The effect of intravenous perfluorocarbon emulsions on whole-body oxygenation after severe decompression sickness

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Abstract

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Introduction: Decompression sickness (DCS) results from a decrease in ambient pressure leading to supersaturation of tissues with inert gas and bubble formation. Perfluorocarbons (PFCs) are able to dissolve vast amounts of non-polar gases. Intravenous (IV) PFC emulsions reduce both morbidity and mortality associated with DCS, but the mechanism of this protective effect has not yet been demonstrated.

Methods: Juvenile Dorper-cross sheep ($n = 31$) were anaesthetised and instrumented for physiological monitoring, IV fluid administration and blood sampling. Animals were compressed in air in a hyperbaric chamber to 608 kPa for 30 minutes and then rapidly decompressed. Upon decompression, animals were randomly assigned to receive 6 mL kg^{-1} of PFC or saline over 10 minutes beginning immediately after chamber exit. Arterial and mixed venous bloods were drawn at 5, 10, 15, 30, 60 and 90 minutes to examine pH, partial pressures of oxygen and carbon dioxide, oxygen saturation and electrolytes.

Results: Compared to saline, PFC administration increased arterial oxygen content (16.33 ± 0.28 vs. 14.68 ± 0.26 ml dL^{-1}, $P < 0.0001$), mixed venous oxygen content (12.56 ± 0.28 vs. 11.62 ± 0.26 ml dL^{-1}, $P = 0.0167$), oxygen delivery (14.83 ± 0.28 vs. 13.39 ± 0.26 ml min^{-1} kg^{-1}, $P = 0.0003$) and tissue oxygen consumption (3.30 ± 0.15 vs. 2.78 ± 0.13 ml min^{-1} kg^{-1}, $P = 0.0149$) but did not increase the extraction ratio (0.22 ± 0.012 vs. 0.21 ± 0.011, $P = 0.5343$).

Conclusions: It is likely that the improved oxygenation explains, at least in part, the previously-observed therapeutic effects of PFCs in DCS.

Introduction

Breathing compressed air increases the amount of nitrogen (N_2) dissolved in body fluids.[1–3] Factors such as ambient pressure and time at depth are the primary determinants of the amount of N_2 absorbed.[1–4] As ambient pressure decreases, dissolved gas tensions in tissue can exceed ambient pressure. This supersaturated state may lead to the formation and growth of gas bubbles, resulting in venous gas emboli (VGE) and possible arterial gas emboli (AGE).[4,5] It is believed that these bubbles within the vasculature and tissues are the root cause of decompression sickness (DCS).[4,5] There are likely multiple pathophysiological mechanisms at play in DCS, including impairment of microcirculation by inert gas bubbles, increased blood viscosity, endothelial damage and complement activation.[6–10] The physicochemical discontinuity of the gas-blood interface can also denature proteins promoting the release of fatty acids from cell membranes leading to the formation of fat emboli.[4,5] When bubbles obstruct capillaries or venules, ischaemia ensues followed by reperfusion-induced oxidative tissue damage.[11]

Perfluorocarbon emulsions (PFCs) are emulsions of fluorinated hydrocarbons within phospholipid micro-particle micelles.[12] PFCs have been developed in medicine as intravenous oxygen (O_2) therapeutics.[12] However, compared to how whole blood carries the majority of its O_2, the transport of O_2 by PFCs is fundamentally different. O_2 carried by PFCs is not bound, as with haemoglobin, rather it is dissolved in the PFC. Pure perfluorocarbons can dissolve up to 600 ml L^{-1} O_2,[13] whereas plasma can only dissolve 0.031 ml L^{-1} and whole blood at 150 gm L^{-1} haemoglobin can contain up to 210 ml L^{-1} O_2.[12] The O_2 dissolved in PFCs is all available to tissue, whereas that bound by haemoglobin is restricted (arterial pO_2 would need to drop below 40 mmHg for greater than 25% of bound O_2 to be released).[14]

Microcirculatory changes such as oedema, vasospasm, white cell activation and vessel plugging result in decreased erythrocyte delivery of O_2 to watershed tissue beds, yet plasma flow may continue without red cells.[15] PFCs, due to their extremely small particle size (~0.1–0.4 μm), can be delivered in this trickle-flow of plasma.[12,16,17] Plasma O_2 delivery by PFCs is enough to keep tissue alive, as seen with Fluosol DA-20%, a PFC which reduced myocardial infarction and garnered FDA approval.[18,19]

