Ultrasound for bubble detection

Toward automated bubble counts from 2D echocardiography

Ultrasound – the impact of new technology

Estimating sample size for ultrasound studies

Decompression illness treated in Auckland, New Zealand

Biochemical markers of neurological decompression sickness

Does vinegar make box jellyfish stings worse?

PURPOSES OF THE SOCIETIES
To promote and facilitate the study of all aspects of underwater and hyperbaric medicine
To provide information on underwater and hyperbaric medicine
To publish a journal and to convene members of each Society annually at a scientific conference

DIVING and HYPERBARIC MEDICINE
\<www.dhmjournal.com\>

Editorials

The lymphatic pathway for microbubbles

Costantino Balestra

The sites for formation of microbubbles that are routinely detected precordially by Doppler after a decompression are still a matter of debate. Firstly, microbubbles could form on the endothelial wall of capillaries, at specific nanometric sites, but the release mechanism of such small emerging entities remains puzzling. They might also be formed from pre-existing gas nuclei present in the blood when favorable local hydrodynamic/supersaturation conditions generate microcavitation and tribonucleation phenomena. Finally, tissues could represent large pools for microbubble formation and amplification. Nevertheless, it remains unexplained as to what the potential driving pathways might be.[1]

Knowing that the permeability of most of the blood capillary network is quite low, an alternative is proposed for such transport. The lymphatic system, which drains the interstitial fluid to guarantee the fluid balance of tissues, could allow the transfer of micrometric elements, like stabilized microbubbles formed in tissues, over long distances. These might then be reinjected into the bloodstream via the right lymphatic and thoracic ducts. The characteristics of this slow transport, activated by the muscular pump, could explain the detection of vascular gas emboli (VGE) over long periods.

This hypothesis may give credence to a relatively old empirical finding of combat and commercial divers: that one should drive the boat fast to the dive site, but not on the way back, to reduce the risk of decompression sickness. These stories finally interested researchers enough to take a scientific look at why this happens. It was confirmed that 30 minutes of whole-body vibration before a dive (30 min, 30 msw) had preventive effects on post-dive bubble formation.[2] As there was no observed change in flow-mediated dilatation after vibration, the authors concluded that a nitrogen monoxide-mediated mechanism was not involved; rather, a mechanical dislodgement or enhanced lymphatic elimination of gas nuclei was hypothesized.

There are several possible explanations for this effect. Firstly, the vibrational force transmission to the whole-body should interact with the blood flow as well as the endothelium in order to eliminate the gas nuclei. In addition, vibrations may increase the blood friction forces on the endothelium favoring the detachment of gas micronuclei from the vascular wall. Vibrations should induce, by force transmission, a modification of endothelial spatial conformation. This modification should be responsible for a higher exposition of gas nuclei to the blood flow drag forces. Finally, the increase of lymphatic circulation, induced by vibration,

Figure 1
Accelerated peripheral elimination of radioactive tracer during vibration ($n = 5$); Tc99-labelled albumin was injected subcutaneously into the first dorsal interosseous space; the gamma camera was positioned over the axilla and the arm vibrated at 30Hz using a physiotherapeutic vibrator
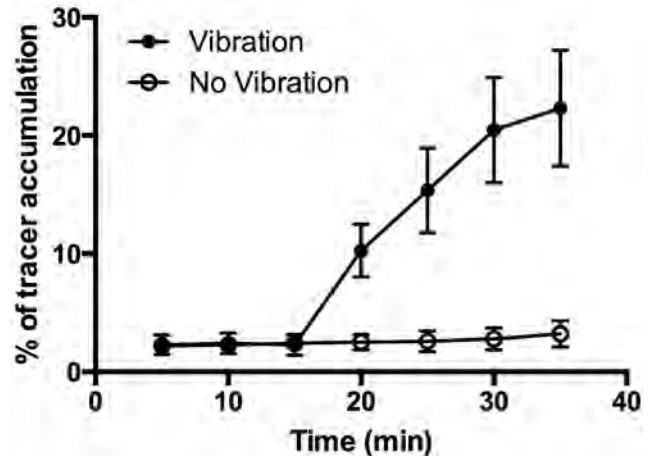
would allow the elimination of a part of intercellular tissue micronuclei (Figure 1).[3]

In conclusion, the effectiveness of vibration on VGE elimination might be explained by the mechanical action of vibration on the endovascular and tissue localization of micronuclei. Other preconditioning situations showing positive effects on the number of post-dive vascular gas emboli also can be explained by increased lymphatic activity.

References

1   Hugon J, Barthelemy L, Rostain JC, Gardette B. The pathway to drive decompression microbubbles from the tissues to the blood and the lymphatic system as a part of this transfer. *Undersea Hyperb Med.* 2009;36:223-36.
2   Germonpré P, Pontier JM, Gempp E, Blatteau JE, Deneweth S, Lafère P, et al. Pre-dive vibration effect on bubble formation after a 30-m dive requiring a decompression stop. *Aviat Space Environ Med.* 2009;80:1044-8.
3   Leduc A, Lievens P, Dewald J. The influence of multidirectional vibrations on wound healing and on regeneration of blood- and lymph vessels. *Lymphology.* 1981;14:179-85.

Costantino Balestra, PhD
President, EUBS
Professor of Integrative Physiology, Haute Ecole Paul Henri-Spaak, Brussels
E-mail: <costantino.balestra@eubs.org>

Key words
Doppler, bubbles, venous gas embolism, physiology, editorials

Front page photo of a rebreather diver at the Cod Hole on Ribbon Reef Number 10 at the northern end of the Great Barrier Reef was taken by Dr Simon Mitchell

Ultrasonic detection of decompression-induced bubbles

Neal W Pollock and Ron Y Nishi

Detection of gas emboli (bubbles) using ultrasound is a principle tool for monitoring decompression stress short of symptom development. Decompression-induced bubbles were first observed 47 years ago at the Virginia Mason Research Center as audible signals from sheep being monitored with a Doppler ultrasonic flowmeter.[1] Bubbles were later observed in human divers following decompression.[2] Aural detection of decompression-induced bubbles usually employs continuous-wave Doppler ultrasonic bubble detection (DUBD) using transcutaneous transducers to monitor a three-dimensional volume of blood in the precordial region (pulmonary artery or right ventricle of the heart) or peripheral veins such as the subclavian. Pulsed DUBD may provide more sensitivity and reduce background noise since 'range-gating' can be used to look at a specific distance from the transducer where bubbles are expected. However, it is more difficult to use, particularly with multiple subjects who are measured serially, and not widely applied in decompression studies. In either case, the portability of the instruments makes them useful for both laboratory and field studies.

The use of two-dimensional (2D) echocardiography to look for bubbles in the chambers of the heart is a more recent development.[3] 2D systems can provide a cross-sectional view along a single plane of all four chambers of the heart. Thus, unlike DUBD systems that assess only blood prior to pulmonary filtration, 2D imaging systems can also assess blood that will be sent systemically. Initially, 2D scanning devices were of sufficient bulk to be limited to laboratory studies. However, within the last 15 years, battery-operated portable units with sufficient resolution have become available for field studies. Technological advances, particularly harmonic processing, which allows analysis of less noisy signals at a harmonic frequency than at the return of the fundamental frequency sent out by the device, have made it possible to achieve image resolution close to that of standard clinical laboratory instruments. While transoesophageal echocardiography offers better resolution, transthoracic echocardiography is more appropriate for the relatively prolonged and repeated sampling used in decompression studies and is generally adequate to identify highly reflective gas bubbles.

DUBD requires observers who have the aural skills (and aptitude) to identify and semi-quantify bubbles in the complex signals arising from blood flow and heart motion artifacts. Bubbles are usually graded with one of two common scales. Disparities in technician skill, technician bias, signal quality and the grading scales used create a degree of inherent subjectivity in grading. Automated detection and counting systems, whether hardware-based or software-driven, have long been desired but difficult to produce in a robust form. 2D echocardiography, on the other hand, can produce visual representations of bubbles, potentially more easily assessed with automated counting algorithms. It remains to be seen how such systems can address the confounding introduced by bubbles in the blood volume either not passing through or repeatedly passing through the imaging plane.

Other major challenges are the estimation of bubble size and total gas volume when direct measurement is not available for confirmation. While dual frequency ultrasound holds potential for future bubble sizing (the first pulse excites bubbles of a diameter related to the ultrasound frequency and the second pulse identifies vibrating bubbles; a sweep of frequencies could identify a range of bubble sizes), the issues are complex. The shape of bubbles, for example, particularly larger bubbles, can be substantially distorted, potentially affecting size estimates. While current efforts can be valuable, any size and volume estimates must be considered very critically and with substantial restraint.

A final practical challenge is the comparability of different methods of grading bubbles. While there has been some evaluation of sequential DUBD and 2D scans, such efforts have been completed with very few of the many devices available. Questions of comparability are likely to increase as technology evolves and resolution continues to improve. The evolution of 2D imaging has become apparent in recent reports documenting a greater than expected frequency of bubbles in the left heart. Classically, left heart bubbles have been associated with an elevated risk of serious decompression sickness (DCS) since they have bypassed pulmonary filtration and are about to be sent forth systemically; the jump in observations with current devices (in asymptomatic subjects) suggests that their impact in decompression stress likely requires a more nuanced assessment.

While the relationship between bubbles and DCS is not simple, there is a clear association. Practically, bubbles occur far more frequently than DCS, sometimes following exposures that have very good safety records. The great utility of bubble assessment is likely to remain, not in determining absolute decompression risk, but in assessing relative decompression stress, in studies with a repeated-measures design. Bubble studies can be useful in developing and validating dive tables and/or in evaluating and modifying dive profiles and procedures. Repeated-measures design is very important given the marked inter-individual variability in bubble expression. Intra-individual variability will remain a concern, moderated by the tightest controls feasible.

In this issue, two papers consider 2D ultrasound systems to detect and quantify decompression stress. Blogg et al provide a review of the comparability of Doppler and 2D imaging technologies and evaluate the impact of harmonic processing and estimates of bubble load by obtaining paired 2D ultrasound images made using conventional and harmonic

imaging.[4] Germonpré et al look at 2D imaging procedures, bubble grading, statistical methodologies for determining inter- and intra-rater agreement, and how a frame-based bubble counting system can improve agreement. The frame-based system allows bubbles to be treated as a continuous variable and may, perhaps, ultimately lead to computer-based algorithms for real-time analysis.[5]

A third paper in this issue, by Doolette et al, analyzes sample sizes required for sufficient statistical power to assess the differences in DCS risk between two decompression schedules when using observations of bubbles (that may have substantial variability) as an endpoint.[6] Paired samples (from subjects monitored with 2D echocardiography) of different sizes were investigated. The considerations raised in this paper may provide guidance in estimating appropriate sample sizes for future studies using observed bubbles for comparison of different dive profiles. While these authors employed a somewhat novel scale, it is possible that the methods described can be applied as a general standard to a variety of scales.

The common thread in these three papers is 2D imaging. They reflect a trend in decompression research towards a greater reliance on these techniques. Key benefits are their increased sensitivity and the ability to assess both sides of the heart. Still, despite these benefits, the relatively high cost of 2D systems and the extensive record of DUBD studies will undoubtedly keep DUBD technology in play, demanding ongoing attention to comparability.

References

1   Spencer MP, Campbell SD. Development of bubbles in venous and arterial blood during hyperbaric decompression. *Bull Mason Clinic.* 1968;22:26-32.
2   Spencer MP, Campbell SD, Sealey JL, Henry FC, Lindbergh J. Experiments on decompression bubbles in the circulation using ultrasonic and electromagnetic flowmeters. *J Occupational Med.* 1969;11:238-44.
3   Powell MR, Spencer MP, von Ramm O. Ultrasonic surveillance of decompression. In: Bennett PB, Elliott DH, editors. *The physiology and medicine of diving*, 3rd ed, San Pedro, CA: Best Publishing; 1982. p. 404-34.
4   Blogg SL, Gennser M, Möllerlökken A, Brubakk AO. Ultrasound detection of vascular decompression bubbles: the influence of new technology and considerations on bubble load. *Diving Hyperb Med.* 2014;44:35-44.
5   Germonpré P, Papadopoulou V, Hemelryck W, Obeid G, Lafère P, Eckersley RJ, Tang M-X, Balestra C. The use of portable 2D echocardiography and 'frame-based' bubble counting as a tool to evaluate diving decompression stress. *Diving Hyperb Med.* 2014;44:5-13.
6   Doolette DJ, Gault KA, Gutvik CR. Sample size requirement for comparison of decompression outcomes using ultrasonically detected venous gas emboli (VGE): power calculations using Monte Carlo resampling from real data. *Diving Hyperb Med.* 2014;44:14-9.

Neal W Pollock[1] and Ron Y Nishi[2]

[1] *Center for Hyperbaric Medicine and Environmental Physiology, Duke University Medical Center, and Divers Alert Network, Durham NC, USA*
E-mail: *<neal.pollock@duke.edu>*
[2] *Defence R&D Canada – Toronto (retired), Toronto, ON, Canada.*
E-mail: *<nishir@rogers.com>*

The Editor's offering

This issue has a strong focus on decompression and decompression illness. Despite almost 50 years of Doppler studies, the relationship between circulating bubbles after diving or hypobaric exposure and symptomatic decompression sickness (DCS) is still not clear-cut. New technology and better statistical methods will undoubtedly change our understanding of these phenomena.

Noticeable in the clinical report from Auckland are the long delays to presentation for treatment of recreational divers in New Zealand.[1] This is reflected in my own unit in Christchurch (unpublished observations), but is in sharp contrast to series such as that from the West of Scotland.[2]

The often frustrating search for clinically useful markers of DCS to guide management and prognosis continues with a French report that suggests a limited utility for neuron-specific enolase and none for S100B protein.[3]

Vinegar has been central to the first-aid treatment of box jellyfish stings, but sometimes worsen the often severe pain. A neat in-vitro study suggests a mechanism for this: that there may be partially discharged nematocysts present, which discharge more venom when vinegar is applied.[4]

References

1   Haas RM, Hannam JA, Sames C, Schmidt R, Tyson A, Francombe M, Richardson D, Mitchell SJ. Decompression illness in divers treated in Auckland, New Zealand, 1996-2012. *Diving Hyperb Med.* 2014;44:20-5.
2   Sayer MDJ, Ross JAS, Wilson CM. Analysis of two datasets of divers with actual or suspected decompression illness. *Diving Hyperb Med.* 2009;39:126-32.
3   Gempp E, Louge P, De Maistre S, Emile L, Blatteau J-E. Neuron-specific enolase and S100B protein levels in recreational scuba divers with neurological decompression sickness. *Diving Hyperb Med.* 2014;44:26-9.
4   Welfare P, Little M, Pereira P, Seymour J. An in-vitro examination of the effect of vinegar on discharged nematocysts of *Chironex fleckeri*. *Diving Hyperb Med.* 2014;44:30-4.

Michael Davis

The SPUMS President's page

Michael Bennett, President SPUMS

This is my final column as the SPUMS President, a good time to reflect on the changes the organisation has gone through and to speculate on the future. The 2008 ASM in Kimbe Bay, West New Britain, when I took over from Chris Acott, seems a surprisingly long time ago. I was suffering from high fevers and limb pains, later diagnosed as Dengue Fever, and my memories of that meeting are a little hazy.

In fact, 2008 turned out to be a watershed year for the Society. We formally amalgamated the *SPUMS Journal* and the *European Journal of Underwater and Hyperbaric Medicine* to form *Diving and Hyperbaric Medicine* (DHM), currently the most cited journal in our field. That is certainly one of the most satisfying achievements of our Society in recent years. By 2009, DHM was listed by SciSearch® (having been on Embase/Scopus since 2001). Finally in 2011, DHM was indexed by the National Library of Medicine (Medline).

In 2010, we made another big move by reorganizing how we manage the ASM each year. The most obvious change was that we no longer employed a travel agent. A significant departure from the past, this was a traumatic event for the Society and has caused injuries that may never heal. While the meeting would not have been possible without the tireless efforts of our convener that year, Glen Hawkins, I want to make it clear that, whether for good or ill, the impetus to move away from a travel agent-orientated approach was mine. I felt we needed to leave the easy attractiveness of total travel/meeting/accommodation packages behind us, allowing delegates more flexibility to make their own arrangements. This leaves no doubt about where the responsibilities of the Society begin and end. I argued then, and continue to believe, that a medical society is responsible for organizing a scientific meeting, but should avoid any direct involvement or responsibility for flights and accommodation. The latter risks a slippery slope into arrangements that are not in the best interests of the members. Well, my time is over now and the Society is, of course, free to review our decisions.

2011 may have been the year we got on the journalistic map, but it was a difficult year for our convener, with arrangements to return to Palau mysteriously thwarted by our inability to secure a suitable venue, resulting in a late move to Guam. Sarah Lockley handled this with aplomb, however, and it was a very happy meeting. Similarly in 2012, we reprised our visit to Madang, and despite a few wrinkles with flights and dive weights, the resort did their very best for us, and we had two truly motivational speakers in Jamie Seymour and Richard Fitzpatrick.

More worryingly, for some years our membership has shrunk, although this trend seems to have stabilised recently. The decline is multifactorial: the combining of the two journals meaning the loss of any reason to belong to both societies, as was the case with some members; disenchantment with the change in arrangements for the ASM and the general tendency for the elderly to die and young people not to join clubs and societies. Currently we are running at about 500 members and, encouragingly, the average age of ASM attendees is falling rather than rising. Meanwhile, our treasurers (Shirley Bowen and Jan Lehm) have been battling hard against considerable difficulty to keep our books in line, and our financial position remains strong and stable.

At the same time, our tireless Education Officer, David Smart, having worked vigorously to get our potential diplomates in order, to re-accredit the appropriate courses in our region, and to appraise the appropriate training schemes available, has announced his imminent departure. No-one has given more to SPUMS in the last 13 years (I recall talking him into being Chair of the ANZHMG in about 2001). Whoever takes over both David's major roles in SPUMS can be sure they will find a more ordered structure than he did.

Where do we go from here? Well, I am very optimistic about the Society. We have a great journal, an excellent ASM and a fascinating field of medicine to investigate. We have a much improved means of communication through our website and Facebook, our membership database is pared down from 13,000 entries to a more useful 1,000 or so records of current and recent members and our dues no longer need to be paid by cheque and snail mail. We seem to have reached the late 20th Century! There is plenty left to do, however, and some big decisions to be made. How and when do we move to electronic publishing? How will we be affected by free access journals? Should we find ways of making the ASM more attractive to the general membership (around 10% attend our meetings)? I look forward to participating as Past President in what the new team does to further the aims of the most useful medical society to which I belong.

Finally I have to thank all those who have contributed their time and effort so generously during my time as President. I have named some above, but there are many others equally deserving and I apologize to those I will miss! Sarah Lockley and Karen Richardson as Secretary have brought such energy to the table, full of bright ideas and hard work, Guy Williams who has advised us so wisely on treasury and constitutional matters, all the conveners, our Editor, Mike Davis, Steve Goble for his total reliability and Cathy Meehan for keeping an eye on the future for us. At times you all managed to make me look good!

Key words
Medical society, general interest

Original articles

The use of portable 2D echocardiography and 'frame-based' bubble counting as a tool to evaluate diving decompression stress

Peter Germonpré, Virginie Papadopoulou, Walter Hemelryck, Georges Obeid, Pierre Lafère, Robert J Eckersley, Ming-Xing Tang and Costantino Balestra

Abstract

(Germonpré P, Papadopoulou V, Hemelryck W, Obeid G, Lafère P, Eckersley RJ, Tang M-X, Balestra C. The use of portable 2D echocardiography and 'frame-based' bubble counting as a tool to evaluate diving decompression stress. *Diving and Hyperbaric Medicine*. 2014 March;44(1):5-13.)

Introduction: 'Decompression stress' is commonly evaluated by scoring circulating bubble numbers post dive using Doppler or cardiac echography. This information may be used to develop safer decompression algorithms, assuming that the lower the numbers of venous gas emboli (VGE) observed post dive, the lower the statistical risk of decompression sickness (DCS). Current echocardiographic evaluation of VGE, using the Eftedal and Brubakk method, has some disadvantages as it is less well suited for large-scale evaluation of recreational diving profiles. We propose and validate a new 'frame-based' VGE-counting method which offers a continuous scale of measurement.

Methods: Nine 'raters' of varying familiarity with echocardiography were asked to grade 20 echocardiograph recordings using both the Eftedal and Brubakk grading and the new 'frame-based' counting method. They were also asked to count the number of bubbles in 50 still-frame images, some of which were randomly repeated. A Wilcoxon Spearman *rho* calculation was used to assess test-retest reliability of each rater for the repeated still frames. For the video images, weighted kappa statistics, with linear and quadratic weightings, were calculated to measure agreement between raters for the Eftedal and Brubakk method. Bland-Altman plots and intra-class correlation coefficients were used to measure agreement between raters for the frame-based counting method.

Results: Frame-based counting showed a better inter-rater agreement than the Eftedal and Brubakk grading, even with relatively inexperienced assessors, and has good intra- and inter-rater reliability.

Conclusion: Frame-based bubble counting could be used to evaluate post-dive decompression stress, and offers possibilities for computer-automated algorithms to allow near-real-time counting.

Key words

Echocardiography, Doppler, bubbles, venous gas embolism, arterial gas embolism, decompression sickness, risk assessment, diving research

Introduction

Underwater diving on compressed air or other breathing gases exposes the diver to so-called 'decompression stress', caused by the release of nitrogen and/or other inert gases from the body tissues during and after ascent from depth, resulting in bubbles forming in tissues and (more commonly observable) in blood. In order to minimise this stress and decrease the risk of decompression sickness (DCS), decompression algorithms, summarised in dive tables or incorporated into dive computers, have been developed. These algorithms are not completely successful in the avoidance of every instance of DCS and, to this day, a major research effort is directed to identifying factors and interventions (pre dive, during the dive and post dive) that could make decompression safer.[1]

Evaluation of these algorithms and of the efficacy or inefficacy of other preventive measures has been done primarily on the basis of the presence or absence of clinical symptoms of DCS, as well as on the detection of bubbles in the vascular system using Doppler ultrasonic bubble detectors. Doppler bubble 'grades' were first defined by Spencer et al. in 1974, and classified into 5 grades (0 to 4), depending on the number of acoustic bubble signals audible in the precordial region:[2]

Grade 0 – Complete lack of bubbles;
Grade 1 – Occasional bubble signal, vast majority of cardiac cycles bubble-free;
Grade 2 – Many, but less than half, of cardiac cycles contain bubbles, singly or in groups;
Grade 3 – All cardiac cycles contain bubbles in showers, but not overriding heart signals;
Grade 4 – Bubbles sounding continuously during systole and diastole, overriding amplitude of normal heart signals.

In 1976, Kisman and Masurel defined a scale using three parameters (frequency, amplitude and duration) allowing for more precise classification but rendering acquisition and evaluation more complicated.[3,4] Both these scales require a skilled, experienced Doppler technician in order to be reproducible.[5,6] In 2004, Divers Alert Network (DAN) Europe Research proposed a simplified 'bubble score',

distinguishing only low, medium, high and very high bubble grades based on precordial Doppler, but this scale has not been widely adopted by others.[7,8] Modifications of the original Spencer scale have likewise been proposed, resulting in the 'Expanded Spencer Scale', with a larger number of categories and thus a more incremental grading.[7,8] Whilst the original Spencer scale has been by far the most frequently used in diving research, the Kisman-Masurel scale has been preferred for large, well-controlled, laboratory decompression research studies, and an association between bubble grade and risk for decompression sickness has been developed that can equally be used for the Spencer scale.[2] Generally, it is accepted that the higher the number of bubbles detected precordially, the higher the statistical risk for DCS after a dive.[4,6,9]

Using echocardiography, Eftedal and Brubakk in 1997 proposed a bubble score of six grades based on visual analysis of 2D precordial echo images:[10]

Grade 0 – No observable bubbles;
Grade 1 – Occasional bubbles;
Grade 2 – At least one bubble every four cardiac cycles;
Grade 3 – At least one bubble every cardiac cycle;
Grade 4 – At least one bubble per cm² in every image;
Grade 5 – 'White-out', single bubbles cannot be discriminated.

This allows a semi-quantitative evaluation in a reproducible manner, with minimal intra- and inter-observer variability. However, the scoring system as proposed does not discriminate well in the medium range of bubble scoring, with a large jump between grade 3 and grade 4, making this score less adapted for the evaluation of low to medium levels of decompression stress (classifying into either 'low' or 'severe'). Also, the use of echocardiography made this method less practical for deployment in real-life diving situations (e.g., on a dive boat with a humid, sometimes cold environment and possible lack of AC power). Only recently have good-quality, portable echocardiographs become available, that make on-site evaluation (at the waterfront) possible, by visualising decompression VGE. The use of 'harmonic imaging' (HI) decreases noise in the cardiac cavities, and Color Map application ('gold' setting instead of standard 'grey') provides better image contrast.[11,12] Thus, the detection of VGE in divers' heart cavities and large veins is easier and visualisation of smaller VGE than were detectable by older echography machines is possible.[13] Of note, this use of HI improves the signal-to-noise ratio and increases contrast, but does not aim to make VGE oscillate to emit their own harmonic frequencies, as much lower scanning frequencies would be needed for this to happen.[14–17] For a useful review of HI the reader is referred to references 11 and 12.

In this paper, we describe a newly developed method of evaluation of decompression-induced VGE, using transthoracic 2D echocardiography, which may offer significant advantages compared to current methods.

Methods

A standardised technique for evaluation of decompression stress by means of counting the number of VGE is described, using a portable echocardiography device, with hard-disk recording and *a posteriori* (off-line) evaluation of cardiac images. The technique was developed using a Vivid-i portable echograph (GE Healthcare, UK) and subsequently applied successfully using a Vivid 7 echograph (GE Healthcare, UK), both in a controlled environment (beside a swimming pool) and in the field (dressing room of a Belgian quarry dive site).

A GE 3S-RS sector array ultrasound probe (GE Healthcare, UK) is used; the machine is used in harmonic imaging mode (2.0/4.0 MHz). A four-chamber view is obtained by placing the probe at the level of the left fifth intercostal space. It is necessary to modify the standard four-chamber view by rotating the probe slightly ventrally (in the direction of the xyphoid process) so the right atrium and ventricle can be fully visualised. Three 'landmark points' are identified to aid proper positioning of the ultrasound probe: both transsections of the tricuspid ring and the top of the right ventricle should be visible in the image (Figure 1). A series of at least 15 cardiac cycles are recorded onto the internal hard disk of the echograph while keeping the probe immobile. With practice, each recording can be done in less than 3 minutes (positioning of the diver, attachment of three ECG electrodes, obtaining a good view, recording, detaching the electrodes), allowing for serial measurements on up to 10 divers within a 30-minute interval between measurements of the same diver. At the completion of the measuring period, all videos are saved onto external hard disk or USB thumb drive in the 'wmv' format (Windows Media Video, at 30 frames per second), for which GE Healthcare provided a proprietary video player (MPEGVue Player).

At a later stage, the recordings stored on portable hard disk are reviewed using the MPEGVue software (GE Healthcare, UK), which allows for easy patient and examination selection, frame-by-frame advancing of the video frames using the keyboard arrow keys and freezing of the video frames while maintaining good still-image quality. First, the pre-dive echography loops are reviewed in order to identify intra-cardiac structures that may mimic VGE (e.g., papillary muscles, valve leaflets, Chiari network, Valsalva sinus). Then, the post-dive echography is reviewed and played in a loop at real-time speed in order to rapidly assess the presence or not of circulating bubbles. In cases where bubbles are seen, a formal bubble counting procedure is performed. Using the pause button, the loop is frozen at the start, and then with the forwards and backwards buttons, an image frame is selected in end-diastolic/proto-systolic position (where the tricuspid valve leaflets are fully opened and almost invisible) (Figure 2) and bubbles are counted in both the right atrium and ventricle (Figure 3). In case the chosen view does not contain any bubbles, but bubbles are clearly present in the heart cycle, the forwards and

Figure 1

Landmark structures in the right heart echography image: the upper circle identifies the 'top' of the right ventricle (RV) while the lower two circles identify the section through the tricuspid annulus on either side of the right atrium and constitute the 'upper' border of the RA. (N.B., echocardiograph images are inverted)
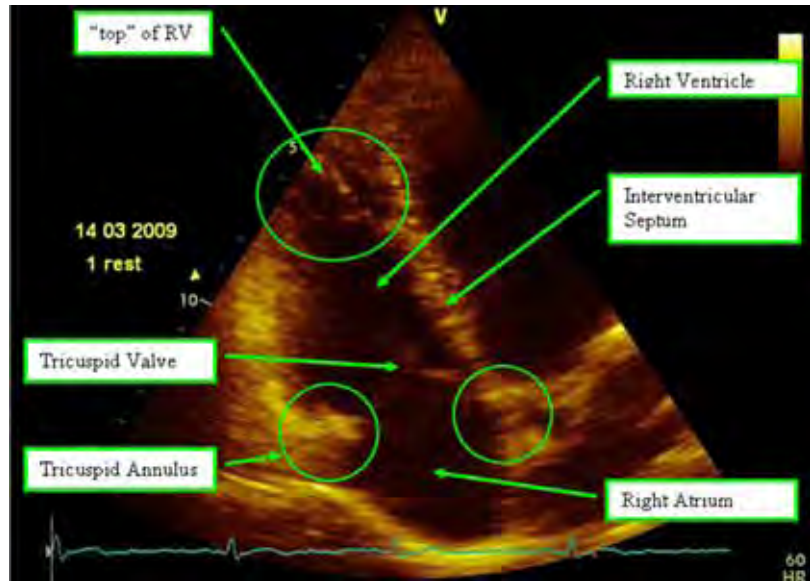
Figure 2

Choice of frame to analyse: the three landmark circles are drawn as in Figure 1. The frame chosen for analysis is indicated by the red marker on the electrocardiography trace (marked by the small green circle, bottom right). Both leaflets of the tricuspid valve are fully open and visible against the ventricular wall (points of green arrows); the right atrium and ventricle form a single cavity
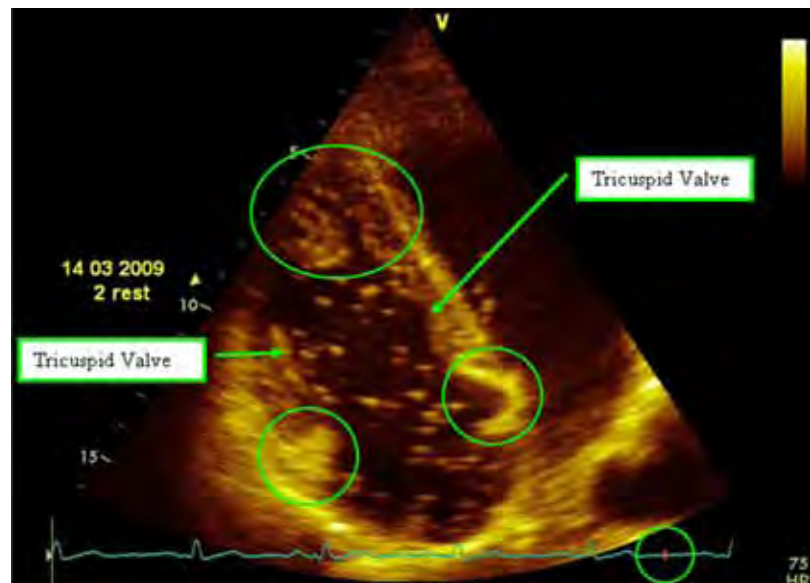
Figure 3

Bubble counting: bubble signals are identified as bright spots and counted individually; tricuspid valve leaflets and other fixed structures (e.g., papillary muscles in the top of the right ventricle) are not counted
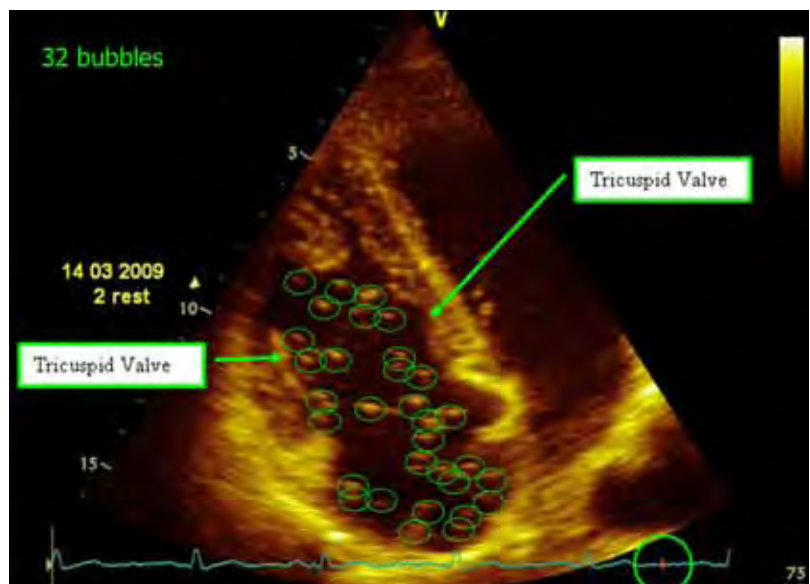
backwards buttons are used to select another frame, within two to three frames of the frame originally chosen. Ten consecutive frames are analysed and the bubble count is averaged over these 10 frames.

