Aortic Function in Rats After Decompression Without Ultrasonically Detectable Bubble Formation

KIM-VIDAR RASDAL, ASTRID HJELDE, ANDREAS MØLLERLØKKEN, NINA LUNDSET, AND ALF OTTAR BRUBAKK

RASDAL K-V, HJELDE A, MØLLERLØKKEN A, LUNDSET N, BRUBAKK AO. *Aortic function in rats after decompression without ultrasonically detectable bubble formation.* Aviat Space Environ Med 2009; 80:1006–11.

Background: Several studies have demonstrated an adverse effect of bubbles on endothelial function. The degree of dysfunction appears to be related to the number of bubbles present. The aim of the study was to determine whether decompression without bubble formation visible by ultrasound had any effect on arterial endothelial function. ***Methods:*** We decompressed 21 Sprague-Dawley rats weighing 215–260 g from 700 kPa (≈6.9 ATA) in a dry hyperbaric chamber followed by a 1-h observation period and measured aortic endothelial-dependent relaxation to acetylcholine. Later, we determined the specific weight of the brain as a measure of edema formation and vascular bubbles in the arterial circulation. ***Results:*** No bubbles were seen in the pulmonary arteries of seven rats. We found a significant lower vasodilatory response to acetylcholine in the decompressed rats (44% ± 14%) compared to the control rats (58% ± 12%) as a sign of endothelial dysfunction. There was no significant difference between the two groups in the specific gravity of the brain. ***Conclusion:*** We conclude that measurable arterial dysfunction in the aorta can occur even if no visible venous bubble formation is seen. There are no results in this study suggesting that these rats had damaged blood-brain barriers or brain edema.

Keywords: diving, endothelial function, specific gravity.

IT IS GENERALLY assumed that bubbles grow from preformed bubble nuclei (≈1.0 μ), since we can detect vascular bubbles after dives with far lower partial pressure than what is required for de novo bubble formation from supersaturation alone (1,16). These bubble nuclei are highly unstable in liquid, but can stay nearly indefinitely on a surface, such as the endothelium. Once a bubble is stabilized, its size will change according to the tension of dissolved gas and the bubble gas pressure. The vascular environment influencing bubble formation and growth is complex and several biochemical factors are known to influence bubble formation. Nitric oxide (NO) has been shown to reduce bubbling while high blood lipids give more bubble formation by reducing surface tension (5). The properties of the endothelium may play a key role in both bubble formation and subsequent damage.

Endothelial dysfunction is characterized by deterioration of endothelial vasodilatory function (34) and nitric oxide is the key endothelial-derived relaxing factor (31). Bubbles detected by ultrasound in the pulmonary artery have been used as a sign of decompression stress (12). It has been demonstrated that even a few gas bubbles from decompression may lead to endothelial damage and dysfunction in the pulmonary artery both in animals and man (9,27,28). The damage seems to be related to the amount of bubbles present, although the clinical significance of this damage is still unknown. Bubbles may also enter the arterial circulation through an intrapulmonary shunt or through a right-to-left shunt in the heart in the presence of a persistent foramen ovale. Such shunts may open after even light exercise. These bubbles have been related to neurological decompression illness (14). It has, therefore, been hypothesized that arterialization of bubbles is one of the main mechanisms leading to neurological decompression sickness (DCS). Even short contact between bubbles and the endothelium in the brain's vascular system can lead to a break in the blood-brain barrier (3). Hjelde et al. have shown in decompressed rats that even a low bubble score in the pulmonary artery may result in brain edema and gas bubble retention when measured with a specific weight 1 h after surfacing (21). A recent study in man indicated that arterial endothelial dysfunction may be present even if few pulmonary artery bubbles were present and no left ventricular bubbles or large patent foramen ovale were seen (6). The bubbles in that study were detected using an ultrasonic frequency of 2 MHz and it is possible that small bubbles might have been missed. The aim of the present study is to see whether we can detect damage in the arterial system even if there are no detectable bubbles using an ultrasonic frequency of 10 MHz. We also wanted to investigate whether we could find any break in the blood-brain barrier in rats with no detectable

From the Department of Circulation and Medical Imaging Faculty of Medicine, Norwegian University of Science and Technology, Trondheim, Norway.

