Short oxygen prebreathing and intravenous perfluorocarbon emulsion reduces morbidity and mortality in a swine saturation model of decompression sickness

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Dainer H, Nelson J, Brass K, Montcalm-Smith E, Mahon R. Short oxygen prebreathing and intravenous perfluorocarbon emulsion reduces morbidity and mortality in a swine saturation model of decompression sickness. *J Appl Physiol* 102: 1099–1104, 2007. First published November 9, 2006; doi:10.1152/japplphysiol.01539.2005.—Disabled submarine (DISSUB) survivors will achieve inert gas tissue saturation within 24 h. Direct ascent to the surface when saturated carries a high risk of decompression sickness (DCS) and death, yet may be necessary during rescue or escape. O_2 has demonstrated benefits in decreasing morbidity and mortality resulting from DCS by enhancing inert gas elimination. Perfluorocarbons (PFCs) also mitigate the effects of DCS by decreasing bubble formation and increasing O_2 delivery. Our hypothesis is that combining O_2 prebreathing (OPB) and PFC administration will reduce the incidence of DCS and death following saturation in an established 20-kg swine model. Yorkshire swine (20 ± 6.5 kg) were compressed to 5 atmospheres (ATA) in a dry chamber for 22 h before randomization into one of four groups: *1*) air and saline, *2*) OPB and saline, *3*) OPB with PFC given at depth, *4*) OPB with PFC given after surfacing. OPB animals received >90% O_2 for 9 min at depth. All animals were returned to the surface (1 ATA) without decompression stops. The incidence of severe DCS < 2 h after surfacing was 96%, 63%, 82%, and 29% for *groups 1, 2, 3*, and *4*, respectively. The incidence of death was 88%, 41%, 54%, and 5% for *groups 1, 2, 3*, and *4*, respectively. OPB combined with PFC administration after surfacing provided the greatest reduction in DCS morbidity and mortality in a saturation swine model. O_2-related seizure activity before reaching surface did not negatively affect outcome, but further safety studies are warranted.

disabled submarine; nitrogen

A DIVER'S BODY absorbs inert gases as a function of the depth and duration of dive, breathing mixture composition, individual diver characteristics, and environmental conditions (9, 19, 27). After remaining at depth for extended periods, a diver's body becomes saturated, with equal amounts of inert gas entering and leaving the body. Divers will achieve inert gas tissue saturation within 24 h at any given depth (5). Saturation diving operations permit divers to live and work at depth with a singular decompression obligation at the conclusion of the dive (25). However, decompression from saturation conditions is lengthy, an obligation in excess of 16 h when saturated at only 50 feet of seawater (fsw) (14). Direct ascent to the surface [1 atmosphere (ATA) of pressure] from saturation conditions carries a high risk of decompression sickness (DCS) and death (37) and yet may be necessary in certain situations such as commercial diving accidents, equipment malfunction, and disabled submarine (DISSUB) escape or rescue.

While there is no definitive etiology of DCS, bubbles are believed to form from inert gas exiting supersaturated tissues during rapid decompression (17). Bubbles may be directly responsible for DCS symptoms or may initiate a biochemical cascade with serious indirect effects, including leukocyte adhesion, platelet aggregation, and complement activation (13, 24, 28). In controlled environments, optimal decompression follows established schedules, allowing the time needed to slowly eliminate dissolved gas and minimize bubble formation (2, 35, 37). This is normally achieved by one of the following methods: *1*) controlled ascent within the water column; or *2*) after surfacing, rapid recompression in a hyperbaric chamber to the depth of saturation followed by slow measured decompression. However, operational scenarios, remote locations, or emergency situations may prohibit these options, and alternative means to prevent, attenuate, or treat DCS are required.

The known ability of oxygen (O_2) to reduce DCS morbidity and mortality has been attributed to enhanced elimination of dissolved inert gas and improved tissue oxygenation (3). Hyperbaric O_2 (HBO) has been shown to decrease leukocyte adhesion, decrease platelet aggregation, and affect complement activation (8, 33). Therefore, the use of O_2 at depth holds the promise for benefits beyond inert gas elimination that may further improve outcomes in situations of rapid ascent from saturation to 1 ATA.

