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Effects of Perfluorocarbon Infusion in an Anesthetized Swine Decompression Model

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Introduction. **Decompression illness (DCI) results from sudden changes in ambient pressure leading to super-saturation and bubble formation in tissues and the blood stream. Perfluorocarbon emulsions (PFC) increase both oxygen and nitrogen solubility when infused into the blood stream. This study hypothesized that PFC would increase N_2 removal as well as O_2 delivery to tissues.**

Materials and Methods. **Juvenile swine (20kg) were anesthetized and highly instrumented with arterial monitoring, pulmonary artery catheterization, EDAC ultrasound bubble detection, and end tidal N_2 by mass spectrometry. Blood gases were monitored in both the mixed venous and arterial circulation. Full hemodynamics were calculated using standard equations. Four groups of animals were randomized to be either sham controls or compressed and to receive either saline or PFC at 4.5 ml/kg. Animals were dry compressed to 6.8 ATA for 30 minutes of time on the bottom and then rapidly decompressed. Animals were monitored for 120 minutes after surfacing, then euthanized.**

Results. **DCI was created by the dive profile but the severity was variable. Sham animals had no significant changes except that those who received PFC developed significant pulmonary hypertension and decreased cardiac output. This held true for those that also underwent DCI. Respiratory N_2 washout was not significantly different with and without PFC. However, O_2 delivery to tissues was improved with PFC and EDAC bubble count was dramatically less with PFC.**

Conclusions. **PFC decreased bubble generation but the data was confounded by a species specific pulmonary hypertensive response. Even with this as a problem O_2 delivery to tissues was enhanced by PFC. Future work with PFC in different species will help to further understand the contribution of these two mechanisms to treatment efficacy by PFC in DCI.**

Key Words: **decompression sickness; DCS; perfluorocarbon; PFC; bubble generation; oxygen therapeutic; blood substitute; buddle detection.**

INTRODUCTION

Decompression sickness (DCS) is caused by a sudden decrease in ambient pressure. This is most commonly encountered either when surfacing from diving under water or in association with high altitude loss of pressurization, such as in an airplane or space vehicle [1–3]. DCS has also occurred in mine accidents and in caisson workers who surface without appropriate decompression [4]. DCS creates bubbles within tissues and the circulatory system, leading to venous gas emboli (VGE). Potentially, arterial gas emboli (AGE) may result either from gas bubble formation within arteries or from bubbles crossing through a right-to-left shunt, such as a patent foramen ovale, or from a pulmonary shunt. AGE is also a catastrophic event upon decompression if the glottis is closed and expanding air in the lungs is unable to escape [5]. When breathing air, the bubbles formed are largely nitrogen (N_2), which is poorly soluble in plasma, but other respiratory gases may contribute. Formation and dissolution of bubbles within the circulation have been widely studied [6–10].

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Sport diving DCS mortality is believed to be 3 to 9 deaths per 100,000 dives in the United States. Paresis and other injuries are even more common [3]. DCS is less common in professional diving and almost nonexistent in military dives wherein rigorous and controlled dive protocols are followed. However, even in the military, accidents and unforeseen changes in mission may cause some dives to become much more hazardous.

Submarines operate at 1 atmosphere absolute (ATA) internal pressure. Although rare, submarine accidents have occurred with loss of ability to maintain the vessel at 1 ATA [11–14]. If power to run environmental systems is lost, ambient pressure inside the submarine will slowly increase, potentially reaching ambient pressure outside the ship. An acute hull breach would increase the pressure of the internal environment to that of the surrounding seawater. Thus, it is possible that if a submarine were to become disabled, more than 100 sailors inside may require rescue, all of whom may be in need of treatment for DCS. Because some submarines are now operating in the shallower waters of continental shelves, as opposed to the deep water of the open ocean, the rescue of submariners from a disabled submarine (DISSUB) is now a more realistic scenario than in the past.

The present treatment for DCS involves recompression, the administration of hyperbaric oxygen, and a slow, controlled decompression [5]. A recompression chamber could be many hours of travel time away from a remote DCS rescue site. Ascent to an even higher altitude in an evacuation aircraft will worsen the DCS. In a DISSUB rescue, the number of potential victims would certainly exceed the locally available resources for recompression. Therefore, alternate nonrecompression DCS treatments are needed. In addition to their occurrence in DCS, AGE and VGE cause significant morbidity and mortality in surgery [15–17]. Therefore, DCS treatment methods have direct applicability to civilian and military surgeries that produce VGE and AGE.

Perfluorocarbon emulsions (PFCs) are oxygen therapeutics ("blood substitutes") developed as intravenous agents in medicine to carry oxygen [18]. Such pharmaceuticals are now undergoing human Phase II testing. A first generation PFC technology, Fluosol DA-20%, was approved by the United States Food and Drug Administration to reduce myocardial infarction during coronary angioplasty [19]. PFCs not only dissolve oxygen but can carry other nonpolar gases, such as nitrogen, argon, xenon, and helium. Prior work has shown that PFC emulsions, given prior to AGE/VGE, can decrease the physiological effects of those experimental emboli [20–24]. In several prior studies of DCS, the use of PFC after surfacing showed decreased mortality [25–27]. In recent swine models of cardiopulmonary DCS, mortality associated with DCS was dramatically reduced in animals treated with PFC [28–30]. Unfortunately, those studies were survival studies and, although the data were provocative, there were no data gathered that could reveal the mechanism by which the PFC preserved life. The following study was proposed to observe closely a number of physiological events in a large animal model of severe cardiopulmonary DCS. It was hypothesized that PFC infusion after surfacing would: (1) increase N_2 washout through the lungs, (2) preserve or improve cardiac output in animals with DCS, (3) improve O_2 uptake and delivery to tissues, (4) decrease bubble formation by absorbing and removing N_2.

MATERIALS AND METHODS

The following protocol was approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee. All records and procedures were followed in accordance with University standards for care of animal research subjects.

Subjects and Surgical Preparation

Juvenile farm raised Yorkshire swine $n = 20$, 18 t 23 kg, of either gender were used for this study. The animals were shipped to our research facility and given 5 to 7 days for acclimation. Prior to the day of study, each animal was fasted overnight with water eliminated for 12 h. In the morning, a subject animal was sedated with an intramuscular injection of ketamine/acepromazine (40/0.5 mg/kg) while in the animal care facility. After sedation, the animal was transported within the institution to the DCS laboratory, a 23-gauge intravenous cannula was inserted into an ear vein, and general anesthesia was induced and maintained with ketamine/xylazine 20/0.5 mg/kg/h. This infusion rate was increased as necessary to maintain a surgical plane of anesthesia. Intravenous glycopyrrolate (0.01 mg/kg) was given as an antisialagogue. A tracheotomy was performed and a 7 mm endotracheal tube was inserted and sutured into place. All animals were ventilated (but not paralyzed) on a Siemens-Elema 900C ventilator breathing a 20% O_2/80% N_2 mixture with the end tidal CO_2 targeted to be 35 mmHg $\pm$ 5 mmHg. Respiratory gases were continuously monitored at the endotracheal tube ventilator interface via mass spectrometry (MGA-1100; Perkin-Elmer, Norwalk, CT).

