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Rapid (0.5°C/min) minimally invasive induction of hypothermia using cold perfluorochemical lung lavage in dogs

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Abstract

Objective: Demonstrate minimally invasive rapid body core and brain cooling in a large animal model. *Design:* Prospective controlled animal trial. *Setting:* Private research laboratory. *Subjects:* Adult dogs, anesthetized, mechanically ventilated. *Interventions:* Cyclic lung lavage with FC-75 perfluorochemical (PFC) was administered through a dual-lumen endotracheal system in the new technique of 'gas/liquid ventilation' (GLV). In Trial-I, lavage volume (V-lav) was 19 ml/kg, infused and withdrawn over a cycle period (tc) of 37 s. (effective lavage rate V'-lav = 31 ml/kg/min.) Five dogs received cold ($\sim$4 °C) PFC; two controls received isothermic PFC. In Trial-II, five dogs received GLV at V-lav = 8.8 ml/kg, tc = 16 s, V'-lav = 36 ml/kg/min. *Measurements and main results:* Trial-I tympanic temperature change was -3.7 ± 0.6 °C (SD) at 7.5 min, reaching -7.3 ± 0.6 °C at 18 min. Heat transfer efficiency was 60%. In Trial-II, efficiency fell to 40%, but heat-exchange dead space (VDtherm) remained constant. Lung/blood thermal equilibration half-time was <8 s. Isothermic GLV caused hypercapnia unless gas ventilation was increased. At necropsy after euthanasia (24 h), modest lung injury was seen. *Conclusions*: GLV cooling times are comparable to those for cardiopulmonary bypass. Heat and CO_2 removal can be independently controlled by changing the mix of lavage and gas ventilation. Due to VDtherm of $\sim$6 ml/kg in dogs, efficient V-lav is >18 ml/kg. GLV cooling power appears more limited by PFC flows than lavage residence times. Concurrent gas ventilation may mitigate heat-diffusion limitations in liquid breathing, perhaps via bubble-induced turbulence.

Keywords: Resuscitation; Hypothermia (induced); Brain ischemia; Spinal cord injury; Perfluorocarbons; Fluorocarbons; Respiration (artificial/methods); Dogs; Dead space (respiratory); Lavage

Resumo

Objectivo: Demonstrar a redução rápida da temperatura central e cerebral num modelo de animal de grande porte com um método minimamente invasivo. *Desenho:* estudo controlado e prospectivo em animais. Contexto: laboratório de pesquisas privado. *Sujeitos:* Cães ventilados mecanicamente e anestesiados. Intervenção: lavagem cíclica dos pulmões com FC-75 penfluorado (PFC) através de um tubo traqueal de duplo lumen, recorrendo à nova técnica de ventilação gás/líquido (GLV). No ensaio I, o volume da lavagem (V-lav.) foi de 19 ml/kg, introduzido recuperado em períodos cíclicos de 37 s. Taxa de lavagem efectiva V-lav. = 31 ml/kg/min. A cinco dos quais foi feita PFC a cerca de 4 °C. Dois grupos de controlo receberam PFC isotérmico. No ensaio II cinco dos cães receberam GLV a V-lav. = 8.8 ml/kg, tc = 16s, V'-lav. = 36 ml/kg por minuto. *Medições e resultados principais:* No ensaio I a temperatura no tímpano baixou-3.7 ± 0.6 °C aos 7.5 min e a -7.3 ± 0.6 °C. A eficiência de transferência de calor foi de 60%. No ensaio II, a eficiência reduziu-se a 40% mas os humidificadores, o espaço morto(Vdtherm) permaneceram constantes. O tempo de equilíbrio térmico do conjunto coração/pulmão foi <8 s. A GLV com normotermia provocou hipercapnia a menos que o volume corrente fosse aumentado. À autópsia, depois de sacrificar os cães, as lesões pulmonares encontradas eram escassas. O tempo de arrefecimento é comparável aos dos 'bypass' cardiopulmonar. A mudança da forma de fazer a lavagem e da composição dos gases permitem controlar de forma independente o calor e a CO_2. Como nos cães a ''Vdtherm'' é de cerca de 6 ml/kg, a V-lav é >18 ml/kg. A capacidade de arrefecer da GLV parece ser mais limitada pelo fluxo

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do PFC, do que o momento da lavagem A utilização em simultâneo de ventilação por gás pode dificultar o arrefecimento, provavelmente pela turbulência provocada pelas bolhas de gás.

Palavras chave: Reanimação; Hipotermia (induzida); Isquemia cerebral; Lesão da espinal medula; Penfluorcarbonados; Fluorcarbonados; Cães; Espaço morto (respiratório); Lavagem

1. Introduction

Mild hypothermia ($\Delta T = -2$ to -6°C) during ischemia [1] and reperfusion has been called the gold standard against which neuroprotective strategies must be measured in the research setting [2]. However, despite excellent results in controlled animal models, clinical application of post-insult hypothermia has been problematic, due primarily to the logistics of achieving very rapid systemic cooling after injury [3]. The optimum therapeutic window for the treatment of CNS injury using hypothermia remains unknown, however results from hypothermia treatment of burns and sports injury suggest by analogy that the therapeutic window for all post-injury hypothermic treatment may be narrow. Indeed, it has been reported that in one dog model of cardiac arrest, even a 15 min delay after injury negates most of the considerable CNS-protective effect of post-insult hypothermia induction [4,5]. The utility of mild hypothermia treatment for human CNS injury may therefore require the ability to very rapidly cool the CNS and body core [6].

Several systemic cooling modalities are available. The most rapid and invasive of these is cardiopulmonary bypass (CPB). CPB is limited to cooling rates of approximately 1°C/min, due to RBC aggregation and the danger of gas embolism as chilled gas-saturated blood contacts warmer tissues [7]. Technical constraints also limit CPB's application to the hospital setting, where it is available only after transport and operative delay. Less invasive modalities with potential for field use, such as surface cooling and cold saline lavage of body cavities, typically produce cooling at 0.10–0.15°C/min. The experimental technique of 'total liquid ventilation' (TLV) with chilled, oxygenated liquid perfluorochemicals (PFCs) uses the >20 m^2 surface area of the lungs for heat exchange, but thus far has been reported to cool little faster than surface techniques [8].

The ideal modality for rapid induction of systemic hypothermia would achieve cooling rates comparable to CPB, yet also be minimally invasive, easily implemented, and portable. With these goals we investigated a PFC lung-lavage technique combining some features of partial liquid ventilation (PLV) and cold saline lavage. At high PFC infusion rates and shorter cycle periods, the implementation of PFC lung-lavage begins to resemble TLV-cooling (or warming). In practice however, certain significant differences remain. In the technique we have termed 'gas/liquid ventilation' (GLV), the critical element of gas ventilation is retained. This gas ventilation component allows for flexibility in selecting ventilation parameters independently for heat and gas-exchange, and allows for liquid-mediated heat-exchange to be easily integrated into existing ventilation systems. It may also play a role in the surprisingly good thermal efficiency of GLV as compared with TLV.

The present study introduces GLV [9], explores the performance of GLV using a prototype automated liquid-delivery device, and finally discusses the basic mechanics and intrinsic limitations of heat-exchange using PFC lung-lavage.

2. Materials and methods

Trials described were approved by our Institutional Animal Care and Use Committee and were in compliance with the Animal Welfare Act and the National Research Council's Guide for the Care and Use of Laboratory Animals. Fifteen mongrel dogs weighing 13.8–25.7 kg were used (Table 1). Dogs were pre-medicated with I.M. acepromazine (1.0 mg/kg) and atropine (0.02 mg/kg) prior to induction of general anesthesia using sodium pentobarbital (30 mg/kg I.V., with maintenance dosing). Anesthetized dogs were intubated with a reinforced 10.0 mm I.D. (Willy Rüsch AG, Kernen, Germany) endotracheal tube (E.T.), and ventilated on room air using a Bennett MA1 or Siemens Servo 900 C ventilator. Ventilator parameters, unless otherwise noted, were 12 gas-breaths/min, gas tidal-volume of 15 ml/kg, I:E ratio of 1:3, and a maximal positive inspiratory pressure (PIP) limit of 26 cm H_2O (2.5 kPa). Gas pressures were measured at the E.T. adapter. Gas minute-volume ($\dot{V}_g$) was adjusted to maintain $PaCO_2$ between 35 and 40 torr. Animals were maintained at $\sim$37.5°C prior to GLV, using a temperature-controlled water blanket. Rectal and bilateral tympanic temperatures (T_{tym}) were monitored continuously using a type-T thermocouple system (Cole-Parmer, Vernon Hills, IL) with a response time constant (t_o) of 5 s.

Combination pressure, blood sampling, and temperature-probe catheters were constructed from rigid polyethylene pressure-monitoring catheters, threaded centrally with 0.05 in. O.D. Teflon-sheathed type-T thermocouples ($t_o = 0.3$ s, Physitemp Instruments, Clifton, N.J.). In order to reduce the risk of catheter-as-

sociated clot formation, I.V. sodium heparin was given to adjust activated clotting times to 300–500 s, prior to central line placement. Femoral vessels were isolated surgically, and arterial and venous catheters placed and advanced to a level above the renal vessels, as confirmed by X-ray. During surgery, bupivacaine (0.5%) was infiltrated into wounds to mitigate post-operative pain. In one dog (Trial I-2), a femorally-placed pulmonary artery thermodilution catheter replaced the venous combination catheter. Blood and ventilator pressures were acquired through a Hewlett Packard 78532-B monitor/transducer system.

