TISSUE OXYGENATION WITH GRADED DISSOLVED OXYGEN DELIVERY DURING CARDIOPULMONARY BYPASS

Background: Intravascular perfluorochemical emulsions together with a high oxygen tension may increase the delivery of dissolved oxygen to useful levels. The hypothesis of this study is that increasing the dissolved oxygen content of blood with incremental doses of a perfluorochemical emulsion improves tissue oxygenation during cardiopulmonary bypass in a dose-related fashion. *Methods and Results:* Oxygen utilization was studied in a profoundly anemic canine model of hypothermic cardiopulmonary bypass. Forty-two dogs underwent normovolemic hemodilution to a hematocrit of 15.8% ± 0.6% (mean ± standard error of the mean). Cardiopulmonary bypass was begun and resulted in a hematocrit of 9.4% ± 0.6%. A standard priming solution was used in the control group ($n = 12$), and the test groups received 1.35 gm perfluorochemical · kg^{-1} ($n = 10$ dogs), 2.7 gm perfluorochemical · kg^{-1} ($n = 10$ dogs), or 5.4 gm perfluorochemical · kg^{-1} ($n = 10$ dogs) through the venous return cannula. Each animal underwent a series of randomized pump flows (0.25, 0.5, 1.0, 1.5, 2.0, and 3.0 L · min^{-1} · m^{-2}) at 32° C. After the randomized flows were completed at 32° C, the temperature was raised to 38° C and cardiopulmonary bypass was discontinued. Mortality from cardiac failure on separation from cardiopulmonary bypass was 42% in the control group and 20% in perfluorochemical-treated groups. The mean perfluorochemical dose was higher in survivors than in nonsurvivors (2.9 ± 0.4 versus 1.3 ± 0.5 gm perfluorochemical · kg^{-1}; $p < 0.05$). No differences in oxygen consumption or transbody lactate gradient were found between groups during cardiopulmonary bypass. Analysis of mixed venous oxygen tension (a surrogate measure for tissue oxygenation) as a function of cardiopulmonary bypass flow normalized to body surface area showed that the control group had significantly lower mixed venous oxygen tension ($p < 0.05$) than the perfluorochemical emulsion–treated groups. Furthermore, the differences were related to the perfluorochemical emulsion dose. These differences in mixed venous oxygen tension continued after termination of cardiopulmonary bypass. The coronary sinus oxygen tension and cardiac arterial-venous oxygen content differences during and after cardiopulmonary bypass were similar among the control and perfluorochemical emulsion–treated animals. Dissolved oxygen consumption during and after cardiopulmonary bypass was calculated. Dissolved oxygen consumption increased in the perfluorochemical-treated animals in a perfluorochemical dose–related manner and was significantly higher in perfluoro-

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chemical-treated animals than in the control animals ($p < 0.05$). *Conclusions:* Graded increases in mixed venous oxygen tension during cardiopulmonary bypass were observed in response to graded increases in the dissolved oxygen delivery. These data suggest that enhancing oxygenation with perfluorochemical-dissolved oxygen is an effective temporary substitute for the use of hemoglobin-bound oxygen during cardiopulmonary bypass. Perfluorochemical-dissolved oxygen may be particularly beneficial in the setting of multiple hypoxic stresses. (J THORAC CARDIOVASC SURG 1995;110:774-85)

Oxygen delivery during total cardiopulmonary bypass (CPB) is dependent on perfusate oxygen content and pump flow. Perfusate oxygen content is primarily dependent on the concentration of hemoglobin with only a small contribution from physically dissolved oxygen in the plasma.

Perfluorochemicals (PFCs) have a high intrinsic solubility for oxygen. The recent development of novel high-concentration (e.g., 90% to 100% wt/vol) PFC emulsions in theory can substantially increase the volume of physically dissolved oxygen that is carried in the blood.[1, 2] Dissolved oxygen in plasma obeys Henry's law, which states that the mass of gas that dissolves in a liquid is directly proportional to the partial pressure of that gas. The relationship of dissolved oxygen content in blood and Po_2 is therefore linear, in contrast to the sigmoidal relationship for hemoglobin oxygen content and Po_2. In addition, unlike for hemoglobin-bound oxygen, the availability of dissolved oxygen for diffusive transport to tissues is unaffected by changes in pH or temperature.

The purpose of this study was to measure tissue oxygenation, as represented by the Po_2 in mixed venous blood, during hypothermic CPB with graded concentrations of dissolved oxygen in the perfusate. The hypothesis tested was that increasing the dissolved oxygen content of blood with incremental doses of a PFC emulsion improves tissue oxygenation during CPB in a dose-related fashion.

Methods

Oxygen metabolism was studied in a profoundly anemic (i.e., hematocrit of approximately 10% during bypass) canine model of hypothermic CPB. The experimental protocol we describe was approved by the University of Alabama at Birmingham Animal Use Review Committee and met the standards outlined by the American Physiological Society and the National Institutes of Health in the "Guide for the Care and Use of Laboratory Animals" (NIH publication No. 85-23, revised 1985).

Experimental variables. The biochemical variables measured in this study included (1) systemic arterial, mixed venous, and coronary sinus blood gas values, oxygen content, and hemoglobin saturations; and (2) systemic arterial, mixed venous, and coronary sinus lactate concentration. Oxygen content was measured with a Lex-O_2-Con device (Hospex Fiberoptics, Inc., Chestnut Hill, Mass.). Blood gas and hemoglobin saturation determinations were made with a pH–blood gas analyzer (model 238, Ciba-Corning Diagnostics Ltd., Halstead, Essex, England) and a co-oximeter (IL model 282, Instrumentation Laboratory Co., Norcross, Ga.). Lactate determinations were made with an analyzer (COBAS-FARA II, Roche Diagnostic Systems, Montclair, N.J.).

The hemodynamic variables included (1) systemic and pulmonary artery blood pressures (phasic and mean), (2) left atrial pressure (mean), and (3) cardiac output (thermodilution). Hemodynamic and electrocardiographic data were digitized and stored on hard drive or magneto-optical disk for subsequent analysis.