An infusion of normal saline was begun immediately at a rate of 1 mL/kg/h to prevent dehydration during surgery, compression, and decompression. The right internal jugular vein was isolated and cannulated with a pulmonary artery catheter (93A-095-7F, proximal port 15 cm; Edwards Lifesciences) for measurement of cardiac output (CO) (American Edwards Laboratories, Irvine, CA). The right femoral artery was isolated and cannulated for continuous measurement of arterial blood pressure and for obtaining blood gas samples. All catheters were flushed with heparinized saline (4 IU/cc) to prevent clotting. The left external jugular vein was exposed via a cut down. A probe from a new and unique ultrasound bubble detection system (Emboli Detection and Classification [EDAC]; Lockheed Martin, Orincon Division, Arlington, VA) was sutured into place directly on the surface of the vein to record venous bubble load [31]. The EDAC technology had been validated in cardiopulmonary bypass but not yet tested in DCS.

Baseline Monitoring

As soon as the surgical preparations were completed, all animals were given a 10-min stabilization period before measurements were

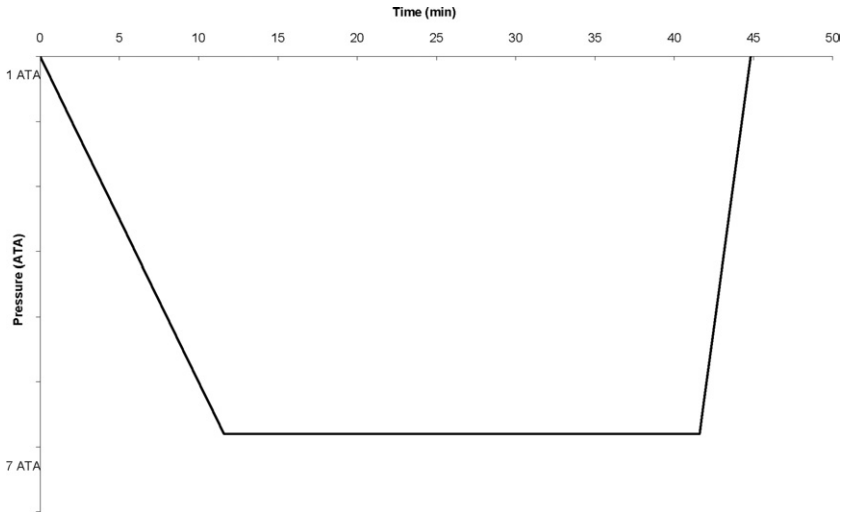


FIG. 1. Air dive profile. Swine were compressed at 0.5 ATA/min to 6.8 ATA and maintained at the bottom level for 30 min. They were then decompressed at 2 ATA/min.

made for femoral arterial pressure, central venous pressure (CVP), pulmonary arterial pressure (PAP), and pulmonary arterial occlusion pressure (PAOP or wedge pressure). Systemic vascular resistance (SVR) and pulmonary vascular resistance (PVR) were subsequently calculated offline. The BioPAC analogue-to-digital system (MP-150; BioPAC Systems, Inc., Santa Barbara, CA) was used to acquire hemodynamic data at a frequency of 500 Hz. Blood samples (0.3 cc) from the femoral artery, vein, and pulmonary artery (mixed venous samples) were taken for analysis of arterial and venous blood gases, electrolytes and selected metabolites (glucose and lactate). An ABL 700 blood gas analyzer and OSM3 hemoximeter (Radiometer America, Westlake, OH) were used for all blood gas and biochemical measurements.

Animals were randomly assigned to one of four experimental groups:

1. Control saline without compression-sham/saline, (n = 3)
2. Control saline with compression, compression/saline (n = 10)
3. PFC-treated without compression, PFC/sham (n = 3)
4. PFC-treated with compression, compression/PFC (n = 10)

Air Dive Procedures

Before the animals were moved into the chamber, dexamethasone (1 mg/kg, i.v.) was given as a bolus. All animals received dexamethasone whether they were sham (without compression) with or without PFC. Also, sham animals were placed in the chamber but not compressed. This was done in an attempt to block a species-specific pulmonary macrophage degranulation that is known to occur in swine on exposure to PFC emulsions [31]. The animals were weaned off the ventilator so they would breathe spontaneously inside the hyperbaric chamber. The animals were placed supine inside a custom-built, large animal hyperbaric chamber (Reimers Systems, Inc., Arlington, VA). The hyperbaric chamber has a large number of through-hull fittings for physiological monitoring. During the compression phase, anesthesia was maintained with a syringe pump, continuously infusing ketamine/xylazine anesthesia as described above. Arterial pressure, pulmonary artery pressure, and central venous pressure were continuously monitored the entire time the animals were in the hyperbaric chamber. All other lines were disconnected. The chamber was compressed with air at a rate of 0.5 ATA/min (7.35 psi/min or 16.5 fsw/min) to the target pressure of 6.8 ATA (85 psi, 191.4 fsw) (Fig. 1) and maintained at this pressure (6.8 ATA) for 30 min. Decompression then followed at a rate of 1.8 ATA/min (26.5 psi/min, or 60 fsw/min) to 1 ATA. Not only were vital signs monitored by computer attachments through the hull but the glass observation window of the chamber allowed the observation of any movements of the animal. If the animals exhibited any movements, signaling a "light" plane of anesthesia, the anesthesia infusion rate was increased. The experimenters were ready to abort the experiment and euthanize the animal immediately, if any animal were to exhibit an intractable seizure, but this never occurred.