2.1. Gas/liquid ventilation (GLV)

Immediately prior to GLV, dogs were assessed for adequacy of general anesthesia, then given Pancuronium Bromide (2 mg) to inhibit shivering and spontaneous breathing. FIO_2 was increased to 100% and external temperature control discontinued. To serve as a cannula for both infusion and removal of PFC liquid, a 19-Fr. flat-wire reinforced Bio-Medicus® venous catheter (Medtronic, Eden Prairie, MN) was introduced through the suction port of the E.T. adapter, and advanced $\sim$45 cm to approximately the level of the carina (as confirmed by X-ray). This cannula was connected to the GLV apparatus described below.

GLV was performed using the PFC liquid 'FC-75' (3M Corporation, St. Paul, MN), a perfluorinated butyl-tetrahydrofuran isomer mixture [10,11]. A two-reservoir circuit (Fig. 1) was used to infuse and remove PFC from the lungs via the cannula, in cycle periods of 37 s (Trial I) or 16 s (Trial II). During timed PFC infusions ($t_{in} = 20$ s for Trial I, or 10 s for Trial II), PFC was pumped through the cannula by a continuously-engaged Travenol CPB roller-pump (Sarns, Ann Arbor, MI). A bypass loop, open during suction, allowed the roller-pump to divert (recirculate) PFC flow back into the storage reservoir whenever flow was not directed by line clamps V1–V3 into the animal. PFC was pumped continuously through an in-line 0.2 μm 'pre-bypass' filter, a primary heat-exchanger (Torpedo-T, Sarns, Ann Arbor, MI), and a combination silicone-membrane oxygenator/heat-exchanger (SciMed II-SM35, SciMed Life Systems, Minneapolis, MN). The oxygenator was supplied with 5–6.5 l/min O_2 (maximal device design rate), and the reservoir PFC was allowed to circulate and equilibrate with heat-exchangers and O_2, before GLV was initiated. The circuit tubing was constructed of S-50 HL TYGON® 3/8 and 1/2 in. I.D. class VI tubing (Norton/Performance Plastics, Akron, OH) save for a length of silicone tubing (Masterflex® 96410-73, Barrant Co., Barrington, IL) used in the roller-pump head in order to retain flexibility at cold temperatures. PFC suction was driven by a vacuum pump (model 107CAB18B, Thomas Compressors, Sheboygan, WI), and suction reservoir negative pressure was limited to -35 torr by a vacuum relief valve.

2.2. Gas/liquid ventilation protocol

Concurrent FC-75 lavage and gas ventilation (GLV) was performed for 18 min in Trials I and II ($n = 12$). This time was chosen, on the basis of preliminary work (data not shown), to achieve rapid systemic-cooling of greater than 5°C. For Trials I and II, the PFC recirculation rate within the GLV device (= PFC infusion rate, $\dot{V}_{inf}$) was set at 50 ml/kg per min. Immediately after a timed infusion of PFC into the lungs, PFC was removed as rapidly as the system vacuum allowed. Infusion of PFC for the next cycle began immediately after suction was discontinued. In 'cold' lavage experiments, PFC was chilled to $\sim$4°C prior to lung infusion (Table 1), whereas in normothermic (control) dogs, isothermic PFC was delivered to the dog within $\sim$2°C of tympanic temperature (T_{tym}). The PFC inflow and outflow temperature was measured continuously by a thermocouple inserted into the PFC path at the base of the delivery/removal cannula. Temperature data was collected throughout GLV, and for 22 min after GLV was completed. Arterial blood gas (ABG) samples were taken from the femoral arterial line before the start of GLV, and every 2 min during GLV. Following the post-GLV equilibration period, monitoring devices were removed and incisions closed.

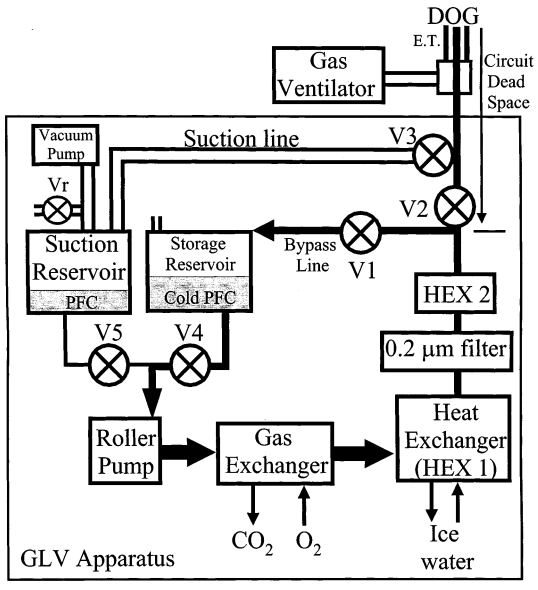

Fig. 1. The GLV lung lavage system. The GLV system was connected to a catheter inserted into the suction port of the E.T. adapter. Circuit PFC flows were directed by manual or mechanical clamps at V1–3. During the suction phase, PFC from the lungs was removed into a sealed 'suction' reservoir, for later addition to the primary circuit (via adjustment of V4 and V5), while 'infusion ready' PFC was re-circulated through a bypass loop. Negative pressure was limited by a vacuum relief valve (Vr).

Table 1
Summary of controlled variables in GLV[a]

	Dog mass (kg)	Initial tym. temp. (°C)	Total GLV duration (s)	PFC infusion temp. (°C)	No. of PFC cycles (n)	GLV cycle length (s)	Total PFC infused in GLV (ml/kg)	Flow in PFC infusion phase (V_{inf}) (ml/kg per min)
Trial I								
I-1 (cold)	20.7	38.5	1063	2.4	28	38	553	50.7
I-2 (cold)	16.4	37.6	1056	2.9	25	42	548	50.7
I-3 (cold)	22.0	38.6	1076	2.8	29	37	494	49.4
I-4 (cold)	25.0	37.7	1111	5.9	30	37	549	50.8
I-5 (cold)	18.7	38.4	1083	6.2	34	32	609	49.7
Mean	20.6	38.2	1078	4.0	29	37	551	50.3
SD	3.3	0.42	21	1.8	3.2	3.7	41	0.6
I-6 (warm)	16.9	37.7	1046	37.4	30	35	523	50.5
I-7 (warm)	21.5	36.7	1066	37.1	27	39	452	48.8
Trial II								
II-1 (cold)	19.0	36.9	1092	5.7	63	17	589	56.8
II-2 (cold)	13.8	35.7	1086	2.1	83	13	692	50.0
II-3 (cold)	18.2	36.4	1075	5.9	76	14	682	50.0
Mean	17	36.3	1084	4.6	74	15	654	52.3
SD	2.8	0.6	9	2.1	10	2.2	57	4.0
II-4 (warm)	17.5	37.1	1077	38.0	67	15	561	50.3
II-5 (warm)	17.6	36.1	1073	38.2	54	20	573	63.6
Additional experiments								
A[b] (cold)	21.8	37.0	600	4.4	1	600	76	32.4
B (cold)	25.7	36.8	6040	−0.3	15	403	354	6.07
C (cold)	24.5	39.7	4933	−0.5	21	235	682	6.00–16.7

[a] PFC, perfluorocarbon; GLV, gas/liquid ventiaaaaalation; tym., tympanic; temp., temperature; SD, standard deviation.
[b] Animal A was given the PFC FC-84 which has a heat capacity that does not differ significantly from FC-75.

Table 2
Summary of thermal results of GLV[a]

Trial	Maximum tym. ΔT (°C)	Net tym. ΔT_e (°C)	Total heat removed ΔQ_T (kJ/kg)	Mean heat transfer efficiency E_f	Mass-specific heat capacity C_m cal/g per °K (kJ/kg per °K)	Thermal dead space V_{Dtherm} ml/kg
Trial I						
I-1 (cold)	−7.5	−6.3	21.9	0.68	0.83 (3.5)	5.9
I-2 (cold)	−7.8	−6.6	19.8	0.67	0.72 (3.0)	6.2
I-3 (cold)	−7.5	−6.2	19.3	0.63	0.74 (3.1)	6.9
I-4 (cold)	−6.9	−5.8	14.7	0.53	0.61 (2.5)	8.9
I-5 (cold)	−7.7	−6.2	15.4	0.50	0.59 (2.5)	9.5
Mean	−7.5	−6.2	18.2	0.60	0.70 (2.9)	7.5
SD	0.35	0.28	3.1	0.09	0.1 (0.42)	1.6
I-6 (warm)		+0.5	N/A	N/A	N/A	N/A
I-7 (warm)		+0.2	N/A	N/A	N/A	N/A
Trial II						
II-1 (cold)	−6.1	−5.1	22.6[b]	0.74[b]	1.1 (4.4)[b]	4.5
II-2 (cold)	−5.1	−4.3	23.8[b]	0.61[b]	1.3 (5.5)[b]	5.9
II-3 (cold)	−5.3	−4.2	19.0[b]	0.59[b]	1.1 (4.5)[b]	5.5
Mean	−5.5	−4.5	21.8[b]	0.65[b]	1.2 (4.8)[b]	5.3
SD	0.5	0.5	2.5[b]	0.08[b]	0.15 (0.61)[b]	0.7
II-4 (warm)		+0.4	N/A	N/A	N/A	N/A
II-5 (warm)		+0.2	N/A	N/A	N/A	N/A
Additional experiments						
A (cold)	−1.9	−1.5	4.71	1.0	0.75 (3.1)	0.0
B (cold)	−8.4	−7.3	19.4	0.89	0.63 (2.7)	2.6
C (cold)	−12.5	−12.1	33.8	0.84	0.66 (2.8)	5.2
Mean	N/A	N/A	N/A	N/A	0.68 (2.9)	N/A
SD					0.06 (0.26)	

[a] PFC, perfluorocarbon; GLV, gas/liquid ventilation; tym., tympanic; SD, standard deviation; N/A, not applicable.
[b] See text Appendix A.3.

