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Cardiopulmonary changes with moderate decompression in rats

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Butler BD, Robinson R, Little T, Chelly JE, Doursout M-F. Cardiopulmonary changes with moderate decompression in rats. *Undersea Hyperbaric Med* 1996; 23(2):83-89.—Sprague-Dawley rats were compressed to 616 kPa (a) for 120 min then decompressed at 38 kPa/min to assess the cardiovascular and pulmonary responses to moderate decompression stress. In one series of experiments the rats were chronically instrumented with Doppler ultrasonic probes for simultaneous measurement of blood pressure, cardiac output, heart rate, left and right ventricular wall thickening fraction, and venous bubble detection. Data were collected at baseline, throughout the compression/decompression protocol, and for 120 min post decompression. In a second series of experiments the pulmonary responses to the decompression protocol were evaluated in non-instrumented rats. Analyses included blood gases, pleural and bronchoalveolar lavage (BAL) protein and hemoglobin concentration, pulmonary edema, BAL and lung tissue phospholipids, lung compliance, and cell counts. Venous bubbles were directly observed in 90% of the rats where immediate post-decompression autopsy was performed and in 37% using implanted Doppler monitors. Cardiac output, stroke volume, and right ventricular wall thickening fractions were significantly decreased post decompression, whereas systemic vascular resistance was increased suggesting a decrease in venous return. BAL Hb and total protein levels were increased 0 and 60 min post decompression; pleural and plasma levels were unchanged. BAL white blood cells and neutrophil percentages were increased 0 and 60 min post decompression and pulmonary edema was detected. Venous bubbles produced with moderate decompression profiles give detectable cardiovascular and pulmonary responses in the rat.

decompression illness, venous bubbles, air embolism, cardiovascular, pulmonary

Decompression can result in bubble formation in veins and tissues, in a dose-related fashion depending on the rate and degree of pressure reduction (1-2). Symptoms of decompression illness (DCI) include pain-only manifestations as well as circulatory or neurologic events, linked with the site of the bubbles, especially whether they are in an intravascular or extravascular location (3). A common conclusion is that extravascular bubbles are associated with pain-only symptoms (most often joint pain), although intravascular bubbles are commonly detected and reported in the evaluation of decompression stress (4).

Animal models have offered numerous insights into the mechanisms and etiologies of the symptoms of DCI. The smaller animal models usually require more extreme decompression exposures before more typical symptoms are manifested. These symptoms may also be far more severe than occurs routinely in the human clinical situation, especially where actual mortality rates are reported. The rat, for example, is a model commonly used in experimental DCI, but often requires greater extremes in decompression protocols (5-7). As a result, the more subtle effects of DCI are often overshadowed by the onset of severe circulatory or pulmo-

nary complications. Large animals such as the goat and sheep have been useful in evaluating the effects of decompression profiles in terms of limb pain (8-9) or respiratory complications (10), whereas the dog and swine have been used for hemodynamic assessment (11-13). Flynn and Lambertsen (14) reported in a number of animal models a log-log relationship between body weight and the nitrogen dose required to produce DCI. Because of a difference in relative susceptibility in symptoms, they did not recommend making direct projections to man.

Of equal importance in drawing conclusions from one species or measurement to another is the type and accuracy of symptom or measurement being made. The purpose of the present study was to examine the effects of moderate decompression stress in terms of cardiovascular function and pulmonary responses in chronically instrumented, conscious rats using a profile that was well below a threshold of significant mortality.

METHODS

Sprague-Dawley rats were used to study the cardiac function and pulmonary changes with decompression. The rats

were divided into two groups, one using chronically instrumented, awake rats for cardiovascular function assessment and the second using non-instrumented rats for pulmonary measurements. The pulmonary group was subdivided into groups 2–4 (2 controls, 3 those monitored 0 min post decompression, and 4 those monitored 60 min post decompression).