This manuscript was received for review in October 2008. It was accepted for publication in August 2009.

Address reprint requests to: Kim-Vidar Rasdal, Det medisinske fakultet, Olav Kyrres g. 9, Medisinsk teknisk forskningssenter (MTFS), 7489 Trondheim, Norway; kim-vidar.rasdal@ntnu.no.

DOI: 10.3357/ASEM.2458.2009

bubble formation and relate it to possible endothelial damage in the arterial circulation.

METHODS

A total of 21 adult female rats (Sprague-Dawley, 215-280 g, Møllegaard, Denmark) were used in the study. The rats were acclimatized for a minimum of 4 d before the start of the experiments, as described below, and were randomized into two groups. Group I contained 7 control rats weighing 255.7 ± 16.2 g, and Group II consisted of 14 decompressed rats weighing 248.6 ± 18.4 g. Light was controlled on a 12-h dark/12-h light cycle. Temperature was 21.5 ± 1.5°C and humidity 55.6 ± 4.0%. The rats were maintained 3 to 6 per cage and fed a pellet rodent diet ad libitum and had free access to water. No signs of illness were detected during the study period. The study was performed in accordance with the Norwegian State Commission for Animal Experimentation and approved by the Norwegian Committee for Animal Experiments.

Decompression Model and Anesthesia

Rats from Group II were placed in a dry hyperbaric chamber (Sira Engineering, Trondheim, Norway). The pressure was increased by 200 kPa · min^{-1} up to 700 kPa (≈6.9 ATA) and the rats maintained for 45 min at 700 kPa breathing air. The rats were then decompressed at a rate of 50 kPa · min^{-1} to the surface (100 kPa). This decompression protocol has been shown to produce few bubbles in rats weighing less then 300 g (37). Immediately after reaching the surface the rats were anesthetized with midazolam (Dormicum, Roche, Oslo, Norway)/fentanyl/fluanison (Hypnorm) (0.4 ml · 100 g^{-1}, S.C.). The right and left ventricle were scanned transthoracically using a Vivid 5 ultrasonic scanner (GE Vingmed Ultrasound AS, Horten, Norway) with a 10-MHz transducer. At this ultrasonic frequency, a bubble will have a resonant size on the order of 0.3 μm. The reflected signal will increase proportionally to bubble size above this level and potential stable bubbles in the blood stream should hence be detectable. Due to the high heart rate in rats it is difficult to detect bubbles if there are only a few present in real time, but potential bubbles would appear as high-intensity white dots (**Fig. 1**) when looking at slow playback of the recording. We also used the Doppler mode to further identify bubbles in the blood stream (**Fig. 2**), which could be heard as loud "chirps."

The bubble images were graded on a scale from 1–5 according to a previously described method (11) which has been demonstrated to be accurate independent of the operator's experience. Grade 0 is no bubbles, 1 is occasional bubbles, 2 is at least one bubble every fourth heart cycle, 3 is at last one bubble every heart cycle, 4 is continuous bubbling, and 5 is massive bubbling. The scoring system is approximately exponential compared with the number of bubbles in the right ventricle and pulmonary artery. The rats were decompressed in pairs and we scanned continuously for 60 min, scanning each rat for 2-3 min at a time. Rats from Group I were kept at 100 kPa breathing air and were given anesthesia and kept under anesthesia for 60 min.