Other measured variables included perfusate and esophageal temperatures, bypass flow rate (calibrated roller pump), wet and dry myocardial tissue weights (obtained post mortem), hematocrit-fluorocrit (perfluorocarbon emulsion settles as a distinct layer at the bottom of a spun blood sample), and blood samples at end of bypass and at end of study (3 to 5 ml whole blood in ethylenediaminetetraacetic acid, stored frozen) for perfluorocarbon content analysis by gas chromatography.

Derived variables included cardiac arterial-venous oxygen content difference (AVO_2 difference), total body oxygen consumption, cardiac and total body lactate gradient, and partitioning of total oxygen consumption into hemoglobin-bound and dissolved oxygen components.

Surgical and pump-oxygenator protocol. A total of 42 mixed-breed dogs weighing from 25 to 30 kg were included in this study. The initial surgical and anesthetic management was as follows. The animals were anesthetized with sodium pentobarbital, placed on a heating blanket, and intubated, and their lungs were ventilated with 100% oxygen. Pancuronium bromide was given after an adequate level of anesthesia was assured, and an infusion of sodium pentobarbital and pancuronium was used to maintain anesthesia.

Limb electrocardiographic leads and an esophageal temperature probe were placed. The femoral vessels, carotid artery, and external jugular vein were exposed. An arterial pressure–monitoring catheter, a thermodilution right heart catheter, and fluid infusion lines were placed. A laparotomy was performed. Epinephrine was injected through the splenic artery; then the contracted spleen was removed. The laparotomy was closed, and a median

sternotomy was made. Catheters for blood sampling were placed in the main pulmonary artery and in the mid portion of the coronary sinus. A pressure monitoring catheter was placed in the left atrium.

The baseline hematocrit was measured, and the dog's total blood volume (TBV) was calculated by using the formula:

$$TBV = [92.6 \text{ ml} \cdot \text{kg}^{-1}] [\text{Weight (kg)}]$$

Heparin was administered (300 U/kg), and the animal was normovolemically hemodiluted to a hematocrit of approximately 15% by using a constant mean left atrial pressure as an indicator of vascular volume. The volume of crystalloid solution administered was calculated as

$$\text{Amount diluent added (ml)} = -\text{TBV} \cdot \ln\left[\frac{\text{Hct}}{\text{Hct}_{base}}\right]$$

where Hct = final hematocrit and Hct_{base} = initial hematocrit. The replacement fluid for blood was 38° C normal saline solution with 50 $mEq \cdot L^{-1}$ $NaHCO_3$ and 5 $mEq \cdot L^{-1}$ KCl added.

The pump oxygenator circuit used right atrial and femoral arterial cannulation. The left ventricle was vented. The oxygenator was a hollow fiber device (Maxima, Medtronic, Inc., Minneapolis, Minn.). A hollow fiber oxygenator design was chosen on the basis of a previous study that demonstrated significantly higher oxygen transfer rates to PFC emulsions for hollow fiber and true membrane oxygenators than for bubble oxygenators.[3]

The oxygenator was gassed with oxygen at twice the blood flow, and CO_2 was blended with the sweep oxygen as needed to maintain Pco_2 within physiologic range. The pump priming solution contained 660 ml Normosol-R, pH 7.4 (Abbott Laboratories, North Chicago, Ill.), 330 ml 5% dextrose in water, and 10 ml 1 $mEq \cdot ml^{-1}$ $NaHCO_3$.

Experimental data acquisition protocol. After the splenectomy and line placement were completed, prehemodilution data were acquired. The animals were then normovolemically hemodiluted, and posthemodilution data were obtained. CPB was initiated at 38° C, a Po_2 >500 mm Hg, and a flow of 2.0 $L \cdot min^{-1} \cdot m^{-2}$. At this time, control animals (n = 12) had no addition to the oxygenator, whereas the test groups had PFC 1.35 $gm \cdot kg^{-1}$ (n = 10 dogs), PFC 2.7 $gm \cdot kg^{-1}$ (n = 10 dogs), or PFC 5.4 $gm \cdot kg^{-1}$ (n = 10 dogs) added through the venous return cannula. The PFC emulsion was a 90% wt/vol emulsion based on perflubron (perfluorooctyl bromide) (Oxygent [AF0142], Alliance Pharmaceutical Corp., San Diego, Calif.).

Initial blood samples were drawn 5 minutes after randomization and PFC dosing. The water bath was cooled to 32° C; after 10 minutes a series of randomized pump flows (0.25, 0.5, 1.0, 1.5, 2.0, and 3.0 $L \cdot min^{-1} \cdot m^{-2}$) was begun. Each flow was run for 5 minutes before blood samples and hemodynamic data were obtained. After the randomized flows were completed at 32° C, flow was resumed at 2.0 $L \cdot min^{-1} \cdot m^{-2}$, and the temperature was raised to 38° C for 20 minutes. Hemodynamic data and blood samples were obtained. CPB was terminated. By protocol restriction, no inotropic agents were used. The pre-CPB mean left atrial pressure was used as a guide for the post-CPB left atrial pressure; however, if the systemic blood pressure and cardiac output were inadequate, the left atrial pressure was increased as high as 20 mm Hg in an attempt to discontinue CPB. Blood samples and hemodynamic data were acquired at the termination of CPB and every 15 minutes for 1 hour after CPB. After CPB, crystalloid or whole blood was administered as needed to maintain a hematocrit of 10% to 12% and a constant mean left atrial pressure. No additional PFC emulsion was given during the study even if the fluorocrit decreased. The animals were then killed. The hearts were removed and weighed, and sections of the left ventricle were obtained for weighing and desiccation.

Statistical analysis. The data were analyzed by using SAS-PC software (SAS Institute, Inc., Cary, N.C.), then displayed by using Sigma Plot software (Jandel Scientific, Corte Madera, Calif.). Statistical comparisons of hemodynamic data, hematocrit, fluorocrit, and biochemical data were made with analysis of variance designs (i.e., Duncan's multiple-range test and least-squares means test) contained in the General Linear Models procedure of SAS-PC. Duncan's multiple-range test was used to statistically define differences between groups when multiple groups were being simultaneously compared.[4] The least-squares means test was used for individual between-group comparisons.[4] The level of significance chosen for this study was $p < 0.05$.