Another means to mitigate the effects of DCS may be the use of perfluorocarbons (PFCs). PFCs are synthetic straight-chain and aromatic hydrophobic hydrocarbons first developed for industrial use but later adapted for biological use through combination with organic emulsification agents (10, 11, 20). PFCs dissolve more N_2 and O_2 than plasma and therefore may decrease inert gas bubble formation and enhance tissue O_2 delivery (4, 36). In this experiment, we report the findings of a short O_2 prebreathing (OPB), both with and without intravenous PFC emulsion, in a 20-kg swine model saturated at 132 fsw for 22 h before direct ascent to surface pressure.

MATERIALS AND METHODS

The experiments reported were conducted according to the principles set forth in the *Guide for the Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council, National Academy Press (1996). Before commencing, our Institutional Animal Care and Use Committee reviewed and approved all aspects of this protocol. The institutional animal care facility is fully AALAC accredited, and staff members are familiar with our swine saturation model (7).

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Subjects. All animals were neutered male Yorkshire swine (20 ± 6.5 kg). On receipt, each was examined by a veterinarian, fitted with an adjustable chest harness, and housed in individual runs with freely available water. Daily feedings consisted of 2% by body weight of laboratory animal feed (Harlan Teklad, Madison, WI). The animals remained in the care facility for a minimum of 72 h, adjusting to their new surroundings before any experiments.

Predive preparation. Animals were moved in plastic transport kennels to the surgical suite of the animal housing facility, placed in a Panepinto sling, and anesthetized by intramuscular injection of 20 mg/kg ketamine and 1 mg/kg xylazine. Under sterile conditions, eight-gauge catheters were placed into the left exterior jugular vein and tied in place. All animals received a dose of chloramphenicol (25 mg/kg) intravenously to prevent infection. Following anesthetic recovery, animals were returned to the housing facility and received no further interventions that day.

Dive protocol. Three animals were compressed during each saturation test dive. The morning after intravenous catheterization, animals were transported to the chamber building, weighed, and placed into clear Plexiglas observation crates modified to allow gas switching at depth. Intravenous lines were connected through hull penetrators to pumps outside the chamber, allowing the administration of medications at depth. Drinking water was available ad libitum while at depth.

The chamber was progressively compressed to 132 fsw (5 ATA) equivalent with air. Compression began at a rate of 0.15 ATA/min. If no animal showed distress or other evidence of middle ear barotraumas at 2 ATA, the descent rate was increased to 0.30 ATA/min to 4 ATA, and then further increased to a rate of 0.45 ATA/min until reaching test pressure. During the dive, animals were monitored and recorded via closed-circuit television cameras through observation ports. Atmospheric samples in all boxes and in the chamber were continuously monitored and recorded. Temperature was maintained between 26.7 and 29.4°C, humidity between 50 and 75%, and CO$_2$ concentration <0.3% (Abbeon certified hygrometer and temperature indicator, model HTAB169B, Abbeon Cal, Santa Barbara, CA).

The experiments in this study used Oxygent, an intravenous PFC emulsion (60% weight per volume perflubron-based emulsion, Alliance Pharmaceutical, San Diego, CA). Oxygent is a second-generation emulsion with good safety and tolerability (18, 26). After 22 h at depth, animals were randomized to receive one of four breathing mixtures and intravenous infusion treatments: *1)* air and normal saline (Air); *2)* O$_2$ and saline (OPB); *3)* O$_2$ and 6 ml/kg PFC administered at depth (B-PFC); and *4)* O$_2$ at depth with 6 ml/kg PFC given on surface after decompression (S-PFC). All saline infusions were equal in volume to PFC infusions, and each group was held to the same timeline of events (Fig. 1). The pressure required to pump fluid through the intravenous tubing at depth limited the speed of PFC

emulsion boluses to 20 min. PFC emulsion administered on the surface required 10 min to complete. Animals were switched from air to O$_2$ following PFC infusion; O$_2$ was >90% in <3 min of gas switch, and the animal remained on O$_2$ for the remainder of the hyperbaric exposure and decompression. Animals were monitored continuously for signs of seizure activity. After 9 min on >90% O$_2$ (except for the air group), animals remained on the specified randomized breathing mixture during decompression to 1 ATA at a steady rate of 30 fsw/min (0.91 ATA/min).

