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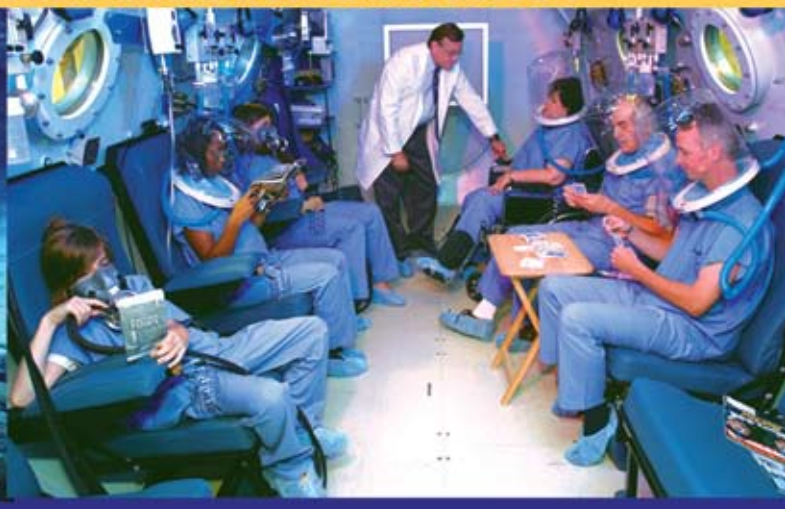
UNDERSEA & HYPERBARIC MEDICAL SOCIETY



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UNDERSEA AND HYPERBARIC MEDICINE

The Journal of the Undersea & Hyperbaric Medical Society Inc.

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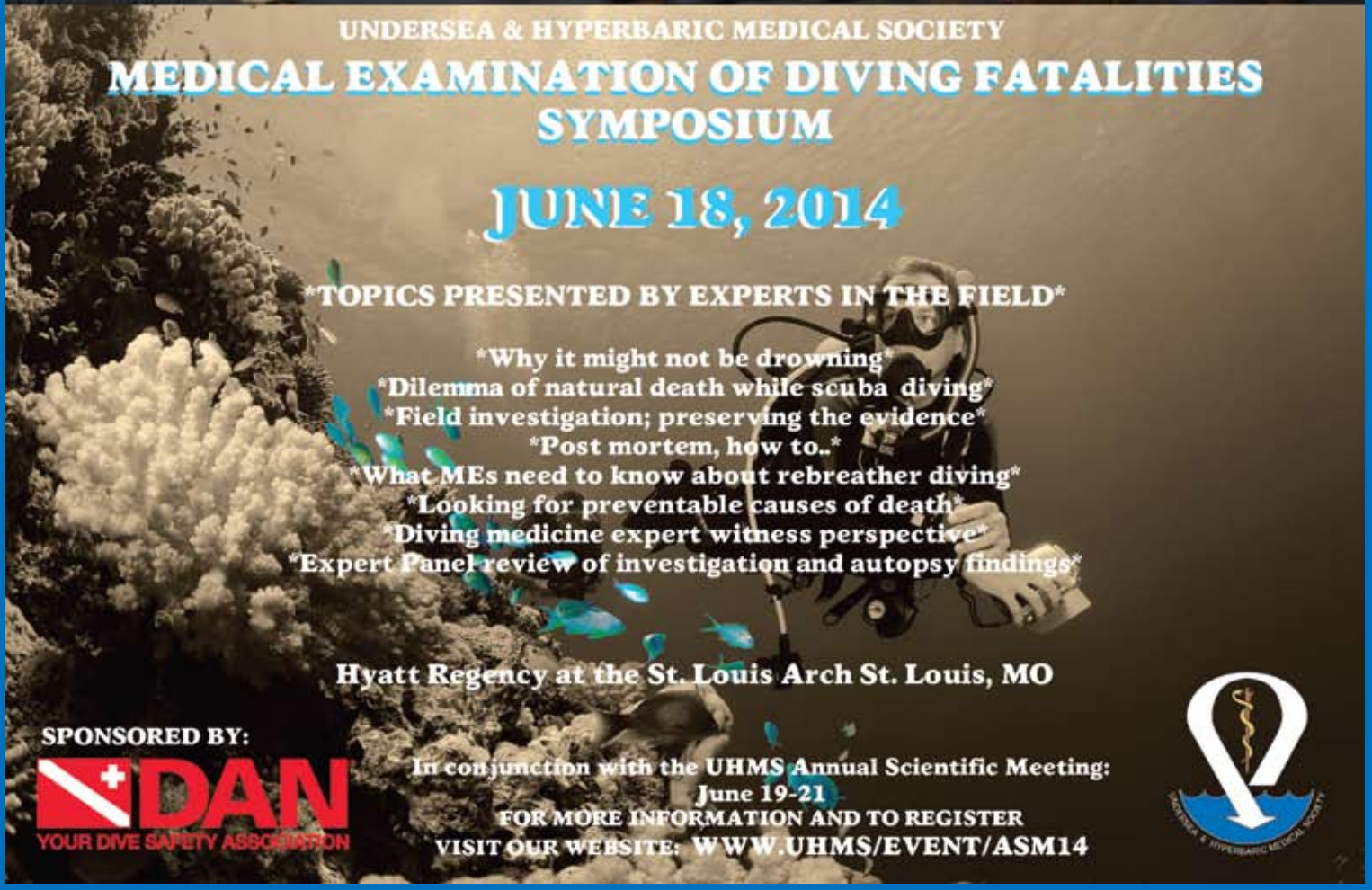
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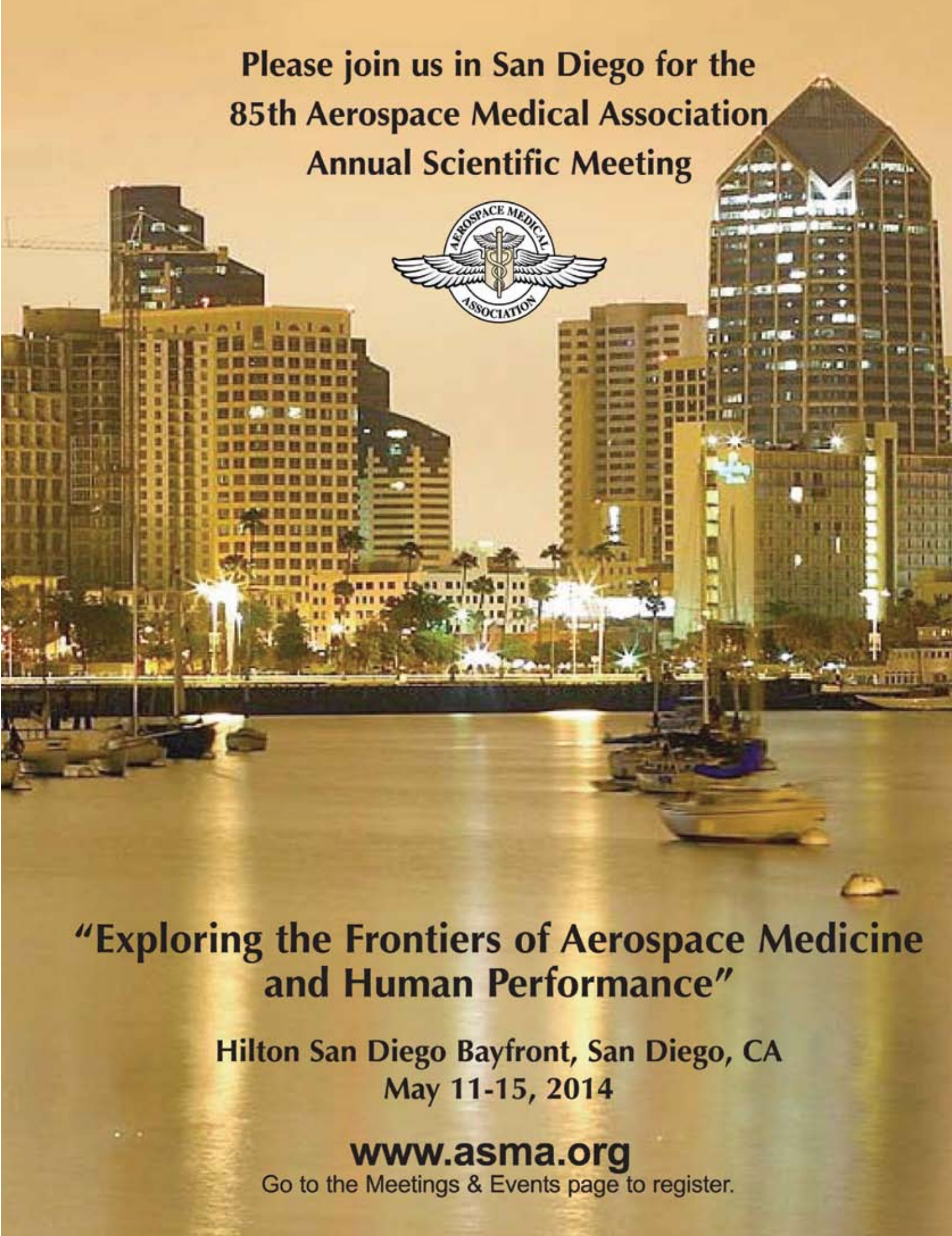

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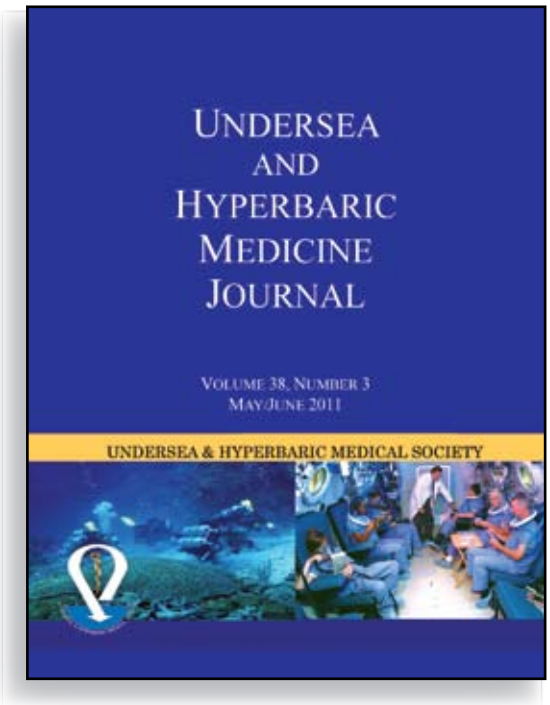

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How and why hyperbaric oxygen therapy can bring new hope for children suffering from cerebral palsy – *An editorial perspective*

Shai Efrati [1,2,3,4], *Eshel Ben-Jacob* [1,2,4,5,6]

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CORRESPONDING AUTHORS: Dr. Shai Efrati – *efratishai@013.net* and Prof. Eshel Ben-Jacob – *eshel@rice.edu*

Cerebral palsy (CP) is generally considered a non-progressive condition resulting from neurological injury in the antenatal or perinatal period. The increased survival rates of premature infants due to advances in neonatal intensive care has led to increased incidence of CP, which is now higher than three in 1,000 births. Perinatal hypoxic-ischemic (HI) events resulting in cellular necrosis, neuronal inactivation and cerebral white matter injury are the most common causes of severe neurological handicaps in children with CP.

The challenge

Physiologically, hypoxic-ischemic brain injury could be defined as acute oxygen and nutrient deprivation to the brain caused by faulty cerebral circulation, resulting in cellular bioenergetics failure and neurological dysfunction. As in stroke, traumatic brain injury (TBI) and age-related metabolic brain disorders, there is no effective treatment/metabolic intervention in routine clinical practice for children with CP. Intensive therapy and rehabilitation programs are valuable tools for improving the quality of life for these unfortunate children, but they offer, at best, only partial relief.

New results

In this current issue of *UHM*, Mukherjee *et al.* present convincing evidence that hyperbaric oxygen (HBO_2) therapy in combination with standard intensive rehabilitation (SIR) could be the coveted neurotherapeutic method for children suffering from neurological dysfunctions due to CP [1]. The idea that HBO_2 therapy can provide a valuable brain repair tool for CP is not new and has been investigated in several earlier clinical trials, but the results were conflicting [2-6]. What makes the current findings persuasive is the methodical, multifaceted comparison: The short-term and long-term outcomes of SIR in conjunction with normal air (21% oxygen) HBO_2 sessions at 1.3 atmospheres absolute (atm abs) were compared with those of SIR in conjunction with:

(a) 100% oxygen HBO_2 sessions at 1.5 atm abs and
(b) 100% oxygen HBO_2 sessions at 1.75 atm abs.

For long-term follow-up, patients were evaluated two and eight months after the beginning of treatment. Interestingly, significant long-term beneficial effects were observed for all combined treatments, including the case of normal oxygen at 1.3 atm abs, compared to SIR alone.

A call for consensus

While the findings support the idea that "low-dose" HBO_2 can provide new hope for children with cerebral palsy, additional, larger-scale clinical studies are needed to further confirm the findings and determine the most effective and personalized treatment protocols. Furthermore, before initiating future clinical trials, some issues associated with the optimal practice of HBO_2 therapy for children with CP should be explored:

- proper sham control;
- the optimal dose-response curve (oxygen and pressure levels);
- the optimal treatment duration/number of HBO_2 sessions; and
- the proper selection criteria of the study cohort.

Further below we reflect on the optimal HBO_2 therapy practice in light of the recent findings by Mukherjee *et al.* – of new understanding of the brain damage

associated with CP and of new understanding regarding the neurotherapeutic effects of hyperbaric oxygen. We hope that our reflections will ignite in-depth discussions within the hyperbaric medicine community, to help reach consensus on whether, why and how HBO_2 therapy can give hope to children with cerebral palsy.

