Intravenous perfluorocarbon emulsion increases Nitrogen washout after venous gas emboli in rabbits.

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Zhu J, Hullett JB, Somera L, Barbee RW, Ward KR, Berger BE, Speiss BD. Intravenous Perfluorocarbon Emulsion Increases Nitrogen Washout after Venous Gas Emboli in Rabbits. Undersea Hyperb Med 2007; 34(1):7-20. Intravenous perfluorocarbon emulsion (IV-PFC) has been shown to provide hemodynamic protection from gas embolism (Venous-VGE or arterial-AGE). The objective of this study was to investigate the mechanism of PFC protection from controlled VGE by quantifying the effects of IV-PFC emulsion on pulmonary elimination of nitrogen (N₂). All rabbits received an intravenous pretreatment of PFC emulsion (Oxygent™, 2.7 g /kg) or saline, then either a continuous room air infusion (0.25 ml/kg for 10 minutes) or a bolus of air (0.8 ml/kg within 10 seconds) through the femoral vein. Expiratory N₂ peaked higher with PFC infusion immediately after air injection. The recovery to baseline of end tidal N₂ was faster for PFC-treated animals (40 ± 4.7 vs. 58± 6.5 minutes). In PFC-treated animals, expired CO₂, O₂, arterial pressure and central venous pressure returned to baseline faster than the saline group. This study demonstrated that PFC increased pulmonary N₂ washout. Correspondingly, PFC treatment better preserved the animals’ hemodynamics after VGE injury. The use of IV-PFC promises to be a breakthrough non-recompression therapy for gas embolism in the treatment of Decompression Sickness (DCS) and in surgery.

INTRODUCTION

Gas embolism (venous-VGE or arterial-AGE) is a consequence of decompression sickness (DCS) and several types of surgical procedures (1, 2, 3). DCS occurs in sport and professional diving accidents. DCS is an impediment to rescue from an otherwise survivable disabled submarine (DISSUB) accident (4, 5). In DISSUB, the internal environment of the submarine could change from approximately one atmosphere pressure to that of the surrounding sea water, if the inner hull has been breached. In DISSUB, the loss of atmosphere control capabilities might lead to a slow increase of internal pressure, possibly in the range of 2-5 ATA. Submariners rescued would be at risk for DCS. In civilian medicine, VGE or AGE are present in cardiac surgery, neurosurgery, gynecologic procedures and orthopedic surgery. The presence of a patent foramen ovale in up to 20% of the population means that VGE can become AGE as they cross from right to left sides of the heart through a patent foramen ovale.

VGE may be subclinical or cause a gradation of cardiovascular changes, dependent upon the amount of embolized air. Small
amounts of VGE may be tolerated by the cardiopulmonary system. Large boluses of air (3-8 ml/kg) can cause acute right ventricular outflow obstruction with cardiogenic shock and circulatory arrest. Intermediate amounts of air collect in the pulmonary circulation and produce a pulmonary vascular injury manifested by pre- and post-capillary pulmonary vasoconstriction, pulmonary hypertension, endothelial injury, pulmonary edema, protein changes, inflammation, and diffuse intravascular coagulation and a propensity for VGE to travel to the arterial side (6, 7). Blockage of blood flow through the pulmonary vasculature and the pulmonic valve contributes to depressed cardiac output as well as tissue hypoxia. (4, 5, 8, 9).