Cardiovascular function (group 1)

($n = 19$, $wt = 424 \pm 41$ g)

Instrumentation: Our evaluation was based on simultaneous measurement of arterial blood pressure, cardiac output, heart rate, left and right ventricular wall thickening fraction, and venous bubble detection. For surgical placement of the probes, the rats were anesthetized with halothane, intubated, and ventilated. The pericardium was exposed and sectioned through a left parasternal thoracotomy. To measure cardiac output (CO), a 20-MHz pulsed Doppler flow probe was placed around the ascending aorta. To measure left and right ventricular wall thickening fraction, a 20-MHz wall thickness epicardial probe was sutured to the left ventricular wall and a 10-MHz epicardial probe was sutured to the right ventricular wall. Silastic catheters were placed into the abdominal aorta via the femoral artery and the jugular vein for venous access and blood pressure measurement. An 18-gauge thoracic drain was positioned until closure of the chest. The ultrasound probe leads and catheters were tunneled subcutaneously to the dorsum of the neck for externalization. The lungs were then reinflated under vacuum, and the surgical wound infiltrated with bupivacaine (0.5%) for post-operative analgesia. Antibiotics were given for 5–7 days (gentamycin, 5 mg/kg i.m.).

The Doppler recordings were made with a Hartley pulsed Doppler flow/dimension system (15) and continuously displayed on a six-channel recorder. The wall thickening fraction (TF) was measured by velocity integration of the range-gated ultrasonic signal as it passed through the myocardial layers at a fixed distance from the epicardial surface where the probe was attached. The range depth was 2–3 mm. TF has been previously demonstrated to be an index of myocardial contractility also dependent on preload and afterload (16). The pulsed Doppler system for determining cardiac output in rats has been described in detail (17). The pulsed Doppler resolves frequency shifts of up to 62.5 KHz (PRF/R) at a maximum range (R) from 0.6 to 6 mm and a spatial range of 0.3 mm.

The right ventricular Doppler probe was sutured to the myocardial wall such that the signal could also be range-gated across the pulmonic valve to detect venous bubbles circulating into the pulmonary artery. Optimal audio signals were verified by rapid injection into the venous catheter of small volumes of sterile saline.

Hemodynamic measurements: Hemodynamic variables, including heart rate (HR), mean arterial blood pressure (MAP), CO, and left and right ventricular wall TF were processed with the multichannel Doppler flow/dimension system and continuously recorded. The TF was determined as the maximum excursion recorded during systole (SE), divided by the sample volume depth; $\%TF = 100 (SE/R)$, where SE is expressed in millimeters and R is the range gate depth in millimeters. Systemic vascular resistance (SVR) was calculated as MAP/CO .

Procedure: The conscious rats were placed in a rodent restraint apparatus and acclimated to the chamber environment before collection of baseline data. The chamber was then compressed to 616 kPa (a) at 34 kPa/min for a bottom time of 120 min, then decompressed to sea level at 38 kPa/min. Hemodynamic monitoring continued throughout the compression/decompression procedure and for 120 min after. The chamber environment was maintained at room temperature ($\pm 4^\circ\text{C}$) during both compression and decompression with a constant fresh air flow.

Pulmonary measurements (groups 2–4 $n = 60$)

Group 2 rats (440 ± 59 g) consisted of non-decompressed controls, group 3 rats (421 ± 57 g) were decompressed and measurements taken immediately after the decompression (0 min post decompression), and group 4 rats (406 ± 27 g) were decompressed and measurements taken 60 min post decompression. At 0 or 60 min post decompression the rats were anesthetized (pentobarbital sodium, 50 mg i.p.) and an arterial blood sample was collected anaerobically from the abdominal aorta for blood gas, plasma protein, and white blood cell analysis. Pulmonary artery blood samples were collected by direct puncture of the pulmonary artery following thoracotomy. The animals were then exsanguinated, pleural fluid collected, and the lungs removed for measurement of compliance, bronchoalveolar lavage (BAL) collection, and extravascular lung water (pulmonary edema) measurement. Not all measurements were obtainable from each rat. The number of rats/measurement are listed in the tables.

Bronchoalveolar lavage: Upon removal, the lungs were weighed, degassed under vacuum, and lavaged via tracheal cannula with 10 ml cold, normal saline (5°C) 6 times for a total volume of 60 ml. Recovered BAL volume was $93 \pm 0.03\%$.