Underlying repair mechanisms

It is now understood that the recently observed restoration of neuronal activity in the metabolically dysfunctional stunned areas following HBO_2 treatments is accomplished via an assortment of intricate mechanisms. The combined action of hyperoxia and hyperbaric pressure leads to significant improvement in tissue oxygenation and affects both oxygen-sensitive and pressure-sensitive genes. HBO_2 therapy can initiate vascular repair and improve cerebral vascular flow, induce regeneration of axonal white matter, stimulate axonal growth, promote blood-brain barrier integrity, and reduce inflammatory reactions as well as brain edema [7-12].

At the cellular level, HBO_2 can improve cellular metabolism, reduce apoptosis, alleviate oxidative stress and increase levels of neurotrophins and nitric oxide through enhancement of mitochondrial function in both neurons and glial cells, and may even promote neurogenesis of endogenous neural stem cells [7-13]. It is important to note that, as in stroke and TBI, the hypoxic-ischemic conditions following cerebral palsy engender depolarization of the mitochondria membrane and induction of mPTP (mitochondrial permeability transition pore), which reduces the efficiency of energy production and elevates the level of reactive oxygen species (ROS).

Tissue oxygenation via HBO_2 can inhibit mPTP and thus has the potential to reverse this abnormality [8]. However, it must be applied carefully to ensure that the increased tissue oxygen does not cause cellular toxicity due to overly high ROS levels.

The control group dilemma

There are inherent ethical and logistic difficulties in handling the sham-control in HBO_2 therapy trials. The standard requirement for proper sham-control is: *"Medically ineffectual treatment for medical conditions intended to deceive the recipient from knowing which treatment is given."*

Hyperbaric oxygen therapy includes two active ingredients: pressure and oxygen. The pressure is being utilized for increasing plasma oxygen, but the pressure change by itself may have significant effects on the cellular level. The pressure effect may be of greater significance in human tissues that are under tight autoregulation pressure control, such as the brain and kidneys [14-18]. The intracranial pressure, the pressure within the skull and thus in the brain tissue and cerebrospinal fluid (CSF), is normally 0.0092-0.0197 atm (7–15 mm Hg). Any increase in cranial pressure may have a significant effect on neurons, glial cells and the function of endothelial cells [14,15, 18].

A classical example that highlights the significance of small changes in pressure is acute mountain sickness (AMS) and high-altitude cerebral edema (HACE). In AMS and HACE, even a small increase in ambient air pressure – less than a sixth of an atmosphere – may reverse the pathology [19]. Put together, the observations imply that any increase in pressure, even with reduced oxygen percentage, cannot serve as a placebo since it exerts at least one of the two active ingredients of HBO_2 therapy.

Elevated pressure with low oxygen can be an effectual treatment

To generate the sensation of pressure, the chamber pressure must be 1.3 atm abs or higher. However, breathing normal air, even at 1.3 atm abs, cannot serve as a proper sham-control since it is not an *"ineffectual treatment,"* as required by the placebo definition; it leads to significant physiological effects resulting from the elevated pressure and the tissue oxygenation. Therefore, as we discuss below, such doses should be regarded as a dose-comparison study, as was correctly done by Mukherjee *et al.*, who demonstrated that it is effective in the treatment of children with CP [1]. Other clinical trials also found that patients treated with low oxygen showed improvements similar to patients treated with higher dosages [2,4,20,21]. However, in those trials, the low-dose treatments were mistakenly regarded as sham-control, leading to incorrect conclusions. In studies 4, 20 and 22, room (21% oxygen) air at 1.3 atm abs was used as a sham-control to test the HBO_2 effect on CP and patients with mild TBI (mTBI) treated with 100% oxygen at 2.4 atm abs. Another study used lower-than-normal (14% oxygen) air at 1.5 atm abs to test the effect of hyperbaric

oxygen on children with cerebral palsy who were treated with 100% air at 1.5 atm abs [2]. In all of those studies, the treated group and the low-oxygen group, which the authors mistakenly considered to be sham-control, show similar improvements [2,4, 20,21]. Consequently, the authors in both studies concluded that the observed improvements were merely placebo effects and therefore that HBO_2 therapy had no neurotherapeutic effects on mTBI and CP.

Their conclusions are clearly challenged by the findings of Mukherjee *et al.* published in this volume and by recent clinical trials testing the effect of HBO_2 on post-stroke and mTBI patients [1,23,24]. Changes in brain activity that were assessed by SPECT imaging, as described next, further support this understanding [23,24].

HBO_2 therapy can activate neuroplasticity and revitalize brain functions: New trials provide convincing evidence that hyperbaric oxygen can induce neuroplasticity, leading to repair of chronically impaired brain functions and improved quality of life in post-stroke and mTBI patients with prolonged post-concusssion syndrome, even years after the brain insult [23,24].

These trials adopted the crossover approach in order to overcome the inherent sham-control constraints of HBO_2 therapy. In this approach, the participants are randomly divided into two groups. One, the trial group, receives two months of HBO_2 treatment while the other, the control group, goes without treatment during that time. The latter are then given the same treatment two months later. The advantage of the crossover approach is the option for a triple comparison:

- between treatments of two groups,
- between treatment and non-treatment periods of the same group, and
- between treatment and non-treatment periods in different groups.

The study endpoint included blinded detailed computerized clinical evaluations that were blindly compared for all patients, with single-photon emission computed tomography (SPECT) scans. HBO_2 sessions led to similar significant improvements in tests of cognitive function and quality of life in both groups. No significant improvements occurred by the end of the non-treatment period in the control group. What made the results particularly persuasive was that the results of SPECT imaging were well correlated with clinical improvements and revealed restored activity mostly in metabolically dysfunctional stunned areas. Those observations indicate hyperbaric oxygen as a potent means of delivering to the brain sufficient oxygen to activate neuroplasticty and restore impaired functions that are accomplished via an assortment of intricate mechanisms, some of which were mentioned earlier.

Rethinking the HBO_2 dose-response curve

The aforementioned recent trials provide convincing evidence that HBO_2 can repair brain damage in post-stroke and mTBI patients. These results, and in particular the remarkable agreement between clinical improvements and SPECT imaging, imply that the observed improvements following HBO_2 therapy in the earlier studies on mTBI patients and children with CP were due to the neurotherapeutic effect of hyperbaric oxygen rather than being a placebo effect.

By the same token, the observed improvements following either normal air at 1.3 atm abs (on patients with mTBI) or 14% air at 1.5 atm abs (on children with CP) imply that HBO_2 sessions can have significant neurotherapeutic effects even at low dosage, provided there is pressure elevation. Therefore, as we mentioned earlier, such doses should be considered as dose-comparison studies rather than sham-control, as was correctly done by Mukherjee *et al.*, who demonstrated normal air at 1.3 atm abs to be an effective treatment for children with CP rather than a placebo effect [1]. These results are also in agreement with the earlier findings by Collet *et al.* [4] that were perceived as puzzling for more than a decade. Yet, as stated by Collet *et al.* (Collet *et al.* 2001): *"The improvement seen in both groups for all dimensions tested deserves further consideration."* The results by Mukherjee *et al.* clearly responded to this suggestion by considering room air at 1.3 atm abs as dose-comparison. Their findings could have been even more persuasive had they included metabolic imaging as part of their evaluations. Since they did not, this issue should be further addressed in future studies.

Clearly, large-scale, well-controlled, pressure dose-response studies are required to determine the optimal HBO_2 therapy protocol for different conditions. Until such information is available, any treatment involving change in the environmental pressure should be considered as a dose-comparison rather than a sham-control study. Moreover, since at a young age, brain protection is stronger (reflected by high ROS levels associated with CP) and neuroplasticity is more potent, it is reasonable to expect that optimal efficacy will be achieved by lower

tissue oxygenation. Along such line of reasoning, the previously described trials used 2.0 atm abs for post-stroke patients and 1.5 atm abs for patient with mTBI with an intact macrovascular bed [23,24]. Due to the high diversity in the manifestation of cerebral palsy and in its severity, future efforts should also be directed towards a personalized dose-response curve. For example, it is likely that higher tissue oxygenation will be the practice of choice for children with a high expression of ApoE4, which is an inhibitor of mitochondrial respiration.

Treatment duration and monitoring protocols:

Treatment duration is another elusive issue that needs to be resolved by future studies. It is quite clear that weeks to months would be necessary for brain tissue r egeneration and angiogenesis, but the upper time limit from which no further improvements are expected remains unknown. The first clinical evaluation (not metabolic/physiological evaluation) should be done after a sufficient number of HBO_2 sessions and should expect sizable changes. One must bear in mind that children with CP suffer neurological deficiency since birth, so it will take time for the brain repair to become clinically apparent. For example, it is not reasonable to administer 20 daily HBO_2 sessions to children with pervasive developmental disorders (PDD) and expect to see significant clinical progress within a time frame of less than a month [25].

On the other hand, it is important to perform frequent metabolic/physiological evaluations, which may provide valuable information for adjusting the dose-response curve. More studies are needed to determine the minimal effective dosage and the treatment duration for specific brain injuries. Non-invasive, in-chamber measurements that are currently being developed, specifically EEG and DTI, may shed some light on this important question.

It is also crucial to perform long-term post-treatment evaluation, as done by Mukherjee *et al.*, who performed evaluations after two and eight months [1]. Especially, when children are concerned, one expects that HBO_2 therapy will ignite the brain's innate repair system so that improvements will continue long after the treatment. As Mukherjee *et al.* have found, different doses may generate similar short-term improvements but can lead to different long-term post-treatment effects. In other words, dose-response curves should be assessed based on long-term effects. Clearly, there is an urgent need for larger-scale, prospective studies with long-term follow-up.

Optimal candidates for HBO_2 therapy

Brain insults may result in a variety of brain injuries. The most severe is necrosis, which cannot be reversed. However, as was mentioned earlier, necrotic foci are often surrounded by metabolically dysfunctional, stunned areas, which manifest as regions of high anatomy-physiology mismatch. Current imaging technologies reveal that the stunned brain areas may persist for months and years after an acute brain event [24, 26-28] and this is where metabolic intervention can be most effective [23,24]. For this reason, the optimal candidate for hyperbaric oxygen is a patient with unrecovered brain injury where tissue hypoxia is the limiting factor for the regeneration processes. In this patient, HBO2 may induce neuroplasticity in the stunned regions where there is a brain anatomy/physiology (e.g., SPECT/CT) mismatch [23, 24]. Unfortunately, in many – if not most – clinical studies done with hyperbaric oxygen on brain-injured patients, including those with cerebral palsy, the stunned areas have not been assessed by imaging. The anatomical/physiological imaging should be incorporated as an essential part of the basic evaluation of every candidate for hyperbaric oxygen therapy. In a similar manner, transcutaneous oximetry at the ulcer bed serves as a basic evaluation for patients suffering from peripheral non-healing wounds [29,30}.

An urgent call

In conclusion, we call on the hyperbaric community to rethink the neurotherapeutic effects of HBO_2 therapy and to agree on common and scientifically sound guidelines to best conduct prospective, controlled HBO_2 clinical trials. Reaching a consensus on the way to handle the control group, dose *vs.* efficacy, selection criteria of the study cohort and duration of treatment will pave the way for future studies that will explore the full potential of neurotherapeutic HBO_2.

We envision future studies that will demonstrate the effectiveness of HBO_2 therspy for a wide spectrum of syndromes that currently have partial or no solutions, such as central sensitization (fibromyalgia), radiation damage, vascular dementia and other metabolic aging effects.

The authors report that no conflict of interest exists with this submission.

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Intensive rehabilitation combined with HBO_2 therapy in children with cerebral palsy: A controlled longitudinal study

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ABSTRACT

Objective: The present study aimed to assess the effect of intensive rehabilitation combined with hyperbaric oxygen (HBO_2) therapy on gross motor function in children with cerebral palsy (CP).

Methods: We carried out an open, observational, platform-independent study in 150 children with cerebral palsy with follow-up over eight months to compare the effects of standard intensive rehabilitation only (control group $n = 20$) to standard intensive rehabilitation combined with one of three different hyperbaric treatments. The three hyperbaric treatments used were:

- air ($FiO_2 = 21\%$) pressurized to 1.3 atmospheres absolute/atm abs ($n = 40$);
- 100% oxygen pressurized at 1.5 atm abs ($n = 32$); and
- 100% oxygen, pressurized at 1.75 atm abs ($n = 58$).

Each subject assigned to a hyperbaric arm was treated one hour per day, six days per week during seven weeks (40 sessions). Gross motor function measure (GMFM) was evaluated before the treatments and at two, four, six and eight months after beginning the treatments.

Results: All four groups showed improvements over the course of the treatments in the follow-up evaluations ($p < 0.001$). However, GMFM improvement in the three hyperbaric groups was significantly superior to the GMFM improvement in the control group ($p < 0.001$). There was no significant difference between the three hyperbaric groups.

Conclusion: The eight-month-long benefits we have observed with combined treatments *vs*. rehabilitation can only have been due to a beneficial effect of hyperbaric treatment.

INTRODUCTION

Cerebral palsy (CP) is due to a lesion of the developing brain, characterized by inadequate muscle tone and control, often associated with other types of neurodevelopmental delay involving cognitive, communication and psychosocial skills. Treatments are mainly focused on exploiting residual cerebral function, and intensive rehabilitation is recognized to have demonstrated its efficacy in achieving better function and autonomy, thus creating a better quality of life [1].