PFCs are also effective in treating DCS, AGE and VGE.[20–26] Using a swine saturation dive model with direct ascent to the surface, it was found that administration of intravenous (IV) PFCs and 100% O_2 post-decompression decreased mortality, the incidence of DCS and the number of neurological events compared to animals administered just 100% O_2 or room air.[21] Also, PFC and 1 hour of 100% O_2 given at the onset of DCS significantly decreased mortality observed 24 hours post-dive compared to animals treated with saline and 100% O_2 in a swine model of rapid decompression.[27]

Similarly it was found that IV PFCs improve outcomes after massive VGE, cerebral AGE, and coronary AGE.[23,24,28] IV PFCs have also been shown to increase N_2 washout after VGE.[26] PFC administration is of benefit in the treatment of decompression illnesses, but the mechanism of this benefit has not been elucidated. Is it the PFCs' ability to increase N_2 washout and remove bubbles obstructing circulation, a product of improving O_2 supply and metabolic state of tissue, or some combination of these? The research described here was designed to investigate the effect of IV PFCs administered acutely after surfacing on whole-body oxygenation in an ovine model of severe DCS.

Materials and methods

All experiments were performed in accordance with the National Institutes of Health *Guide for the care and use of laboratory animals*, and were approved by the Department of Defense and the Virginia Commonwealth University Institutional Animal Care and Use Committees. Juvenile Dorper-cross sheep of either sex (Robinson Services, Inc., Mocksville, NC) weighing 18.5 ± 2.6 kg were housed in United States Department of Agriculture and Association for Assessment and Accreditation of Laboratory Animal Care International approved facilities in social flocks with free access to food and water on a 12-hour light/dark cycle. Sheep were allowed a minimum of three days for acclimatisation and veterinary inspection prior to use in any experiment.

PREPARATION AND INSTRUMENTATION

Prior to the experiment, sheep were muzzled for a period of 48 hours in order to prevent access to food but to provide free access to water while remaining with their flock to limit animal stress. Sheep were sedated with ketamine/xylazine (20.0/2.0 mg kg⁻¹ IM) and placed supine on the surgical table. Animals were intubated with a 9.0 mm internal diameter cuffed endotracheal tube (Hudson RCI, Temecula, CA) and ventilated with 50/50 N_2/O_2 using a Siemens 900C ventilator (Siemens Corp., New York, NY) with a tidal volume of approximately 10 ml kg⁻¹ and a rate of approximately 15 breaths per minute adjusted to maintain arterial pCO_2 at 40 ± 5 mmHg. An orogastric tube fashioned from TYGON® R-3603 tubing (Satin-Gobain Performance Plastics Corp., Akron, OH) was advanced into the rumen to allow for fluid drainage and to allow gas accumulated in the gut during the air dive to vent upon decompression. A MAC® 2-port introducer sheath (Arrow International Inc., Reading, PA) was placed in the right external jugular vein to allow for the administration of fluids and anaesthetic cocktail.

Once IV access was secured, administration of 'triple drip' anaesthetic cocktail (ketamine/xylazine/guaifenesin 2.0/0.1/50.0 mg ml⁻¹ in 5% dextrose) was begun immediately at 1.0–2.0 ml kg⁻¹ hr⁻¹ titrated to maintain a surgical plane of anaesthesia using a Harvard Apparatus PHD 2000 syringe pump (Harvard Apparatus, Holliston, MA). The left

femoral artery was cannulated with an 18-gauge femoral arterial catheter (Arrow International Inc., Reading, PA) for monitoring of arterial pressure (AP) and arterial blood sampling. The right femoral vein was cannulated with a 4-French double-lumen catheter (Arrow International Inc., Reading, PA) for the anaesthetic administration while in the hyperbaric chamber and for study drug administration after exiting the chamber. The left femoral vein was cannulated for the placement of a 7.5 Fr CCOmbo® continuous cardiac output (CCO) pulmonary artery catheter (Edwards Lifesciences, Irvine, CA) to allow for CCO, central venous (CVP) and pulmonary arterial pressure (PAP) monitoring and mixed venous blood sampling. Respiratory gases were monitored continuously using an MGA 1100 respiratory mass spectrometer (Perkin-Elmer, Norwalk, CT).

Following surgical manipulations, all animals were allowed to stabilise for 30 minutes. After stabilisation, animals were weaned off the ventilator until capable of spontaneously breathing prior to being placed inside the hyperbaric chamber. Normal saline was administered intravenously at a rate of approximately 1 ml min⁻¹ throughout the surgical procedure in order to ensure proper hydration of all animals.