The technique was developed for use during a series of standardised test dives organised by DAN Europe Research (Roseto, Italy and Brussels, Belgium), in an indoor swimming pool of 34 metres' fresh water (mfw) depth (Nemo33, Brussels, Belgium). The dives were designed to evaluate the effect of several pre-dive interventions on the number of VGE post dive. For this purpose, each diver performed one (identical) dive per week, to 33 mfw for 20 minutes. This 'standard' dive was performed at least three times under 'normal' conditions, and several times under 'experimental' conditions, when the effects of several methods of preconditioning were measured. The order of the experimental dives was randomised. Each diver was evaluated with, among other tests, precordial echocardiography at three time points: before the dive, at 30 minutes and at 90 minutes after surfacing. The study was approved by the Academic Bioethical Committee of the Free University of Brussels (CE/2008/66); all divers were unpaid volunteers who provided written informed consent.

In order to verify the internal (intra-rater) and external (inter-rater) consistency of this frame-based counting method, nine observers were asked to perform analysis of the same set of images. Three were trained cardiologists, at various times involved in diving research performed by DAN Europe. All had performed one or more image acquisition sessions during the experimental pool dives. Three were medical doctors from the Centre of Hyperbaric Oxygen Therapy of the Military Hospital Brussels, who had no formal cardiology training but were present during some or all of the diving experiments, and had some experience in viewing echocardiographic images. The third group consisted of DAN Europe researchers or certified hyperbaric technicians (CHT) from the Centre of Hyperbaric Oxygen Therapy, who had various degrees of paramedical training, allowing them to identify the major intra-cardiac structures after some instruction. All received written instructions detailing the evaluation procedure (and containing the same pictures as in this report) and a short period of hands-on training in the use of the MPEGVue software, which is simple and intuitive to use.

First, a test was administered to verify the reliability and repeatability of the VGE counting by itself. A set of 50 still-frame images was presented for static bubble counting. These images were extracted by the authors from the available video loops, and chosen so as to represent a mix of better- and worse-quality images containing between 0 and 40 VGE signals. Images were presented as a Microsoft PowerPoint presentation. No identifying elements (such as name, birthdate, acquisition date) were displayed on the images, only the slide number. No time limit was given for viewing the slides. Unknown to the test persons, several of

the slides were in fact identical but spread out randomly over the presentation. Then, a selection of 20 post-dive video sequences were presented, together with their baseline pre-dive echocardiographic loop (no bubbles present) and the observers were asked to evaluate these video loops, using first the Eftedal and Brubakk score, then using frame-based counting as described above.

As there is no way to determine the exact number of VGE in the images, obviously a true 'gold standard' cannot be determined. The need to set a standard by which to compare the data from this study prompted us to define a 'reference score' as the number of visible bubbles in each image and video loop, agreed on by a priori consensus by the main authors of the study.

STATISTICAL METHODS

Internal consistency was verified on the static images; external consistency was verified on the static and video images with both scoring systems, using the following statistical methods.

Eftedal and Brubakk score

The weighted kappa statistic was chosen to evaluate the inter-rater agreement, in accordance with the discussion on the appropriateness of statistical methods to this effect by Sawatzky.[5] Cohen's kappa (κ) statistic is used to calculate the coefficient of agreement between raters for nominal grades where the outcome of agreement is binary: either agreement or disagreement.[18–20] For ordinal scales, the degree of agreement should be taken into account and this is done using the weighted kappa statistic instead. Both the kappa and weighted kappa are completely corrected for chance agreement.[18] The weights chosen to weight disagreements were defined in the same manner as the original Eftedal and Brubakk method to allow direct comparison. Since the data are ordinal (but not continuous) for the Brubakk and Eftedal method, a disagreement is 'stronger' if one rater assigns a score of 4 and another a score of 1, compared to 1 and 2 respectively. This is taken into account by using weights for characterising the degree of disagreement. In the usual contingency tables for two raters, the weights were specified as:

$$\omega_{ij} = 1 - \frac{|i - j|}{k - 1} \qquad (1)$$

where i and j index the rows and columns and k is the maximum number of possible ratings. The weighted kappa is then calculated from the proportional observed and expected agreements:[18,21]

$$Po(\omega) = \frac{1}{k} \sum_{i=1}^{k} \sum_{j=1}^{k} \omega_{ij} f_{ij} \qquad (2)$$

and

$$Pe(\omega) = \frac{1}{k^2} \sum_{i=1}^{k} \sum_{j=1}^{k} \omega_{ij} r_i c_j \qquad (3)$$

where f_{ij} is the number of recordings graded i by one rater

and j by the other, r_i is the row total for grade i and c_j is the column total for grade j, such that:

$$weighted\ kappa = \frac{Po(\omega) - Pe(\omega)}{1 - Pe(\omega)} \quad (4)$$

The kappa-statistic measure is a value between -1 and 1, with 0 corresponding to the value expected by chance and 1 perfect agreement. The interpretation of the values as suggested by Landis and Koch are given as:[22,23]

    below 0.00 – Poor
    0.00–0.20 – Slight
    0.21–0.40 – Fair
    0.41–0.60 – Moderate
    0.61–0.80 – Substantial
    0.81–1.00 – Almost perfect.

Frame-based counting method
For the frame-based counting method, both on still images and on the average over 10 video frames, the data are also ordinal but this time continuous (video) or discrete (units of bubbles). The same weighting applies and the added possibilities are factored in through the use of k so the kappa scores are comparable. The weighted kappa statistic cannot be used for continuous variables.[24] Therefore, another statistical test has to be chosen. For continuous data the intra-class correlation coefficient should be used as a measure of reliability, or Bland-Altman plots for limits of agreement and bias.[24,25] The intra-class correlation coefficient or ICC gives a measure of the proportion of total variance due to the difference between raters by penalising systematic error. For ordinal data, the intra-class correlation coefficient is comparable to the weighted kappa statistic if quadratic weights are used, which is why both weighted kappas (linear as in Sawatzky, and quadratic for comparing with the ICC) are quoted in this paper.[5,26] Note that it is exactly equivalent only for uniform marginal distributions.[25,27] The ICC scale goes from 0 to 1, with 1 representing perfect agreement and 0 no agreement. The Bland-Altman plot displays for two assessors (or groups of assessors) the difference for each assessment against the mean of each assessment.[21,28] The confidence interval is also displayed, calculated as the 95% percentiles such that the upper and lower bounds are given by:

    Means of differences ± 1.96 (std of differences) (5)
As such, the Bland-Altman plot shows any bias and the limits of agreement between two raters.

Intra-rater reliability (internal consistency)
The intra-rater reliability was assessed on the still-images test for the repeated images by the Wilcoxon signed-rank test, calculating the Spearman *rho* (rank correlation coefficient ρ) for every rater on the repeated images counts (taking the maximum discrepancy for the one image repeated three times). The value of ρ lies between -1 and 1, a higher number indicating a better reliability. The calculation of the weighted kappa statistic and ICC was performed offline using the standard statistical package Stata (StataCorp. 2011. *Stata Statistical Software: Release 12*. College Station, TX: StataCorp LP). All other data processing and plotting was

Table 1
Static images bubble counting – identical image pairs scores
Spearman ρ between raters and a reference score (see text);
all comparisons non-significant (Wilcoxon test-retest $P > 0.05$)
C – cardiologist, MD – physician, O – other (paramedic or
hyperbaric chamber attendant)

Rater	Category	Spearman rho
1	C	0.9733
2	C	0.9487
3	C	0.2052
4	MD	0.9211
5	MD	0.7632
6	MD	0.9211
7	O	0.7632
8	O	0.9747
9	O	0.8922

done by calculating the appropriate values offline as defined above directly in the commercial software package MatLab (MATLAB 6.1, The MathWorks Inc., Natick, MA, 2000).

Results

After some practice runs with the frame-based method, all observers reported bubble counting to be relatively easy and rapid, although the process of scrolling through video files was found to be somewhat tedious and slow (approximately 5 minutes for a video file evaluation). The static images were less confidently scored because, as the raters reported, no video images were available to help discriminate between intracardiac structures and VGE. However, the number of bubbles counted was not significantly different between observers (absolute number of bubbles 0 to 40 bubbles). As expected, a larger standard deviation was observed for larger bubble numbers. The ICC between the reference score and all raters was 0.96 (95% confidence interval (CI) from 0.92 to 0.99).

Calculated differences in scoring for identical image pairs (intra-rater or internal consistency) were non-significant (Wilcoxon test-retest, $P > 0.05$) with excellent Spearman ρ (0.76 to 0.97) except for one cardiologist, rater C3 ($\rho = 0.21$, Table 1). Further analysis showed that this observer consistently scored approximately 5 bubbles higher than the average, suggesting that a systematic error was present (see Bland-Altman plot, Figure 4). However, even in the case of this cardiologist with lower Spearman ρ, the Wilcoxon test-retest P-value showed that the differences in the test-retest counts were non-significant.

For the video sequences, the Eftedal and Brubakk scoring gave a weighted kappa of $\kappa = 0.5815$ with linear weights and $\kappa = 0.7634$ with quadratic weights, which shows a moderately good external consistency. It was found to be slightly lower than reported in the original publication ($\kappa = 0.6796$ using linear weights);[10] this may be a reflection of our study design testing and how easy the grading

Figure 4

Bland-Altman plot showing systematic over-estimating by cardiologist 3 as compared to the mean number of VGE counted by all others; X-axis: number of VGE in the image, Y-axis: difference of count vs. mean; horizontal lines – 95% confidence intervals as 1.96 (std of differences); LoA – limits of agreement

Figure 5

Bland-Altman plot showing the good consistency between the reference score (see text for explanation) and all observers for frame-based counting in the video sequences; X-axis: number of VGE counted in the video sequences (average of 10 frames); Y-axis: difference of count vs. mean; horizontal lines – 95% confidence intervals as 1.96 (std of differences); LoA – limits of agreement
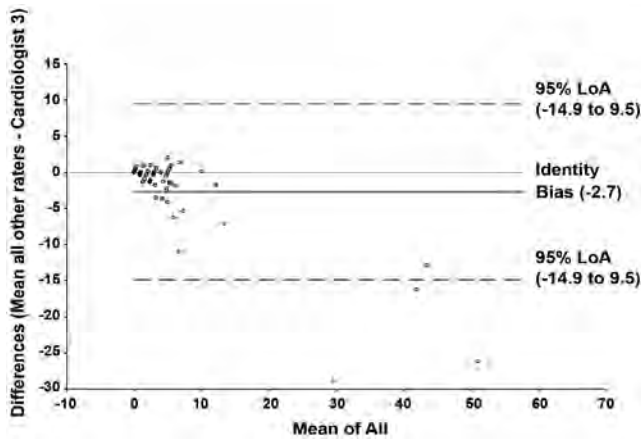
methods are to learn (use of non-expert raters with only written instructions). As indicated in the methods section, all raters received only minimal instructions in the various methods: a three-page document and a short hands-on training session on the use of the video player software. Therefore, the lower external consistency may well reflect the lesser experience in grading according to this score, as none of the nine raters had ever performed an Eftedal and Brubakk scoring before. The ICC for the Eftedal and Brubakk scoring gives 0.79 (95% CI 0.54 to 1.05); as this method is similar to the weighted κ with quadratic weights, it shows a very good inter-rater agreement.

Frame-based counting gave a higher external consistency, with an ICC of 0.84 (95% CI 0.77 to 0.92). There was no significant difference between all observers and the reference score (see Bland-Altman plot, Figure 5); however, here again, the same cardiologist scored consistently approximately 5 bubbles higher on every occasion.

Discussion

(Semi-)quantitative determination of VGE is an important, if not still the only tool available for evaluation of diving decompression stress. Currently used methods suffer from either the necessity of highly skilled observers, a complicated evaluation method (Spencer and Kisman-Masurel scales) or a semi-quantitative visual evaluation that fails to discriminate well in the mid-range of VGE (Eftedal and Brubakk score), exactly the range that most interventions to improve decompression safety for recreational divers would act upon. Also, bubble counting takes place only at certain points in time after the dive, and the accuracy of estimating the total bubble load is dependent on the number of measurements and their timing. One method of estimating the bubble load out of a number of discrete bubble evaluations is the Kisman
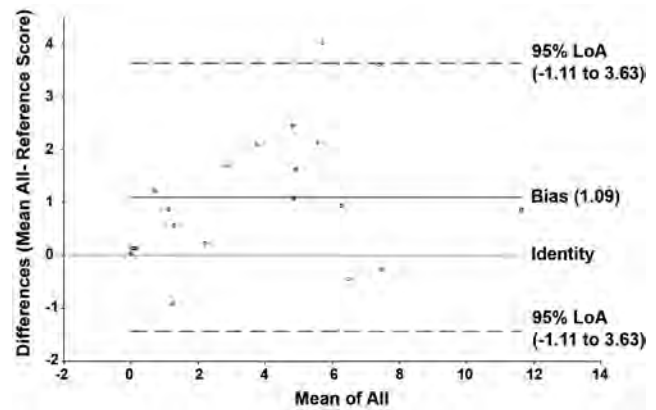
integrated severity score (KISS), which integrates bubble grades from a number of observations over a given time period into a single value; it can be considered an estimate of the 'area under the bubble grade curve', and is a relative value that can be used for comparative purposes.[29–31]

Using frame-based counting, a continuous-scale (more quantitative) evaluation of VGE presence can be done in a relatively quick, easy way, with good reproducibility. Using the bubble counts for 10 consecutive frames allows for small beat-to-beat variations in bubble numbers to be averaged out. A current drawback is that bubble counting must be done manually at a later stage, which requires additional steps (exporting the video loops in MPEGVue format) and takes some time for counting. Thus, it is not real-time analysis. However, taking into account the echogenicity of the different surrounding structures and using intelligent learning algorithms, computerised automatic counting may become possible. This would permit real-time and continuous counting of VGE, and thus make VGE evaluation independent of the timing of observations after the dive. These algorithms are currently under development.[32–34]

As 2D echocardiography permits viewing the cardiac cavities in a single plane only, the choice of plane may be of some importance. The standard four-chamber view, as used in echocardiography, shows only the basal part of the right ventricle, with the top of the right ventricular cavity out of view. This is not a problem in cardiac evaluation, as most emphasis lies on the morphology and function of the left atrium and ventricle, but may obscure significant parts of the right heart cavities, where VGE are primarily visible after the dive. To overcome this, the method described requires slight tilting of the echo probe to point more in the direction of the xyphoid region, permitting identification of the three landmarks: the top of the right ventricle, the tricuspid ring

and the left and right tricuspid valve leaflet bases, in order to maximally expose the right heart cavities (Figure 1).

The selection of the freeze frame where counting will be done is somewhat arbitrary, but based on the following considerations:
- The end-diastolic/proto-systolic time point is when atrial contraction has finished and ventricular contraction has yet to begin. This is the moment in the cardiac cycle when there is the least flow of blood. Although small areas of turbulence cannot be ruled out, there is at least no rapid movement driven by cardiac contraction.
- It is also the moment when the tricuspid valve leaflets are fully open and almost invisible, making the right atrium and ventricle into a single blood-filled cavity; this decreases the chance of erroneously interpreting valve leaflets as bubble signals.
- This moment is identified easily using the electrocardiographic trace, when recorded with the images.

Although it may be possible theoretically to analyse other frames in the cardiac cycle, these considerations make it unlikely that a better estimation of the number of bubbles might be obtained. In any case, it is important to count the same frame consistently.

Dynamic evaluation such as the Eftedal and Brubakk method seems to slightly over-estimate VGE numbers as compared to actual counting on freeze frames. This can be explained by the fact that vortices of blood exist both in the atrium and ventricle, by which VGE may be swept several times through the plane of vision.[35,36] These blood-flow patterns account for the fact that in some instances, the 'correct' freeze frame chosen for frame-based counting does not show any VGE at all, whereas the previous or next frames do show a significant number (up to 9 or 10) VGE. The procedure therefore allows choosing a frame slightly 'off' if there are obviously VGE in the heart cycle but none can be seen in the initially chosen frame. With automated computerised counting, it will be possible, using three to five frames around the optimal frame, to eventually average out these turbulence effects. Currently, the manual method is too slow to reasonably permit counting of more than 10 to 20 frames in a video loop, as a certain degree of 'observer fatigue' eventually sets in.

The counting method described here makes use of a proprietary video file player on the PC (MPEGVue) which is offered as a package by the echograph's manufacturer (GE). This offers the possibility of viewing echocardiography video files off-line on any Windows PC while offering an easy patient selection menu and the possibility to smoothly step forwards and backwards through the video file, making frame-accurate selection of the images possible. Although a large range of video-playing software that can play back 'wmv' video files on a PC is available, none of them offer this frame-accurate playback. The major drawback here is that the MPEGVue videoplayer can only play back files if the file structure is organised in a certain way – in practical

terms, it limits the application to using GE echographs for acquisition and storage of the videos. All of those echographs offer MPEGVue export of the digital (DICOM) files, and once in the MPEGVue format, video files can be shared using either USB disk or sent by e-mail, with the player installation files added to the export package. Automated software will not suffer from this limitation, as it will be able to digest the individual frames of a video stream or file using proprietary software, e.g., MatLab software (MathWorks, Natick, MA, USA).

The inter-rater agreement for frame-based counting is high (ICC of 0.84), indicating there is no major difference between the individual observers and the reference score. This would permit pooling of data from different observers within the same experimental data set. As the VGE counts are an ordinal and continuous variable, mean and average VGE numbers can be calculated, which represents an obvious advantage over the use of discrete variables such as bubble grade scores for evaluating decompression stress. However, the almost perfect (ICC 0.96) intra-rater consistency for this method means that having the same assessor count VGEs for a set of experimental data would give extremely reliable results with regards to the evolution of VGE numbers post dive. Of course, it will be necessary to verify the (intra-rater) consistency of the computer automated counting software which is being developed. If confirmed, this software could be used either for off-line analysis of large numbers of files or, perhaps, directly on an ultrasound scanner (real-time evaluation). At present, the time-consuming process of counting individual bubbles and moving back and forth between frames to discriminate bubbles from their paths and movement prohibits large-scale use of the method.

It has been correctly pointed out that newer echocardiographic techniques are able to detect much smaller bubbles and that, as a result, it is impossible to compare published research using counted bubbles on echography unless exactly the same settings are used. Specifically, Eftedal and Brubakk scores will be impossible to compare among different studies, and it will be impossible to compare the effect of (pre-) diving interventions on VGE production with previous data from similar dives because of this. Recent case reports have indeed described divers with Eftedal and Brubakk grade 5 cardiac echograms (initially thought to be almost impossible without resulting severe DCS), without any symptoms of DCS.[37] This is undoubtedly a result of the better spatial resolution of modern echography, and the use of second harmonics imaging.[13]

The same applies for frame-based bubble counting; it is important to obtain baseline, control dive and post-intervention images on the same group of divers. However, the continuous-scale nature of this method will permit a quantitative evaluation of the effect of the intervention on VGE production. This way, even if the echographic method per se changes and becomes more sensitive, the relative effect observed in different studies may be compared.

Finally, using echocardiography, it may also be possible to evaluate (de)hydration state (by the degree of respiratory collapse of the inferior vena cava, IVC) and, in some subjects, decompression bubbles may even be detected in the IVC and the portal veins.[38–40] Incorporation of this information may provide additional insights into the influence of factors unrelated to the dive profile itself on the production of VGE after the dive. Using solely the degree of VGE after a dive as a measure of dive profile safety without at least trying to standardize these individual (diver-related) factors that may make a diver, either constitutionally or temporarily, less or more prone to the production and liberation of VGE after a dive, disregards a mass of scientific information already available on this subject.[41–45] The presence of VGE in the left cardiac cavities after a dive, be it by passage through a patent foramen ovale or through pulmonary arteriovenous shunts, may indicate a higher risk for cerebral or high-spinal DCS in the individual diver.[46,47] This may guide a decision as to whether a particular diver should be excluded from further participation in diving studies, especially if high risk.

Conclusions

As opposed to existing methods of evaluation, a frame-based counting method permits the investigator to define bubbles as a continuous variable, allowing more flexible and powerful statistical evaluation of the presence of VGE as an indicator of decompression stress. The method presented here shows excellent inter- and intra-rater consistencies, which can be achieved with minimal training by non-experts. Because of the linear, continuous-scale nature of the evaluation, a better discrimination of VGE levels can be achieved in the important intermediate range of bubble load. Therefore, the method seems well suited for use in interventional human diving experiments, where it is ethically impossible to subject volunteer divers to dive profiles generating extreme bubble grades. Moreover, the method is suitable for the development of automated counting software.

References

1   Pollock NW, Vann RD, Denoble PJ, Freiberger JJ, Dovenbarger JA, Nord DA, et al. *Divers Alert Network Annual Diving Report 2007 (based on 2005 data).* Durham, NC: Divers Alert Network; 2007.

2   Spencer MP, Johanson A. *Investigation of new principles for human decompression schedules using Doppler ultrasound blood bubble detection. Technical Report to ONR on Contract N00014-73-C-0094.* Seattle: Institute for Environmental Medicine and Physiology, 1974.

3   Kisman K, Masurel G. *Method for evaluating circulating bubbles detected by means of the Doppler ultrasonic method using the "K.M. code" (English translation of 283 CERTSM 1983).* Toulon, France: Centre d'Etudes et de Recherches Techniques Sous-Marines; 1983

4   Nishi RY, Brubakk AO, Eftedal OS. Bubble detection. In: Brubakk AO, Neuman TS, editors. *Bennett and Elliott's physiology and medicine of diving.* 5th ed. Philadelphia, PA: WB Saunders; 2003. p. 501-29.

5   Sawatzky KD, Nishi RY. Assessment of inter-rater agreement on the grading of intravascular bubble signals. *Undersea Biomedical Research.* 1991;18:373-96.

6   Sawatzky KD. *The relationship between intravascular Doppler-detected gas bubbles and decompression sickness after bounce diving in humans.* MSc Thesis. Toronto, ON: York University; 1991.

7   Marroni A, Bennett PB, Cronje FJ, Cali-Corleo R, Germonpré P, Pieri M, et al. A deep stop during decompression from 82 fsw (25 m) significantly reduces bubbles and fast tissue gas tensions. *Undersea Hyperb Med.* 2004;31:233-43.

8   Bennett PB, Marroni A, Cronje FJ, Cali-Corleo R, Germonpré P, Pieri M, et al. Effect of varying deep stop times and shallow stop times on precordial bubbles after dives to 25 msw (82 fsw). *Undersea Hyperb Med.* 2007;34:399-406.

9   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.

10   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.

11   Choudhry S, Gorman B, Charboneau JW, Tradup DJ, Beck RJ, Kofler JM, et al. Comparison of tissue harmonic imaging with conventional US in abdominal disease. *Radiographics.* 2000;20:1127-35.

12   Uppal T. Tissue harmonic imaging. *AJUM.* 2010;13:29-31.

13   Daniels C, Weytjens C, Cosyns B, Schoors D, De Sutter J, Paelinck B, et al. Second harmonic transthoracic echocardiography: the new reference screening method for the detection of patent foramen ovale. *Eur J Echocardiogr.* 2004;5:449-52.

14   Eftedal O. *PhD Thesis: Ultrasonic detection of decompression induced vascular microbubbles.* Trondheim, Norway: Norwegian University of Science and Technology; 2007.

15   Dejong N, Hoff L, Skotland T, Bom N. Absorption and scatter of encapsulated gas filled microspheres - theoretical considerations and some measurements. *Ultrasonics.* 1992;30:95-103.

16   Tang MX, Mulvana H, Gauthier T, Lim AK, Cosgrove DO, Eckersley RJ, et al. Quantitative contrast-enhanced ultrasound imaging: a review of sources of variability. *Interface Focus.* 2011;1:520-39.

17   Eatock BC, Nishi RY, Johnston GW. Numerical studies of the spectrum of low-intensity ultrasound scattered by bubbles. *J Acoust Soc Am.* 1985;77:1692-701.

18   Cohen J. Weighted kappa: nominal scale agreement with provision for scaled disagreement or partial credit. *Psychol Bull.* 1968;70:213-20.

19   Cohen J. A coefficient of agreement for nominal scales. *Educ Psychol Meas.* 1960;20:207-16.

20   Kraemer HC. Extension of the kappa coefficient. *Biometrics.* 1980;36:207-16.

21   Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet.* 1986;1:307-10.

22   Landis JR, Koch GG. An application of hierarchical kappa-type statistics in the assessment of majority agreement among multiple observers. *Biometrics.* 1977;33:363-74.

23   Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics.* 1977;33:159-74.

24   Rousson V, Gasser T, Seifert B. Assessing intrarater, interrater and test-retest reliability of continuous measurements. *Stat Med.* 2002;21:3431-46.

25   Shrout PE, Fleiss JL. Intraclass correlations: uses in assessing rater reliability. *Psychol Bull.* 1979;86:420-8.

26   Fleiss JL, Cohen J. Equivalence of weighted kappa and intraclass correlation coefficient as measures of reliability. *Educ Psychol Meas.* 1973;33:613-9.

27 Fleiss JL, Shrout PE. Approximate interval estimation for a certain Intraclass Correlation-Coefficient. *Psychometrika.* 1978;43:259-62.

28 Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Int J Nurs Stud.* 2010;47:931-6.

29 Kisman KE, Masurel G, Guillerm R. Bubble evaluation code for Doppler ultrasonic decompression data. *Undersea Biomedical Research.* 1978;5:28.

30 Nishi RY, Kisman KE, Eatock BC, Buckingham IP, Masurel G. Assessment of decompression profiles and divers by Doppler ultrasonic monitoring. In: Bachrach AJ, Matzen MM, eds. *Underwater physiology VII. Proceedings of the 7th Symposium on Underwater Physiology.* Bethesda, MD: Undersea Medical Society; 1981. p. 717-27.

31 Kisman K, Masurel G, LaGrue D, Le Pêchon J. [Evaluation of the quality of decompression using ultrasound bubble detection]. *Méd Aéro Spat Méd Sub Hyp* 1978;67:293-7. French

32 Parlak IB, Egi SM, Ademoglu A, Balestra C, Germonpr P, Marroni A. Intelligent bubble recognition on cardiac videos using Gabor wavelet. *International Journal of Digital Information and Wireless Communications.* 2011;1:195-203.

33 Parlak IB, Egi SM, Ademoglu A, Balestra C, Germonpre P, Marroni A, et al. A neuro-fuzzy approach of bubble recognition in cardiac video processing digital information and communication technology and its applications. In: Cherifi H, Zain JM, El-Qawasmeh E, editors. *Digital information and communication technology and its applications; communications in computer and information science.* Berlin Heidelberg: Springer; 2011. p. 277-86.

34 Papadopoulou V, Hui J, Balestra C, Hemelryck W, Germonpré P, Eckersley R, et al. Evaluating counting of venous gas emboli on post-SCUBA dive echocardiographs, ID 464. *2013 IEEE Joint UFFC, EFTF and PFM Symposium.* Prague; 2013.

35 Fredriksson AG, Zajac J, Eriksson J, Dyverfeldt P, Bolger AF, Ebbers T, et al. 4-D blood flow in the human right ventricle. *Am J Physiol Heart Circ Physiol.* 2011;301:H2344-50.

36 Wigstrom L, Ebbers T, Fyrenius A, Karlsson M, Engvall J, Wranne B, et al. Particle trace visualization of intracardiac flow using time-resolved 3D phase contrast MRI. *Magnet Reson Med.* 1999;41:793-9.

37 Bakovic D, Glavas D, Palada I, Breskovic T, Fabijanic D, Obad A, et al. High-grade bubbles in left and right heart in an asymptomatic diver at rest after surfacing. *Aviat Space Environ Med.* 2008;79:626-8.

38 Romero-Bermejo FJ, Ruiz-Bailen M, Guerrero-De-Mier M, Lopez-Alvaro J. Echocardiographic hemodynamic monitoring in the critically ill patient. *Curr Cardiol Rev.* 2011;7:146-56.

39 Butler BD, Fife C, Sutton T, Pogodsky M, Chen P. Hepatic portal venous gas with hyperbaric decompression: ultrasonographic identification. *J Ultrasound Med.* 1995;14:967-70.

40 Bird N. CT finding of VGE in the portal veins and IVC in a diver with abdominal pain: a case report. *Undersea Hyperb Med.* 2007;34:393-7.

41 Papadopoulou V, Eckersley RJ, Balestra C, Karapantsios TD, Tang MX. A critical review of physiological bubble formation in hyperbaric decompression. *Adv Colloid Interface Sci.* 2013;191-192:22-30.

42 Gempp E, Blatteau JE, Pontier JM, Balestra C, Louge P. Preventive effect of pre-dive hydration on bubble formation in divers. *Br J Sports Med.* 2009;43:224-8.

43 Germonpré P, Pontier JM, Gempp E, Blatteau JE, Deneweth S, Lafère P, et al. Pre-dive vibration effect on bubble formation after a 30-m dive requiring a decompression stop. *Aviat Space Environ Med.* 2009;80:1044-8.

44 Blatteau JE, Gempp E, Balestra C, Mets T, Germonpré P. Predive sauna and venous gas bubbles upon decompression from 400 kPa. *Aviat Space Environ Med.* 2008;79:1100-5.

45 Jankowski LW, Nishi RY, Eaton DJ, Griffin AP. Exercise during decompression reduces the amount of venous gas emboli. *Undersea Hyperb Med.* 1997;24:59-65.

46 Germonpré P, Dendale P, Unger P, Balestra C. Patent foramen ovale and decompression sickness in sports divers. *J Appl Physiol.* 1998;84:1622-6.

47 Germonpré P, Hastir F, Dendale P, Marroni A, Nguyen AF, Balestra C. Evidence for increasing patency of the foramen ovale in divers. *Am J Cardiol.* 2005;95:912-5.

Peter Germonpré[1,2], Virginie Papadopoulou[3,4], Walter Hemelryck[1], Georges Obeid[5], Pierre Lafère[2,6], Robert J Eckersley[7], Meng-Xing Tang[4], Costantino Balestra[2,3]
[1] *Centre for Hyperbaric Oxygen Therapy, Military Hospital, Brussels, Belgium*
[2] *Divers Alert Network Europe, Roseto, Italy and Brussels, Belgium*
[3] *Biophysiology and Environmental Physiology Laboratory, Haute Ecole Paul Henri Spaak, University of Brussels, Belgium*
[4] *Department of Bioengineering, Imperial College London, UK*
[5] *Department of Cardiology, Military Hospital, Brussels, Belgium*
[6] *Department of Anesthesiology and Hyperbaric Medicine, Hôpital de la Cavale Blanche, Brest, France*
[7] *Biomedical Engineering Department, Division of Imaging Sciences, King's College London, UK*

Address for correspondence:
P Germonpré MD
Centre for Hyperbaric Oxygen Therapy
Military Hospital Brussels
Belgium
Phone: *+32-(0)2-264-4868*
Fax: *+32-(0)2-264-4861*
E-mail: *<peter.germonpre@mil.be>*

Sample size requirement for comparison of decompression outcomes using ultrasonically detected venous gas emboli (VGE): power calculations using Monte Carlo resampling from real data

David J Doolette, Keith A Gault and Christian R Gutvik

Abstract

(Doolette DJ, Gault KA, Gutvik CR. Sample size requirement for comparison of decompression outcomes using ultrasonically detected venous gas emboli (VGE): power calculations using Monte Carlo resampling from real data. *Diving and Hyperbaric Medicine*. 2014 March;44(1):14-19.)

Introduction: In studies of decompression procedures, ultrasonically detected venous gas emboli (VGE) are commonly used as a surrogate outcome if decompression sickness (DCS) is unlikely to be observed. There is substantial variability in observed VGE grades, and studies should be designed with sufficient power to detect an important effect.