The leading causes for cerebral palsy stem from a critical reduction of oxygen (O_2) delivery to a part of the developing brain in the perinatal period [2]. The site of the brain lesion can be localized with cerebral blood flow measurements using brain single-photon emission computerized tomography (SPECT) [3,4] because impaired brain cell nutrition and oxygen delivery are related to inadequate blood flow. While hypoxia may cause neuronal death, there is a well-known phenomenon called the "ischemic penumbra," which defines a volume of tissue surrounding a zone of infarction where cells receive enough oxygen to survive in an "idling state," but not enough to function normally [5]. It has been suggested that these neurons might be viable much longer than previously believed [6,7,8], and this is where regenerative medicine is trying to play a role. Hyperbaric oxygen (HBO_2) treatment has shown reproducible benefits for more than two decades in hundreds of

children with CP around the world [9]. Using high-quality SPECT imagery, several studies of children with CP and of adults after a stroke have shown that HBO_2 therapy may regenerate or revive cells in the ischemic penumbra in the brain [7,10,11]. This increased vascular activity would allow the reactivation of "idling" neurons [6,10,11, 12], as HBO_2 therapy is known to increase neovascularization in wound healing. The higher tissue oxygen levels provided by HBO_2 therapy might also favor better metabolism and function of unaffected cells [13,14].

To date, despite several reports of benefit, the use of HBO_2 therapy for CP has met opposition, which has even polarized the field of clinical HBO_2 therapy [15-18]. The first pilot study [19] reported the positive effects of HBO_2 therapy on 25 carefully selected children with the form of CP known as spastic diplegia. The improvements were measured both on gross and fine motor function. Based on the results of this pilot study, a double-blind randomized multicenter trial (n = 111) of HBO_2 therapy for children with CP was conducted by Collet *et al.* [20]. This study included only two groups of children: one treated at 1.75 atmospheres absolute (atm abs) with 100% O_2, while the other breathed air at 1.3 atm abs. Some involved in the statistical analysis of the results regarded the use of compressed air at 1.3 atm abs to be an inactive placebo, although this was opposed by the clinicians.

The controversy required the appointment of an independent adjudicator by the *Lancet*, who agreed that such a change in pressure and increase in the level of oxygen could not be referred to as a "sham" treatment. In fact, exposure to 1.3 atm abs increases the arterial plasma oxygen concentration (PaO_2) by nearly 50% [21]. It was little recognized at the time that blood flow in the physiological range of oxygen concentrations is controlled by the interaction between nitric oxide and hemoglobin [22]. Changes in oxygen levels also regulate genes involved in angiogenesis and neutrophil activity in inflammation [23]. As the best dosage of oxygen for the treatment of children with CP is not known, a sham control group should have been included to ensure an adequate experimental design. The controversy was highlighted by an editorial comment entitled "Hype or hope" published in the same issue of the *Lancet* journal [24].

After the courses of treatment, the improvements on gross motor function were impressive and equivalent in both groups. Improvements in language and neuropsychological functions were also recorded in both treatment groups. There are two ways of interpreting the results: either the two treatments were equally effective, or the improvements were all caused by a "participation effect." Based on the major improvements reported, the latter interpretation is inappropriate [25] but has, unfortunately, been promoted as evidence that hyperbaric treatment is ineffective in CP children [26] restricting further research on the subject. The aim of this present study is to answer the questions raised by the study by Collet *et al.* [20] by assessing the effect of different dosages of hyperbaric treatment combined with intensive rehabilitation on motor function in children with CP.

METHODS

Participants

A total of 150 children with CP were selected for the study among those attending rehabilitation at the Foundation for Spastic and Mentally Handicapped Persons-UDAAN (FSMHP-UDAAN) center in Delhi, India. All participants had to meet the following inclusion criteria: children up to teen age of either sex with all types of CP, any cognitive and motor development level.

Children were excluded if there were other developmental or genetic disorders, uncontrolled epilepsy or asthma, as well as ear, nose or throat disorders. Forty percent of all of our participants had minor to moderate epilepsy due to their injured brain. Half of them were significant enough to be on antiepileptic medication. It was the parents' decision to include their children in the HBO_2 therapy groups. Participants who were not assigned to HBO_2 therapy groups were assigned to the control group. All participants were engaged in the same intensive rehabilitation program at FSMHP-UDAAN. Only the children who did not default on at least six months of standard therapies were assessed. Quality, magnitude and type of care were uniform across all four groups. Participants' characteristics are described in Table 1. The study was approved by the ethics committee of Apollo Hospital, Delhi, and the parents' informed voluntary written consent was required after medical clearance.

Treatments

The study covers a 10-year span of treatments during which the three different dosages of hyperbaric oxygen were used. The different dosages were not implemented at the same time, and the children were offered the HBO_2 therapy available at the time of their inclusion in the protocol, which means that no selection bias occurred in the choice of dosage.

Table 1: Participants' characteristics

Groups	Diagnostics	Gender (M/F)	Age (yrs) Mean (range)	GMFM baseline score Mean (SD)
Control (*n*=20)	Athetoid CP, *n*=2 Hemiplegic CP, *n*=2 Diplegic CP, *n*=4 Quadriplegic CP, *n*=12	13/7	3.5 (1 to 17)	29.6 (13.0)
1.3 atm abs (*n*=40)	Athetoid CP, *n*=3 Hemiplegic CP, *n*=0 Diplegic CP, *n*=16 Quadriplegic CP, *n*=12	29/11	4.9 (1 to 11)	29.6 (14.8)
1.5 atm abs (*n*=32)	Athetoid CP, *n*=3 Hemiplegic CP, *n*=1 Diplegic CP, *n*=15 Quadriplegic CP, *n*=13	23/9	4.3 (1 to 12)	34.3 (15.6)
1.75 atm abs (*n*=58)	Athetoid CP, *n*=6 Hemiplegic CP, *n*=2 Diplegic CP, *n*=19 Quadriplegic CP, *n*=31	40/18	4.3 (1 to 13)	32.5 (11.8)

atm abs = atmosphere absolute; CP = cerebral palsy; F = female;
GMFM = gross motor function measurement; M = male.

Every child received the same intensive rehabilitation care by the same therapist team, at the same center, using the same protocol, and the same duration of follow-up. The rehabilitation program was applied for two hours/day, six days/week over six months, and consisted of a half-hour of individual therapies of physical therapy, occupational therapy, speech therapy and special education.

For hyperbaric therapy, the children were assigned to four groups:

A- No hyperbaric treatments, rehabilitation only (control group), *n*=20;
B- 40 sessions, one hour/day, six days/week at 1.3 atm abs air, 21% O_2 (room air), *n*=40;
C- 40 sessions, one hour/day, six days/week at 1.5 atm abs HBO_2, 100% O_2, *n*=32;
D- 40 sessions, one hour/day, six days/week at 1.75 atm abs HBO_2, 100% O_2, *n*=58.

All hyperbaric treatments were given six days/week during seven weeks. In all treatment sessions, the total amount of time spent in the hyperbaric chambers was 90 minutes, as 15 minutes for either compression and decompression was taken. HBO_2 using 100% oxygen was delivered through a hood inside a multiplace hyperbaric chamber at a local tertiary care hospital, using pressures of 1.75 or 1.5 atm abs. Hyperbaric air treatment at 1.3 atm abs using room air at 21% oxygen was carried out using a soft chamber. We carried out initial and periodic assessment of lung and ENT passages and temporarily stopped hyperbaric therapy whenever there was any air passage obstruction or inflammation. Children with a previous history of epilepsy were referred to a pediatric neurologist, and the anti-epileptic dosages were increased marginally during the hyperbaric treatments period.

Evaluation procedures

In all children, gross motor function was systematically evaluated before the treatments and at four and six months after the beginning of the treatments by the same therapists, who were accustomed to undertaking the evaluations. To have more data, and when possible, we were often able to evaluate the children at two and eight months after the beginning of treatments. The gross motor function measure (GMFM66) [27] was applied to every child. It is a criterion-based observational measure (66 items) that assesses motor function in five dimensions: A-lying and rolling, B-sitting, C-crawling and kneeling, D-standing and E-walking, running and jumping.

Table 2: GMFM observed mean before and after HBO_2 therapy

	GMFM observed mean (SD)				
	Before HBO_2	**2 months after beginning HBO_2**	**4 months after beginning HBO_2**	**6 months after beginning HBO_2**	**8 months after beginning HBO_2**
Control	29.6 (13.0)		31.0 (12.8)	32.4 (12.8)	
1.3 atm abs 21% O_2	29.6 (14.8)	33.4 (13.1)	36.2 (13.6)	38.6 (14.3)	40.8 (14.2)
1.5 atm abs 100% O_2	34.3 (15.6)		39.3 (15.4)	42.5 (15.3)	46.4 (17.0)
1.7 atm abs 100% O_2	32.5 (11.8)		37.2 (10.8)	42.1 (10.4)	46.7 (9.7)

atm abs = atmosphere absolute; GMFM = gross motor function measurement

Each item is scored on a four-point scale, and the test gives numeric results for each dimensions as well as a total score. The score is reported as a percentage of the maximum score (100%) generally obtained in a normal 5-year-old child.

Data analysis

Linear mixed models were used to analyze the GMFM data. Such models permit the data to exhibit correlations and non-constant variances. These models, therefore, provide the flexibility of modeling not only the means of the data but also their variances and co-variances. Treatments were considered as fixed factors, and month and age were considered as co-variables. Month was time-dependent, while age was time-independent. Random components were introduced to depict individual trajectories over months with separate intercepts and slopes. A maximum likelihood approach was used to estimate the coefficients, and an unstructured random effect covariance matrix was utilized. Linearity for month and interactions (treatment *x* month) were tested. Information criteria (such as the Akaike criterion and the -2ln (likelihood)) and residual values were used to verify the quality of adjustment. Pearson product-moment correlation coefficient (r) was calculated to quantify the interrelationship among the GMFM variation and GMFM level before HBO_2 therapy.

RESULTS

As expected, groups were similar on the GMFM level at baseline ($p = 0.429$) and each group, including the control group, showed improvement in the GMFM scores over the course of the treatments ($p < 0.001$). As depicted in Table 3, there were statistically significant interactions between group and month ($p < 0.001$) and a statistically significant age effect ($p = 0.003$). To better understand these results, fixed-effect linear models are presented in Table 4 for each group. We observe that the GMFM score increases by 0.46 unit per month in the control group as compared to values ranging from 1.36 to 1.50 unit per month in the experimental groups; and these slopes are significantly different from the control group slope ($p < 0.001$). These results are visualized in Figure 1. GMFM variation, which is the average monthly improvement in the GMFM results over the course of the follow-up, was correlated with GMFM level before HBO_2 therapy ($r = -0.33$, $p < 0.001$).

DISCUSSION

This is the first study that has compared the effects of different hyperbaric dosages combined with rehabilitation in children with CP to a control group receiving only rehabilitation. As expected with intensive therapies, all four groups improved substantially. However, our findings demonstrate that the three groups treated with different dosages of HBO_2 improved much more than the control group, as their GMFM variations were on average three times higher.

In the present study, the three treatments were equally effective in producing gross motor improvement. This reproduces the impressive results obtained in the two groups (1.5 atm abs HBO_2, 100% O_2 and 1.3 atm abs air) in the study of hyperbaric treatment for CP children by Collet *et al.* [20]. Mychaskiw has pointed out in a UHM editorial that children treated with compressed air at 1.3 atm abs cannot be regarded as a control group [28]. It is obvious that giving more oxygen for neurologic conditions is not an all-or-none phenomenon. We find it disconcerting that such a flawed study has been used to claim a lack of efficacy of hyperbaric treatment in cerebral palsy when Collet *et al.* [20] actually stated: "The improvements in GMFM scores in both groups are

Table 3: Fixed effects estimation for GMFM

Variable	Coefficient (B)	SE(B)	T	*p*-value
Constant	24.65	3.31	7.45	0.000
1.3 atm abs	-1.91	3.65	-0.52	0.602
1.5 atm abs	2.91	3.73	0.78	0.437
1.75 atm abs	1.42	3.39	0.42	0.675
Month	0.46	0.18	2.52	0.013
LnAge	4.96	1.66	2.99	0.003
1.3 atm abs* month	0.90	0.22	4.14	0.000
1.5 atm abs* month	0.94	0.23	4.16	0.000
1.75 atm abs* month	1.04	0.210	4.95	0.000

atm abs = atmospheres absolute; GMFM = gross motor function measurement

Table 4: Predicted GMFM from fixed effects models in each group

Group	Model
Control group	GMFM = 24.65 + 0.46 Month + 4.96 LnAge
1.3 atm abs group	GMFM = 22.75 + 1.36 Month + 4.96 LnAge
1.5 atm abs group	GMFM = 27.56 + 1.40 Month + 4.96 LnAge
1.75 atm abs group	GMFM = 26.07 + 1.50 Month + 4.96 LnAge

atm abs = atmospheres absolute; GMFM = gross motor function measurement

clinically important… The improvement seen in all other outcomes is also striking." Moreover, the U.S. Agency for Healthcare Research and Quality (AHRQ) analyzed the results of the study and arrived at the same conclusions [25]. The AHRQ report mentioned that "The possibility that pressurized room air had a beneficial effect on motor function should be considered the leading explanation."

However, our study has, like that of Collet *et al.* [20], clearly demonstrated the benefit of treatment with compressed-air at 1.3 atm abs, because we included a control group; thus the effect of hyperbaric conditions cannot be attributed to a participation or placebo effect. In fact, the placebo effect is a temporary phenomenon that lasts for a few weeks [29] and not for the eight months we have found benefit in our follow-up. Human physiology works within a narrow band for optimal activity. In this context, the increase of almost 50% in plasma oxygenation achieved by compressed air at 1.3 atm abs was of significance.