SHEEP DRY-DIVE PROCEDURES

Once weaned from the ventilator, monitoring equipment was disconnected and sheep (*n* = 31) were placed into a Reimers Systems model #17-48-100 Research Hyperbaric Chamber (Reimers Systems, Inc., Springfield, VA). During the dry dive all animals breathed room air and general anaesthesia was maintained using a continuous infusion of 'triple drip' as described above. Sheep were subjected to the following dive profile. The chamber was pressurised at a rate of 101.3 kPa min⁻¹ to a pressure of 203 kPa. From 203 kPa the chamber was pressurised at a rate of 203 kPa min⁻¹ to a pressure of 608 kPa. The pressure of 608 kPa was maintained for 27 minutes, after which sheep were immediately decompressed to ambient pressure at a rate of 203 kPa min⁻¹.

POST-DECOMPRESSION MONITORING

Upon complete decompression (time = 0) all animals were quickly removed from the hyperbaric chamber and monitoring equipment was reconnected, as was the ventilator with settings and breathing gas unchanged from pre-compression/decompression settings. Animals were randomised using a computer-generated block randomisation sequence such that for each eight animals, four were assigned to receive IV infusion of 6.0 ml kg⁻¹ PFC (*n* = 15, 60% w/v *tert*-butyl perfluorocyclohexane) and four were assigned to receive saline control (*n* = 16) as an infusion over 10 minutes. All animals were monitored for 90 minutes after decompression, during which time both arterial and mixed venous blood samples were drawn and analysed using a Radiometer OSM 3 Hemoximeter and a Radiometer ABL

700 blood gas analyser (Radiometer America, Westlake, OH) at 5, 10, 15, 30, 60 and 90 minutes after decompression. Data from all instruments were recorded directly to hard drive using the BioPac system with Acqknowledge 3.90 software (BioPac Systems Inc., Goleta, CA). After 90 minutes all animals were euthanased.

Later offline analyses were performed to determine arterial and mixed venous blood oxygen content (C_aO_2, $C_{\bar{v}}O_2$), oxygen delivery ($\dot{D}O_2$), tissue oxygen consumption ($\dot{V}O_2$), and oxygen extraction ratio (ER). The formulae used for the calculations are listed in Table 1.

Statistical methods

Unless otherwise stated, all data were analysed using repeated measures analysis of variance (ANOVA) with cardiac index and PFC administration included as model effects. If the ANOVA was found to be significant, *post hoc* least squares means Student's t-tests were applied to determine if the PFC treatment and saline control groups were significantly different. Data are presented as least squares (LS) means ± SEM. Differences were considered statistically significant with *P* values of less than 0.05. All statistical calculations were performed using the JMP 8 from SAS Institute (Cary, NC).

Results

Prior to diving the sheep, all physiological parameters under investigation were compared to ensure that differences between PFC and saline-treated sheep observed post-dive were not the result of pre-dive surgical manipulations. One-way ANOVA performed on baseline data obtained during the stabilisation period post-surgery and pre-dive indicated that there were no significant differences between the PFC-treated group and the saline controls on any of the variables of interest (PFC vs. saline – C_aO_2, $C_{\bar{v}}O_2$, $\dot{D}O_2$, $\dot{V}O_2$, ER and cardiac index (indexed to body weight, CI)).

Since previous studies have indicated that split-hoofed species can develop pulmonary hypertension severe enough to interfere with CI after PFC administration, we examined the effect of PFC administration on CI in this model.[29] Figure 1A shows CI changes in saline- and PFC-treated sheep during the 90-minute period post-dive (repeated measures ANOVA, *P* < 0.0001). In PFC-treated animals, CI was lower compared to saline and trended towards increasing over time while remaining stable in saline-treated sheep. When LS means were compared, CI was found to be significantly lower by 19.4% in the PFC-treated group vs the saline control group (Figure 1B). Because of the significant effect PFC administration had on CI, CI was included as a model effect in all further analyses.

Figure 2A illustrates the changes in C_aO_2 over the time course of the experiment following the return to surface (repeated measures ANOVA, *P* < 0.0001). C_aO_2 increased in both PFC- and saline-treated animals. Likewise, C_aO_2 is higher in the PFC-treated group vs. the saline control. Figure 2B shows the results of the LS means post-hoc comparison. C_aO_2 was significantly increased over saline control by 10.5%.

