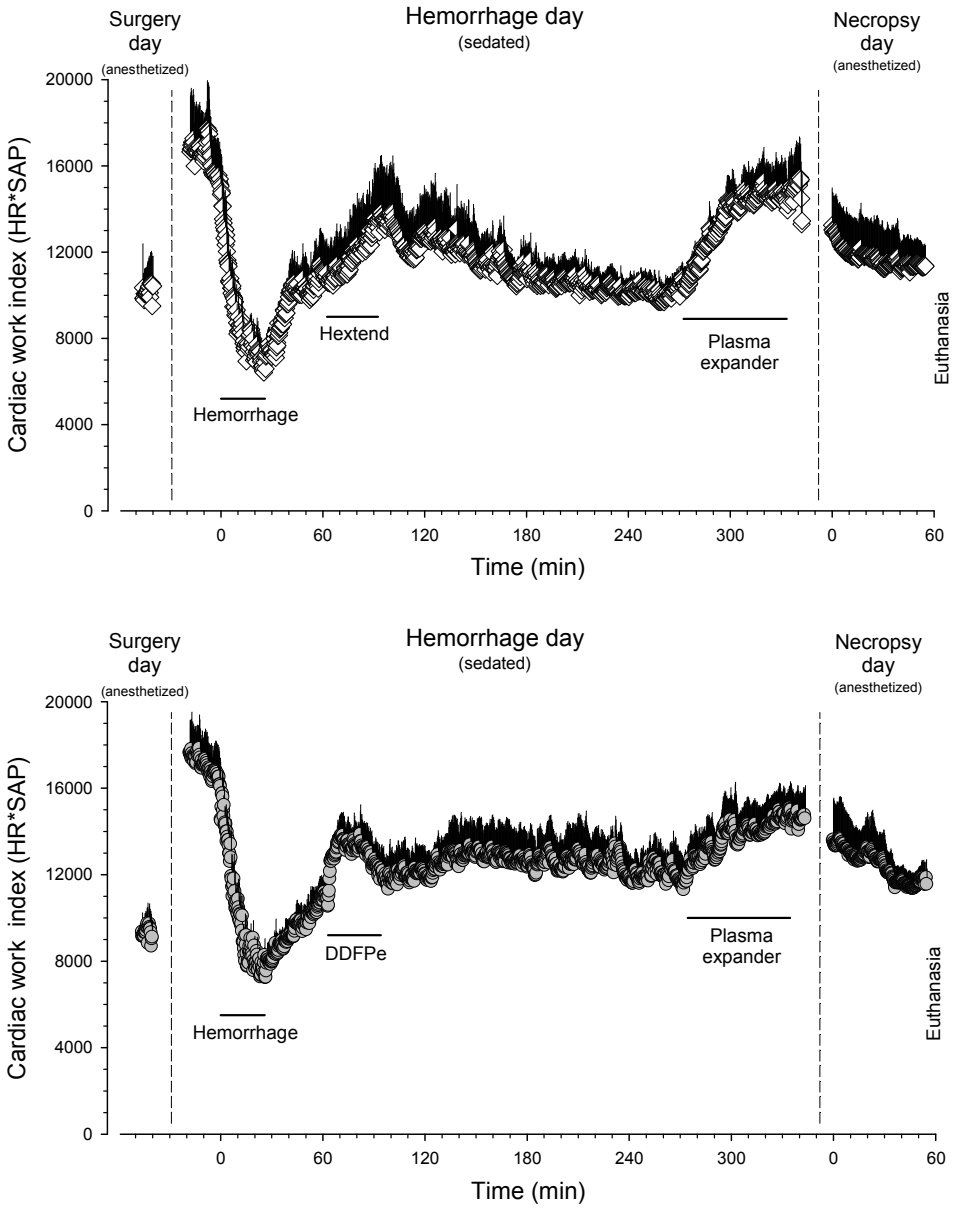


Figure 6: The effects on cardiac work indexes, calculated as heart rate times systolic arterial pressure, of hemorrhage and treatments there of are demonstrated in two groups of sedated pigs. Hextend (n=6) treated pigs are shown in upper panel and DDFPe (n=6) treated ones in lower panel. For other details see Figure 1 and 2.

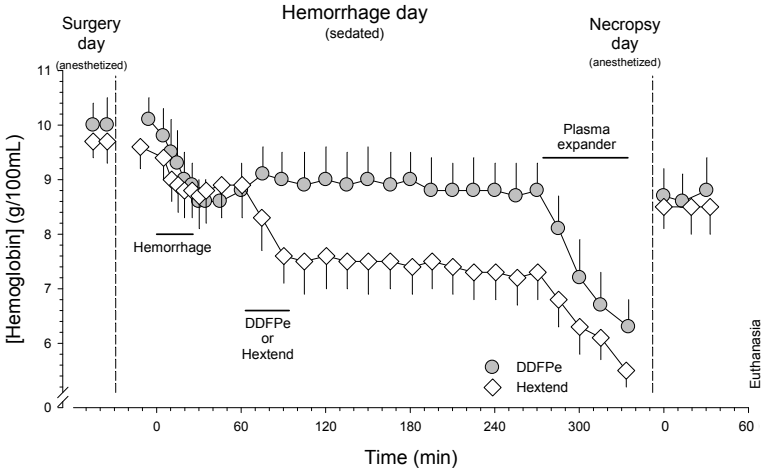


Figure 7: The variations in arterial blood hemoglobin concentration during severe hemorrhage and treatment thereof are shown in two groups of sedated pigs. Hextend (n=6) treated pigs are presented as white diamonds while DDFPe (n=6) treated ones are presented as grey circles. Note the significant fall in hemoglobin concentration after the start of Hextend infusion ($P<0.01$). For other details see Figure 1 and 2.

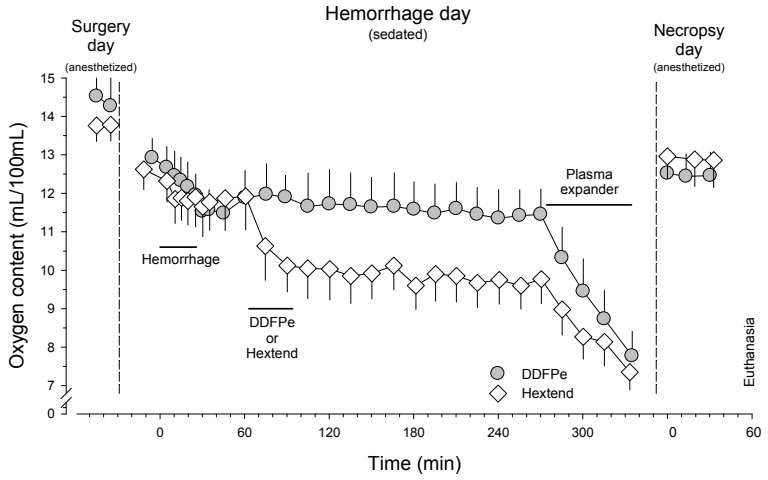


Figure 8: The effects on arterial blood oxygen content of hemorrhage and treatments there of are shown in two groups of sedated pigs. Hextend (n=6) treated pigs are presented as white diamonds while DDFPe (n=6) treated ones are presented as grey circles. There is a significant difference between the two groups after treatment (P<0.01). The oxygen content in the DDFPe pigs is most likely underestimated during the next hours after treatment starts. The "blood gas" machine used for measurements is able to measure oxy-hemoglobin (done at 2 ATA) and oxygen tension in plasma, but not likely able to measure oxygen content in the microbubbles. For other details see Figure 1 and 2.