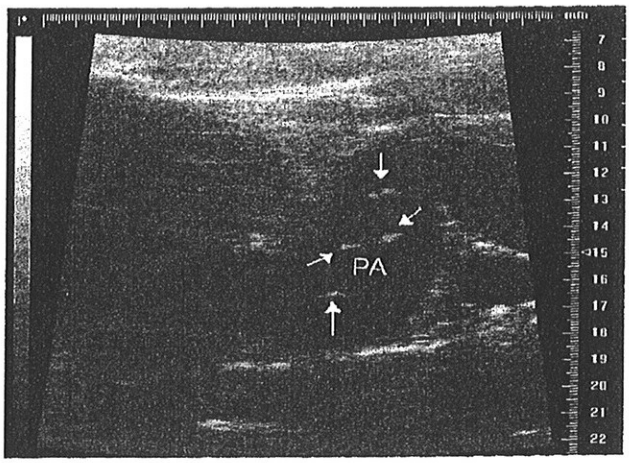

Fig. 1. Illustration of ultrasonic detection of bubbles in the pulmonary artery (PA); bubbles appear as high intensity dots within the vascular lumen (arrows).

Excision of Material

After 60 min of observation the rats in both groups were killed while still under anesthesia and the abdominal aorta was immediately removed and stored in a cold oxygenated (5% CO_2:95% O_2) Na-Krebs buffer solution with the following composition: 119 mmol · L^{-1} NaCl; 10 mmol · L^{-1} $NaHCO_3$; 1.2 mmol · L^{-1} MgCl; 4.6 mmol · L^{-1} KCl; 1 mmol · L^{-1} NaH_2PO_4; 1.5 mmol · L^{-1} $CaCl_2$; and 11 mmol · L^{-1} glucose. Within 3–4 min after decapitation the brains, including the medulla oblongata and the cerebellum, were excised and immersed in kerosene.

Specific Gravity Measurements

The specific gravity of the brains was determined using a translucent brombenzene/kerosene graduated

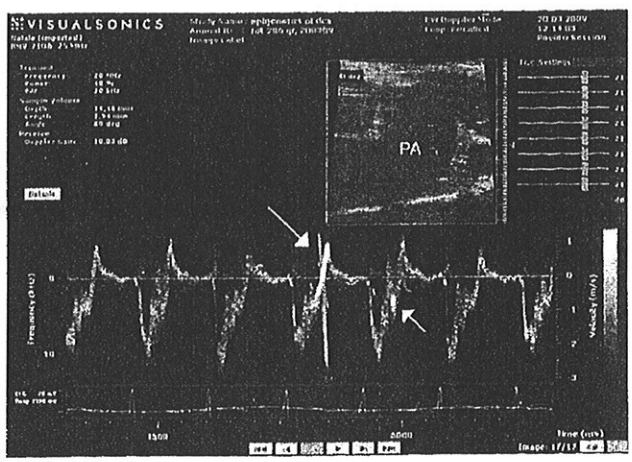

Fig. 2. Illustration of bubbles detected in the pulmonary artery by Doppler sonography; the bubbles induced high intensity "spikes" (arrows).

column as described elsewhere (21). The column was made by adding a lighter mixture (1.040 g · ml^{-1}) of brombenzene (52.2 ml) and kerosene (97.8 ml) to a heavier mixture (1.075 g · ml^{-1}) of brombenzene (59.8 ml) and kerosene (90.2 ml). Thus the column had a continuous gradient with densities from 1.040 g · ml^{-1} to 1.075 g · ml^{-1}. To calibrate the column we used drops of K_2SO_4 with a known specific gravity ranging from 46.6 g · L^{-1} to 73.9 g · L^{-1}. Calibration of the column was performed prior to each brain specific gravity measurement and we used the same column for a maximum of seven recordings. The brains were transferred to the column within 30 min after decapitation and equilibrated 2 min before we recorded their position in the column. We then pressurized the column to 300 kPa, let the brains equilibrate to the new pressure for 2 min, and recorded their new position in the column. By interpolating from the position of K_2SO_4 solutions of known specific gravity we could then graphically estimate the specific weight of the brains. This method has been used in several studies with high sensitivity and small standard deviations (19,21), making this method appropriate for studying brain edema in small study populations. Furthermore, a study on rats with the same weight using the same technique for specific weight measurement showed the ability to detect possible brain edema otherwise masked by simultaneous gas retention in the brain tissue by pressurizing the column to 300 kPa and thereby dissolving possible gas bubble retention (21).