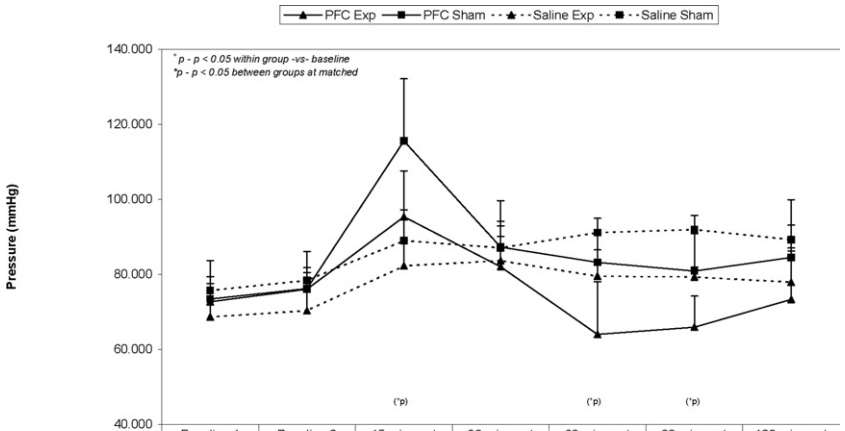
Postdecompression Monitoring and Data Acquisition

After decompression, the animals were removed from the hyperbaric chamber (considered time zero) and all monitoring technologies were immediately reconnected. The animals were ventilated with 100% oxygen, and paralyzed with an intravenous bolus of pancuronium (0.1 mg/kg). An additional dexamethasone bolus (1 mg/kg) was administered intravenously. Hemodynamic data, along with left jugular venous emboli counts and blood gas data were collected. PFC (Oxygent, 4.5 mL/kg, i.v.) or placebo (4.5 mL/kg normal saline) was administered 5 min after exit from chamber. Saline has been used in other PFC studies as it most closely represents the volume load of the PFC. Oxygent (Alliance Pharmaceutical Corp, San Diego, CA) is a 60% wt/vol emulsion of perfluorooctyl bromide.

Measurements of arterial, venous and mixed venous blood gases, pulmonary artery wedge pressure, central venous pressure, and bubble count (EDAC) were repeated at 15 and 30 min and then every 30 min until 120 min following decompression. At the same time points, cardiac output was measured using the thermodilution technique. Measurements were made by injecting ice-chilled saline into the central venous circulation via the proximal injection port of the pulmonary arterial catheter. The catheter was connected to an American Edwards Laboratories COM-1 cardiac output computer (Edwards Laboratories, Exeter, CA). The cardiac output computer is able to track the change in blood temperature and, from this information, calculate cardiac output. All cardiac output measurements were made in triplicate and averaged. Expired nitrogen levels were continuously monitored by mass spectroscopy. At 120 min after decompression, animals were sacrificed with Euthasol euthanasia solution (100 mg/kg, i.v.).

RESULTS

The hemodynamic data are presented in Figs. 2–8. The mean arterial blood pressure (MAP) did not vary



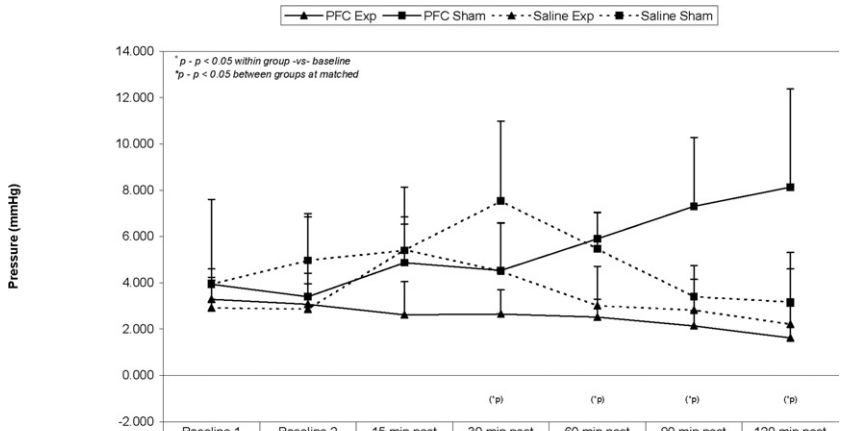
	Baseline 1	Baseline 2	15 min post	30 min post	60 min post	90 min post	120 min post
PFC Exp	72.667	76.000	95.367 ('p)	81.983	63.983	65.900	73.300
PFC Sham	73.400	76.200	115.600('p)	87.267	83.200	80.867	84.467
Saline Exp	68.613	70.278	82.244 ('p)	83.611 ('p)	79.478	79.267	77.900
Saline Sham	75.667	78.400	89.000	87.067	91.133 ('p)	91.933 ('p)	89.233

FIG. 2. MAP before compression and after decompression. At 15 min after decompression, MAP of PFC groups and saline experimental group were significantly increased and back to baseline at 30 min after decompression.

among the four groups or over time (Fig. 2). However, at 15 min after decompression, the PFC sham group had an isolated single higher MAP than the other groups. At 60 and 90 min, the PFC experimental group had a slightly lower mean arterial blood pressure than the other groups.

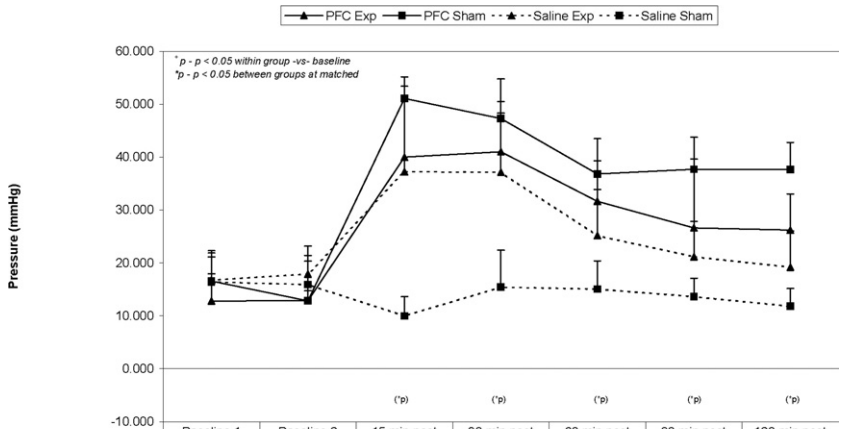
Volume load alone had a small and transient effect upon pre-load of the heart. The saline sham group demonstrated these findings, with a stable PAP over time (Fig. 4). The CVP increased immediately after infusion and at 30 min but returned to baseline by 90 min (Fig. 3). In the groups that received PFC, analysis of the PAP and CVP demonstrated both the isolated and combined effects of DCS as well as PFC treatment (Figs. 3, 4). This rise in PAP was also reflected in the CVP for that group. The use of PFC in the sham (no DCS) group demonstrated a dramatic rise in PAP by 15 min and a sustained, high level of PAP throughout the 120 min (Fig. 4). Of note, the PFC-induced rise in PAP was greater than that produced by DCS alone (Fig. 4).