Methods: Data for estimating sample size requirements for studies using VGE as an outcome is provided by a comparison of two decompression schedules that found corresponding differences in DCS incidence (3/192 [DCS/dives] vs. 10/198) and median maximum VGE grade (2 vs. 3, $P < 0.0001$, Wilcoxon test). Sixty-two subjects dived each schedule at least once, accounting for 183 and 180 man-dives on each schedule. From these data, the frequency with which 10,000 randomly resampled, paired samples of maximum VGE grade were significantly different (paired Wilcoxon test, one-sided $P \leq 0.05$ or 0.025) in the same direction as the VGE grades of the full data set were counted (estimated power). Resampling was also used to estimate power of a Bayesian method that ranks two samples based on DCS risks estimated from the VGE grades.

Results: Paired sample sizes of 50 subjects yielded about 80% power, but the power dropped to less than 50% with fewer than 30 subjects.

Conclusions: Comparisons of VGE grades that fail to find a difference between paired sample sizes of 30 or fewer must be interpreted cautiously. Studies can be considered well powered if the sample size is 50 even if only a one-grade difference in median VGE grade is of interest.

Key words

Decompression, diving, echocardiography, venous gas emboli, decompression sickness, statistics, research

Introduction

Decompression sickness (DCS) is thought to be caused by intracorporeal bubble formation. Venous bubbles (venous gas emboli, VGE) are sometimes used as an outcome in studies of decompression procedures because they can be easily detected by ultrasonic methods and graded, and because VGE grades have a general correlation with the incidence of DCS in large compilations of data.[1,2] This correlation may arise in part because VGE can cause some manifestations of DCS, but an increase in detectable VGE is also presumed to be correlated with an increase risk of bubble formation at other DCS sites. VGE grades are used to augment DCS incidence data or as a surrogate outcome if DCS is unlikely to be observed, for instance in anesthetized animals, or in studies of low-risk human procedures.

VGE occur commonly without DCS (which is rare); therefore, VGE data are potentially more information-rich than low-incidence DCS data. This additional information is counterbalanced by the facts that, owing to poor specificity, VGE grades have poor diagnostic value for DCS, and there is substantial inter- and intra-individual variability in VGE grades observed following identical exposures.[3–6] These latter facts impose a lower limit on sample size for studies of low-risk human procedures that use VGE as a surrogate outcome measure.

A common design of such studies is for two different procedures to be performed on separate occasions by the same subjects, and to test for a difference in VGE outcome using a paired statistical test such as the Wilcoxon signed-rank test. The power of a statistical test to detect a particular effect size at a particular statistical significance criterion (α) depends on the sample size, so power calculations may be used when designing an experiment to select an appropriate sample size. This study provides estimates of power for various sample sizes for human studies that use paired comparisons of VGE grades following decompression.

Methods

Monte Carlo experiments analyze outcomes in multiple computer-generated random samples. For instance, the probability of an outcome is estimated by the proportion of samples in which the outcome occurs. Monte Carlo experiments can be used to examine the properties of statistical hypothesis tests, for instance, the probability of rejecting a false null hypothesis (power) for a test procedure which produces a P-value and then rejects the null hypothesis if the P-value is less than or equal to a particular α-level. Monte Carlo estimation of the power involves computing the proportion of rejections in many random samples. Typically the random samples would be simulations generated from

parametric distributions and, in the case of a two-sample test, hypothetical effect sizes. However, in this report, samples were generated by resampling subsets of real data.

DATA

A recently published, large-scale comparison of two air decompression schedules provides unique data for estimating sample size requirements, finding corresponding statistically significant differences in DCS incidence and median peak VGE grade.[7] Eighty-one US Navy divers participated in a total of 390 man-dives, performing work during 30 minutes' bottom time at 622 kPa absolute (170 feet of sea water gauge, fsw). They were at rest and cold during either of two decompression schedules that differed only in the distribution of 174 minutes' total decompression stop time among stop depths: a shallow stop (A1) schedule and a deep stop (A2) schedule. The study reached an early stopping criterion at midpoint analysis, which found a lower incidence of DCS on the A1 than the A2 schedule at one-sided $\alpha = 0.05$ (an early 'opposite tail' finding relative to a final result that would have motivated changing US Navy procedures). DCS was diagnosed by the duty diving medical officer and full descriptions are given in the original report. During re-evaluation of the cases according to the criteria described in Temple et al,[8] one case with symptom onset 27 hours after surfacing from the A2 schedule was re-classified as not DCS. This resulted in 3/192 (DCS/dives) and 10/198 ($P = 0.0489$, one-sided Fisher's exact test), on the A1 and A2 schedules, respectively.

As a secondary outcome measure, subjects were monitored for VGE with trans-thoracic cardiac 2-D echo imaging at 30 minutes and two hours post dive. While the subjects reclined with left side down, the four heart chambers were imaged with the subject at rest and then, in turn, while they flexed each elbow and knee. VGE were graded according to the Table 1 scale, adapted from Eftedal and Brubbak.[9] The same ultrasound technician conducted all the examinations and all observed VGE grades are documented elsewhere.[7] However, in this report, only the maximum VGE grades observed at any time (rest or limb flexion, any examination) after each dive were used and will be referred to as 'VGE grade' without qualification. The median VGE grades were 2 and 3 (two-sided $P < 0.0001$, Wilcoxon rank sum test), on the A1 and A2 schedules, respectively. VGE data were missing for three man-dives: two subjects were recompressed to treat DCS before VGE examination, and results for a subject without symptoms were inadvertently not recorded. In each case, the same subject undertook the same schedule (for which data was missing) and had VGE recorded, on at least one other occasion.

The original study was not designed as a paired comparison, but of the 81 subjects who participated in the original trial, 62 dived each schedule at least once. The VGE outcome of all dives undertaken by these 62 subjects was designated the

Table 1
Venous gas embolism grading (modified from reference 9)

Grade	Description
0	No bubble seen
1	Rare (< 1/s) bubble seen
2	Several discrete bubbles visible per image
3	Multiple bubbles visible per image but not obscuring image
4	Bubbles dominate image, may blur chamber outlines

paired data set and was used to generate random samples of paired data (VGE grade after A1 and A2 schedules in the same subject). The paired data set contained 363 records, each representing one man-dive, and each comprised of a subject identifier, a schedule identifier, and the VGE grade. The distribution of VGE grades in the paired data set is given in Table 2. Median VGE grade was 2 (interquartile range [IQR] 1–3) following the A1 schedule and 3 (IQR 2–4) follow the A2 schedule. These VGE grades were significantly different (Wilcoxon rank sum test, two-sided $P < 0.0001$), and A1 less than A2 will be considered as the true outcome for power estimation. Many subjects dived the A1 and A2 schedules more than once. The mean number of dives per subject on the A1 schedule was 3 (range 1–9) accounting for a total of 183 man dives. The mean number of dives per subject on the A2 schedule was 3 (range 1–8) accounting for a total of 180 man-dives. There was no requirement in the original study for subjects to dive A1 and A2 schedules an equal number of times; however, the differences between the number of A1 and A2 schedules undertaken by each subject were relatively symmetrically distributed around zero with the absolute value of the difference/number of subjects: 0/25; 1/20; 2/12; 3/3; 4/2. Subjects refrained from any hyperbaric or hypobaric exposure for three days prior to any of the dives in the paired data set and the most common interval between these dives was seven days.

RESAMPLING

For each of a range of paired sample sizes ($n = 10$ to 60 subjects), Monte Carlo resampling and testing of paired VGE grades was performed in the following manner. First, a subset of n subjects was randomly selected without replacement from a vector containing the 62 subject identifiers. Second, for each subject in this subset, one VGE grade was randomly selected from among the A1 schedules and one from among the A2 schedules that subject had completed. The resulting subset contained an A1-A2 pair of VGE grades for n different subjects. VGE grades from different subjects were considered independent and the resampling scheme took advantage of subjects who dived a schedule more than once by allowing different A1-A2 pairs for that subject in different subsets (there are more than 10^{41} possible such combinations in the paired data set for each value of n). Finally, for each

Table 2
VGE grades, paired data set (taken from reference 7)

	0	1	2	3	4
A1	27	36	36	53	31
A2	9	25	29	45	72

subset, the *P*-value of a paired Wilcoxon signed-rank test, with alternative hypothesis A1 less than A2 (in accord with the true outcome) was recorded. This three-step procedure was repeated 10,000 times for each value of *n*. The frequency with which *P*-values from the 10,000 subsets were less than or equal to a particular α-level provides an estimate of the probability of an α-level test on sample size of *n* subjects detecting the true one-grade difference in VGE in the paired data set (power). Power estimates are given for one sided α = 0.05 because this level was an early stopping criterion for difference in DCS incidence in the original study that generated the data set, and for one-sided α = 0.025 because this level is equivalent to two-sided α = 0.05 that would commonly be used for comparisons where there is no justification for a one-sided test.

Within-subject variability in VGE grade for the same schedule was considered to be random since dives were sufficiently spaced so as not to influence each other either in terms of residual nitrogen or acclimatization. This assumption was not a requirement of the nonparametric statistical analysis. Some variability may result from measurement precision and, in particular, VGE measurements in the original study were infrequent (30 and 120 min post dive) and may not have consistently captured the peak VGE grade that occurred after each dive. To examine the consequence of possible frequent failure to record the peak VGE grade, a modified data set was drawn from the paired data set. The modified data set comprised only the maximum VGE grade observed among each repetition of the A1 schedule and each repetition of the A2 schedule for each of the 62 subjects (no intra-individual variability). The modified data set had median VGE grades of 3 (IQR 2.25–4) following the A1 schedule and 4 (IQR 3–4) following the A2 schedule (paired Wilcoxon signed-rank test, two-sided *P* = 0.0056). For each of a range of paired sample sizes (*n* = 10 to 50), a subset of *n* A1-A2 pairs of VGE grades was randomly selected without replacement from the 62 in the modified data set and tested with a paired Wilcoxon signed-rank test, with alternative hypothesis A1 less than A2. This resampling procedure was repeated 10,000 times and the power estimated as described for the paired data set. There are more than 10^{12} combinations of 50 from 62 subjects, but only 1,891 combinations of 60 from 62 subjects, so estimating power for *n* = 60 subjects by resampling from the modified data set was not considered meaningful.

Recently, a Bayesian method has been proposed to estimate the probability of DCS of a decompression procedure from maximum observed VGE grades and test for a difference in risk between two procedures.[10] We estimated the power

Table 3
Power estimated from frequency of observed *P*-values of Wilcoxon test, paired data set

	Number of subjects					
	10	20	30	40	50	60
Power						
one-sided *P* ≤ 0.05	0.27	0.48	0.65	0.78	0.88	0.94
one-sided *P* ≤ 0.025	0.15	0.34	0.50	0.66	0.78	0.87

of this latter test for comparison with the Wilcoxon test. Briefly, the method constructs posterior distributions of the probability of DCS given VGE grade (for instance based on the data given by Sawatzky[1]) and the probability of VGE grade given the test procedure, and then the total probability of DCS of a procedure is estimated by Monte Carlo simulation from these posteriors. Two procedures are tested for a difference in DCS risk by counting the frequency with which one procedure is estimated as riskier than the other (estimated confidence of the difference) in parallel Monte Carlo simulations. Using the same prior distributions as originally described[10] to produce posterior distributions from the present paired data set resulted in an estimated 99.98% confidence that the A2 schedule was riskier than the A1 schedule. Again using the same prior distributions, posterior distributions were produced from resampled subsets of the present paired data set. For each resampled subset, the confidence that the A2 schedule was riskier than the A1 schedule (in accord with the true outcome of both the Bayesian and Wilcoxon tests) was estimated. The frequency with which this confidence was greater than 95% in resampled subsets is comparable (but not identical) to the power estimate for the Wilcoxon rank sum test at one-sided α = 0.05. Only sample sizes *n* = 20 and *n* = 50 were examined, and resampled 500 times, because the Bayesian method itself requires Monte Carlo simulations and is highly computing intensive.

Data analysis was performed using R version 2.14.2 (Vienna, Austria: R Development Core Team; 2012) and MATLAB version 7.8.0.347 (R2009a) (Natwick, MA: The MathWorks Inc; 2009).

Results

Table 3 shows the power for various sample sizes for the Wilcoxon rank sum test, estimated by resampling from the paired data. These values are the probabilities of a significant test (*P* ≤ 0.05 and *P* ≤ 0.025) in accord with the true outcome. The fraction of results not in accord with the true outcome were usually failure to find a difference between A1 and A2 VGE grades (type II error) – the opposite tail finding of higher VGE grades on A1 than A2 was extremely rare, the highest frequency of this result was 0.0016 for *n* = 10 and *P* ≤ 0.05, and otherwise zero. The choice of power depends on the consequences of making a type II error, but

Table 4
Power estimated from frequency of observed *P*-values of
Wilcoxon test, modified data set

Power	Number of subjects				
	10	**20**	**30**	**40**	**50**
one-sided *P* ≤ 0.05	0.22	0.39	0.56	0.75	0.95
one-sided *P* ≤ 0.025	0.11	0.24	0.37	0.55	0.78

one convention is to design experiments with two-sided α = 0.05 and 80% power. From the one-sided *P* ≤ 0.025 row (equivalent to two-sided α = 0.05) in Table 3, it can be seen that VGE grades from a paired sample size of about *n* = 50 subjects would have 80% power to detect a difference of one VGE grade. Power dropped quickly with sample size so that at *n* = 30 subjects (*P* ≤ 0.025) there was equal probability of a true answer and a type II error.

Table 4 shows the power for various sample sizes for the Wilcoxon rank sum test, estimated by resampling from the modified data comprising only the highest VGE scores from repeated dives on the same schedules. Although there are some differences from the results of the paired data set, a sample size of about *n* = 50 is required for 80% power at two-sided α = 0.05.

Power estimates for the Bayesian test were similar to those of the Wilcoxon rank sum test. The frequency of predicting the A2 schedule to be riskier than A1 schedule with 95% confidence was 0.40 for *n* = 20 resampled subsets and 0.80 for *n* = 50 resampled subsets. These power estimates are comparable to the values for these sample sizes in the *P* ≤ 0.05 row of Table 3. The opposite tail finding (A1 riskier than A2 with 95% confidence) never occurred.

Discussion

Statistical power (or sensitivity) is the probability of rejecting a false null hypothesis (not making a type II error). In the current context, this is the probability of finding a difference (rejecting the null hypothesis of no difference) between paired samples of VGE grades for each schedule given that the VGE grades are different for each schedule in the population. The power of a statistical test depends on the magnitude of the effect to be detected, the α-value of the test, and the sample size. Power calculations are used to select appropriate sample sizes when designing experiments and Table 3 provides guidelines for designing paired comparisons using VGE as an outcome. For instance, a paired sample size of about 50 subjects is required for 80% power to detect a one-grade difference in median VGE at one-sided α = 0.025 (equivalent to two-sided α = 0.05) in this relatively homogenous group of subjects diving under rigidly controlled conditions.

The present results are only relevant to a one-grade difference in VGE. For instance, analysis of a simulated data set with a two-grade difference in median VGE (not shown) found a paired sample size of about 20 was required for 80% power to detect the difference at two-sided α = 0.05. Nevertheless, the present guidelines are broadly applicable for two reasons: one VGE grade is the precision that is common across the most frequently used grading systems and many published studies report one-grade or less difference in VGE. With respect to grading precision, the present VGE grading system was a modification of the Eftedal-Brubakk system for grading VGE in 2D echocardiographic images, and the Eftedal-Brubakk grading system is broadly similar to the Spenser and Kisman-Masurel systems for aural grading of VGE detected by ultrasonic Doppler shift, in that they all grade human VGE data on an approximately equivalent zero to four ordinal scale (although the Kisman-Masurel system reports "+" and "−" intergrades and the Eftedal-Brubakk system has a grade 5 which has not been reported in humans).[2,9,11] Sample size guidelines based on the minimum measurable difference in peak VGE grade (e.g., Table 3) are useful if there is no reason to expect or require a greater difference.

The estimated power to detect a one-grade difference in median VGE is relevant to many published studies. A Medline search for the 10 years up to 2012 identified 23 publications that were paired comparisons of VGE following diving (68% of all publications found concerning VGE and diving in humans in this period). Of these, 16 reported the individual or summary statistics of the observed VGE grades (Eftedal-Brubakk, Spencer or Kisman-Masurel systems).[12–27] Only three of these 16 papers reported more than a one-grade difference in median VGE.[17,24,27] Sample sizes in these studies ranged from 6 to 28 subjects and only four of these papers reported a significant difference in VGE grades. Four papers reported no significant difference in VGE grades, and eight reported significant difference in transformations of the data. The most common transformations were to bubble count·cm⁻² and to the Kisman-Masurel integrated severity score.[2,5] Bubble count·cm⁻², if a transformation from peak VGE grades (i.e., not measured directly), is subject to the same power constraints as the underlying VGE grades. The current power calculations are not applicable to the Kisman-Masurel integrated severity score which includes additional time-course information. If the Kisman-Masurel integrated severity score were demonstrated to have a stronger correlation with DCS incidence than has maximum VGE grades, sample size guidelines would be useful, but the present data did not include sufficiently frequent VGE measurements to calculate a meaningful score.

Power estimates are dependent on the precision of measurement. A limitation of the present estimates is that the paired data set may have unnecessary variance because infrequent measurements of VGE may not have always captured the true peak VGE grade. Any such aliasing may

not have been severe because the two VGE examinations (at 30 and 120 minutes) span the period during which peak VGE are typically recorded following bounce dives and VGE grades were similar at these two examinations.[28] There was no difference in VGE grades between examination times following the A1 schedule; however, there was a significant difference in VGE grades between examinations following the A2 schedule (Wilcoxon rank sum test two-sided, $P = 0.0006$) but the estimated location shift was only one-half a VGE grade. Also, the modified data set, which had no intra-individual variability in VGE scores, produced similar power estimates to those extracted from the paired data set.

The concordance between VGE grades and DCS incidence in the present data is of interest since VGE grades are often used as a surrogate for DCS (although not in the original study). The dives in the present data set were relatively risky air decompression dives; for instance, in the US Navy Diving Manual, an air dive to 170 fsw for 30 minute bottom time requires the use of oxygen decompression, and the two air schedules had a measurable difference in DCS incidences.[29] The original study planned 375 man-dives on each schedule, which would have had approximately 80% power to detect the actually observed difference in DCS incidences (a difference which was larger than expected) at two-sided $\alpha = 0.05$. This is compared with a paired sample size of about $n = 50$ subjects to detect the observed one-grade difference in median VGE at the same power and significance. While this comparison is interesting in hindsight, the objective of the original comparison of decompression procedures was to discern any practical difference in the DCS incidence, not VGE grades per se.

The concordance of differences in VGE grades and differences in DCS risk (estimated from observed DCS incidence) in the present data will not necessarily hold for all experiments. In the largest compilation of VGE and DCS incidence following diving, there was no DCS associated with Kisman-Masurel grade 0 (0 DCS/819 dives) and DCS incidence was indistinguishable between grades I (3 DCS/287 dives) and II (2 DCS/183 dives) or between grades III (27 DCS/365 dives) and IV (9 DCS/72 dives), although the DCS incidence does differ between these low and high VGE grades.[1] Therefore, an experiment that demonstrates a statistically significant difference between, for instance, median VGE grades I and II using a Wilcoxon signed rank test, may not reflect a demonstrable difference in DCS risk. Misinterpretation is less likely with the Bayesian method of Eftedal and colleagues.[10] This Bayesian method compares estimates of the probability of DCS derived from information about the distribution of DCS incidence with VGE grades, in this case a prior distribution from the data compilation noted above.[1] Because the Bayesian method incorporates this prior, it is unlikely to find a difference between a sample dominated by VGE grade I and a sample dominated by VGE grade II, unless there is also substantial difference in the distribution of other VGE grades between the samples.

Conversely, any analysis of VGE may fail to identify a true difference in DCS risk between two samples dominated by grade IV VGE, since this is the highest grade observable, irrespective of DCS risk. The similarity of power and sample size estimates between the Wilcoxon and Bayesian test on the present data arises because the median VGE grades on the A1 and A2 schedule were 2 and 3 (equivalent to Kisman-Masurel grades II and III), respectively, and there is a significant difference in DCS incidence between these grades in the prior distribution.

Conclusions

Comparisons of two decompression procedures using only VGE as an endpoint that fail to find a difference between paired sample sizes of 30 or fewer must be interpreted cautiously. Studies can be considered well powered if the sample size is above 50 even if only a one-grade difference in median VGE is of interest. Maximum VGE grades can provide more power than DCS incidence to distinguish between two decompression procedures; however, a difference in VGE grades does not necessarily reflect a difference in DCS risk. If the purpose of the study is to infer a difference in DCS risk from VGE grades alone, VGE data must be interpreted cautiously, and the Bayesian method incorporating appropriate prior information about the distribution of DCS incidence with VGE grades is preferred over simple statistical tests such as the Wilcoxon signed-rank test.

References

1   Sawatzky KD. *The relationship between intravascular Doppler-detected gas bubbles and decompression sickness after bounce diving in humans* [MSc Thesis]. Toronto, ON: (Canada): York University; 1991.
2   Nishi RY, Brubakk AO, Eftedal OS. Bubble detection. In: Brubakk AO, Neuman,TS, editors. *Bennett and Elliott's physiology and medicine of diving*. 5th ed. Edinburgh: Saunders; 2003. p. 501-29.
3   Kumar VK, Billica RD, Waligora JM. Utility of Doppler-detectable microbubbles in the diagnosis and treatment of decompression sickness. *Aviat Space Environ Med*. 1997;68:151-8.
4   Gerth WA, Ruterbusch VL, Long ET. *The influence of thermal exposure on diver susceptibility to decompression sickness*. Technical Report. Panama City, FL: Navy Experimental Diving Unit; 2007 Nov. Report No.: NEDU TR 06-07. Available at http://archive.rubicon-foundation.org/xmlui/handle/123456789/5063.
5   Nishi RY, Kisman KE, Eatock BC, Buckingham IP, Masurel G. Assessment of decompression profiles and divers by Doppler ultrasonic monitoring. In: Bachrach AJ, Matzen MM, editors. *Underwater physiology VII. Proceedings of the 7th Symposium on Underwater Physiology*. Bethesda, MD: Undersea Medical Society; 1981. p. 717-27.
6   Eckenhoff RG, Hughes JS. Acclimatization to decompression stress. In: Bachrach AJ, Matzen MM, editors. *Underwater physiology VIII. Proceedings of the 8th Symposium on Underwater Physiology*. Bethesda, MD: Undersea Medical Society; 1984. p. 93-100.

7   Doolette DJ, Gerth WA, Gault KA. *Redistribution of decompression stop time from shallow to deep stops increases incidence of decompression sickness in air decompression dives*. Technical Report. Panama City, FL: Navy Experimental Diving Unit; 2011 Jul. Report No.: NEDU TR 11-06. Available at http://archive.rubicon-foundation.org/xmlui/handle/123456789/10269.

8   Temple DJ, Ball R, Weathersby PK, Parker EC, Survanshi SS. *The dive profiles and manifestations of decompression sickness cases after air and nitrogen-oxygen dives*. Technical Report. Bethesda, MD: Naval Medical Research Center; 1999. Vol 1. Report No.: 99-02. Available at http://archive.rubicon-foundation.org/xmlui/handle/123456789/4975.

9   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.

10   Eftedal OS, Tjelmeland H, Brubakk AO. Validation of decompression procedures based on detection of venous gas bubbles: a Bayesian approach. *Aviat Space Environ Med*. 2007;78:94-9.

11   Brubakk AO, Eftedal O. Comparison of three different ultrasonic methods for quantification of intravascular gas bubbles. *Undersea Hyperb Med*. 2001;28:131-6.

12   Dujic Z, Duplancic D, Marinovic-Terzic I, Bakovic D, Ivancev V, Valic Z, et al. Aerobic exercise before diving reduces venous gas bubble formation in humans. *J Physiol*. 2004;555:637-42.

13   Marroni A, Bennett PB, Cronje FJ, Cali-Corleo R, Germonpre P, Pieri M, et al. A deep stop during decompression from 82 fsw (25 m) significantly reduces bubbles and fast tissue gas tensions. *Undersea Hyperb Med*. 2004;31:233-43.

14   Blatteau JE, Gempp E, Galland FM, Pontier JM, Sainty JM, Robinet C. Aerobic exercise 2 hours before a dive to 30 msw decreases bubble formation after decompression. *Aviat Space Environ Med*. 2005;76:666-9.

15   Dujic Z, Palada I, Valic Z, Duplancic D, Obad A, Wisloff U, et al. Exogenous nitric oxide and bubble formation in divers. *Med Sci Sports Exerc*. 2006;38:1432-5.

16   Blatteau JE, Boussuges A, Gempp E, Pontier JM, Castagna O, Robinet C, et al. Haemodynamic changes induced by submaximal exercise before a dive and its consequences on bubble formation. *Br J Sports Med*. 2007;41:375-9.

17   Blatteau JE, Pontier JM. Effect of in-water recompression with oxygen to 6 msw versus normobaric oxygen breathing on bubble formation in divers. *Eur J Appl Physiol*. 2009;106:691-5.

18   Bosco G, Yang ZJ, Di Tano G, Camporesi EM, Faralli F, Savini F, et al. Effect of in-water oxygen prebreathing at different depths on decompression-induced bubble formation and platelet activation. *J Appl Physiol*. 2010;108:1077-83.

19   Jurd KM, Thacker JC, Seddon FM, Gennser M, Loveman GA. The effect of pre-dive exercise timing, intensity and mode on post-decompression venous gas emboli. *Diving Hyperb Med*. 2011;41:183-8.

20   Blatteau JE, Hugon J, Gempp E, Pény C, Vallée N. Oxygen breathing or recompression during decompression from nitrox dives with a rebreather: effects on intravascular bubble burden and ramifications for decompression profiles. *Eur J Appl Physiol*. 2012;112:2257-65.

21   Schellart NA, Sterk W. Venous gas embolism after an open-water air dive and identical repetitive dive. *Undersea Hyperb Med*. 2012;39:577-87.

22   Gennser M, Jurd KM, Blogg SL. Pre-dive exercise and post-dive evolution of venous gas emboli. *Aviat Space Environ Med*. 2012;83:30-4.

23   Risberg J, Englund M, Aanderud L, Eftedal O, Flook V, Thorsen E. Venous gas embolism in chamber attendants after hyperbaric exposure. *Undersea Hyperb Med*. 2004;31:417-29.

24   Marinovic J, Ljubkovic M, Breskovic T, Gunjaca G, Obad A, Modun D, et al. Effects of successive air and nitrox dives on human vascular function. *Eur J Appl Physiol*. 2012;112:2131-7.

25   Castagna O, Brisswalter J, Vallee N, Blatteau JE. Endurance exercise immediately before sea diving reduces bubble formation in scuba divers. *Eur J Appl Physiol*. 2011;111:1047-54.

26   Dujic Z, Palada I, Obad A, Duplancic D, Bakovic D, Valic Z. Exercise during a 3-min decompression stop reduces postdive venous gas bubbles. *Med Sci Sports Exerc*. 2005;37:1319-23.

27   Møllerløkken A, Breskovic T, Palada I, Valic Z, Dujic Z, Brubakk AO. Observation of increased venous gas emboli after wet dives compared to dry dives. *Diving Hyperb Med*. 2011;41:124-8.

28   Blogg SL, Gennser M. The need for optimisation of post-dive ultrasound monitoring to properly evaluate the evolution of venous gas emboli. *Diving Hyperb Med*. 2011;41:139-46.

29   Naval Sea Systems Command. *US Navy Diving Manual*. Revision 6, NAVSEA 0910-LP-106-0957/SS521-AG-PRO-010. Arlington, VA: Naval Sea Systems Command; 2008.

David J Doolette[1], Keith A Gault[1] and Christian R Gutvik[2]

[1] *Navy Experimental Diving Unit, Panama City, Florida, USA*
[2] *Technology Transfer Office, Norwegian University of Science and Technology (NTNU)*

Address for correspondence:
David Doolette, PhD
Navy Experimental Diving Unit
321 Bullfinch Road
Panama City, FL 32407, USA
Phone: *+1-(0)850-230-3100*
E-mail: *<david.doolette.as@navy.mil>*

Decompression illness in divers treated in Auckland, New Zealand, 1996–2012

Rachel M Haas, Jacqueline A Hannam, Christopher Sames, Robert Schmidt, Andrew Tyson, Marion Francombe, Drew Richardson and Simon J Mitchell

Abstract

(Haas RM, Hannam JA, Sames C, Schmidt R, Tyson A, Francombe M, Richardson D, Mitchell SJ. Decompression illness in divers treated in Auckland, New Zealand, 1996–2012. *Diving and Hyperbaric Medicine*. 2014 March;44(1):20-25.)

Introduction: The treatment of divers for decompression illness (DCI) in Auckland, New Zealand, has not been described since 1996, and subsequent trends in patient numbers and demographics are unmeasured.

Methods: This was a retrospective audit of DCI cases requiring recompression in Auckland between 01 January 1996 and 31 December 2012. Data describing patient demographics, dive characteristics, presentation of DCI and outcomes were extracted from case notes and facility databases. Trends in annual case numbers were evaluated using Spearman's correlation coefficients (ρ) and compared with trends in entry-level diver certifications. Trends in patient demographics and delay between diving and recompression were evaluated using regression analyses.

Results: There were 520 DCI cases. Annual caseload decreased over the study period ($\rho = 0.813$, $P < 0.0001$) as did entry-level diving certifications in New Zealand ($\rho = 0.962$, $P < 0.0001$). Mean diver age was 33.6 (95% confidence limits (CI) 32.7 to 34.5) years and age increased ($P < 0.0001$) over the study period. Median (range) delay to recompression was 2.06 (95% CI 0.02 to 23.6) days, and delay declined over the study period ($P = 0.005$).

Conclusions: Numbers of DCI cases recompressed in Auckland have declined significantly over the last 17 years. The most plausible explanation is declining diving activity but improvements in diving safety cannot be excluded. The delay between diving and recompression has reduced.

Key words

Diving, embolism, decompression illness, hyperbaric oxygenation therapy, air/diagnosis/etiology/therapy, decompression sickness/diagnosis/epidemiology/etiology/physiopathology/therapy

---

Introduction

Decompression illness (DCI) may occur following compressed gas dives if intra-corporeal bubbles form from dissolved inert gas, or if air is introduced to the arterial circulation by pulmonary barotrauma. The definitive treatment of DCI involves recompression and oxygen administration in a hyperbaric chamber.[1] Recompression facilities in New Zealand are located in Auckland and Christchurch and these have, in general, served divers from the North and South Islands respectively, although lower North Island divers are sometimes evacuated to Christchurch for recompression. The recompression facility (the Slark Hyperbaric Unit, SHU) in Auckland has been based at the Royal New Zealand Navy Hospital (RNZNH). Another unit, operated by Hyperbaric Health (a private company), has offered treatment for DCI since 2006. The caseload of the SHU was last reported for the 1996 calendar year.[2]

We undertook this study to describe the numbers and characteristics of DCI cases treated in Auckland from 1996 to the present time. In particular, we set out to document any trends in case numbers, and in relevant parameters such as patient demographics, type of diving, and latency between the incident dive and recompression.

Methods

The study was approved by the University of Auckland Human Participants Ethics Committee (Reference: 9287). Locality approval was given by the Royal New Zealand Navy and Hyperbaric Health Limited. This was a retrospective, longitudinal audit of DCI cases treated in Auckland between 01 January 1996 and 31 December 2012. We chose 1996 as the start point because from this year forward the Christchurch unit was in continuous operation and patient numbers were not influenced by the need for evacuations from the South Island. A small number of cases were treated at the Hyperbaric Health Unit from 2006 and so these were also included in the audit.