Vessel Tension Measurement

The recording equipment was custom made and is described by Nossum et al. (28). It is based on the method for tension measurements of isolated vessels described by Edvinsson et al. (10) and the tissue bath technique originally described by Högestätt et al. (22). The aorta was carefully cut under a microscope in about 1.5-2.0 mm long segments, mounted on two L-shaped metal prongs, and lowered into a temperature-controlled (37°C) tissue bath containing Na-Krebs buffer with the composition described above. The pH was maintained at 7.35 ± 0.1 by bubbling the buffer continually with 5% CO_2:95% O_2. The tension applied to the aorta segments were gradually increased to a level of 6.5–8.0 mN and the vessels were allowed to stabilize at this level for 0.5–1 h. To test the contractile capacity of the vessels we exposed them to a 60 mmol · L^{-1} K-Krebs buffer solution for 3 min before precontracting the vessels with noradrenalin (NA) in the Na-Krebs buffer previously described. The endothelial-dependent relaxation response was tested by adding cumulative doses of acetylcholine (ACh) (10^{-9}–10^{-4} M). The response depended upon how much of the endothelial layer was damaged. The maximum relaxation response was defined as the maximal dilatory response regardless of the concentration induced by an agonist and is expressed as a percentage of the precontraction induced by a precontracting agent. Endothelial-independent relaxation was tested by adding cumulative doses of sodium nitroprusside (SNP) (10^{-12}–10^{-7}M).

Drugs

±NA, ACh, and SNP were diluted in small volumes of distilled water. All concentrations given are the final molar concentrations in the tissue bath. NA acts on alpha-1 and alpha-2 adrenergic receptors on smooth muscle cells in the blood vessels and causes vasoconstriction. ACh causes endothelium-dependent vasorelaxation. When submitted to ACh, the endothelial cell will produce NO through a G protein, which in turn relaxes vascular smooth muscle cells. SNP diffuses into the vascular smooth muscle, causing an endothelial-independent vasorelaxation and subsequent vasodilatation.

Statistics

Data are given as mean ± SD. Due to the small number of rats and lack of normal distribution on a Q-Q plot, nonparametric statistics were used. The Mann-Whitney U-test was applied to evaluate the differences between groups both when assessing endothelial function and specific weight. $P < 0.05$ was considered statistically significant.

RESULTS

A total of 14 rats underwent a simulated dive in the present study: 7 had no bubbles detected; 4 had detectable bubbles and were excluded from the study; 3 were excluded due to technical problems. In the control group, one rat was excluded from the specific weight measurements due to technical problems. This left seven rats in the experimental group, six rats in the control group for specific weight measurements, and seven rats in the control group for vessel tension measurements.

There was a significant difference in the maximum relaxation response induced by acetylcholine ($P < 0.05$) between the two groups, with a mean vasodilatation of 44 ± 14% in the group of decompressed rats versus 58 ± 12% in the control group (**Fig. 3**). SNP gave no significant difference in response, indicating that the lower vasodilatation in the decompressed rats was related to endothelial dysfunction. We found no difference in specific gravity between the two groups at 100 kPa (**Table I**), suggesting no brain edema following the de-

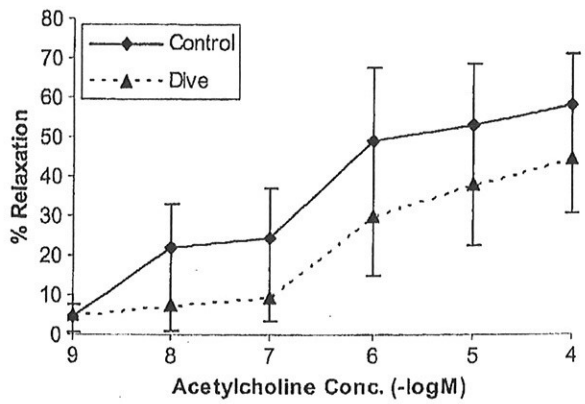

Fig. 3. Dose-response (% relaxation) to ACh in the abdominal aorta in the control group ($N = 7$) and the decompressed group ($N = 7$). Each data point symbolizes mean ± SD.