Observations at depth and through decompression. Approximately 30 min before the intravenous infusion at depth, a dedicated observer began independently monitoring each animal. Respiratory distress, agitation, seizure activity, and any abnormal behavior were recorded. Sustained seizure activity was treated with intravenous Diazepam (2.5 mg). Observations continued for all animals with recorded seizures, and these animals were included in the data analysis.

Surface observations. On return to surface pressure, the breathing mixture was switched from O$_2$ back to air. Observers entered the chamber to continue observations and recording for an additional 2 h. Animals remained inside separate Plexiglas containers throughout the surface observation period. Neurological DCS was defined as motor weakness (limb weakness, repeated inability to stand after being righted by the investigator), paralysis (complete limb dysfunction, areflexia, hypotonia), sensory compromise (lack of retraction from painful stimuli), or cranial nerve dysfunction. Cardiopulmonary DCS was defined as a visually observed respiratory rate of 60 breaths/min combined with respiratory distress, as evidenced by open-mouthed, labored breathing, central cyanosis, or the production of frothy white sputum. The onset of severe DCS (neurological or cardiopulmonary dysfunction) and all behavioral signs and symptoms were recorded to the nearest minute. Subjects with signs of severe DCS were given intravenous diazepam (2.5 mg) through the indwelling catheter as necessary to alleviate their distress. Skin DCS and behavioral features indicative of mild DCS (e.g., limb lifting) were recorded but not classified as positive cases for this study.

After the 2-h observation period, survivors were thoroughly examined by the principal investigator and euthanized with 5 ml bolus of Euthasol. The natural history of morbidity and mortality has been shown to plateau by 1 h in the Naval Medical Research Center 20-kg swine saturation model (7). The 2-h observation period was chosen to provide additional time for possible increased latency due to partially effective treatment while continuing to limit observations to what would be used for triage of survivors during a DISSUB scenario.

Statistics. Mean weights before the hyperbaric exposure were compared with ANOVA and post hoc with Dunnett's test against air controls. Development of DCS and death were displayed in the Kaplan-Meier method and then compared with the log rank test. Next,
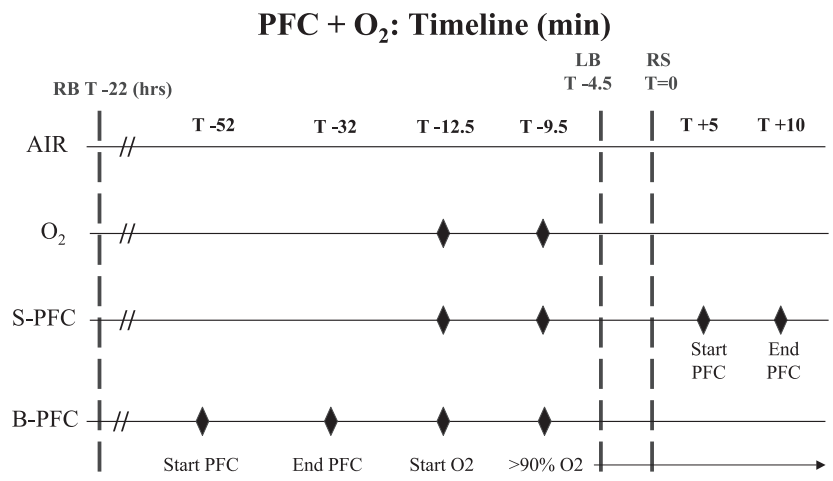
PFC + O$_2$: Timeline (min)