Intravenous perfluorocarbon emulsions (IV-PFC) have been investigated as O₂ therapeutics (blood substitutes) and contrast media (10, 11, 12, 13, 14, 15, 16). IV-PFCs are halogen (mostly fluorine)-substituted hydrocarbon chains in which solubility for respiratory gases is enhanced (13, 15, 17, 18). There is now an extensive literature on the beneficial effects of IV-PFC’s on VGE and AGE compromised cardiovascular dysfunction (19,20) indicating therapeutic promise for the emulsions, but they have not been widely embraced by medical researchers. Nevertheless, progress continues with each set of studies continuously showing that IV-PFC can be utilized to enhance tissue O₂ delivery and decrease the effects of air emboli. IV-PFC’s increase brain oxygenation (21) and decrease infarct volume in experimental stroke (22, 23, 24). In addition, IV-PFC pre-treatment provides protection from systemic cerebral and coronary air emboli in rabbits and dogs (25, 26, 27, 28). Daugherty et al. (29) and Kwon, TH et al. (30) have reported that PFC treatment improved the cerebral oxygenation and mitochondrial function following traumatic brain injury. PFC treatment also increased survival rate in both large and small animals with DCS, and prevented neurologic DCS (31, 32). In the swine model of severe cardiopulmonary DCS, animals received IV-PFC within 10 minutes of reaching surface (31). Those that received IV-PFC had a reduced mortality and those that did die, died suddenly, possibly from intra-coronary gas embolism. No animal that received IV-PFC at surface developed either seizures or ataxia. Neurologic DCS was a common finding in control animals.

Since PFC emulsions are able to enhance gas solubility, we hypothesized that the pre-treatment with the PFC emulsion, Oxygenet™ (Alliance Pharmaceuticals, San Diego, CA), prior to experimental VGE, would increase the rate of pulmonary N₂ washout.

**MATERIALS AND METHODS**

The animal procedures for this study followed national guidelines, and the protocol was approved by The Institutional Animal Care and Use Committee (IACUC) at Virginia Commonwealth University.

**Subjects and groups**

Male New Zealand White rabbits (2.0 ±0.5 kg, Burleson Enterprises, Inc, Unionville, VA) were housed singly in an environmentally controlled room at 22°C with food and water ad libitum and were acclimatized for 3 to 7 days prior to experimentation. Each animal was randomly assigned to one of four groups: VGE bolus group receiving either saline or IV-PFC (n= 10 per subgroup); VGE continuous air infusion group receiving either saline or IV-PFC (n= 10 per subgroup). IV-PFC or saline was administered to the experimental animals (4.5 ml/kg, intravenous injection, IV over 15 minutes) at 10 minutes prior to air delivery. This dose was chosen because it was the maximum dose under study in humans at the time.
Surgical procedures and monitoring

The rabbits were anesthetized with intramuscular ketamine plus xylazine (35/2 mg/kg, mixture; 0.45 ml/kg) and maintained throughout the study with hourly doses. Toe pinch and corneal reflexes were assessed every 10 minutes. Core temperature was maintained at 39.0 ± 0.50° C, using an auto-controlled animal heating system (Harvard Apparatus, Inc).

A tracheotomy was performed and the animals were mechanically ventilated (Harvard Rodent Ventilator, Model 683) with 100% oxygen, to establish a stable end tidal CO₂ of 35 ± 5 mmHg. The end-tidal respiratory gases (CO₂, O₂, N₂) were continuously measured by mass spectrometry (MGA 1100, Perkin-Elmer Corp, MA Tech Services Inc. St Louis, MO). The right common carotid artery and the right external jugular vein were isolated to allow for mean arterial blood pressure (MAP) and central venous pressure (CVP) monitoring. The right femoral artery and vein were catheterized for PFC/saline treatment, air infusion, and blood sampling. Electrocardiogram (ECG, lead III) and electroencephalogram (EEG, temporal lobe) were recorded using surface needle electrodes. Pancuronium, 0.15 mg/kg IV infusion, was administered for muscle relaxation.

Physiological data (MAP, CVP, ECG, and EEG) and the end-tidal gases data (CO₂, O₂, and N₂) were continuously recorded using a (BIOPAC System Inc, Model: MAP150, Goleta, CA). Arterial and venous blood samples, 0.2 ml, before and after emboli were analyzed (ABL725, RADIOMETER AMERICA, Inc, Westlake, OH). All blood removed was replaced with an equivalent volume of 6% hydroxyethyl starch (Hetastarch®) solution in normal saline to insure stable intravascular volume.

PFC emulsion and treatment

Oxygent, (AF0144, Alliance Pharmaceuticals, San Diego, CA) is a second generation, perflubron-based, sterile IV-PFC emulsion for intravenous infusion, which contains 60% PFC per ml. The PFC is emulsified with egg yolk phospholipids, and the emulsion has a median particle size of 0.16 to 0.18 μm.