The effect of PFC treatment on $C_{\bar{v}}O_2$ over time post-chamber was also investigated and found to be significant as described by repeated measures ANOVA (Figure 3, *P* = 0.0159). Both PFC- and saline-treated sheep displayed a non-significant trend towards increasing over time. The

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Table 1
Equations used to determine arterial and mixed venous blood oxygen (O_2) content (ml dl^{-1}), O_2 delivery (L min^{-1} kg body weight^{-1}), tissue O_2 consumption (L min^{-1} kg body weight^{-1}), and oxygen extraction ratio

Arterial O_2 content:
$$C_aO_2 = (1.34 \times Hb \times S_aO_2) + [(0.0031 \times P_aO_2 \times a) + (0.01997 \times P_aO_2 \times \beta)] \quad (1)$$

Mixed venous O_2 content:
$$C_{\bar{v}}O_2 = (1.34 \times Hb \times S_{\bar{v}}O_2) + [(0.0031 \times P_{\bar{v}}O_2 \times \alpha) + (0.01997 \times P_{\bar{v}}O_2 \times \beta)] \quad (2)$$

O_2 delivery:
$$\dot{D}O_2 = \frac{[CO \times (C_aO_2 \times 10)]}{weight} \quad (3)$$

O_2 consumption:
$$\dot{V}O_2 = \frac{CO \times [(C_aO_2 - C_{\bar{v}}O_2) \times 10]}{weight} \quad (4)$$

Extraction ratio:
$$ER = \frac{\dot{V}O_2}{\dot{D}O_2} \quad (5)$$

Where Hb = haemoglobin concentration in mg dL^{-1}; S_aO_2 = arterial O_2 saturation fraction; P_aO_2 = arterial O_2 tension in mmHg; CO = cardiac output in L min^{-1}; 0.0031 = O_2 solubility coefficient in plasma in ml dL^{-1}; 0.01997 = O_2 solubility coefficient in 60% w/v tert-butyl perfluorocyclohexane emulsion in ml dL^{-1}; a = blood fraction of circulation volume; ß = PFC fraction of circulating volume and 1.34 = O_2-haemoglobin binding coefficient in ml g^{-1}.

Figure 1
The effect of perfluorocarbon administration on cardiac index (CI)
A: Cardiac index plotted against time; solid line represents PFC, dashed line represents saline; chamber exit at time = 0
B: Least squares means of saline- and PFC-treated groups; PFC significantly decreased CI vs saline control (* $P < 0.0001$)
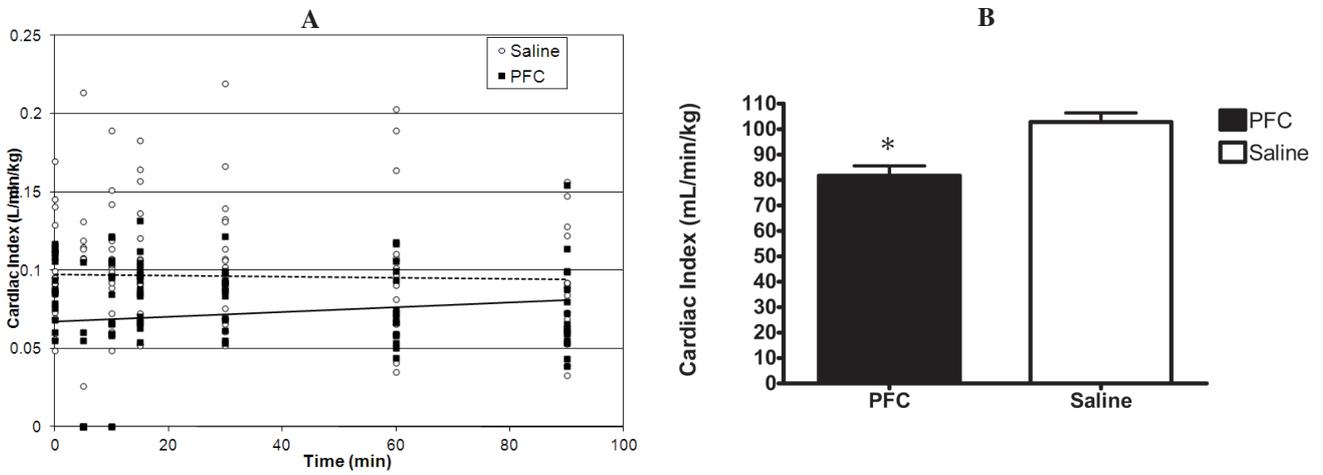