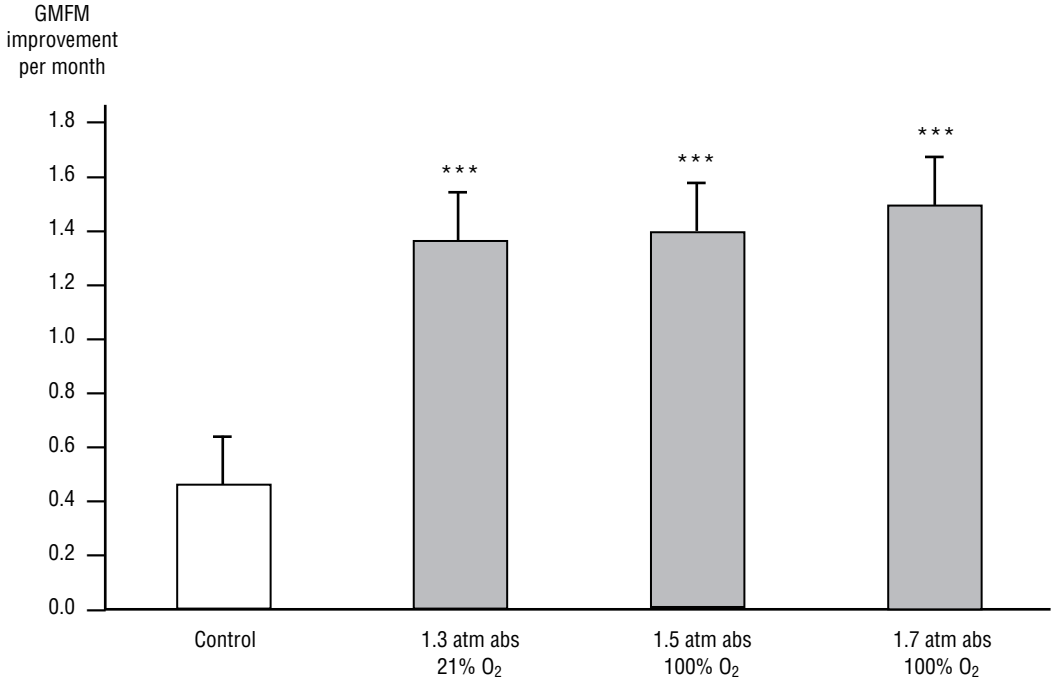
A study on patients with advanced lung disease has been undertaken in Jerusalem. While maintained on supplemental oxygen, they were taken down to the Dead Sea, where they breathed only ambient air. A statistically significant increase in walking distance was recorded, which persisted for a month after returning to Jerusalem. The increase in pressure achieved by descending to the Dead Sea was just 0.06 atm abs [30]. Compressed air at a pressure 0.3 atm abs over ambient cannot be considered a placebo; and a recent paper discussed the osmotic effects of a sudden increase in pressure [31]. In addition, most of the children included in our series were barely in a position to have the mental maturity to understand what was being done for them.

The results of the present study strongly support the fact that HBO_2 therapy, even in small dosage, can improve motor function and increase the effects of standard rehabilitation. The amount and quality of changes observed in our study are also in accordance with the results obtained in other studies on HBO_2 therapy in CP [10,19,20]. The authors are aware that Lacey *et al.* [32] have recently conducted a randomized control study in which they compared two different hyperbaric treatments, one of which (14% O_2 at 1.5 atm abs) has never been used on CP children before, and was considered by these authors as a control group. These authors present their study as a definitive answer to hyperbaric therapy inefficacy in children with CP even if major concerns can be addressed and explain the discrepancy with the present study.

First, despite the fact that in the control group, the condition simulated 21% oxygen at room air, this treatment must not be considered as a placebo treatment because no one knows exactly the potential physiologic effects of this hyperbaric treatment. Secondly, the change in GMFM in the HBO_2 group was 1.5 in two months, which is more than most changes measured with recognized treatments in CP [9]. Thirdly, Lacey *et al.* included only 20 participants per group and stopped the study prematurely, which avoided possibility of the results reaching a threshold for significance. These concerns have been addressed in a letter to the Annals of Neurology [33].

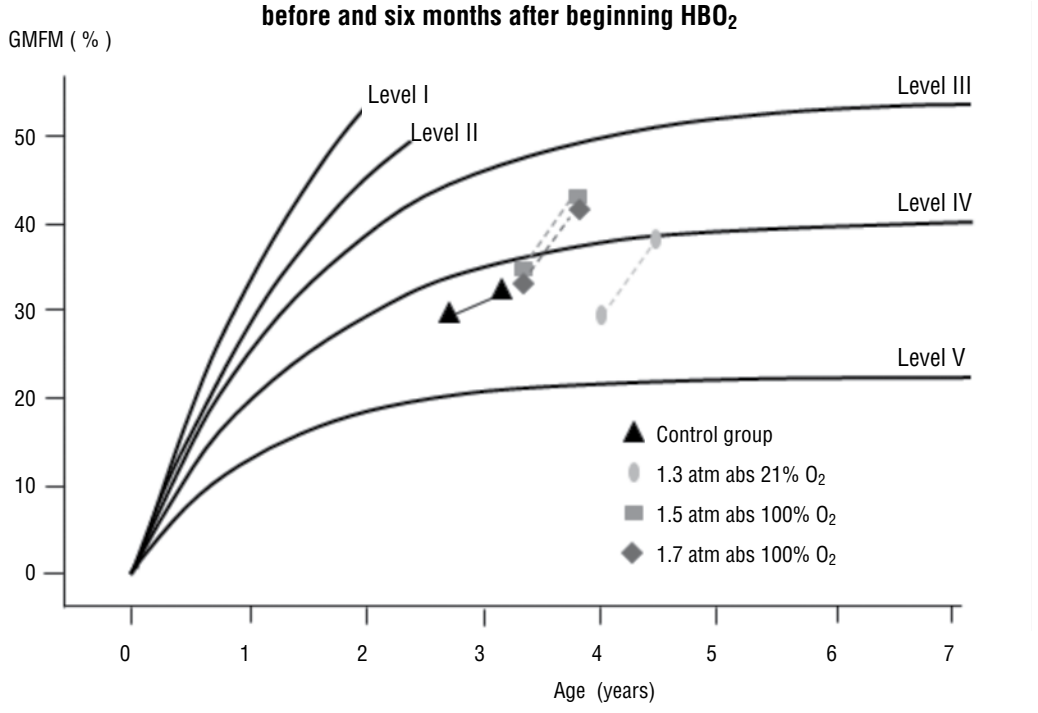
The Gross Motor Function Classification Scale (GMFCS) classifies CP disabilities into five levels based on the GMFM measurement at a given age. The natural gross motor progression of children with CP usually

Figure 1: Rate of gross motor function measurement improvement



*** = significantly different from the control group, $p<0.001$; atm abs = atmospheres absolute

Figure 2: Gross motor function classification scale values before and six months after beginning HBO_2



atm abs = atmosphere absolute; GMFM = gross motor function measurement

follows a curve similar to a logarithmic curve [27]. The children with the highest level of abilities are classified in Level 1, while Level 5 regroups the children with the most severe form of motor disability (Figure 2). The progression of children with CP should naturally follow the curves corresponding to their level of disabilities [27]. Figure 2 shows that the mean initial GMFM values of the four groups would classify them between Level 4 and Level 5 of the GMFCS. By end of six months of therapy, all three hyperbaric groups had improved to Level 4, whereas the control group did not change its disability level.

There are risks associated with the high oxygen pressures used in diving, but they are not relevant to the much lower pressures used in this study. The rate of change of pressure was slow, as the pressurization took 15 minutes, and only three children were excluded because of ear pain on compression. None of the participants needed ear canal grommet use. There were no other side effects.

Our study shows that HBO_2 therapy, when combined with rehabilitation, has many more positive effects than rehabilitation alone. As seen on SPECT imaging, hyperbaric treatment appears to reactivate certain damaged areas of the brain. It is, however, obvious that the recovering brain must be trained to work to its full potential to gain the best results. This highlights the importance of rehabilitation after or during HBO_2 therapy. Further research is needed to explore the cerebral plasticity processes that follow hyperbaric treatment. Improvement in function, comfort and the independence of children with disabling neurological conditions could lead to better health and quality of life as well as important cost savings in the long term.

LIMITATIONS

There were several limitations inherent to this study. First, participant repartition between groups was not randomized. It was the parents' decision to include their children in HBO_2 therapy groups, and participants who were not assigned to HBO_2 groups were automatically assigned to the control group. The different dosages of HBO_2 were not implemented at the same time over a 10-year period, which means that no selection bias occurred in the treatment or dosage choice.

Secondly, the evaluations were not blinded. We certainly recognize that it was not ideal, but it was difficult for us, in a longitudinal study conducted in a relatively small center and involving human interaction and evaluation by the same therapists, for blinded evaluations to have been undertaken.

CONCLUSION

A longitudinal study in children with cerebral palsy has been conducted. The study compared three different dosages of hyperbaric oxygen, combined with intensive rehabilitation with a control group receiving only rehabilitation. The rate of improvement in GMFM score was significantly superior in the three hyperbaric groups compared to the control group, There was no difference between the three HBO_2 therapy groups. The amount of changes are similar to the results obtained in the multiple studies on HBO_2 therapy in CP that have been published and are more important than the improvements measured with standard recognized therapies alone in CP. The very important difference observed in treated *vs.* controlled children can only be a genuine beneficial effect of HBO_2 therapy. Based on the results of this and other studies of HBO_2 therapy in CP children, HBO_2 combined with rehabilitation should be recommended for children with CP.

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The authors report that no conflict of interest exists with this submission. ■

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Hyperbaric oxygen treatment for post-radiation central nervous system injury: A retrospective case series

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ABSTRACT

Increased use of radiation therapy and increasing life spans following radiation treatment has led to an increase in the finding of post-radiation central nervous system injury in patients who have previously undergone radiation treatments. At this time, information regarding treatment for patients suffering from this serious side effect is limited and not readily available. It is imperative to examine possible treatment options, complications and success rates for these patients. This retrospective review will look at 10 patients who underwent hyperbaric oxygen therapy for post-radiation injury to the central nervous system. Review and investigation of the subjective, clinical and radiologic outcomes of these patients was conducted. It was determined that for patients with post-radiation central nervous system injury it is important to distinguish the exact diagnosis for each patient. For those patients with radiation necrosis, conclusion was made that hyperbaric oxygen (HBO_2) therapy does lead to improvement in subjective, clinical and radiologic outcomes. However, the results were not consistent across all patients. For those patients with non-specific delayed radiation injury, findings showed that HBO_2 does not lead to any improvement. Therefore, we conclude that for those patients who have been diagnosed with radiation necrosis of the central nervous system, we recommend HBO_2 therapy as a potential treatment option for some patients.

INTRODUCTION

Hyperbaric oxygen (HBO_2) therapy involves the use of high levels of pressurized oxygen in an effort to increase blood oxygen levels. The use of hyperbaric oxygen treatment for soft tissue radionecrosis has been well established [1]. However, there has been very limited research into the effects of HBO_2 therapy on post-radiation injury to the central nervous system on a large patient population [2]. Since the central nervous system is another form of soft tissue, it is logical to believe that HBO_2 will have the same effect as seen at other soft tissue sites. For example, brain tissue does not regenerate in the same way that skin does. The brain may be devoid of blood flow after radiation injury, and may be viewed as a chronically ischemic penumbra that may restore with HBO_2.

There are many effects of HBO_2 that we believe will lead to improved outcomes. First, HBO_2 will provide a neuroprotective effect to the cells by decreasing ischemia and edema. Second, HBO_2 will decrease apoptosis at the site of ischemia due to activation of anti-apoptotic factors, such as Bcl-2. Third, the increased vessel formation from HBO_2 therapy will lead to better perfusion of ischemic area. Fourth, HBO_2 has been shown to increase the stability of the blood-brain barrier, which will decrease the transfer of neurotoxic metabolites. Lastly, HBO_2 will decrease the amount of inflammation at the site of necrosis by decreasing myeloperoxidase activity and the level of ICAM-1 [3]. In this study we share our experience with HBO_2 therapy in patients with post-radiation central nervous system injury.

Prior reports regarding radiation treatment of the central nervous system have shown that there is approximately a 90% incidence of neurotoxic injury [4]. Kuffler *et al.* cite previous studies showing the possible

changes seen on MRI from necrosis following radiation therapy, as well as possible side effects. The reports cited described neurologic deficits such as progressive dementia, memory loss, vision and hearing change, and ataxia. However, due to limited information regarding neurotoxicity following radiation of the central nervous system [2,5,6,7,8], it is imperative that more studies and case reports be conducted on possible treatment modalities.

METHODS

Retrospective analysis of 10 patients with post-radiation central nervous system injury treated from 2000 to 2011 at a hyperbaric unit in a tertiary care referral hospital was conducted (Table 1). All patients were scheduled and ordered to receive 100% oxygen (O_2) for 30 treatments, as the majority of soft tissue radionecrosis conditions are treated in the range of 30-60 treatments – based upon physician training and experience – at 2.4 atmospheres absolute (atm abs) for 90 minutes each session. Deviations from this protocol were due to individual patient cases. Patients who did not complete all 30 treatments either declined further treatment, were unable to complete the treatment course due to disease burden or other factors, or it was decided that the treatments were not efficacious.

Patients who did not receive 2.4 atm abs of pressure received 2.0 atm abs. These patients all had contraindications to using higher pressures in the hyperbaric chamber, such as a history of seizures or were receiving concurrent chemotherapy. It is a well-known and common practice to decrease atmospheric pressure while using concurrent chemotherapeutic agents due to the possibility of increased neurotoxicity. Chemotherapeutic agents that tend to show increased toxicity include, but are not limited to, adriamycin, cisplatin and bleomycin.

The study was approved by the Medical College of Wisconsin Investigational Review Board (IRB). Records from the electronic charts and hyperbaric unit with ICD codes for soft tissue radionecrosis were obtained. Patient information was de-identified and study records kept in a locked cabinet at the principal investigator's laboratory. A full chart review of the 10 patients who qualified for our study was then conducted, taking specific note of the patients' pre- and post-clinical assessments and radiologic imaging studies. Descriptive statistics were then used to populate a spreadsheet with pertinent information in order to analyze the post-HBO_2 therapy outcomes (Table 2).