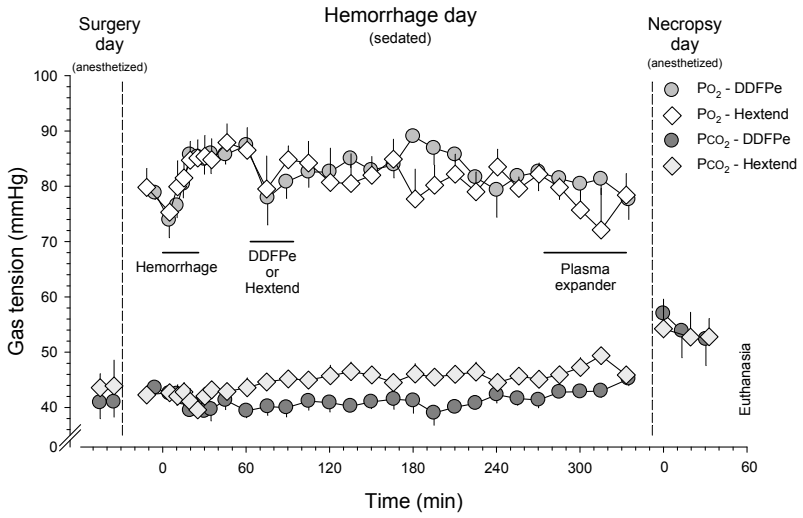


Figure 9: Arterial oxygen and car bon dioxide tensions shown in two groups of sedated pigs during severe hemorrhage and treatment there of. Gas tensions for He xtend (n=6) treated pigs are presented as diamonds while DDFPe (n=6) treated ones ar e presented as circles. For other d etails see Figure 1 and 2.

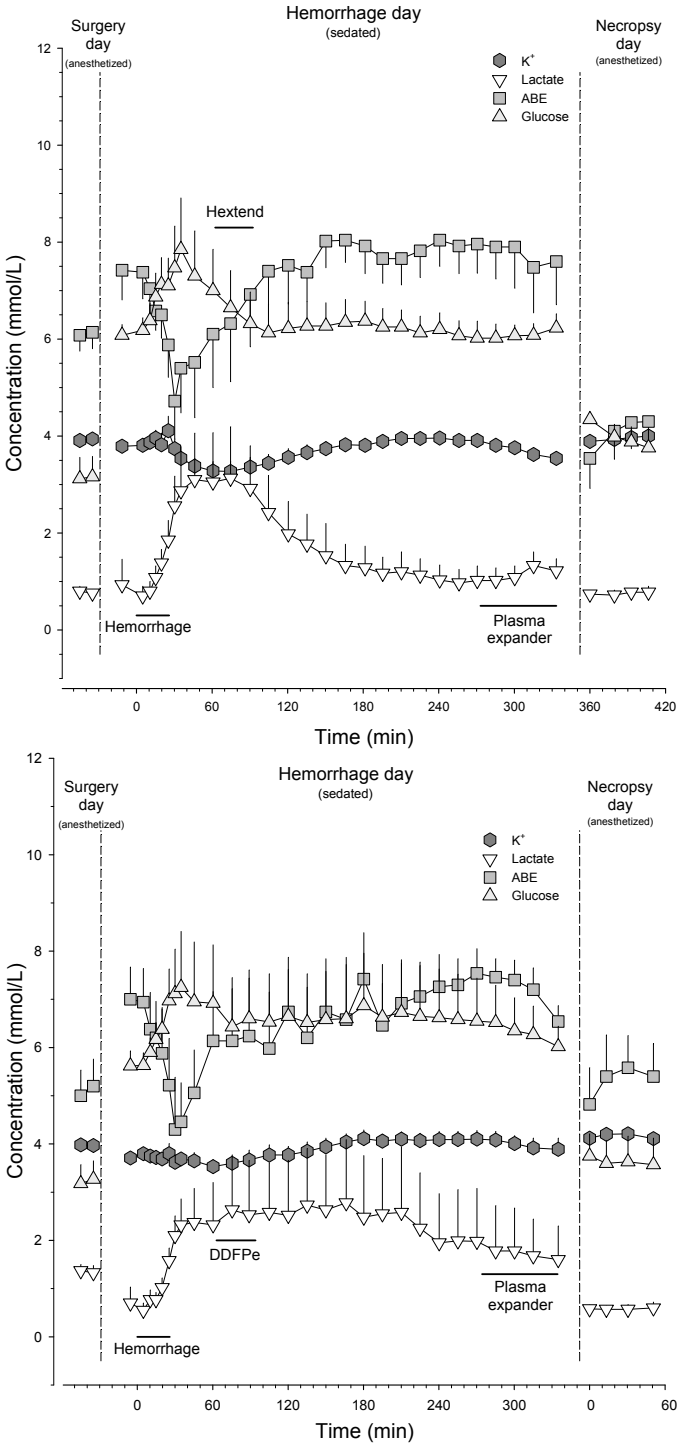


Figure 10: The effe cts on pot assium, glu cose, la ctate and base-access concentration s of hemorrhage and treatments there of are shown in two groups of sedated pigs. Hext end (n=6) treated pigs are shown in uppe r panel and DDFPe (n=6) treated o nes in lower panel. For other details see Figure 1 and 2.