Scuba divers who were recompressed and given a discharge diagnosis of DCI, probable DCI, or possible DCI were included. Cases considered 'unlikely' to have DCI or given alternative discharge diagnoses were excluded. At the SHU, case data were accessed by the principal author from two sources. The primary source was a Microsoft® Access 2 database maintained by the hyperbaric technicians and updated with each new patient's data during or soon after their admission. Where available, original case notes were also accessed for comparison against the database and extraction of missing or additional parameters. Data for cases treated at the Hyperbaric Health unit were extracted directly

Figure 1
Temporal trends in the number of divers treated for decompression illness in Auckland between 1996 and 2012 ($P < 0.0001$); the temporal trend in the number of newly certified divers (all New Zealand) is also displayed for 2000 to 2012 ($P < 0.0001$)
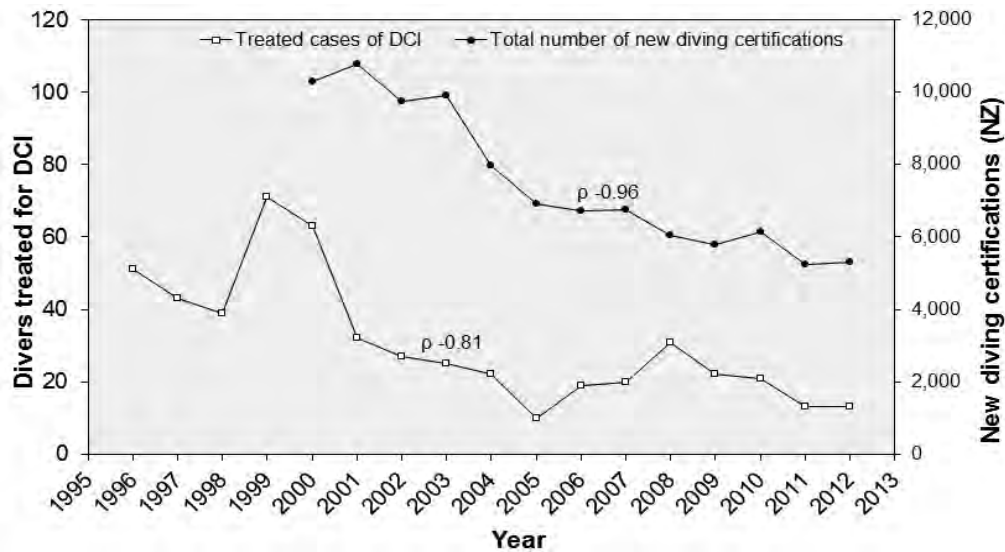
from case notes by a Hyperbaric Health clinician. Data from both units were combined into a single spreadsheet. Each case was given a unique study identifier. No data were collected that could identify patients or hospital staff.

The following data were extracted for each case: age; gender; height; weight; diver certification level; number of previous dives; maximum depth of incident dive or dive series; method of assessing decompression status during incident dive (dive table, dive computer, nil); breathing gas used (air or nitrox/mixed gases); equipment used (open-circuit scuba, surface supply or rebreather); latency between last dive and symptoms; nature of first-aid treatment; latency between last dive and recompression; qualitative nature of the symptoms; the presence or absence of objective signs on examination; putative risk factors for DCI; initial recompression treatment table; number of follow up recompressions and the recovery status at discharge (categorically divided as complete or incomplete recovery).

In an attempt to compare trends in annual case numbers against an indirect index of diving activity, annual numbers of new diver certifications in New Zealand over the years 2000–2012 were obtained by courtesy of a major global and national provider of diver training, the Professional Association of Diving Instructors (PADI).

This was a descriptive study rather than an investigation of hypotheses. Nevertheless, we identified the measurement of any trend in annual cases recompressed between 1996 and 2012 as the primary endpoint. Secondary endpoints were the trends over time in: maximum depth of the incident dive or dive series; breathing gases used; latency between the incident dive or dive series and recompression and in

diver demographics such as age, body mass index (BMI) and gender.

STATISTICAL ANALYSIS

Trends in annual case numbers were investigated using Spearman's *rho* (ρ) correlation coefficients. Trends in secondary outcomes were investigated using regression analyses with year as a covariate. Linear regressions were conducted using normal distributions where appropriate, and Poisson distributions for count data. Binary data were investigated using logistic regression. A *P* value of < 0.05 is usually considered to indicate statistical significance; however, a total of eight analyses were conducted in this study and therefore the predefined criterion for statistical significance was adjusted using a Bonferroni correction (to $P < 0.00625$). All analyses were conducted using SPSS Statistics V. 19.

Results

NUMBER OF CASES

A total of 522 divers recompressed for DCI were identified. Two cases were excluded (one was an erroneous entry in the RNZNH database, and a second case was eventually diagnosed as feigned or 'factitious' DCI[3]), leaving a total cohort of 520 cases of which 506 were treated at the SHU and 14 at Hyperbaric Health. The annual DCI case load has trended downward over this period (Spearman's $\rho = 0.813$, $P < 0.0001$). Similarly, new diving certifications issued in New Zealand by PADI have also trended downward over a similar period (2000–2012) (Spearman's $\rho = 0.962$, $P < 0.0001$) (Figure 1).

Figure 2

Age of divers recompressed over the study period; the box plot shows the median (horizontal line inside boxes), interquartile range (boxes), and 10th-90th percentile (vertical lines). Outlier data are indicated by black dots. A significant upward trend in age is shown ($P < 0.0001$).
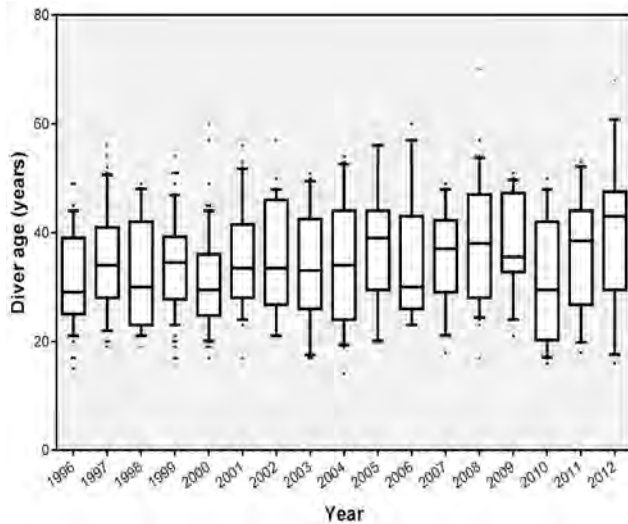
DIVER AGE, GENDER AND BMI

Demographics and diving characteristics of the recompressed divers are summarised in Table 1. Mean age (95% confidence limits, CI) across the cohort was 33.6 (32.7 to 34.5) years and age increased over the study period ($P < 0.0001$) (Figure 2). No significant trends were identified for gender or BMI over time.

DIVER EXPERIENCE

Certification levels among recreational divers covered a spectrum from no formal certification to instructor. There were also a number of so-called recreational 'technical divers' and occasional divers from professional groups such as commercial and military divers. Fifty-four per cent of divers for whom the previous number of dives was recorded had completed fewer than 100 dives at the time of injury, and 19% had undertaken more than 500 (Table 1).

NATURE OF DIVING

The vast majority of cases of DCI occurred in divers using standard open-circuit scuba equipment (95%), with six (~1%) using rebreathers, and six (~1%) using surface-supply equipment. In 13 cases (~2%) the equipment was not recorded. Of the 496 cases where the diving activity was explicitly recorded, 460 (93%) were diving recreationally, with only three involved in military diving and 33 (~6%) in occupational diving. Note, this distribution of activity does not intuitively match the certification data because some occupational diving (such as diving instruction) is undertaken by divers with recreational qualifications.

Table 1

Demographics and diving experience of 520 divers treated for decompression illness in Auckland between 1996 and 2012

Diver demographics	*n*	mean	range
Age (yr)	512	34	14–70
Male:Female	419:101		
Weight (kg)	384	83	45–198
Height (cm)	379	178	140–210
BMI (kg m^{-2})	377	26.2	16.5–59.1

Certification level	*n*	%	
No certification	24	4.6	
Training	14	2.7	
Basic open water	274	52.7	
Advanced/Rescue/ Dive-master	98	18.8	
Instructor	49	9.4	
Commercial/Military/ Technical	4	0.8	
Unknown	57	11.0	

Experience level	*n*	%	
No previous dives	8	1.9	
≤ 5	25	5.8	
≤ 10	29	6.8	
≤ 100	169	39.4	
≤ 500	118	27.5	
> 500	80	18.6	

Breathing gas used	*n*	%	
Air diving	488	96.6	
Mixed-gas diving	17	3.4	

The depth of incident dives (or dive series) ranged from 1.8 to 80 metres, with a mean (95% CI) of 25.8 (24.74 to 26.92) m. There were no significant trends over time for depth of incident dive or use of air versus nitrox and mixed gas. There was an apparent increase over time in the proportion of recompressed divers who used a dive computer as the primary method of depth and time control. For example, in 1996 45% of incident dives were controlled according to a table plan whereas 18% were controlled by a computer (37% of divers either used nothing or the planning tool was not recorded). By 2012, this situation had reversed and 46% of divers were controlled by a computer, and 15% according to a table. Unfortunately over the period of the study, many data were missing in relation to this parameter, and we did not attempt to analyse the trend.

RISK FACTORS

In addition to provocative depth/time profiles, a number of putative risk factors for DCI have been proposed. The most prevalent of these among cases in this study was repetitive diving (57%). Rapid ascents (30%), consecutive days' diving

Figure 3

Putative risk factors for DCI among divers recompressed over the study period; data are the percentage of the total cases in which the risk factor was reported

Figure 4

Presenting symptoms of the divers treated over the study period (percentage of the total cases). S.O.B – short of breath. L.O.C – loss of consciousness; 'Cognitive' refers to complaints of dysexecutive problems such as poor memory and difficulty concentrating; 'Weakness' refers to subjective perceptions of weakness (frequently associated with pain but not always associated with objective signs of weakness)

(26%) and subjectively 'strenuous' diving (11%) were also features in many cases (Figure 3).

PRESENTATION OF DCI

The latency of symptom onset ranged from "*present on surfacing*" to 168 hours after diving, with a median time of 1.5 hours. The most frequently reported symptom was musculoskeletal pain (65% of cases), followed by cutaneous tingling (45%), headache (35%), fatigue (32%), weakness (31%), numbness (28%) and dizziness (22%). Objective signs were seen in 180 (36%) of the 499 divers for whom symptoms and objective signs were explicitly recorded. Objective signs included an abnormal sharpened Romberg test.[4] The percentage of cases in which each reported symptom occurred is given in Figure 4.
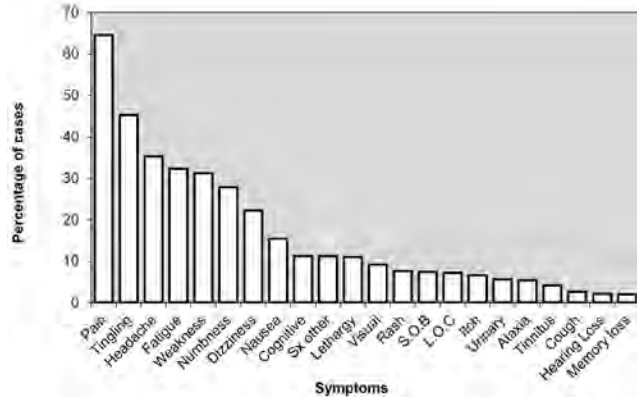
FIRST AID, REFERRAL AND TREATMENT

In 60% of cases, whether first-aid oxygen was administered was not recorded. Of the 210 (40%) recorded cases, only 87 (41%) received oxygen prior to recompression. Divers were referred mainly by their local doctor (31%), a hospital (30%), or were self-referred (28%). For the entire cohort, the median (range) latency between the incident dive and the time to recompression was 2.06 (0.02–23.6) days. This declined to a small but significant extent over the 17 years audited ($P = 0.005$).

RECOMPRESSION PROTOCOL

In accordance with widely accepted practice, divers underwent an initial recompression prescribed by a protocol chosen according to perceived DCI severity and physician preference. Most commonly this was the US Navy Treatment Table 6, used in 338 (65%) of cases. A 4 ATA (405 kPa) table utilising 50:50 oxygen-helium breathing, the so-called

'RNZN 1A', was prescribed in a further 109 (21%) cases which were generally of a more serious nature. Divers with residual symptoms after the first recompression underwent further once-daily recompressions until there was either full recovery or no sustained improvement over two consecutive days. These follow-up treatments were conducted according to a shorter protocol specifying oxygen breathing at either 284 or 203 kPa for 60 or 90 minutes respectively. The mean number of re-treatments was 1.27.

DIVER OUTCOMES

At discharge, 438 divers (84%) were either without sequelae or with an expectation that minor subjective symptoms would resolve within one month. Though this was usually confirmed by follow-up, these latter cases were deemed to have experienced a complete recovery. Sixty-one (12%) patients were considered to have had an incomplete recovery. Outcome data were not recorded for 21 (4%) divers.

Discussion

We have described the caseload of DCI patients treated in Auckland between 1996 and 2012. The most striking feature of these data is the significant decline in annual case numbers that has occurred over the 17-year period. The mid- to late-1990s was characterised by high numbers of DCI cases treated in Auckland. Indeed, 100 cases were treated in 1995 though this cohort included patients from the South Island because the Christchurch chamber was not operational.[5] Whereas annual numbers above 50 were typical in the 1990s, these have dwindled to fewer than 30 in recent years. There are few published accounts of comparable data from other centres but it is notable that a similar decline in the numbers of divers recompressed for DCI in Australia also occurred

between 1995 and 2007.[6] The number of calls from within Australia to the Australian Diver Emergency Service hotline also declined between 1991 and 2007.[7] Thus, the decline in the number of DCI cases treated at Auckland is confluent with the Australian experience. The cause of this decline is unknown.

One potential explanation is that it reflects a regional decrease in diving activity, but the latter has not been measured and it would be difficult to do so.[6] We have reported annual numbers of entry-level certifications issued in New Zealand by the predominant diver training organisation as one plausible index of diving activity over an approximately corresponding period. There has been a significant decline in certification numbers (Figure 1). Similar data were provided by PADI to estimate the incidence rate of scuba diving fatalities for a previous New Zealand study.[8] Although this lends some strength to the hypothesis that the decline in DCI is owing (at least in part) to a decline in diving activity, the observation deserves cautious interpretation. There are other training organisations operating in New Zealand whose training numbers were not obtained, and the number of new certifications cannot be assumed to directly equate with diving activity because this could also be influenced by fluctuations in the activity of previously trained divers, due to factors such as changing economic conditions, or by changes in diving tourism activity.

Another potential explanation is that diving has become safer. The imposition of regulation and safety strategies can produce dramatic declines in DCI cases in high-risk populations, but it is debateable whether there were any pivotal positive influences on diver safety in New Zealand over the reference period.[9] One possibility might be that an increasing proportion of divers adopted the use of dive computers instead of tables for planning and controlling of their depth/time profiles. Computers have several potential advantages such as: ensuring the diver at least uses some means of controlling depth and time; the monitoring of ascent rates and provision of alarms when safe rates are exceeded and avoidance of the errors that divers frequently make when performing dive table calculations.[10] It is known that computer use has increased markedly over the last 20 years to such an extent that, whereas dive table instruction was previously mandatory, the PADI entry level course now offers the option of only learning to use a computer. Little can be deduced from our finding of a trend to increasing computer use among DCI patients without accurate data describing the relative use of computers and tables in the community. The apparent trend in our data probably reflects increasing use of dive computers in the community, and it is possible that dive computer users are actually under-represented in our cohort. Other plausible contributors to improved decompression safety over the audit period include the progressive inculcation of divers in the use of a 'safety stop' for 3 minutes at 5 metres' depth as a routine on every dive. Similarly, relevant educational initiatives such as the

SAFE Dive (Slowly Ascend From Every Dive) campaign have become ubiquitous in the instructional and training pedagogy.

A third potential explanation is that fewer divers suffering symptoms of DCI are choosing to present for recompression treatment. This would seem unlikely in the face of serious manifestations, but divers with mild symptoms might invoke the findings of the 2004 remote DCI workshop to justify such a choice.[11] Specifically, a workshop consensus statement reads: *"The workshop acknowledges that some patients with mild symptoms and signs after diving can be treated adequately without recompression. For those with DCI, recovery may be slower in the absence of recompression."*[11] We doubt this has had significant, if any impact on divers' choices in respect of seeking recompression in New Zealand. Awareness of the workshop's findings among divers is not widespread. In addition, the SHU policy of offering recompression to all local divers diagnosed with DCI has not changed. Moreover, this explanation is not consistent with our data which show that the trend to declining numbers was well established prior to publication of the workshop proceedings in 2005. Finally, if declining case numbers reflected an increasingly frequent choice not to present for mild symptoms, we would expect to see serious cases making up a greater proportion of the total. In fact, the proportion of cases (36%) with objective signs (which tend to be seen in the serious neurological events) is less in this series than the 45% recorded for the 100 cases treated in 1995.[5]

There were several other significant trends revealed by our data. First, the average age of divers treated for DCI increased over the study period. The most plausible explanation for this is that it simply reflects the demographic of the diving population. It is certainly possible that if fewer new divers are being trained (as the PADI data indicate) then a greater proportion of the total diving is being conducted by an aging population of established divers. Second, the median latency between incident dive and recompression also declined over the study period. It is more difficult to generate a plausible hypothesis to explain this trend. The most obvious (but entirely speculative) explanation would be that divers are becoming better educated, such that the diagnosis of DCI has become 'de-stigmatised' and, combined with better understanding on the potential benefits of timely treatment, this has resulted in earlier reporting of symptoms. In respect to evacuation for treatment and since first-aid oxygen can improve the early response to recompression, it was disappointing that in those cases where first-aid strategies were recorded, less than half received first-aid oxygen.

The clinical aspects of the cases in this series were confluent with those reported from a 1995 cohort treated at the SHU.[5] The most common symptoms were pain and patchy paraesthesiae, with objective signs in only 36% of cases. The choice of a higher pressure oxygen/helium table for

cases of greater perceived severity is consistent with practice among hyperbaric units in Australia.[12] Most cases (84%) were considered to have made a complete recovery. This was higher than for the 1995 SHU cohort (70%), but the difference is probably explained by changes in the definition of complete recovery at the point of discharge rather than a change in actual outcomes.[5] Over the period considered in the present study mild residual symptoms thought likely to resolve within a month were not considered in determining categorisation as 'incomplete' recovery.

This study has several weaknesses that should be acknowledged. The retrospective method placed reliance on the accuracy and completeness of data recorded in the patient notes and SHU patient database. In some cases, the notes were not available for reconciliation with the database, mandating total reliance on the database. Not surprisingly, there were some missing data. The retrospective design also precluded the accurate application of potentially useful severity scoring systems to individual cases which would have helped inform some of the preceding discussion.[13] Finally, many symptoms of DCI are non-specific and there is an undeniable potential for cautious practitioners to over-apply the diagnosis resulting in contamination of our dataset by non-DCI cases. Such conservative practice is widespread. The 'marginal' cases included in our dataset were recompressed and discharged with the diagnosis of DCI, and by definition they constitute part of the case load. They are, therefore, included in our report. Despite these limitations, our study describes one of the larger single-centre cohorts of DCI patients reported to date in Australia and New Zealand. In addition, the longitudinal design has facilitated identification of several interesting and potentially important trends in the number and nature of cases.

We conclude that the annual number of cases of DCI recompressed at Auckland has declined significantly over the past 17 years. A decrease in diving activity is the most plausible explanation, but other factors cannot be excluded.

References

1   Vann RD, Butler FK, Mitchell SJ, Moon RE. Decompression illness. *Lancet*. 2011;377:153-64.
2   Richardson K, Mitchell SJ, Davis M, Richards M. Decompression illness in New Zealand divers: the 1996 experience. *SPUMS Journal*. 1998;28:50-5.
3   Kenedi C, Sames C, Paice R. A systematic review of factitious decompression sickness. *Undersea Hyperb Med*. 2013;40:267-74.
4   Fitzgerald B. A review of the sharpened Romberg test in diving medicine. *SPUMS Journal*. 1996; 26:142-6.
5   Gardner M, Forbes C, Mitchell SJ. One hundred divers with DCI treated in New Zealand during 1995. *SPUMS Journal*. 1996;26:222-6.
6   Lippmann J. Review of scuba diving fatalities and decompression illness in Australia. *Diving Hyperb Med*. 2008;38:71-8.
7   Wilkinson D, Goble S. A review of 17 years of telephone calls to the Australian Diver Emergency Service (DES). *Diving Hyperb Med*. 2012;42:137-45.
8   Davis FM, Warner M, Ward B. Snorkelling and scuba deaths in New Zealand, 1980–2000. *SPUMS Journal*. 2002;32:70-80.
9   Xu W, Liu W, Huang G, Zou Z, Cai Z, Xu W. Decompression illness: clinical aspects of 5278 consecutive cases treated in a single hyperbaric unit. *PloS one*. 2012;7:e50079.
10   Wilks J, O'Hagan V. Queensland scuba divers and their tables. *SPUMS Journal*. 1991;21:12.
11   Mitchell SJ, Doolette DJ, Wacholz CJ, Vann RD, editors. *Management of mild or marginal decompression illness in remote locations*. Workshop Proceedings. Divers Alert Network. 2005: Sydney, Australia. p. 240.
12   Bennett MH, Mitchell SJ, Young D, King D. The use of deep tables in the treatment of decompression illness: the Hyperbaric Technicians and Nurses Association 2011 Workshop. *Diving Hyperb Med*. 2012;42:171-80.
13   Mitchell SJ. Severity scoring in decompression illness. *SPUMS Journal*. 2005;35:199-205.

Haas Rachel M[1], Hannam Jacqueline A[2], Sames Christopher[3], Schmidt Robert[3], Tyson Andrew[4], Francombe Marion[3], Richardson Drew[5], Mitchell Simon J[2,3,6]

[1] *School of Medicine, University of Auckland, New Zealand*
[2] *Department of Anaesthesiology, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand*
[3] *Slark Hyperbaric Unit, Waitemata District Health Board, Auckland, New Zealand*
[4] *Hyperbaric Health, Quay Park, Auckland, New Zealand*
[5] *Professional Association of Diving Instructors, Rancho Santa Margarita, CA, USA*
[6] *Department of Anaesthesia, Auckland City Hospital, 2 Park Rd, Grafton, Auckland, New Zealand*

Address for correspondence:
Associate Professor Simon Mitchell
Department of Anaesthesiology,
Faculty of Medical and Health Sciences,
University of Auckland,
Auckland, New Zealand
E-mail: <sj.mitchell@auckland.ac.nz>
Phone: +64-(0)9-373-7599
Fax: +64-(0)9-373-7970

Neuron-specific enolase and S100B protein levels in recreational scuba divers with neurological decompression sickness

Emmanuel Gempp, Pierre Louge, Sébastien De Maistre, Loïc Emile and Jean-Eric Blatteau

Abstract

(Gempp E, Louge P, De Maistre S, Emile L, Blatteau J-E. Neuron-specific enolase and S100B protein levels in recreational scuba divers with neurological decompression sickness. *Diving and Hyperbaric Medicine*. 2014 March;44(1):26-29.)

Introduction: Neuron-specific enolase (NSE) and S100B protein are brain-origin proteins commonly described to assess the presence and severity of neurological injury. To date, there are limited data examining the influence of scuba diving on these biomarkers, particularly when symptoms of decompression sickness (DCS) occur. The purpose of this controlled study was to determine whether these serum neurochemical markers could be used as 1) indicators of neurological DCS and 2) predictors of incomplete recovery.

Methods: Fifty-nine divers with neurological DCS and 37 asymptomatic divers admitted for inadequate decompression, serving as controls, were consecutively enrolled between 2010 and 2012. Blood samples were collected at initial presentation up to 6 hours after dive completion (controls) or onset of symptoms (DCS divers). Biomarkers were quantified in non-haemolysed samples only. Clinical outcome was assessed at 6 months post-injury.

Results: The two groups did not differ regarding the variables examined, except for the total dive time which was slightly shorter in the control group. NSE, but not S100B protein, was higher in the DCS group than in controls ($P < 0.0001$). An NSE level $> 15.9 \, \mu g \, L^{-1}$ determined by ROC analysis predicted DCS development with a specificity of 100% (95% confidence interval (CI) 90 to 100) and a sensitivity of 24% (95% CI 14 to 36). There was a trend towards a higher likelihood of residual neurological deficits above this cut-off value ($P = 0.08$).

Conclusions: Early determination of NSE was found to be useful for the diagnosis of neurological DCS with a high specificity. However, its clinical applicability in decision making for determining treatment as well as its prognostic value remains to be established. Reliability of S100B protein was not demonstrated in the present study.

Key words

Decompression sickness, central nervous system, brain injury, proteins, severity, outcome, diving research

---

Introduction

Neurological decompression sickness (DCS) in scuba divers is a rare event with an incidence estimated between 0.02 and 0.03% per dive.[1] This disorder is the leading cause of morbidity with potential residual deficits of around 30% reported in the literature.[2,3] While there have been a number of clinical scoring systems devised for acute neurological DCS that have proved reliable for the prediction of incomplete recovery, little in the way of research into biological markers in humans has been conducted to test their value in diagnosing DCS and assessing prognosis.[4–6] Numerous studies have documented a variety of haematological and biochemical changes associated with decompression stress or the occurrence of DCS, but their utility as diagnostic tools has not yet been proven.[7–10] Particular attention has focused on the measurement of haematocrit, which has been noted to rise in severe cases of neurological DCS.[11] However, normal values have also been observed commonly in patients with a poor outcome, limiting the prognostic performance of this test in routine clinical use. Recent work also showed that elevated plasma D-dimer levels during the acute phase of neurological DCS was associated with the occurrence of sequelae at three months but the sensitivity of the test is still rather low.[12]

Neuron-specific enolase (NSE), a glycolytic enzyme predominantly localized in the cytoplasm of neurons and cells with neuro-endocrine differentiation, and S100B, a calcium-binding protein found in abundance in astroglial and Schwann cells, are commonly elevated during the acute phase of neurological damage after global cerebral ischaemia, stroke and traumatic brain injury.[13–15] The value of these neurochemical biomarkers in spinal cord injury is still unknown with very few investigations conducted in this field of study.[16]

To date, there are limited data examining the influence of scuba diving on these biomarkers, particularly when symptoms of DCS occur.[17–19] The purpose of this retrospective observational study in a large cohort of divers was to determine whether serum NSE and S100B protein levels could be used as 1) supplementary indicators to a clinical diagnosis of neurological DCS and 2) predictors of incomplete recovery.

Methods

The ethics committee of Saint Anne's Military Hospital approved the study, and all patients gave their informed consent. Between January 2010 and February 2012, 94 recreational divers with clinical signs of neurological DCS and 38 asymptomatic divers referred for inadequate decompression (i.e., fast ascent, omitted decompression

Figure 1
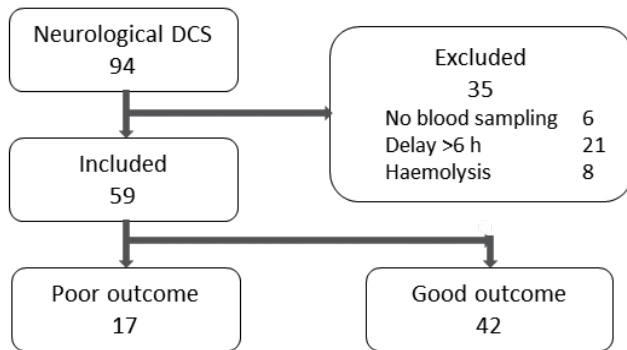Flow diagram describing the selection of DCS divers

Table 1
Characteristics of DCS divers and control divers; mean (SD);
* means $P < 0.05$

Characteristics	DCS divers $n = 59$	Controls $n = 37$	P-value
Age (years)	46 (10)	49 (12)	0.16
Gender (M/F)	43/16	27/10	0.82
Mean depth (msw)	40.5 (10.5)	41.5 (12.5)	0.65
Mean dive time (min)	35 (10)	30 (14)	0.02*
Repetitive dive	12/59	7/3	0.9
Delay for blood collection (min)	170 (70)	156 (34)	0.27

stops), serving as controls, were admitted to our hyperbaric facility (Toulon, France). Cases suspected of cerebral arterial gas embolism, patients with incomplete data or those who presented more than 6 hours after onset of symptoms (DCS divers) or more than 6 hours after surfacing (controls) were excluded. Demographics, diving parameters and delay between blood collection and dive completion were recorded in each group. Clinical outcome was classified as poor (presence of residual neurological manifestations defined as persistent objective sensory, motor or urinary disorders) or good (full recovery) after clinical evaluation at six months post injury.

Venous blood samples were collected from all divers on initial presentation and drawn in dry and EDTA tubes (8 ml). Serum NSE and S100B were obtained by centrifugation (5000 rpm for 10 min at 4°C) and stored at -80°C until measurement of both biomarkers with commercially available electrochemicoluminescence immunoassay kits (Elecsys, Roche Diagnostics). All samples with visible haemolysis were discarded from analysis to avoid any falsely elevated values for NSE.

STATISTICAL ANALYSIS

Data were expressed as mean ± SD or median with range for nonparametric variables. Differences between groups were compared using the unpaired Student's t-test or the Mann-Whitney U test where appropriate. Correlations between continuous variables were evaluated by calculating Spearman's coefficient (ρ). Associations between categorical variables were measured by the Fisher exact test. A receiver operating characteristics (ROC) curve was performed to discriminate the highest measurement of NSE levels in predicting DCS development while specificity (Sp) and sensitivity (Se) were obtained with the use of predefined thresholds. Odds ratios with 95% confidence intervals (CI) were calculated when needed and P-values lower than 0.05 were considered significant. Statistical calculations were performed with Graphpad Prism 5.00 (GraphPad Software, San Diego, CA).

Results

Fifty-nine DCS divers and 37 controls (after exclusion of 1 diver with haemolysis) were eligible for this study (Figure 1). Both groups were similar regarding the variables examined, except for total dive time which was shorter in the control group compared with DCS divers (35 ± 10 min vs. 30 ± 14 min, $P = 0.02$, Table 1). There was no significant difference in the mean delay to collection of blood between the groups (170 ± 70 min vs. 156 ± 34 min for DCS divers and controls, respectively).

Of the 59 injured divers, 17 were found to have incomplete recovery after follow-up evaluation and were considered the severe group. Among them, four had disabling sequelae including urinary or bowel disturbance, ataxia due to sensory spinal myelopathy and mild degrees of limb spasticity. The remaining 42 (of 59) DCS divers did not exhibit neurological residual symptoms, and thus belonged to the benign group.

Serum NSE was higher in the DCS group than in controls (12.5 ± 4.3 µg L^{-1} vs. 8.8 ± 3.2 µg L^{-1}, $P < 0.0001$) (Figure 2). The level with the highest specificity and sensitivity was 12.1 µg L^{-1} (Sp = 89%, 95% CI 75 to 97; Se = 44%, 95% CI 32 to 58). A cut-off value of 15.9 µg L^{-1} predicted DCS development with a specificity of 100% (95% CI 90 to 100) and a sensitivity of 24% (95% CI 14 to 36).

The mean NSE level was significantly higher among patients in the severe group than those with a good outcome (14.5 ± 5.2 µg L^{-1} vs. 11.7 ± 3.6 µg L^{-1}; $P = 0.02$). However, association between NSE ≥ 15.9 µg L^{-1} and DCS severity did not reach statistical significance although there was a trend towards a poorer outcome above this cut-off value (OR = 3.5, 95% CI 0.99 to 2.3, $P = 0.08$).

There was no difference in the median S100B levels between injured divers and controls (0.087 ng L^{-1}, 95% CI 0.010 to 0.270 vs. 0.083 ng L^{-1}, 95% CI 0.045 to 0.260, respectively) or between severe DCS divers and those with

Figure 2
NSE concentrations in the serum of diver subgroups;
* $P < 0.0001$
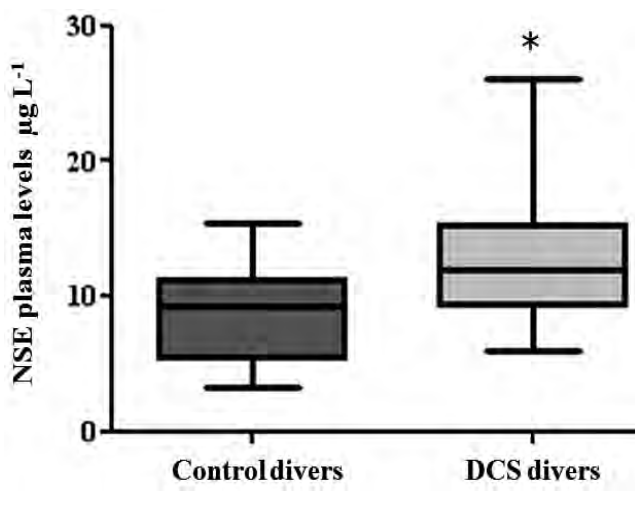
benign evolution (0.081 ng L^{-1}, 95% CI 0.036 to 0.227 vs. 0.087 ng L^{-1}, 95% CI 0.010 to 0.272, respectively). In addition, there was no statistically significant correlation ($\rho = 0.08$) between NSE and S100B levels.