Comparisons of mixed venous Po_2 (Pvo_2), serum lactate, and total body oxygen consumption during CPB were made by first plotting the experimental variable as a function of oxygen delivery rate or CPB flow normalized to the animal's body surface area.[4, 5] The data were then fit to functions, and the parameters of the equations were compared between the four experimental groups by using Duncan's multiple-range test for comparisons between multiple groups and the least-squares means test for individual between-group comparisons. (See Appendix 1 for a description of methods used to determine optimal curve fit and calculate variance.)

Results

Normovolemic hemodilution before CPB resulted in a decrease in the hematocrit to 15.8% ± 0.6% (all values are given as mean ± SEM*). Initiation of CPB with a crystalloid priming solution further decreased the hematocrit to 9.4% ± 0.6% (Table I). No statistically significant differences were found in hematocrit between groups throughout the study. The fluorocrits of the treated groups reflect the dose of PFC emulsion the animals received (Table II). The gas chromatographic analysis of blood PFC content corroborated the relative amounts of PFC detected by the fluorocrit in the four experimental groups (Table III). The fluorocrit remained constant during CPB and decreased after CPB.

The animals responded to hemodilution with an increase in cardiac output. No significant differences

*Standard error of the mean.

Table I. *Hematocrits in the three groups before and after hemodilution and during and after CPB*

	Hematocrit (%)				
Groups	*Before HD*	*After HD*	*During CPB*	*After CPB*	*60 min after CPB*
Control	51.5 ± 1.9	16.0 ± 0.6	9.9 ± 0.4	13.4 ± 0.6	11.9 ± 1.0
1.35 gm PFC·kg^{-1}	55.2 ± 1.4	16.5 ± 0.5	9.8 ± 0.3	12.5 ± 0.4	11.6 ± 0.5
2.7 gm PFC·kg^{-1}	45.4 ± 2.6	15.8 ± 0.5	9.1 ± 0.7	13.7 ± 1.1	11.8 ± 0.8
5.4 gm PFC·kg^{-1}	50.0 ± 1.7	14.8 ± 0.7	8.6 ± 0.8	13.5 ± 0.7	12 ± 1.1

Values are expressed as mean ± standared error of the mean. There were no significant differences between groups. *Before HD,* Before hemodilution; *After HD,* after hemodilution before CPB; *After CPB,* at termination of CPB; *60 min after CPB,* 60 minutes after termination of CPB.

Table II. *Fluorocrits of the three groups before and after hemodilution and during and after CPB*

	Fluorocrit (%)				
Groups	*Before HD*	*After HD*	*During CPB*	*After CPB*	*60 min after CPB*
Control	0	0	0	0	0
1.35 gm PFC · kg^{-1}	0	0	1.1 ± 0.1	1.2 ± 0.1	1.0 ± 0.1
2.7 gm PFC · kg^{-1}	0	0	2.0 ± 0.1	1.9 ± 0.1	1.25 ± 0.2
5.4 gm PFC · kg^{-1}	0	0	3.1 ± 0.1	3.1 ± 0.2	2.1 ± 0.2

Values are expressed as mean ± standard error of the mean. The fluorocrits of the PFC emulsion groups remained stable throughout CPB and decreased during the initial 60 minutes after CPB. *Before HD,* Before hemodilution; *After HD,* after hemodilution before CPB; *After CPB,* at termination of CPB; *60 min after CPB,* 60 minutes after termination of CPB.

Table III. *Blood perfluorocarbon concentration after CPB as determined by gas chromatography*

	Blood PFC concentration ($\mu g \cdot ml^{-1}$)		
Time off CPB (min)	*1.35 gm PFC · kg^{-1} group*	*2.7 gm PFC · kg^{-1} group*	*5.4 gm PFC · kg^{-1} group*
0	8,394 ± 474 (n = 10)	15,257 ± 1,149 (n = 8)	32,420 ± 2,032 (n = 5)
60	6,631 ± 237 (n = 5)	12,801 ± 1,002 (n = 6)	25,156 ± 5,212 (n = 4)

Values are expressed as mean ± standard error of the mean. Actual number of determinations for each sampling group is given in parentheses. Perfluorocarbon content of blood in control group was below detectable limit.

were observed between groups in pre-CPB or post-CPB cardiac index. During the post-CPB period, five of the untreated animals died and five of the animals that received an emulsion dose of 1.35 gm PFC · kg^{-1} died. Only two of the animals given a 2.7 gm · kg^{-1} PFC emulsion dose and one given a 5.4 gm · kg^{-1} PFC emulsion dose died. All deaths were a result of post-CPB circulatory failure (i.e., low cardiac output). Fig. 1 shows the Kaplan-Meier estimate for death during the first hour after CPB. A trend was found toward a lower incidence of death at 1 hour after CPB as the dose of PFC increased (p = 0.065). Furthermore, the mean PFC emulsion dose in survivors was significantly higher than in nonsurvivors (PFC 2.6 ± 0.4 gm · kg^{-1} versus PFC 1.2 ± 0.5 gm · kg^{-1}; $p < 0.05$).

Measurements of total hemoglobin content and oxyhemoglobin saturation were complicated somewhat by PFC emulsion turbidity. This effect has been noted by others and can cause errors of ±10% in readings.[6] Rather than using measurements of hemoglobin saturation from the oximeter, we calculated oxyhemoglobin saturation with equations developed by Rossing and Cain.[7] These calculated values were used to evaluate venous Po_2 as a function of hemoglobin oxygen delivery. Consistent between-group differences in the functions defining Pvo_2 versus hemoglobin oxygen delivery were not demonstrated when the method of Rossing and Cain was used to calculate oxyhemoglobin saturation.