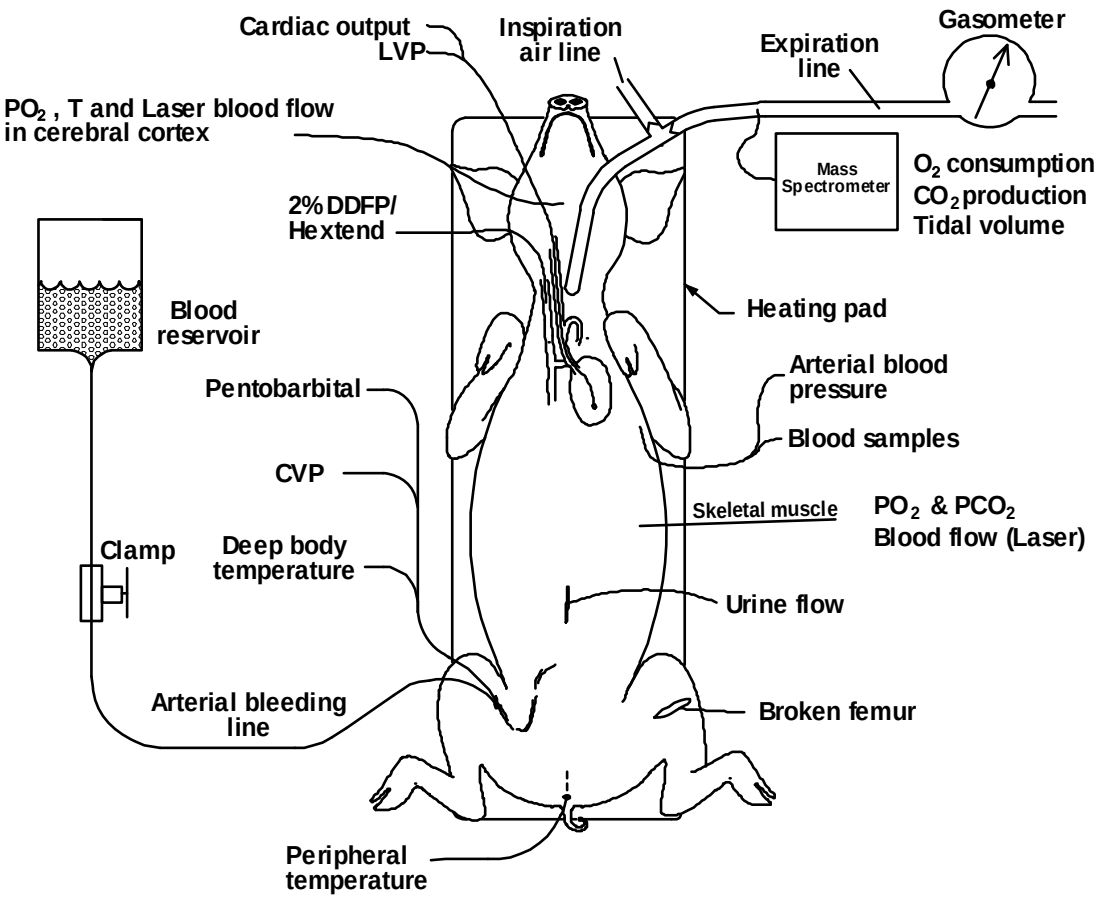


Figure 11: A schematic drawing of an anesthetized pig in supine position prepared for experimental hemorrhagic shock (Model III). Catheters for bleeding and infusion are shown as well as connections to different recording equipments. These pigs had lost 33+/-1 mL /kg blood at the end of the experiments.

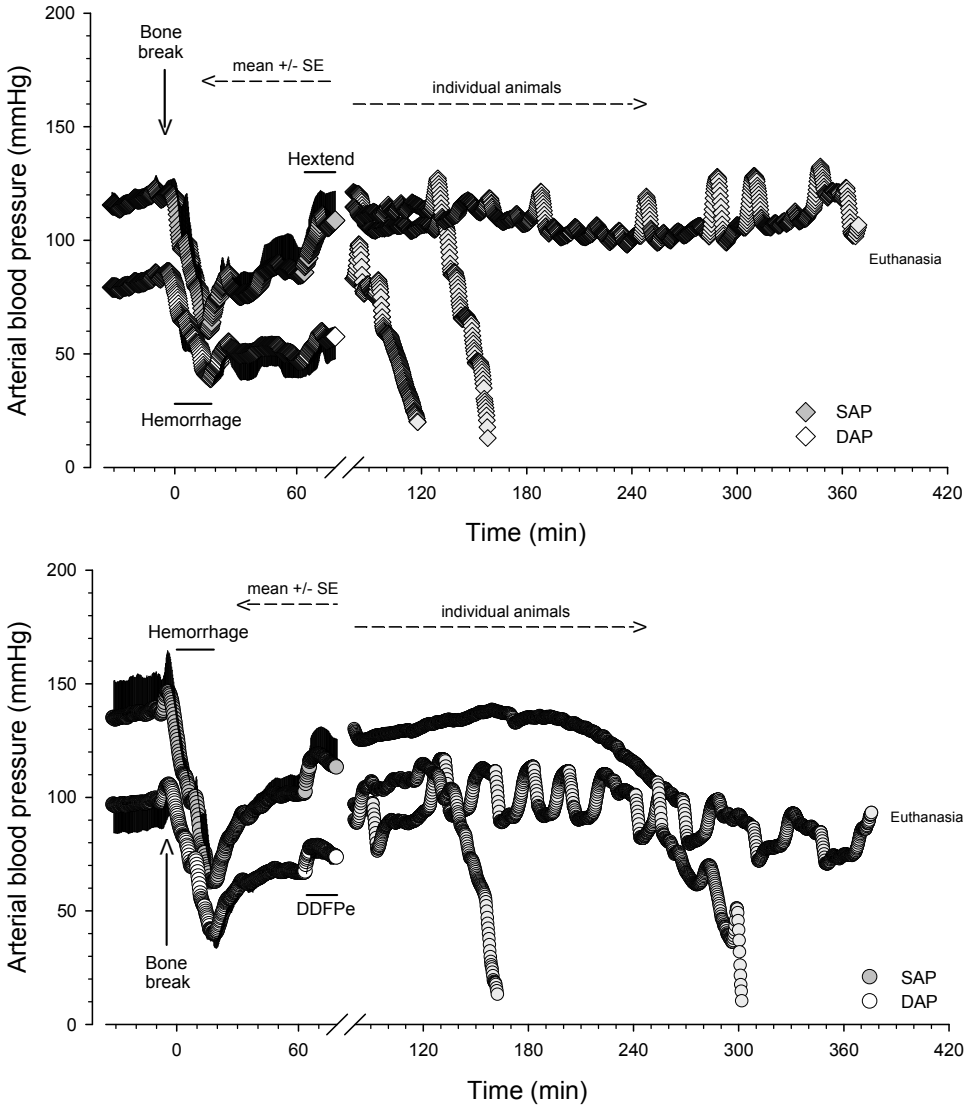


Figure 12: The figure displays the effects of bone fracture and hemorrhage on arterial blood pressures in two groups of pentobarbital anesthetized pigs (n=3 in each) weighing 25.6 ± 0.9 kg. The femur was broken 5 min prior to hemorrhage. Within 18 min the pigs bled 28.1 ± 0.0 mL/kg. Treatment, lasting 15 min, was started 45 min post hemorrhage. Upper panel shows the effect of Hextend infusion (7 mL/kg) and lower panel the effect of DDFPe (0.6 mL/kg). Until the end of treatment both panels displays SAP (systolic arterial pressure) and DAP (diastolic arterial pressure) as mean ± SE, after treatment however, only SAP are shown for individual pigs. The mean survival time of the two Hextend pigs that died within 6 hrs of hemorrhage was 123 min, while the mean survival time for the two DDFPe pigs that died was 233 min. Note: One of these DDFPe pigs had a hole between left and right side of the heart ("arterial blood" drawn from the right atrium, the hole found during necropsy). This kind of anatomical shunt has previously been noticed to reduce the survival time, independent of treatment modality in our shock pigs, but always with DDFPe pigs living the longest. Also note the instability in the pressure recordings from one animal in each group.