2.3. Trial I (manually-controlled GLV)

Trial I was designed to investigate the variability in individual animal response to GLV, and to investigate the physiological effects of GLV delivered with and without thermal stress. Either isothermic (near-body temperature) or cold PFC lavage was administered using a manually-controlled system (V1–V5 in Fig. 1 represent CPB tubing-occluders in this Trial). One lavage cycle (period $t_c \sim 37$ s) was composed of a timed PFC infusion ($t_{inf} = 20$ s), followed by PFC suction ($t_s \sim 17$ s). Suction was stopped when PFC liquid return became sparse, or gas pressure in the ventilator circuit fell below -5 cm H_2O (-0.5 kPa). Five dogs received cold PFC (Trial I-1–5), while two controls received the same protocol using isothermic PFC (Trial I-6 and 7).

2.4. Trial II (machine-controlled GLV)

Trial II assessed the utility of using an automated device (custom manufactured by Korr Medical, Inc., Salt Lake City, UT) to perform rapid-cycle GLV. Computer-controlled solenoid clamp-valve occlusion of circuit lines at V1–V3 allowed smaller lavage volumes (V_{lav}) and smaller t_c. While t_{inf} was decreased to 10 s in Trial II, $\dot{V}_{inf}$ remained constant, and the effective PFC lavage rate ($\dot{V}_{lav}$) remained in the range of $\dot{V}_{lav}$ for Trial I. Table 1 gives relevant trial parameters. In Trial II, suction removal of PFC from the lungs began immediately after infusion, and was automatically stopped whenever ventilator circuit pressure of -5 cm H_2O was reached ($t_s \sim 6$ s, giving $t_c \sim 16$ s). Three dogs received cold PFC (Trial II-1–3), while two controls (Trial II-4 and 5) received isothermic PFC.

2.5. Animals A, B and C

Selected data from three dogs in an earlier method-development series was used. These dogs had been prepared as above, then manually given 1, 15 and 21 lavages, respectively with cold PFC, at much slower rates than in Trials I and II (Table 1). Data from these animals allowed independent measurements of lavage-volume heat-contents and temperatures, and thus heat

capacities and heat transfer efficiencies, by a more thorough thermal accounting method (Table 2, Appendix A).

2.6. Data collection and correction, statistical methods, graphical display and presentation

Temperature and pressure data were collected using a PCI E series data acquisition board and LabView™ software (National Instruments, Austin, TX). Graphical analysis and display of temperature data, and curve fitting, was done using the software package Origin™ (Microcal Software, Northhampton, MA). Statistical comparison of Trial group values was done using GraphPad Prism (GraphPad Software, San Diego, CA). Group means are reported ± standard deviation (SD) except as otherwise noted. For each animal, the T_{tym} from whichever probe cooled most rapidly, was used (right probe in 12/15 dogs). In order to facilitate comparison of cooling rates between sites in the same animal, temperatures at all probe sites were corrected to the baseline aortic temperature (T_{art}), as measured immediately prior to the start of GLV. For ease of description, GLV-cooling is presented in terms of thermal-deficit ('cold') moving from the lungs into successive body compartments. A compartmental analysis of thermal transfer in this model, and a glossary of notation and equations used, is given in Appendix A.

3. Results

The GLV technique allowed cyclic liquid lung-lavage of dogs undergoing concurrent gas ventilation. Suction from a submerged catheter tip at the carina allowed direct distal collection of PFC even during forced gas inspiration. We found that a long suction catheter was necessary to insure that higher suction pressures could be used to directly withdraw the dense PFC throughout the liquid removal phase, without prolonged exposure of the gas filled portion of the airways to the negative pressure of the suction system/reservoir. The relief valve limited negative pressures in the suction reservoir, and also in the lungs, for the relatively brief time after liquid flow no longer blocked the suction line. Suction in this manner was efficient, although lavage volume measurements showed that the lungs chronically retain ~12 ml/kg PFC (approximately the pulmonary residual capacity).

The PFC pump circulation/infusion rate ($\dot{V}_{inf}$), measured volumetrically preceding and following GLV, was stable to within 1% over the duration of GLV, and was not significantly different between trials ($P = 0.28$). The $\dot{V}_{lav}$, calculated as $t_{inf}\,\dot{V}_{inf}/t_c$, was 30.7 ± 2.3 ml/kg per min (Trial I) and 36.4 ± 3.2 ml/kg per min (Trial II). The $\dot{V}_{lav}$ was significantly ($P = 0.023$) larger in Trial II because machine-controlled suction made more efficient use of available non-infusion time, resulting in faster net PFC removal.

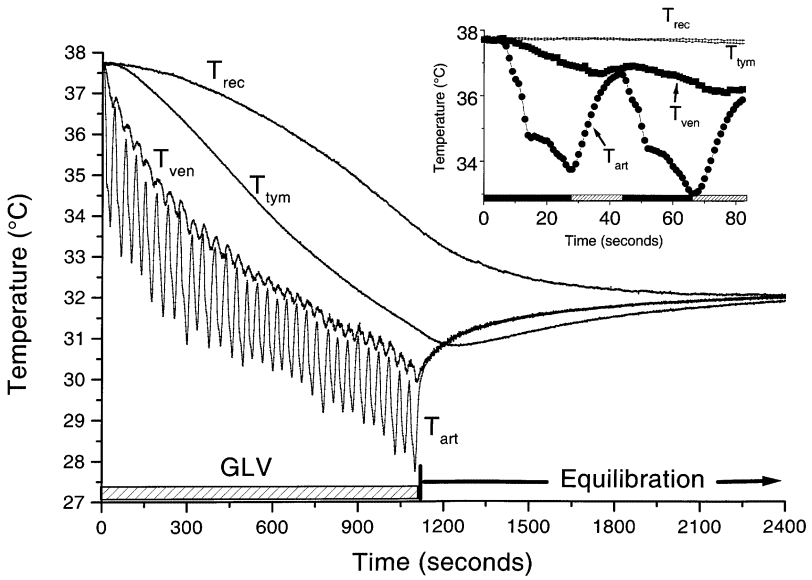

Fig. 2. Body temperature changes observed during GLV (Method of Trial I). In this illustrative experiment from Trial I (I-4), lavages of cold (4°C) FC-75 were infused (~20 s) and removed (~17 s) from the lungs. GLV was performed for 18 min (hatched bar), then stopped to allow thermal equilibration (22 min). Arterial temperature (T_{art}), central venous temperature (T_{ven}), tympanic temperature (T_{tym}), and rectal temperature (T_{rec}) are shown. *Inset:* Enlarged view of temperature changes recorded during the first two cycles of PFC infusion (dark bar) and removal (hatched bar).

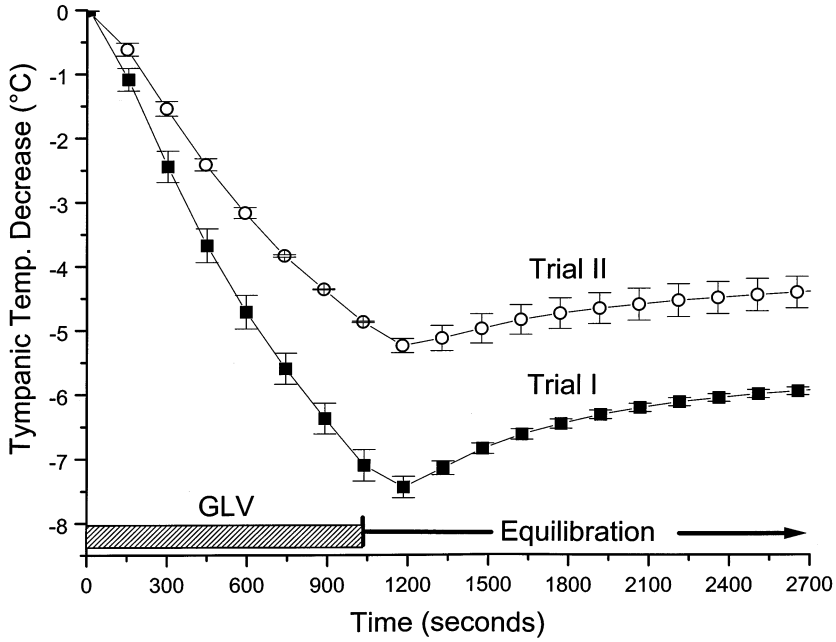

Fig. 3. Body temperature changes during manual and mechanical GLV (Trial I vs. Trial II). The relative rates of core body cooling in dogs undergoing 18 min (hatched region) of manual (Trial I, solid squares) or machine-driven (Trial II, open circles) cold GLV, were assessed by comparing changes in group mean $T_{tym.}$ Symbols represent the mean and SEM ($n = 5$ for manual, and 3 for machine groups).

3.1. Thermal results of GLV

3.1.1. Cooling time delay

Fig. 2 illustrates GLV cooling in a representative dog (I-4) from Trial I. The T_{art} began to decrease 3–6 s after the start of each PFC lavage. Since this delay included circulation delay from lungs to aorta, the transfer of thermal-deficit from newly-introduced PFC to pulmonary blood was very rapid.