Total oxygen delivery and oxygen consumption were calculated from perfusate oxygen content as measured by a fuel cell (i.e., the Lex-O_2-Con device). The fuel cell is insensitive to the turbidity of PFC emulsions because it measures oxygen content by a chemical reaction. No differences were found in oxygen consumption (Fig. 2) between groups during

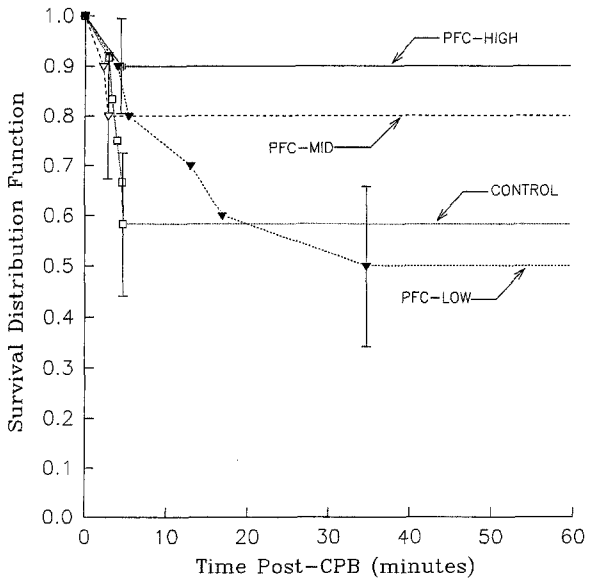

Fig. 1. Survival function estimate for groups. Kaplan-Meier depiction for death after CPB. A higher incidence of death was seen at 1 hour after CPB as the dose of PFC decreased ($p = 0.065$ by logistic regression analysis). *PFC-HIGH,* PFC 1.35 gm · kg^{-1} ($n = 10$ dogs); *PFC-MID,* PFC 2.7 gm · kg^{-1} ($n = 10$ dogs); *PFC-LOW,* PFC 5.4 gm · kg^{-1} ($n = 10$ dogs).

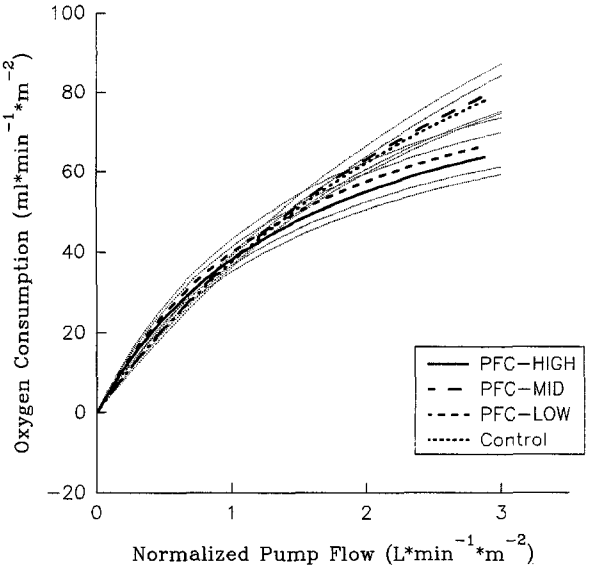

Fig. 2. Total body oxygen consumption during CPB was not significantly different between groups. Normalized pump flow is CPB flow normalized to body surface area. *PFC-HIGH,* PFC 1.35 gm · kg^{-1} ($n = 10$ dogs); *PFC-MID,* PFC 2.7 gm · kg^{-1} ($n = 10$ dogs); *PFC-LOW,* PFC 5.4 gm · kg^{-1} ($n = 10$ dogs).

CPB, as measured by fuel cell determinations of AVO_2 difference and pump flow.

Analysis of Pvo_2 as a function of CPB flow normalized to animal body surface area is shown in Fig. 3. The regression equation that describes this function is shown in Appendix 2. The control group was significantly different ($p < 0.05$) from the PFC emulsion–treated groups. Furthermore, the differences were dose related.

The Pvo_2 values for the four groups were then expressed as a function of oxygen delivery during CPB (Fig. 4 and Appendix 2). This analysis accounts for the effects of additional dissolved oxygen in the PFC emulsion–treated animals and for any animal-to-animal variance in hemoglobin concentration; thus it was anticipated that the four curves would be similar. The *b* coefficients were similar; however, there is a higher intercept (i.e., *a* value) for the three PFC emulsion–treated groups as compared with the control group. The *p* values ranged from 0.025 to 0.09 for individual PFC emulsion group versus control group comparisons. If the data for the three PFC emulsion groups are combined and compared with data for the control animals to examine for a PFC emulsion effect, a significant difference is seen between control and PFC emulsion animals ($p < 0.002$) (Fig. 5). This finding suggests that PFC-dissolved oxygen exerted a sparing effect on hemoglobin-bound oxygen.

The portion of total oxygen delivery and consumption attributable to dissolved oxygen was calculated. This calculation was based on blood PFC content as measured by gas chromatography (Table III), Po_2, and the solubility coefficient for oxygen in perfluorooctyl bromide (oxygen 50 ml · dl^{-1} at 38° C and 1 atm pressure). A depiction of dissolved oxygen consumption during and after CPB shows a significant increase in dissolved oxygen consumption for PFC emulsion–treated dogs (Fig. 6). The increase in dissolved oxygen consumption was related to the dose of PFC emulsion.

Significant differences were seen in Pvo_2 between the control and PFC emulsion groups at 0, 15, 30, and 45 minutes after CPB (Table IV). The Pvo_2 values measured 60 minutes after termination of CPB in the control and PFC emulsion groups were insignificantly different ($p = 0.099$). This insignificant difference at 60 minutes after CPB occurred contemporaneously with a decrease in blood PFC content in all three PFC groups. The differences in Pvo_2 between control and PFC emulsion–treated animals at 0 through 45 minutes after CPB could not