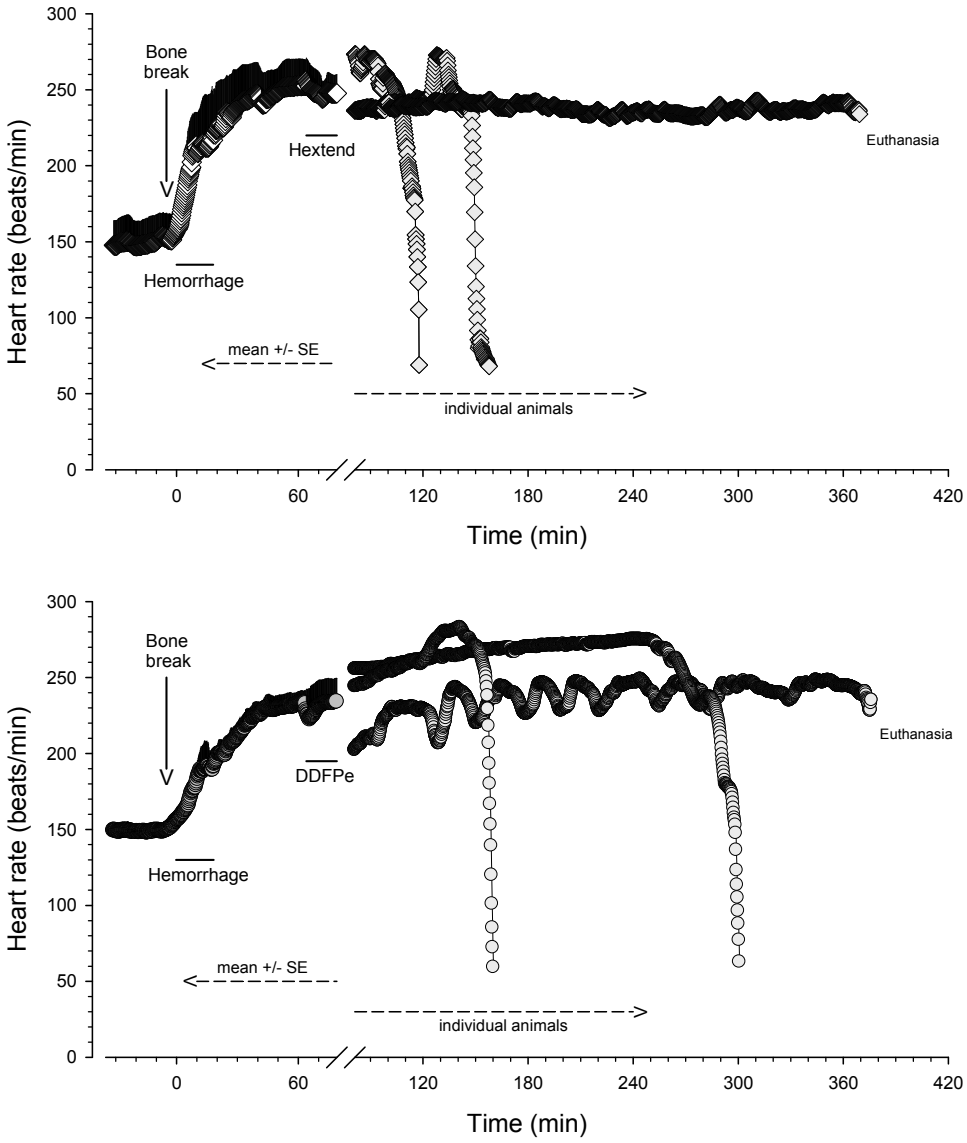


Figure 13: The effects of bone fracture and hemorrhage on heart rate in two groups of pentobarbital anesthetized pigs are displayed in this figure. Upper panel shows the effect of Hextend treatment (7 mL/kg) while lower panel the effect of DDFPe treatment (0.6 mL/kg). For other details see Figure 11.

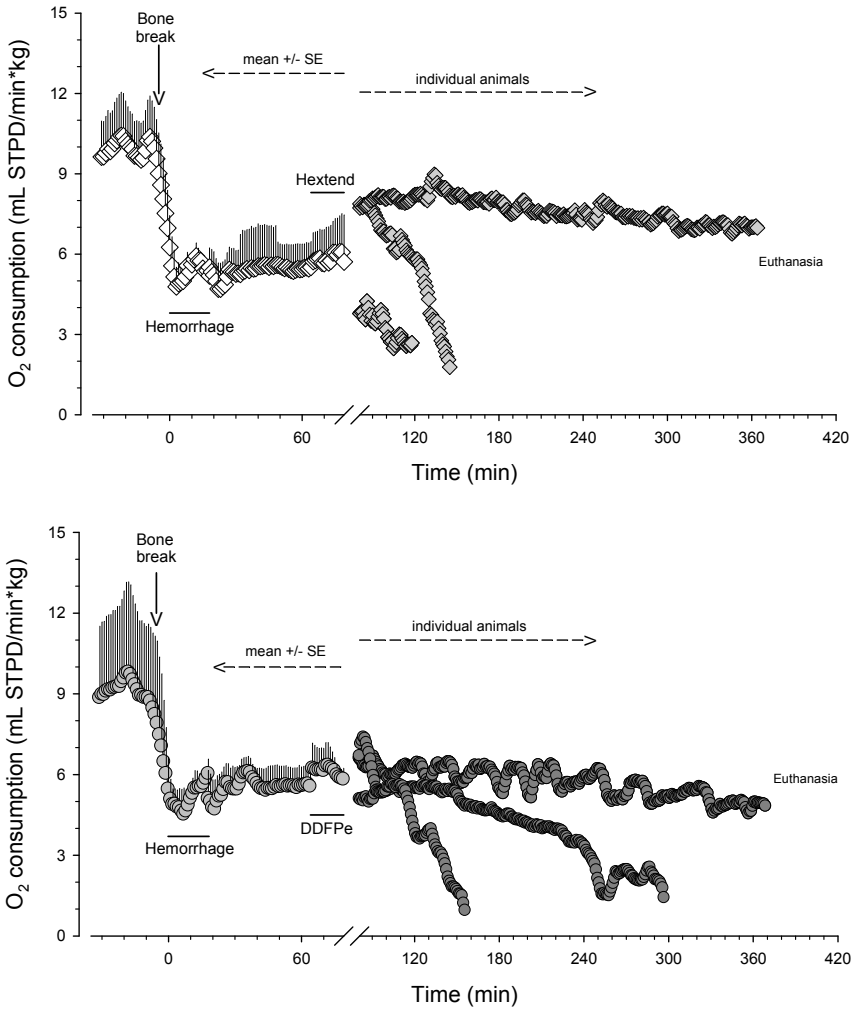


Figure 14: Effects of bone fracture and hemorrhage on oxygen consumption in two groups of pentobarbital anesthetized pigs are displayed in this figure. Upper panel shows the effect of Hextend treatment (7 mL/kg) while lower panel the effect of DDFPe treatment (0.6 mL/kg). Note the abrupt fall in O_2 consumption immediately after femur was broken and before the hemorrhage was started. For other details see Figure 11.