A total of 10 patients, (five male, five female) were treated between the years 2000 to 2011 for post-radiation injury to the central nervous system. At the tertiary center where this study was conducted, post-radiation injury to the central nervous system comprised a very small number of the total number of patients who received HBO_2 therapy. All patients were treated under a compassionate use basis. The mean age group of the patients was 58 years. All patients had pre-HBO_2 therapy diagnoses of post-radiation injury to the central nervous system.

The average radiation dose received was 63 gray (Gy) (only those treatments received between 2000 and 2011 were used in calculation, as some patients had received treatments prior to this time period). The average duration before presentation of encephalopathy was 39.8 months, and the most common symptoms were headaches, loss of coordination or balance, vision changes, and neuropsychiatric changes. The trial of HBO_2 was begun after consent, and other standard of care treatments such as steroids and chemotherapy were continued.

Table 2 displays the diagnosis and descriptive results for each patient. The diagnoses were determined based on review of the radiologic images and reports. Those patients who had T1 enhancement on MRI were diagnosed with radiation necrosis; PET and MRI spectroscopy results were not noted in any patient records, and there was no indication that biopsies were conducted for any of the 10 patients.

Patients without T1 enhancement were diagnosed with radiation encephalopathy. Subjective outcome was determined by a careful review of the patients' follow-up notes. These outcomes are reported as "improved" or "worsened," or as "N/A" if there were not enough data to determine an outcome.

Clinical outcomes are shown by the patients' pre- and post-HBO_2 clinical assessment scores. These scores were reported by the patients' physicians throughout their care and follow-up. The two clinical assessments used by the physicians were the Karnofsky Performance Status Scale index score and the ECOG Performance Status score (even though performance scales were useful in this case, it is believed that system-specific assessments would have proved more useful).

TABLE 1: Patient ages, diagnoses, dosages, time to treatment and co-morbidities

Case	Age at HBO_2 treatment	Original diagnosis	Radiation dose (gray)	Time to treatment (months)	History of seizures	Received chemo-therapy	Steroid use
1	71	Posterior fossa meningioma	62, 50*	24-36	-	-	-
2	54	ACTH secreting pituitary adenoma	50.4	64	+	+	+
3	50	Right cerebellar hemangioblastoma	70	25	-	-	-
4	68	Recurrent atypical meningioma	54, 35*	32	+	-	+
5	81	Esophageal carcinoma	50.4	12	-	+	+
6	56	Lung carcinoma with brain mets	50.4, 10.8*	9	-	+	+
7	43	Lung carcinoma with brain mets	61	24	+	+	+
8	64	Ovarian carcinoma with brain mets	30.6, 38.5*	204	-	+	+
9	38	Left hemisphere AVM	21	12	+	-	-
10	43	Optic nerve sheath meningioma	40	19-31	-	-	+

*Patient received radiation treatment twice during their lives. The dates for each treatment have been included in the individual case descriptions.

TABLE 2: Chart reporting patient outcomes

Case	Diagnosis	Subjective outcome	Pre-HBO_2 Pre-clinical assessment	Post-HBO_2 clinical assessment	Radiologic outcome
1	Radiation necrosis	Improved	KPS - 60% ECOG - 2	KPS - 50-60% ECOG 2-3	Worsened
2	Radiation encephalopathy	No change	KPS - 70% ECOG - 2	KPS - 30% ECOG - 3	No change
3	Radiation necrosis	Worsened	KPS - 70% ECOG - 2-3	KPS - 50-60% ECOG 2-3	Worsened
4	Radiation necrosis	Worsened	KPS - 50-60% ECOG - 2	KPS - 30% ECOG - 2	Improvement
5	Radiation myelopathy	No change	N/A	KPS - 50%	Worsened
6	Radiation necrosis	Worsened	KPS - 90-100% ECOG - 0-1	N/A	No change
7	Radiation necrosis	N/A	KPS 80-90%	N/A	Worsened
8	Radiation encephalopathy	Improved	N/A	N/A	No change
9	Radiation necrosis	Improved	N/A	N/A	Improvement
10	Radiation necrosis	Improved	N/A	N/A	No change

Only a single pre- and post-HBO_2 score is reported, if available. For those patients who did not have a score reported for either pre- or post-HBO_2 assessment, their scores were reported as "N/A." These clinical assessment tools are used to objectively follow a patient's progress through a treatment regimen. Radiologic outcome was determined by a careful review of the patients' radiology reports. These outcomes are reported as "improved" or "worsened" or "N/A" if there was not enough data to determine an outcome.

RESULTS

The main goal of this retrospective research study was to determine what, if any, significant improvements could be offered to patients with post-radiation injury to the central nervous system through hyperbaric oxygen treatments. With this goal in mind, it was determined that attention would be focused upon three categories of "outcomes." These categories include: subjective outcome; clinical outcome; and radiologic outcome. Prior case studies involving the treatment of radiation necrosis from tumor resection [5], radiation myelitis [7] and radiation following AVM treatment [8] provided the background basis for including these patients in this study.

Since these patients were followed by a neurologist certified in the use of hyperbaric medicine, all of the subjective and clinical outcome data come from review of their notes for all pre- and post-HBO_2 therapy issues. While not every patient had reports that included a clinical assessment tool, the primary tools that were used were the Karnofsky Performance Status Scale score and/or ECOG Performance Status assessments. The Karnofsky score classifies a patient's functional impairment and ability to care for him- or herself. The scores range from 100% (no evidence of disease) to 0% (dead) [9]. The ECOG score also assesses patient function, but focuses more on the impact of the disease on patient function: Scores range from 0 (fully active) to 5 (dead) [10]. Both of these clinical assessment tools can be used to follow a patient's progress to objectify the effect of a treatment regimen. See Table 2 for the listing of patient outcomes.

All radiologic outcomes were determined by viewing MRI imaging of the patient's central nervous system before and after treatment and reviewing the pertinent radiologic reports.

Of these 10 patients, two (#2 and #8) had radiation encephalopathy, and one (#5) had radiation myelopathy. While one of the patients with radiation encephalopathy reported improvement in symptoms, none of the three showed an improvement in clinical or radiologic outcomes.

Of the seven patients who were diagnosed with radiation necrosis, three (#1, #9, #10) reported subjective improvement in their symptoms. For Patient #1, his clinical assessments remained stable following HBO_2 treatment. Patients #1 and #9 also showed improvement in their radiologic imaging, while Patient #10 had no change in imaging. Of the remaining four patients with radiation necrosis (#3, #4, #6, #7), all reported worsening of their symptoms. Patient #4 had improved radiologic images, while Patient #6 had no change. Of note, only two of these four patients (#6 and #7) underwent a full course of HBO_2 therapy.

Five of the 10 patients (#2, #4, #7, #8, #9) had a history of seizure disorders. This may have affected these patients' outcome because of possible previous central nervous system damage. Having had a history of seizure disorder, these patients also underwent HBO_2 therapy at 2.0 atm abs rather than 2.4 atm abs, which may have affected the efficacy of the treatment.

Seven of the 10 patients (#2, #4, #5, #6, #7, #8, #10) were receiving concurrent steroid treatment at the time they underwent HBO_2 therapy. Steroid treatment has been recommended as a treatment option for radiation injury to the central nervous system. Therefore, these patients were receiving co-treatment with steroids and HBO_2 This may have influenced the outcome for these patients and makes it more difficult to determine the true effect of their HBO_2 treatments, although common practice dictates that the use of steroids is not known for resolution of central nervous system pathology, but rather only amelioration of symptoms.

Five patients (#2, #5, #6, #7, #8) were receiving concurrent chemotherapy during their HBO_2 treatments. Patients #5 and #6 received chemotherapy that was a relative contraindication to HBO_2, and therefore underwent treatment pressures of 2.0 atm abs instead of 2.4 atm abs. This may have influenced their outcomes by changing from the standard procedure.

These 10 patients represent 1.3% of all the patients treated with HBO_2 therapy during the time frame studied.

Following we describe the cases and their salient features.

CASE 1: A 71-year-old male with recurrent posterior fossa meningioma

He underwent resection and radiation therapy on two occasions, 1999 and 2004 (62 and 50 Gy). He later presented with progressive dysarthria, headache and decreased left arm coordination. Karnofsky score of 60% and ECOG score of 2 at this time. MRI imaging of the brain showed "multifocal and confluent white matter changes ... in bilateral frontoparietal and right temporal regions," described as leukoencephalopathy. He was diagnosed with radiation necrosis of the brain.

At a time approximately 24 to 36 months after receiving radiation, he underwent three 30-treatment courses. At the end of three rounds of treatment, patient showed improvement with motor, oral and gait function. Following the HBO_2 he did have improvement in his ability to ambulate and use his upper arms. His Karnofsky score was 50-60% and ECOG score was 2 to 3. MRI after his treatment showed stable mass effect. He died from what was determined to be progressive tumor formation 25 months after completion of his hyperbaric treatment.

CASE 2: A 54-year-old male with an ACTH-secreting pituitary adenoma

The tumor was resected and the patient received external radiation treatment (50.4 Gy). He underwent 30 HBO_2 treatments at 2.0 atm abs (due to a history of seizures) for vision loss in his left eye in 2006, with reported improvement. He underwent repeated tumor resection twice more. Following resection he continued to have decreasing vision in his left eye. An MRI of his brain showed an enhancing mass with possible ischemia. Sixty-four months after his radiation treatment, he underwent two treatments for 90 minutes each at 2.0 atm abs. After his two sessions, he noted that his vision had not changed. An MRI showed "encephalomalacia in the left temporal pole." Because of the lack of improvement, the patient did not feel that HBO_2 therapy was adequate and decided to cease treatment, thus not undergoing a full treatment course. In the following months, his Karnofsky score decreased from 70% to 30% and his ECOG score increased from 2 to 3. The patient died seven months after receiving therapy, without a reported cause of death.

CASE 3: A 50-year-old male with von Hippel-Lindau syndrome

The patient underwent gamma knife therapy for a right cerebellar hemangioblastoma (70 Gy). He later reported left hand ataxia and paresthesia, left leg paresthesia and right arm ataxia. He also complained of intermittent dizziness and light-headedness. His Karnofsky score was 70% and ECOG score was 2 to 3. MRI showed enhancing lesions in the right pons and posterior-superior right cerebellum and stable lesions in a second right cerebellar lesion. 25 months after his gamma knife therapy, he underwent 28 treatments of HBO_2 at 2.4 atm abs for 90 minutes. After his therapy ended, he reported worsening left hand paresthesia and weakness, as well as worsening difficulty in walking. His physician noted "[Patient] is unsure if HBO_2 therapy really helped." His Karnofsky score decreased to 50-60% and his ECOG score remained 2 to 3. MR imaging showed that a posterior lateral right pons lesion was larger, with a cystic component, as well as a second lesion in the posterior right lateral fourth ventricle that had become larger. There was also a new lesion in the lateral right cerebellar hemisphere. However, the scan also showed that a different lesion in the right cerebellar hemisphere was smaller. He died 25 months after receiving HBO_2 therapy, and a cause of death was not reported.

CASE 4: A 68-year-old male with a recurrent atypical meningioma

The patient underwent resection of a left-sided meningioma with subsequent re-resection 15 years later. His surgery was complicated by infections and required revision. He had an additional recurrence that was treated with tomotherapy and radiation on two occasions, 2005 and 2007 (54 and 35 Gy). He later presented for decreased mental status and weakness. His clinical assessment showed a Karnofsky score of 50-60% and an ECOG score of 2. An MRI showed "increased irregular and heterogeneously enhancing mass in left frontal lobe, extending to the ventricular surface, probably due to radiation necrosis." At 32 months after completing radiation treatments, the patient underwent 19 treatments of HBO_2 therapy at 2.0 atm abs (history of seizures) for 90 minutes each. After his treatment, his function continued to decline. He could no longer bear weight or feed himself and his language was declining. Repeat Karnofsky score was 30% and ECOG score was 4. MRI revealed "hyperintense on long-TR and hypointense on T1 lesion ... unchanged from the prior scan." At 52 months after his treatment concluded the patient was noted to be alive. While performance status and subjective assessment could have easily been evaluated with a patient visit or phone call for this patient – and others who were noted as being alive at the time of the study – the IRB protocol stipulated against this.

Case 5: An 81-year-old female with esophageal carcinoma

She underwent chemotherapy and external radiation treatment (50.4 Gray). She developed sensory changes in her lower extremities, weakness and difficulty walking, and bowel and bladder incontinence. MRI of her cervical and thoracic spine revealed "diffuse fatty marrow replacement consistent with radiation changes from T5 through T12 levels." Twelve months after receiving radiation, she underwent 30 treatments of HBO_2 for 90 minutes at 2.0 atm abs (concurrent chemotherapy treatment with carboplatin and taxol). After her treatment she reported that her lower extremity numbness and weakness had remained the same, and she continued to have bowel and bladder incontinence. Post-HBO_2 Karnofsky score was 50%. Repeat MRI during the course of her treatment showed expansion of the radiation myelitis with "diffuse fatty marrow replacement of the vertebral bodies from T5 through L1 consistent with radiotherapy changes." She died four months after her therapy ended.

CASE 6: A 56 year-old female with metastatic lung cancer to the brain

The patient underwent whole-brain radiation with gamma knife treatment, and repeated whole-brain radiation (50.4 and 10.8 Gy, respectively). She developed bilateral vision loss. Karnofsky score was 90-100% and ECOG score was 0-1. MRI showed "focal enhancement without appreciable swelling or T2 signal abnormality within the intracanalicular portion of the left optic nerve anterior to the chiasm." Nine months after her gamma knife treatment, she was referred for HBO_2 therapy for radionecrosis of the right and left optic nerves. She underwent two sets of 30 HBO_2 treatments at 2.0 atm abs (concurrent chemotherapy with carboplatin, pemetrexed, gematabine and taxol) for 90 minutes, one set for each eye. After completing her therapy, she developed dysphagia and dyspnea. No post-HBO_2 Karnofsky score was reported, but she was admitted to the hospital for pneumonia. MRI showed stable changes in the periventricular white matter and decreasing enhancement and size of the right cerebellar lesion. 54 months after her treatment concluded the patient is noted to be alive.