The BAL, total plasma, and pleural fluid protein was assayed using a modified Lowry method (18), and Hb levels were measured using a modified benzidine assay.

Phospholipid analysis: Lung phospholipids were assayed from both BAL and freshly extracted lung tissue. Fifteen milliliters of pooled BAL or 0.9 g tissue homogenate were

extracted, using the chloroform/methanol procedure of Folch et al. (19), and spotted onto silica gel thin-layer chromatographic plates for two-dimensional development. Phospholipid amounts were determined by measuring the phosphorous content of each developed spot.

Lung compliance: Before BAL collection, quasi-static lung compliance was measured in the isolated lungs. The lungs were degassed under vacuum, put into a heated/humidified chamber, and connected via a tracheal cannula to an infusion/withdrawal pump and airway pressure transducer. They were inflated to 30 cmH₂O (20 ml/min) and allowed to equilibrate (15 min) while maintaining 30 cmH₂O pressure. Inflation/deflation pressure-volume curves were determined, from which compliance values were derived.

Pulmonary edema measurement: The amount of blood-free extravascular lung water (edema) was measured using a modified method of Pearce et al. (20) which accounted for the blood volume in the tissue. The extravascular lung water (EVLW) was expressed as the extravascular fluid to dry-weight ratio.

Cell counts: BAL, pleural fluid, and blood (arterial and pulmonary) white cell counts were performed using a Neubauer hemacytometer with EDTA stabilization. Differential cell counts were performed with microscopic survey using Wright-Geimsa and esterase stains.

Data analysis

Data were analyzed using analysis of variance (ANOVA) (repeated measures for hemodynamic data). Individual comparisons were analyzed using Student's *t* test with Bonferroni correction. Significance was considered at $P < 0.05$.

These studies were approved by the Institutional Animal Care and Use Committee.

RESULTS

The decompression profile used in this study was selected to produce saturation conditions with moderate bubbling and symptoms. Venous bubbles were visually observed in 90% of the rats examined immediately after decompression (group 3), while only 3% (2/60) demonstrated gross symptoms of DCI (hindlimb paralysis, severe respiratory complications, etc.). Doppler bubble detection in the instrumented rats (group 4) was less conclusive, demonstrating bubbles in 37% (7/19) of rats in which the probe functioned properly. The insensitivity of the Doppler data may be attributable to the placement of the probe on the right ventricular wall vs. the more common precordial site where effective sampling of all venous blood is more assured. Quantitation of the bubbles in terms of size or count was not undertaken in these studies.

Blood gas measurements: (Table 1) Arterial PO₂ values were decreased in group 3 rats (nonsignificant) and elevated

in group 4 (significant). PaCO₂ values were significantly increased (44.9 ± 2.4 to 51 ± 1.8 mmHg) in group 3 ($P < 0.05$) and were unchanged in group 4. Increased respiratory frequency with decreased tidal volumes was observed visually in 68% of the rats 0 min post decompression. These rats had PaCO₂ values significantly higher (53 ± 8 mmHg) than those without respiratory symptoms (47 ± 11 mmHg) or from the control group (44.9 ± 2.4 mmHg).

Pulmonary measurements: (Table 2) BAL hemoglobin values were increased in groups 3 and 4 by 7.5 and ($P < 0.05$) 17-fold, respectively. BAL total protein was increased in groups 3 and 4 (36 and 77%; group 4, $P < 0.05$). The BAL protein:plasma protein ratio was also significantly increased in group 4 compared to controls. Plasma protein levels were unchanged. Pleural protein and Hb values were unchanged. Lung compliance was decreased by 10% in group 3 and 16% in group 4 (non-significant). BAL phosphatidyl choline (PC) levels were unchanged in groups 3 and 4 compared to controls, whereas BAL lysophosphatidylcholine (LPC) levels were significantly increased in both groups. Tissue PC and LPC levels were unchanged in both groups (Table 2)

Cell counts: Pulmonary blood white cell counts (WBC) were increased in group 4 (Fig. 1, *bottom*). The percentage of arterial and pulmonary WBC that were neutrophils was slightly elevated (nonsignificant) in both groups (Fig. 1, *top*). Pleural fluid WBC was decreased in group 3, a dilutional effect, whereas the percentage of neutrophils was increased in group 4 ($P < 0.05$). BAL WBC increased significantly in both groups 3 and 4 (121 and 212%, respectively), as did the percentage of neutrophils in group 4 (Fig. 1, *top*).