Fig. 1. Timeline for administration of perfluorocarbon (PFC) and O$_2$ for all groups. RB, reached bottom; LB, left bottom; RS, reached surface. Air, control group; OPB, O$_2$ prebreathing; S-PFC, O$_2$ prebreathing and PFC on surface; B-PFC, O$_2$ prebreathing and PFC given at depth.

paired groups were compared using a Cox proportional-hazards regression; 95% confidence intervals (CIs) were recorded and a $P < 0.05$ was considered significant. The Peto test was used to explore differences in DCS latency, both among all groups and between specific groups. The relationship of seizure activity and mortality outcome were compared using the Fisher's exact test. Minitab or Statistix (version 8.0) software packages were used for all calculations.

RESULTS

Baseline weight. Mean weights (Table 1) before dive exposure (16.2–26.6 kg) were not different among groups by ANOVA ($P = 0.396$).

DCS. In the Air group (Table 1), 25/26 animals (96%) suffered DCS <2 h after returning to surface pressure. In the B-PFC group, which received PFC at depth in addition to OPB, 18/22 (82%) suffered DCS <2 h after returning to surface pressure. In the O₂ (OPB-only) group and S-PFC groups, 17/27 (63%) and 6/21 (29%) experienced DCS within the 2-h observation period, respectively. Log-rank testing of the Kaplan-Meier method (Fig. 2) showed a significant treatment effect among the combined groups with a $P < 0.0001$. Paired groups were then analyzed by Cox proportional-hazards regression. The relative risk for DCS in the Air vs. OPB groups was 3.22 (95% CI 1.70, 6.1; $P < 0.0001$). The relative risk for DCS in the Air vs. S-PFC groups was 12.1 (95% CI 4.58, 32.0; $P < 0.0001$). We also determined that the relative risk for OPB vs. S-PFC (Table 2) was 3.23 (95% CI 1.27, 8.20; $P < 0.05$). The relative risk for Air vs. B-PFC did not reach statistical significance and was 1.59 (95% CI 0.87, 2.92; $P = 0.132$).

DCS latency. Initial review of the Kaplan-Meier plots (Fig. 2) appeared to show an increase in the average time of onset of DCS in the non-Air group animals. This was explored with the Peto test, which showed that among all groups there was a significant difference in the latency of symptoms ($P < 0.0001$). Further evaluations were then performed with the Peto test to compare differences in latency (results rounded to the nearest minute) between individual groups (Table 3). Censoring of animals that did not experience DCS prevented skewing of the mean times of latency for groups toward the maximum value of 120 min for animals that did not exhibit DCS symptoms. The B-PFC group did not differ significantly in latency compared with the air group ($P = 0.075$). However, both O₂ and S-PFC groups demonstrated a significant increase in time to onset of DCS compared with the Air group ($P < 0.001$ and $P < 0.0001$, respectively). Additionally, the S-PFC group was found to have significantly increased latency compared with the B-PFC group ($P < 0.0001$).
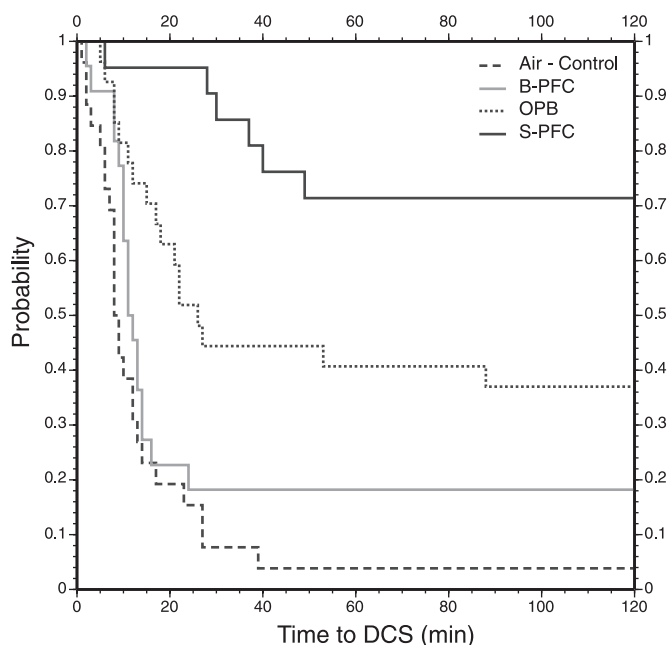