TABLE I. SPECIFIC GRAVITY OF THE BRAIN INCLUDING CEREBELLUM AND MEDULLA OBLONGATA.

	N	Mean	SD	P
100 kPa				0.53
Control	6	1.04525	0.00033	
Dive	7	1.04550	0.00069	
300 kPa				0.63
Control	6	1.04542	0.00033	
Dive	7	1.04565	0.00069	

One rat in the control group was taken out of the statistics since the measurement had an extreme value and a low R^2. The R^2 in the Control group was 0.981 ± 0.018 and the R^2 in the Dive group was 0.985 ± 0.013.

compression, nor was there a change in specific gravity when pressure increased to 300 kPa ($P = 0.38$), indicating no gas phase retention.

DISCUSSION

The present study demonstrated that arterial endothelial function was significantly reduced in rats following decompression, even if no venous gas bubbles could be detected using ultrasound. Hence, this study confirms a previous study in man (6). Even though we measured an impaired endothelial response, we found no signs of brain edema or gas bubble retention in the brain detectable by a reduction in specific gravity. This would indicate that the changes in endothelial function did not lead to a break in the blood-brain barrier. Previous studies have shown that such breaks can occur after decompression (8,13,20). It is possible that this damage, followed by brain edema, develops at a later stage and, therefore, was not observed in our acute study, which only had a 1-h observation period. However, one study by Hjelde et al. showed that rats with detectable bubbles get both brain edema and gas bubble retention when killed 1 h after surfacing (21); this was also true in the nine animals in that study with a low bubble score. This indicates that even a low number of vascular bubbles may lead to a break in the blood-brain barrier, while bubbles too small to be detected do not. If any damage did occur, it was too subtle to be detected using this methodology. The study by Hjelde et al. showed that the edema/free-gas ratio decreased with increasing bubble score (21). They found no significant difference in gas retention between the rats with a low bubble score and the control group. To our knowledge there are no studies demonstrating how much gas must be retained in the brain before we can detect it using specific weight measurements.

Our study was performed using female rats and it has been previously demonstrated that cerebrovascular inflammation is suppressed by estrogen but increased by progesterone and it has also been suggested that sex steroids may modulate blood-brain barrier permeability (29). Chryssanthou and coworkers found no sex differences when studying blood-brain barrier permeability by altitude decompression (8). Estrogen appears to enhance NO production through endothelial NO synthase and increases NO bioavailability (29,30). The effect of progesterone on the vascular endothelium seems to be tissue specific and possible antagonizing effects on the protective effects of estrogen have been found in, for example, the coronary artery (30). Still, several studies have found a protective effect from female sex hormones with regard to endothelial-dependent vasorelaxation in the aorta in rats with cardiovascular diseases and we can not exclude the possibility of a similar effect in the present study (23,24,30,35).

There seems to be a correlation between the amount of venous bubbles observed after decompression and the risk of developing DCS (13,15,37), although DCS can occur even when small amounts of bubbles are present. When studying endothelial damage caused by bubbles in the pulmonary arteries of pigs, Nossum et al. found that the damage was related to bubble score in a dose dependent manner, where high bubble score induced a more severe endothelial dysfunction. They further showed that a short exposure to many bubbles injures the endothelium more than a longer duration of exposure to fewer bubbles (28).