When end-tidal respiratory nitrogen (ETN₂) was stabilized at about 0 +/-1 mmHg for 10 minutes, either IV-PFC emulsion or 0.9% saline (4.5 ml/kg, containing 2.7 g/kg of IV-PFC) was administered intravenously over 15 minutes. The carrier compounds which are combined with PFC to form the emulsion are not used as “controls” because, in the emulsification process, the chemical and physical characteristics of the carrier compounds change during multiple heating and mixing stages of emulsification. Any effects the unaltered carrier may have are, therefore, irrelevant.

VGE and post VGE monitoring

VGE commenced through the femoral vein catheter at 10 minutes after IV-PFC/Saline treatment. For the bolus VGE group, air (0.8 ml/kg) was given in 10 seconds and for the continuous air infusion group, air (0.25 ml/kg/minute) was infused for 10 minutes. All animals were monitored for 60 minutes after VGE and euthanized (intravenous injection of Euthasol® 0.5 ml/kg).

Data analysis

Statistical analysis of the group data was subjected to a single-factor ANOVA. If significance (p ≤ 0.05) was observed with ANOVA, a post hoc assessment was made with the Bonferroni test. All analyses were implemented in SPSS v.11, and a significance level of P value ≤ 0.05 was used for all tests.

RESULTS

Body weights and, therefore, volumes
of IV-PFC or saline were the same among groups. The volume of air infused was the same: saline and IV-PFC treated group (1.78 ml ± 0.04 versus 1.79 ml ± 0.03) in bolus VGE subgroups; 5.83 ml ± 0.25 versus 5.86 ml ±
0.22 in continuous infusion VGE subgroup). The total air volume in bolus administration was significantly less than that in continuous infusion [P<0.001].

After IV-PFC or saline pre-treatment, all hemodynamic values (AP, CVP, ECG) and blood gases monitored were not significantly different from the baseline measurements (Tables 1, 2, 3 and 4; please see pages 12-15).

**End-tidal gases**

Nitrogen (N$_2$): There was a small but detectable increase in ETN$_2$ just after the IV-PFC was infused. This was not seen with saline infusion. ETN$_2$ dramatically increased in all groups when air administration commenced (P=0.001, Fig 1; Table 1). In the continuous air infusion group, the maximum N$_2$ level in PFC-treated animals was significantly higher than the level in the saline group (P=0.001, Table 1). There was a trend towards a higher peak value in the bolus air group with IV-PFC treatment but it did not reach significance. ETN$_2$ levels returned to baseline faster in the PFC-treated air infusion groups than did the corresponding ones treated with saline. This reached statistical significance in the infusion group (42.22 minutes ± 4.72 vs. 64.11 ± 6.53; P=0.01) and was a strong trend in the bolus air group (45.22 minutes ± 3.97 in PFC group vs. 51.9 minutes ± 4.14 in saline group; P=0.08).

The areas under the ETN$_2$ curves for PFC vs. saline treated animals were the same. Interestingly, the curves for IV-PFC-treated animals shifted the peak of ETN$_2$ higher, earlier, and shortened the time to return to baseline.

Carbon dioxide (CO$_2$): Mean end-tidal CO$_2$ decreased significantly after VGE in all groups and reached its lowest levels at approximately 2.5-5 minutes after air injection. The traces of end tidal CO$_2$ appeared to indicate less overall decrease and faster recovery for all animals that received PFC treatment. End-tidal CO$_2$ returned to baseline level at or between 25-65 minutes after air injection, and the IV-PFC group returned to baseline faster. In the continuous air infusion groups, IV-PFC-treated animals showed a significantly higher expired CO$_2$ level than did the saline group at 5, 10, 15, 30, and 45 min post air (P(5) =0.02, P(10) =0.03, P(15)=0.04, P(30)=0.01, P(45)=0.01). Although the overall trace of end tidal CO$_2$ was higher in the IV-PFC animals for bolus air VGE group, the CO$_2$ difference reached significance between groups only at 2.5 minutes post injection. The CO$_2$ clearance went above baseline, showing that during the time of peak VGE obstruction, the animals had acquired a CO$_2$ load that was subsequently cleared from the circulation.