Fig. 2. Kaplan-Meier plots for decompression sickness (DCS) occurrence.

Death. Death (Table 1) was observed during the 2-h surface observation in 23/26 (88%) of the Air group, 12/22 (54%) of the B-PFC group, 11/27 (41%) of the OPB group, and 1/21 (5%) of the S-PFC group. Log-rank testing of the Kaplan-Meier method (Fig. 3) showed a significant difference among the four groups ($P < 0.0001$). Cox proportional-hazards regression between paired groups showed that the relative risk of death for animals receiving Air vs. B-PFC was 2.10 (95% CI 1.04, 4.24; $P < 0.05$); OPB was 3.28 (95% CI 1.59, 6.79; $P < 0.001$); and Air vs. S-PFC was 39.2 (95% CI 5.21, 294.0; $P < 0.001$). We also compared OPB vs. S-PFC (Table 2) and determined that the relative risk was 10.02 (95% CI 1.32, 77.69; $P < 0.05$).

Seizure activity. Seizure activity was observed in 5/26 (19%) of the Air group, 9/22 (41%) of the B-PFC group, 12/27 (44%) of the OPB group, and 2/21 (9.5%) of the S-PFC group. When reviewed with attention to the time of onset, seizure activity appeared to be separated into two groups: *1*) seizures occurring below the surface (BS) with lower mortality, and *2*) seizures occurring after reaching the surface (AS) with higher mortality. The Fisher exact tests (Table 4) showed a significant difference between BS and AS seizures ($P < 0.01$). The odds ratio of death following AS vs. BS seizure was 15.13 (95% CI 2.28, 100.3).

Seizure activity below the surface occurred in all three groups of animals with exposure to $>90\%$ O₂ at depth (B-PFC, OPB, and S-PFC). Animals in these groups were subdivided

Table 1. *Baseline weights, overall occurrence of DCS and death, and relative risk for Air group vs. other groups*

Group	n	Mean Wt, kg	SD	Severe DCS	RR	Death	RR
Air	26	20.2	2.6	25	N/A	23	N/A
B-PFC	22	20.3	1.6	18	1.59	12	2.10*
OPB	27	19.9	2.0	17	3.22‡	11	3.28†
S-PFC	21	19.4	1.7	6	12.1‡	1	39.2†

n, no. of animals. Air, control group; OPB, O₂ prebreathing; S-PFC, O₂ prebreathing and perfluorocarbon (PFC) on surface; B-PFC, O₂ prebreathing and PFC given at depth. N/A, not applicable; DCS, decompression sickness; RR, relative risk. *$P < 0.05$, †$P < 0.001$, ‡$P < 0.0001$.

Table 2. *Baseline weights, overall occurrence of DCS and death, and relative risk of OPB vs. S-PFC group*

Group	n	Mean Wt, kg	SD	Severe DCS	RR	Death	RR
OPB	27	19.9	2.0	17	N/A	11	N/A
S-PFC	21	19.4	1.7	6	3.23*	1	10.0*

n, no. of animals. *$P < 0.05$.