Discussion

To our knowledge, this is the first study investigating the concomitant use of NSE and S100B in divers with neurological DCS and comparing them to a control population. Our findings indicate that NSE, but not S100B, is elevated in the serum of divers presenting with neurological decompression sickness as compared to asymptomatic divers who had performed a dive with inadequate decompression. It appears that NSE is a specific biomarker which allows ruling in the diagnosis of neurological DCS with a very good reliability when the values exceed 12.1 µg L^{-1}. We also identified a cut-off value for NSE (> 15.9 µg L^{-1}) predicting the development of DCS with no false positives. However, the clinical usefulness of this test is hampered by its low sensitivity, meaning that a negative result does not necessarily rule out the occurrence of neurological DCS. In addition, the assay procedures make the clinical applicability difficult for the acute evaluation of the severity of DCS and consequently, for the choice of hyperbaric treatment regimen.

To our knowledge, there are only two reports assessing the influence of scuba diving on these two humoral indicators of neuronal damage.[17,18] Although no cumulative effect of repetitive dives on serum S100B levels was found in either study, there were small but significant post-dive increases in S100B concentrations in one study.[18] However, the concomitant rise in creatine kinase activity following each dive led the authors to suggest a skeletal muscle origin for this protein, as already observed after swimming.[19] In addition to S100B, NSE release did not seem be affected

by four days of consecutive diving despite detection of significant amounts of vascular bubbles post dive.[18] These findings may indicate that uneventful no-decompression scuba dives do not cause discernable neuronal damage. On the other hand, a previous study in rats demonstrated a rise in serum S100B following simulated dives, with a strong correlation between S100B expression, bubble formation and/or the extent of hyperbaric exposure, suggesting a potential influence of decompression stress severity on alterations of the blood brain barrier.[20]

In a study of divers with neurological DCS, S100B also did not appear to be of clinical use in diagnosis as this marker did not increase over the next few days following the onset of symptoms.[21] Our data are in agreement with these findings, although the blood samples were drawn at different times, with an average time of less than 3 hours in the present study. To date, no clinical study has focused on the analysis of NSE concentration in DCS divers, hence making direct comparison with our findings difficult. Further research is warranted to evaluate the potential role of this biomarker in predicting outcome, in particular, after serial measurements over time since it has been reported that the release of NSE may reach a peak value at 48 to 72 h following acute ischaemic stroke.[22]

Conclusion

The present study reveals that plasma NSE concentrations in divers with neurological DCS exceed the levels found in control subjects who had performed dives with inadequate decompression. Our findings suggest that an increase of NSE level above a cut-off value of 15.9 µg L^{-1} measured early on admission appears to have a specificity of 100% but a low sensitivity for neurological DCS. The clinical relevance of this test in the acute assessment of divers with suspected neurological DCS remains to be established, considering the relatively long time needed to perform the biomarker analysis. The combined measurement of S100B with NSE does not add diagnostic or prognostic information, suggesting that damage of neurones is more involved in neurological DCS than glial alterations.

References

1   Vann RD, Butler FK, Mitchell SJ, Moon RE. Decompression illness. *Lancet.* 2011;377:154-64.
2   Gempp E, Blatteau JE. Risk factors and treatment outcome in scuba divers with spinal cord decompression sickness. *J Crit Care.* 2010;25:236-42.
3   Vann R, Freiberger JJ, Caruso JL, Denoble P, Pollock NW, Uguccioni DM, et al. *DAN report on decompression illness, diving fatalities and project dive exploration.* Durham, NC: Divers Alert Network; 2005. p. 63-5.
4   Blatteau JE, Gempp E, Simon O, Coulange M, Delafosse B, Souday V, et al. Prognostic factors of spinal cord decompression sickness in recreational diving: retrospective and multicentric analysis of 279 cases. *Neurocrit Care.* 2011;15:120-7.

5   Boussuges A, Thirion X, Blanc P, Molenat F, Sainty JM. Neurologic decompression illness: a gravity score. *Undersea Hyperb Med*. 1996;23:151-5.

6   Dick APK, Massey EW. Neurologic presentation of decompression sickness and air embolism in sport divers. *Neurology*. 1985;35:667-71.

7   Nyquist P, Ball R, Sheridan MJ. Complement levels before and after dives with a high risk of DCS. *Undersea Hyperb Med*. 2007;34:191-7.

8   Ersson A, Walles M, Ohlsson K, Hekholm A. Chronic hyperbaric exposure actives proinflammatory mediators in humans. *J Appl Physiol*. 2002;92:2375-80.

9   Pontier JM, Gempp E, Ignatescu M. Blood platelet-derived microparticule release and bubble formation after an open-sea air dive. *Appl Physiol Nut Metab*. 2012;37:888-92.

10   Philp RB. A review of blood changes associated with compression decompression: relationship to decompression sickness. *Undersea Biomedical Research*. 1974;1:117-50.

11   Boussuges A, Blanc P, Molenat F, Bergmann E, Sainty JM. Haemoconcentration in neurological decompression illness. *Int J Sports Med*. 1996;17:351-5.

12   Gempp E, Morin J, Louge P, Blatteau JE. Reliability of plasma D-dimers for predicting severe neurological decompression sickness in scuba divers. *Aviat Space Environ Med*. 2012;83:771-5.

13   Martens P, Raabe A, Johnsson P. Serum S100 and neuron-specific enolase for prediction of regaining consciousness after global cerebral ischemia. *Stroke*. 1998;29:2363-6.

14   Bloomfield SM, McKinney J, Smith L, Brisman J. Reliability of S100B in predicting severity of central nervous system injury. *Neurocrit Care*. 2007;6:121-38.

15   Anand N, Stead LG. Neuron specific enolase as a marker for acute ischemic stroke: a systematic review. *Cerebrovasc Disord*. 2005;20:213-9.

16   Pouw MH, Hosman AJF, Van Middendorp JJ, Verbeek MM, Vos PE, Van de Meent H. Biomarkers in spinal cord injury. *Spinal Cord*. 2009;47:519-25.

17   Stavrinou LC, Kalamatianos T, Stavrinou P, Papasilekas T, Koutsarnakis C, Psacholia C, et al. Serum levels of S100B after recreational scuba diving. *Int J Sports Med*. 2011;32:912-5.

18   Bilopavlovic N, Marinovic J, Ljubkovic M, Obad A, Zanchi J, Pollock NW, et al. Effect of repetitive SCUBA diving on humoral markers of endothelial and cerebral nervous system integrity. *Eur J Appl Physiol*. 2013;113:1737-43.

19   Dietrich MO, Tort AB, Schaf DV, Farina M, Goncalves CA, Sousa DO, et al. Increase in serum S100B protein level after a swimming race. *Can J Appl Physiol*. 2003;28:710-6.

20   Havnes MB, Hjelde A, Brubbak AO, Møllerløkken A. S100B and its relation to intravascular bubbles following decompression. *Diving Hyperb Med*. 2010;40:210-2.

21   Poff DJ, Wong R, Bulsara M. Acute decompression illness and serum S100B levels: a prospective observational pilot study. *Undersea Hyperb Med*. 2007;34:359-67.

22   Brea D, Sobrino T, Blanco M, Cristobo I, Rodriguez-Gonzalez R, Rodriguez-Yanez M, et al. Temporal profile and clinical significance of serum neuron-specific enolase and S100 in ischemic and hemorrhagic stroke. *Clin Chem Lab Med*. 2009;47:1513-8.

Emmanuel Gempp[1], Pierre Louge[1], Sébastien De Maistre[1], Loïc Emile[2], Jean-Eric Blatteau[3]

[1] *Department of Diving and Hyperbaric Medicine, Ste Anne's Military Hospital, Toulon, France*
[2] *Department of Biochemistry, Ste Anne's Military Hospital, Toulon*
[3] *ERRSO, Institute of Biomedical Research of the Armed Forces Health Service, Toulon*

Address for correspondence:
Emmanuel Gempp, MD
Department of Diving and Hyperbaric Medicine
Ste Anne's Military Hospital
83800 Toulon cedex 9, France.
Phone: *+33-(0)4-8316-2320*
E-mail: *<gempp@voila.fr>*

An in-vitro examination of the effect of vinegar on discharged nematocysts of *Chironex fleckeri*

Philippa Welfare, Mark Little, Peter Pereira and Jamie Seymour

Abstract

(Welfare P, Little M, Pereira P, Seymour J. An in-vitro examination of the effect of vinegar on discharged nematocysts of *Chironex fleckeri*. *Diving and Hyperbaric Medicine*. 2014 March;44(1):30-34.)

Objective: To determine the effect acetic acid (vinegar) has on discharged nematocysts in a simulated sting from *Chironex fleckeri*.

Method: This research was performed in 2 parts:

1   *C. fleckeri* tentacles placed on amniotic membrane were electrically stimulated, and venom washings collected before and after application of vinegar. Lyophilised venom washings were run through a fast-performance protein liquid chromatography column to confirm the venom profile, with a quantitative measure of venom from each washing calculated using UNICORN™ software.

2   The toxicity of the washings was determined by application to human cardiomyocytes, with percentage of cell detachment providing a measure of cell mortality, and hence toxicity.

Results: Part 1: There was a 69 +/- 32% (F = 77, $P < 0.001$) increase in venom discharge after vinegar was applied compared to post electrical stimulation.

Part 2: Venom collected after the administration of vinegar demonstrated the same toxicity as venom from electrically stimulated *C. fleckeri* tentacles and milked venom, causing cell mortality of 59 +/- 13% at 10 μg ml^{-1} compared to 57 +/- 10% and 65 +/- 7% respectively.

Conclusion: This in-vitro research suggests that vinegar promotes further discharge of venom from already discharged nematocysts. This raises concern that vinegar has the potential to do harm when used as first aid in *C. fleckeri* envenomation.

Key words

Jellyfish, envenomation, clinical toxicology, toxins, research, first aid

Introduction

Jellyfish envenoming is a major and increasing issue worldwide with numerous envenomations occurring each year, many of which require medical treatment.[1] *Chironex fleckeri* envenomations whilst relatively rare can be fatal, with more than 60 recorded deaths within Australian waters.[2] If stung by *C. fleckeri* (the large box jellyfish) in tropical Australia, the Australian Resuscitation Council (ARC) recommendation is to *"liberally douse/spray the sting area with vinegar (4–6% acetic acid) for 30 seconds."*[3] In the USA, vinegar is recommended as first aid for all jellyfish stings by the American Heart Association (AHA) and American Red Cross.[4]

The use of vinegar originated from laboratory studies on the tentacles from *C. fleckeri* in which vinegar was found to permanently inactivate all undischarged nematocysts, with later work attempting to isolate other compounds that may have a similar effect.[5,6] This reaction has been shown in several other cubozoans.[7–9] However, vinegar is also known to cause nematocyst discharge in other jellyfish species.[10–13] The inclusion of vinegar into resuscitation protocols is because of its beneficial action in inactivating undischarged cubozoan nematocysts, and through this process preventing further discharge and envenomation.[5] There is no dispute about vinegar's effectiveness in inactivating undischarged nematocysts of *C. fleckeri*; however, there are no published

data to demonstrate vinegar has any benefit when applied to nematocysts which have already discharged. Discharged nematocysts are not innocuous; they are able to release further venom, for example, when pressure is applied.[14]

It is widely recognised that the application of vinegar does not reduce the symptoms of envenomation.[15] Vinegar has even been reported to worsen pain immediately after application and anecdotal reports from Cairns Base Hospital include increased analgesic needs in patient who have used vinegar on their sting site compared to those who had not been treated with vinegar.[16]

As a sting victim must have discharged nematocysts present, vinegar could be having a different effect on discharged nematocysts compared to its inactivation of undischarged nematocysts. This research was designed to ascertain how discharged nematocysts react to vinegar by quantitatively simulating human envenomation from *C. fleckeri*, with the application of 4% acetic acid to determine whether active venom is released after the application of vinegar and to determine its toxicity towards human cardiomyocytes.

Method

The study was approved by the Cairns Base Hospital Ethics Committee (approval number 287). This research was performed in two parts.

PART 1: VENOM PROFILES GENERATED FROM STIMULATED *C. FLECKERI* TENTACLES BEFORE AND AFTER THE APPLICATION OF VINEGAR

Venom Collection

To collect venom, we applied tentacles of *C. fleckeri* onto human amniotic membrane in an experimental procedure described previously.[14] In brief, human amniotic membrane was secured across one end of a sterile container from which the base had been removed to form an open-ended cylinder. Isotonic sterile saline (0.9% NaCl, 3.5 ml) was washed over the inside of the amniotic membrane five times to remove any extraneous proteins or foreign material, with the final washing kept for analysis (W1 – control).

Following this, 10 cm of tentacles from a freshly caught adult *C. fleckeri* were placed onto the outer surface of the amniotic membrane and partial discharge with adherence of the tentacles was observed. To maximise nematocysts discharge, we applied a 6-volt, 3-ampere direct current charge across the tentacle pieces for two seconds (Figure 1). Such electrical augmentation is currently used to collect venom from *C. fleckeri* for commercial anti-venom production.[17] Contraction and frosting of the tentacles were observed and taken as confirmation of successful nematocyst triggering and discharge.

Venom from the under surface of the membrane was then collected via washing in the following manner: the cylinder was inverted and 3.5 ml of 0.9% NaCl was placed into the cylinder, rinsing venom from the side of the membrane with the penetrating nematocyst shafts. The cylinder was agitated for 15 seconds, re-inverted and the washing collected (W2 – after voltage). To ensure clearance of discharged venom, the under surface was washed three times with 3.5 ml of 0.9% NaCl with the final washing kept for analysis (W3 – after wash).

The cylinder was re-inverted and 1 ml of 4% acetic acid (commercially available vinegar) was then applied to the adherent tentacles and left untouched for 30 seconds to simulate the current first-aid treatment guidelines recommended by the ARC.[3] Following this, the underside of the amniotic membrane was rewashed with 3.5 ml of 0.9% NaCl and the washing collected (W4 – after vinegar).

This entire experimental procedure was repeated twice, on new sections of the same amniotic membrane and with fresh tentacles from the same *C. fleckeri*, giving a total of three replicates. All washings were then lyophilised, weighed and stored at -80°C.

Venom profiling

Known weights of each lyophilised sample were individually rehydrated to give solutions with protein concentrations of 0.27 mg ml⁻¹ (to allow comparison with previously published data).[18] For some of the samples (W1 and W3), this required the majority of the sample to be rehydrated.

Figure 1

Electrical stimulation (6-volt, 3-amp DC) to discharge nematocysts of *C. fleckeri* tentacles placed on human amniotic membrane; toxin was obtained with washings of the underside of the membrane (see text for details)

Between 200 and 500 μl of reconstituted venom was then passed through a 0.22 μm filter and individually run over an ÄKTA™ fast-performance protein liquid chromatography (FPLC) (Superdex ™ 10/200GL; Tricorn; 13 μm, 10 mm x 200 mm) at a flow rate of 0.3 ml min⁻¹ and wavelength set at 280 nm, using degassed Dulbecco's phosphate buffered saline as a running buffer, and fractionated. This allows rapid purification of the proteins present within the venom, and the specific combination of proteins form a venom profile which can be compared to known data on *C. fleckeri* milked venom.[19]
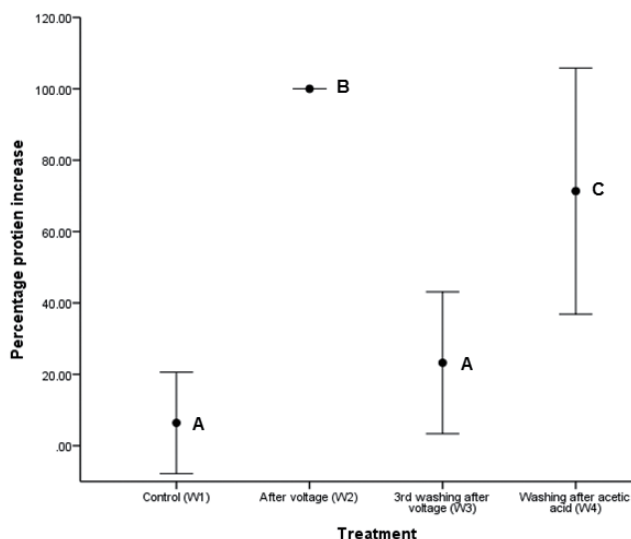
Using UNICORN™ software, the area under the curve for the protein profile for each washing (listed above) was calculated and the total volume of venom expressed in each washing was back-calculated using the initial weight of lyophilised washing. These areas were then converted to percentages relative to the amount of protein collected after voltage was applied for each sample. Statistical analyses (one-way ANOVA) were performed on these percentages (transformed by arcsin square-root to normalise the distribution) to determine if the quantities of venom varied between the different washings. *Post hoc* analysis (using least-significant difference - LSD) was performed to determine which washings were statistically different to one another.

PART 2: APPLICATION OF VENOM TO HUMAN CARDIOMYOCYTES TO MEASURE TOXICITY

To allow the toxicity of the washings (W1, W2, W3 and W4) to be determined and compared to published results on *C. fleckeri* venom toxicity, the lyophilised washings were rehydrated. The lethality of these rehydrated washings was then tested using the Roche Applied Science and ACEA Biosciences Incorporated xCELLigence system. This system

Figure 2
Percentage increase in protein concentration found in washings
(W1–W4); the protein (venom) found in the washing after electrical
stimulation (W2) was significantly higher than any other washing.
Vinegar applied after electrical discharge was associated with
further protein expression (69 +/- 32% (F = 77, $P < 0.001$));
means with the same letter are not statistically different

Table 1
Percentage toxicity (95% confidence limits) of different dilutions
of washings from *Chironex fleckeri* envenomed amniotic membrane
against human cardiomyocytes compared to extracted (milked)
whole *C. fleckeri* venom (data taken from reference 18)

	% cell death at 10 min (10 mg ml⁻¹)	% cell death at 10 min (1 mg ml⁻¹)
Control washing (W1)	n/a	0
1st washing after voltage (W2)	57 (47, 67)	21 (12, 30)
3rd washing after voltage (W3)	n/a	0
Washing after vinegar (W4)	59 (46, 72)	16 (7, 25)
Milked venom	65 (58, 72)	19 (11, 27)

There was a significant difference between the protein
concentrations of the different treatments (F = 77.12_{3x82} ,
$P < 0.001$). The protein (venom) found in the washings after
voltage was applied (W2) was significantly higher than all
other treatments (LSD, $P < 0.001$). Similarly, the percentage
increase in protein (venom) found in the washings after
vinegar was applied (W4) was significantly higher than
controls (W1, W3) (LSD, $P < 0.001$) but not as high as in
washings after electrical stimulation (W2) (LSD, $P = 0.001$) .

PART 2

Washings post electrical stimulation (W2), and post
application of vinegar (W4) were toxic to human
cardiomyocytes. These washings had activity similar to
previously published studies using whole extracted *C.
fleckeri* venom (Table 1).[18] This activity decreased to the
levels of the control (W1) in subsequent washings (W3)
and recrudesced in washings collected after the application
of vinegar (W4).

Discussion

Previous research has shown that application of weak
(3–10%) acetic acid for 30 seconds to *C. fleckeri* tentacles
does not trigger discharge of nematocysts, and that
nematocyst discharge from undischarged nematocysts is
irreversibly inhibited.[5] It is unknown why this occurs, but it
is postulated to be due to the terminal carboxyl group.[5] This
action is not refuted by this study. Instead, we have confirmed
earlier findings that triggered (or discharged) nematocysts
are incompletely discharged of venom.[14]

More importantly, the application of vinegar was associated
with further discharge of venom. We are unsure as to
why this occurs, but given there is evidence that vinegar
completely inactivates undischarged nematocysts we
can only postulate that this additional venom has been
expressed from discharged nematocysts, perhaps through
a chemically mediated process of nematocyst wall

quantifies cell survival by measuring cell attachment over
time and has been used previously to show cell cytotoxicity
of *C. fleckeri* venom.[18]

In brief, human cardiomyocytes were cultured in 75 cm²
flasks. Once cell culture had reached 80% confluence, cells
were lifted using a bovine trypsin solution. The number of
cells in this solution was calculated using a haemocytometer
and approximately 2000 cells with 150 µl of media were
then seeded in each well of a 96 well E-Plate. Cells were
incubated in the E-Plate for 24 hours at 37°C and 5%
carbon dioxide to ensure all cells were properly attached to
the base of the wells before commencing experimentation.
Reconstituted washings were applied to individual wells
and cell survival was determined as described previously.[18,19]
(N.B. Washings were tested at a concentration of 10 µg ml⁻¹
for direct comparison with a previous study.[18] However, the
control (W1) and W3 had insufficient protein to achieve
this and instead were tested at a concentration of 1 µg ml⁻¹.

Results

PART 1

The venom profile obtained from the FPLC was similar
to that reported previously for *C. fleckeri*.[19] Venom was
detected post electrical stimulation and after application
of vinegar. This study demonstrated that the application of
vinegar to a *C. fleckeri* tentacle that had been electrically
discharged was associated with further protein (venom)
expression of 69 +/- 32% more protein (venom) (Figure 2).

contraction. Furthermore, this venom exhibited the same cardiomyotoxic activity as the initial venom discharged. This finding may explain why the application of vinegar gives no symptom relief and may in certain cases (e.g., when a large proportion of discharged nematocysts is present compared to undischarged) actually exacerbate pain. However other causes, e.g., the application of a mild acid to already damaged skin, could also increase pain experienced by the patient.

This raises concern that vinegar may be harmful when applied as first aid to a sting victim who has both discharged and undischarged nematocysts present on their skin. It is unknown what the proportion of discharged to undischarged nematocysts is on a patient with envenomation, and ideally undischarged nematocysts should be inactivated with vinegar. However, if vinegar causes further discharge in already discharged nematocysts in vivo, vinegar may or may not have an overall benefit.

Therefore, it may be time to reconsider first-aid options for tropical Australian jellyfish stings. Heat (hot water) can lessen the pain experienced from bluebottle *(Physalia)* stings, and other types of box jellyfish (*Carybeda alata*).[20–23] In animal models, hot water has been shown to stop the lethal effects of *C. fleckeri* venom when heated above 43°C.[24] However, the ability to obtain hot water in a timely fashion, and the high temperatures required limit its feasibility as a first-aid measure. Topical lidocaine has been shown to be an effective analgesic in stings from the box jellyfish *Chiropsalmus quadrumanus* (sea wasp).[10] Lidocaine is also proven to inhibit nematocyst discharge in *Chrysaora quinquecirrha* (sea nettle) and *Physalia physalis* (Portuguese man-of-war).[10] To date, there are no studies into the use of lidocaine on *C. fleckeri*.

Conclusion

This in-vitro research demonstrates that vinegar promotes further discharge of venom (approximately a further 69% of venom load released) from already electrically discharged nematocysts of *C. fleckeri*. This in turn raises concern that vinegar may have the potential to do harm by exacerbating envenomation from *C. fleckeri*. Further investigations are required to elucidate the mechanism(s) of this secondary release of toxin and to identify first aid measures which will reduce both pain and the risk of cardiac arrest.

References

1　Fenner PJ, Williamson JA. Worldwide deaths and severe envenomation from jellyfish stings. *Med J Aust*. 1996;165:658-61.

2　Tibballs J. Australian venomous jellyfish, envenomation syndromes, toxins and therapy. *Toxicon*. 2006;48:830-59.

3　Australian Resuscitation Council [Internet]. *Guideline 9.4.5 Envenomation: Jellyfish Stings* July 2010. Available from: http://www.resus.org.au/policy/guidelines/section_9/guideline-9-4-5july10.pdf.

4　Epstein J, Gonzales L, Herrington RA, Pellegrino JL, Ratcliff N, Markenson D, et al. Part 17: First aid: American Heart Association and American Red Cross guidelines. *Circulation*. 2010;122:S934-46.

5　Hartwick R, Callanan V, Williamson J. Disarming the box-jellyfish: nematocyst inhibition in *Chironex fleckeri*. *Med J Aust*. 1980;1(1):5-20.

6　Currie B, Ho S, Alderslade P. Box-jellyfish, Coca-Cola and old wine. *Med J Aust*. 1993;158:868.

7　Fenner PJ, Fitzpatrick PF, Hartwick RJ, Skinner R. "*Morbakka*", another cubomedusan. *Med J Aust*. 1985;143:550-1, 554-5 .

8　Mianzan HW, Fenner PJ, Cornelius PF, Ramírez FC. Vinegar as a disarming agent to prevent further discharge of the nematocysts of the stinging hydromedusa *Olindias sambaquiensis*. *Cutis*. 2001;68:45-8.

9　Fenner PJ, Williamson JAH. Experiments with the nematocysts of *Carybdea rastoni*. *Med J Aust*. 1987;147:258-9.

10　Birsa LM, Verity PG, Lee RF. Evaluation of the effects of various chemicals on discharge of and pain caused by jellyfish nematocysts. *Compar Biochem Physiol*. Part C. 2010;151:426-30.

11　Ward NT, Darracq MA, Tomaszewski C, Clark RF. Evidence-based treatment of jellyfish stings in North America and Hawaii. *Ann Emerg Med*. 2012;60:399-414.

12　 Fenner PJ, Fitzpatrick PF. Experiments with the nematocysts of *Cynea capillata*. *Med J Aust*. 1986;45,174.

13　Fenner PJ, Williamson JA, Burnett JW, Rifkin J. First aid treatment of jellyfish stings in Australia. Response to a newly differentiated species. *Med J Aust*.1993;158:498-501.

14　Pereira PL, Carrette T, Cullen P, Mulcahy RF, Little M, Seymour J. Pressure immobilisation bandages in first aid treatment of jellyfish envenomation: current recommendations reconsidered. *Med J Aust*. 2000;173:650-2.

15　Nomura JT, Sato RL, Ahern RM, Snow JL, Kuwaye TT, Yamamoto LG. A randomised paired comparison trial of cutaneous treatments for acute jellyfish (*Carybdea alata*) stings. *Am J Emerg Med*. 2002;20:624-6.

16　Beadnell CE, Rider TA, Williamson JA, Fenner PJ. Management of a major box jellyfish (*Chironex fleckeri*) sting. Lessons from the first minutes and hours. *Med J Aust*. 1992;158:655-7.

17　Barnes JH. Extraction of *Cnidaria* venom from living tentacle. In: Russell FE, Saunders PR, editors. *Animal toxins*. London: Pergamon Press;1967. p.115-29.

18　Saggiomo SLA, Seymour JE. Cardiotoxic effects of venom fractions from the Australian box jellyfish *Chironex fleckeri* on human myocardiocytes. *Toxicon*. 2012;60:391-5.

19　McClounan S, Seymour J. Venom and cnidome ontogeny of the cubomedusae *Chironex fleckeri*. *Toxicon*. 2012;60:1335-41.

20　Loten C, Stokes B, Worsley D, Seymour J, Jiang S, Isbister GK. Randomised controlled trial of hot water (45°C) immersion versus ice packs for pain relief in bluebottle stings. *Med J Aust*. 2006;184:329-33.

21　Bowra J, Gillett M, Morgan J, Swinburn E. Trial comparing hot showers and ice packs in the treatment of *Physalia* envenomation. *Emerg. Med*. 2002;14:A22.

22　Thomas CS, Scott SA, Galanis DJ, Goto RS. Box jellyfish (*carybdea alata*) in Waikiki: their influx cycle plus the analgesic effect of hot and cold packs on their stings to swimmers on the beach: A randomised, placebo-controlled clinical trial. *Hawaii Med J*. 2001;20:100-7.

23　Atkinson PRT, Boyle A, Hartin D, McAuley D. Is hot water immersion and effective treatment for marine envenomation? *Emerg Med J*. 2006;23:503-8.

24   Carrette TJ, Cullen P, Little M, Pereira PL, Seymour JE. Temperature effects on box jellyfish venom: a possible treatment for envenomed patients? *Med J Aust*. 2002;177:654-5.

Philippa Welfare[1], Mark Little[1,2,3], Peter Pereira[1,2], Jamie Seymour[2,3]
[1] *Emergency Department Cairns Base Hospital, Queensland, Australia*
[2] *Queensland Emergency Medical Research Foundation (QEMRF), Australia*
[3] *Queensland Tropical Health Alliance, School of Public Health and Tropical Medicine, Centre for Biodiscovery and Molecular Development of Therapeutics, Faculty of Medicine, Health and Molecular Sciences, James Cook University, Queensland, Australia*

Address for correspondence:
P Welfare, MBChB
Department of Emergency Medicine, Cairns Base Hospital
PO Box 902, Cairns
Queensland 4870, Australia
Phone: *+61 -(0)7-4226-8227*
E-mail: *<pipwelfare@hotmail.com>*

The Rubicon Foundation

Upon crossing the Rubicon River in 49 BC, Caesar said "*The die has been cast*". For the Rubicon Foundation, the point of no return was reached when three divers walked into a bar. This sounds like a bad joke but how many pivotal projects in undersea medicine must have started this way? "*Martini's Law*" as a way of describing inert gas narcosis to divers came from a similar beginning at the Key West "O" club.[1] In 2002, two dive buddies and I realized that our access to literature on diving and hyperbaric medicine was becoming increasingly difficult. Popular magazines like *AquaCorps,* which provided a bridge between science and the technical diving community had disappeared. Who would answer our questions? While some organizations were doing an amazing job reaching out to answer questions for recreational divers, had anyone picked up the reins with respect to trimix breathing gases or decompression modelling?

That same year, the US Office of Naval Research evaluated their long-term needs in undersea medicine.[2] The panel found that 60% of young researchers in the field left within less than 10 years, and that many senior scientists were close to retirement (52% in less than 10 years and 96% retiring within the next two decades). Furthermore, the Navy had not trained any investigators in the previous decade. With our own needs and the needs of a community established, we initially set out to make research easier for those in the community. In doing so, we would also be making sure that knowledge that was known to the senior scientists would be available to those entering the field. This is where the Rubicon Research Repository (RRR) began.

We have been fortunate in forming collaborations to grow the RRR with organizations like SPUMS, and this has had a substantial impact for newcomers to the field. Many young scientists and graduate students have expressed how the RRR has proven to be a valuable resource to their work. New literature reviews published in the field now cite the RRR as one of the databases searched more frequently.

In 2008 we began to harness the power of Wikipedia to increase the exposure of the documents we hold. At that time, diving and hyperbaric articles existed on Wikipedia but had almost no references to support their content. To date, the RRR holds over 9,500 documents and abstracts and over 1,700 links from Wikipedia references are in place. Since Wikipedia has become a likely source for medical information, embracing the tool has enabled exposure to go beyond clinicians and students to our patients as well.

The second phase of our growth has been to initiate our own research, such as utilizing mathematical models developed by Dr Wayne Gerth to assess risk associated with various decompression planning tools and operational procedures.

The final phase of our growth is intended to establish long-term financial sustainability for the organization. With the decline in funding that has been available for our field and with increasing global economic tensions, we are bracing ourselves for what may very well become a forced independence. Throughout 2013, we have been working to combine new technologies and professional skill sets to launch our sustainability model.

We look forward to collaborating with *Diving and Hyperbaric Medicine* for many years to come.

References

1   Rosenberg LC, Ramsdell RC. *Changes in intellectual function associated with nitrogen narcosis*. US Navy Submarine Medical Officer's Thesis. 1957. Available from: http://archive.rubicon-foundation.org/10268
2   Undersea and Hyperbaric Medical Society. *An assessment of a national naval need for undersea research*. Office of Naval Research, report in response to 5000 Ser 341/270 20 Feb 02.

Gene Hobbs
Rubicon Foundation, Inc.
E-mail: *<gene@rubicon-foundation.org>*

Review article

Ultrasound detection of vascular decompression bubbles: the influence of new technology and considerations on bubble load

S Lesley Blogg, Mikael Gennser, Andreas Møllerløkken and Alf O Brubakk

Abstract

(Blogg SL, Gennser M, Møllerløkken A, Brubakk AO. Ultrasound detection of vascular decompression bubbles: the influence of new technology and considerations on bubble load. *Diving and Hyperbaric Medicine*. 2014 March;44(1):35-44.)

Introduction: Diving often causes the formation of 'silent' bubbles upon decompression. If the bubble load is high, then the risk of decompression sickness (DCS) and the number of bubbles that could cross to the arterial circulation via a pulmonary shunt or patent foramen ovale increase. Bubbles can be monitored aurally, with Doppler ultrasound, or visually, with two-dimensional (2D) ultrasound imaging. Doppler grades and imaging grades can be compared with good agreement. Early 2D imaging units did not provide such comprehensive observations as Doppler, but advances in technology have allowed development of improved, portable, relatively inexpensive units. Most now employ harmonic technology; it was suggested that this could allow previously undetectable bubbles to be observed.