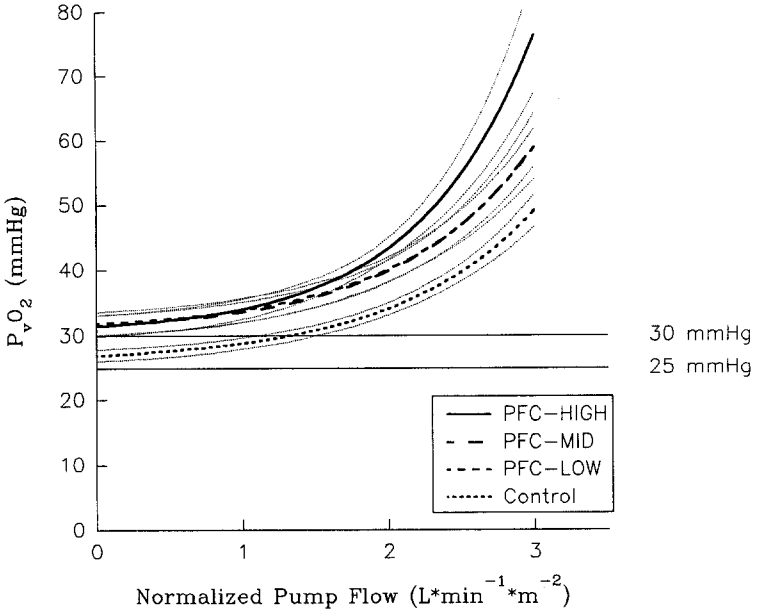

Fig. 3. Pvo_2 expressed as a function of CPB flow was higher ($p < 0.05$) in the 2.7 and 5.4 gm · kg^{-1} PFC emulsion dose groups than in the control group. Pvo_2 in the range of 25 to 30 mm Hg represents the level below which oxygen transfer rates into tissues begin to decrease. The addition of a PFC emulsion to the perfusate allows a lower CPB flow rate before this range is entered. *PFC-HIGH,* PFC 1.35 gm · kg^{-1} (n = 10 dogs); *PFC-MID,* PFC 2.7 gm · kg^{-1} (n = 10 dogs); *PFC-LOW,* PFC 5.4 gm · kg^{-1} (n = 10 dogs). There is considerable overlap of the PFC-MID and PFC-LOW data.

be explained by post-CPB differences in cardiac index, hematocrit, arterial Po_2, or arterial oxygen content. Consistent, albeit insignificant, differences in the coronary sinus Po_2 were observed between the control and PFC emulsion groups (i.e., the coronary sinus Po_2 was consistently higher in the PFC emulsion groups) (Table IV). The cardiac AVO_2 differences during and after CPB were similar among the control and PFC emulsion–treated animals.

Myocardial wet-to-dry ratios measured at the termination of the study were similar between the four experimental groups (wet-to-dry ratios: control group, 0.81 ± 0.002; PFC 1.35 gm · kg^{-1} group, 0.82 ± 0.003; PFC 2.7 gm · kg^{-1} group, 0.81 ± 0.003; and PFC 5.4 gm · kg^{-1} group, 0.81 ± 0.003). Blood and esophageal temperatures were similar between groups during the study.

Discussion

Our model of hypothermic CPB in an anemic animal represents an extreme condition as evidenced by the high post-CPB mortality rates in the control and lowest-dose PFC emulsion groups. Oxygen delivery during CPB in this model is limited by flow (stagnation hypoxia), anemia (anemic hypoxia), and an increase in hemoglobin oxygen affinity caused by hypothermia (affinity hypoxia).

There are advantages to an experimental model that combines several hypoxic stresses. First, it is clinically relevant because multiple hypoxic stresses also occur during CPB in human beings, although these stresses are less severe. Second, our model defeated the body's usual mechanisms of compensating for an individual hypoxic stress. This feature allowed us to detect the effect of PFC-dissolved oxygen on post-CPB mortality.

The experimental model used in this study also has disadvantages. For instance, it is impossible to dissect out the effect of PFC-dissolved oxygen on individual hypoxic stresses during CPB because multiple hypoxic stresses were operative simultaneously. Most important among these differentiations is defining the effect of dissolved oxygen on tissue oxygenation in the setting of anemic hypoxia. This information is necessary to equate dissolved oxygen (i.e., Po_2 and PFC emulsion dose) with oxygen transported by hemoglobin.

In this study, total body oxygen consumption was not increased by the addition of PFC-dissolved oxygen, although Pvo_2 during CPB was increased by

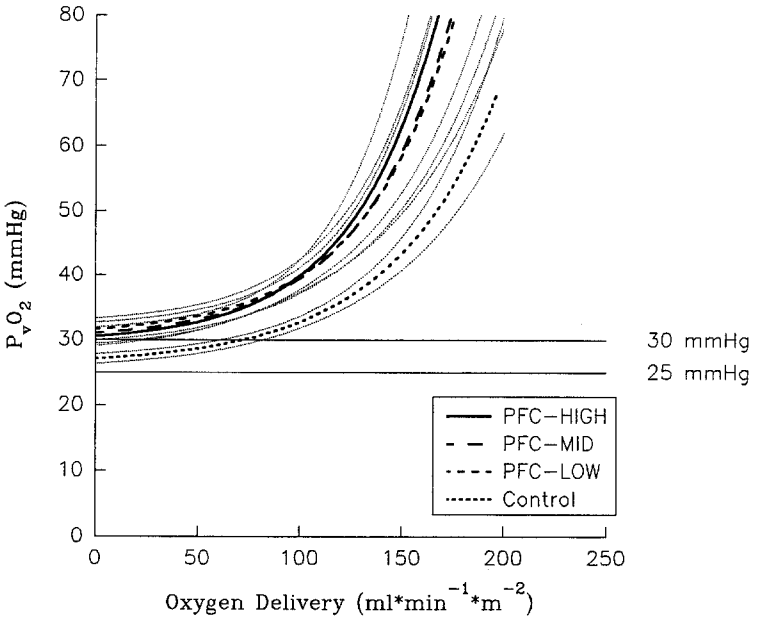

Fig. 4. Pvo_2 values for the four groups were expressed as a function of oxygen delivery. This analysis accounts for the effect of dissolved oxygen in the PFC emulsion–treated animals and any animal-to-animal variance in hemoglobin concentration. The *b* coefficients (i.e., slopes) were similar; however, there are higher *a* values (i.e., intercepts) for the three PFC emulsion–treated groups as compared with the control group (*p* value range 0.025 to 0.09 for individual PFC emulsion groups versus control group comparisons). *PFC-HIGH,* PFC 1.35 gm · kg^{-1} (n = 10 dogs); *PFC-MID,* PFC 2.7 gm · kg^{-1} (n = 10 dogs); *PFC-LOW,* PFC 5.4 gm · kg^{-1} (n = 10 dogs).