Pulmonary edema measurement: Extravascular lung water (wet:dry weight ratio) was significantly elevated in group 4, 60 min post decompression (Table 2). Cut lung surfaces often revealed perivascular cuffs of edema fluid, especially around larger vessels. Excess airway fluid was not observed.

Cardiovascular measurements (Fig. 2): Mean arterial blood pressure was significantly elevated at the end of the compression period and unchanged post decompression. HR was unchanged throughout the compression/decompression protocol. CO (Fig. 3) was unchanged throughout the compression period, decreased upon return to sea level pressure (21%, $P < 0.05$), and remained decreased for 60 min post decompression (21%, $P < 0.05$). Comparable changes occurred with stroke volume, with a greater degree of recovery, however (18% from baseline), 120 min post decompression. Systemic vascular resistance (Fig. 2) was unchanged during the period of compression, increased significantly 0 and 60 min post decompression (65 and 70%), and remained elevated for 120 min (34%). Left ventricular wall TF was unchanged for all groups, whereas right ventricular TF (Fig. 3) was decreased significantly post decompression.

Table 1: Lung Function and Blood Gas Data^a

	Control	Post Decompression	
		0 min	60 min
Compliance, ml/cmH ₂ O	1.10 ± 0.02 (4)	1.00 ± 0.05 (5)	0.92 ± 0.06 (9)
TLC, ml	22.1 ± 0.04 (5)	21.6 ± 1.14 (6)	20.8 ± 0.56 (9)
OaO ₂ , mmHg	91.8 ± 3.53 (20)	81.2 ± 4.34 (25)	101.2 ^b ± 2.30 (35)
PaCO ₂ , mmHg	44.9 ± 2.35 (23)	51.0 ^b ± 1.77 (26)	42.6 ± 1.31 (35)
pH	7.36 ± 0.016 (23)	7.32 ± 0.012 (26)	7.37 ± 0.012 (35)

^aData are mean ± SE. Numbers in parentheses are *n*. ^b*P* < 0.05 vs. controls. TLC = total lung capacity.

Table 2: BAL Hb, BAL:Plasma Protein, EVLW Ratio, Phospholipids^a

	Control	Post Decompression	
		0 min	60 min
BAL Hb, ml/100 ml	0.02 ± 0.014 (11)	0.167 ± 0.097 (11)	0.375 ^b ± 0.077 (12)
Plasma protein, g/100 ml	4.83 ± 0.20 (13)	5.03 ± 0.23 (14)	5.22 ± 0.11 (14)
BAL protein, g/100 ml × 10 ⁻³	12.87 ± 1.20 (15)	17.45 ± 2.60 (15)	22.81 ^b ± 2.22 (16)
BAL protein/plasma protein	2.67 ± 0.26 (13)	3.49 ± 0.53 (14)	4.40 ^b ± 0.58 (14)
EVLW	4.18 ± 0.05 (18)	4.27 ± 0.08 (22)	4.53 ^b ± 0.13 (24)
Phosphatidylcholine, μg/lung			
Tissue	3,094 ± 551 (7)	4218 ± 605 (12)	4,478 ± 747 (12)
BAL	750 ± 101 (11)	668 ± 85 (10)	745 ± 104 (11)
LPC, μg/lung			
Tissue	364 ± 36 (7)	408 ± 36 (12)	356 ± 18 (11)
BAL	2.0 ± 0.4 (10)	4.2 ^b ± 0.8 (10)	7.4 ^b ± 1.3 (9)

^aData are mean ± SE. Numbers in parentheses are *n*. ^b*P* < 0.05 vs. controls. LPC = lysophosphatidyl choline