The elevations in PAP were not always transmitted through the right heart to the CVP (Figs. 3, 4). The CVP in the PFC sham group continued to rise during the 120 min. The CVP in the PFC experimental DCS group did not show the same rise (Fig. 3). The PAOP (wedge pressure) did not follow the changes seen either with PAP or CVP (Fig. 5). The saline sham and saline experimental (DCS) groups showed few changes in PAOP pressures over time. The PFC sham (no DCS) quadrupled its PAOP at 15 min with a return to baseline by 120 min. When the effects of DCS and PFC



	Baseline 1	Baseline 2	15 min post	30 min post	60 min post	90 min post	120 min post
PFC Exp	3.283	3.067	2.617	2.650	2.517	2.133	1.617 ('p)
PFC Sham	3.933	3.400	4.867	4.533	5.900	7.300	8.133
Saline Exp	2.913	2.856	5.433 ('p)	4.500	3.011	2.822	2.211
Saline Sham	3.933	4.967	5.400	7.533	5.467	3.400	3.167

FIG. 3. Mean central venous blood pressure (MCVP) before compression and postdecompression. At 15 min after decompression, the saline experimental group showed a significantly higher MCVP than other groups.



	Baseline 1	Baseline 2	15 min post	30 min post	60 min post	90 min post	120 min post
PFC Exp	12.800	12.850	40.000 (*p)	41.000 (*p)	31.650 (*p)	26.617	26.217
PFC Sham	16.567	12.833	51.100 (*p)	47.300 (*p)	36.833 (*p)	37.700 (*p)	37.667 (*p)
Saline Exp	16.750	17.900	37.244 (*p)	37.133 (*p)	25.156	21.167	19.178
Saline Sham	16.400	15.900	9.967	15.433	15.033	13.600	11.800

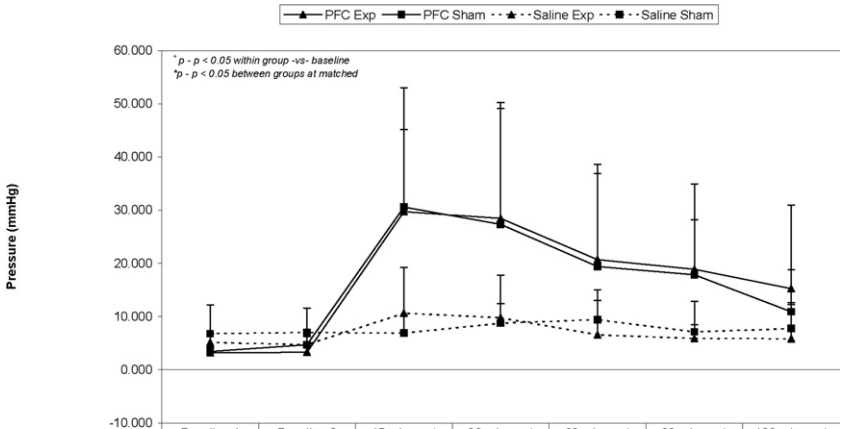
FIG. 4. Mean pulmonary arterial pressure (MPAP) before compression and after decompression. At 15 min after decompression, MPAP of PFC groups and saline experimental group were significantly increased compared with their baseline levels. Also, all PFC groups and the experimental saline group showed a significantly higher MPAP than the saline sham group after decompression.

infusion were combined, the highest rise (6-fold) in PAOP was noted with a parallel fall over time.

CO was unchanged in the saline sham group (Fig. 6). It appeared to fall from baseline in the saline experimental group by 120 min approaching only 50% of its own baseline. PFC infusion, however, caused a drop in cardiac output. From time 0 until 15 min after infusion, the CO dropped over 50% and stayed at that level until 120 min. The level of CO reduction was the same in both groups treated with PFC, whether they were compressed or not.

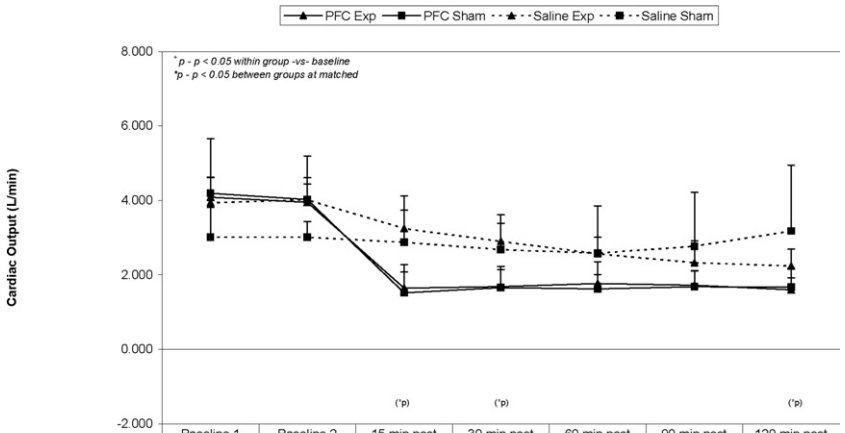
Both SVR and PVR were calculated. (Figs. 7, 8). A slow rise in SVR over time was noted in both saline treated groups. The PFC-treated animals had a spike of high SVR at 15 min after infusion, which tailed off toward baseline and was similar to that seen in the saline groups by 60 to 120 min. It did not appear that DCS either raised SVR in the saline or the PFC-treated group, independent of the time effect. PVR showed no change over time in the saline sham group (control). With DCS, by 15 min there was a tripling effect of PVR in the saline experimental group. The effect at 120 min on PVR was still at least double the PVR seen at baseline in the saline experimental (DCS) group.

The effect of PFC upon PVR was large (Fig. 8). PVR in the PFC sham group increased more than 5-fold. PVR in PFC-treated animals with DCS did not rise as high and trended toward the saline control (no DCS) group. PFC in the face of DCS did not cause the PVR rise seen in the PFC sham group. Furthermore, the



	Baseline 1	Baseline 2	15 min post	30 min post	60 min post	90 min post	120 min post
PFC Exp	3.175	3.292	29.733 (*p)	28.467 (*p)	20.692	18.892	15.250
PFC Sham	3.450	4.717	30.575	27.350	19.400	17.875	10.925
Saline Exp	5.129	4.702	10.646	9.799	6.584	5.871	5.822
Saline Sham	6.767	7.000	6.900	8.767	9.417	7.117	7.750

FIG. 5. Mean pulmonary artery occlusion pressure (MPAOP) before compression and after decompression. At 15 min after decompression, the MPAOPs of PFC groups were significantly increased compared to baseline.