The venous bubbles damaging the abdominal aorta either have to go through the lungs or a persistent foramen ovale. Since the lungs are considered a good filter for gas bubbles (7) and we could not detect any bubbles in the right ventricular chamber, it is unlikely that a significant number of bubbles in the aorta caused the endothelial damage measured in the present study. Furthermore, it is unlikely that bubbles form de novo in large arteries (33). A recent study showed impaired endothelial function in man evaluated in vivo, according to the method of Raitakari and Celemajer (6), with minimal bubble formation. In that study a 1.5-3.3 MHz ultrasound probe was used to measure bubble grade. We used an ultrasonic frequency of 10 MHz. Since there is a proportionality between the size of the gas bubble and the amplitude of the reflected signal, as long as the bubbles are larger than the resonant size for the ultrasonic frequency used, we should be able to detect small bubbles even with transthoracic recordings due to the short distance from the probe to the right ventricle when testing rats. Using an ultrasonic frequency of 10 MHz at 1 ATA we will get a resonant size on the order of 0.3 μm, while a frequency of 2 MHz has a resonant size of 1.5 μm (4). Still, available commercial ultrasound technology has several limitations, particularly when measuring in animals with low bubble scores and/or very small bubbles. Even if potential bubbles were larger then resonance size, and although bubbles are strong reflectors of the ultrasonic beam, there is a limitation of size in detectable bubbles. Hills and Grulke predicted, for example, a minimum detection size in the heart or large arteries of 20-25 μm (17). We could not set a certain detection limit in this study, although we should have been able to detect bubbles with a diameter of 20-50 μm depending on the signal/noise ratio when scanning each rat. We should, therefore, have been able to detect the majority of stable vascular bubbles. In addition, we used Doppler to further increase the probability of detecting bubbles, if

any were present. Still, we cannot conclude that there were no bubbles present in the decompressed rats in this study and we cannot rule out such bubbles as a cause of the endothelial damage observed. If there were only few bubbles present they could have escaped detection by available ultrasound technology if they were not within the focus of the ultrasonic beam or were located near the ventricle wall. It is further possible for small bubbles to pass undetected during Doppler measuring due to noise artifacts. It is therefore possible that undetected bubbles entered the arterial circulation and mechanically caused the endothelial damage we observed.

Another hypothesis for the dysfunction is biochemical influence from damaged endothelium in the venous system. Venous bubbles that dissolved or got trapped before reaching the heart may have damaged or activated endothelial cells in the veins, which then started an inflammatory process that affected the endothelium in the arterial circulation. A possible pathway is via shedding of endothelial microparticles from the venous system that pass the lung filter and directly affect endothelium-dependent vasorelaxation in the arterial system via a mechanism that involves diminished production and/or bioavailability of NO as shown by Brodsky et al. (2). Maden et al. recently showed that EMP levels rise after a simulated dive in accordance with the USN standard air decompression tables (280 kPa for 80 min) (25). Unfortunately, they did not monitor for bubbles in their study.

A third possibility is that a reduction in endothelial function may have been caused by the elevated oxygen tension during the dive at 700 kPa breathing air, leading to vasoconstriction independent of the sympathetic system (6,26). This may have been due to an increase in the deactivation of NO by O_2-depending mechanisms, such as superoxide anion production (36). However, the mechanism by which elevated oxygen tension influences systemic arterial endothelial function remains unclear and several vasoconstrictor agents have been suggested. For example, Hink et al. performed an in vivo study on two groups of rats that were exposed to 100% oxygen at 2.8 ATA for 45 min (18) and the aorta were cut out, respectively, immediately and 17 h after the exposure. In that study they could not detect any changes in maximal vasodilatation to acetylcholine in the thoracic aorta in vitro 0 h or 17 h after the exposure. Another study by Täepõld et al. found a higher endothelial-dependent relaxation to ACh in rat aortic rings immediately after a 1-h exposure to 95% oxygen (32), an effect that was almost completely reversed 24 h after the exposure. Although the rats in our study were not killed until 1 h after the simulated dive, neither of these studies using similar rats and methodology indicates that the lower endothelial vasorelaxation to ACh in this present study could have been caused by elevated oxygen tension during the simulated dive. Since the rats were under anesthesia 1 h before the aorta were excised, it is unlikely that sympathetic stress influenced the results in this study.

In conclusion, this study, along with a previous study in man, indicates that following decompression, endothelial arterial dysfunction can be detected even if no bubbles are observed in the venous system. Furthermore, if no bubbles are detected, there is little evidence of a break in the blood-brain barrier. Thus, the assumption that bubble transfer from the right to the left heart is not needed for arterial dysfunction following decompression is supported.