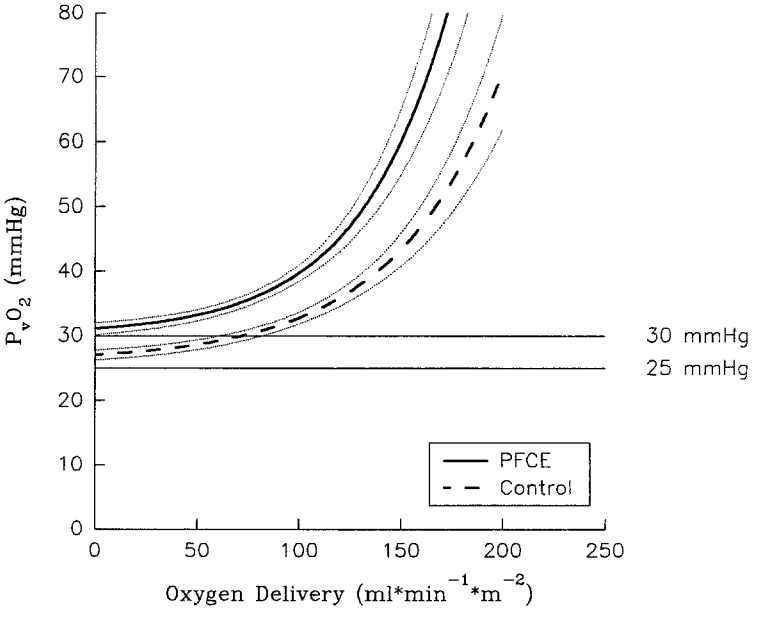

Fig. 5. Pvo_2 values for PFC emulsion groups *(PFCE)* were combined and expressed as a function of oxygen delivery during CPB. PFC emulsion–treated groups had significantly higher ($p < 0.05$) Pvo_2 values than the control group. This finding suggests sparing of hemoglobin-bound oxygen by dissolved oxygen.

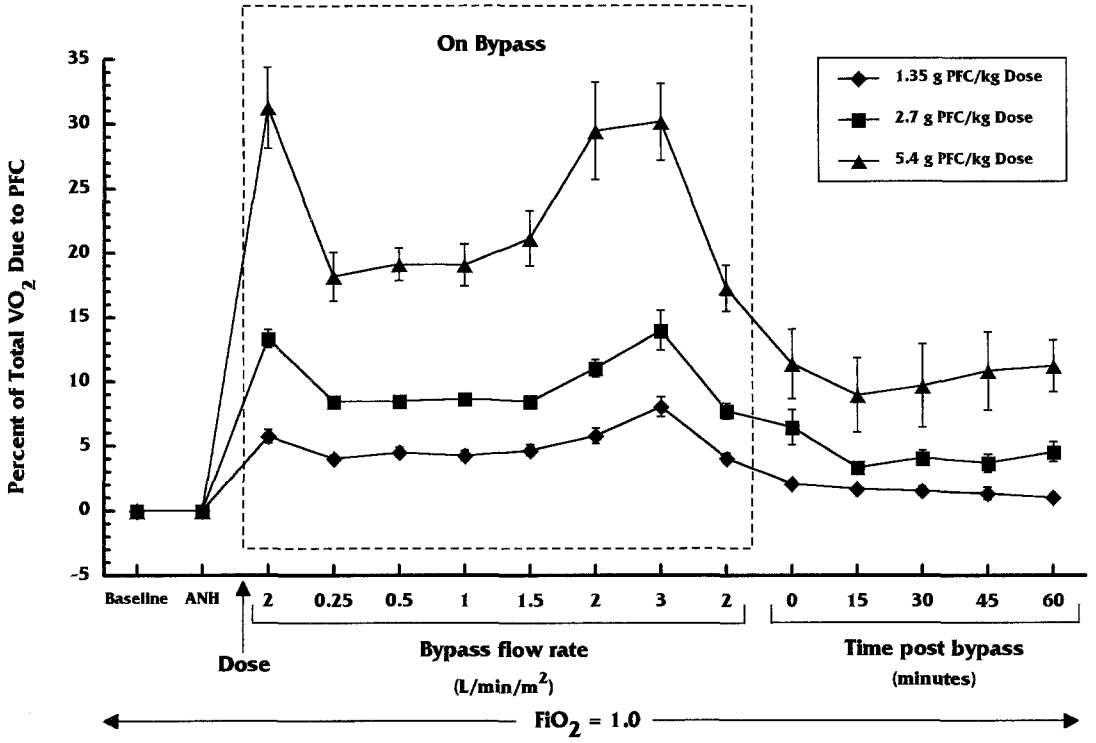

Fig. 6. PFC concentrations in blood were used to calculate PFC-dissolved oxygen consumption during the experiment. This value is expressed as a percentage of the total oxygen consumption on the ordinate. Vo_2, Total body oxygen consumption; *Baseline,* prehemodilution control value; *ANH,* prebypass control value after normovolemic hemodilution; *Dose,* administration of PFC emulsion to experimental groups; *boxed area,* time during which animals were undergoing CPB.

Table IV. *Po_2 measured in mixed venous and coronary sinus blood samples after CPB compared in control animals and PFC emulsion-treated animals*

	Po_2 (mm Hg)				
Groups	*T = 0 min*	*T = 15 min*	*T = 30 min*	*T = 45 min*	*T = 60 min*
Mixed venous control	31.5 ± 1.9	34.7 ± 2.2	30.7 ± 5.1	30.1 ± 1.2	31.9 ± 2.1
Mixed venous PFC emulsion	38.4 ± 1.5*	40.0 ± 1.4†	39.5 ± 1.6*	35.4 ± 1.2*	36.2 ± 1.3
Coronary sinus control	27.0 ± 2.6	27.6 ± 2.1	25.9 ± 2.1	28.1 ± 2.3	26.1 ± 1.8
Coronary sinus PFC emulsion	28.9 ± 1.3	30.4 ± 1.1	29.9 ± 1.1	32.3 ± 2.6	29.9 ± 1.4

Values are expressed as mean ± standard error of the mean. *Mixed venous,* Mixed venous blood samples; *Coronary sinus,* coronary sinus blood samples; *T,* time after CPB.

*$p < 0.05$ control compared with PFC emulsion-treated animals.