	Baseline 1	Baseline 2	15 min post	30 min post	60 min post	90 min post	120 min post
PFC Exp	4.083	3.948	1.646 ('p)	1.686 ('p)	1.768 ('p)	1.721 ('p)	1.597 ('p)
PFC Sham	4.190	4.023	1.518 ('p)	1.655 ('p)	1.623 ('p)	1.682 ('p)	1.672 ('p)
Saline Exp	3.938	4.019	3.244	2.898 ('p)	2.566 ('p)	2.327 ('p)	2.238 ('p)
Saline Sham	3.010	3.008	2.873	2.678	2.582	2.768	3.180

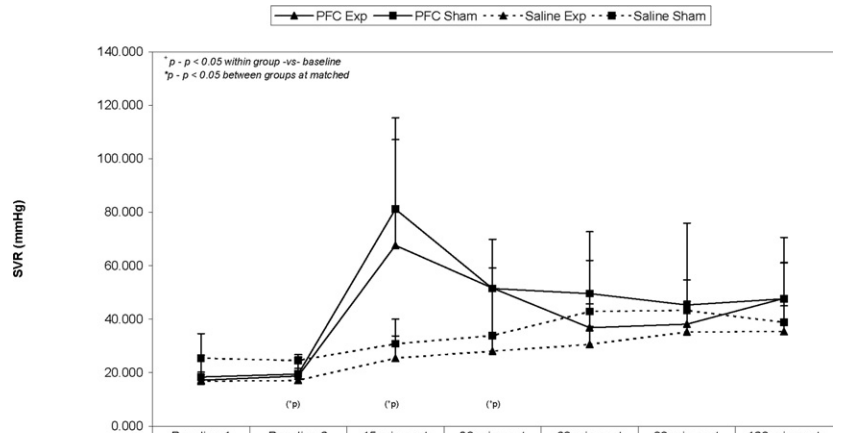
FIG. 6. Mean cardiac output before compression and postdecompression. CO of PFC groups and saline experimental animals dropped significantly after decompression, which also were lower than the saline group at the same postdecompression time measurement.

PFC treatment of DCS seemed to produce a lower PVR than the saline experimental (DCS) group.

The blood gas data showed no differences in the saline or PFC sham animals over time and between groups at any one time. The data presented here compare the effects of saline *versus* PFC treatment in DCS animals (Figs. 9–11). The pH in both arterial and mixed venous samples was similar between groups at baseline and at 15 min after surfacing (infusion of treatment). From 30 to 120 min, the arterial and mixed venous pH were lower in the PFC group. PaO_2 was similar among groups at 15 min after decompression. The arterial PaO_2 of the PFC group did drop over time and was slightly below that seen in the saline treatment group. However, mixed venous (PvO_2) in both the saline and PFC-treated groups with DCS was the same over time. PCO_2 was significantly increased by DCS in all groups compared with saline controls and PFC controls. These changes are common in experimental decompression sickness and are believed to be directly reflective of the deleterious effects of DCS [32, 33] (Fig. 12).

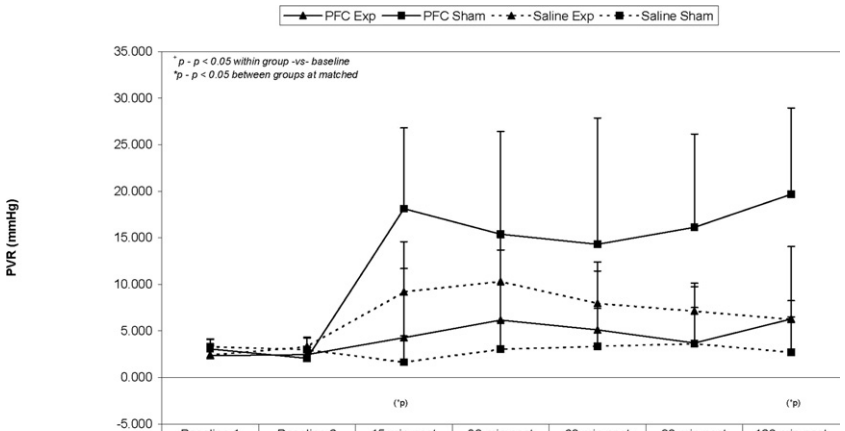
Respiratory end tidal N_2 washout revealed no significant differences in any group over time with respect to N_2 washout curves (Fig. 13). The fact that the PFC decreased CO (Fig. 6) and increased PVR (Fig. 8), compared with the saline, may have affected N_2 washout (Fig. 13).

The EDAC system counted bubbles within the external jugular vein (Fig. 14). Raw EDAC data are reported



	Baseline 1	Baseline 2	15 min post	30 min post	60 min post	90 min post	120 min post
PFC Exp	17.150	18.736	67.605 ('p)	51.625 ('p)	36.818	38.162	47.795 ('p)
PFC Sham	18.398	19.489	81.200 ('p)	51.499	49.581	45.341	47.621
Saline Exp	16.813	17.093	25.451	28.019 ('p)	30.546 ('p)	35.196 ('p)	35.369 ('p)
Saline Sham	25.381	24.535	30.796	33.827	42.828	43.295	38.794

FIG. 7. SVR was significantly increased after decompression in PFC groups and returned toward baseline level at 30 min after decompression. The saline experimental group had a long lasting increase in SVR, from 30 min to 120 min after decompression.



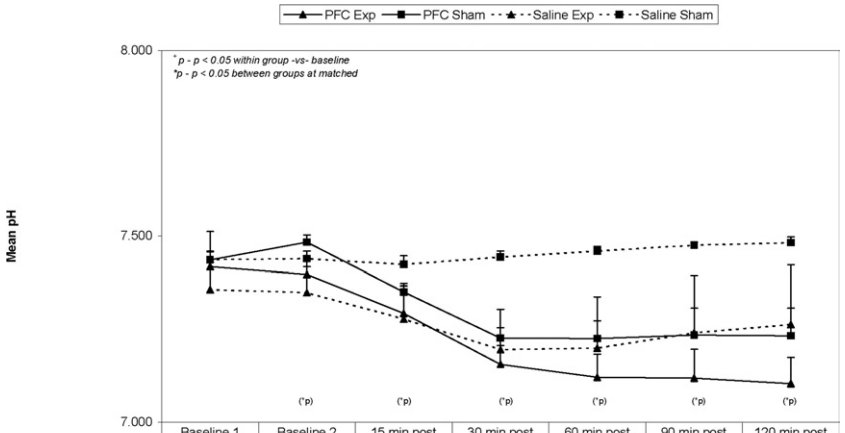
	Baseline 1	Baseline 2	15 min post	30 min post	60 min post	90 min post	120 min post
PFC Exp	2.342	2.450	4.269	6.164	5.118	3.697	6.276
PFC Sham	3.059	2.054	18.130	15.379	14.301	16.118	19.670
Saline Exp	2.410	3.294	9.205 (*p)	10.290 (*p)	7.953 (*p)	7.108 (*p)	6.225
Saline Sham	3.297	2.970	1.632	3.026	3.332	3.625	2.692