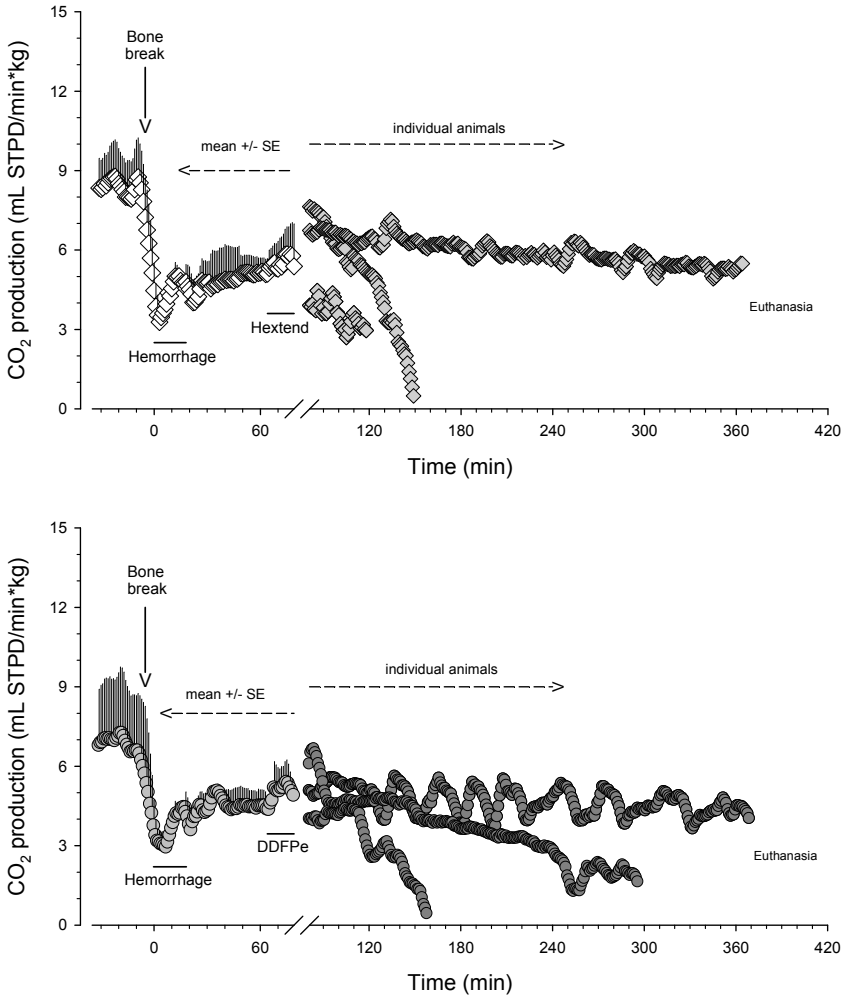


Figure 15: Changes in carbon dioxide (CO_2) production after bone fracture and hemorrhage in two groups of pentobarbital anesthetized pigs are presented in this figure. Upper panel shows the effect of Hextend treatment (7 mL/kg) while lower panel the effect of DDFPe treatment (0.6 mL/kg). Note the abrupt fall in CO_2 production immediately after femur was broken and before the hemorrhage was started. For further details see Figure 11.

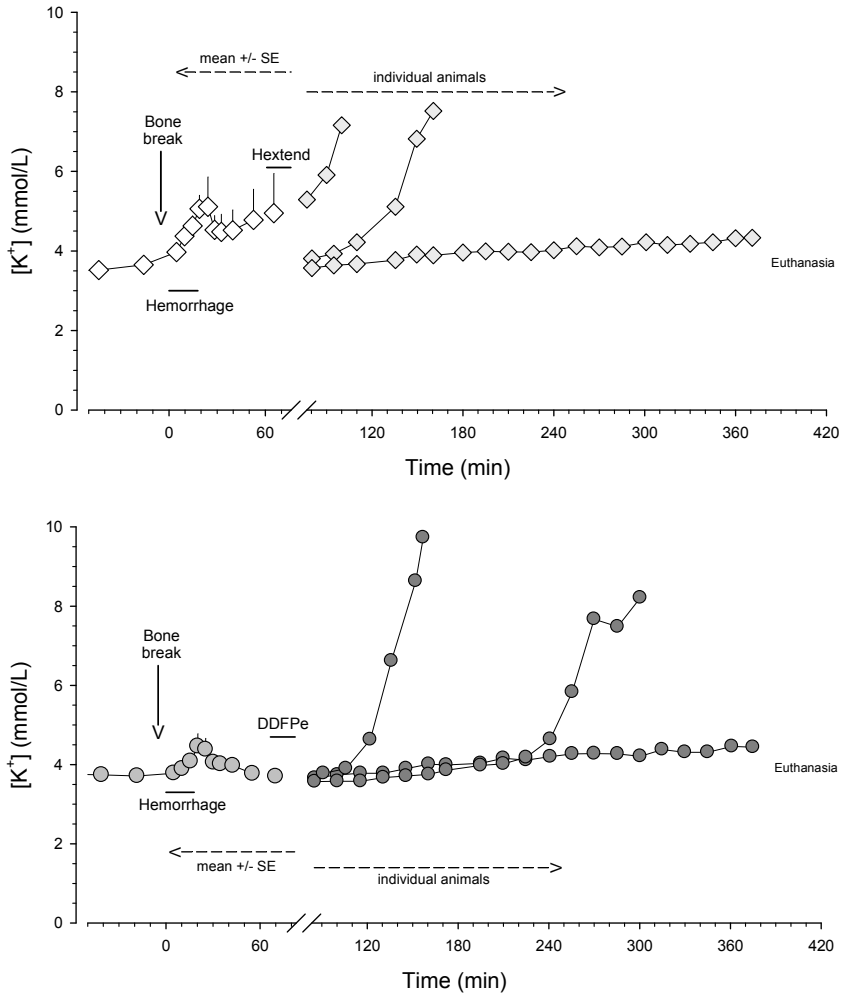


Figure 16: Changes in arterial blood potassium concentrations after bone fracture and hemorrhage in two groups of pentobarbital anesthetized pigs are demonstrated in this figure. Upper panel shows the effect of Hextend treatment (7 mL/kg) while lower panel the effect of DDFPe treatment (0.6 mL/kg). For additional details see Figure 11.

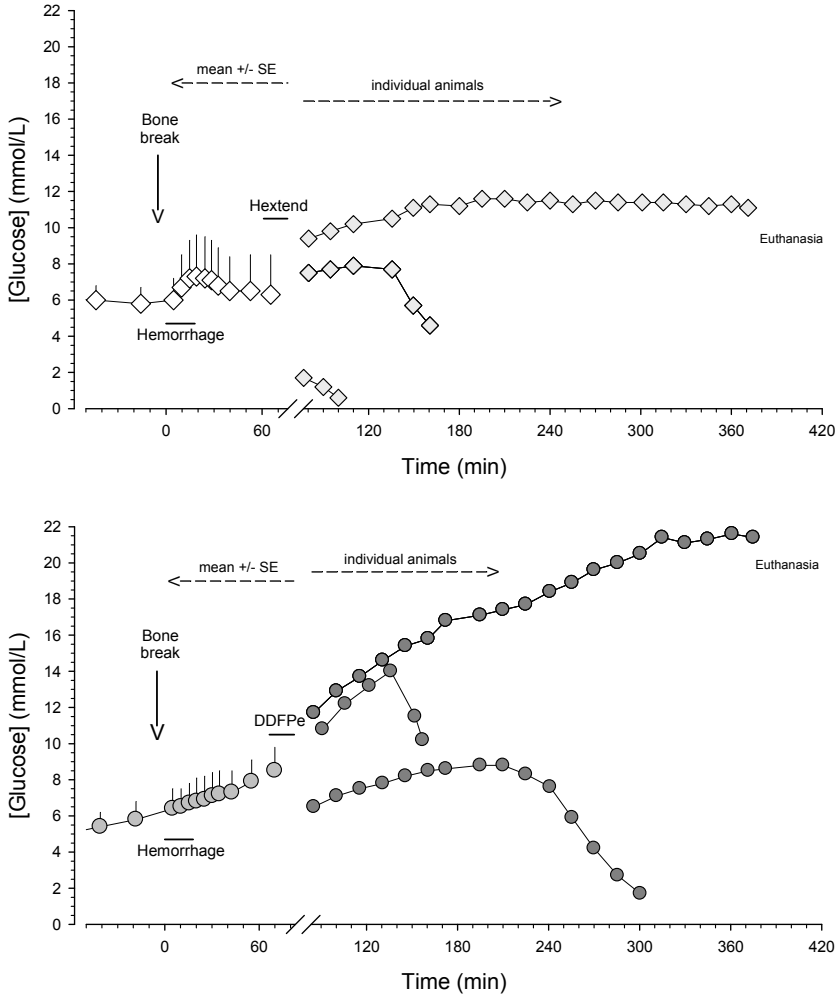


Figure 17: Changes in glucose concentration in arterial blood after bone fracture and hemorrhage are presented in two groups of pentobarbital anesthetized pigs in this figure. Upper panel shows the effect of Hextend treatment (7 mL/ kg) while lower panel the effect of DDFPe treatment (0.6 mL/kg). For additional details see Figure 11.