†$p < 0.05$ for 2.7 and 5.4 gm · kg^{-1} PFC emulsion group compared with control animals.

the PFC emulsion in a dose-related fashion. An analysis of tissue oxygen consumption in a Krogh cylinder, published previously by Biro,[8] provides an explanation for this phenomenon. Biro's model shows that as the dissolved oxygen content of blood increases, small regions of tissue hypoxia resolve and Pvo_2 increases even though total tissue oxygen consumption remains constant.

Another way to view the importance of increasing the Pvo_2 during CPB is to consider Pvo_2 as a surrogate measure of global tissue Po_2.[9-13] Using this line of reasoning, we can conclude that increasing the perfusate-dissolved oxygen content during CPB increases tissue Po_2. If a Pvo_2 range of 25 to 30 mm Hg is assumed to represent the lowest Pvo_2 consistent with adequate tissue oxygenation, the contribution of additional PFC-dissolved oxygen to maintaining adequate tissue oxygenation during

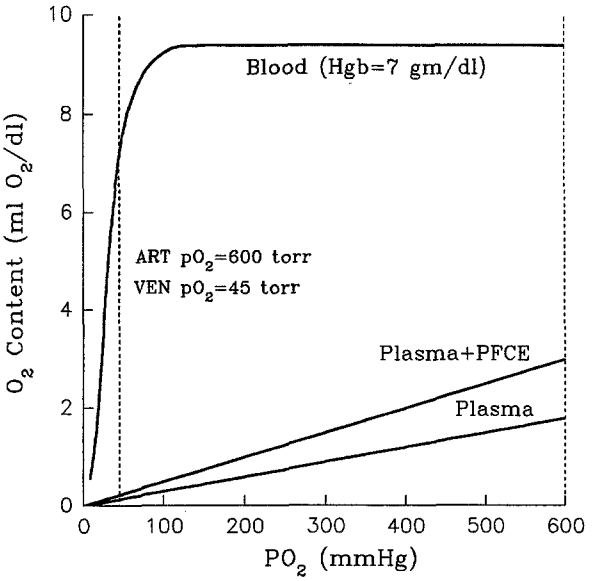

Fig. 7. Oxygen delivery and availability during CPB at 38° C and pH 7.40. Conditions of this model include: hemoglobin *(Hgb)* content, 7 $gm \cdot dl^{-1}$; arterial *(ART)* Po_2, 600 mm Hg; and mixed venous *(VEN)* Po_2, 45 mm Hg. The arterial-venous difference for bound oxygen is 2.3 ml oxygen per deciliter of perfusate, and the arterial-venous difference for plasma-dissolved oxygen is 1.7 ml oxygen per deciliter of perfusate. Adding PFC 5.4 $gm \cdot kg^{-1}$ increases the solubility coefficient of oxygen in the plasma–PFC emulsion *(PFCE)* mixture from 0.003 $ml \cdot dl^{-1} \cdot mm\ Hg^{-1}$ to 0.005 $ml \cdot dl^{-1} \cdot mm\ Hg^{-1}$ and increases the arterial-venous difference for dissolved oxygen to 2.8 ml oxygen per deciliter of perfusate. As the temperature of CPB decreases or as pH increases, the arterial-venous difference for hemoglobin-bound oxygen *decreases* because of a leftward shift of the oxyhemoglobin dissociation curve. In contrast, the arterial-venous difference for dissolved oxygen *increases* as the temperature decreases.

CPB can be equated with additional hemoglobin-bound oxygen or a higher pump flow (Figs. 3 and 4).

The contribution of PFC-dissolved oxygen to survival after separation from CPB was noted even though the total amount of PFC-dissolved oxygen in the blood was smaller than the total amount of hemoglobin-bound oxygen. The beneficial effect of PFC-dissolved oxygen is due, in part, to the highly efficient transfer of dissolved oxygen to tissues. The finding that relatively small volumes of oxygen can have important effects on cardiac function also corroborates results of previously published studies. These studies demonstrated abrupt deterioration in regional contractile function during progressive anemia in myocardium distal to a stenotic coronary artery.[14-17] This functional deterioration was reversed by a small increment in blood oxygen content.[17]

Our study shows that increasing the dissolved oxygen content of blood by using a PFC emulsion and a high Po_2 improves tissue oxygenation during CPB. There are two possible mechanisms for this effect. The first mechanism involves the mass transport of oxygen dissolved in a PFC emulsion. PFCs have extremely high solubility coefficients for oxygen and other gasses, and the total volume of oxygen carried in the dissolved state during CPB can be further increased by the appropriate choice of oxygenator device to provide a consistently high arterial Po_2.[3] The total dose of a PFC emulsion that can be given during CPB has a limit; however, this dose limitation is counterbalanced by the greater availability of dissolved oxygen than of hemoglobin-bound oxygen for diffusive transport into tissue. Dissolved oxygen is not chemically bound to plasma water or the PFC emulsion. Thus utilization of dissolved oxygen by tissues is highly efficient (90% to 95% consumption of arterial dissolved oxygen) (Fig. 7). Furthermore, larger volumes of oxygen dissolve in water and PFC emulsions as the temperature decreases, and the availability of this dissolved oxygen is not affected by changes in pH or temperature.

The second mechanism by which PFC emulsions may improve tissue oxygenation is acceleration of oxygen diffusion from hemoglobin to tissues. This putative mechanism is based on a consideration of diffusion barriers in the microcirculation. Plasma in capillaries forms a boundary layer around erythrocytes. Smaller particles such as platelets and PFC emulsion particles tend to segregate from the erythrocytes into the plasma layer. Diffusion of oxygen across this boundary layer represents the greatest barrier to the passage of oxygen from hemoglobin to tissues.[18-20] One previously published report suggested that a PFC emulsion in blood accelerates the diffusion of oxygen from erythrocytes to tissues[21]; however, others have not been able to document this effect. In vitro studies that quantify the kinetics of oxyhemoglobin dissociation with or without a PFC emulsion in the surrounding fluid may resolve this question.