FIG. 8. Pulmonary vascular resistance (PVR) was significantly increased in saline experimental group after decompression.

in this study exactly as the machine recorded the data. A separate paper will deal with an in-depth analysis of EDAC data and DCS. The control (sham) saline and control (sham) PFC animals had a zero bubble count at all times in their external jugular veins. Over time, in the saline control animals, the bubble count rose and was approximately 10-fold baseline by 60 min postsurfacing. This level continued to rise throughout the time of the experiment (Fig. 14). The PFC-treated DCS animals experienced a far lower bubble load with an absolute rise of 2- to 4-fold over that same time period ($P < 0.05$).

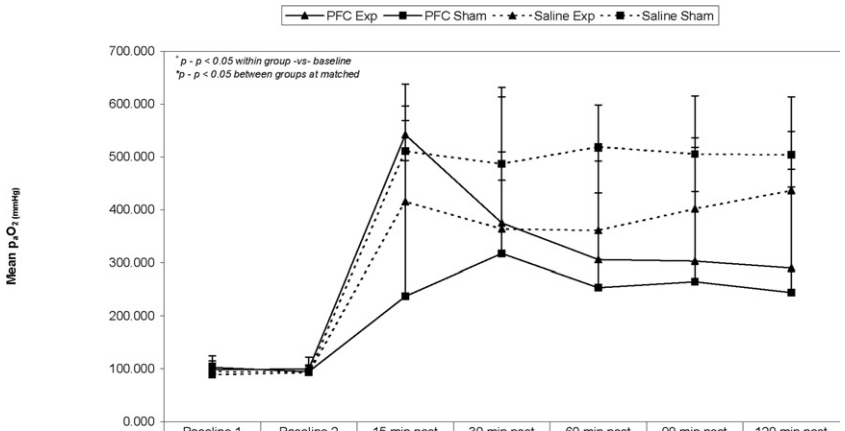
DISCUSSION

In this instrumented and anesthetized swine model of severe acute cardio-pulmonary DCS, we have created a reproducible model for hemodynamic and physiological monitoring of DCS. The extensive placement of monitoring devices makes it impossible to accomplish these measurements in conscious animals undergoing compression and decompression. Maintenance of a large animal preparation in a stable state, anesthetized, yet spontaneously breathing (normal $PaCO_2$), during a compression and decompression experiment, is not a small task. Considerable pilot work and trial and error testing were involved in developing and refining this particular dive profile. Numerous technical engineering problems had to be overcome to maintain stability and control of the anesthesia administration during compression and decompression. Much effort was required in assembling and coordinating the large amount of car-



	Baseline 1	Baseline 2	15 min post	30 min post	60 min post	90 min post	120 min post
PFC Exp	7.418	7.397	7.293 (*p)	7.155 (*p)	7.121 (*p	7.118 (*p)	7.104 (*p)
PFC Sham	7.436	7.484	7.350	7.226 (*p)	7.225 (*p	7.234 (*p)	7.232 (*p)
Saline Exp	7.356	7.348	7.277	7.195 (*p)	7.199 (*p	7.240	7.263
Saline Sham	7.437	7.439	7.424	7.443	7.460	7.475	7.482 (*p)

FIG. 9. Mean arterial pH was significantly decreased in saline experimental group and PFC-treated groups after decompression.

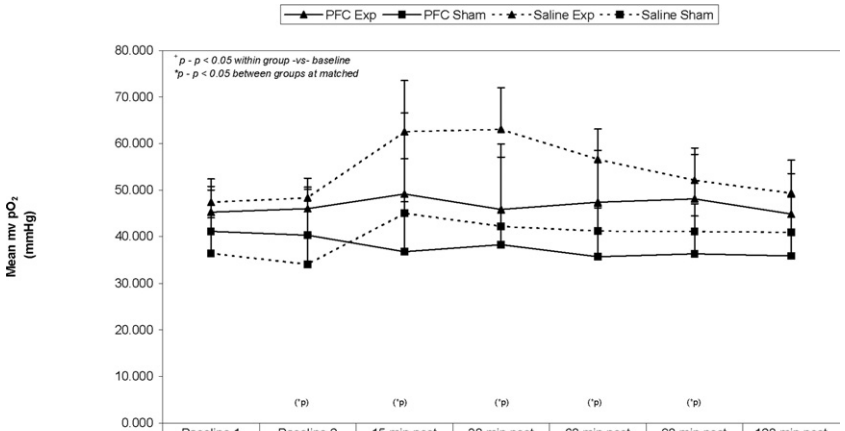


	Baseline 1	Baseline 2	15 min post	30 min post	60 min post	90 min post	120 min post
PFC Exp	98.225	99.533	542.000 (*p)	375.000 (*p)	306.000 (*p)	303.000 (*p)	290.167 (*p)
PFC Sham	102.533	94.400	237.100	317.533	253.367	264.600	244.167
Saline Exp	94.744	93.744	415.556 (*p)	363.556 (*p)	361.111 (*p)	401.778 (*p)	436.556 (*p)
Saline Sham	88.767	93.133	511.000 (*p)	487.000 (*p)	519.333 (*p)	505.667 (*p)	504.000 (*p)

FIG. 10. Mean arterial pO_2 in all groups was significantly increased because of breathing 100% oxygen after decompression.

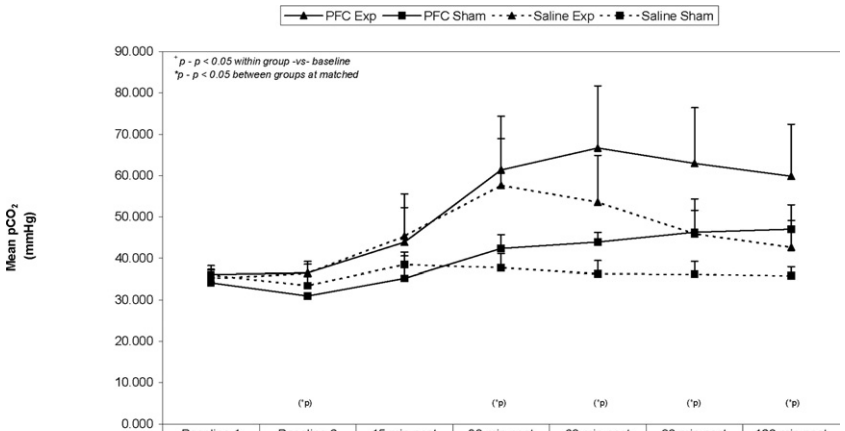
diopulmonary physiological monitoring equipment and data collection systems involved.