Oxygen (O$_2$): Expired O$_2$ levels rose with air infusion. Inspired oxygen was constant. Therefore, there were changes in the uptake of oxygen (O$_2$ (I-E)) after air injection. There were significant decreases of O$_2$ (I-E) in all saline-treated animals, compared with IV-PFC-treated animals (air bolus and air infusion) at 2.5 minutes after air injection (P(bolus air) =0.04, P(infusion air) =0.01). IV-PFC-treated animals in the air infusion group showed significantly higher O$_2$ extraction at 5, 30, and 45 minutes after air injection as compared to saline-treated animals (P(5) =0.01, P(30) =0.01, P(45) =0.03).
**Fig. 1a.** The effect of PFC on end-tidal \( N_2 \) levels after venous gas embolism (VGE). End-tidal \( N_2 \) level increased significantly after VGE compared to baseline level. The peak end-tidal \( N_2 \) level in PFC-treated group was significantly higher than saline-treated group \((p<0.05)\) and returned faster to the baseline level compared to saline-treated animals in the air-infusion group. The mean time of the end-tidal \( N_2 \) level back to baseline was significantly faster in PFC-treated animals than that of saline-treated animals \((40 \pm 4.72 \text{ minutes} \pm \text{SE} \text{ vs.} \ 58 \pm 6.53, p<0.05 \text{ in air infusion group})\). End-tidal \( N_2 \) level in bolus-air VGE showed a similar curve pattern as air-infusion VGE. PFC-treated animals showed a trend to washout \( N_2 \) faster than saline-treated animals in bolus-air VGE.

**Fig. 1b.** The effect of PFC on end-tidal \( CO_2 \) levels after venous gas embolism (VGE). End-tidal \( CO_2 \) level decreased significantly after VGE compared to baseline level. The end-tidal \( CO_2 \) level in PFC-treated group was significantly less reduced than saline-treated group and returned faster back to the baseline level compared to saline-treated animals in the air-infusion group \((p<0.05)\). In bolus-air VGE, end-tidal \( CO_2 \) level showed a similar curve pattern as air-infusion VGE. PFC-treated animals showed a trend of less end-tidal \( CO_2 \) reduction than saline-treated animals in bolus-air VGE.

**Fig. 1c.** The effect of PFC on end-tidal extraction \( O_2 \) levels after venous gas embolism (VGE). End-tidal extraction \( O_2 \) level decreased significantly after VGE compared to baseline level indicating that reduced oxygen usage. The end-tidal \( O_2 \) level in PFC-treated animals was significantly less reduced than saline-treated animals and returned faster towards the baseline level compared to saline-treated animals in the air-infusion group \((p<0.05)\). In bolus-air VGE, the end-tidal \( O_2 \) level showed a curve pattern similar to air-infusion VGE. PFC-treated animals showed a trend of less end-tidal \( O_2 \) reduction than saline-treated animals in bolus-air VGE. \([\text{Extraction: Inspired } O_2 (760 \text{ mmHg; 100\% } O_2) - \text{Expired } O_2]\)
Table 1. End tidal gas effects

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tx</th>
<th>100% O₂</th>
<th>5 min post</th>
<th>15 sec post</th>
<th>45 min post</th>
<th>60 min post</th>
<th>90 min post</th>
<th>120 min post</th>
<th>150 min post</th>
<th>180 min post</th>
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</table>

Note: Averages are the relative values compared with baseline values. Median ± standard error (SE). P ≤ 0.05 when compared to saline group.
### Table 2. Blood Pressure Effects