Methods: This paper provides a review of current methods of bubble measurement and how new technology may be changing our perceptions of the potential relationship of these measurements to decompression illness. Secondly, 69 paired ultrasound images were made using conventional 2D ultrasound imaging and harmonic imaging. Images were graded on the Eftedal-Brubakk (EB) scale and the percentage agreement of the images calculated. The distribution of mismatched grades was analysed.

Results: Fifty-four of the 69 paired images had matching grades. There was no significant difference in the distribution of high or low EB grades for the mismatched pairs.

Conclusions: Given the good level of agreement between pairs observed, it seems unlikely that harmonic technology is responsible for any perceived increase in observed bubble loads, but it is probable that our increasing use of 2D ultrasound to assess dive profiles is changing our perception of 'normal' venous and arterial bubble loads. Methods to accurately investigate the load and size of bubbles developed will be helpful in the future in determining DCS risk.

Key words

Doppler, bubbles, venous gas embolism, arterial gas embolism, decompression sickness, diving research, review article

Introduction

ULTRASOUND MEASUREMENT OF VASCULAR BUBBLES: RECENT OBSERVATIONS

It is well accepted that divers commonly develop venous gas emboli (VGE) on decompression. Most will never be aware of their presence, as the bubbles are often 'silent', without accompanying symptoms of decompression sickness (DCS). Bubbles form from supersaturated gases in the tissues or blood upon decompression and can occur after surprisingly shallow hyperbaric exposures.[1] For example, in one study, it was concluded that 50% of humans would be expected to develop VGE upon decompression after saturation at only 135 kPa (3.5 metres' sea water, msw).[2]

The significance of VGE is their relationship to the risk of DCS. Studies have shown that the absence of VGE correlates well with the absence of DCS; in other words, if bubbles cannot be detected, then it is unlikely that symptoms of DCS will occur.[3–5] It also appears that the number of bubbles is proportional to decompression stress and the higher the venous bubble load, the more likely DCS is to occur,

although the relationship is not direct.[6] Large numbers of VGE imply a very high free gas load, increasing the risk of clinical symptoms.[6]

In order to assess the number or load of bubbles in the body, two methods have been used: aural Doppler ultrasound monitoring and visual two-dimensional (2D) ultrasound imaging. Both methods most often focus on the cardiac region, observing venous bubbles as they return from the body to the right heart and into the pulmonary artery, though an important benefit of 2D imaging is that it also provides a simultaneous view of the left heart and any bubbles present there. Doppler methods remain essentially the same as when they were first developed in the 1960s. However, 2D ultrasound imaging has progressed; while conventional ultrasound processes only one returning signal, the more recently introduced harmonic imaging increases resolution and contrast of the images and allows differentiation between smaller objects.

In 2011, a study was presented comparing the link between VGE load and DCS risk.[7] Sixty-nine no-decompression dives were performed by 12 divers, all ranging in depth

between 18 and 33 msw. Harmonic ultrasound imaging was used to assess bubble loads in the divers after they exited the water. The dives produced a considerable number of VGE in all divers, with most dives resulting in an Eftedal-Brubaak grade 4 (55 of 69 dives).[8,9] Five of the 12 divers also had arterial bubbles following 11 of 69 dives.[7]

We were surprised that so many VGE had been observed and, at the 2010 meeting of the European Undersea and Baromedical Society, we speculated that this was owing to the harmonic ultrasound technology and the greater resolution it afforded. Did this new technique allow smaller bubbles, previously invisible to conventional ultrasound, to be seen? It was also noted that left-heart bubbles were found in a greater percentage of the subjects than might have been expected. It is highly unlikely that more bubbles (arterial or venous) are being produced by today's divers; a simpler explanation is that we now have the ability to discover them via ever-improving technology.

This study examines the present techniques and equipment that are commonly used in decompression ultrasound, via a review of the literature. In order to test the hypothesis that harmonic imaging might reveal bubbles that were previously 'invisible' using conventional imaging, it also includes a simple study comparing images made with 'harmonics switched off' (conventional mode) and 'harmonics on' and goes on to discuss the relevance of bubble size and load to the risk of DCS.

Current methods of bubble measurement

DOPPLER ULTRASOUND

Doppler ultrasound was the original method, first reported by Spencer and Campbell in 1968, to detect VGE in the body associated with decompression.[9] Despite some improvements in methodology and transducer technology, the technique and equipment have remained relatively similar to the present day, whereby a well-trained operator applies an ultrasound transducer to the body that transmits a signal at a particular frequency. The operator then listens to the difference in frequency between the transmitted and received signal (that has been Doppler-shifted in frequency by moving objects such as red blood cells and bubbles in the blood). Gas bubbles are more efficient scatterers of ultrasound waves than red blood cells and are thus easily discernable.

The Doppler technique has been used by many workers across the years to detect bubbles and the information collected from both animal and human subjects forms a large data bank that may be used to compare the severity of decompression profiles, giving the method continuing relevance today. Smaller, more portable Doppler units with longer battery life also make the technique attractive for use on dive boats and the like. Limitations include the difficulty

and time investment involved in training operators correctly, that information can be obtained only from one site at a time (for example the presence of arterial bubbles cannot be investigated whilst monitoring venous sites) and not being able to quantitatively assess the size of the bubbles.

Doppler measurements are usually evaluated using the Kisman-Masurel (KM) bubble evaluation code, or the Spencer code.[6,10] These methods are relatively subjective, and rely heavily on the operator having a good ear for the signal and being well trained and practised in using the grading scale. The KM code is generally preferred for its greater flexibility and sensitivity in grading scores, as it takes into account three components of the bubble signal. The first component assesses the number of bubbles produced per cardiac cycle (frequency), graded on a scale of 1 to 4 and is noted over at least 10 heart beats. The second component assesses the proportion of cardiac heart beats containing the bubbles (percentage), and the third considers the 'loudness' (amplitude), of the bubble signal using the background blood flow sounds as a reference. Once the three-part code has been determined, it is transformed into a KM grade, from 0 to IV, which aims to give a sense of physiological severity to the data. It should be noted that the scale is highly non-linear in nature, both in regard to the number of bubbles and to the corresponding risk of DCS; the resulting data should be handled with that in mind. Measurements are also often taken after movement, for example, a deep knee bend which, if bubbles are present, will produce a surge back to the heart that is easy for the operator to identify and helps to remove any ambiguity. Resting and movement measurements are always made separately and denoted when reporting results.

2D VISUAL ULTRASOUND IMAGING (ECHOSONOGRAPHY)

The second method of evaluating decompression bubbles is via the use of visual 2D ultrasound (echosonography) imaging systems. This is a comparatively young art in the field of decompression physiology, but it offers a number of benefits over Doppler, including an immediate impression of the bubble load in both the left and right heart (Figure 1), and ease of monitoring. It has been demonstrated that relatively little training is needed to accurately perform grading of 2D images although it is harder to reliably capture high-quality scans.[8] In contrast, learning to grade Doppler data may take a considerable time (months) to perfect. The 2D ultrasound technique has been used to assess human decompression bubbles in the heart since the late twentieth century and is growing in popularity, particularly as the once prohibitive price and size of imaging units is decreasing and, importantly, their image quality is improving.

Initially, the quality of the 'conventional ultrasound' images was such that 2D ultrasound was not as effective in assessing bubble loads as the use of Doppler ultrasound operated by experienced personnel. As for 2D visual data, a grading

Table 1
Relationship between Kisman-Masurel (KM) grades, Eftedal-Brubakk (EB) grades and bubble counts[3]

KM grade	0	I-	I	I+	II-	II	II+	III-	III	III+	IV-	IV
EB grade	0	1	1	1	2	2	2	3	3	4	4	5
Bubble count (bubbles cm^{-2})	0	0.01	0.05	0.1	0.15	0.2	0.3	0.5	1	2	5	10

Figure 1

2D ultrasound image showing bubbles in both sides of the heart of a degree (EB Grade 4C / 5) such that the outlines of the right ventricle (RV) and atrium (RA) cannot be discerned; bubbles (VGE and AGE) can be seen in both the left ventricle (LV) and atrium (LA)
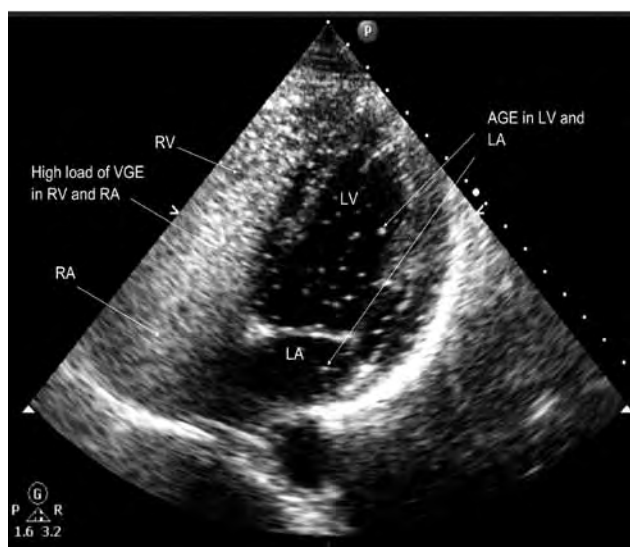
system is necessary to evaluate the images and the most commonly used, the EB grade, was developed by Eftedal and Brubakk in 1997:[8]

0 – No bubbles;
1 – Occasional bubbles;
2 – At least one bubble every four cardiac cycles;
3 – At least one bubble every cardiac cycle;
4 – At least one bubble per cm^2 in every image;
5 – White out, single bubbles cannot be discriminated.

This simple system also relies on a degree of subjectivity. The agreement of the KM Doppler and the EB visual grading scales has been assessed for comparative purposes and was found to be good generally, though direct conversion from one scale to another should probably be avoided.[11] A bubble-counting system has also been developed, based on the number of visible bubbles in the observed field, providing a quantifiable measure of bubbles per square cm.[12] The relationship between these three techniques is shown in Table 1.[12]

The next stage in the development of the technology, harmonic ultrasound, was commonly introduced in the late 1990s.[13] It has recently been introduced to decompression physiology, although investigations into its use for bubble detection began as far back as the 1970s. Originally developed for use with ultrasound contrast microbubbles for clinical purposes, it was observed that the images acquired prior to the arrival of the contrast medium were of better quality than the fundamental signal (as processed by conventional instruments). Because of harmonic technology, many improvements to the quality of images have been made in recent years, including visualisation of smaller objects and improved contrast resolution, meaning that layers of grayscale in the image can be visually differentiated more easily. In simple terms, smaller bubbles should become more apparent and the image should be clearer.[14] The majority of modern ultrasound imaging systems now employ this harmonic principle. That this technology can be contained in small, portable, less expensive, dive-research-friendly units may go some way to explain why more bubbles, both venous and arterial, are being observed.

DOPPLER VERSUS IMAGING.

Comparing the relationship between the aural and visual grading methods with the bubble-count method (Table 1), it becomes obvious how highly non-linear the KM and EB grading systems are, particularly at the higher end of the grading scales.[3] For example, a single move from I+ to II- on the KM scale, or 1 to 2 on the EB scale, equates to a jump from 0.1 to 0.15 bubbles cm^{-2} in terms of bubble count. Moving from a KM III+ grade to IV-, both equating to a grade 4 on the EB scale, is comparable to a much larger move from 2 to 5 bubbles cm^{-2}.

In a recent investigation, KM Doppler grades using a Doppler Bubble Monitor, DBM9008 (Techno Scientific, Ontario, Canada) and harmonic 2D images (from a Philips CX50, Philips Healthcare, Stockholm, Sweden) of precordial VGE graded on the EB scale were compared directly (unpublished observations). The study was carried out to determine whether the harmonic technology would now render Doppler and 2D ultrasound non-comparable, whereas previously, conventional ultrasound imaging and Doppler were found to be comparable.[11] It was suggested that any smaller bubbles detectable to harmonics might be the reason for the perceived increase in observed venous and arterial bubbles, as described by others.[15] If this hypothesis were

correct, then the EB grades should be lifted up a level or two against the KM grades. However, this was not the case. The harmonic imaging and Doppler data collected over 2 h post-decompression, from subjects who had been placed in a dry hyperbaric chamber compressed to 283 kPa for 100 min (RN Table 11[16]) were still generally found to be in agreement and in accordance with Table 1, both in the discovery and grading of bubbles. Hence, the findings also suggested that the majority of bubbles produced following decompressions in the study fell within the size range (circa 30 μm in diameter and above) of Doppler detection (these observations were reported as an abstract at the 2010 Undersea and Hyperbaric Medical Society meeting).

Conventional ultrasound versus harmonic ultrasound – an experimental study

INTRODUCTION

In light of the above unpublished observations, a study was carried out to compare paired harmonic and conventional images on the EB scale. In this way, the possibility that harmonic ultrasound could reveal bubbles previously invisible to conventional imaging post hyperbaric exposure was investigated.

METHODS

The study used ultrasound images that were recorded following a number of different dives made in the autumn of 2012. Subjects included male divers from the Swedish and Danish Navy and the study complied with the Declaration of Helsinki (2008). Images were recorded after open-water trimix dives with closed-circuit rebreathers and after trimix dives with semi-closed rebreathers in a hyperbaric chamber attached to a wet-pot. Dive profiles varied considerably, ranging from a dry introductory chamber dive, a wet dive to 30 m for 25 min, and deep-water dives made to 90 m with a bottom time of 20 min. The profiles are not included here, as it is the comparison of the paired grades that is of interest to this study, rather than the bubble loads provoked by the varying dive profiles.

On surfacing, 2D ultrasound measurements were made from five minutes to two hours post surfacing. When bubbles were present and the images were of reasonable quality, two recordings were made in a randomised order, one with harmonics switched on and another with harmonics switched off. The unit used, a Philips CX50 (Philips Healthcare, Best NL), allows the switch from harmonic to conventional ultrasound to be made easily, using a toggle switch. An attempt to include at least one set of recordings from each subject was made and the time taken between paired measurements was kept to a minimum. Each image was recorded after the subject was asked to make a move from the left lateral decubitus position, roll onto their back and then return to their starting posture, in order to try to

standardise the bubble load returning to the heart on each of the measurements.

In total, 69 paired images were included in the study, taken from different subjects. The recordings were then played back for grading on the EB scale by a single, experienced operator. It was impossible to carry out blind grading of the data, as it is obvious as to which mode, conventional or harmonic, is being played back. The quality of the image (in terms of contrast and grayscale) is far superior in the harmonic mode and is instantly recognisable.

STATISTICAL ANALYSIS

Fisher's exact test was used to compare whether mismatched pairs of EB grade were more common with high (EB ≥ 3) or low (EB < 3) bubble loads ($P < 0.05$).

RESULTS

Of the 69 paired measurements, 54 matched; that is, both the harmonic and the conventional images were graded as the same on the EB scale. Of the 54 pairs that matched, 39 were of an EB grade of 3 or above (high grade), while 15 were graded at 2 or below (low grade). Of the 15 pairs that were not matched, 10 involved harmonic EB grades of 3 or above, while the remaining 5 pairs were mismatched when the harmonic EB grade was 2 or below. There was no significant difference in the frequency of the high/low grade split between matching and non-matching observations (matching low grade – 15, high grade – 39; mismatch low grade – 5, high grade – 10; Fisher's exact test, $P = 0.45$). However, in 14 of the 15 mismatched pairs, the score was higher when harmonics was used. In 10 of these observations, the use of harmonics translated the result from an EB < 3 to an EB > 3. In no case did the converse occur. The median mismatch in those 15 pairs was 1 (range -1 to 3) EB grade. It should be noted that in one subject, imaging was difficult; the quality of both the harmonic and particularly the conventional images made them difficult to score, and this subject accounted for a number of the mismatched pair grades.

Discussion

EXPERIMENTAL STUDY OF HARMONICS VERSUS CONVENTIONAL ULTRASOUND

In this study, 2D harmonic ultrasound images made post-decompression were compared with conventional ultrasound images, to reveal whether any extra bubble load information could be gained by using the former (Figure 2). It should be noted that the study may be limited by the use of a single machine; the effectiveness of harmonic and no-harmonic settings might differ between models used in decompression studies. Harmonic imaging is known to increase grayscale resolution, so improving the sharpness of the image. It should also have the capability to reveal

Figure 2
Comparison of a conventional 2D ultrasound image (A) with a harmonic 2D image (B); the images are from the same subject taken sequentially, so at a very similar time point; note the improved contrast of B and clarity of the structures and bubbles
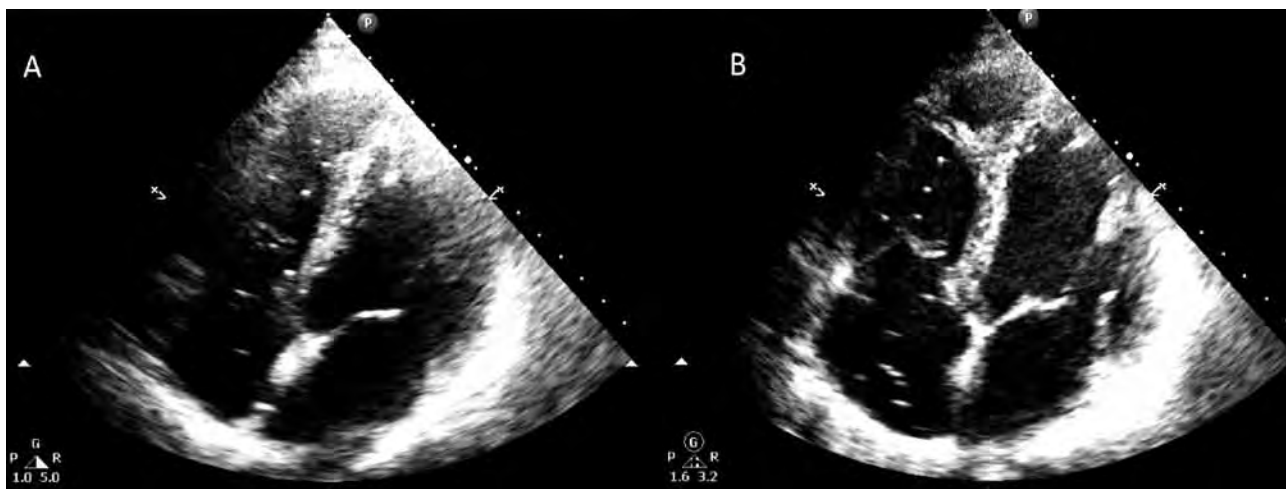
smaller bubbles, should any be present, because of improved spatial resolution.

Over three-quarters of the paired images produced the same EB grades; and the only obvious difference was in the greyscale quality of the image; the images were much sharper and of greater contrast (Figure 2). Although there was no significant difference between the ratio of matching and non-matching pairs with low and high grades, a clear majority (14/15) of the non-matching pairs showed more bubbles when harmonics was used. In small samples and particularly if subjects are difficult to image clearly, there is a possibility that the harmonic technology will skew the results towards higher bubble grades. However, unless the median of the bubble grades is close to a given study cut-off point, for instance between grades 2 and 3, it is unlikely that the results will produce a significant difference between studies made using old or new ultrasound imaging technology.

Of the 54 grades that did match, 39 of these were of an EB grade of 3 or above, meaning that there was at least one bubble seen in every cardiac cycle. An EB grade 3 approximates to a KM III- or III on the KM Doppler scale and has been shown to carry a higher risk of DCS in comparison to KM grade II or below in a number of studies.[5,17,18] It is at this higher end of the grading scale that bubbles visible only with harmonics would be of importance. For example, it was the presence of high ultrasound grades in several studies that prompted the suggestion that harmonic ultrasound was raising reported grades with small bubbles that were previously invisible.[7,15] However, the present results show a good level of agreement at EB grade 3 or over and so would seem to refute that argument. Perhaps this should have been expected; it has been noted that harmonics may have additional sensitivity to bubbles with resonance close to the driving frequency of the device only,

so very small bubbles would be detected only by medical grade equipment.[19]

In some cases, alternative explanations for unexpectedly high bubble loads might be relatively straightforward. Using the V-planner software and the VPM-B algorithm (variable permeability model – HHS Software Corp., Kingston, ON, Canada) to derive the dive profiles for trimix dives, large numbers of VGE and AGE were observed.[15] This may have been caused by the critical level of the algorithm being set too high and so, simply, more decompression was needed to reduce the bubble load and DCS risk. However, the large number of bubbles produced following the no-decompression dives was not expected, as those dive profiles were based on standardised and conventionally tested tables that were thought to be relatively conservative.[7,20]

The classical method of testing a dive table is to use DCS as a binomial yes/no endpoint. Interestingly, and as above, when ultrasound measurements have been made recently following such tabled dives, results suggest that they are often not as conservative as might have been expected. For example, the UK Royal Navy's Table 11 (their standard air diving table, based on Haldanian principles) has been used in a number of studies to test the effect of different prophylactic regimes on VGE production, as this table is known to be 'bubble producing'.[16,21,22] The resulting bubble grades usually range across the entire scale. Perhaps the occurrence of high VGE grades and occasional arterial bubbles when using traditionally tested dive tables is 'normal'. Is it simply the fact that ultrasound monitoring is now more common that makes us increasingly aware of their presence? That conventionally tested tables do appear safe in terms of DCS risk also further highlights the uncertain relationship between high bubble loads and DCS occurrence.

Although the majority of images did match in the present study, a 100% record was not observed. At low levels of bubbling, the inability to make simultaneous measurements will pose a disadvantage when trying to make comparisons. The mismatch in higher EB grades above 3, where at least one bubble must be present every cardiac cycle, is likely to be explained by the ability of harmonic imaging to improve resolution, particularly in those subjects where imaging of any kind is difficult owing to their individual anatomy. Clearly this is where harmonic technology could make a difference in post-decompression bubble monitoring.

TECHNICAL DISCUSSION: BUBBLE SIZE AND CONSEQUENCES

If bubbles detectable by harmonic ultrasound are not responsible for a perceived increase in bubble loads, it does not mean that gaining an impression of the size distribution of decompression bubbles is not still of use. Indeed, one aspect of imaging that may aid in investigating the aetiology of DCS is the increasing ability to gain a quantitative measure of the size of the bubbles present. Knowing the distribution in size of intravascular bubbles is desirable, as size plays an important part in how far a single bubble can travel in the arterial system.[23] Those bubbles with the smallest radii have the shortest lifespan. If passing from the venous to the arterial circulation, they will immediately become subject to higher mechanical pressures that should mean that they are crushed very swiftly.[24] The suggestion has also been made that arterial bubbles usually have venous origins, in which case the lung would act as a filter for bubbles over a certain size.[23]

The approximate diameter of a pulmonary capillary is around 10 μm.[25] Bubbles of this diameter or less can cross the lung under 'normal' conditions. Should any of these very small bubbles be present and cross to the arterial circulation, they would likely collapse quickly. Indeed the majority of VGE reaching the lungs are excreted to the atmosphere by molecular diffusion across the arteriolar wall into the alveolar spaces, where the rate of washout is related to the mean pulmonary artery pressure and right ventricular performance.[26,27] It is during adverse conditions, such as emergency surfacing or surface recompression for example, that problems might occur. In the presence of large VGE loads (KM grades III and above) or under the influence of other factors that can combine with VGE load, the pulmonary capillary filter might be overwhelmed. Larger bubbles may deform and elongate to pass through to the arterial circulation, while smaller bubbles might find conditions that would allow them to grow, perhaps then leading to neurological DCS.[24,28] So the ability to assess both bubble size and bubble load with ultrasound would be beneficial in terms of gauging DCS risk.

Doppler cannot make a quantitative assessment of bubble size, although technically it has been found that the amplitude of the reflected signal could be considered approximately proportional to the radius of the bubble, so a qualitative assessment of bubble size could be derived.[29] It is not possible with ultrasound imaging to assess absolute bubble size using either conventional or harmonic technology, although a relative idea of size may be gained. However, recently developed dual-frequency technology may allow us to accurately size bubbles in the future.[30] The dual frequency device emits a 'pump' and an image 'signal' at two frequencies. The pump signal causes appropriately sized bubbles to resonate, so that when the image signal hits a resonating bubble, a 'mixing' signal is returned. A study in swine has shown that such mixing signals can be detected in the right atrium and histograms of estimated bubble sizes produced from the data, while stationary bubbles may also be monitored in the tissue post decompression.[30–32] This may lead to a new understanding of bubble evolution and another method through which to evaluate DCS at multiple sites around the body, once the technology becomes more commonplace.

At normal pressure, conventional 2D ultrasound has been reported to be able to detect bubbles in vivo at a diameter with a lower limit of 10 to 20 μm although, if packed together closely, groups of bubbles may be identified as one large bubble.[33] Noise in the images will also influence detection. The size of the bubble detected is dependent on the operating frequency of the probe used; usual transmitted frequencies range from 1 to 10 MHz, where 1–3 MHz is used in the heart and 5–10 MHz is used in smaller vessels closer to the surface of the body.

The lower limit of detection for Doppler will be higher than that of 2D imaging. In vitro studies have shown that bubbles of a minimum 30 μm in diameter could be detected by a 2 MHz probe in the presence of red blood cells flowing through a 9.6 mm diameter cannula though, in vivo, the minimal detectable size might be larger.[34] In the pulmonary artery or right ventricle for example, where the volumes of blood present are far greater, only signals from larger bubbles may be great enough to overcome the higher background scattering signal produced by the millions of red blood cells present.[34] Overall, Doppler is limited by its inability to detect bubbles below a certain threshold.[3] This is determined by a number of factors including driving frequency, transducer configuration and the scattering properties of the moving objects in the ultrasonic field (red blood cells and bubbles) that are needed to produce a Doppler shift. So, is Doppler able to give a relatively complete representation of bubble load following decompression in humans?

In a study of the size distribution of intravenous bubbles formed by severe decompression in the dog, it was found that they ranged in size from 19–700 μm in diameter, so above the size that could normally pass through the pulmonary filter.[23] At five minutes post decompression, most bubbles measured between 24–32 μm, with the size increasing

with time to range from 50 to 170 µm at 35 minutes. The measurements were made by drawing venous bubbles from the dog through a cannula, so the range of bubble sizes may have been altered and not be completely reflective of in vivo bubble distribution. In theory, Doppler measurements made using a 2 MHz probe should be able to report the majority of the bubbles in this range. However, at the onset of bubbling, when it would seem that smaller bubbles are produced, it might not be possible to report the entire bubble load over the entire period of bubble evolution. Nevertheless, if these results from the dog could equate to humans, then Doppler should be able to describe a relatively complete illustration of post-decompression bubble load in the diver.

It may be that bubbles small enough to pass the pulmonary capillary bed filter (< 10 µm) without deformation are relatively uncommon. However, arterial bubbles are now reported in studies more frequently than might be expected.[7,15] This observation poses a number of questions, not least whether the subjects in these studies represent a group particularly predisposed to arterial bubble production by their environment, lifestyle or physiology, e.g., the presence of a patent foramen ovale (PFO). Perhaps the most important question is why are these bubbles being produced and what is their level of pathophysiological risk? This question is pertinent as, globally, DCS incidence rates remain low at around 0.03% (derived from a sample of 135,000 dives made by 9,000 divers).[35]

Historically, the observation or awareness of arterial gas bubbles has always created apprehension, as they may potentially lodge, sludge and then grow in the arterial blood supply to organs and tissues, particularly the brain and spinal cord. For example, in a paper on Doppler ultrasound for monitoring haemodynamic changes and bubbles, it was noted that a large number of bubbles were found in the aorta and carotid artery of the human subjects, but no signs of serious DCS accompanied them.[36] At the time (early 1980s), these findings were met with general disbelief and concern, prompting a lively discussion. Today, the role of arterial bubbles in the onset of DCS remains unresolved.

VGE LOADS: THE ASSOCIATED RISK OF DCS AND AGE DEVELOPMENT

In the KM grading system, the highest VGE load is represented by grade IV (Table 1); a signal given this grade indicates that individual bubble sounds cannot be differentiated; instead a continuous sound is heard in 100% of heartbeats and is clearly perceptible against the cardiac blood flow. A KM grade IV is equivalent to an EB grade 5; Figure 1 depicts the huge bubble load associated with these grades. Of the 1,726 human air dives where Doppler data were collected during a safe dive limits survey, only three precordial KM IV grades were recorded at rest.[37] There was no concomitant DCS in these subjects. It should be noted that in controlled experimental diving trials, when

a subject presents with DCS, he is usually then lost to Doppler monitoring as medical treatment commences and takes precedence over further measurements. Therefore, it becomes impossible to determine what the maximum bubble grade might have been, should monitoring have been able to continue. If, for example, symptoms appeared before the first measurement was made, the subject may well have had very high bubble grades (Nishi R, personal communication, 2014). KM III grades were more common (191) and of these, 21 subjects had symptoms of DCS (11% incidence). In total, 35 subjects were reported to have DCS, giving an overall incidence rate of 2% from 1,726 dives. That such a small number of maximal bubble grades and cases of DCS were observed indicates that the dives performed in this study had adequate decompression.

However, when maximal grades are provoked by more extreme dive profiles, it seems the risk of DCS is raised. In an early study, five of 174 participants had Spencer grade IV bubbles (equivalent to KM grade IV) and, of these five, four developed DCS.[6] Some of these dives were extremely provocative and outside of normally accepted limits. This is again reflected by the fact that in the same study, of 14 subjects with grade III scores, six (vs. 21 of 191 [11%] in the previous study[37]) developed DCS. In another study, also using 'higher risk' dives, six cases of DCS were observed from 19 subjects with grade IV bubbles.[38] Although the relationship between bubble grade and DCS occurrence is not clearly defined, it is probable that there is an increased risk for DCS with higher bubble grades (III and IV). Of course not all DCS will be reported and bubble grading is subjective, so some latitude must be given to these comparisons.

The standard treatment for DCS is recompression. Altitude exposure studies are often allowed to progress until DCS occurs, with the subject then simply being recompressed to normal atmospheric pressure to resolve the problem, unless severe symptoms require further hyperbaric treatment. Thus, hypobaric studies may help to define the relationship between very high bubble grades and DCS, as grade IV scores occur more often. In a study investigating VGE as a predictive measure of hypobaric DCS, 121 of 249 subjects with grade IV scores developed DCS (49%), while in another, DCS presented in 391 of 633 subjects with grade IV scores (62%).[4,39] However, in describing this relationship, the differences between hyperbaric and hypobaric exposure should be considered. During hypobaric exposure, grade IV scores may persist for some time before DCS symptoms occur. In hyperbaric studies, where bubbling is usually measured post decompression, the bubble load might fall away from grade IV before the onset of symptoms, and this might also be true during altitude exposures. The duration of high levels of bubbling may determine if and when DCS occurs, influencing the mode or type of DCS that develops. For example, it is thought that neurological DCS is closely linked to high bubble loads immediately post dive, while

limb bends are associated with more prolonged bubbling.[40]

Another consideration when assessing bubble data obtained with both Doppler and 2D ultrasound is that the precordial site (cardiac) is not always the most effective in which to monitor VGE. Although less fashionable, it is acknowledged that bubbles may be heard with Doppler in the subclavian vein when they are not obvious in the precordial area. This is because of the diminished background noise at this site; in the heart, noise is ever present, created by the valves, heart wall and greater blood flow, all of which mask the signal. In the safe dive limits survey study mentioned earlier, seven subjects with zero precordial bubble grades presented with DCS.[5,17] However, when subclavian measurements were taken into account, these subjects were seen to have bubbles. If no subclavian bubbles were present, no cases of DCS were seen (*n* = 819). So this study, the largest of its kind, demonstrated that DCS was always accompanied by VGE when both precordial and subclavian measurements were taken into account. This presents a good argument for both sites to be measured as a standard. It also illustrates that methodology and protocol play an important role when considering and comparing data, particularly from different laboratories, as measurements may have been made, for instance, with varying frequency or from different sites.