Figure 2
The effect of perfluorocarbon administration on arterial oxygen content (C_aO_2)
A: Arterial oxygen content vs time; solid line represents PFC, dashed line represents saline; chamber exit at time = 0
B: Least squares means of saline- and PFC-treated groups; PFC significantly increased C_aO_2 vs saline control (* $P < 0.0001$)
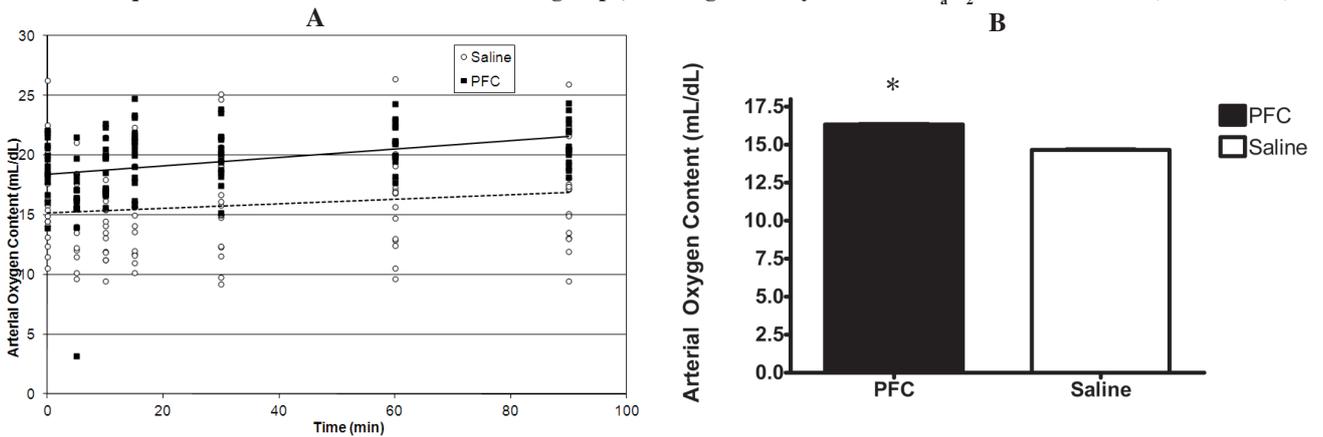

Figure 3
The effect of perfluorocarbon administration on mixed venous oxygen content
A: Mixed venous oxygen content vs time; solid line represents PFC, dashed line represents saline; chamber exit at time = 0
B Least squares means of saline- and PFC-treated groups; PFC significantly increased $C_{\bar{v}}O_2$ vs saline control (* $P = 0.0159$)
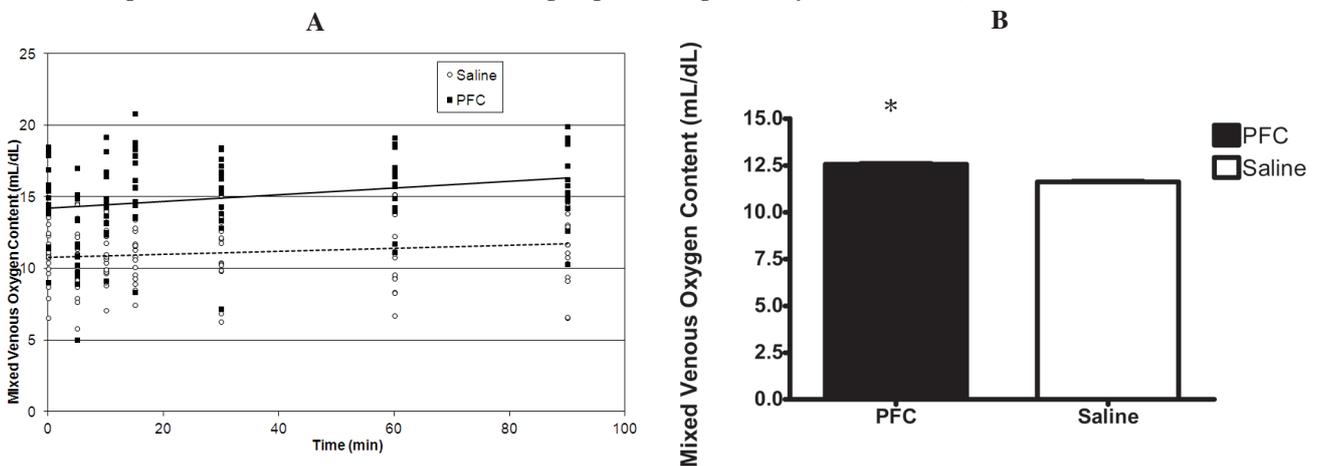
Figure 4
The effect of perfluorocarbon administration on oxygen delivery ($\dot{D}O_2$)
A: Oxygen delivery vs time; solid line represents PFC, dashed line represents saline; chamber exit at time = 0
B: Least squares means of the saline- and PFC-treated groups; PFC significantly increased $\dot{D}O_2$ vs saline control (* P = 0.0002)
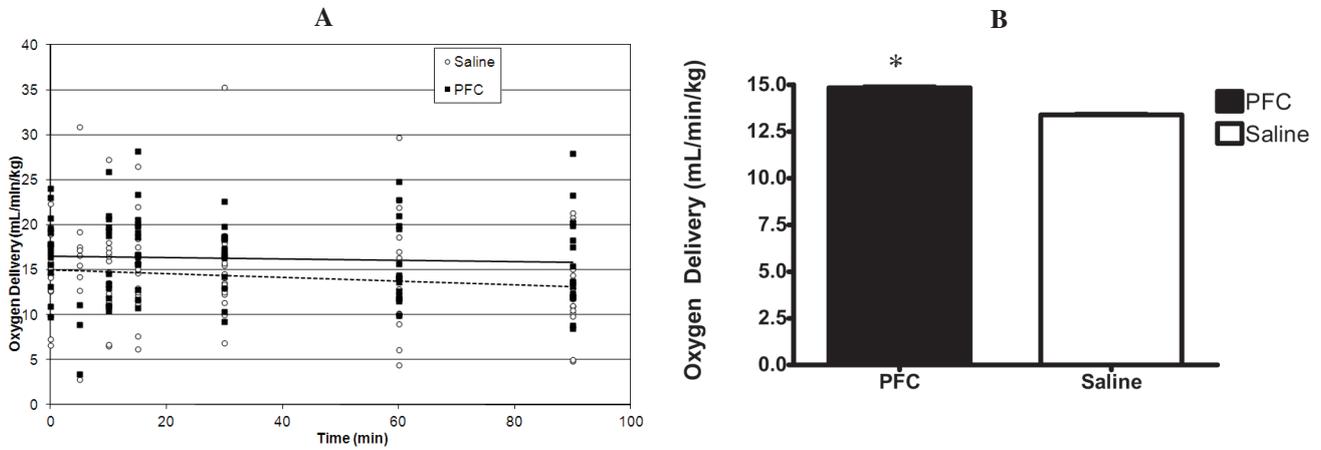