CASE 7: A 43-year-old female with metastatic lung cancer to the brain

The patient underwent whole-brain radiation and gamma knife treatment (40 and 21 Gy, respectively). She had increasing headaches and gait imbalance, as well neuropsychiatric changes, which included depression, insomnia and forgetfulness. Karnofsky score was 80-90%. MRI showed stable metastases in the right frontal and occipital lobes, as well as increased vasogenic edema around both lesions. These findings were diagnosed as radiation necrosis of the brain. At 24 months after receiving radiation, she underwent 30 HBO_2 treatments at 2.0 atm abs (history of seizures) for 90 minutes. There is no known data on her current clinical situation and no reported assessment scores. MRI revealed increasing size of a lesion in the right anterior frontal lobe, and increased mass effect on the right frontal horn with increased compression. At 66 months after completing her treatment course, the patient is noted to be alive.

CASE 8: A 64-year-old female with metastatic ovarian carcinoma to the brain

The patient underwent two rounds of whole-brain radiation, one of them occurring in 1991 (outside the study parameters) and the other in 2010 (30.6 and 38.5 Gy, respectively). She later developed seizures, dysautonomia, worsening dementia, changes in cognition and speech, and difficulty with coordination. No clinical assessment tools were noted in her chart by her physician. An MRI revealed stable encephalomalacia in the right cerebellar hemisphere and "multiple scattered lesions are also noted in the brainstem." She was diagnosed with radiation necrosis of the brain. At 24 months after receiving radiation therapy, she underwent 30 treatments of HBO_2 therapy for 90 minutes each at 2.0 atm abs (history of seizures). Following treatment, she reported some improvements in gait and cognition. However, she continued to have decreased cognition, seizures and dysautonomia. Her neurologist did not report any clinical assessment scores, but the progress note stated "no apparent positive effects of hyperbaric treatment." MRI showed that the lesions in her cerebral hemispheres and pons were stable. She died seven months after her HBO_2 treatments concluded, with cause of death annotated in the record as "likely due to mass effect of the tumor."

CASE 9: A 38-year-old female with an arteriovenous malformation of the left MCA

The patient underwent gamma knife treatment (21 Gy). She developed seizures, progressive headaches, gait imbalance, dysphagia, right-sided weakness and memory decline. MR spectroscopy reported "worrisome for radiation necrosis at the site of prior radiation therapy," and she was diagnosed clinically with radiation necrosis of the brain. 12 months after her gamma knife therapy she received 30 HBO_2 treatments at 2.0 atm abs (history of seizures). Throughout her treatments, she continued to report that she was having "seizure-like" episodes, but no seizures were ever observed in the hyperbaric chamber. Repeat MRI showed "maturation and involution of the post-treatment effects with decreasing T1 shortening and postcontrast enhancement in the left posterior frontal lobe." While she reported some resolution of her symptoms, she continued to have seizures, headaches and gait imbalance. It was determined that these symptoms were not caused by worsening radiation necrosis because of improved imaging findings. At 65 months after treatment concluded, the patient is noted to be alive.

CASE 10: A 63-year-old male with a right optic nerve sheath meningioma

The patient was treated with external beam radiation (40 Gy). He developed blurry vision, proptosis and increased intracranial pressure. He was diagnosed with radiation optic neuropathy. He underwent 30 HBO_2 treatments at 2.4 atm abs for 90 minutes at a time, 19-31 months after completing radiation treatment. Following therapy he reported that he was having fewer symptoms. However, his ophthalmologist reported "ongoing ischemic disc swelling and secondary retinal edema." The ophthalmologist also noted that the patient continued to have increased intracranial pressures, as well as difficulty with color plates. Repeat MRI reported no change to the right optic nerve sheath meningioma. At 74 months after receiving treatment, the patient was noted to be alive.

DISCUSSION

Nearly the entire population of patients who have been diagnosed with radiation-induced central nervous system injury are those who have undergone radiation to the head and neck for central nervous system tumors. As the field of radiation oncology continues to grow and the use of radiation expands, it will be important to have treatments in place for patients who suffer from the side effects of their treatments. Each of these patients adds new data regarding radiation necrosis of the central nervous system. Therefore, any information that we are able to glean from these patients is useful and viable information to the fields of radiation oncology and hyperbaric medicine. It is important to continue to add data to the research banks in order to determine what the best treatment options are for our patients.

The only major therapy currently available to those with radiation-induced central nervous system injury is steroids [4]. Steroid treatments have a very long history of serious side effects and disadvantages, including weight gain, soft tissue breakdown and immunodeficiency. As this option may not be available to many patients with other co-morbidities, there is a definite need to find a new or adjunct treatment option.

There are two types of injuries that we must consider when discussing post-radiation injury to the central nervous system: radiation encephalopathy, often seen as leukoencephalopathy, and radiation necrosis. We found that HBO_2 therapy proved useful for those patients with radiation necrosis of the central nervous system, but was not beneficial in cases of radiation encephalopathy of the central nervous system. Given this difference, it is imperative to distinguish between these two diagnoses, as the pathologic cause and radiologic findings are significantly different.

Radiation encephalopathy of the central nervous system is a disease characterized on swelling and edema of the brain [11]. On radiologic imaging, this disease process may be noted by a lack of enhancement on MRI, although white matter changes are often visible. Because encephalopathy is not due to tissue degradation and inflammation, we believe that patients would not receive any benefit from HBO_2 therapy. Looking at the patients in this study and their outcomes, we see that this was indeed the case.

Radiation necrosis of the central nervous system, on the other hand, has a completely different underlying pathology. The inflammatory reaction and subsequent necrosis of the soft tissue in radiation necrosis is an ongoing problem [2]. Once a single brain cell undergoes necrosis, signals from that cell will cause other cells nearby to start dying also. Radiologically, necrosis of the central nervous system may appear as enhancement on T1-weighted images, but it is indeed more common for enhancement on T1 to be related to active cancer. Because this process is mediated by

inflammation, we believe that HBO_2 therapy would be of benefit to these patients. As our results show, those patients who had a diagnosis of radiation necrosis of the central nervous system did show, at the very least, significant subjective improvements.

We believe that HBO_2 therapy can be a useful new treatment option for patients. One important benefit of HBO_2 therapy is the fact that the treatment regimen can be implemented on an outpatient basis. This makes the regimen easier to work around, especially for a treatment regimen that requires 30 days. Also, because there is no admission to the hospital, the cost of treatment is kept to a minimum. Lastly, for most patients HBO_2 treatments are covered by insurance plans, which also make the treatment more tolerable to patients from a financial standpoint.

One must take a moment to understand the importance of oxygen in rectifying diseased tissue. Oxygen is the element that drives angiogenesis and fibroplasia in radiated tissue and it does so in a gradient-dependent manner. This was determined by earlier studies in which non-radiated but wounded tissue was treated with various oxygen dosing [12]. Radiated tissue does not spontaneously revascularize, as do other wounded tissues because of the unique physics and pattern of tumoricidal radiation delivery [12]. This is, in fact, due to only shallow oxygen gradients being created. Therefore, the physiochemical response which identifies an injured area as a wound does not develop, and the body, in a sense, does not recognize the radiation injury as a wound [12]. Thus, oxygen at clinically defined pressures may ultimately serve to produce gradients that are substantial for angiogenesis to occur even in tissue that originally was irreversibly damaged.

In light of what has been discovered during this study and other studies that have preceded the findings of these 10 patients, it is important to consider the course of radiation injury without HBO_2 treatment. Radiation-induced central nervous system injury, which is most often labeled as radiation necrosis of the brain, is a most feared complication associated with the treatment of brain tumors. From a neurological standpoint, the signs and symptoms that result are often progressive and can be difficult to distinguish from tumor recurrence. Radiation injury, as has been previously stated, can develop immediately after treatment, commonly resulting in transient worsening of neurologic symptoms and headaches [12]. This is presumed to be due to both leaky capillaries and edema in the tumor bed, which is frequently reversible with steroid therapy. The symptoms that develop within a few weeks of radiation treatments are usually reversible, often without treatment. However, radiation necrosis, which tends to develop months to years after radiation exposure, is usually irreversible with steroid treatment alone. Thus, if steroid treatment cannot reverse these complications, an alternative therapy such as hyperbaric oxygen should be given consideration. Given that endothelial cell damage and microvascular ischemia are considered to be part of the injury cascade seen in radiation necrosis, hyperbaric oxygen as therapy is valid [12]. If in fact this alternative therapy does not reverse the findings of necrosis, a substantial outcome that is of great advantage is the ability to prolong life of the patient. The question here, and that should be explored in future studies, is not simply the prolonging of life, but rather the quality of that prolonged life.

Time is also a factor that must be considered when deciding to begin hyperbaric medicine therapy. There have been studies that have postulated that treatment after radiation exposure should be delayed [13]. This is concerning and argumentative on many fronts, and there is not a large reservoir of research that proves immediate or delayed treatment is more advantageous. Future studies that include mechanism of control would be of great benefit, but the ethical consideration of which patients to delay treatment for and which to begin immediate treatment on would be a valid point of contention. One must continue to consider the fact that radiation treatment causes damage to arterioles, and thus blood flow and tissue health, and that this process is not immediately over once radiation treatments end [12]. In lieu of this, the argument arises in wanting to do the best one can for a patient at any given time. To date, there has not been any determination that immediate hyperbaric oxygen therapy after radiation treatment is not warranted, and our foreseeable contention is that it should be instituted for cases of radiation necrosis. If the patient is not harmed, and signs and symptoms that were manifested due to radiation damage are progressively relieved, then hyperbaric treatment is a modality that should be implemented. One must understand that one planned course of hyperbaric oxygen therapy may not be enough because of the lasting and progressive effects of radiation-induced central nervous system injury, and that future courses of hyperbaric oxygen therapy may need to be implemented.

The future of radiation-induced central nervous system injury is surely to be augmented with alternative treatments, and it appears that hyperbaric oxygen has the potential to be the standard protocol of choice when available due to its nearly non-existent side effect profile when compared to current steroid and antithrombotic use.

While these 10 patients will add valuable information to the knowledge base on radiation necrosis, more research must be generated. One area where we feel our research could be improved would be to investigate the effects of time on HBO_2 treatment outcomes. In our study, all of the patients received HBO_2 therapy after a significant period of time had passed since their radiation treatment. In a new study, we would propose to investigate whether HBO_2 therapy delays the onset of neurologic symptoms from radiation necrosis. This study would involve the use of HBO_2 in the immediate post-radiation period. We would then be able to compare the results from these patients to those in this study to determine if those patients who received HBO_2 therapy in the immediate post-operative period had improvement in neurologic function. If those patients did develop symptoms, we would be also be able to determine if those symptoms developed at a later time than for those patients who did not receive HBO_2 treatments immediately after their radiation exposure.

If it would be possible to create a randomized controlled trial of HBO_2 treatments in cases of radiation injury to the central nervous system, some changes from our protocol would be necessary. These changes would include separating out those patients with encephalopathy from necrosis using standard brain MRI as well as PET scans for all patients in order to decrease the variability of the disease pathology. We would also use a standard dosage of pressure for each patient, making certain to decrease co-morbidities such as seizure history, steroid use and chemotherapy treatments. Lastly, we would make certain that all patients had standard clinical assessments with Karnofsky and ECOG scores both pre- and post-HBO treatment, and a neurologist or neuroscientist blinded to the study would be used so that a more consistent evaluation process could be implemented.

Based on our results, we conclude that HBO_2 therapy can be used as a viable treatment option or as an adjunct treatment for patients with radiation necrosis of the central nervous system. However, patient selection is an important part of determining which patients are suitable candidates for HBO_2 treatments. All patients who are being evaluated for post-radiation central nervous system injury should undergo MR imaging to determine the specific nature of their disease. Those patients who do not have enhancement on their imaging should be diagnosed with radiation encephalopathy, and not recommended for HBO_2 therapy. Those patients whose imaging does reveal enhancement need to undergo further testing, as these changes can signify either necrosis or recurrent tumor growth. Because an MRI cannot distinguish between tumor recurrence and necrosis, these patients should ideally undergo a biopsy of the lesion to determine its origin. If this option is not available, the patients should undergo PET scanning. If the lesion is a tumor, then the PET scan will show hypermetabolic findings at the site; if the lesion is necrosis, the PET scan will show hypometabolic findings. Once the patient is confirmed either by biopsy or PET scan to have radiation necrosis, he or she can be referred for HBO_2 therapy. Any discussion involving HBO_2 therapy for post-radiation central nervous system injury must include a detailed discussion regarding the success of HBO_2 therapy and that the therapy may not be a completely effective tool. All patients who undergo HBO_2 treatments should be followed closely by their physicians for changes in subjective, clinical and radiological findings.

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Effects of scuba diving on vascular repair mechanisms

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ABSTRACT

A single air dive causes transient endothelial dysfunction. Endothelial progenitor cells (EPCs) and circulating angiogenic cells (CAC) contribute synergistically to endothelial repair. In this study (1) the acute effects of diving on EPC numbers and CAC migration and (2) the influence of the gas mixture (air/nitrox-36) was investigated. Ten divers performed two dives to 18 meters on Day (D) 1 and D3, using air. After 15 days, dives were repeated with nitrox-36. Blood sampling took place before and immediately after diving. Circulating EPCs were quantified by flow cytometry, CAC migration of culture was assessed on D7. When diving on air, a trend for reduced EPC numbers is observed post-dive, which is persistent on D1 and D3. CAC migration tends to improve acutely following diving. These effects are more pronounced with nitrox-36 dives. Diving acutely affects EPC numbers and CAC function, and to a larger extent when diving with nitrox-36. The diving-induced oxidative stress may influence recruitment or survival of EPC. The functional improvement of CAC could be a compensatory mechanism to maintain endothelial homeostasis.