If the magnitude of the bubble load post decompression is important with respect to the development of arterial gas emboli, then the risk of bubbles passing to the left heart is further heightened in some people owing to the presence of a right–to-left shunt across a PFO. Approximately 25–30% of the population, irrespective of gender, have these well documented, inter-atrial communications that persist after birth.[41] PFO may vary greatly in size from person to person and in certain circumstances, including the high pressures created by large amounts of venous gas in the right heart, which may lead to right-to-left shunting of blood.[41–43] When VGE move across the septum, the arterial circulation will become victim to embolization. Scanning for PFO is not routinely performed for commercial, military or recreational divers, as the associated risk of DCS derived from the condition is relatively low. The mean estimated incidence of neurological DCS (Type II) is 2.28 cases per 10,000 dives across the diving population, while the odds ratio increases only 2.5 times in divers with a PFO.[44]

A more recent study found the risk of serious DCS in subjects with a PFO was more than five times that without PFO and the severity of the DCS increased in parallel with the size of the PFO.[45]

As divers have a one-in-three risk of having a PFO and an even higher chance of producing venous bubbles following a normal, incident-free air dive, it is very likely that at some point during their diving career, contributing factors such as repetitive diving, environment, health issues, high-risk dive profiles or dive accidents will provoke a large bubble load to

form and they may be exposed to arterial gas bubbles. The aforementioned Norwegian study is evidence of this: five out of 12 divers performing successful no-decompression air dives exhibited arterial bubbles upon 2D ultrasound monitoring post dive.[7] The results of that study were unexpected, as the Norwegian no-decompression tables used were thought to be relatively conservative.[20] Arterial bubbles were also present in five out of seven subjects, and nine out of 21 dives, following trimix profiles calculated using V-planner, and they have also been observed following heliox saturation dives from 300 and 250 msw.[15,46] In all of these cases, there was a concomitant high level of bubbles present in the right heart, but importantly, no clinical symptoms or signs of DCS.

It is probably because of the increasing use of 2D visual ultrasound, allowing us a view of all four chambers of the heart, that we are becoming more aware of such unexpectedly high left-heart bubble loads. How they should be approached, in terms of risk of DCS and the subsequent management of divers, remains speculative. Perhaps a cautious attitude would still be recommended, despite our increasing awareness of their presence and relatively low worldwide DCS incidence.

Given that the detection of bubbles is now easier to carry out, present and future technology should provide us with more information on the size and load of bubbles in both the venous and arterial circulations. This will be helpful in exploring the links with and determining the risks of DCS. Moreover, as the increased observation of arterial bubbles has not gone hand in hand with an increase in DCS, future advances in technology should help us understand further the mechanics of bubble formation and then to unravel their role in initiating DCS.

Conclusions

Doppler ultrasound remains a useful tool for decompression research although it is constrained by the difficulty of training operators and its limited window of observation. Portable, more affordable user-friendly ultrasound imaging units have become more widely used in diving research; this might help to explain the seemingly increased observation of VGE and left-heart bubble loads. Unlike Doppler, 2D imaging allows us to view both sides of the heart concurrently, which may explain the apparent increase in incidence of observations of left-heart and arterial bubbles. However, there has not been a concomitant increase in the incidence of DCS. If the frequent occurrence of low numbers of left heart bubbles is 'normal', should this change our perception of their importance to the risk of DCS?

Harmonic technology does not seem to have altered findings relating to post-decompression bubble loads as some have postulated; our study found a good level of agreement between the grades of images made with both conventional

and harmonic imaging technology. Thus, for the most part, harmonic imaging does not seem to impart any fundamental benefit in terms of improving the detection of decompression bubbles or conventional grading of such bubbles. However, the present study did not find a 100% match between harmonic and conventional images. This deficit is most apparent in a subject whose heart is difficult to scan. It is in these cases that the most benefit can be gained by technological improvements: bubbles that might have been missed can often be observed using harmonics, because of the improved resolution it affords. For this reason, harmonic technology does make imaging easier overall and helps to improve the accuracy of grading.

References

1   Tikuisis P, Gerth WA. Decompression theory. In: Brubakk AO and Neuman TS, editors. *Bennett and Elliott's physiology and medicine of diving,* 5th ed. Edinburgh: Saunders; 2003. p. 419-54.

2   Eckenhoff RG, Olstad CS, Carrod G. Human dose-response relationship for decompression and endogenous bubble formation. *J Appl Physiol*. 1990;69:914-8.

3   Nishi RY, Brubakk AO, Eftedal O. Bubble detection. In: Brubakk AO and Neuman TS, editors. *Bennett and Elliott's physiology and medicine of diving,* 5th ed. Edinburgh: Saunders; 2003. p. 501-29.

4   Conkin J, Powell MR, Foster PP, Waligora JM. Information about venous gas emboli improves prediction of hypobaric decompression sickness. *Aviat Space Environ Med*. 1998; 69:8-16.

5   Sawatzky K, Nishi RY. Intravascular Doppler-detected bubbles and decompression sickness. *Undersea Biomedical Research*. 1990; 17(Suppl):34-5.

6   Spencer M, Johanson D. *Investigation of new principles for human decompression schedules using the Doppler ultrasonic blood bubble detector.* Seattle, WA: Institute for Environmental Medicine and Physiology; 1974.

7   Ljubkovic M, Dujic Z, Möllerlökken A, Bakovic D, Obad A, Breskovic T, Brubakk AO. Venous and arterial bubbles at rest after no-decompression air dives. *Med Sci Sports Exerc*. 2011;6:990-5.

8   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.

9   Spencer MP, Campbell SD. Development of bubbles in venous and arterial blood during hyperbaric decompression. *Bull Mason Clin*. 1968;22:26-32.

10 Kisman K, Masurel G. *Method for evaluating circulating bubbles detected by means of the doppler ultrasonic method using the 'K.M. code'.* Toulon, France: Centre d'Etudes et Recherches Techniques Sous-Marines; 1983.

11 Brubakk AO, Eftedal O. Comparison of three different ultrasonic methods for quantification of intravascular gas bubbles. *Undersea Hyperb Med*. 2001;28:131-6.

12 Eftedal O, Brubakk AO. Detecting intravascular gas bubbles in ultrasonic images. *Med Biol Eng Comput*. 1993;31:627-33.

13 Burns PN. Harmonic imaging with ultrasound contrast agents. *Clin Radiol*. 1996;51(Suppl 1):50-5.

14 Desser TS, Jeffrey RB. Tissue harmonic imaging techniques: physical principles and clinical applications. *Semin Ultrasound CT MR*. 2001;22:1-10.

15 Ljubkovic M, Marinovic J, Obad A, Breskovic T, Gaustad SE, Dujic Z. High incidence of venous and arterial gas emboli at rest after trimix diving without protocol violations. *J Appl Physiol*. 2010;109:1670-4.

16 Navy UMR. BR2806 - *UK Military Diving Manual*. In: CINCFLEET/FSAG/P2806, Royal Navy, UK; 1999.

17 Sawatzky K. *The relationship between intravascular Doppler-detected gas bubbles and decompression sickness after bounce diving in humans*. Toronto, (ON): York University; 1991.

18 Powell M, Johanson D. Ultrasound monitoring and decompresison sickness. In: Shilling CW, Beckett MW. *Underwater Physiology VI*: Proceedings of the Sixth Symposium on Underwater Physiology; Bethesda, MD: FASEB; 1978. p. 503-10.

19 Eatock B, Nishi R, Johnston G. Numerical studies of the spectrum of low-intensity ultrasound scattered by bubbles. *J Acoust Soc Am*. 1985;77:1692-701.

20 Arntzen A, Eidsvik S, Risberg J. *Norwegian dive and treatment tables*. Bergen: Barotech AS; 2004.

21 Gennser M, Jurd KM, Blogg SL. Pre-dive exercise and post-dive evolution of venous gas emboli. *Aviat Space Environ Med*. 2012;83:30-4.

22 Jurd KM, Thacker JC, Seddon FM, Gennser M, Loveman GA. The effect of pre-dive exercise timing, intensity and mode on post-decompression venous gas emboli. *Diving Hyperb Med*. 2011;41:183-8.

23 Hills BA, Butler BD. Size distribution of intravascular air emboli produced by decompression. *Undersea Biomedical Research*. 1981;8:163-70.

24 Neuman TS. Arterial gas embolism and decompression sickness. *News Physiol Sci*. 2002;17:7-81.

25 West J. *Respiratory physiology - the essentials*. 6th ed. Baltimore: Lippincott Williams and Wilkins; 2000.

26 Verstappen FT, Bernards JA, Kreuzer F. Effects of pulmonary gas embolism on circulation and respiration in the dog. III. Excretion of venous gas bubbles by the lung. *Pflugers Arch*. 1977;370:67-70.

27 Irwin RS, Rippe JM, editors. *Irwin and Rippes' intensive care medicine*. Baltimore: Lipincott Williams and Wilkins; 2007.

28 Butler BD, Hills BA. The lung as a filter for microbubbles. *J Appl Physiol*. 1979;47:537-43.

29 Moulinier H, Masurel G. Detection of bubbles in blood vessels and the evaluation of their flow. *Med Biol Eng Comput*. 1978;16:585-8.

30 Buckey JC, Knaus DA, Alvarenga DL, Kenton MA, Magari PJ. Dual-frequency ultrasound for detecting and sizing bubbles. *Acta Astronaut*. 2005;56:1041-7.

31 Bollinger BR, Wilbur JC, Donoghue TG, Phillips SD, Knaus DA, Magari PJ, et al. Dual-frequency ultrasound detection of stationary microbubbles in tissue. *Undersea Hyperb Med*. 2009;36:127-36.

32 Swan JG, Bollinger BD, Donoghue TG, wilbur JC, Phillips SD, Alvarenga DL, et al. Microbubble detection following hyperbaric chamber dives using dual-frequency ultrasound. *J Appl Physiol*. 2011;111:1323-8.

33 Daniels S, Paton WD, Smith EB. Ultrasonic imaging system for the study of decompression-induced gas bubbles. *Undersea Biomedical Research*. 1979;6:197-207.

34 Lubbers J, Van den Berg JW. An ultrasonic detector for microgasemboli in a bloodflow line. *Ultrasound Med Biol*. 1976;2:301-10.

35 Pollock NW, Dunford RG, Denoble PJ, Dovenbarger JA, Causo JL. *Annual diving report: 2008 edition*. Durham, NC: Divers Alert Network; 2008. p. 139.

36 Brubakk A, Grip A, Holand B, Onarheim J, Tonjum S. Pulsed Doppler ultrasound for studying haemodynamic changes and bubbles during simulated diving. In: Elliott D, editor. *Proceedings of the European Underwater and Baromedical Society Annual Scientific Meeting 1981*. Cambridge, UK: European Underwater and Baromedical Society; 1981. p. 462-84.

37 Nishi RY, Kisman KE, Eatock B. Assessment of decompression profiles and divers by Doppler ultrasonic monitoring. In: Bachrach AJ, Matzen MM, editors. *Underwater Physiology VII*: Proceedings of the Seventh Symposium on Underwater Physiology. Bethesda, MD: Undersea Medical Society; 1974. p. 717-27.

38 Neuman TS, Hall DA, Linaweaver PG Jr. Gas phase separation during decompression in man: ultrasound monitoring. *Undersea Biomedical Research*. 1976;3:121-30.

39 Pilmanis AA, Kannan N, Krause K, Webb T. Relating venous gas emboli (VGE) scores to altitude decompression sickness (DCS) symptoms. *Aviation Space Environ Med*. 1999;4:364.

40 Blogg SL, Gennser M, Loveman GAM, Seddon FM, Thacker JC, White MG. The effect of breathing hyperoxic gas during simulated submarine escape on venous gas emboli and decompression illness. *Undersea Hyperb Med*. 2003;30: 163-74.

41 Hagen PT, Scholz DG, Edwards WD. Incidence and size of patent foramen ovale during the first 10 decades of life: an autopsy study of 965 normal hearts. *Mayo Clin Proc*. 1984;59:17-20.

42 Wilmshurst PT, Byrne JC, Webb-Peploe MM. Relation between interatrial shunts and decompression sickness in divers. *Lancet*. 1989;334:1302-6.

43 Moon RE, Camporesi EM, Kisslo JA. Patent foramen ovale and decompression sickness in divers. *Lancet*. 1989;333:513-4.

44 Bove AA. Risk of decompression sickness with patent foramen ovale. *Undersea Hyperb Med*. 1998;25:175-8.

45 Torti SR, Billinger M, Schwerzmann M, Vogel R, Zbinden R, Windecker S, Seiler C. Risk of decompression illness among 230 divers in relation to the presence and size of patent foramen ovale. *Eur Heart J*. 2004;25:1014-20.

46 Brubakk AO, Peterson R, Grip A, Holand B, Onerheim J, Segadal K, et al. Gas bubbles in the circulation of divers after ascending excursions from 300 to 250 msw. *J Appl Physiol*. 1986;60:45-51.

S Lesley Blogg[1], Mikael Gennser[2], Andreas Møllerløkken, Alf O Brubakk[3]

[1] *SLB Consulting, Winton, Cumbria, UK*
[2] *Department of Environmental Physiology, School of Technology and Health, Royal Institute of Technology, Stockholm, Sweden*
[3] *Barophysiology Group, Department of Circulation and Medical Imaging, Norwegian University of Science and Technology, Trondheim, Norway*

Address for correspondence:
SL Blogg, PhD
SLB Consulting
C/O The Barn
Manor House Wynd, Winton
Cumbria, CA17 4HL, UK
Phone: *+44-(0)771-442-2042*
E-mail: *<lesley@chapelclose20.fsnet.co.uk>*

The database of randomised controlled trials in hyperbaric medicine maintained by Michael Bennett and his colleagues at the Prince of Wales Hospital Diving and Hyperbaric Medicine Unit, Sydney is at:
<http://hboevidence.unsw.wikispaces.net/>

Assistance from interested physicians in preparing critical appraisals is welcomed, indeed needed, as there is a considerable backlog. Guidance on completing a CAT is provided. Contact Associate Professor Michael Bennett: <M.Bennett@unsw.edu.au>

Case report

Cutaneous decompression sickness

Konstantinos Tasios, Georgios G Sidiras, Vasileios Kalentzos and Athina Pyrpasopoulou

Abstract

(Tasios K, Sidiras GG, Kalentzos V, Pyrpasopoulou A. Cutaneous decompression sickness. *Diving and Hyperbaric Medicine*. 2014 March;44(1):45-47.)
A probable case of decompression illness with associated *cutis marmorata* is presented, which regressed over a few hours with oxygen breathing and after intravenous methylprednisolone and fluid resuscitation without recompression. He was eventually transferred for hyperbaric treatment some 10 hours post dive. Cutaneous decompression illness is not associated with high mortality per se, but prompt and accurate recognition is warranted, as it may represent a prodromal feature of potentially life-threatening complications. However, in this case, as differential diagnosis, an allergic reaction remains possible.

Key words

Introduction

Decompression illness (DCI) is a major complication of diving. It is caused by intravascular and/or extravascular bubbles that are formed as a result of a reduction in environmental pressure (decompression). Severity of the syndrome may vary, with manifestations ranging from arthralgias and skin rashes to paralysis and death. Due to its rarity, experience of the emergency room physician in the recognition and treatment of this syndrome is limited. Cutaneous manifestations, when present, are usually a transient feature of the disease and thus rarely captured. We report a diver with prominent skin manifestations typical of decompression sickness (DCS).

Case report

A 52-year-old male patient was brought to the emergency department with circulatory collapse and confusion following the ascent after diving. The patient had remained at 18 metres' sea water (msw) depth for a total of 1.5 hours, with intermittent ascents to the surface about every 20–30 min. His medical history was unremarkable apart from mild chronic obstructive pulmonary disease, which he had developed possibly secondary to diving.

The patient was found by the notified medical team on his sailing boat; he was confused, with severe hypotension (systolic blood pressure 60 mmHg). Inhaled oxygen and intravenous fluid administration were initiated. The patient's state of consciousness improved rapidly, without obvious neurological deficit. On presentation in the emergency department, the patient was fully alert and orientated but remained hypotensive (BP 105/80 mmHg) and was anuric. Other vital signs were normal (temperature 36.0°C, oxygen saturation 97% on 100% inhaled O_2). The patient's skin was

remarkable for *cutis marmorata* ('marble skin') of the torso, as well as the thighs and knees (Figures 1 and 2) and a fine, confluent macular rash of the upper extremities. The patient did not report any fish bite or any perceived sting.

Bolus methylprednisolone (125 mg) and 2 L normal saline (over 4 hours) were administered intravenously. The patient gradually improved haemodynamically, urination was restored, and the skin rash regressed over 3 hours. Mild leukocytosis (total white blood cell count 15,500 μl^{-1}, 86.6% neutrophils) was noted, but haematological and biochemical profiles were otherwise normal. After communication with a specialized hyperbaric unit, the patient was transferred for further evaluation and management.

The patient arrived at the Diving and Hyperbaric Medicine Unit (DHMU) approximately 10 hours post dive. He had a mild recurrence of the rash on his trunk, could not fully control his bladder, and his white blood cells remained elevated (18,280 μl^{-1}, 94.3% neutrophils). He received five hyperbaric oxygen treatments (HBOT) over four days (1st: 200 kPa/60 min – 180 kPa/60 min – 150 kPa/60 min, 2nd: 200 kPa/20 min – 180 kPa/50 min – 150 kPa/10 min, 3rd: 180 kPa/70 min, 4th and 5th: 200 kPa/60 min – 180 kPa/15 min – 150 kPa/10 min) and had complete regression of his symptoms and haematological values.

Discussion

The estimated number of injured divers who need recompression treatment in European hyperbaric facilities varies between 10 and 100 per year per facility depending on the number of divers in the population, number of dives performed annually, and the number of hyperbaric centres in the country.[1] Because of its rarity, the experience of the emergency doctor in the recognition and treatment of this

Figure 1
Cutis marmorata of the right groin and upper thigh

Figure 2
Cutis marmorata of the right thigh

syndrome is often limited. Decompression illness is caused by intravascular and/or extravascular bubbles that are formed as a result of reduction in environmental pressure (decompression) in situ and the introduction of gas bubbles into the arterial system via intra- or extra-cardiac shunts.[2] It usually presents in the context of underwater diving but may be experienced in other depressurisation events, such as in caisson workers, flying in unpressurised aircraft, and extra-vehicular activity from spacecraft.[3] Severity of the syndrome may vary, with manifestations ranging from arthralgias and rashes to paralysis and death, and appears to be inversely related to the time interval to the manifestation of symptoms.[4] Onset of symptomatology in the vast majority of cases occurs within 6 h after surfacing.[5]

First-aid treatment of decompression illness includes breathing 100% O_2, intravenous fluid administration, and transfer for hyperbaric treatment.[6] Adjunctive treatments (non-steroidal anti-inflammatory agents and the use of steroids) have been tested but their benefit remains to be proven.[7] In a recent study to determine the potential risk factors associated with the development of severe diving-related spinal cord decompression illness, the time to recompression and the choice of initial hyperbaric procedure did not appear to significantly influence recovery; however, clinical symptoms of spinal cord decompression syndrome and their initial course before admission to the hyperbaric centre were identified as major prognostic factors in recovery.[8]

Cutaneous manifestations of decompression sickness are usually a transient feature of the disease and thus rarely captured. They do not appear to be directly related to the severity of the syndrome; however, prompt and accurate recognition is important, as they may represent a prodromal

feature of potentially life-threatening complications.[9,10] Most divers who suffer cutaneous decompression illness also have a right-to-left shunt. The shunt is usually across a patent foramen ovale, but some have pulmonary shunts.[11] Skin manifestations typically include erythema accompanied by pruritus; the rash spreads irregularly and deepens in color, developing a mottled appearance, with areas of pallor surrounded by cyanotic patches (*cutis marmorata*).[12] Analogous lesions in pigs revealed abnormalities in 20 of 22 animals, mainly vascular congestion, focal areas of vasculitis, perivascular neutrophil infiltrates, oedema and occasional haemorrhage.[13]

In this case, the possibility of an allergic reaction cannot be excluded. However, the severity of the symptomatology (including neurological signs) compared to the small dose of corticosteroids administered, the gradual restoration of haemodynamic stability and the nature of the rash, which rather resembled areas of impaired perfusion, rendered the initial diagnosis of DCI more likely. The management in our case followed the DHMU guidelines.[14] DHMU is considered the national centre for diving accidents in Greece. Each treatment is individualised, commonly developed on site by the hyperbaric physician on duty. The choice of duration, pressure, breathing mixtures, intravenous medications, and fluid replacement depends on various factors, including the clinical manifestations.[14] HBOT was ceased one day after our patient showed no further improvement.

References

1   Kot J, Sićko Z, Michałkiewicz M, Lizak E, Góralczyk P. Recompression treatment for decompression illness: 5-year report (2003–2007) from National Centre for Hyperbaric Medicine in Poland. *Int Marit Health*. 2008;59:69-80.

2   Vann RD, Butler FK, Mitchell SJ, Moon RE. Decompression illness. *Lancet*. 2011;377:153-64. doi: 10.1016/S0140-6736(10)61085-9.
3   Butler WP. Epidemic decompression sickness: case report, literature review, and clinical commentary. *Aviat Space Environ Med*. 2002;73:798-804.
4   Türkmen N, Akan O, Cetin S, Eren B, Gürses MS, Gündoğmuş UN. Scuba diver deaths due to air embolism: two case reports. *Soud Lek*. 2013;58:26-8.
5   Xu W, Liu W, Huang G, Zou Z, Cai Z, Xu W. Decompression illness: clinical aspects of 5278 consecutive cases treated in a single hyperbaric unit. *PLoS One*. 2012;7:e50079. doi: 10.1371/journal.pone.0050079. Epub 2012 Nov 21.
6   Antonelli C, Franchi F, Della Marta ME, Carinci A, Sbrana G, Tanasi P, et al. Guiding principles in choosing a therapeutic table for DCI hyperbaric therapy. *Minerva Anestesiol*. 2009;75:151-61.
7   Bennett MH, Lehm JP, Mitchell SJ, Wasiak J. Recompression and adjunctive therapy for decompression illness. *Cochrane Database of Systematic Reviews*. 2012. May 16;5:CD005277. doi: 10.1002/14651858.CD005277.pub3.
8   Blatteau JE, Gempp E, Simon O, Coulange M, Delafosse B, Souday V, et al. Clinical symptoms of spinal cord DCS and their initial course before admission to the hyperbaric center should be considered as major prognostic factors in recovery. *Neurocrit Care*. 2011;15:120-7.
9   Ozyigit T, Egi SM, Denoble P, Balestra C, Aydin S, Vann R, et al. Decompression illness medically reported by hyperbaric treatment facilities: cluster analysis of 1929 cases. *Aviat Space Environ Med*. 2010;81:3-7.
10   Bledsoe BE, Loptien M, Berkeley RP. A desert rash. *West J Emerg Med*. 2011;12:563-4. doi: 10.5811/westjem.2010.9.2075
11   Wilmshurst PT, Pearson MJ, Walsh KP, Morrison WL, Bryson P. Relationship between right-to-left shunts and cutaneous decompression illness. *Clin Sci* (Lond). 2001;100:539-42.
12   Kalentzos VN. Images in clinical medicine. *Cutis marmorata* in decompression sickness. *N Engl J Med*. 2010;362:e67.
13   Buttolph TB, Dick EJ Jr, Toner CB, Broome JR, Williams R, Kang YH, Wilt NL Cutaneous lesions in swine after decompression: histopathology and ultrastructure. *Undersea Hyperb Med*. 1998;25:115-21.
14   Sidiras G. Management of diving accidents using hyperbaric oxygenation. *Polish Journal of Emergency Medicine*. 2008;1:19-25.

Acknowledgement

Konstantinos Tasios[1], Georgios Gr Sidiras[2], Vasileios Kalentzos[2], Athina Pyrpasopoulou[1]

[1] *Emergency Department, Hippokration General Hospital, Thessaloniki, Greece*
[2] *Diving & Hyperbaric Medicine Unit, Athens Naval Hospital, Athens, Greece*

Address for correspondence:
Athina Pyrpasopoulou, PhD
Hippokration General Hospital
Konstantinoupoleos 49
54642 Thessaloniki, Greece
Phone: +30-(0)2310892072
Fax: +30-(0)2310892117
E-mail: <a.pyrpasopoulou@doctors.org.uk>

Continuing professional development

Transcutaneous oximetry, problem wounds and hyperbaric oxygen
Öle Hyldegaard

Accreditation statement

INTENDED AUDIENCE

The intended audience consists of all physicians subscribing to *Diving and Hyperbaric Medicine* (DHM), including anaesthetists and other specialists who are members of the Australia and New Zealand College of Anaesthetists (ANZCA) Diving and Hyperbaric Medicine Special Interest Group (DHM SIG). However, all subscribers to DHM may apply to their respective CPD programme coordinator or specialty college for approval of participation.

This activity, published in association with DHM, is accredited by the ANZCA Continuing Professional Development Programme for members of the ANZCA DHM SIG under Learning Projects: Category 2 / Level 2: 2 credits per hour.

OBJECTIVES

The questions are designed to affirm the takers' knowledge of the topics covered, and participants should be able to evaluate the appropriateness of the clinical information as it applies to the provision of patient care.

FACULTY DISCLOSURE

Authors of these activities are required to disclose activities and relationships that, if known to others, might be viewed as a conflict of interest. Any such author disclosures will be published with each relevant CPD activity.

DO I HAVE TO PAY?

All activities are free to subscribers.

Key words
Hypoxia, hyperoxia, inflammation, transcutaneous oximetry, chronic wounds,

Recommended background reading

Practitioners are referred to the following background references and reading.

1 Smart D, Bennett MH, Mitchell SJ. Transcutaneous oximetry, problem wounds and hyperbaric oxygen therapy. *Diving Hyperb Med.* 2006;36:72-86.
2 Fife CE, Smart DR, Sheffield PJ, Hopf HW, Hawkins G, Clarke D. Transcutaneous oximetry in clinical practice: consensus statements from an expert panel based on evidence. *Undersea Hyperb Med.* 2009;36:43-53.
3 Löndahl M, Katzman P, Nilsson A, Hammerlund C. Hyperbaric oxygen therapy facilitates healing of chronic foot ulcers in patients with diabetes. *Diabetes Care.* 2010;33:998-1003.
4 Löndahl M. Hyperbaric oxygen therapy as adjunctive treatment of diabetic foot ulcers. *Med Clin N Am.* 2013;97:957-80.
5 Game FL, Hinchliffe RJ, Apelqvist J, Armstrong DG, Bakker K, Hartemann A, et al. A systematic review of interventions to enhance the healing of chronic ulcers of the foot in Diabetes. *Diabetes Metab Res Rev.* 2012;28(Suppl 1):119-41.
6 Fife CE, Buyukcakir C, Otto GH, Sheffield PJ, Warriner RA, Love TL, Mader J. The predictive value of transcutaneous oxygen tension measurement in diabetic lower extremity ulcers treated with hyperbaric oxygen therapy: a retrospective analysis of 1,144 patients. *Wound Repair Regen.* 2002;10:198-207.

How to answer the questions

Please answer all responses (A to E) as True or False.
Answers should be posted by e-mail to the nominated CPD co-ordinator.
For EUBS members for this CPD issue this will be Lesley Blogg, **E-mail:** <lesley.blogg@eubs.org>
For ANZCA DHM SIG and other SPUMS members, this will be Suzy Szekely, **E-mail:** <Suzy.Szekely@health.sa.gov.au>
If you would like to discuss any aspects with the author, contact him at: <ole.hyldegaard@regionh.dk>

On submission of your answers, you will receive a set of correct answers with a brief explanation of why each response is correct or incorrect. A correct response rate of 80% or more is required to successfully undertake the activity. Each task will expire within 24 months of its publication to ensure that additional, more recent data has not superseded the activity.

Question 1. During measurements of transcutaneous partial

pressure of oxygen (TcO$_2$), it is an established procedure to evaluate the presence of peripheral arterial disease (PAD):

A. In TcO$_2$ measurements, the regional perfusion index (i.e., the ratio of the PtcO$_2$ (of the extremity in question divided by that of the chest reference), is a predictor of PAD.
B. A TcO$_2$ < 40 mmHg during air breathing is a standard predictor of PAD.
C. Leg elevation test to 30 degrees for 5 min with > 10 mmHg decrease in TcO$_2$ with no sign of recovery may be considered significant for the presence of PAD.
D. If a hypoxic wound during an oxygen provocation test does not reach 35-40 mmHg TcO$_2$ the test is indicative of PAD.
E. All of the above.

Question 2. Transcutaneous oximetry (TcO$_2$M) is considered a gold standard in predicting ulcer healing during hyperbaric oxygen treatment (HBOT) because:

A. Of its ability to predict responders to hyperbaric hyperoxia.
B. It measures directly blood flow reduction due to HBO-induced vasoconstrictive effects.
C. It identifies the presence of hypoxia in wounded tissue.
D. It determines when HBO treatment is complete.
E. TcO$_2$ measurements are better to predict failure to heal rather than the likelihood of healing.

Question 3. The ankle-brachial index (ABI) and toe blood pressure (TBP) are used in patient evaluation and planning of treatment schedules. With respect to HBOT in diabetic foot ulcer healing:

A. ABI and TBP measurements have proven to be predictive of wound healing or amputation during HBOT.
B. They exclude the need for vascular surgery before HBOT is initiated.
C. Neither ABI nor TBP is a predictor of wound healing in the assessment of HBOT as adjunctive therapy.
D. ABI and TBP evaluate blood supply to the extremities rather than predicting wound healing during HBOT.
E. They predict the presence of peripheral arterial disease (PAD).

Question 4. The criteria for the use of adjunctive HBOT for

healing of diabetic foot ulcer may be based on the following guidelines:

A. Any diabetic foot ulcer, Wagner grades 1–5 should be treated with HBO.
B. Diabetic full-skin foot ulcers, not healing despite the best available multidisciplinary care in a diabetic foot clinic for at least 6 to 12 weeks, without need for or no ability of vascular surgical intervention, should be considered for HBOT.
C. The inclusion criteria or the clinical selection standard for HBOT may be based on studies with the highest Jadad scores.
D. The criteria are based on selection by means of ABI and TBP.
E. Both B and C may be considered correct.

Question 5. Although TcO$_2$ measurement is considered the best evaluation method for predicting wound healing prior to HBOT, if the oxygen provocation test is inconclusive, in-chamber testing may be recommended based on the following observations:

A. An in-chamber oxygen breathing test may predict a healing potential only if TcO$_2$ rises above > 200 mmHg.
B. As the studies done on TcO$_2$ in-chamber measurements are done using a variety of treatment modalities, a definitive statement regarding healing prediction cannot be made on in-chamber oxygen provocation testing alone. Thus, a trial of HBOT is recommended on a case-by-case basis.
C. If during normobaric air breathing, a wound is hypoxic, but reaches 200 mmHg during in-chamber oxygen breathing at 203–243 kPa, it has a 75% likelihood of healing; if the in-chamber test is below 100 mmHg the wound is likely to fail (predictive accuracy 89%).
D. In-chamber oxygen testing may only need to be performed if the normobaric testing including oxygen provocation test and leg elevation are inconclusive.
E. All of the above.

Critical appraisal

Pre-conditioning with hyperbaric oxygen treatment (HBOT) may induce cerebral and cardiac protection in patients undergoing on-pump coronary artery bypass graft (CABG) surgery

Bottom line:
1. Pre-conditioning with HBOT resulted in improved clinical outcomes for patients undergoing on-pump CABG surgery.
2. There were also potential cerebral and cardiac protective effects as determined by proxy outcomes.
3. No protective effects were noted in off-pump CABG.

Citations:
1. Yang L, Dong H, Chen M, Liu J, Yang L, Chen S, Xiong L. Preconditioning with repeated hyperbaric oxygen induces myocardial and cerebral protection in patients undergoing coronary artery bypass graft surgery: a prospective, randomized, controlled clinical trial. *Journal of Cardiothoracic and Vascular Anesthesia*. 2011;25:908-16.

Lead author's name and e-mail:
Yang Li: <lxiong@fmmu.edu.cn>

Three-part clinical question:
For patients undergoing CABG, does the addition of hyperbaric oxygen to standard care confer any myocardial and/or cerebral protection?

Search terms:
CABG, preconditioning, cardiopulmonary bypass

The study:
Single-blinded randomized controlled trial without intention-to-treat.

The study patients:
Male patients under 80 years having CABG either on cardiopulmonary bypass or off-pump.

Control group:
(*n* = 16; 15 analysed)
Full orthodox pre-operative care for CABG; no sham treatment.

HBOT group:
(*n* = 15; 14 analysed)
As above, but patients were given five daily HBOT at 203 kPa, breathing 100% oxygen for 70 minutes over five days before surgery.

The evidence:
See Table 1.

Comments:
1. Exclusion of female subjects and small numbers may limit external validity of the study.
2. Only two biochemical markers were analyzed to measure cerebral injury; they may not be fully representative of actual cerebral damage.
3. Early post-operative outcomes may not be representative of long-term morbidity and mortality.
4. Values of mean and standard deviation were not provided for non-significant results in off-pump patients.