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tx</th>
<th>baseline (100% O₂)</th>
<th>10 min post treatment</th>
<th>2.5 min post air injection</th>
<th>5 min post air injection</th>
<th>15 min post air injection</th>
<th>30 min post air injection</th>
<th>45 min post air injection</th>
<th>60 min post air injection</th>
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<tbody>
<tr>
<td><strong>MAP</strong></td>
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<td></td>
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<tr>
<td>air infusion</td>
<td>PFC</td>
<td>83.9±1.20</td>
<td>80.3±0.36</td>
<td>53.4±4.19 (^a)</td>
<td>31.7±3.50 (^\dagger)</td>
<td>36.3±6.14 (^\ddagger)</td>
<td>64.6±7.77 (^*)</td>
<td>59.7±5.04 (^\ast)</td>
<td>64.7±2.63 (^\ast)</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>83.1±1.72</td>
<td>80.1±0.16</td>
<td>52.2±6.37</td>
<td>26.4±4.13</td>
<td>23.6±4.41</td>
<td>44.6±10.18</td>
<td>57.5±4.14</td>
<td>66.5±2.47</td>
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<tr>
<td>bolus air</td>
<td>PFC</td>
<td>80.4±1.59</td>
<td>82.4±1.65</td>
<td>25.4±2.72</td>
<td>10.3±3.84</td>
<td>16.1±1.80</td>
<td>54.8±2.28</td>
<td>58.7±3.09</td>
<td>58.9±1.05</td>
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<td>Saline</td>
<td>76.7±4.79</td>
<td>79.2±3.79</td>
<td>27.3±4.42</td>
<td>30.4±4.66</td>
<td>37.2±6.93</td>
<td>49.3±8.83</td>
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<td><strong>CVP</strong></td>
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<tr>
<td>air infusion</td>
<td>PFC</td>
<td>1.37±0.23</td>
<td>1.37±0.22</td>
<td>2.50±0.31 (^b)</td>
<td>3.81±0.30</td>
<td>3.59±0.29</td>
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<td>2.25±0.63</td>
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<td>3.91±0.36</td>
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<td>1.28±0.79</td>
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<tr>
<td>bolus air</td>
<td>PFC</td>
<td>1.60±0.16</td>
<td>1.50±0.18</td>
<td>4.20±0.27</td>
<td>3.98±0.22</td>
<td>3.64±0.46</td>
<td>2.86±0.37</td>
<td>2.11±0.36</td>
<td>1.30±0.53</td>
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<td>Saline</td>
<td>1.68±0.12</td>
<td>1.71±0.12</td>
<td>4.50±0.39</td>
<td>4.38±0.44</td>
<td>3.60±0.35</td>
<td>2.65±0.45</td>
<td>1.93±0.41</td>
<td>1.44±0.39</td>
</tr>
</tbody>
</table>

PFC = perfluorocarbon; MAP = mean arterial blood pressure; CVP = central venous pressure
All other data are mean ± standard error of the mean, \(^*\) P < 0.05, \(^\ast\) P < 0.01 when compared to saline group
\(^a\). \(^b\): All data at 2.5 minutes after air injection are significantly different from their baseline data (P < 0.05).
<table>
<thead>
<tr>
<th>Group</th>
<th>0 min post</th>
<th>15 min post</th>
<th>30 min post</th>
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<td>air infusion</td>
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<tr>
<td>Saline</td>
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</table>

Table 3. Arterial Blood Gas Effects
Blood gases

Arterial and Venous Oxygen (PaO₂ and PvO₂): Both PaO₂ and PvO₂ decreased significantly after air injection. In the continuous air infusion groups, IV-PFC-treated animals showed a significantly higher level of PaO₂ and PvO₂ at 45 min and 60 min than that found in saline-treated animals (Table 3).

Arterial and Venous Carbon Dioxide (PaCO₂ and PvCO₂): Both PaCO₂ and PvCO₂ increased significantly after air injection. There were no significant differences in blood CO₂ levels between IV-PFC- and saline-treated animals. However, PFC-treated animals showed an increased blood CO₂ level in the early phase (<30 minutes post air injection) but the blood CO₂ began to fall towards baseline more quickly in the late phase (>30 minutes post air injection) compared with saline-treated animals (Tables 3 & 4).

pH: There were significant decreases in blood pH in both arterial and venous blood samples after air injection (Tables 3 & 4). There were no significant differences between PFC- and saline-treated groups.