INTRODUCTION

Healthy endothelial cells fine-tune vascular homeostasis by regulating vascular tone, cellular adhesion, thromboresistance, smooth muscle cell proliferation, and vessel wall inflammation [1]. Prolonged and/or repeated damage will ultimately exhaust protective mechanisms, resulting in loss of integrity and endothelial dysfunction [2].

Circulating endothelial progenitor cells (EPCs) are recruited from the bone marrow in response to endothelial damage and differentiate into mature cells with endothelial characteristics. There is substantial evidence that EPCs mediate vascular repair and prevent the progression of atherosclerosis [3]. Circulating angiogenic cells (CACs) are derived from the monocyte-macrophage lineage and are involved in endothelial repair [4]. Although CACs have low proliferative capacity and fail to form blood vessels *in vivo*, they contribute to vascular homeostasis in a paracrine fashion by producing angiogenic cytokines [5]. Patients at risk for [6] or with established coronary artery disease [7] have lower numbers of EPCs. In addition to this quantitative deficit, dysfunction of CACs correlates with impaired endothelial function [8]. Interventions that improve endothelial function, such as physical exercise and medical therapy with statins, are considered to have a potent EPC-mobilizing effect and to increase the angiogenic capacity of CACs [9-11].

Decompression sickness (DCS) is a potential problem for a growing number of professional and recreational divers. During diving, compressed air is taken up at high pressure and saturates tissues. When divers ascend to the surface, especially after a brisk rise, this gas is partly released into the circulation and forms bubbles. The latter are commonly considered the main cause of DCS [12]. However, divers with no bubbles or a low bubble score, assessed with ultrasonic scanning, have been shown to develop DCS, whereas – conversely – divers with high bubble scores may have no symptoms.

Madden LA, *et al.* hypothesized that these gas bubbles are not the causal mechanism of DCS, but should rather be regarded as an exacerbating factor [13]. Indeed, a single scuba dive (self-contained underwater breathing apparatus) with compressed air acutely induces vascular oxidative stress, leading to transient endothelial dysfunction [14]. Breathing oxygen at higher-than-normal pressure increases the oxidant status in the circulation [15-17]. Superimposed on oxidative stress, bubbles may aggravate endothelial dysfunction by damaging the vascular endothelium through ischemia/reperfusion through physical contact with the endothelial cell layer or by an increase in shear stress. During recovery after hyperoxia, an increase of hypoxia-induced factor expression occurs in human umbilical vein endothelial cells, associated with an increase of matrix metalloproteinases activity, suggesting that endothelial cells "interpret" the return to normoxia after hyperoxia as a hypoxic stimulus [18].

The nitrogen concentration in compressed air (78%) limits bottom time, requires a longer surface interval between dives and subsequently reduces the number of dives that can be performed in a day. Other gas mixtures, such as enriched air, also called nitrox, contain higher-than-normal oxygen levels and, consequently, less nitrogen. Diving with nitrox reduces nitrogen uptake in tissues and thereby extends dive time and reduces bubble formation during decompression. On the other hand, stringent depth limits need to be respected to avoid oxygen toxicity while diving with nitrox.

In this study, the aim was to determine the acute effects of consecutive deep dives on endothelial repair mechanisms (EPC numbers and CAC migratory capacity). Secondly, the effects of air and nitrox dives by using similar no-decompression diving profiles in the same cohort of divers were compared.

METHODS

Study population

Ten male non-smoking military scuba divers participated in this study, which is part of a larger project and described in detail elsewhere [19]. All subjects were highly experienced divers with more than 1,000 hours of diving with both air and technical gases (nitrox or trimix). Each method and potential risks were explained to the participants in detail, and all subjects gave their written informed consent. The experimental procedures were conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the University of Split School of Medicine.

Dive protocol and timeline of measurements

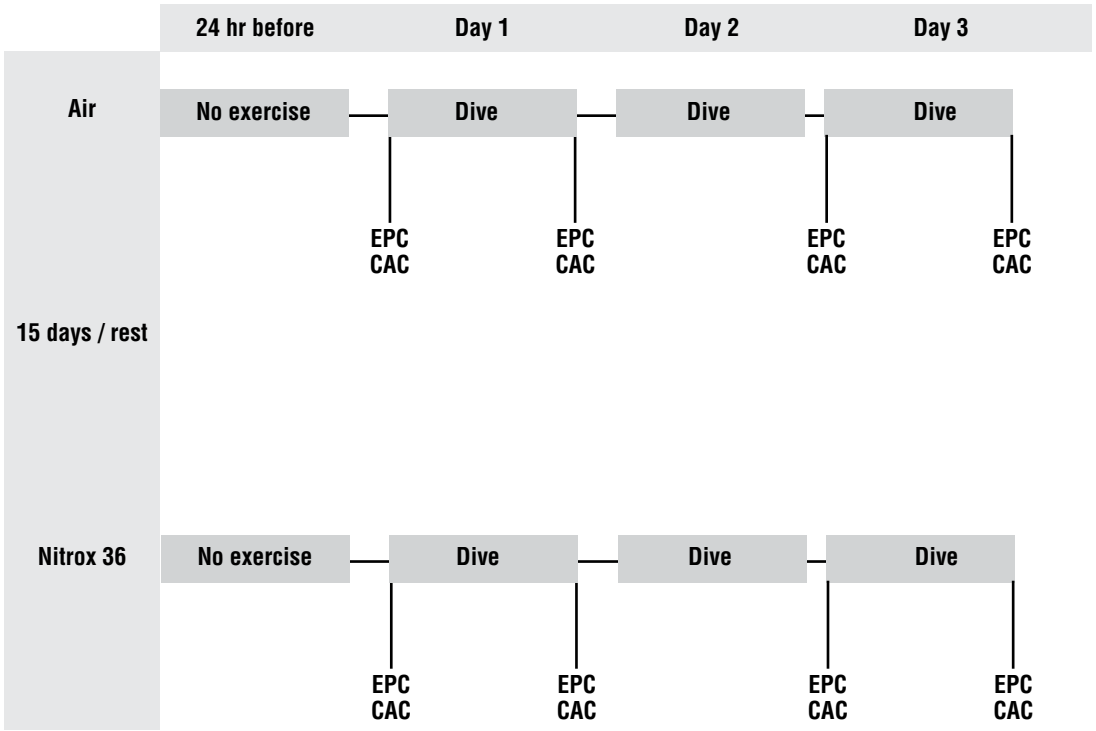
Divers performed three consecutive dives with air and three consecutive dives with nitrox-36 (36% oxygen and 64% nitrogen) as breathing gases, with two weeks' break between the air and nitrox-36 dives (Figure 1). All dives were no-decompression to 18 meters of sea water, with 47 minutes of bottom time. Water temperature was 20 ± 3°C at the surface and 16 ± 1°C at the bottom. The divers refrained from exercise 24 hours before diving since pre-dive exercise was shown to influence physiological parameters post-dive [20].

In vitro assessment of endothelial function was performed on the first and third dive of each diving series. Venous blood was sampled pre- and post-dive at an onshore diving facility. Blood samples (20 ml) were collected in acid citrate dextrose tubes. The first 3 ml of blood were discarded in order to prevent contamination with circulating endothelial cells due to vascular trauma [21]. Samples were processed immediately or stored at 4°C and analyzed within two hours.

Quantification of CD34+/KDR+/CD45- progenitor cells

The number of circulating EPCs (defined as CD34+/KDR+/CD45- cells) was determined by flow cytometry in whole blood samples. Briefly, 200 µl of whole blood was pre-treated with an FcR blocking reagent (Miltenyi Biotec, Bergisch Gladbach, Germany) and incubated with anti-KDR-PE (R&D Systems, Minneapolis, USA), anti-CD34-PC5 (Beckman Coulter, Marseille, France) and anti-CD45-ECD (Beckman Coulter, Marseille, France) antibodies for 30 minutes at 4°C. A dump channel was created on CD45+ cells to exclude leukocytes. SYTO 13 (Molecular Probes, Invitrogen, Eugene, USA) was used to eliminate non-nucleated cells. After red blood cell lysis with ammonium chloride (Stem Cell Technologies, Vancouver, Canada), 106 events were recorded on a Coulter Epics XL flow cytometer (Beckman Coulter Corporation, Miami, USA). Fluorochrome and isotype-matched controls as well as unstained cell samples were measured and processed as negative controls. The numbers of CD34+/KDR+/CD45- cells were analyzed in the lymphocyte region using FACS Diva software and expressed as positive events/10^6 total events.

FIGURE 1: Study design



Divers performed three consecutive dives with air and three consecutive dives with nitrox-36. In between these diving series, they refrained from diving for 15 days. The divers avoided strenuous exercise 24 hours before diving. Numbers of circulating endothelial progenitor cells (EPC) and function of circulating angiogenic cells (CAC) were evaluated pre and post-diving, on the first and third dive of each diving series.

CAC culture and characterization

Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation with Histopaque (Sigma-Aldrich, Saint Louis, Mo. USA) from 20 ml of peripheral blood. PBMC were cultured in Endothelial Growth Medium-2-MV (EGM-2-MV, Clonetics, Lonza, Walkersville, Md. USA), supplemented with 20% fetal bovine serum (FBS, Invitrogen, Eugene, Ore. USA). EGM-2-MV is composed of Endothelial Basal Medium (EBM)-2, supplemented with hydrocortisone, hEGF, VEGF, hFGF, R3-IGF-1, ascorbic acid and gentamicin-amphotericin B. Cells were seeded on 24-well dishes (BD, Franklin Lakes, N.J. USA), manually coated with human fibronectine (Invitrogen, Eugene, Ore. USA), at density of $1x106$/well. After 48 hours in culture, non-adherent cells were removed by thorough washing with phosphate buffer saline (PBS), and the adherent cells were maintained for five additional days. On Day 7, CAC migratory activity was evaluated.

CAC migratory capacity

At Day 7 of culture in a 24-well dish, growth medium was changed to EBM-2 supplemented with 0.5% bovine serum albumin (BSA, Invitrogen, Eugene, Ore. USA) in order to starve CACs. After three hours, CACs were harvested using PBS/ EDTA by repeated pipetting. In the upper chamber of a transwell with a pore size of 5 μm (Corning Costar, New York, N.Y. USA) $2x105$ cells were placed. In the lower chamber, vascular endothelial growth factor (VEGF, 50 ng/ml, R&D systems, Minneapolis, Minn. USA) and stromal cell-derived factor (SDF)-1α (100 ng/ml, R&D systems, Minneapolis, Minn. USA) were added to EBM-2/0.5% BSA. After four hours, viable CACs that had migrated to the lower chamber were counted manually using a hemacytometer. CACs counted in the negative control transwell (no addition of chemo-attractants) were subtracted from this number. Migrated CACs were expressed as percentage of total cell number added to the transwell (positive control).

Table 1: Subject characteristics

	n = 10
Male (%)	100
Age (yrs)	36.6 ± 2.3
Height (cm)	181 ± 1.7
Weight (kg)	94.4 ± 2.4
Heart rate (bpm)	61.6 ± 3.5
Systolic blood pressure (mmHg)	119 ± 2
Diastolic blood pressure (mmHg)	77 ± 2

Data are mean ± SEM

Statistical analysis

Continuous data are expressed as mean (± standard error of the mean, SEM). Normality of data was assessed using the one-sided Kolmogorov-Smirnov test. Due to non-normal distribution, EPC measures were normalized by natural logarithmic transformation prior to analysis. Pre- and post-exercise data were compared using paired sampled Student t-test. All tests were two-sided and a p-value < 0.05 was considered statistically significant. All analyses were performed using PASW Statistics 18.0 (SPSS Inc., Chicago, Ill., USA).

RESULTS

Characteristics of subjects and dives

Ten male non-smoking military divers with a mean age of 36.6 years were enrolled (Table 1). During participation in this study, none of the divers developed DCS.

Effect on numbers of circulating endothelial progenitor cells (EPCs)

Figure 2A shows the evolution of CD34+/KDR+ EPCs for each gas mixture separately. Diving with air led to a decrease in EPC numbers on Day 1, but this difference did not reach statistical significance (- 36%; p=0.39). The number of EPCs was not affected after diving with air on Day 3. Although diving with nitrox-36 had no effect on Day 1, EPCs clearly decreased following the dive on Day 3 (- 56%; p=0.019).

Effect on function of circulating angiogenic cells (CACs)

In Figure 2B, diving-induced changes in migratory capacity of CACs are depicted for air and nitrox-36 separately. Diving with air did not affect the chemotactic response of CACs towards VEGF and SDF-1α, neither on Day 1 nor on Day 3. A significant increase of 36% (p=0.036) could be shown when divers used nitrox-36 on Day 1. Repetitive diving with nitrox-36 did not lead to a further improvement.

DISCUSSION

The present study is the first to describe the acute effects of scuba diving on the numbers of circulating CD34+/KDR+ EPCs and on the functional capacity of CACs. Immediately after diving, numbers of EPCs were decreased, while the migratory capacity of CACs improved acutely. Both these effects were more pronounced when a nitrox-36 mixture was used compared to compressed air.