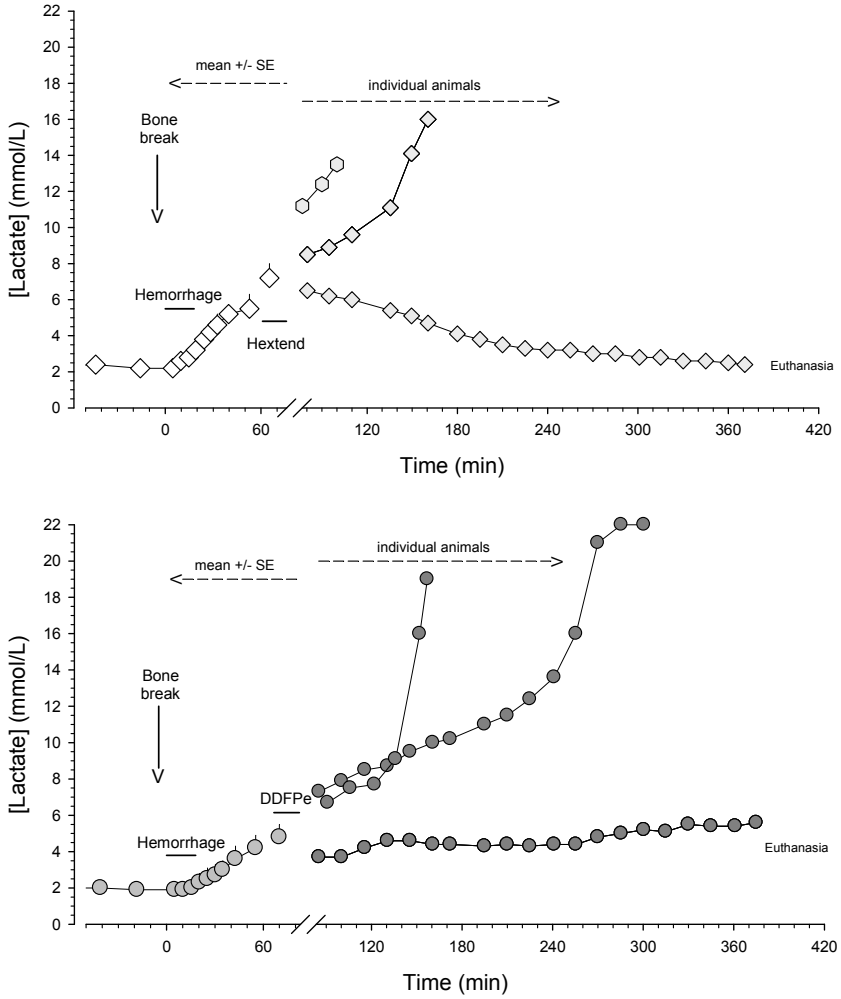


Figure 18: Changes in lactate concentration in arterial blood after bone fracture and hemorrhage are presented in two groups of pentobarbital anesthetized pigs in this figure. Upper panel shows the effect of Hextend treatment (7 mL/kg) while lower panel the effect of DDFPe treatment (0.6 mL/kg). For additional details see Figure 11.