ACKNOWLEDGMENT

Authors and affiliations: Kim-Vadar Rasdal, M.D., Astrid Hjelde, Ph.D., M.Sc., Andreas Møllerløkken, Ph.D., M.Sc., Nina Lundset, M.Sc., and Alf Ottar Brubakk, Prof., Ph.D., Department of Circulation and Medical Imaging Faculty of Medicine, Norwegian University of Science and Technology, Trondheim, Norway.

REFERENCES

1. Blatteau JE, Souraud JB, Gempp E, Boussuges A. Gas nuclei, their origin, and their role in bubble formation. Aviat Space Environ Med 2006; 77:1068–76.
2. Brodsky SV, Zhang F, Nasjletti A, Goligorsky MS. Endothelium-derived microparticles impair endothelial function in vitro. Am J Physiol Heart Circ Physiol 2004; 286:H1910–5.
3. Broman T, Branemark PI, Johansson B, Steinwell O. Intravital and post-mortem studies on air embolism damage of the blood brain-barrier. Acta Neurol Scand 1966; 42:146–52.
4. Brubakk AO. Ultrasonic methods for detection of gas bubbles . In: Supersaturation and bubble formation in fluids and organisms. The Royal Norwegian Society of Sciences and Letters, The Foundation. Trondheim: Tapir; 1989:353–86.
5. Brubakk AO, Eftedal O, Wisløff U. Endothelium and diving. In: Aird WC. Endothelial biomedicine. New York: Cambridge University Press; 2007:497–505.
6. Brubakk AO, Duplancic D, Vilic Z, Palada I, Obad A, et al. A single air dive reduces arterial endothelial function in man. J Physiol 2005; 566(Pt. 3):901–6.
7. Butler BD, Hills BA. The lung as a filter for microbubbles. J Appl Physiol 1979; 47:537–43.
8. Chryssanthou C, Palaia T, Goldstein G, Stenger R. Increase in blood-brain barrier permeability by altitude decompression. Aviat Space Environ Med 1987; 58:1082–6.
9. Dujic Z, Obad A, Palada I, Valic Z, Brubakk AO. A single open sea air dive increases pulmonary artery pressure and reduces right ventricular function in professional divers. Eur J Appl Physiol 2006; 97:478–85.
10. Edvinsson L, Nielsen KC, Owman C. Influence of initial tension and changes in sensitivity during amine-induced contractions of pial arteries in vitro. Arch Int Pharmacodyn Ther 1974; 208:235–42.
11. Eftedal O, Brubakk AO. Agreement between trained and untrained observers in grading intravascular bubble signals in ultrasonic images. Undersea Hyperb Med 1997; 24:293–9.
12. Eftedal OS. Ultrasonic detection of decompression induced vascular microbubbles [Dissertation]. Trondheim, Norway: Norwegian University of Science and Technology; 2007.
13. Eftedal OS, Lydersen S, Brubakk AO. The relationship between venous gas bubbles and adverse effects of decompression after air dives. Undersea Hyperb Med 2007; 34:99–105.
14. Foster PP, Boriek AM, Butler BD, Gernhardt ML, Bové AA. Patent foramen ovale and paradoxical systemic embolism: a bibliographic review. Aviat Space Environ Med 2003; 74(6, Suppl.)B1–64.
15. Foster PP, Feiveson AH, Boriek AM. Predicting time to decompression illness during exercise at altitude, based on formation and growth of bubbles. Am J Physiol Regul Integr Comp Physiol 2000; 279:R2317–28.
16. Hemmingsen EA, Hemmingsen BB. Lack of intracellular bubble formation in microorganisms at very high gas supersaturations. J Appl Physiol 1979; 47:1270–7.
17. Hills BA, Grulke DC. Evaluation of ultrasonic bubble detectors in vitro using calibrated microbubbles at selected velocities. Ultrasonics 1975; 13:181–4.