Figure 5
The effect of perfluorocarbon administration on oxygen consumption ($\dot{V}O_2$)
A: Oxygen consumption vs time; solid line represents PFC, dashed line represents saline; chamber exit at time = 0
B: Least squares means of saline- and PFC-treated groups; PFC significantly increased $\dot{V}O_2$ vs the saline control (* P = 0.0122)
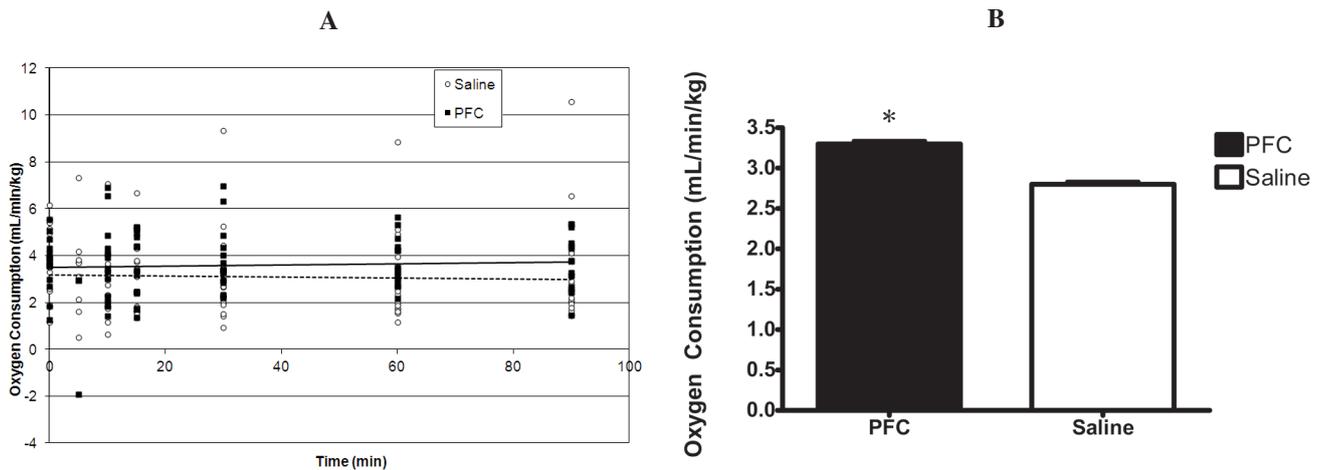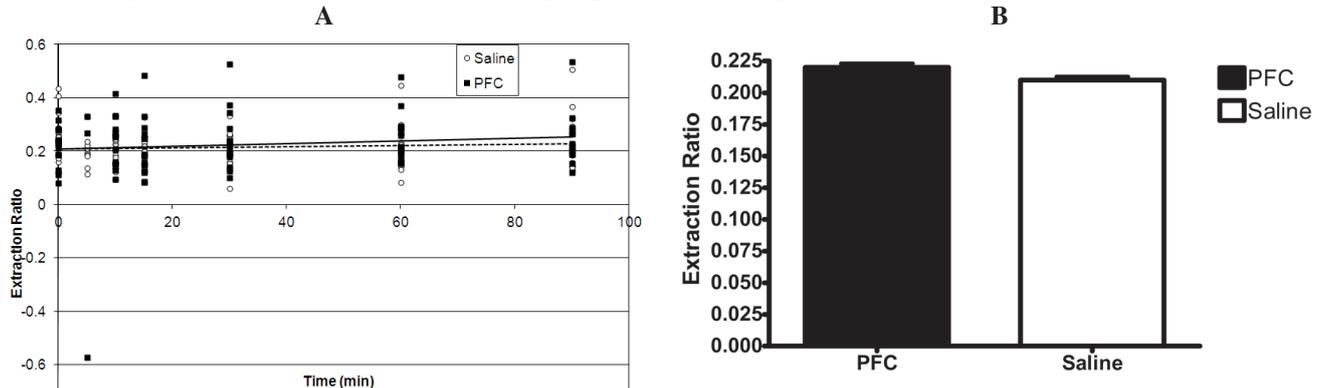