DISCUSSION

The data reported in this study further demonstrate the utility of the rat model in the evaluation of cardiovascular and pulmonary responses to moderate decompression stress. Changes in cardiovascular parameters are consistent with those reported in other species such as the dog or the pig (11–13). MAP values are commonly increased (often nonsignificant) with moderate decompression exposures in the larger species. We observed an increase in MAP during compression only with no changes post decompression. Decreases in HR have been reported during compression, whereas increases lasting up to several hours post decompression or no change at all have also been reported (12, 13). In our study, the increase in HR observed 60 min post de-

compression was not significant. Changes in HR may be attributable to sympathetic stimulation or changes in PaCO₂ and concomitant increases in SVR may also be a result of direct sympathetic stimulation or a result of catecholamine release (21,22).

Previous studies have reported a decrease in CO following an increase in pulmonary vascular resistance (23). Others have suggested that decompression-induced decreases in CO may be due to changes in venous return, increased load on the right ventricle as a result of the obstructed pulmonary circulation, or to increased afterload on the left ventricle due to the elevated SVR (11,12, 23).

Our data suggest that a decrease in venous return may be responsible for the decrease in CO recorded after decompres-

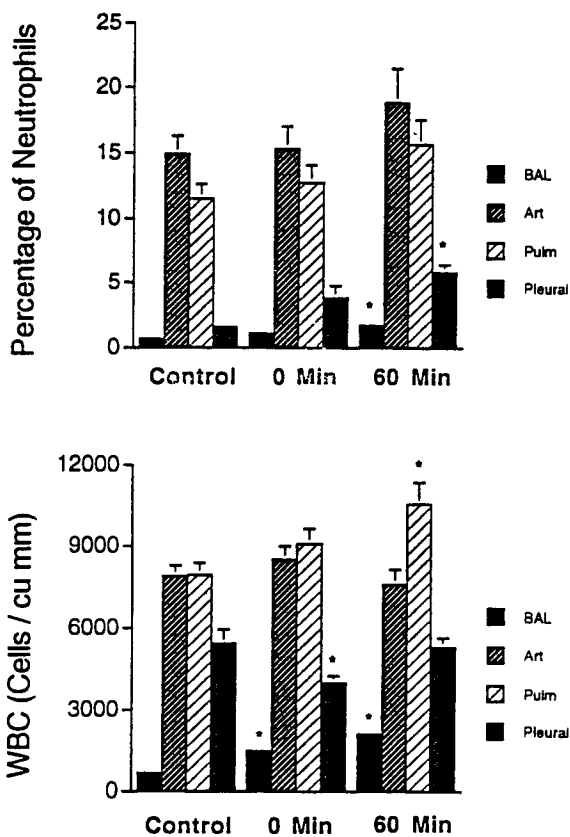


FIG. 1—*Top*, white blood cell count; percentage of neutrophils in three groups. Significant increase in percentages were observed in the bronchoalveolar lavage (BAL) and pleural fluids, 60 min post decompression. *Bottom*, white blood cell counts. Significant increases were observed in the bronchoalveolar lavage (BAL) at 0 min. and 60 min post decompression and in pulmonary blood 60 min post decompression. Pleural WBC counts were decreased 0 min, returning to control values 60 min post decompression.

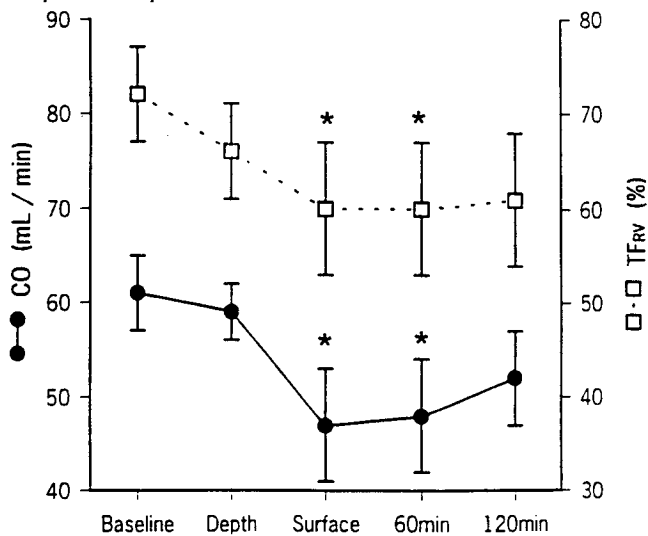


FIG. 2—Mean arterial blood pressure (MAP) and systemic vascular resistance (SVR) changes with decompression. MAP increased at depth while SVR increased immediately upon surface and 60 min. post decompression.