Table 3. *Latency in onset of DCS in minutes*

Group	n	Severe DCS	Censored	Average DCS Onset, min	P vs. Air
Air	26	25	1	11	N/A
B-PFC	22	18	4	11	0.075
OPB	27	17	10	22	<0.001
S-PFC	21	6	15	32	<0.0001

n, no. of animals.

into two groups on the basis of observation of seizure activity at depth: *1*) BS seizure and *2*) no seizure activity or AS only (NBS). With preliminary review, the presence or absence of BS seizure activity did not appear to alter mortality rate. This was assessed further with the Fisher exact tests (Table 4), which demonstrated no significant difference between the BS and NBS seizure groups ($P = 0.19$). The odds ratio of death following BS seizure vs. NBS seizure was also not significant with a value of 0.28 (95% CI 0.06, 1.43).

DISCUSSION

In this study we demonstrate that OPB with or without an intravenous PFC emulsion substantially decreases the rates of both DCS and death within a 2-h observation period after rapid decompression from saturation. Reduction in DCS was most marked when 10 min OPB at depth was combined with PFC administration after animals had returned to surface pressure.

OPB has previously been demonstrated to decrease DCS from hypobaric exposure and is a standard procedure in high-altitude operations to include high-altitude aircraft and extravehicular space missions (38). The mechanisms underlying the beneficial effects of short-term OPB may be related to both the direct physical effects of a partial washout of inert gases (31) and indirectly to the known pharmacological properties of HBO. HBO may decrease DCS by inhibition of neutrophil adherence (33), decreased platelet aggregation (8), and enhanc-

ing nitric oxide formation (32). These effects have the potential to maintain benefit beyond the period of HBO exposure.

The effectiveness of PFC both with and without hyperoxic exposure has been previously demonstrated in animal models (6, 23, 29). However, we were impressed by the marked reduction in DCS when OPB at depth and PFC administration at surface pressures was combined. Our results also demonstrate that the combination reduces rates of DCS and death below those achieved in a similar swine saturation study that used surface administration of PFC without an OPB (6). Known properties of PFCs make them a logical adjunctive therapy for DCS.

PFCs are synthetic straight-chain and aromatic hydrophobic hydrocarbons first developed for industrial use but later adapted for biological use through combination with organic emulsification agents (10, 11, 20). The solubility of O$_2$ in PFC is 20–25 times greater than that of water or blood plasma and approaches 60 vol% (whole blood carries 20 vol%). The N$_2$-carrying capacity of PFC emulsions approaches 50 vol% (by comparison, plasma N$_2$ solubility is 0.015 vol%) (6, 29). While biological molecules, such as hemoglobin, form chemical bonds with gases, PFCs do not bind with dissolved gases. Instead, gases dissolve into and come out of solution in a linear fashion on the basis of partial pressure. Both of these factors make PFC emulsions ideal candidate compounds for reducing DCS risk and severity through elimination of N$_2$ and improved O$_2$ delivery.

Recently a PFC emulsion-based contrast agent for ultrasound imaging was shown to enhance N$_2$ tissue elimination in an anesthetized swine (21). Importantly, in this model, the N$_2$ elimination rate was highest early after administration of the PFC emulsion. The resultant decreased inert gas load could decrease the amount of bubbles formed. Given that increased bubble counts are associated with increased pulmonary artery pressure (34), the early N$_2$ elimination with PFC would likely lead to decreased pulmonary artery pressure and a reduced incidence of cardiopulmonary DCS. This may explain the delay in onset of severe DCS observed in this study.

Mechanical vascular blockage of regional blood flow, associated bubble formation, clotting, and an inflammatory cascade compromise tissue perfusion in DCS (1). This decreased perfusion results in focal ischemia and the clinical deficits mani-
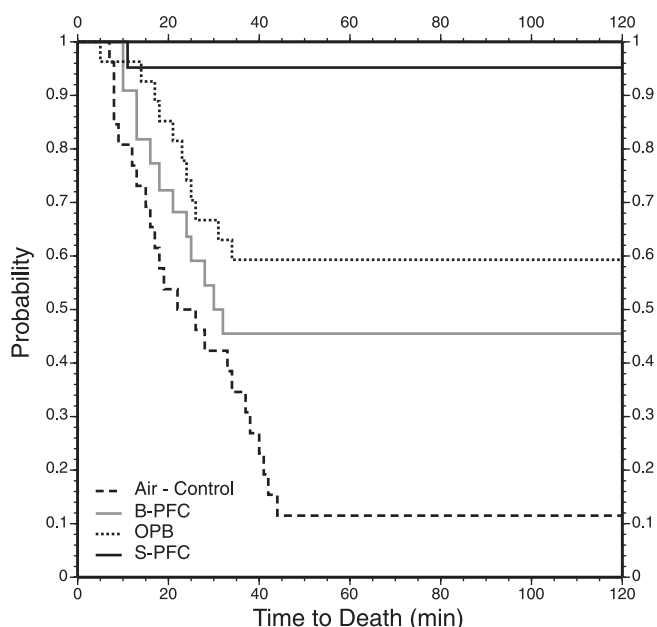