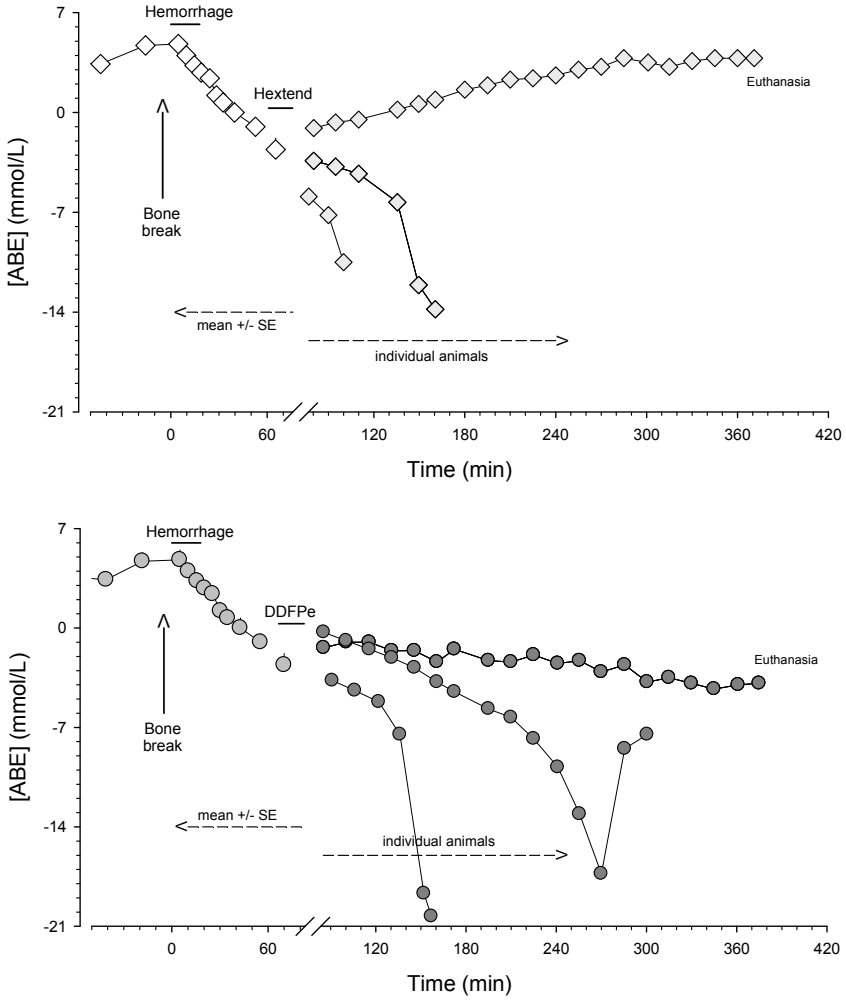
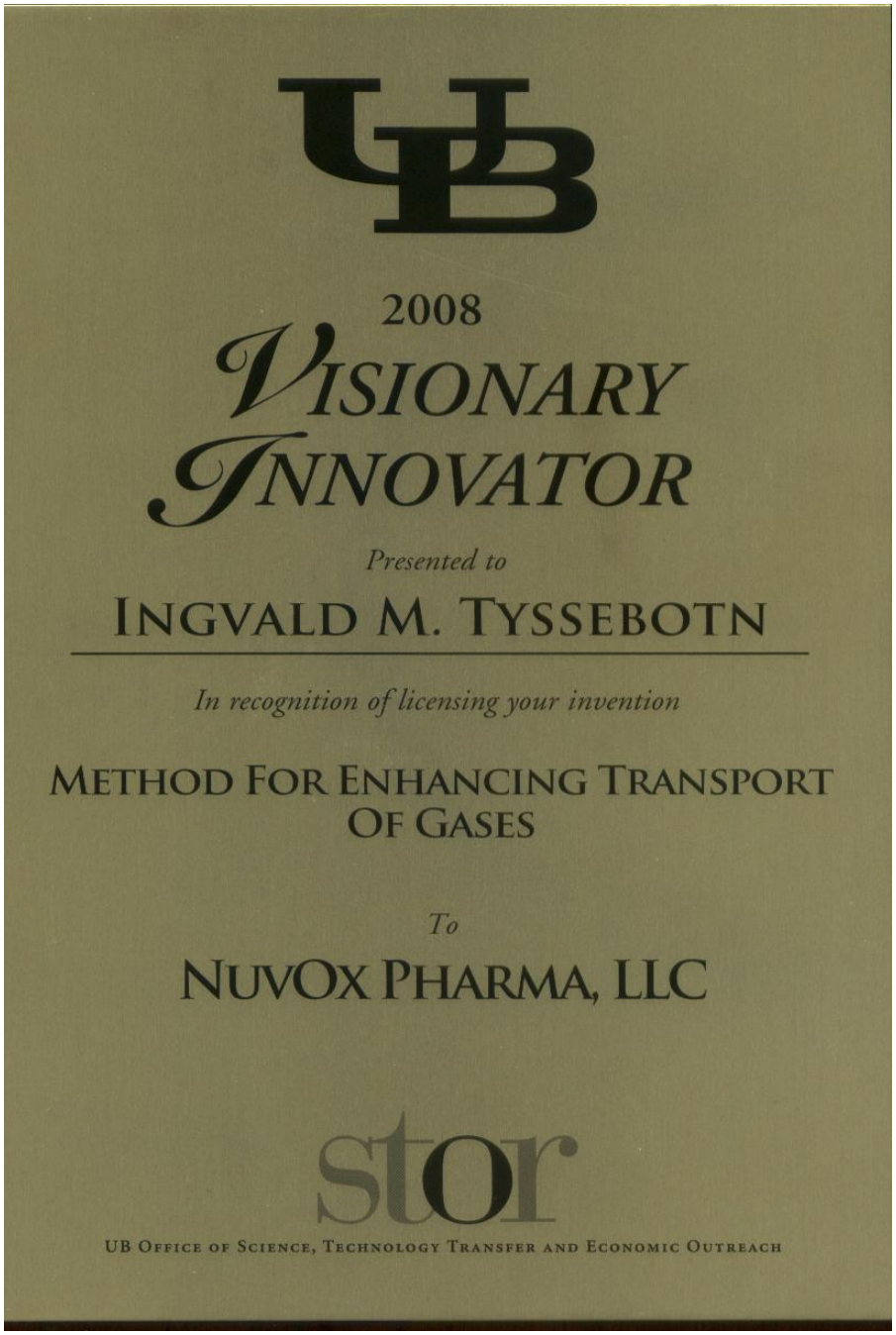


Figure 19: Effects of bone fracture and hemorrhage on actual base excess (ABE) are displayed in two groups of pentobarbital anesthetized pigs. Upper panel shows the effect of Hextend treatment (7 mL/kg) while lower panel the effect of DDFPe treatment (0.6 mL/kg). For further details see Figure 11.

2 – PROOF OF LICENSING

The following US patents were licensed from UB to NuvOx Pharma, LLC in January 2008:

Van Liew, H., Burkard, M.E., Lundgren, C.E .G., Ty ssebotn, I.M. Method for enhancing transport of gases to tissues. US Patent # 5,869,538 (Feb. 9, 1999).
Van Liew, H., Burkard, M.E., Lundgren, C.E .G., Ty ssebotn, I.M. Method for enhancing transport of gases to tissues US Patent # 6,127,428 (2000).



3 - CURRICULUM VITAE (for Principal Investigator)

Ingvald Mikal Tyssebotn

CITIZENSHIP: Norway
VISA STATUS: Permanent Resident

ACADEMIC HISTORY:

1968: Medical School (Cand Med = M.D.), University of Bergen, Norway
1/5/70: Licensed Physician (Norway, Sweden, Denmark)
1/1/71: Licensed Diving Physician from Norwegian Health Directorate (licensed in Norway and Great Britain for Examination and treatment of North Sea Divers)
12/17/80: Ph.D. in Physiology. Thesis title: Intra-renal Blood Flow in Circulatory Shock and during Infusion of Vasoactive Agents

FUTHER EDUCATION:

1963-1965: Basic mandatory military training (The Royal Norwegian Navy – 4 months)
1969: Critical Medical Care, Central Hospital in Sør-Trøndelag, Trondheim, Norway
1969: Adrenals and Its Illness, Aker Hospital, Oslo, Norway
1970: Navy Divers Education, The Royal Norwegian Navy.
1970: Certified instructor for Theoretical and Practical Diver Education, The Royal Norwegian Navy
1970: Officers training (Lieutenant) for Medical Duty in the Royal Norwegian Navy, Sjøkrigsskolen, Bergen, Norway
1971: Experimental Renal Physiology, University of Oslo
1971-1972: Diving Medicine Courses in: Decompression Treatment, Hyperbaric Oxygen Treatment for Diving Accidents and Clinical Illness, The Royal Norwegian Navy and Haukeland Hospital, Bergen, Norway
1972: Pedagogic Course and Lecture Training, University of Bergen, Norway
1972: Clinical Renal Physiology, University of Oslo, Norway
1973: Measurements with Radioactive Isotopes and Radiation protection, The Netherlands-Norwegian School for Isotopic Work, Kjeller, Norway
1974: Radioimmunoassays and Connected Methods, University of Oslo, Norway
1978: Light- Microscopy, EM- and SEM- Microscopy, University of Bergen, Norway.
1979: Clinical Respiratory Physiology, University of Bergen, Norway
1981-1996: Several courses in Diving Medicine, Hyperbaric Oxygen Treatment, and Evaluation of Divers Fitness (Professional and Amateur), approved by the Undersea and Hyperbaric Medical Society and European Undersea Biomedical Society
1994: Hyperbaric Oxygen Treatment, Dept of Anesthesiology, Karolinska Sjukhuset, Stockholm, Sweden
1998: Responsible Care and Use of Laboratory Animals Certification Program, modules 1-4, Institutional Animal Care & Use Committee, Laboratory Animal Facility SUNY at Buffalo.

STUDY TOURS including invited lectureships:

1968: Several Universities in Germany (Erlangen, Goettingen, Kiel and Wuertzburg)
1976: Research Fellow at the Queen Elisabeth Hospital, University of Birmingham, Great Britain (6 months), with short Visits to the Universities of Cambridge, Southhampton, and London, Lecturing all Places.