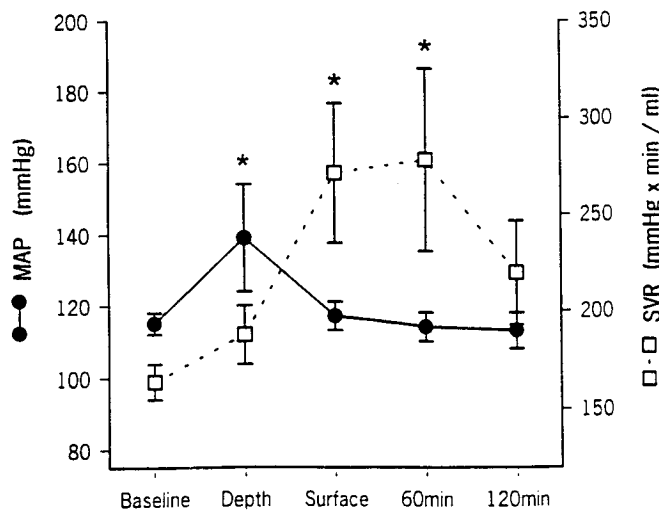


FIG. 3—Cardiac output (CO) decreased at surface and 60 min. post decompression while right ventricular thickening fraction (TF_{RV}) showed parallel responses.

tion. Evidence supporting this concept includes:

- The decrease in right TF related to either a decrease in an intrinsic cardiac inotropism or a decrease in venous return. Both mechanisms have been previously described. Right ventricular failure may occur because of myocardial ischemia resulting from a decrease in coronary blood flow due to a decrease in coronary perfusion pressure (e.g., decrease in aortic diastolic pressure associated with elevated right ventricular end-diastolic pressures) (24) or a failure of the right ventricle to compensate for the increase in pulmonary vascular resistance due to the presence of bubbles following decompression. However, it is unlikely that a decompression-induced right ventricular failure was involved in the decrease in TF. Thus, the TF decreased only in the right ventricle after decompression. Furthermore, the changes cited above are reported with more severe cases of DCI or with air embolization where right ventricular pressures are greatly increased.

- In the absence of cardiac failure, a decrease in venous return is associated with a decrease in preload of the left ventricle.

- It is established that in the absence of preload changes, an increase in afterload (e.g., systemic vasoconstriction) is associated with a compensatory increase in cardiac contractility to maintain cardiac output. This is reflected as an increase in left TF (Starling's Law).

In our experimental conditions, left TF remained unchanged despite an increase in SVR and decrease in CO. Therefore the lack of expected left TF increase also represents an argument in favor of a decrease in venous return occurring after decompression. Any additional effects of venous bubbles or mediator-induced changes in venous resistance or capacitance

or both may also have contributed to a decrease in venous return.