In the saline-treated DCS animals, a picture of increased PVR (100% increase over baseline) and dramatic elevation of venous bubble count (10-fold) was seen. This translated into a drop in expired CO_2 and a rise in $PaCO_2$ corresponding to a cardiopulmonary bubble load. CO was reduced progressively over time in the saline DCS group by approximately 50%. At 120 min, the CO was still trending downward and the bubble count in the venous circulation was continuing upward. One could conclude that DCS, even at 120 min after decompression, was continuing, as evidenced by bubble formation and bubble movement in the venous, central venous, and pulmonary circulations. We did not continue this experiment beyond the 120-min time window so the ultimate course of the bubble generation and CO changes are unknown. It is intriguing that N_2 washout through the lungs concluded rapidly yet the hemodynamic data and EDAC data show that bubble generation and movement were continuing within the circulation. Perhaps our model is so severe that we can only monitor the movement and release of dissolved nitrogen coming out of the lungs. Once the CO is depressed and a substantial bubble load has blocked many of the pulmonary arterioles, the ability to dissolve and export these N_2 bubbles is severely depressed. The use of respiratory mass spectrometry has not been applied before to experimental models of severe cardiopulmonary DCS. Further research needs to be conducted using these techniques to learn about bubble resolution and N_2 dissipation via the pulmonary vascular bed.



	Baseline 1	Baseline 2	15 min post	30 min post	60 min post	90 min post	120 min post
PFC Exp	45.240	45.980	49.140	45.840	47.367	48.083	44.850
PFC Sham	41.100	40.267	36.733	38.233	35.667	36.267	35.800
Saline Exp	47.367	48.333	62.578 (*p)	63.067 (*p)	56.589 (*p	52.111	49.322
Saline Sham	36.367	34.000	45.000	42.167	41.167	41.067	40.900

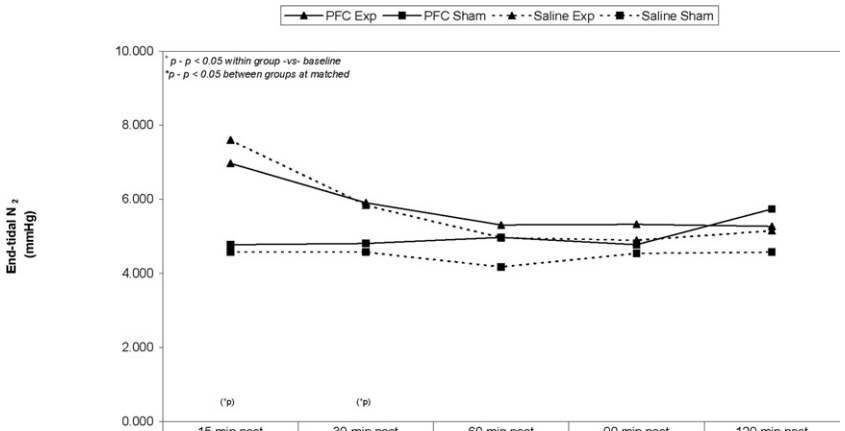
FIG. 11. Mean mixed venous pO_2. Breathing 100% oxygen after decompression resulted in a short increase of mean mixed venous pO_2 in saline experimental group.



	Baseline 1	Baseline 2	15 min post	30 min post	60 min post	90 min post	120 min post
PFC Exp	36.125	36.500	43.880	61.340 (*p)	66.650 (*p	62.933 (*p)	59.833 (*p)
PFC Sham	34.133	30.950	35.200	42.367	43.900	46.267 (*p)	47.000 (*p)
Saline Exp	35.133	36.389	45.311	57.644 (*p)	53.556 (*p	45.922 (*p)	42.600
Saline Sham	35.967	33.433	38.467	37.700	36.233	36.200	35.800

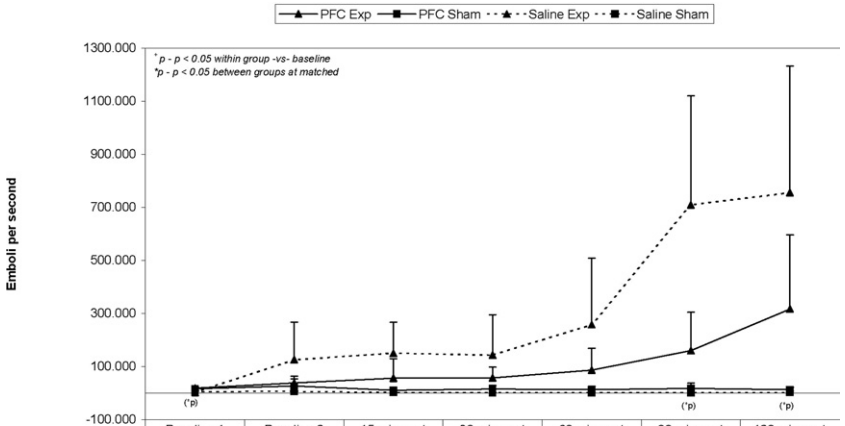
FIG. 12. Mean arterial pCO_2 was increased in all experimental groups at 30 min after decompression.