Hemodynamics

After injection of air, the mean arterial blood pressure (MAP) decreased significantly in all groups by 70-80%. The lowest level of MAP was observed between 5 min and 15 min after air injection, followed by a return of MAP towards the baseline (Table 2). IV-PFC-treated animals had significantly higher MAPs’ during the period from 5 min to 30 min than those corresponding saline-treated animals and appeared to return towards baseline more quickly. In the IV-PFC-air-bolus group, the MAP had recovered approximately 50% of its loss after 5 minutes, whereas, the control groups recovery of baseline blood pressure lagged (only approximately 35% recovered at
The central venous blood pressure (CVP) more than doubled in all groups after injection of air. The highest CVP level was observed at the period from 5 min to 15 min after air injection and then returned towards back to baseline (Table 2). In the air-infusion group, PFC-treated animals showed that CVP level was significantly lower than those animals treated with saline at 45 min after air injection. There were no significant differences in CVP between PFC- and saline-treated animals in the air-bolus group.

**ECG and EEG**

There were no significant differences in the ECG and EEG activities within the frequency band distribution between PFC-treated and saline-treated animals after air injection in all groups.

**DISCUSSION**

Our study demonstrated that pre-treatment with a second-generation IV-PFC at a dose of 4.5 ml/kg (2.7g PFC /kg) increased respiratory N\textsubscript{2} elimination. Furthermore, IV-PFC pre-treatment induced recovery of end-tidal gases and hemodynamics more quickly in rabbits that received an infusion of air rather than the bolus. These findings support the prior work on VGE with first-generation IV-PFCs (19, 20). Second generation IV- PFC’s are at least four to six times more concentrated than first generation. The present study was unique in that it quantified N\textsubscript{2} clearance through the pulmonary vascular bed. The rate of pulmonary elimination of experimental VGE has been examined using similar techniques (33). Mass spectrometry allows for sensitive measures of gas elimination through the airways. Although the mass spectrometer picked up a significant increase in N\textsubscript{2} elimination with IV-PFC, the maximum amounts were not as high as expected.

VGE is a hallmark of DCS (4, 34). AGE is universally observed during cardiopulmonary bypass surgery, where the pressure and heating changes of the extracorporeal circuit cause micro gas bubble formation. Such microbubbles have been proposed as a cause of post cardiac surgery brain dysfunction (16). VGE occurs in a number of other surgical situations: neurosurgery, orthopedic surgery, trauma, and gynecologic surgery. Mass spectrometers have historically been utilized as monitors of ETN\textsubscript{2} for detection of VGE in the operating room (1). They have also been used to observe the N\textsubscript{2} elimination after human diving (33, 35). Experimental studies have demonstrated that the response time (time to maximum change following VGE) was significantly more rapid for ETN\textsubscript{2} and PAP than for ETCO\textsubscript{2} (37, 38, 39). Prior work has demonstrated that a threshold dose of VGE for ETCO\textsubscript{2} and ETN\textsubscript{2} to reach significant change was about 0.25 ml/kg. Such a bolus dose of air led to immediate ETCO\textsubscript{2} decreases greater than 0.2% and ETN\textsubscript{2} increases greater than 0.04% (37, 38). In our studies, VGE was induced by giving 0.8 ml/kg in the bolus group and 2.5 ml/kg in the air infusion group (10 minutes infusion). Significant increase of ETN\textsubscript{2}, decreases of the mean of ETCO\textsubscript{2}, and increased right to left shunting of blood leading to decreased PaO\textsubscript{2} were measured in all experimental groups within 15 seconds following VGE. The maximum changes were observed from 2.5 min to 15 min after VGE, corresponding with VGE-induced pulmonary circulation and gas exchange dysfunction.