The venous temperature (T_{ven}) began to decrease 10.4 ± 6.9 s after T_{art} decline, representing the minimum systemic circulation time. Though exhibiting delay, damping, and broadening behavior (presumably due to peripheral heat-exchange and varying systemic blood-return path lengths), T_{ven} transients from lavages mirrored T_{art} transients. T_{tym} temperatures, presumably reflecting brain and viscera temperatures, were non-oscillatory.

The T_{tym} did not begin to decrease until ~ 24 s after the start of GLV. This decrease occurred in three phases: an initial phase lasting ~ 100 s, an exponential phase lasting for ~ 900 s, and a final linearly-decreasing phase lasting until the end of GLV. Core cooling as measured by T_{tym} continued for about 120 s after the end of GLV (Fig. 3), then exhibited a marked rebound effect [12] with exponential dampening ($t > 20$ min, Figs. 2–4). These phases of cooling and equilibration were consistent with a five-compartment thermal model, in which the three compartments representing animal tissues corresponded roughly with (1) the blood and vasculature; (2) the classical thermal core; and (3) the classical thermal periphery (Fig. 5). Modeling equations and estimation of compartment sizes are given in the Appendix A.

3.1.2. Cooling rate

Crude cooling rates were determined numerically from appropriate T vs. t graph segments. The mean cooling rate from GLV initiation, or $\Delta T_{tym}/\Delta t$, reached a maximum value in Trial I at -0.49 ± 0.09°C/min ($t = 6.6$ min). The differential cooling rate $d(\Delta T_{tym})/dt = dT_{tym}/dt$ reached a maximum (max) value of -0.59 ± 0.13°C/min at $t \cong 100$ s, near the end of the initial heat exchange development region. (This value is comparable to analytic $d(\Delta T_{tym})/dt$ (max) from (Eq. (1)) $= \Delta T_k/t_o = -0.63$°C/min). Corresponding cooling rates in Trial II were $\Delta T_{tym}/\Delta t$ (max) $= -0.33 \pm 0.02$°C/min (at $t = 7.3$ min) and dT_{tym}/dt (max) $= -0.37 \pm 0.06$°C/min (at $t = 100$ s).

3.1.3. Mean cooling power

The mean heat removal rate (cooling-power) P over the entire duration of GLV, for each animal, was estimated from ΔT_e according to $P = m\ C_m\ \Delta T_e/t$ (total). Here t (total) is the entire GLV application time $= \sim 1080$ s. (Note: for this calculation, the more accurate Trial I mean C_m is used for all Trial II animals.) The mean cooling power of Trial I was 336 ± 60 watts, while that of Trial II (using the Trial I value of C_m) was 207 ± 49 watts ($P = 0.02$). Variation in animal size was the major source of intra-group variability.

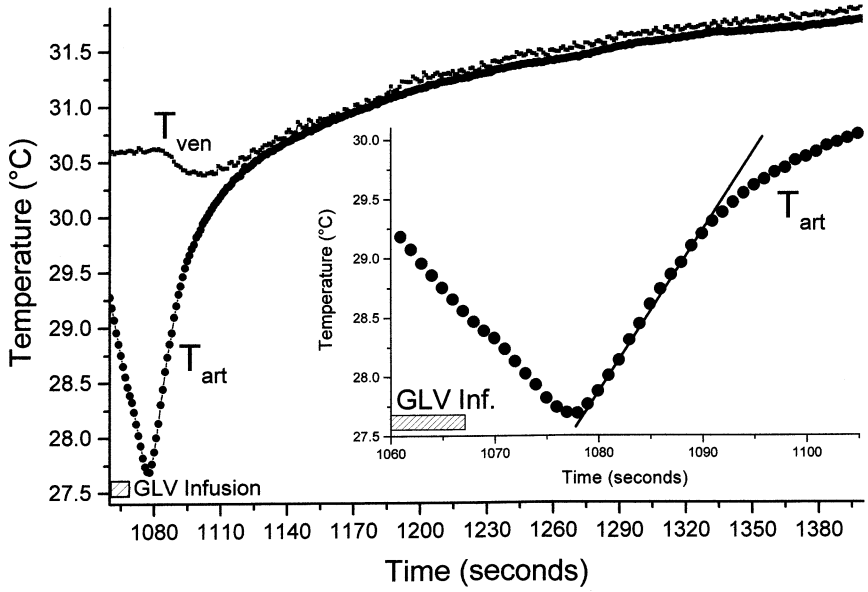

Fig. 4. Thermal equilibration after GLV. Mean T_{art} and T_{ven} values (Fig. 2) are shown for Trial I, dogs 1–5. To highlight equilibration changes, T_{art} curve nadirs ($n = 5$) were superimposed before calculation of means, and T_{ven} data ($n = 4$) for each dog was adjusted with its corresponding T_{art} curve. Incompatible T_{ven} data from a pulmonary artery thermodilution catheter in I-2 has been omitted. *Inset:* The sigmoidal mean ($N = 5$) T_{art} recovery during the first ~12 s after final GLV infusion halt is approximated by linear fitting.

3.2. Gas exchange

ABG measurements demonstrated that infusion of cold PFC stabilized PaO_2 and $PaCO_2$ during GLV. In contrast, GLV using isothermic PFC failed to maintain pre-treatment PaO_2 or $PaCO_2$ levels (Fig. 6). In Trial II-4, hypercapnia during the first 13 min of isothermic GLV was abolished by increasing the tidal volume from 15 to 25 ml/kg (final $\dot{V}_g = 375$ ml/kg per min). In Trial II-5, $\dot{V}_g$ was pre-set to 375 ml/kg per min in an attempt to avoid hypercapnia, and no significant ABG changes were observed.

3.3. Clinical observations and gross pathology

With the exception of one dog, animals subjected to GLV displayed mild tachypnea and increased expiratory sounds, but otherwise exhibited unremarkable recovery from anesthesia, including the ability to walk and drink. The exception was an eosinophilic animal (Trial II-1) which had normal oxygenation during GLV, but developed severe hypoxia shortly after GLV. Chest X-ray pre- and post-procedure showed no comparatively remarkable features. This dog was sacrificed at 9 h. Necropsy revealed a mass of *D. immitis* (heart-

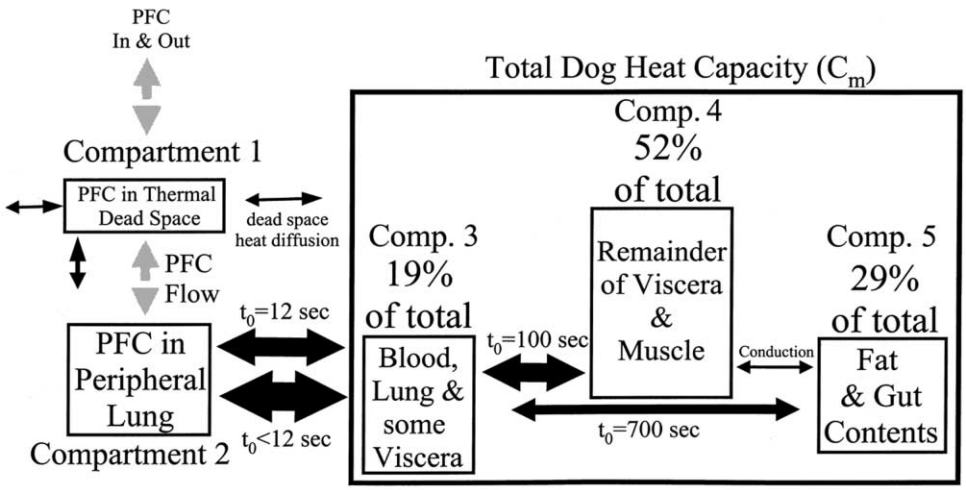

Fig. 5. Heat transfer among body compartments during GLV. Heat transfer during GLV in the dog may be modeled using 5 thermal compartments. Heat transfer between compartments (which is by blood circulation, except as noted) is shown in the box diagram as double-headed arrows. The pair of arrows connecting Compartments 2 and 3 represent the different processes of lung equilibration with (1) pulmonary artery flow; and (2) with the complete blood volume and selected viscera.