Fig. 3. Kaplan-Meier plots for death occurrence.

Table 4. *Fisher exact tests of seizure groups and of >90% O$_2$ groups*

	Seizure Groups		
	Death	Survival	Total
AS	11	4	15
BS	2	11	13
Total	13	15	28

	>90% O$_2$ Groups		
	Death	Survival	Total
BS	2	11	13
NBS	22	35	57
Total	24	46	70

AS, above-surface seizure activity; BS, below-surface seizure activity; NBS, no seizure activity or AS only; Death and Survival are within 2-h observation period.

fested in DCS. PFC compounds enhance O_2 delivery in circumstances where regional blood flow is compromised, such as severe hemodilution (7) and sickle cell vascular obstruction (15). In fact, the only FDA-approved PFC emulsion was for increased regional myocardial O_2 delivery in conjunction with coronary angioplasty (16). PFC's ability to increase O_2 delivery is based on its O_2 linear dissociation curve and small particle size. Emulsified PFC compounds can be <0.2 μm and have a limited diffusion distance unhampered by vascular obstruction (30).

Contrary to other studies (23), PFC administered before decompression (B-PFC) did not demonstrate a beneficial effect within the 2-h observation period. Because of technical limitations in overcoming the ambient pressure while animals were at saturation depth, the administration of PFC could not be completed while the animal was breathing O_2. It is likely that additional inert gas was accumulated in the PFC during this time, thus negating the benefit of its N_2-dissolving characteristics on ascent.

DISSUB relevance. While there has not been a U.S. Navy DISSUB event requiring rescue since the *USS Squalus* (1939), preparedness presents a significant concern and logistical challenge (22). In any DISSUB scenario catastrophic enough to prevent a submarine from surfacing under its own power, increased interior air pressure is nearly inevitable. Internal compartment flooding, high-pressure air leaks from ruptured supply lines, and the use of the Emergency Air Breathing System may all increase the ambient pressure (12). Survivors in remote areas may have to wait several days for rescue, becoming saturated with inert gas and at high risk for DCS (7, 37). Furthermore, a poisonous submarine atmosphere, rapid rescue vehicle turnover (disembarking a group of survivors to load the next group), and treatment of physical injuries necessitate rapid rescue. Each of these would interfere with the ability of emergency responders to follow current Navy standards requiring >30 h to safely decompress from saturation at 132 fsw (12, 25).

The dramatic reduction in both morbidity and mortality and the increased latency of DCS onset demonstrated by the combined use of a short OPB at depth and intravenous PFC emulsion at the surface suggests that incorporating these treatment modalities into operational protocols has the potential to safely accelerate DISSUB rescue.

In conclusion, OPB at depth is effective in reducing the morbidity and mortality of severe DCS associated with direct ascent from saturation conditions. Further reductions of both morbidity and mortality were observed when OPB was combined with PFC emulsion administered on surfacing, and these beneficial effects were independent of hyperoxic seizures. We conclude that OPB at depth in combination with PFC at the surface is especially effective in reducing the morbidity and mortality of severe DCS, even when O_2 exposure is sufficient to cause hyperoxic seizures. This strategy may be useful in DISSUB rescue planning and commercial diving operations.

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GRANTS

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