1978: Defense and Civil Institute of Environmental Medicine, Downsview, Ontario, Canada and The Center for Research and Education in Special Environments, State University of New York at Buffalo, USA
1978: Aviation and Naval Medicine Laboratory, Lund University, Sweden
1982, 1986, 1987, 1988, 1989, 1992, 1994: Karolinska Institutet, University of Stockholm, Sweden
1988: Center for Research & Education in Special Environments (CRESE), State University of New York at Buffalo
1988 Schools of Medicine, University of Pennsylvania, Philadelphia, PA

TEACHING:

1965-1968: Teaching microscopical and macroscopical anatomy to students of Medical and Dental School, University of Bergen. Supervising research in anatomy. Establishing the medical library for macro and microscopical anatomical preparations used for demonstration, teaching, and examination purposes at Department of Anatomy, University of Bergen
1968-1969: Medical diseases, nurses education, Orkdal Sanitetsforenings Hospital, Orkanger, Norway
1970-1973: Lecturing Diving Medicine and Diving Physics, Royal Norwegian Navy Diving Courses (including training for Physicians working in the Navy) and Military Diving Units, Fire-Fighters, Oslo, Norway. Lecturing and supervising training of Health Professionals, Royal Norwegian Navy
1970-1974: Lecturing physiology, lung diseases and cardiovascular problems, School for Nurses, Betanien sykepleie-skole, Bergen, Norway
1971-1998: Lectured at the Department of Physiology, University of Bergen on the following topics:

Renal Physiology: lectures and supervising courses for medical, dental, and science students. Revision of all laboratory journals/manuals
Barophysiology: low and high pressure physiology for medical and science students
Respiratory Physiology: lectures and training courses for medical, science and dental students. Revised all laboratory journals/manuals
Acid-Base Chemistry: lectures and training course for medical, science and dentist students. Revised all experimental journals/manuals
Fluid and Electrolyte Physiology: lectures and training course for medical, science and dental students. Revised laboratory journals/manuals
Basal Metabolism: lectures and training course for medical, science and dental students. Revised the laboratory journals/manuals
Exercise Physiology: lectures and training courses for medical, science and dental students. Revised the experimental journals/manuals
Hemostasis and the Physiology of Erythrocytes: lectures and training courses for medical, science and dental students. Specialty courses for dentists. Revised all journals/manuals
Supervision of master and doctoral students in renal physiology, cardiovascular, hypo- and hyperbaric physiology
Lectured respiratory physiology for physicians with specialty in "Clinical Physiology"
1985-1997: Lectured hyperbaric physiology and diving medicine: nitrogen narcosis, oxygen toxicity, high pressure nervous syndrome, work tolerance at pressure, hyperbaric oxygen treatment for decompression sickness and clinical use at the Norwegian Governmental Diver Training School, Bergen, Norway
1975-1997: Responsible for testing of medical fitness and certification of scientific divers working at the University of Bergen

1988: Organized specialty cou rses in hyperbaric oxygen treatme nt and diving medicine for Norwegian surgeons at The Central Hospital in Aust-Agder, Kristiansand, Norway
1980-1998: Supervisor for use of hyperbaric oxygen for several hospitals in Norway
1978-present: Supervised scien ce students studying for th e Masters Degree, and Science students and M.D.'s studying for Ph.D Degree. Supervised a total of 16 Masters Degrees an d 7 Ph.D. Degrees
1985-1994: Supervisor and consult ant for research fello ws in the Deep Divin g Program, the Norwegian Council for Science and the Hu manities. Top ics: circu lation, respiration, oxygen toxicity, gas density.
1992: Head of Professor Evaluation Committee, University of Bergen.
1992: Organized specialty courses in Hyperbaric Oxygen Treatments for Norwegian Surgeons at Haukeland Hospital, Bergen, Norway.
1997-present: Organized PGY50 3 entitled " Physiological measurements in small animals" for graduate students within the School of Medicine & Bio medical Scie nces, SUNY at Buffalo.
2001-present: Respiratory Physiology, PGY 300, University at Buffalo.
Respiratory Diseases, PGY 412, University at Buffalo.
Renal Diseases, PGY 412, University at Buffalo.

PHD EVALUATIONS:
1983: Outside resder Ragnar Vaernes, cand. Psycol. for PhD
1999: Outside rea der for An dreas Østlu nd, MD, for PhD thesis, Karolin ska Institut, Stockolm, Sweden.
2000: Outside rea der for An dreas Fahlman, MSc, for PhD t hesis, Carlt on University, Ottawa, Canada.

PROFESSIONAL EXPERIENCE:
1965-1968: Research Fellow, Department of Anatomy, University of Bergen
7/1/68-6/30/69: Intern at Orkdal Sanitetsforenings Hospital, Orkanger, Norway
7/1/68-12/31/69: Physician in Sel kommune, Norway
1/1/70-2/9/71: The Royal Norwegian Navy, Responsible for training in pr actical divin g and diving medicine at the Navy Diving School, Haakonsvern, Norway
2/10/71-1992: Associate Professor, Department of Physiology, University of Bergen, Bergen, Norway
7/15/91-8/15/92: Visiting Research Professor, State University of NY at Buffalo, Center fo r Research & Education in Special Environments (CRESE)
1/1/93–8/1/97: Professor of Physiology, Departmen t of Physiology, University of Berg en, Bergen, Norway
1/2/94-8/1/97: Consultant, Hyperbaric Medicine, Royal Norwegian Navy
1/2/94-8/1/97: Professor of Hyperbaric Medical Research, University of Bergen, Norway
1/2/94-8/1/97: Consultant in Hyperbaric Medicine, Haukeland University Hospital, Bergen, Norway
8/1/95-8/1/97: Visiting Research Professor, State University of NY at Buffalo, Center fo r Research & Education in Special Environments (CRESE)
8/97-7/31/01: Senior Bertha H. & Henry C. Buswell Fellowship awarded t hrough State University of NY at Buffalo, School of Medicine and Biomedical Sciences
8/1/01-present: Research Professor of Physiology and Biophysics, State University o f New York at Buffalo.

AWARDS:
1972-1995: Yearly grants from The Norwegian Council for Research in the Scien ces and the Humanities

3/14/2000: First Place (with co-inventors) Niagara Frontier Inventor of the Year Award in Science. Nomination for this award was made by the University of Buffalo Technology Transfer and Licensing Office and sponsored by the Niagara Frontier Intellectual Property Law Association.

PATENTS:

1. Van Liew, H., Burkard, M.E., Lundgren, C.E.G., Tyssebotn, I.M. Method for enhancing transport of gases to tissues. US Patent # 5,869,538 (Feb. 9, 1999).
2. Van Liew, H., Burkard, M.E., Lundgren, C.E. G., Tyssebotn, I.M. US Patent # 6,127,428 (2000).

PUBLICATIONS:

Articles in peer reviewed journals:

1. Aukland, K., Kirkeboe, A., Løyning, E. and Tyssebotn, I. Effect of hemorrhagic hypotension on the distribution of renal cortical blood flow in anaesthetized dog. Acta Physiol Scand, 87, 514-525, 1973.
2. Clausen, G. and Tyssebotn, I. Intrarenal distribution of glomerular filtration in conscious rats during isotonic saline infusion. Acta Physiol Scand, 89, 289-295, 1973.
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