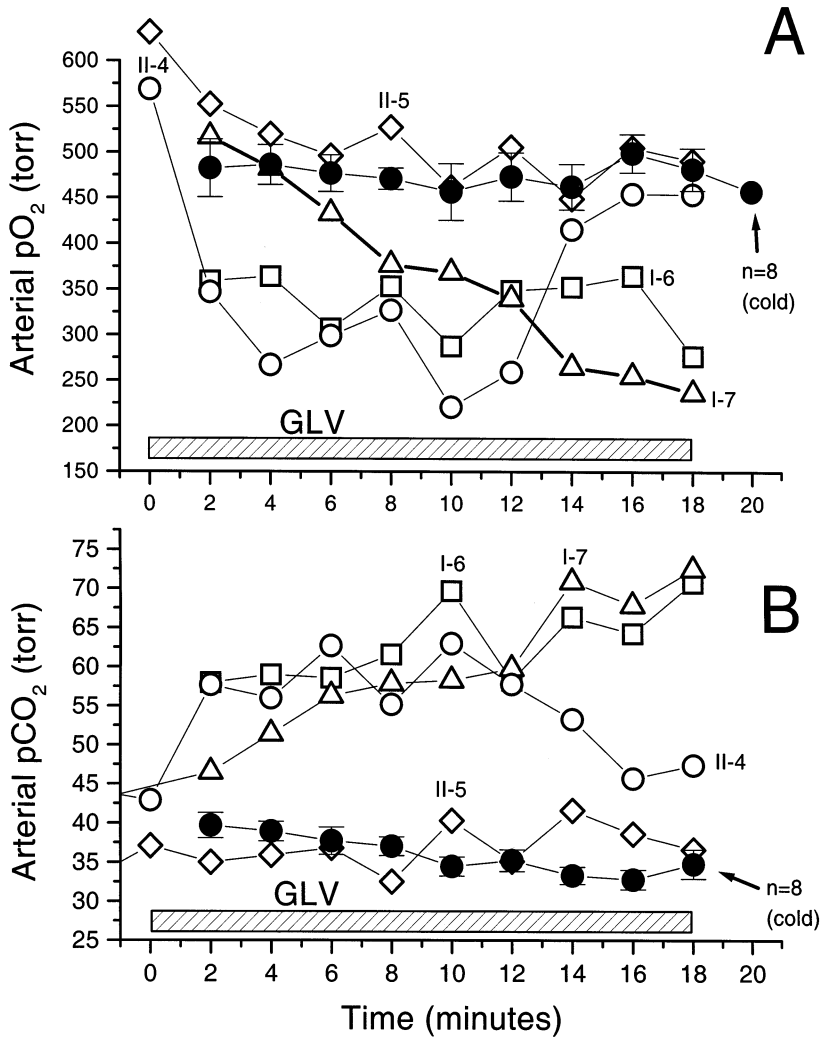

Fig. 6. GLV does not maintain normocapnia at isothermic temperatures without altering gas ventilator parameters. Animals in Trials I and II underwent GLV using either cold (closed symbols) or isothermic (open symbols) FC-75. Both arterial PaO_2 (Panel A) and $PaCO_2$ (Panel B) levels were affected by lavage temperature. Cold lavage data from Trials I and II were very similar in magnitude, and therefore, have been combined ($n = 8$). Isothermic GLV is shown as four separate experiments (Trial I-6 and 7, and Trial II-4 and 5). Gas tidal volume was increased from 15 to 25 ml/kg in Trial II-4 at $t = 13$ min, and at $t = 0$ in Trial II-5, normalizing PaO_2 and $PaCO_2$ in both animals. Declining PaO_2 in Trial I-7 was due to inadvertent failure to pre-oxygenate PFC.

worm) embolized into the pulmonary arterial circulation, possibly as a result of cold GLV (this animal had been heartworm seronegative). Necropsies performed on nine remaining Trial I and II animals sacrificed 24 h post-procedure revealed diffuse spongy, resilient 'foam rubber' lung lesions typical of lungs exposed to a high-vapor pressure PFC at high PIP pressures. This was noted especially in anterior portions of lung lobes. This trapped intraparenchymal PFC was thought to be the cause of broncho-constriction and wheezing found typical of post-GLV animals. There was gross-level evidence of dependent-lung damage with alveolar-filling in both isothermic and cold PFC-lavaged animals. Other organ systems in this series were grossly normal. Two animals in Trial II (II-3 and II-4) were not sacrificed, and were held for long term evaluation. They were neurologically normal at 1 year post-GLV.

4. Discussion

4.1. Apparent effect of temperature on gas exchange

Isothermic GLV in our model was surprisingly poor at removing CO_2, considering that the CO_2 carrying capacity in FC-75 decreases by only $\sim 23\%$ from 0 to 40°C (extrapolated from [11]). A useful observation was that even pO_2 values decreased in isothermic animals, indicating an extreme influence on total ventilation. Capnographic analysis of GLV in Trials I and II (data

not shown) indicated that isothermic GLV had a much larger negative effect on pressure-limited total gas ventilation $\dot{V}_g$, as compared to cold GLV using the same technique and the same gas ventilator settings. Since GLV at a $\dot{V}_{lav}$ of 30–36 ml/kg per min relies on gas ventilation $\dot{V}_g$ for $>50\%$ of total alveolar ventilation, a differential loss of pressure-limited $\dot{V}_g$ with temperature appeared to be the basis of CO_2 retention in isothermic GLV. The mechanism of the implied differential change in lung compliance is unclear. However, gas ventilation adjustments similar to those in Trial II- 4 and 5 may be required if GLV is used as a re-warming technique.

4.2. Thermal transfer efficiency and kinetics

The optimal GLV cooling (or warming) protocol remains unknown. However, the finding that the thermal equilibration of non-dead space PFC and local pulmonary blood flow proceeds very rapidly ($t_o < 12$ s) suggests that PFC liquid infusion times need to be no longer than this time scale. When PFC-bolus lung residence times exceed this duration, the lavage bolus is in place longer than is required to transfer the most labile part of its thermal potential to the pulmonary blood and parenchyma. Since PFC ventilation rates ($\dot{V}_{lav}$) in the present study are already at least a third of the maximal rates possible in TLV, it seems probable that PFC infusion rates and pressures, rather than heat transfer rates from PFC to lung, will be the fundamentally limiting factor to power transfer in GLV. Our observations suggest that, as least to $\dot{V}_{lav}$ rates of 30 ml/kg per min and $\dot{V}_{inf}$ rates of 50 ml/kg per min, the total cooling power (cooling rate) in GLV will be greatest if no lavage dwell time is allowed, and all available time during the respiratory cycle is used to either introduce, or remove, PFC.

4.3. Question of diffusion dead space in GLV

Mammalian lungs depend on simple gas diffusion for CO_2 transport through acinar airways during normal tidal ventilation. An intractable problem in experimental TLV has been that simple diffusion is not sufficient to similarly move CO_2 through liquid PFC at physiologic CO_2 partial pressure gradients. This limitation appears physiologically in liquid ventilation as a 'CO_2 diffusion dead space' which effectively lowers alveolar ventilation. In part due to such extra physiologic dead space, TLV of adult humans has been estimated to require liquid minute-volumes near 70 ml/kg per min [14]. This value is at the upper bound of realistically attainable liquid flow rates [15,16], and leaves little leeway for treating hypermetabolic or acidotic states, or lung disease. Such difficulties are not a theoretical limitation in GLV, however, since GLV does not require high liquid flow rates for ventilation. In the most rapid-cooling GLV protocol used in this trial, $\dot{V}_{lav}$ was 31 ml/kg per min—a low baseline value which permitted the addition of 10 times this minute-volume of gas ventilation (see Fig. 4, Trial II-4,5). Moreover, since normal gas minute volumes were required to maintain normocapnia in Trial I, there is as yet no evidence in GLV for any CO_2 diffusion limitations caused by PFC in the lungs. Possible reasons for this are discussed below.

Thermal-diffusion limits in TLV have not been studied per se, but their presence is suggested by the results of Shaffer and coworkers [8]. In the cat TLV model using a $\dot{V}_{lav}$ of 75 ml/kg per min, a decrease in PFC inspiration temperature from 20 to 10°C (increasing the thermal gradient by a factor of 1.6) increased the cooling rate from -0.13 to -0.15°C/min. This small rate change represented a significant loss of efficiency. By contrast, in the present GLV study using PFC at 4°C, there was no evidence of a thermal-diffusion limit at rates up to 4 'liquid-breaths'/min. Notably, in Trial I, where 100% of the V_{lav}, and 40% of the $\dot{V}_{lav}$ of the cat TLV model was used, cooling rates for GLV were more than three times those reported for cats on TLV at 4.5 liquid breaths/min at 10°C [8].

The possible quantitative presence of a thermal diffusion limit for GLV at 4 liquid breaths/min may be evaluated using a modified version of the concept of gas-exchange dead space (V_D). The respiratory system of a dog undergoing GLV heat-exchange may be considered, by analogy with gas exchange dead space (V_D), to also contain a 'thermal exchange dead space' (V_{Dtherm}). Each thermal lavage volume V_{lav} (analogous to a liquid breath) of PFC then also contains a V_{Dtherm}, which by definition does not participate in heat-exchange. Thus, cycle thermal transfer efficiency E_f may be expressed as $(V_{lav} - V_{Dtherm})/V_{lav}$, and any measured value of mean E_f may be expressed as an equivalent mean $V_{Dtherm} = V_{lav}(1 - E_f)$. For Trial I ($E_f = 0.6$, Appendix A for calculation), mean V_{Dtherm} was then seen to be 7.5 ± 1.6 ml/kg, and in Trial II (using Eq. (6) E_f value $= 0.40$), V_{Dtherm} was 5.3 ± 0.8 ml/kg ($P = 0.072$). The absence of an increase in V_{Dtherm} in Trial II vs. Trial I indicated that the size of V_{Dtherm} in these GLV protocols was non-dynamic at time-scales of one lavage period, providing evidence against the presence of a 'thermal diffusion dead space' (analogous to a CO_2 diffusion dead space) at these lavage rates.

In absolute terms, it may be useful to compare calculated V_{Dtherm} in the GLV dog model to the expected physiologic gas-exchange dead space, V_{DCA}, which in healthy animals is close to the dog anatomic $V_D = \sim 6.5$ ml/kg [17]. In thermal diffusion as for gas diffusion, diffusion physiologic dead space would be expected to significantly add to anatomical dead space. However, the sum of mechanical-V_D in the GLV circuit (~ 1.5 ml/kg) plus the anatomic V_D for dogs, is found

to be more than the calculated V_{Dtherm} in either trial in this study, leaving little room for a large heat-diffusion contribution to V_{Dtherm}. For these reasons it is suggested that the loss of cooling power observed in Trial II was not due to heat diffusion limitations, but instead due to a loss of efficiency effect similar to that seen with low tidal volumes in ordinary gas ventilation. In these terms, low lavage volumes in GLV result in an increase in 'thermal dead space ventilation' at the expense of PFC flow involved in active heat-exchange, resulting in a larger 'wasted' lavage fraction V_{Dtherm}/V_{lav}.