This in vivo whole-animal study of oxygenation during CPB has limitations that should be recognized. The measurements of oxyhemoglobin saturation and oxyhemoglobin content by optical densitometry were imprecise because of the turbidity of

the PFC emulsion. This imprecision made it impossible to determine by direct measurement the portion of total oxygen consumption that was derived from hemoglobin-bound oxygen. Thus sparing of hemoglobin-bound oxygen by the increased delivery of PFC-dissolved oxygen could not be directly demonstrated. The data describing arterial and venous blood oxygen content were acquired by using a fuel cell (i.e., Lex-O_2-Con device) rather than by calculations from blood gas and hemoglobin saturation measurements. The fuel cell data provided a reliable basis for the inferences of this experiment. The various contributions of PFC-dissolved oxygen to total oxygen consumption were calculated from the following direct measurements: PFC concentration as measured by gas chromatography and arterial Po_2 and Pvo_2 as measured on blood gas analysis. The gas chromatography and blood gas measurements are considered highly reliable.

Total body oxygen consumption is a gross measure that can miss potentially important regional variations in tissue oxygen consumption.[22] Similarly, the transcardiac AVO_2 difference cannot detect variations in regional myocardial oxygen delivery or consumption. Information regarding regional myocardial oxygen metabolism is crucial to understanding cardiac adaptation to hemodilution and the role of dissolved oxygen in preventing regional myocardial hypoxia.

The Pvo_2 is an imperfect measure of tissue Po_2. It is insensitive to the regional heterogeneity of tissue Po_2 levels that exist within individual organs. Furthermore, the tissue Po_2 is always lower than the surrounding venous Po_2; how much lower, however, is not known.

We described oxygen metabolism in animals with normal hearts. Additional experiments that measure regional myocardial oxygen metabolism and mechanical function will be necessary to define the limits of hemodilution in normal and diseased hearts. Most of the information currently available regarding adequate levels of perfusate hemoglobin concentration during CPB was generated by empiric observations in patients.[23-26] Currently no method exists to define the lowest acceptable hemoglobin concentration during CPB for individual patients.[27, 28]

It is possible that increasing the dissolved oxygen content of blood will be especially beneficial in diseased hearts. This benefit is primarily because the viscosity of blood is increased less by the addition of a PFC emulsion than by the addition of erythrocytes[29] and because dissolved oxygen is more available than hemoglobin-bound oxygen for diffusive transport into tissues. Keipert and colleagues[30] have published similar observations on the availability of dissolved oxygen in a study that examined oxygen consumption during profound normovolemic hemodilution in a canine model that did not include CPB.

This study examined tissue oxygenation during CPB with use of graded concentrations of dissolved oxygen. Graded increases in Pvo_2 were observed in response to graded increases in the dissolved oxygen content of blood. Dissolved oxygen was useful in ameliorating post-CPB cardiac failure. These data suggest that dissolved oxygen is an effective temporary substitute for hemoglobin-bound oxygen during CPB and that dissolved oxygen may be particularly beneficial in the setting of multiple hypoxic stresses. PFC emulsions used in combination with a high Po_2 during CPB may diminish the need for allogeneic erythrocyte transfusions.

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Appendix 1

The first step in comparing oxygen consumption between groups was to summarize the data with an equation that expresses total body oxygen consumption as a function of CPB flow or oxygen delivery. The total body oxygen consumption data were described by the equation for a rectangular hyperbola,[31]

$$\dot{V}O_2 = \frac{a \cdot x}{b + x}$$

The variable x in the equation was either pump flow normalized to body surface area or the total oxygen delivered to the body (i.e., the x-axis variables). If x is very large ($x \gg b$), the equation tends to a. Thus, the parameter a is the maximum value of the equation, which in this case is the maximum oxygen consumption.

The equation that was chosen is consistent with the physiology of the experimental model and produced correlation coefficients (i.e., r^2 values) >0.50. As CPB flow or oxygen delivery increases, total body oxygen consumption asymptotically approaches a maximum value. The b parameter determines the fraction of maximum oxygen consumption at any given x. This equation was used

previously by Fox and associates[31] to describe total body oxygen consumption during CPB in human beings.

The parameters of the equation were calculated for each animal by using the nonlinear regression procedure of SAS.[4] The data from one animal regressed poorly ($r^2 < 0.50$), and this study was excluded. The calculated parameters a and b were compared between groups by using Duncan's multiple-range test and a least-squares means test. The mean values of a and b for each experimental group were calculated and used to produce the plots of total body oxygen consumption as a function of CPB flow or oxygen delivery.

The variance in the rectangular hyperbola equations was calculated by using the following equation:

$$\mathrm{Var}(y)=\left(\frac{\partial y}{\partial a}\right)^2 \cdot \mathrm{Var}(a)+\left(\frac{\partial y}{\partial b}\right)^2 \cdot \mathrm{Var}(b) + 2 \cdot \frac{\partial y}{\partial a} \cdot \frac{\partial y}{\partial b} \cdot \mathrm{Cov}(ab)$$

where y is total body oxygen consumption. The 70% confidence intervals on the graphs were obtained by using the square root of this equation (±1 SD*).

Similar methods were used to determine the optimal curve fit and variance for the Pvo_2 (see Appendix 2), lactate, and hemoglobin oxygen saturation data. The parameters of the equations were then compared by using Duncan's multiple-range test and a least-squares means test.

*Standard deviation.

Appendix 2

For the Pvo_2 data, the equation that best fit the data was one describing exponential growth:

$$Pvo_2 = a + e^{bx} - 1.$$

The variable x represents either normalized CPB flow or oxygen delivery. By subtracting 1 from the right side of the equation, a becomes the y-axis intercept (i.e., the Po_2 value as x approaches zero). Because by definition $x \geq 0$, the a value at $x = 0$ is the minimum value of the equation. Although an x value of zero is unrealistic in an experimental system, the a value calculated for $x = 0$ is useful for between-group comparisons. The a value quantifies vertical shifts in the curves that describe Pvo_2 as a function of CPB flow or oxygen delivery. The b parameter describes the slope of the curve. A larger b in the equation means that the Po_2 increases more for a given increase in x than if b is a smaller number.

The mean values of a and b for each experimental group were calculated and used to produce the plots of Pvo_2 as a function of CPB flow or oxygen delivery. The data were analyzed by using a nonlinear regression procedure in SAS,[7] followed by between-group comparisons of a and b that used Duncan's multiple-range test and a least-squares means test.