Our findings suggested that IV-PFC was more effective in clearing continuously bubbled VGE as opposed to a single larger bolus of air. Bubbles with smaller diameter/volume should decrease overall cardiac output less than large bubbles. Also, smaller bubbles have a larger ratio of surface to radius to IV-PFC particles in blood. Therefore, it does
seem reasonable that if IV-PFC is effective in absorbing N\textsubscript{2} from bubbles, it could be most effective against the smallest bubbles. DCS may occur when dissolved N\textsubscript{2} or other gases come out of solution and form bubbles in tissue and the circulation. Data from this study support the concept that IV-PFC is effective in absorbing and eliminating air from the circulation. However, we utilized air bubbled into the circulation. And it could be that IV-PFC is more effective when it eliminates N\textsubscript{2} from the circulation prior to DCS bubble formation or when the bubbles are extremely small. Here we had no method of measuring intravascular bubble size, the coalition of bubbles or ultimate bubble behavior. Future work would have to investigate whether IV-PFC can be of use in already formed, coalesced bubbles or not.

VGE causes obstruction of the pulmonary vascular bed and, therefore, causes a decrease in cardiac output and slows clearance of N\textsubscript{2} and CO\textsubscript{2}. We found that CO\textsubscript{2} elimination worsened, PCO\textsubscript{2} increased, and that tissue O\textsubscript{2} uptake decreased while N\textsubscript{2} elimination was ongoing. ETCO\textsubscript{2} elimination returned to baseline and then exceeded baseline once N\textsubscript{2} seemed to have been cleared from the pulmonary circulation. Of interest is that, even though the respiratory gases appeared to come back to normal levels, the mean arterial pressure never fully normalized in any of the treated animals nor did the PaO\textsubscript{2}. Therefore, even though IV-PFC improved the tolerance of animals to experimental VGE, there must have been some residual pulmonary damage or hemodynamic abnormality leading to a decreased pulmonary ability to transport oxygen and/or decreased cardiac output in our model. In this model, cardiac output was not directly measured. It is known that VGE and DCS lead to secondary pulmonary changes as well as far reaching systemic inflammatory changes. It is, therefore, not surprising that, in our model, PFC did not bring hemodynamic parameters all the way back to baseline. One limitation of our study is that we did not directly measure cardiac output. It is quite possible that the animals did better with IV-PFC simply because they were able to maintain better cardiac output, through improved oxygen availability.

IV-PFC has a large solubility capacity for O\textsubscript{2} (7.6 mM; 19.3 vol%), and CO\textsubscript{2} (61.6 mM; 157 vol%) (15). The blood of IV-PFC-treated animals can carry N\textsubscript{2} at a minimum of a four-fold increase over controls (17). IV-PFC treatment will enhance the N\textsubscript{2} absorption of blood and increase body denitrogenation when breathing 100% O\textsubscript{2} (17, 32). Interestingly, we found that even though the animals had achieved complete denitrogenation prior to IV-PFC, the IV-PFC caused a trace amount of N\textsubscript{2} to be eliminated through the lungs. Some of this was due to N\textsubscript{2} present in the IV-PFC that was administered. However, in a small subsequent study, we found the effect was still there, even if the PFC was bubbled with 100% oxygen prior to administration. Perhaps the IV-PFC was able to take up some gut N\textsubscript{2} that would not otherwise be soluble. That means that even when mass spectrometry indicates total body denitrogenation has occurred, there is still residual N\textsubscript{2} in tissues. The addition of a compound to the blood stream that enhances N\textsubscript{2} solubility subsequently allows for removal of even more N\textsubscript{2}. In previous reports, IV-PFCs have demonstrated protective effects on VGE (19, 20) and AGE (25, 26, 28, 40, 41). In the current study, animals pre-treated with IV-PFC showed a significantly higher ETN\textsubscript{2} level than controls (Fig. 1A, Table 1). The time required for ETN\textsubscript{2} to return to baseline level was significantly shorter in IV-PFC-treated animals than those treated with saline. The area under the curves of the IV-PFC-treated animals versus saline-treated animals was about the same. This means N\textsubscript{2} elimination occurs with either treatment but is faster with IV-PFC treatment. It is not however, four times faster. There may be
other ways to further increase the speed of \( N_2 \) elimination, such as increasing cardiac output along with the IV-PFC. Also measuring the rate of \( N_2 \) elimination by mass spectrometry might be a useful physiologic method for assessing DCS treatment.