V_D in heat transfer (V_{Dtherm}) is analogous to V_D in gas-transfer, in as much as all dead space is 'diffusion dead space' at long-enough time-scales. However, some of the mechanisms for diffusion modification of V_{Dtherm} are unique. By contrast with gas molecules, heat diffuses rapidly through device tubing into PFC in the GLV circuit dead space, and also diffuses directly through the tracheal wall into the anatomic-V_D. Thus, heat diffusion from dead space liquid at sufficiently slow lavage rates might be expected to have a pronounced effect on E_f in GLV, due to slow heat-diffusion reduction in $V_{Dtherm.}$

Some evidence for such a process was found, though at lavage dwell times too long to be of interest for rapid cooling. At the relatively small t_c of Trials I and II, the calculated V_{Dtherm} was found to be $\sim V_{DCA}$; but in animal B, with a much longer t_c of 7 min, the V_{Dtherm} was only 2.6 ml/kg. The limit of this process was reached in animal A, in which the V_{Dtherm} of a single retained 'breath' of highly-oxygenated PFC fell to nearly zero after 10 min. Disappearance of V_{Dtherm} by thermal equilibration, estimated from individual cycle E_f variations in animals B and C, was estimated to occur with a half-time of ~ 5 min (data not shown). This process was slow enough to be neglected when GLV lavage periods (t_c) were less than several minutes. Thus, at the lavage rates of Trials I and II, a full-sized V_{Dtherm} of ~ 6 ml/kg appeared, and accounted for significant loss of cooling power at low V_{lav} (e.g. Trial II where V_{lav} was only 8.8 ml/kg).

The characteristic size of V_{Dtherm} at all but the slowest lavage rates (< 1 lavage per 5 min) implies that the only thermally-efficient solution for performing GLV at faster rates is maintenance of [V_{lav}/V_{DCA}] or [V_{lav}/V_{Dtherm}] ratios > 3, in order to avoid excessive 'wasted' V_{Dtherm} ventilation. This requires a V_{lav} of ~ 18 ml/kg in dogs. In humans, where anatomic-V_D is < 3 ml/kg, less than half the value for dogs, both the V_{Dtherm}, and therefore, most-efficient V_{lav} values, might also be expected to be correspondingly less. In any case, it is clear that rapid-cooling GLV techniques cannot wait for the relatively slow thermal equilibration of PFC within the anatomic V_D, since equilibration in the remaining non-V_D parts of the lung is so rapid (i.e. less than Trial II t_c of 16 s).

4.3.1. Possible synergy of combined gas and liquid ventilation in assisting mass (CO_2) and heat transfer

The absence of expected heat-diffusion and gas-diffusion limitations in GLV suggests that some assistive process for both gas and heat transfer through PFC in the peripheral lung may occur in GLV. The authors' fluoroscopic observations (made with the non-brominated and relatively radiolucent FC-75) have been that each gas breath in PLV produces a flash of fine bubbles which spread uniformly throughout the lung. As compared to the more familiar behavior of water, the low surface tension of PFCs (15 dyne-cm for FC-75, about 1/5th that of water) lowers the energy barrier to producing small bubbles in forced gas/liquid flows. Such bubbles moving within small airways may induce eddies and turbulence in laminar liquid flows at small scales, contributing significantly to heat and mass (CO_2) transport though PFC liquid by means other than diffusion. We hypothesize that the lack of bubble-induced turbulence in TLV may account for the large diffusion-dead-space for heat and CO_2 which seems to be present in TLV at even low breathing rates — an effect which is apparently absent in both PLV and GLV.

4.4. Potential development of clinical GLV

Rapid brain cooling has become a goal of resuscitation research. Based on their work, Safar et al. have noted that clinical implementation of mild resuscitative hypothermia, which is effective in large animal models, will depend on the development of rapid mild brain cooling methods [18]. A recent editorial in 'Stroke' [6] commented on the striking ability of combination mild hypothermia and pharmacological pre-treatment to ameliorate ischemic brain damage in the cerebral artery occlusion rat model, then addressed similar concerns:

> A problem for use of this technique for acute stroke therapy is that the time required to induce hypothermia in patients is likely to be considerably longer than for rats. […]. To substantially increase the rate of hypothermia induction in humans, it will almost certainly be necessary to use some sort of invasive procedure, such as a heat-exchanger, to cool the circulation.

The technique of GLV may eliminate the need for such invasive measures. For example, in the cited trial [19], rats were cooled from 37 to 33°C (-4°C) over 40 min, using external ice packs. By contrast, the present study finds cooling of the canine body core and brain by -4°C in less than 10 min.

Development of clinical GLV awaits identification of suitable PFCs for various GLV applications. For example, the pharmaceutical PFC perfluorooctylbromide

(Perflubron®, Alliance Pharmaceuticals) would presumably not be suitable for fast GLV cooling due to its freezing point of +3°C, but might be useful for slower cooling or for GLV re-warming. Some industrial PFCs have pour-points low enough to make them potentially useful as GLV rapid-cooling media; however, some of these also have unsafely-high vapor pressures at 37°C. Such low-boiling point PFCs apparently exacerbate barotrauma injury by adding intraparenchymal PFC-vapor damage to lung pathology. They may also increase the danger of lung compression due to escaped PFC entering the pleural space and vaporizing (so-called 'fluorothorax'). FC-75, (formerly named FX-80) is historically the oldest of the PFC liquid ventilation media [10], but its relatively high vapor pressure probably makes it a less than optimal GLV agent.

Assuming that a PFC with the correct biophysical properties is identified and produced to medical standards, GLV should be easily scalable to the human adult. For example, the viscosity of FC-75 is similar to water [11], and under standard suction a 19 Fr. adult pulmonary toilet catheter will remove FC-75 at ~2 l/min. As in the system described, a GLV system may interface with a conventional gas ventilator system via a simple liquid-carrying catheter which extends through the endotracheal tube adapter suction port.

5. Conclusions

GLV is capable of inducing hypothermia in a fraction of the time that it takes to prepare a patient for cooling via CPB. In addition, automated GLV need not have the spatial and technical restrictions of the hospital setting. Although relatively simple pumpless methods of continuous arteriovenous shunt heat-exchange have been described which might be potentially applicable in the field [20], even these have the drawback of requiring skilled cannulation of a major artery. Since the primary technical skill required to initiate GLV in the field would be endotracheal intubation, GLV by contrast may be a candidate for a much wider range of emergency field-uses in civilian and military settings. GLV holds promise for central warming in severe hypothermia, although an absolute maximal PFC temperature of 42°C would in theory limit the re-warming rate to about one-third of that possible in cooling. GLV has potential as a very rapid treatment for heatstroke and malignant hyperthermia. Whether used inside or outside hospitals, successfully implemented GLV might serve more generally as a neuroprotective bridge [21] in order to gain time for more technical, supportive, or definitive treatment (e.g. neurovascular clot disruption, emergency CPB, cord decompression or hemorrhagic shock/trauma surgery).

Although certain types of liquid breathing are being clinically tested [22], the safety parameters of rapid and cold liquid delivery to the lungs remain to be determined. As noted in this study, GLV can cause lung damage. While the mechanism of such damage is unclear, the placement of lesions suggests that both barotrauma (dependent lung) and volu-trauma (non-dependent lung) may occur. We have generally observed that GLV causes little permanent lung damage in long term survival models. Similar pathology seen in lungs exposed to either isothermic or cold GLV in the present study (data not shown) suggest that thermal/chilling-injury per se is not the major insult in such gross damage. Although more subtle lung biochemical and immune problems (pneumonia) from hypothermia itself are suggested by reports from some longer duration therapeutic hypothermia studies [23,24], it is not clear that the minimal duration treatment necessary for effect in post-resuscitative hypothermia greatly pre-disposes to such problems. We hypothesize that lung pathology in GLV may be reduced with better control of GLV pressure and volume limits, and by use of PFC liquids having more physiologically suitable properties.

Acknowledgements

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Appendix A

A.1. Abbreviations and notation

In the text, volumes (V) are given in ml/kg, and flows ($\mathrm{d}V/\mathrm{d}t = V' = \dot{V}$) in ml/kg per min. Since all V and $\dot{V}$ are expressed in mass-specific (per kg animal) terms, derived quantities ΔQ and C_m are automatically mass-specific. C_m and C_vf are given in calories/(g or ml) per °K for easy comparison with water.

PFC: Perfluorochemical. Hydrogen-free organic molecule in which most peripheral atoms are fluorine. *TLV:* Total liquid ventilation. Modality in which only liquid fills the lungs and ventilator. *PLV:* Partial liquid ventilation. Modality in which all gas exchange is via gas ventilator, with ~1/2 FRC of PFC liquid residing in the lungs to help open dependent alveoli. *GLV:* Gas/liquid ventilation. Heat-exchange modality in which ventilation is by both gas ventilator and PFC lavage.