Figure 6
The effect of perfluorocarbon administration on extraction ratio (ER)
A: Extraction ratio vs time; solid line represents PFC, dashed line represents saline; chamber exit at time = 0
B: Least squares means of saline- and PFC-treated groups; PFC had no significant effect on ER vs saline control (P = 0.5190).

results of the LS means comparison are shown in Figure 3B. $C_{\bar{v}}O_2$ was found to be significantly higher in PFC animals vs saline control by 6.7%.

Figures 4A and 5A demonstrate the changes in $\dot{D}O_2$ and $\dot{V}O_2$ respectively, following decompression (repeated measures ANOVA, $P < 0.0001$ for both). Both $\dot{D}O_2$ and $\dot{V}O_2$ are higher in the PFC sheep compared to the saline-treated animals. Also, $\dot{D}O_2$ and $\dot{V}O_2$ remain stable in animals treated with PFC over the 90 min period while appearing to decrease in sheep administered saline. Figures 4B and 5B show the results of the LS means comparisons of $\dot{D}O_2$ and $\dot{V}O_2$ respectively. It can be seen that $\dot{D}O_2$ is 10.3 % higher and $\dot{V}O_2$ is elevated some 22.1% over saline controls.

Additionally, the effect of PFC treatment on ER was investigated (Figure 6A, repeated measures ANOVA, $P < 0.0001$). The data reveal that ER for PFC-treated sheep was not different to animals given saline during the 90 min observation period. However, ER for both PFC- and saline-treated sheep trended toward increasing throughout the post-chamber examination window. Figure 6B shows the results of the LS means comparison. ER was not significantly increased in the PFC-treated group vs the saline control.

Finally, in order to present a more complete picture of the animals' condition following decompression, several haemodynamic parameters were analysed (Table 2). Arterial pressure (systolic, diastolic, mean), pulmonary arterial pressure, and arterial PCO_2 in both PFC- and saline-treated sheep were all found to be decreasing over time post-chamber (repeated measures ANOVA, $P < 0.0001$ for all except PAP, $P = 0.0007$). LS means comparison showed that all variables were significantly higher in PFC- vs saline-treated animals. Central venous pressure and heart rate in both PFC and saline sheep were stable over time following decompression (repeated measures ANOVA: CVP $P = 0.2696$; HR

$P = 0.2371$ HR). LS means comparison revealed that both variables were significantly lower in PFC- vs saline-treated animals. Arterial pH showed a non-significant trend towards increasing in both PFC and saline sheep over time after the dry dive ($P = 0.0554$), and was significantly lower in PFC- vs saline-treated animals when LS means were compared. Arterial pO_2 was found to be increasing over time in both groups ($P = 0.0188$), but LS means revealed no significant difference between PFC- and saline-treated animals.

Taken together, the data presented show that sheep subjected to decompression stress and treated with PFC immediately following return to surface displayed significantly greater arterial oxygen content, oxygen delivery, and oxygen consumption compared to animals exposed to decompression stress and given saline.

Further analysis using the repeated measures ANOVA model showed both P_aO_2 and S_aO_2 were significantly lower in the PFC-treated vs saline-treated group (200.03 ± 10.77 vs 238.72 ± 9.90 mmHg, $P = 0.0109$ and 93.52 ± 0.89 vs. 97.39 ± 0.81 %, $P = 0.0021$ respectively). Haemoglobin (Hb) was elevated in the PFC-treated group vs saline control after compression/decompression (12.22 ± 0.17 vs. 10.72 ± 0.15 mg dL^{-1}, $P < 0.0001$) but not at baseline (one-way ANOVA 11.19 ± 0.38 vs 10.61 ± 0.37 mg dL^{-1}, $P = 0.2931$).