Appraised by: Bryan Hui and Michael Bennett
Prince of Wales Hospital, Sydney, Friday, 05 July 2013
E-mail: <m.bennett@unsw.edu.au>

Key words
Surgery, cardiovascular, injuries, brain injury, hyperbaric oxygen therapy, outcome, hyperbaric research, critical appraisal

Table 1
Outcomes for patients where coronary artery grafting was performed using formal cardiopulmonary bypass; S100B – S100 calcium binding protein B (μg L^{-1}); NSE – neuron-specific enolase (ng ml^{-1}); HBOT – hyperbaric oxygen treatment

Outcome measured	Control		HBOT		Difference	95% confidence interval
	Mean	(SD)	Mean	(SD)		
Length of ICU stay (h)	85.9	(40.3)	59.4	(20.9)	26.5	1.8 to 51.2
Serum S100B on ICU admission	89.7	(14.6)	60.4	(26.4)	29.3	13.2 to 45.4
Serum NSE at 24 h post-op	38.0	(8.4)	30.3	(6.1)	7.7	2.1 to 13.3

Notices and news

EUBS news now on the website

EUBS notices and news, such as the minutes of the most recent meeting of the Executive Committee and the 2013 General Assembly held during the Tricontinental meeting on Réunion Island, can now be found, along with all other EUBS information, on the society website:

<www.eubs.org>. In order to increase space for original research and educational articles and to minimise the rising costs of publishing *Diving and Hyperbaric Medicine*, the decision has been made to reduce the amount of society business appearing within these pages in future.

European Editor retiring: a successor is needed

EUBS announces with sadness that the European Editor, Peter Müller, will resign at the EUBS 2014 ASM next September. A search has been established to appoint a new European Editor by May 2014, so that (s)he has time to overlap with Peter.

Candidates for the position should send a brief application and their full curriculum vitae, in electronic format, to the Honorary Secretary, Joerg Schmutz at:
E-mail: <joerg.schmutz@eubs.org>.

The 5th Arthur Bornstein Workshop
Diving in Offshore Wind Farms

Date: 23rd September 2014
Venue: Wiesbaden
Chairmen: W Sterk and W Welslau
A satellite of the 40th EUBS ASM 2014

Invited speakers will talk on the current situation in offshore wind energy in Northern Europe and the outlook for tomorrow: a view from the bottom; a view from topside; presentation of current regulations in Northern Europe; myths and facts about surface decompression; mix-gas options; saturation options; system solutions; the need for a joint action to improve offshore shallow divers' safety.

For information please contact: <drfaescke@aol.com>

The

website is at
<www.eubs.org>

Members are encouraged to log in and to keep their personal details up to date

Advertising in *Diving and Hyperbaric Medicine*

Commercial advertising is welcomed within the pages of *Diving and Hyperbaric Medicine*. Companies and organisations within the diving, hyperbaric medicine and wound-care communities who might wish to advertise their equipment and services are welcome.

The advertising policy of the parent societies – EUBS and SPUMS – appears on the journal website: <www.dhmjournal.com>

Details of advertising rates and formatting requirements are available on request from:
E-mail: *<editorialassist@dhmjournal.com>*
Fax: *+64-(0)3-329-6810*

Second Announcement and Call for Abstracts
Dates: 24–27 September 2014
Venue: Wiesbaden, Germany

The 40th EUBS Annual Scientific Meeting will be held in conjunction with the 2014 congress of the German Society for Diving and Hyperbaric Medicine (GTÜeM). The patrons of this event are GTÜeM and the Compression Chamber Centre Rhein-Main-Taunus (HBO-RMT) in Wiesbaden/Germany.

Organising Committee
Peter Müller (Secretary General), Peter Germonpré (EUBS), Karin Hasmiller (EUBS/GTÜeM),
Michael Kemmerer (EUBS/VDD/Wiesbaden), Dirk Michaelis (EUBS/GTÜeM/Wiesbaden), Peter Freitag (HBO-RMT)
Scientific Committee
Costantino Balestra (EUBS), Lesley Blogg (EUBS), Bjorn Jüttner (EUBS/GTÜeM), Claus-Martin Muth (EUBS/
GTÜeM), Lars Perlik (Wiesbaden), Tim Piepho (GTÜeM), Christian Weber (Frankfurt), Christian Werner (Mainz)

Main topics
- Invited lectures: marine biology; carbon monoxide toxicity; stem cells and HBOT
- Diving medicine: physiology; decompression theory; treatment
- HBO medicine: physiology; treatment; technical and safety aspects
- Pro/Con debate
- GTÜeM session: guideline – treatment of diving accidents; checklist – fitness to dive

The meeting format will be the usual EUBS style, with invited keynote lectures,
presentations of free papers (oral and posters) and an industry exhibition.

Call for abstracts
Abstracts for oral and poster presentations should be submitted electronically via <www.eubs2014.org>.
The Organising Committee intends to publish all accepted abstracts in a conference book and encourages all authors
to submit full papers for consideration in *Diving and Hyperbaric Medicine*.

Preliminary timetable
Registration is open via the website: <www.eubs2014.org>
30 April: Deadline for submission of abstracts
01 May: End of early-bird registration period
15 July: Notification of accepted abstracts

A detailed programme will become available on the website <**www.eubs2014.org**> after 01 July 2014.

Language: The official language for all scientific sessions and the International DAN Diver's Day will be English.
The language for the GTÜeM session will be German.

Satellite meetings
23 September	European Code of Practice for Hyperbaric Medicine; authors' meeting
23 September	5th Arthur Bornstein Workshop on Diving in Offshore Wind Farms
27 September	Research meeting on hyperbaric medicine (afternoon)
27–28 September	Rescue Day and International DAN Diver's Day

For further information and hotel bookings see: <www.eubs2014.org>
Conference Secretariat (Peter Freitag) Phone: +49-(0)611-847-27-170
Fax: +49-(0)611-847-27-179
E-mail: <info@eubs2014.org>

SPUMS Notices and news

SPUMS Annual Scientific Meeting 2014

Venue: Alila Manggis Resort, Bali
Dates: 18–25 May 2014

Themes:
Patent foramen ovale (PFO); immersion pulmonary oedema; the older diver

Keynote speaker:
Peter Wilmshurst, Cardiologist, UK

Submission of abstracts:
There are only limited time slots remaining for presenting a paper so please submit abstracts ASAP!

Registration:
The conference is now full and registrations have closed.
Existing registrants may still add an accompanying guest.
A wait list for conference registration and Alila accommodation has been started in case of existing bookings being cancelled. Please contact the Convenor if you wish to be wait-listed.

Registrants and guests are reminded to book their diving via the SPUMS website.

Resort facilities can be viewed at: <http://www.alilahotels.com/manggis>

SPUMS ASM 2014 Convenor: Neil Banham
E-mail: <N.Banham@health.wa.gov.au>

SPUMS news now on the website

SPUMS notices and news, such as the minutes of the November 2012 meeting of the Executive Committee and the 2013 Annual General Meeting held during the tricontinental meeting on Réunion Island, including the officers' and financial reports, can now be found, along with all other information about the Society, on the website <www.spums.org.au>. In order to increase space for original research and educational articles and to minimise the rising costs of publishing *Diving and Hyperbaric Medicine*, the decision has been made by the Executive to reduce the amount of society business appearing within these pages in future and for this to be accessible to members on the website.

The

SPUMS

website is at
<www.spums.org.au>

Members are encouraged to log in and to
keep their personal details up to date

The SPUMS Annual General Meeting 2014, Notice of meeting

The AGM for SPUMS 2014 will be held at Alila Manggis Resort and Spa, Bali at 1800 h on Wednesday 27 May 2014.

Agenda

1. Apologies:
2. Minutes of the previous meeting:
    Minutes of the previous meeting will be posted on the notice board at Alila Resort and have been published on the SPUMS website: <www.spums.org.au>; Minutes of the Annual General Meeting of SPUMS held at La Tamarun Convention Centre, La Saline Les Bains, Réunion Island at 0900 h on Saturday 28th September 2013, along with the officers' and auditor's reports and the financial statements for 2012.
3. Matters arising from the minutes
4. Annual reports:
    President's report
    Secretary's report
    Education Officer's report
    Annual financial statement and Treasurer's report
    Journal Editor's report
5. Subscription fees for 2015:
6. Election of office bearers:
    President
    Secretary
    Treasurer
    Education Officer
    Committee Member
7. Appointment of the Auditor 2015:
8. Business of which notice has been given:
    1. Nominations for office bearers and expressions of interest for the committee member position are to be forwarded to the Secretary by 01 May 2014 (nomination forms may be found on the SPUMS website).
    2. Notices of motions for changes to the Purposes and Rules of the Society are under consideration by the Committee. Once finalized, these will be posted on the SPUMS website and notified to members by e-mail.

New SPUMS Education Officer needed

I will be resigning as the SPUMS Education Officer as of the May 2014 SPUMS AGM. This letter is to broadcast this opening to as wide an audience as possible. I realise we are a small group and everyone is busy with their varied professional and personal commitments. However, we need to pass the baton around and share the load, so we can advance the speciality!

Please contact the Secretary: <secretary@spums.org.au> or myself for further details if you are interested in assuming this very important role within the Society. Candidates must be full members of SPUMS and hold the SPUMS Diploma.

Thank you for your enthusiasm and support.

Associate Professor David Smart, Medical Director, Department of Diving and Hyperbaric Medicine, Hobart Hospital, Hobart, Tasmania.
E-mail: <david.smart@dhhs.tas.gov.au>

Key words
Medical society, research, letters (to the Editor)

SPUMS Diploma in Diving and Hyperbaric Medicine

The full requirements for the SPUMS Diploma and all additional information can be found on the society website: <www.spums.org.au>.

The Education Officer's report of July 2013 may also be found on the website. This contains details of candidates who have registered projects for the Diploma in the past three years and the stage at which each of these has reached.

All enquiries and applications should be sent to:
Associate Professor David Smart
GPO Box 463, Hobart, Tasmania 7001
E-mail: <david.smart@dhhs.tas.gov.au>

SPUMS and Facebook

Remember to 'like' SPUMS at:
<http://www.facebook.com/pages/SPUMS-South-Pacific-Underwater-Medicine-Society/221855494509119>

The
Diving and Hyperbaric Medicine
journal website is at

<www.dhmjournal.com>

Certificate in Diving and Hyperbaric Medicine of the Australian and New Zealand College of Anaesthetists

Eligible candidates are invited to present for the examination for the Certificate in Diving and Hyperbaric Medicine of the Australian and New Zealand College of Anaesthetists.

All details are available on the ANZCA website at:
<http://anzca.edu.au/edutraining/DHM/index.htm>

Suzy Szekely, FANZCA, Chair, ANZCA/ASA Special Interest Group in Diving and Hyperbaric Medicine.
E-mail: *<Suzy.Szekely@health.sa.gov.au>*

Diving and Hyperbaric Medicine Index of contents, Vol 43, 2013

The Index of contents, volume 43, 2013, is now on the journal website <www.dhmjournal.com> and on the SPUMS and EUBS websites.

Capita Selecta Dive Research Seminars 2014 University of Amsterdam, The Netherlands

06 September 2014: *Pulmonology and Diving*
Speakers: Pascal Constantin, diving and hyperbaric physician; Jacques Regnard, sport-diving and hyperbaric physician; Nico Schellart, diving physiologist and medical physicist

29 November 2014: *Breath-hold diving*
Speakers: Rik Roskens; Erika Schagatay, environmental physiologist; Jochen Schipke, medical physiologist and diving physician

For full information contact: <www.duikresearch.org>

DIVING HISTORICAL SOCIETY AUSTRALIA, SE ASIA

P O Box 347, Dingley Village Victoria, 3172, Australia
E-mail:
<deswill@dingley.net>
Website:
<www.classicdiver.org>

Royal Adelaide Hospital Hyperbaric Medicine Unit Courses 2014

Medical Officers' Course
Part 1: 01– 05 December (Lectures)
Part 2: 08–12 December

DMT Full Courses
06–24 October

DMT Refresher Courses
28 April–09 May
22 Sept–03 Oct

All enquiries to:
Lorna Mirabelli, Course Administrator
Phone: +61-(0)8-8222-5116
Fax: +61-(0)8-8232-4207
E-mail: <Lorna.Mirabelli@health.sa.gov.au>

Royal Australian Navy Medical Officers' Underwater Medicine Course 2014

Dates: November 2014, exact date tba
Venue: HMAS PENGUIN, Sydney

The MOUM course seeks to provide the medical practitioner with an understanding of the range of potential medical problems faced by divers. Considerable emphasis is placed on the contra-indications to diving and the diving medical, together with the pathophysiology, diagnosis and management of the more common diving-related illnesses. The course includes scenario-based simulation focusing on management of diving emergencies and workshops covering the key components of the diving medical.

Costs: tba (with or without accommodation at HMAS Penguin)

For information and application forms contact:
Rajeev Karekar, for Officer in Charge,
Submarine and Underwater Medicine Unit
HMAS PENGUIN
Middle Head Rd, Mosman
NSW 2088, Australia
Phone: +61-(0)2-9647 5572
Fax: +61-(0)2-9960 4435
E-mail: <Rajeev.Karekar@defence.gov.au>

Scott Haldane Foundation

The Scott Haldane Foundation is dedicated to education in diving medicine, and has organized more than 150 courses over the past 19 years, both in the Netherlands and abroad. Below is a list of remaining courses for 2014.

The courses Medical Examiner of Diver (part I and II) and the modules of the Diving Medicine Physician course fully comply with the ECHM/EDTC curriculum for Level 1 and 2d respectively and are accredited by the European College of Baromedicine.

Remaining courses for 2014
17–24 May: Basic course (medical examination of divers) Part 2. Al Sawadi, Oman
October: (dates tba): Refresher course. AMC, Amsterdam
08–15 November: Basic course (medical examination of divers) Part 1. Costa Rica
15–22 November: 22nd In-depth course Diving Medicine: case-based diving medicine. Costa Rica
22–29 November: 22nd In-depth course Diving Medicine: case-based diving medicine. Costa Rica

For further information: <www.scotthaldane.org>

German Society for Diving and Hyperbaric Medicine

An overview of basic and refresher courses in diving and hyperbaric medicine, accredited by the German Society for Diving and Hyperbaric Medicine (GTÜeM) according to EDTC/ECHM curricula, can be found on the website: <http://www.gtuem.org/212/Kurse_/_Termine/Kurse.html>

Undersea and Hyperbaric Medicine Society Annual Scientific Meeting 2014

Dates: 19–21 June
Venue: Hyatt Regency St Louis at the Arch
For full information go to: <www.uhms.org>

DAN Europe

DAN Europe has a fresh, multilingual selection of recent news, articles and events featuring DAN and its staff.

Go to the website: <http://www.daneurope.org/web/guest/>

18th International Congress on Hyperbaric Medicine

03–06 December 2014
Buenos Aires, Argentina

The ICHM is a worldwide organization for physicians and scientists interested in diving and hyperbaric medicine. The organization has minimal formal structure and is entirely dedicated to hosting an international scientific congress every three years.

ICHM Committee (2011–2014):
President: Prof Dr Jorge B Pisarello (Argentina)
Executive Director: Dr Alessandro Marroni (Italy)
Secretary: Assoc Prof Michael Bennett (Australia)

Registration: Online registration is now open
Website:
<http://ichm.drupalgardens.com/content/what-ichm-0>

Hyperbaric Oxygen, Karolinska

Welcome to: <http://www.hyperbaricoxygen.se/>.
This site, supported by the Karolinska University Hospital, Stockholm, Sweden, offers publications and free, high-quality video lectures from leading authorities and principal investigators in the field of hyperbaric medicine.

You need to register to obtain a password via e-mail. Once registered, watch the lectures online, or download them to your iPhone or computer for later viewing.

We offer video lectures from:
- The 5th Karolinska PG course in clinical hyperbaric oxygen therapy, 07 May 2009.
- The European Committee for Hyperbaric Medicine *"Oxygen and infection"* Conference, 08–09 May 2009.
- The 17th International Congress on Hyperbaric Medicine, Cape Town, 17–18 March 2011.
-

Also available is the 2011 Stockholm County Council report: *Treatment with hyperbaric oxygen (HBO) at the Karolinska University Hospital*.

For further information contact:
Folke Lind, MD PhD
E-mail: <folke.lind@karolinska.se>
Website: <www.hyperbaricoxygen.se>

Instructions to authors
(updated March 2014)

Diving and Hyperbaric Medicine (DHM) is the combined journal of the South Pacific Underwater Medicine Society (SPUMS) and the European Underwater and Baromedical Society (EUBS) and seeks to publish papers of high quality on all aspects of diving and hyperbaric medicine of interest to diving medical professionals, physicians of all specialties, and members of the diving and hyperbaric industries.

Manuscripts must be offered exclusively to *Diving and Hyperbaric Medicine*, unless clearly authenticated copyright exemption accompanies the manuscript. All manuscripts will be subject to open peer review. Accepted contributions will also be subject to editing.

Address: The Editor, Diving and Hyperbaric Medicine
c/o Hyperbaric Medicine Unit, Christchurch Hospital
Private Bag 4710, Christchurch, New Zealand
E-mail: <editor@dhmjournal.com>
Phone: +64-(0)3-329-6857
Fax: +64-(0)3-329-6810
Website: <www.dhmjournal.com>
European Editor: <euroeditor@dhmournal.com>
Editorial Assistant: <editorialassist@dhmjournal.com>

Contributions should be submitted electronically to:
E-mail: <submissions@dhmjournal.com>

Requirements for manuscripts

Diving and Hyperbaric Medicine welcomes contributions that meet the following requirements:

Original Articles, **Technical Reports and Case Series:** up to 3,000 words is preferred, and 30 references (excluded from word count). These articles should be subdivided into the following sections: a structured **Abstract** of no more than 250 words, **Introduction**, **Methods**, **Results**, **Discussion**, **Conclusions**, **References** (excluded from word count)**. Acknowledgements,** which should be brief, **Funding sources** and any **Conflicts of interest** should be listed after the references.

Review articles: up to 5,000 words is preferred and 60 references (excluded from word count); include an **Abstract** of no more than 300 words (excluded from word count); structure of the article is at the discretion of the author(s).

Case Reports, **Short Communications** and **Work in Progress Reports:** maximum 2,000 words, and 20 references (excluded from word count); include an **Abstract** of no more than 200 words (excluded from word count).

Educational articles, **commentaries** and **case reports** for 'The Diving Doctor's Diary', 'World as it is', 'Opinion'

or '**Historical**' occasional sections may vary in format and length, but should generally be a maximum of 3,000 words and 15 references (excluded from word count).

Letters to the Editor: (maximum 600 words, plus one figure or table and 5 references)

All submissions must comply with the requirements below. Manuscripts not complying with these instructions will be returned to the author for correction before consideration.

Inclusion of more than six authors in any one manuscript requires justification. Authors must have contributed to at least three of the four major components of a study: hypothesis and design; conduct of the study; analysis of data; writing the report. (See DHM website for more information on **Authorship Policy**.)

Documents must be submitted electronically. Multiple or large files may be bundled as a Zip file and sent as an e-mail attachment or using internet services such as <https://www.wetransfer.com> or <www.yousendit.com>, or mailed on a disk.

All articles should include a **Title Page**, giving the title of the paper and the full names of all authors (given names first, followed by the family/surname), their principal qualifications and institutional affiliations at the time of doing the work being reported. One author must be identified as correspondent, with their full postal address, phone number and e-mail address supplied. If this is a different author to the principal (first) author, then full contact details for the first author are also required.

A **Covering Letter** signed by the principal (first) author must accompany all submissions. Authors should complete the proforma cover letter to be found on the DHM website: <http://www.dhmjournal.com/index.php/instructions-to-authors>.

A maximum of seven **Key words** best describing the paper should be chosen from the list on the journal website: <http://www.dhmjournal.com/index.php/instructions-to-authors>. New key words, complimentary with the NLM MeSH (http://www.nlm.nih.gov/mesh/) will be used at the discretion of the Editor. Key words should be placed at the bottom of the title page.

Text: The preferred format is Microsoft Office Word or rich text format (RTF), with 1.5 line spacing, using both upper and lower case throughout. The preferred font is Times New Roman, font size 11 or 12. Headings should conform to the current format in DHM:
Section heading
SUBSECTION HEADING 1
Subsection heading 2

All pages should be numbered, but no other text should appear in the header and footer space of the document. Do not use underlining. No running title is required.

English spelling will be in accordance with the *Concise Oxford Dictionary*, 11th edition revised (or later). Oxford: Oxford University Press; 2006.

Measurements are to be in SI units (mmHg are acceptable for blood pressure measurements) and normal ranges should be included where appropriate. Authors are referred to the online BIPM brochure, International Bureau of Weights and Measures (2006), *The International System of Units* (SI), 8th ed, available at ISBN 9282222136 : <http://www. bipm.org/utils/common/pdf/si_brochure_8_en.pdf>, or Baron DN, McKenzie Clarke H, editors. *Units, symbols and abbreviations. A guide for biological and medical editors and authors*, 6th edition. London: Royal Society of Medicine; 2008. Atmospheric and gas partial pressures and blood gas values should be presented in kPa (ATA/bar/ mmHg may be provided in parenthesis on the first occasion). The ambient pressure should be clearly identified whether it is given in absolute (a) or gauge (g) values. Water depths should be presented in metres' sea (or fresh) water (msw or mfw). Cylinder pressures and inspired gas pressures in a rebreather apparatus may be presented as 'bar'.

Abbreviations may be used once they have been shown in parenthesis after the complete expression. For example, decompression illness (DCI) can thereafter be referred to as DCI. This applies separately to the abstract and main text. Use generally accepted abbreviations rather than neologisms of your own invention.

References

The Journal reference style is based exactly on that of the *International Committee of Medical Journal Editors (ICMJE) Uniform requirements for manuscripts submitted to biomedical journals*. Examples of the formats for different types of references (journal articles, books, monographs, electronic material, etc) are given in detail on the website: <http://www.nlm.nih.gov/bsd/uniform_requirements.html> (last updated 20 August 2013).

Correct formatting and the accuracy of references (verified against the full paper, not simply from the MedLine or PubMed abstract) in a submission are the responsibility of the author(s).

Additional requirements for DHM are:
- References should be numbered consecutively in the order in which they are first mentioned in the text, tables or figures as superscript numbers, preferably at the end of the sentence **after** the full stop.[1,2]
- References appearing in table or figure legends should continue the numbering sequence of references in the

main text of the article in accordance with the position of citing the table/figure in the text.

Use MEDLINE abbreviations for journal names. The *List of Journals Indexed for MEDLINE* publication ceased with the 2008 edition. The Journals database: <http://www.ncbi.nlm.nih.gov/sites/entrez?Db=journals&Cmd=DetailsSearch&Term=currentlyindexed[All]> can be used to obtain a list of currently indexed MEDLINE journal titles.

Abstracts from meeting proceedings may not be used as references unless absolutely essential as they are generally not peer-reviewed.

If EndNote has been used to prepare the references in the document, EndNote field codes should be removed from the text before submission (see EndNote website for advice on how to do this).

Personal communications should appear as such in the text and not be included in the reference list (e.g., Other AN, personal communication, year).

'Long' and 'short' examples of a journal reference in the full ICMJE format are shown below:
   Wilson CM, Sayer MDJ. Transportation of divers with decompression illness on the west coast of Scotland. Diving and Hyperbaric Medicine. 2011 June;41(2):64-69.

If a journal carries continuous pagination throughout a volume (as many medical journals do) then the month and issue number should be omitted and the pagination reduced. Therefore, the **shortened ICMJE version used in DHM** is:
   Wilson CM, Sayer MDJ. Transportation of divers with decompression illness on the west coast of Scotland. Diving Hyperb Med. 2011;41:64-9.

An example book reference is:
Kindwall EP, Whelan HT, editors. Hyperbaric medicine practice, 3rd ed. Flagstaff AZ: Best Publishing Company; 2008.

Examples of all other types of references are to be found on the uniform requirements website.

Illustrations, figures and tables

These must **NOT** be embedded in the word processor document, but submitted as individual, separate electronic files. Each figure and table must be mentioned within the text of the article, e.g., "Rates of decompression illness by demographic are presented in Table 1…", "Differences in rates of decompression illness were not significant (Table 1)", etc. The approximate positions of tables and figures should be identified in the text.

No captions should appear within the body of the table or image, but should be placed in the legend. **Legends** should generally contain fewer than 40 words and must be listed on a separate page at the end of the main text file. Any definition of symbols used in the figures should appear within the white space of the figure to allow the figure to attain maximum size, or be submitted separately or be included in the legend rather than in the figure. Figures should be readable in black and white, with no unnecessary shading, gridlines or box lines. Both markers and lines should be unique to facilitate easy discrimination of the data being presented.

If any figures, images or tables are to be reproduced from previous publications, it is the responsibility of the author to obtain the necessary permissions from the publishers.

Table data should be presented either as tab-spaced normal text or using table format, with tab-separated columns auto-formatted to fit content. No gridlines, borders or shading should be used.

Illustrations, figures and X-rays should be submitted as separate electronic files in TIFF, high resolution JPEG or BMP format. Colour is available only at the author's request and will be at the author's expense (currently approximately AUD600 for a single A4 page). Therefore, authors need to convert figures and images to grayscale to ensure that contrast within the image is sufficient for clarity when printed. Any graphs or histograms created in Excel should be sent within their original Excel file, including the data table(s) from which they were produced. This allows the journal office to edit figures for maximum legibility when printed.

Special attention should be given to ensuring that font sizes within a diagram are sufficiently large to be legible should the diagram be resized for single-column representation. The preferred font is Times New Roman.

Scanned photographs should be submitted as TIFF, JPG or BMP files at a minimum resolution of 300 dpi. Magnification should be indicated for photomicrographs, and consideration given to the positioning of labels on diagnostic material as this can greatly influence the size of reproduction that can be achieved in the published article.

Consent and ethical approval

Studies on human subjects must comply with the Helsinki Declaration of 1975, as revised in 2013, and those using animals must comply with National Health and Medical Research Council Guidelines or their equivalent in the country in which the work was conducted. A statement affirming Ethics Committee (Institutional Review Board) approval should be included in the text. A copy of that approval should be provided with the submission. Patient details must be removed and photographs made unrecognizable unless written consent for their publication has been obtained from the patient(s). When informed consent has been obtained, this should be indicated in the article. Clinical trials commenced after 2011 must have been registered at a recognised trial registry site such as the Australia and New Zealand Clinical Trials Registry <http://www.anzctr.org.au/> or EudraCT in Europe <https://eudract.ema.europa.eu/> and details of the registration provided in the accompanying letter.

For individual case reports, patient consent to publish anonymously images or their clinical details must have been obtained. Case series in which only limited, anonymous summary data are reported, do not require patient consent, but do require ethical approval.

English as a second language

Adequate English usage and grammar are prerequisites for acceptance of a paper. However, some editorial assistance may be provided to authors for whom English is not their native language. English language services can be accessed through the European Association of Science Editors (EASE) website <http://www.ease.org.uk/>. Alternatively, the journal office may be able to put you in touch with a commercial scientific ghost writer.

Copyright

Manuscripts must be offered exclusively to *Diving and Hyperbaric Medicine*, unless clearly authenticated copyright exemption accompanies the manuscript. Authors must agree to accept the standard conditions of publication. These grant DHM a non-exclusive licence to publish the article in printed form in *Diving and Hyperbaric Medicine* and/or in other media, including electronic form; also granting the right to sublicense third parties to exercise all or any of these rights. *Diving and Hyperbaric Medicine* agrees that in publishing the article(s) and exercising this non-exclusive publishing sub-licence, the author(s) will always be acknowledged as the copyright owner(s) of the article.

Articles are embargoed for one year from the date of publication, after which they will be free to access. If authors wish their article to be free to access immediately upon publication, then a fee (determined by the publishers) will be charged for its release.

SPUMS and EUBS Annual Scientific Meetings

DHM has published articles based on many of the presentations from SPUMS annual scientific meetings (ASM). Presenters, including the Guest Speaker(s), are reminded that this is an explicit condition of their participation in the SPUMS meetings, but it is recognized that not all presentations are suitable for publication in DHM. Speakers at EUBS meetings, both those giving keynote

addresses and those presenting previously unpublished research are strongly encouraged to submit manuscripts to DHM. All such articles are subject to the above requirements of standards, presentation and peer review.

Zetterström Award

The author(s) of the scientific poster winning the Zetterström Award at each EUBS ASM explicitly agree(s) to submit an article based on their poster to DHM. This paper is subject to the above requirements of standards and presentation and will be subject to peer review.

Musimu Award

Recipients of the Musimu Award of the EUBS are strongly encouraged to publish their research in DHM.

SPUMS Diploma dissertations

It is the policy of SPUMS that diploma candidates are strongly encouraged to publish their dissertation in DHM. Most dissertations require editing for submission, and these *Instructions to Authors* should be used to guide this process.

Synopses or summaries of master's or doctoral theses will also be considered in order to draw the diving and hyperbaric medical and scientific community's attention to the work of young researchers. Permission to reprint such material may be required from the host institution, and obtaining this is the author's responsibility.

Publication schedule

All submitted manuscripts will be subject to open peer review by a member of the Editorial Board and at least one other reviewer. Reviewer comments will be provided to authors with any recommendations for improvement before acceptance for publication, or if the article is rejected. DHM believes that a transparent review process is indicated in such a small specialty; reviewers are often able to identify the origin of manuscripts and, in the interests of fairness, the authors are therefore provided the names of reviewers of their articles.

The review process typically takes about eight weeks to complete, but can be longer. If additional reviews are needed, this will prolong the process. Papers are generally scheduled for publication in order of final acceptance. The Editor retains the right to delay publication in the interests of the Journal.

Proofs of articles to be published will be sent to authors in PDF format by e-mail close to the time of publication. Authors are expected to check the proofs very carefully and inform the editorial office **within five days** of any minor corrections they require. Corrections should be listed in an e-mail sent to the journal address <editor@dhmjournal.com>, or annotated electronically within the pdf file.

Reprints

Following publication, one complimentary copy of DHM will be sent to the corresponding author, if they are not a current member of SPUMS/EUBS. A PDF copy of articles will also be forwarded to the corresponding author. A limited number of additional print copies of the journal issue containing the article are available for purchase from the SPUMS Administrator: <admin@spums.org.au>.

Editor's note:

The *Instructions to Authors* as printed in this issue are available as a pdf file on the DHM website at: <http://www.dhmjournal.com/index.php/instructions-to-authors>

They are also available on the EUBS and SPUMS websites. A shortened, single-page version, as published in the past, will no longer appear in the printed issue.

DIVER EMERGENCY SERVICES PHONE NUMBERS

AUSTRALIA
1800-088200 (in Australia, toll-free)
+61-8-8212-9242 (International)

NEW ZEALAND
0800-4DES-111 (in New Zealand, toll-free)
+64-9-445-8454 (International)

ASIA
+10-4500-9113 (Korea)
+81-3-3812-4999 (Japan)

SOUTHERN AFRICA
0800-020111 (in South Africa, toll-free)
+27-10-209-8112 (International, call collect)

EUROPE
+39-6-4211-8685 (24-hour hotline)

UNITED KINGDOM
+44-7740-251-635

USA
+1-919-684-9111

The DES numbers (except UK) are generously supported by DAN

DAN ASIA-PACIFIC DIVE ACCIDENT REPORTING PROJECT
This project is an ongoing investigation seeking to document all types and severities of diving-related accidents. All information is treated confidentially with regard to identifying details when utilised in reports on fatal and non-fatal cases. Such reports may be used by interested parties to increase diving safety through better awareness of critical factors. Information may be sent (in confidence unless otherwise agreed) to:

DAN Research
Divers Alert Network Asia Pacific
PO Box 384, Ashburton VIC 3147, Australia
Enquiries to: <research@danasiapacific.org>

DAN Asia-Pacific NON-FATAL DIVING INCIDENTS REPORTING (NFDIR)
NFDIR is an ongoing study of diving incidents, formerly known as the Diving Incident Monitoring Study (DIMS). An incident is any error or occurrence which could, or did, reduce the safety margin for a diver on a particular dive. Please report anonymously any incident occurring in your dive party. Most incidents cause no harm but reporting them will give valuable information about which incidents are common and which tend to lead to diver injury. Using this information to alter diver behaviour will make diving safer.

The NFDIR reporting form can be accessed on line at the DAN AP website:
<www.danasiapacific.org/main/accident/nfdir.php>

DISCLAIMER
All opinions expressed in this publication are given in good faith and in all cases represent the views of the writer and are not necessarily representative of the policies or views of SPUMS or EUBS or the Editor.

CONTENTS

Diving and Hyperbaric Medicine Volume 44 No. 1 March 2014