T_{tym} tympanic temperature
T_{art} arterial temperature
T_{ven} central venous temperature
T_{rec} rectal temperature
ΔT_e net ΔT_{tymp} resulting from GLV, after equilibration at $t = 40$ min
t_{inf} lavage cycle infusion time
t_s lavage cycle suction time
t_c lavage cycle period ($= t_{inf} + t_s$)
V_{lav} single-cycle PFC lavage infusion volume = $t_{inf}\,\dot{V}_{inf}$
V_S single-cycle PFC lavage suction-return volume
V_D ventilatory dead space (any type)
V_{DCA} expected gas ventilation V_D = sum of circuit (mechanical) V_D plus anatomic V_D
V_{Dtherm} thermal or heat-exchange V_D (ml/kg, in reference to liquid PFC infusion)
$\dot{V}_{inf}$ PFC infusion rate (set to $\cong 50$ ml/kg per min in Trials I and II)
$\dot{V}_{lav}$ effective PFC lavage rate = GLV liquid minute-ventilation (ml/kg per min) = V_{lav}/t_c
$\dot{V}_g$ gas minute-ventilation (ml/kg per min)
m animal mass
C_h heat capacity
C_T total heat capacity of the animal ($= mC_m$)
C_m mean mass-specific heat capacity of the animal ($= \Delta Q_T/\Delta T_e$)
C_{vf} volume-specific heat capacity of FC-75 (mean of 0 and 25°C values) = 0.45 cal/ml per °K
ΔQ_T total heat removed during GLV (kJ/kg animal) = $\Sigma\,\Delta Q_c$
ΔQ_c heat removed during one lavage cycle
E_f mean cycle heat transfer efficiency = mean of [ΔQ_c/(theoretic ΔQ_c (max)] for all cycles in a single experiment
n number of lavage cycles in GLV experiment
Σ sum entire quantity following, for all cycles $i = 1$ through n
T_{inf} PFC infusion temperature
T_S PFC suction removal temperature (time-averaged PFC suction flow temperature)
T_{SM} PFC mixed suction return-volume temperature (temperature of mixed V_S)

A.2. Thermal kinetics

During GLV cooling and equilibration, the blood and tympanic temperature changes in the animals were modeled by a simple five compartment model (Fig. 5). During the initial ~ 100 s of GLV (value used as empiric time mark), full development of heat-exchange behavior is established between the lungs, blood volume, and the thermal core of the animal, as suggested by the characteristic half-times for equilibration of these systems (see below).

Modeling of cooling during GLV: After the initial ~ 100 s of cooling, the data for tympanic $\Delta T(t) = \Delta T_{tym}$ during GLV in Trial I and II were modeled by a single time-constant exponential decline. Mean ΔT_{tym} data for each trial from times $t = 100$ to 1080 s were fit using (Eq. (1)).

$$\Delta T(t) = T_{100} + \Delta T_k[1 - \exp(-t/t_o)], \quad (1)$$

$\Delta T(t)$, total T_{tym} change from baseline T_{tym} at start of GLV; t, = time in seconds after empiric time mark, 100 s after start of GLV; T_{100}, observed ΔT at empiric time mark, 100 s after start of GLV; ΔT_k, observed temperature-interval constant, specific to each GLV method; t_o, observed natural-base time-constant, in sec (t_o = half-time/ln 2).

Best-fit values for Trial I data were: $T_{100} = -0.52 \pm 0.02$°C; $\Delta T_k = -11.2 \pm 0.02$°C; and $t_o = 1064 \pm 3$ s. Trial II values were $T_{100} = -0.24 \pm 0.02$°C; $\Delta T_k = -8.14 \pm 0.02$°C; and $t_o = 1107 \pm 5$ s. The relatively long time-constant associated with this thermal phase, which was similar in the two trials, presumably reflects the long time-constant (~ 700 s, see below) associated with heat transfer from the thermal core of the animal to the thermal periphery; thus the exponential phase represents full development of heat exchange between the GLV cooling device and the entire animal. The final linear segments of cooling occurring after this phase, measured at -0.29°C/min (Trial I) and -0.21°C/min (Trial II), represent the final relatively simple state which exists after heat exchange equilibrium between cooling device and animal has been fully established.

Cooling in blood and tympanic sites during GLV, and thermal evolution in these sites during equilibration phase after GLV was discontinued, was in accordance with a five-compartment thermal model (Fig. 5). In this model, the tissues of the animal are divided into three thermal compartments, corresponding loosely with the vascular system, the thermal core, and the thermal periphery.

Modeling of equilibration after GLV: Circulatory forced-convection is the major heat transfer mechanism in very rapid whole-body cooling processes. This fact allowed T_{art} and T_{ven} changes to be used to quantitate some features of heat transfer between body thermal compartments during the equilibration period after GLV. The mean T_{art} curve in Trial I increased nearly linearly ($R^2 = 0.9976$) for 12 s after the end of GLV, rising at a rate of 7.9°C/min. After this initial 12 s, T_{art} departed from linearity (Fig. 4, inset), and was modeled by the sum of three exponential terms with respective time constants (t_0) of 12 ± 0.4, 102 ± 2 and 701 ± 8 s. These t_0 times differed to a large enough extent that

their respective influences could be considered to be controlling over discrete time periods of about twice their value. Thus, the four *equilibration phases* seen after the end of GLV lasted approximately 12, 24, 200, and 1400 s (23 min), respectively and represented 34, 14, 25, and 27% of the 5.1°C rise in T_{art} during equilibration after GLV.

These data may be interpreted as follows: during each phase of the equilibration process, one or more thermal compartments in the animal equilibrated with the next-most closely-connected compartment (Fig. 5). Afterwards, the newly captured compartment(s), as part of a larger unit bound together by blood convection, equilibrated with the next-most closely connected compartment, and so on. The 12 s linear *first equilibration phase* (Fig. 4, inset) most likely represents development of heat transfer from lungs to local pulmonary blood flow. This phase was not associated with blood recirculation since it was seen as a rise in T_{art} but not T_{ven}. The *second equilibration phase* (duration ~ 24 s) was characterized by an increase in dT_{ven}/dt to the value of dT_{art}/dt, indicating that the lungs, blood-volume, and certain other well-perfused viscera, such as the kidneys, were now evolving into a single thermal system. Since the observed t_o for this phase was 12 s, less than the animal's mean circulation time (= cardiac output/blood volume $\cong 30$ s), this process appeared to be driven by blood circulation via the most rapid paths (e.g. renal circulation). Such short paths for circulatory heat transfer were evident in the relatively small lag times (10.4 ± 6.9 s) noted between T_{art} and T_{ven} changes in these animals.

During the first two equilibration processes, the pulmonary circulation added thermal potential to the blood-volume more rapidly than it could be removed by the entire systemic circulation. By the end of the *second equilibration phase*, however, lung-to-blood heat transfer no longer dominated, and the gap between T_{art} and T_{ven} was set by the magnitude of heat transfer from the blood-volume to remaining 'thermal core' systemic tissues. In this *third equilibration phase* (duration ~ 200 s), the viscera and blood-volume, as a unit, equilibrated with the remainder of the 'well-perfused' tissues of the body (thermal core, comprising about 70% of the animal's heat capacity). Heat capacities for thermal compartments are calculated below. The t_o for this process is seen most directly in the ~ 2 min. delay between maximal ΔT_{ven} and maximal ΔT_{tym} (Fig. 3).

Finally, heat transfer within well-perfused tissues fell to a new minimum, and the T_{art} to T_{ven} gap decreased to a value set by the *fourth equilibration phase* (duration ~ 23 min) during which the well-perfused tissues equilibrated, as a unit, with a succession of more poorly-perfused compartments, e.g. gut contents, fat, and other tissues comprising the thermal 'periphery' [12]. These processes could be consolidated into a single exponential term. Due to the long time-scale, heat transfer during phase four was probably partly conductive. Estimates of basal metabolism in the anesthetized, non-shivering dog ($\cong 90$ J/kg per minute) indicate also that as much as 0.6°C of warming per 20 min in this model may be due to metabolism.

A.3. Thermal accounting

Heat transfer efficiency: Although machine-GLV allowed 2.3 times the lavage frequency of the manual method, and resulted in a larger $\dot{V}_{lav}$ by a factor of 1.2, the cooling magnitudes and rates for machine-GLV significantly ($P < 0.001$) fell short of those obtained with manual-GLV (Fig. 3). The strategy of increasing lavage frequency ($1/t_c$) and decreasing V_{lav}, in order to arrive at approximately the same lavage rate ($\dot{V}_{lav}$), therefore, significantly decreased the fraction of thermal potential which was transferred from each lavage (= heat transfer efficiency, E_f).