The infusion of PFC at surface created a substantially different hemodynamic and bubble load picture in our experimental animals. Unfortunately, our attempts to blunt or obliterate the species-specific pulmonary hypertensive response (elevated PAP, CVP, and PAOP with compensatory decreased CO) of swine to PFC emulsions were unsuccessful. This has been described before with the use of steroids or nonsteroidal anti-inflammatory agents [31]. In the group of animals that was not compressed but did receive PFC, the PAP and PVR dramatically rose with PFC infusion. The levels of rise were as much as 5-fold over baseline or levels seen in the saline sham group. A resultant drop in CO was recorded with a corresponding rise in SVR. The drop in CO from the PFC was dramatic and rapid after infusion. It had maximized by 15 min after infusion and stayed at that level throughout the experiment. The drop in CO and rise in PVR were contrasted to the effects of DCS alone (saline DCS group), which demonstrated a slow and continuous drop in CO with a corresponding rise in PVR. We chose to study swine, even though we knew about the species-specific pulmonary changes. The reasons for doing so were based upon the data from prior successful work at the Naval Medical Research Center (NMRC) [28–30]. In the first swine survival study performed there, a saturation dive (4.8 ATA for 22 h) created cardiopulmonary DCS in which PFC treatment at surface reduced 1-h mortality from 85% to 15% (with PFC) [29]. Our goal was to mimic, as closely as possible, the conditions of the NMRC dive profile and survival study, with the understanding that we needed to instrument the animals



	15 min post	30 min post	60 min post	90 min post	120 min post
PFC Exp	6.967	5.900 (*p)	5.300 (*p)	5.317 (*p)	5.267 (*p)
PFC Sham	4.767	4.800	4.967	4.767	5.733
Saline Exp	7.600	5.833 (*p)	4.956 (*p	4.889 (*p)	5.144 (*p)
Saline Sham	4.567	4.567	4.167	4.533	4.567

FIG. 13. End-tidal nitrogen was elevated in animals that experienced DCS but was not different between PFC and saline animals. Expired nitrogen returned to baseline within 60 min.



	Baseline 1	Baseline 2	15 min post	30 min post	60 min post	90 min post	120 min post
PFC Exp	19.000	38.667	56.167 (*p)	57.500	86.833	160.500	316.667
PFC Sham	16.667	27.000	11.000 (*p)	15.667	13.333	18.000	13.333
Saline Exp	2.667	126.000	150.833	143.167	258.167	709.333 (*p)	755.333 (*p)
Saline Sham	6.500	7.000	3.000	1.500	1.500	1.500	3.000

FIG. 14. EDAC. Venous gas emboli count was detected by the Emboli Detection and Classification system (EDAC). After decompression, venous gas bubble count was significantly increased in all groups. The PFC-treated group showed a significantly lower bubble count than the saline-treated group.

fully so as to better understand the mechanisms of PFC treatment and response. We realized that we would need to develop an acute model that would have to be anesthetized from beginning to end.

In the PFC-treated animals that underwent DCS, there were some pulmonary vascular responses as seen with the PFC sham animals. Of interest, the PAP and PVR changes were less intense than with PFC alone (no DCS). Mean PAP was lower than seen with PFC sham and about the same as the saline DCS group. PVR was less in the PFC DCS treatment group than in the saline DCS group throughout all time points, although CO was considerably lower in the PFC DCS group. The pulmonary vasoconstrictive effect of the PFC in the sham portion of this model is confounding and, therefore, limits some of the conclusions that could otherwise be drawn from this experiment. The only deaths (2/7) in our study were in DCS animals that received saline as a treatment. All PFC (DCS and sham) and sham saline animals survived out to 120 min. There were important differences in study design between what is reported here and the prior survival studies conducted by NMRC [28–30]. The animals at NMRC were of larger body weight, awake, spontaneously breathing, not instrumented with central venous or pulmonary artery catheters, and notably, underwent a considerably different dive profile. The effects of general anesthesia and spontaneous respiration *versus* controlled respiration (after decompression in our study) cannot be known. Central venous and pulmonary artery cannulas may well act as a nidus for bubble generation. That factor alone makes our study considerably different than the prior survival study. Both the NMRC studies and ours gave the same (per weight) bolus of the same PFC as soon as possible upon decompression.

The EDAC system found a dramatically lower bubble count in the group treated with PFC. It would be very attractive to think that the large difference seen in the PFC- treated animals was all due to the effect of the PFC in reducing the formation of venous bubbles or the more rapid clearance of N_2 from the body. Unfortunately, we do not have confirmatory evidence of that fact from the N_2 washout curves seen by respiratory mass spectroscopy. The drop in CO found in the PFC animals may have affected the movement of bubbles within the venous circulation and at least been partially responsible for fewer bubbles migrating below the EDAC detector system.

The blood gases and lactate levels are of interest. PFC does not increase PaO_2. PFC acts as a third compartment within the blood stream and dissolves oxygen only in equilibrium with the partial pressure of the gases to which it is exposed. Therefore, the simple addition of PFC does not increase PaO_2 (the partial pressure of O_2 dissolved in plasma) but it will increase total O_2 content. The mixed venous PvO_2 reflects arterial O_2 content, delivery, and metabolic extraction. In our study, the PvO_2 of PFC-treated DCS animals were similar to those seen in the saline DCS animals. However, the PFC-treated animals had a dramatic reduction in CO. As a result, even though their total CO was decreased, they met or exceeded their metabolic O_2 demand. The PFC-treated DCS animals had a stable or rising PvO_2 over time whereas the saline-treated DCS animals had a dropping PvO_2 over time. This fact may well reflect that the saline DCS animals still had bubble formation on-going and the PFC animals had a less

severe bubble load or a beginning resolution of their DCS. Systemic lactate levels are predictive of cellular critical O_2 delivery deficit, organ damage, and organism survival. In our study, the lactate levels were high at 15 min after surfacing in both groups. The PFC animals were able to reduce their lactate load by 33% at 120 min whereas the saline-treated animals' levels remained high and largely unchanged. Again, one has to remember that these lactate levels were observed even in the face of the PFC causing a species-specific drop in CO and an elevation of PVR. The flux of O_2 in the central circulation and peripheral circulation in the face of PFC addition is complex and will be more fully analyzed in a separate paper.

In conclusion, we have established a model wherein we can study acute cardiopulmonary DCS in an anesthetized and hemodynamically instrumented animal model. It is reproducible but may not yet be perfected. PFC, particularly Oxygent, causes a previously reported species-specific pulmonary vasoconstrictive response not seen in humans. Other PFC emulsions may either cause the same, more, or less pulmonary hypertension in a swine model. Each present and future PFC emulsion will need to be dealt with as a new or different pharmaceutical. The PFC treatment of cardiopulmonary DCS immediately at surface seems to decrease the bubble load (EDAC data), as well as improve whole body O_2 delivery (PvO_2 and lactate data). We cannot say, at this time, that the most important mechanism of protection is predominantly enhanced N_2 washout or improved O_2 delivery. Probably both play significant roles. Development of a DCS model in a different large animal (a species that does not develop PFC-related pulmonary hypertension) is required for further mechanistic work regarding PFC and the noted survival improvement. Most importantly, both our study (to some extent) and the prior work have shown that PFC in severe cardiopulmonary DCS improves survival. The swine survival study most probably experienced the same pulmonary hypertensive response that we encountered. More research is required to understand the tissue effects of O_2 delivery, timing of infusion, dose/s of PFC, and best respiratory gas mixture for PFC efficacy in DCS.

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