Discussion

As has been seen before in other split-hoofed animal models, IV administration of PFCs resulted in decreased CI.[29] Previous work conducted in pigs reported pulmonary hypertension to be the cause of the observed decrease in CI and similar observations were made here.[29] When analysed using repeated measures ANOVA, pulmonary arterial pressure was found to be nearly doubled in the PFC-treated animals vs the saline control (see Table 2). This suggests that the problem of pulmonary hypertension leading to decreased CI will likely be present in all split-hoofed species.

It is clear from this study that IV PFC administration results in increased C_aO_2. C_aO_2 was elevated nearly 11% over control with PFC. Even if the oxygen carried directly by the PFC is removed from the calculations, C_aO_2 was still significantly higher in the PFC-treated group ($P = 0.0019$). PFC appears to do more than simply carry more O_2, but exactly what PFC does in addition to its own O_2-carrying ability is unclear. It is possible that the presence of the PFC is inducing the release of erythrocytes from the spleen or other storage, accounting for the higher Hb, and contributing to the higher C_aO_2 in the PFC-treated group. It is also possible that the presence of free gas bubbles in the microcirculation results in some vessel injury, followed by inflammation and leakage of plasma out of the intravascular space resulting in an apparent haemoconcentration. These possibilities warrant further investigation in order to elucidate their exact cause, and could be tested by examining spun haematocrit values,

Table 2
The effect of perfluorocarbon (PFC) administration on haemodynamic parameters and arterial gas partial pressures

Parameter	Least squares means (SEM)		*P* value
	Saline	PFC	
Arterial Pressure (mmHg)			
systolic	93.6 (3.20)	105.3 (3.40)	0.0152
diastolic	76.1 (2.80)	84.9 (2.97)	0.0368
mean	83.6 (2.93)	93.6 (3.10)	0.0245
Central Venous Pressure (mmHg)			
	12.1 (1.17)	7.9 (2.24)	0.0158
Pulmonary Arterial Pressure (mmHg)			
	16.4 (1.11)	25.5 (1.18)	<0.0001
Heart Rate (bpm)	121.7 (2.80)	113.2 (2.97)	0.0452
pH	7.43 (0.008)	7.39 (0.007)	<0.0001
P_aO_2	217.3 (20.08)	209.9 (21.31)	0.8035
P_aCO_2	39.3 (0.65)	43.6 (0.69)	<0.0001

plasma protein content and/or by conducting tagged RBC concentration studies.

The observation that PFC administration results in increases in both $\dot{D}O_2$ and $\dot{V}O_2$ of 10% and 22%, respectively, demonstrates that the PFC was able to not only increase the amount of O_2 present in the blood, but to improve tissue access to that O_2. This suggests that the mechanism whereby IV PFC improves tissue oxygenation is not simply a result of its ability to carry greater quantities of O_2, but that it facilitates O_2 delivery to cells. This may take the form of the PFC extravasating in capillary beds, taking dissolved oxygen with it. Alternatively, the PFC emulsion particles, being approximately 1/100th–1/1000th the size of an erythrocyte, may be able to pass through blood vessels where red cell flow has been blocked by bubbles, but a trickle flow of plasma remains.[21,25] In this case the small amount of O_2 carried in the PFC may be sufficient to keep viable tissues that otherwise might succumb to hypoxic injury.

More interestingly, PFC particles may act as a bridge, facilitating the movement of O_2 from erythrocytes into tissues. This possibility has very intriguing implications. As shown above, the amount of O_2 actually dissolved in PFC is relatively small. Haemoglobin binding O_2 remains the dominant mechanism for O_2 transport. Once in capillary beds, the greatest impediment to the offloading of O_2 from haemoglobin is the plasma.[30] O_2 is very insoluble in plasma, and much more soluble in PFC. Therefore, PFC could act as a transport vessel for O_2, ferrying it from erythrocytes to tissues, a mechanism somewhat akin to facilitated diffusion across cell membranes. These possible mechanisms should be explored further in future studies.

Conclusion

These results demonstrate that improved tissue oxygenation at a whole-body level is likely responsible for at least a portion of the beneficial effects offered by the IV administration of PFC emulsions after decompression sickness.

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Conflict of interest

Travis Parsons is an investor owning 90 shares of stock in Oxygen Biotherapeutics, Inc., less than 0.0001% of public shares available.

Bruce Spiess is an investor owning 10,000 shares of stock in Oxygen Biotherapeutics Inc., less than 0.01% of public shares available.

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