Unexpectedly, when the E_f for each of the 8 GLV-cooled animals of Trials I and II (Table 2) was calculated using (Eq. (2)), the value did not differ ($P = 0.46$) between trials; nor did total heat removed per kg (ΔQ_T), as calculated using (Eq. (3)), differ ($P = 0.14$) between Trials. Both of these quantitative methods were therefore inaccurate for some dogs. Calculation of whole-animal mass-specific heat capacities C_m ($= \Delta Q_T/\Delta T_e$) suggested that the Trial II values of ΔQ_T and E_f principally were inaccurate, since the mean C_m value of 0.70 ± 0.1 cal/g per °K for Trial I was consistent with the C_m reported in the literature for mice and humans [13], whereas C_m values calculated for Trial II using (Eq. (2)) were unrealistic, being greater than the C_m of water.

$$E_f\,(\text{method 1}) = 1/n\sum (T_S - T_{inf})/(T_{ven} - T_{inf}), \tag{2}$$

$$\Delta Q_T\,(\text{method 1}) = V_{lav} C_{vf} \sum T_S - T_{inf}. \tag{3}$$

To independently check the accuracy of Trial I values, we computed ΔQ_T and C_m for three dogs from an earlier study (Tables 1 and 2: dogs A, B, and C) that had been given cold PFC with lavage times sufficiently long to allow the volumes and mixed-temperatures of suction-return liquid to be measured for each lavage. This allowed computation of ΔQ_T and E_f by a more detailed method (Method 2, Eqs. (4) and (5)), which used the extra thermal data (not available for Trials I and II) to more directly estimate lavage heat transfer.

$$\Delta Q_T \text{ (method 2)}$$

$$= C_{vf} \sum V_{inf}(T_{ven} - T_{inf}) - V_S(T_{ven} - T_{SM}), \tag{4}$$

$$E_f \text{ (method 2)} = 1/n \sum 1 - \left[\frac{V_S(T_{ven} - - T_{SM})}{V_{inf}(T_v - T_{inf})} \right] \quad (5)$$

When this was done, the mean C_m for dogs A, B, and C was found to be 0.68 ± 0.06 cal/g per °K, consistent with the C_m in Trial I ($P = 0.80$). Method 1 (Eqs. (2) and (3)) required the assumptions that PFC suction volume equaled infusion volume, that suction flows remained constant, and that thermal hysteresis was negligible. These assumptions apparently held true at the larger V_{lav} and t_c values of Trial I, but not for the smaller values of Trial II.

With this information, a new E_f for Trial II was estimated using method 3 (Eq. (6)), which employed an estimate for the heat required for the observed ΔT_e, vs. the total PFC thermal-deficit theoretically available. This estimate required a presumed value of C_m. However, if the mean Trial II C_m was assumed to be the same as that of Trial I, then the true Trial II E_f could be calculated (Eq. (6)) to be 0.40 ± 0.06.

$$E_f \text{ (method 3)} = \frac{C_m \Delta T_e}{C_{vf} V_{lav} \sum T_{ven} - T_{inf}} \quad (6)$$

This value agreed with the rough estimation that since Trial II achieved only 73% of the ΔT_e of Trial I, despite using 1.19 times more total PFC (Table 1), the E_f in Trial II was expected to be about $73\%/1.19 = 61\%$ that of Trial I.

Thermal compartment size: Heat removal for each cycle (ΔQ_c) in Trial I was calculated from the individual terms of Eq. (3), and individual-cycle mean cooling-power calculated as $\Delta Q_c/t_c$. The latter parameter was useful since thermal compartments in the dog are relatively isolated at short time scales (Fig. 5), and thus the ratio of cooling-power to cooling rate (Eq. (7)) at a given probe site was expected to give the heat capacity (C_h) of the system of thermal compartments that were in equilibrium with each other, and with the site, at the time of the measurement:

$$C_h \text{ (Comp}N) = \frac{m\ [\Delta Q_c/t_c]}{[dT(t)/dt]} \quad (7)$$

C_h (Comp N), total heat capacity of thermal compartments $N = 2 + 3$, or $N = 2 + 3 + 4$.

Both t_c and dT/dt values were picked at a time t, of interest when compartment system N has not yet equilibrated with slower half-time compartment(s). Thus, in Trial I, near the end of lavage cycle $\#1$ ($t = 30$ s), the lavage thermal-deficit had equilibrated within thermal Compartments $2 + 3$ (PFC/viscera/blood-volume), but had not yet significantly reached Compartments 4 or 5. If the cooling rate of T_{ven} at $t = 30$ s (-1.9 ± 0.8°C/min) was then taken as the cooling rate of the system of PFC/viscera/blood-volume, the C_h for this compartment system could be estimated from (Eq. (7)) as $20 \pm 9\%$ of C_T, the total heat capacity of the animal ($C_T = m\ C_m$). Subtracting the C_h contribution of lung PFC ($= m\ V_{lav}\ C_{vf}$) allowed estimation of the remaining tissue C_h for Compartment 3 as $\sim 19 \pm 9\%$ of C_T. Similarly, the C_h of Compartments 2, 3, and 4 together, was estimated at $t = \sim 140$ s (cycle 4) as $71 \pm 17\%$ of C_T, corresponding to the classical whole-body 'thermal core.' The Compartment 5 C_h was then calculated to be the remainder $(100 - 71\%) = 29 \pm 17\%$ of C_T, corresponding to the classical 'thermal periphery.'

References

[1] Barone FC, Feuerstein GZ, White RF. Brain cooling during transient focal ischemia provides complete neuroprotection. Neurosci Biobehav Rev 1997;21:31–44.

[2] Buchan A. Advances in cerebral ischemia: experimental approaches. Neurol Clin 1992;10:49–61.

[3] Thoresen M, Wyatt J. Keeping a cool head, post-hypoxic hypothermia — an old idea revisited [see comments]. Acta Paediatr 1997;86:1029–33.

[4] Kuboyama K, Safar P, Radovsky A, Tisherman SA, Stezoski SW, Alexander H. Delay in cooling negates the beneficial effect of mild resuscitative cerebral hypothermia after cardiac arrest in dogs: a prospective, randomized study [see comments]. Crit Care Med 1993;21:1348–58.

[5] Marion DW, Leonov Y, Ginsberg M, Katz LM, Kochanek PM, Lechleuthner A, Nemoto EM, Obrist W, Safar P, Sterz F, Tisherman SA, White RJ, Xiao F, Zar H. Resuscitative hypothermia. Crit Care Med 1996;24:S81–9.

[6] Zivin J. Editorial comment. Stroke 1999;30:1899.

[7] Reed C, Clark D. Heat exchangers and hypothermia. In: Reed C, Clark D, Houston TX, editors. Cardiopulmonary Perfusion. Texas Medical Press, Inc., 1975:272–8.

[8] Shaffer TH, Forman DL, Wolfson MR. Physiological effects of ventilation with liquid fluorocarbon at controlled temperatures. Undersea Biomed Res 1984;11:287–98.

[9] Darwin M, Russell S, Rasch C, O'Farrell J, Harris S. A novel method of rapidly inducing or treating hypothermia or hyperpyrexia, by means of 'mixed-mode' (gas and liquid) ventilation using perfluorochemicals. In: Society of Critical Care Medicine 28th Educational and Scientific Symposium (vol 27), San Francisco, Critical Care Medicine, 1999. p. A81.

[10] Shaffer TH, Wolfson MR, Clark LC Jr. Liquid ventilation. Pediatr Pulmonol 1992;14:102–9.

[11] Notebook MPI: 3M Performance Fluids. St Paul MN, 3M Company, 1995.

[12] Giesbrecht GG, Bristow GK. A second postcooling afterdrop: more evidence for a convective mechanism. J Appl Physiol 1992;73:1253–8.

[13] Faber P, Garby L. Fat content affects heat capacity: a study in mice. Acta Physiol Scand 1995;153:185–7.

[14] Miyamoto Y, Mikami T. Maximum capacity of ventilation and efficiency of gas exchange during liquid breathing in guinea pigs. Jpn J Physiol 1976;26:603–18.

[15] Koen PA, Wolfson MR, Shaffer TH. Fluorocarbon ventilation: maximal expiratory flows and CO_2 elimination. Pediatr Res 1988;24:291–6.

[16] Matthews WH, Balzer RH, Shelburne JD, Pratt PC, Kylstra JA. Steady-state gas exchange in normothermic, anesthetized, liquid-ventilated dogs. Undersea Biomed Res 1978;5:341–54.

[17] Reece W. Respiration in mammals. In: Swenson M, editor. Duke's Physiology of Domestic Animals, 10th. London: Comstock Publishing Associates, 1987:235–7.

[18] Safar P. Cerebral resuscitation (Letter). Ann Emerg Med 1993;22:759.
[19] Schmid-Elsaesser R, Hungerhuber E, Zausinger S, Baethmann A, Reulen HJ. Combination drug therapy and mild hypothermia: a promising treatment strategy for reversible, focal cerebral ischemia. Stroke 1999;30:1891–9.
[20] Gentilello LM, Cortes V, Moujaes S, Viamonte M, Malinin TL, Ho C, Gomez GA. Continuous arteriovenous rewarming: experimental results and thermodynamic model simulation of treatment for hypothermia. J Trauma 1990;30:1436–49.
[21] Marion DW, Penrod LE, Kelsey SF, Obrist WD, Kochanek PM, Palmer AM, Wisniewski SR, DeKosky ST. Treatment of traumatic brain injury with moderate hypothermia. N Engl J Med 1997;336:540–6.
[22] Cox CA, Wolfson MR, Shaffer TH. Liquid ventilation: a comprehensive overview. Neonatal Netw 1996;1996:31–43; Erratum, Neonatal Netw Jun;15(4):22.
[23] Shiozaki T, Hayakata T, Taneda M, Nakajima Y, Hashiguchi N, Fujimi S, Nakamori Y, Tanaka H, Shimazu T, Sugimoto H. A multicenter prospective randomized controlled trial of the efficacy of mild hypothermia for severely head injured patients with low intracranial pressure. Mild hypothermia study group in Japan. J Neurosurg 2001;94:50–4.
[24] Ebmeyer U, Safar P, Radovsky A, Obrist W, Alexander H, Pomeranz S. Moderate hypothermia for 48 h after temporary epidural brain compression injury in a canine outcome model. J Neurotrauma 1998;15:323–36.