Pulmonary effects of decompression-induced or exogenously introduced venous bubbles include their interactions with cellular components of the blood. Such interactions include platelet and neutrophil activation and the associated release of bioactive mediators including; serotonin, histamine, kinins, prostaglandins, lymphokines, thromboxanes, and leukotrienes (1, 25-27). These agents can alter microvascular membrane and epithelial permeability and vascular tone, leading to pulmonary edema formation (27). This was evident in the present study by an increase in EVLW as well as increases in WBCs in BAL and pulmonary blood as well as neutrophil percentages in pleural and BAL fluid. Ohkuda et al. (28) and others have demonstrated pulmonary edema with venous air infusion suggesting a reversible change in permeability of the microvascular membrane. Catron et al. (29) and Peterson et al. (30) reported pulmonary edema after DCI or venous air infusion without an increase in left ventricular end-diastolic pressures. These observations are consistent with the reported data and suggest that even with moderate decompression, some change in lung fluid balance is possible. Similar changes have been reported with widely variable pressure exposures in animals and man (13,27,29, 31,32). The significant increases in LPC are consistent with other studies suggesting its role in altering lung fluid balance, especially regarding changes in BAL (33). Venous air embolism in dogs has been shown to cause pulmonary endothelial disruption, platelet and leukocyte adherence to bubble surfaces, and increases in leukocyte counts and degranulation (34,35). Similar findings have been reported in mesenteric vessels after decompression in rats (25) and in pulmonary vessels in mice (36). The results of this study demonstrate cardiovascular function and pulmonary changes with moderate DCI in the rat. They further demonstrate that the effects of circulating bubbles can be measured physiologically even in a small animal model at moderate decompression exposures and parallel many of the reported changes that are seen with larger animals and man.

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REFERENCES

1. Elliott DH, Hallenbeck JM. The pathophysiology of decompression sickness. In: Bennett PB, Elliott DK, eds. *The physiology and medicine of diving and compressed air work*. Baltimore, MD: Williams & Wilkins, 1975;435-455.
2. Eckenhoff RG, Olstad CS, Carrod G. Human dose-response relationship for decompression and endogenous bubble formation. *J Appl Physiol* 1990; 69:914-918.
3. Francis TJR, Gorman DF. Pathogenesis of the decompression disorders. In: Bennett P, Elliott D, eds. *The physiology and medicine of diving*, 4th ed. London: WB Saunders, 1993:454-480.
4. Elliott DH, Moon RE. Manifestations of the decompression disorders. In: Bennett P, Elliott D, eds. *The physiology and medicine of diving*, 4th ed. London: WB Saunders, 1993:480-505.
5. Powell MR. Gas phase separation following decompression in asymptomatic rats: visual and ultrasound monitoring. *Aerosp Med* 1972; 43:1240-1244.
6. Lillo RS, MacCallum ME. Decompression comparison of N₂ and O₂ in rats. *Undersea Biomed Res* 1991; 18:317-331.
7. Berghage TE, David TD, Dyson CV. Species differences in decompression. *Undersea Biomed Res* 1979; 6:1-13.
8. Lanphier EH, Lehner CE. Animal models in decompression. In: Lin TC, Shida KK, eds. *Man in the sea*, vol. 1. San Pedro, CA: Best Publications, 1990:273-295.
9. Boycott AE, Damant GC, Haldane JS. Prevention of compressed air illness. *J Hyg (Cambridge)* 1908; 8:342-443.
10. Atkins CE, Lehner CE, Beck KA, Dubielzig RR, Nordheim EV, Lanphier EH. Experimental respiratory decompression sickness in sheep. *J Appl Physiol* 1988; 65:1163-1171.
11. Butler BD, Robinson R, Sutton T, Kemper GB. Cardiovascular pressures with venous gas embolism and decompression. *Aviat Space Environ Med* 1995; 66:408-414.
12. Vik A, Jenssen BM, Eftedal O, Brubakk AO. Relationship between venous bubbles and hemodynamic responses after decompression in pigs. *Undersea Hyperbaric Med* 1993; 20:233-248.
13. Bove AA, Hallenbeck JM, Elliott DH. Circulatory responses to venous air embolism and decompression sickness in dogs. *Undersea Biomed Res* 1974; 1:207-220.
14. Flynn ET, Lambertsen CJ. Calibration of inert gas exchange in the mouse. In: Lambertsen CJ, ed. *Underwater physiology. Proceedings of the fourth symposium on underwater physiology*. New York: Academic Press, 1971:179-191.
15. Hartley C, Lewis R, Ishida T, Chelly J. High frequency pulsed Doppler measurements of blood flow and myocardial dimensions in conscious animals. *Proceedings of the Working Conference on Applicability of New Technology to Biobehavioral Research*. 1982: 95-106.
16. Zhu WX, Meyers ML, Hartley CJ, Roberts R, Bolli R. Validation of a single crystal for measurement of transmural and epicardial thickening. *Am J Physiol* 1986; 251:H1045-H1055.
17. Gardiner SM, Compton AM, Bennett T, Hartley CJ. Can pulsed Doppler techniques measure changes in aortic blood flow in conscious rats. *Am J Physiol* 1990; 259:H448-H456.
18. Lowry O, Rosebrough N. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951;193:265-275.
19. Folch J, Lees M, Sloane-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 1957; 226:497-509.
20. Pearce ML, Yamashita J, Beazell J. Measurement of pulmonary edema. *Circ Res* 1965; 16:482-488.
21. Catron PW, Thomas LB, McDermott JJ, et al. Hormonal changes during decompression sickness. *Undersea Biomed Res* 1987; 14: 331-341.
22. Butler BD, Conkin J, Luehr S. Pulmonary hemodynamics, extra-vascular lung water and residual gas bubbles following low dose venous gas embolism in dogs. *Aviat Space Environ Med* 1989; 60:1178-1182.
23. Neuman TS, Spragg RG, Wagner PD, Moser KM. Cardiopulmonary