The hemodynamic changes after VGE include decrease of systolic and diastolic arterial pressures, increased PAP and CVP (6, 19, 20, 37). Blood gas analysis has demonstrated the decrease of \( PaO_2 \), \( PvO_2 \) and an increase of \( PaCO_2 \) following VGE (19, 20). IV-PFC administration with breathing pure \( O_2 \) before VGE has been demonstrated to prevent or reduce VGE hemodynamic disorders, such as enhanced oxygen delivery, resulting in less decrease in cardiac index and less increase in CVP and PAP, indicating that PFC attenuates some of the detrimental cardiopulmonary effects of venous embolism (19, 20). In the present study, animals with PFC pre-treatment showed a quicker recovery in MAP, CVP, \( PaO_2 \) and \( PaCO_2 \) toward the baseline than controls after air infusion (Tables 2, 3, 4). In the air-bolus group, PFC treatment showed a tendency to have higher MAP and \( PaO_2 \) than control animals.

These results suggest that the absorption of \( N_2 \) from the intravascular bubbles by PFC may shrink the bubble size and reduce the blockage in right heart, thereby right heart dysfunction. On the other hand, several physical characteristics of IV-PFC emulsions are capable of increasing tissue oxygenation. First, IV-PFC can dissolve large amounts of \( O_2 \), resulting in a higher oxygen partial pressure in the microcirculation and augmenting the driving pressure for the diffusion of dissolved oxygen into the tissue. Second, due to their small size (0.16~0.18 µm in diameter), IV-PFC emulsion particles tremendously increase the surface area available for gas exchange and can perfuse even the tiniest capillary (4-5 µm in diameter), while red blood cells may be excluded by size (~ 7.0 µm). Therefore, both enhancement of inert \( N_2 \) removal and increase of tissue oxygenation may be the protective mechanisms of IV-PFC on VGE. Clinical and experimental studies have demonstrated the beneficial effects of the administration of IV-PFC during surgery at hemoglobin concentrations of approximately 9 g/dl (14) by showing a reduction in ischemic damage caused by arterial or venous air emboli (18, 23, 24, 42).

DISSUB is a rare but extremely serious event. Most submarine accidents occur in waters near continental shelves. Therefore, the depths may be shallow enough that even if a hull breach occurred some submariners could survive the initial event. Internal submarine environmental concerns include thermal control, carbon dioxide accumulation, oxygen depletion, toxic gas or fume accumulation and gas pressure changes. In modern submarines, crews of 100-150 men are common. The present procedure for rescue of personnel involves a deep submersible vehicle mating with the DISSUB and transferring the survivors to a second large submarine. This second submarine could be a staging place for first response triage/intervention. At the present time re-compression to several ATA and slow decompression is the only viable method of treatment of DCS. With many victims the ability to triage and treat an entire crew or even a major percentage of the crew is logistically impossible. Primary escape from a DISSUB to surface is theoretically possible with some existing escape suits. Such environmental protection suits, however, will do nothing for treatment or prevention of DCS. They rapidly move the victim from depth to surface. IV-PFC appears to be a promising therapeutic option for treatment of DCS victims potentially without the requirement of re-compression. Since IV-PFC enhances \( N_2 \) washout it may also enhance \( N_2 \) wash in. Therefore, when a victim needs recompression, we must understand
what effects circulating IV-PFC would have on problems O₂ toxicity or driving N₂ back into tissues. Most work to date has focused upon VGE bubble loads or severe cardiopulmonary DCS. Since it appears that IV-PFC is at least partially effective in these circumstances, its use in victims with less severe symptoms may be even more effective. These conjectures will need to be tested by future research.

In summary, we demonstrate that intravenous pretreatment with second generation PFC emulsion (Oxygent 2.7g/kg) enhances N₂ washout. Restoration of O₂ delivery and hemodynamics in PFC-treated animals were temporally correlated with N₂ elimination. Our data fit with the prior work showing that IV-PFC holds great promise for treatment of DCS. The results of this study should fuel interest in surgical applications of IV-PFC as a prevention or treatment for both VGE and AGE. Further investigations are on-going in this laboratory to enhance the cardiac function and accelerate gas offloading which may act synergistically with PFC emulsions. Dose testing of PFC is also being investigated.

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