18. Hink J, Thom SR, Simonsen U, Rubin I, Jansen E. Vascular reactivity and endothelial NOS activity in rat thoracic aorta during and after hyperbaric oxygen exposure. Am J Physiol Heart Circ Physiol 2006; 291:H1988–98.
19. Hjelde A. Surface tension and complement activation: factors influencing bubble formation and bubble effects after decompression [Dissertation]. Trondheim, Norway: Norwegian University of Science and Technology; 2000:30.
20. Hjelde A, Bolstad G, Brubakk AO. The effect of air bubbles on rabbit blood brain barrier. Undersea Hyperb Med 2002; 29:31–8.
21. Hjelde A, Nossum V, Steinsvik M, Bagstevold JI, Brubakk AO. Evaluation of cerebral gas retention and oedema formation in decompressed rats by using a simple gravimetric method. Scand J Clin Lab Invest 2002; 62:263–70.
22. Hogestatt ED, Andersson KE, Edvinsson L. Mechanical properties of rat cerebral arteries as studied by a sensitive device for recording of mechanical activity in isolated small blood vessels. Acta Physiol Scand 1983; 117:49–61.
23. Kauser K, Rubanyi GM. Gender difference in endothelial dysfunction in the aorta of spontaneously hypertensive rats. Hypertension 1995; 25(4, Pt. 1):517–23.
24. Krause DN, Duckles SP, Pelligrino DA. Influence of sex steroid hormones on cerebrovascular function. J Appl Physiol 2006; 101:1252–61.
25. Madden LA, Laden G. Endothelial microparticles in vascular disease and as a potential marker of decompression illness. European Journal of Underwater and Hyperbaric Medicine 2007; 8(1&2):6–10.
26. Molenat F, Boussuges A, Grandfond A, Rostain JC, Sainty JM, et al. Haemodynamic effects of hyperbaric hyperoxia in healthy volunteers: an echocardiographic and Doppler study. Clin Sci (Lond) 2004; 106:389–95.
27. Nossum V, Hjelde A, Bergh K, Brubakk AO. Lack of effect of anti-C5a monoclonal antibody on endothelial injury by gas bubbles in the rabbit after decompression. Undersea Hyperb Med 2000; 27:27–35.
28. Nossum V, Koteng S, Brubakk AO. Endothelial damage by bubbles in the pulmonary artery of the pig. Undersea Hyperb Med 1999; 26:1–8.
29. Orshal JM, Khalil RA. Gender, sex hormones, and vascular tone. Am J Physiol Regul Integr Comp Physiol 2004; 286:R233–49.
30. Qiao X, McConnell KR, Khalil RA. Sex steroids and vascular responses in hypertension and aging. Gend Med 2008; 5(Suppl. A):S46–64.
31. Raitakari OT, Celermajer DS. Testing for endothelial dysfunction. Ann Med 2000; 32:293–304.
32. Tähepõld P, Elfström P, Eha I, Kals J, Taal G, et al. Exposure of rats to hyperoxia enhances relaxation of isolated aortic rings and reduces infarct size of isolated hearts. Acta Physiol Scand 2002; 175:271–7.
33. Yount DE. Application of a bubble formation model to decompression sickness in rats and humans. Aviat Space Environ Med 1979; 50:44–50.
34. Vapaatalo H, Mervaala E. Clinically important factors influencing endothelial function. Med Sci Monit 2001; 7:1075–85.
35. Villar IC, Hobbs AJ, Ahluwalia A. Sex differences in vascular function: implication of endothelium-derived hyperpolarizing factor. J Endocrinol 2008; 197:447–62.
36. Whorton AR, Simonds DB, Piantadosi CA. Regulation of nitric oxide synthesis by oxygen in vascular endothelial cells. Am J Physiol 1997; 272(6, Pt. 1):L1161–6.
37. Wisloff U, Richardson RS, Brubakk AO. NOS inhibition increases bubble formation and reduces survival in sedentary but not exercised rats. J Physiol 2003; 546(Pt. 2):577–82.