- consequences of decompression stress. *Resp Physiol* 1980; 41:143-153.
24. Holt EP, Webb WR, Cook WA, Unal MO. Air embolism: hemodynamics and therapy. *Ann Thorac Surg* 1966; 2:551-559.
 25. Philp RB, Inwood MJ, Warren BA. Interactions between gas bubbles and components of the blood: implications in decompression sickness. *Aerospace Med* 1972; 43:946-953.
 26. Warren BA, Philp RB, Inwood MJ. The ultrastructural morphology of air embolism. Platelet adhesion to the interface and endothelial damage. *Brit J Exper Path* 1973; 54:163-172.
 27. Butler BD, Little T. Effects of venous air embolism and decompression on thromboxane and leukotriene levels in dogs. *Undersea and Hyperbaric Med* 1994; 21s:21-22.
 28. Ohkuda K, Nakahara K, Binder A, Staub NC. Venous air emboli in sheep: reversible increase in lung microvascular permeability. *J Appl Physiol* 1981; 51:887-894.
 29. Catron PW, Flynn ET Jr, Jaffe L, Bradley ME, Thomas LB, Hinman D, Survanshi S, Johnson JT, Harrington J. Morphological and physiological responses of the lungs of dogs to acute decompression. *J Appl Physiol* 1984; 57:467-474.
 30. Peterson BT, Grauer SE, Hyde RW, Ortiz C, Moosavi H, Utell MJ. Response of pulmonary veins to increased intracranial pressure and pulmonary air embolization. *J Appl Physiol* 1980; 48:957-964.
 31. Goodpasture JE, Joki JA, Wells CH. Pulmonary and cardiovascular fluid shifts in experimental dysbarism. *Undersea Biomed Res* 1974; 1:A-19.
 32. Haymaker W, Davison C. Fatalities resulting from exposure to simulated high altitudes in decompression chambers. A clinicopathologic study of five cases. *J Neuropathol Exp Neurol* 1950; 9:29-56.
 33. Butler BD, Davies I, Drake RE. Effect of lysophosphatidylcholine on the filtration coefficient in intact dog lungs. *Amer J Physiol: Heart Circ Physiol* 1989; 26:H1466-H1470.
 34. Moosavi H, Utell J, Hyde RW, Fahey PJ, Peterson BT, Donnelly J, Jensen KD. Lung ultrastructure in noncardiogenic pulmonary edema induced by air embolization in dogs. *Lab Invest* 1981; 45:456-464.
 35. Albertine KH, Weiner-Kronish JP, Koike K, Staub NC. Quantification of damage by air emboli to lung microvessels in anesthetized sheep. *J Appl Physiol* 1984; 57:1360-1368.
 36. Bennet RA. The histology of lesions found in animals following rapid decompression. In: *Proc. 7th Annual Congress of the European Undersea Biomedical Society and Symposium on Decompression Sickness*, vol. 2. Cambridge, UK: European Undersea Biomedical Society, 1981:486-489.