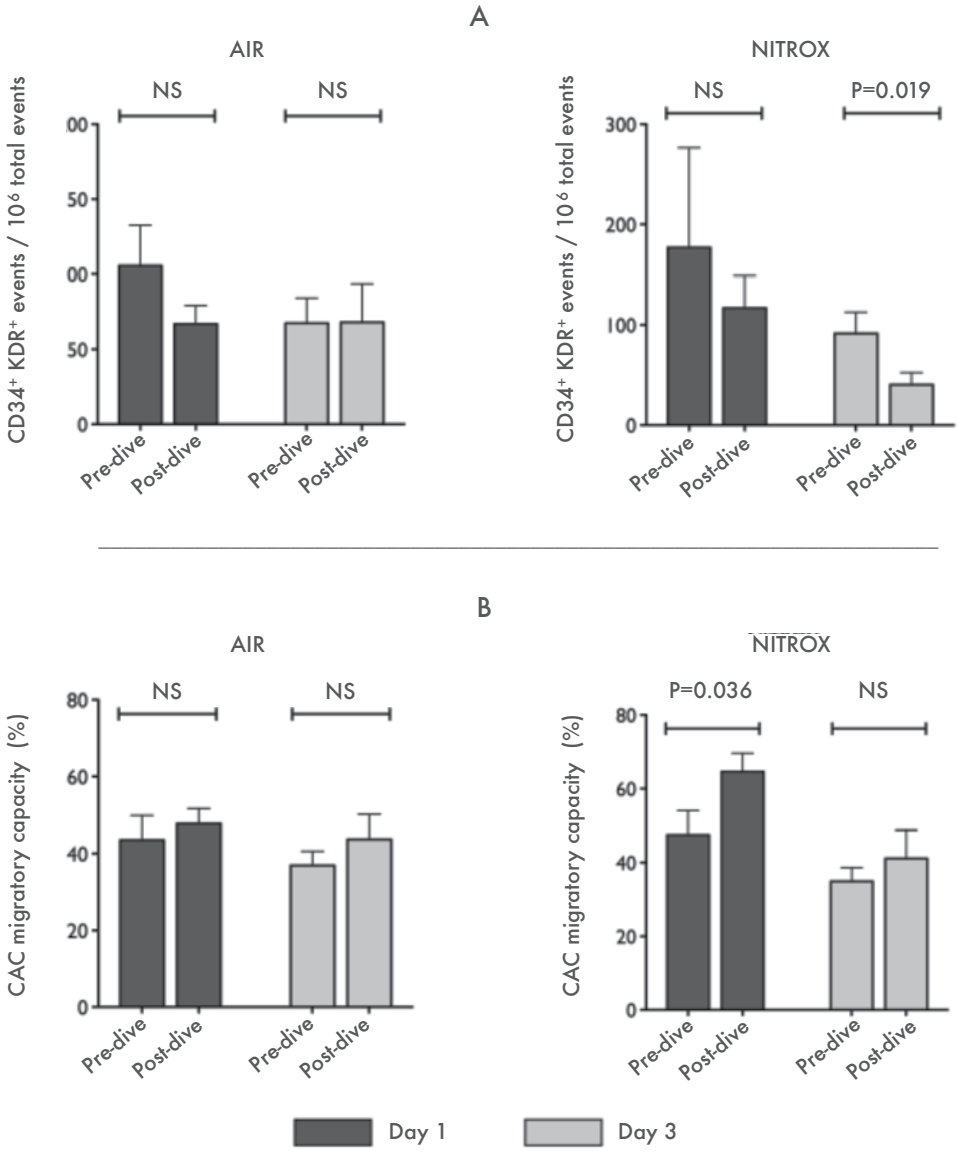
Effects of diving with nitrox on endothelial function

Impaired endothelium-dependent vasodilatation following a single scuba dive [14], does not fully recover after successive dives, indicating that repetitive diving may induce long-term alterations in vascular function [22]. Whereas the exact mechanisms that lead to endothelial dysfunction are still incompletely understood, hyperoxia-induced production of reactive oxygen species (ROS), reduction in the bioavailability of nitric oxide (NO) and direct mechanical damage to the endothelium during decompression, are considered to play an important role [23].

In order to increase dive time and to reduce bubble formation during decompression, a trend toward the preferential use of technical gases, such as nitrox-36, has developed during recent years. Diving with nitrox-36, however, increases the risk of oxygen toxicity, as the oxygen fraction in this mixture is higher compared to air. Breathing higher oxygen concentrations in hyperbaric conditions as well as breath-hold diving enhances the generation of reactive oxygen species [24-26].

These data were collected as a part of a larger study in which the effect of nitrox-36 and air dives on vascular function was assessed [19]. In that study, Marinovic, *et al.* reported that the diving-induced reduction in endothelial function was more prominent following nitrox-36-dives compared to air-dives, despite significantly lower venous gas bubble loads with nitrox-36 [19]. Although speculative, the higher production of reactive oxygen species (ROS) during nitrox dives and the subsequent scavenging of NO could have amplified this effect. However, neither administration of tetrahydrobiopterin (BH4), nor antioxidants, such as vitamin C, decreased pulmonary artery pressure after diving [27].

FIGURE 2: Acute effect of diving on circulating cells with vasculogenic potential



(A) Numbers of circulating EPCs are shown separately for air and nitrox-36 dives. Diving with air led to a decrease in EPC numbers on Day 1 but this difference did not reach statistical significance (- 36%; $p=0.39$). Diving with nitrox-36 had no effect on Day 1, but EPCs were reduced by 56% following the dive on Day 3.

(B) Migratory capacity of CACs was not altered when diving with air. A significant increase of 36% was observed with nitrox-36 on Day 1. Repetitive diving with nitrox-36 did not lead to a further improvement.

Data are mean ± SEM.

Effect of diving on endothelial progenitor cells

Apart from the delicate balance between NO and ROS production, endothelial homeostasis depends on efficient endothelial repair, a process in which bone marrow-derived cells with high vasculogenic potential are involved. Upon stimulation, EPCs are mobilized from the bone marrow into the circulation and are presumed to integrate into dysfunctional/damaged endothelial cell-layer of peripheral tissues.

Our group, as well as others, has shown that a single maximal exercise bout elicits an increase in the numbers of circulating EPCs in both healthy subjects and in cardiovascular patients [28,29]. Guerrero, *et al.* showed beneficial effect of exercise training on endo-thelial function in established hypertension in rats [30]. In the study by Fismen, *et al.*, heat stress, hyperoxia and dive affected several biochemical parameters that may play important roles in the mechanisms protecting against the adverse effects of saturation diving [31]. The high vascular oxidative load of a maximal exercise bout causes a temporary decrease in endothelium-dependent vasodilatation, which is followed by a substantial improvement 12 to 24 hours later [32]. Such an acute period of vascular stress appears to stimulate repair mechanisms, including the mobilization of CD34+/KDR+ EPCs, which could be considered as an adequate physiological response.

In contrast, the present study showed a clear decrease in EPCs immediately after a dive, an effect that was more pronounced when diving with nitrox-36. It is speculative that impaired NO bioavailability, resulting from the abundant generation of superoxide (O_2-) anions following nitrox diving and their reaction with NO to form peroxynitrite ($ONOO^-$), limit the process of EPC recruitment. Indeed, Laufs, *et al.* showed that EPC recruitment was blunted in eNOS knockout mice, confirming the central role of NO [33].

ROS-induced apoptosis of EPCs is another plausible mechanism for the observed decrease in EPCs. Tie, *et al.* demonstrated that addition of oxidized low-density lipoprotein to EPC cultures generates O_2- and H_2O_2, leading to apoptosis by inactivating the phosphoinositide 3-kinase/Akt pathway [34].

In addition, the release of pro-inflammatory cytokines in response to diving, may account for apoptosis in circulating EPCs [35]. Although data on the acute effects of diving on inflammatory cytokines are lacking in humans, it has been reported in rats that a decompression trauma acutely increased levels of interleukin-6 [36].

Effect of diving on circulating angiogenic cells

Several investigators have elucidated the role of CACs in re-endothelialization and neovascularization [5,37]. In the present study, a significant improvement in the functional properties of CACs after a single dive with nitrox-36 was observed, whereas diving with compressed air induced no changes. This could be regarded as a protective effect in an attempt to restore the injury at the level of the endothelium, elicited by a single dive.

CACs have been described to be extremely resistant to vascular oxidative stress. This particular feature may explain why, in contrast to the detrimental effect of ROS on EPC mobilization, the functional properties of CACs are not affected [38]. It has been shown for various cell types that migratory capacity towards SDF-1α is enhanced after priming with TNF-α [39]. This could be due to an increased CAC sensitivity to SDF-1α, by modulating CXCR4 signal transduction without affecting receptor expression [40]. Whether diving with nitrox-36 is associated with a larger release of pro-inflammatory cytokines compared to compressed air will be investigated in future experiments.

LIMITATIONS

The present study is limited by the small number of subjects included, which advocates cautious interpretation of the data. In this particular study design, however, each subject acts as its own control in pre- and post-dive effects and in evaluating the effects of the different gas mixtures, which strengthens the results. Moreover, the effect of diving with nitrox-36 was remarkably consistent in evaluation of endothelial repair mechanisms. Therefore, we think that the effects of diving on endothelial repair merits further study in a larger study cohort.

CONCLUSION

In the current study it is shown that diving reduced EPC numbers immediately after diving. In contrast, CAC migration tends to improve acutely following diving. Recruitment of EPCs may be hampered by reduced NO bioavailability or increased apoptosis. Nevertheless, the immediate functional improvement of CACs could contribute to maintaining endothelial homeostasis. Remarkably, these responses are more pronounced when diving with a nitrox-36 gas mixture, suggesting that such a mixture elicits more endothelial dysfunction.

PERSPECTIVES

Endothelial dysfunction is a risk factor for cardiovascular diseases and an independent prognostic marker of cardiovascular events [41]. Endothelial function diminishes with aging, even in healthy subjects. Since this study included young men, future studies should point out whether there is a differential effect of diving on endothelial repair mechanisms in older subjects.

According to some reports, more than two million people practice scuba diving worldwide. Although manifest cardiovascular disease precludes diving, cardiovascular risk factors (*e.g.*, hypertension, smoking, overweight and diabetes), may be commonly encountered in recreational divers. Numbers of EPCs are already decreased, and function of CACs is impaired in individuals at cardiovascular risk, which contributes to the development of cardiovascular disease [42]. As more patients with cardiovascular risk factors undertake diving, investigation of the implications of diving on endothelial repair in this specific population is of major interest.

The authors report that no conflict of interest exists with this submission. ■

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The relevance of magnetic resonance imaging in spinal cord decompression sickness: A survey of seven cases

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ABSTRACT

To investigate the magnetic resonance imaging (MRI) features of spinal cord decompression sickness (DCS) on compressed-air divers, we hereby report seven cases diagnosed with spinal cord DCS. Only two patients out of seven showed positive MRI findings: A detailed case report will be provided on each. In one of the cases, the MRI revealed extensive high signal within the central gray matter of the spinal cord. The other one showed patchy high signal on T2-weighted images as well as diffusion-weighted images (DWI) in the dorsal column white matter of the spinal cord. The findings in our collective suggest that the MRI focused on the spinal cord is not always appropriate for obtaining a quick diagnosis. The discrepancy between MRI findings and clinical evolution leads to the conclusion that MRI focused on the spinal cord does not always correlate with neurological improvement. Decision for hyperbaric oxygen (HBO_2) treatment should not be based primarily on MRI findings.

INTRODUCTION

Decompression sickness (DCS) is a clinical syndrome caused by an abrupt alteration in environmental pressure that results in the release of inert gas bubbles which had been dissolved previously in tissues into tissue or blood [1]. Spinal cord MRI of DCS has been reported by a few authors [2-5]; however, to our knowledge, few of these reports have described gray matter injury or the ischemia mechanism of the spinal cord on MRI. We encountered two patients out of seven cases who were diagnosed with spinal cord DCS, representing both of the features above and reported as following.

METHODS

We performed a retrospective analysis of seven consecutive divers referred to our hyperbaric center with symptoms indicative of diving-related spinal cord injury between May 2008 and May 2009. Of these patients, all were examined with MRI in our radiology department during the first one to six days after the accident (median four days).

Clinical diagnosis of spinal cord injury was made when the criteria of bilateral sensory and/or motor deficit were recognized after the diver surfaced. If needed, other characteristic symptoms consistent with involvement of the spinal cord in DCS, such as back pain or bladder dysfunction, were recorded. The overview of the 7 divers presenting spinal cord DCS is shown in Table 1.

MRI was performed using a 3.0-Tesla magnet (General Electric Co., Milwaukee, Wisc. USA). All examinations of the whole spinal cord included fast-spin-echo T1-weighted sequences in the sagittal plane, and fast-spin-echo T2-weighted sequences in the sagittal and axial planes. Spinal images were obtained with a CTL spin coil: Parameters were matrix 384 × 192, TE = 26 ms; TR = 2721 ms was used for T1-weighted images. Field of view depended on the the size of the examined area (cervical 24 × 24 cm, thoracic 36 × 36 cm, and lumbar 32 × 32 cm).

TABLE 1: Overview of the seven divers presenting with spinal cord DCS

Case/age	Diving depth	Bottom time	Clinical characteristics	MRI spinal lesion
1/33	23 m	60 minutes	After surfacing, blank out; 30 minutes later, tetraparesis and sensory loss T4 and bladder dysfunction	Positive (Figure 1) two days after the accident
2/38	27 m	150 minutes	After surfacing, loss of consciousness. 30 minutes later, paraparesis and sensory loss T10 and bladder dysfunction.	Negative three days after the accident
3/26	25 m	38 minutes	After surfacing, paraparesis, sensory loss T12 and bladder dysfunction	Negative two days after the accident
4/30	27 m	180 minutes	After surfacing, loss of consciousness. 30 minutes later, paraparesis, sensory loss T4 and bladder dysfunction.	Negative four days after the accident
5/35	38 m	100 minutes	After surfacing, paraparesis, sensory loss T11 and bladder dysfunction.	Negative three days after the accident
6/41	27 m	90 minutes	After surfacing, thoracic back pain, paraparesis, sensory loss T4 and bladder dysfunction.	Negative five days after the accident
7/45	28 m	100 minutes	After surfacing, paraparesis below T8 level and numbness of left upper limb.	Positive (Figure 2) six days after the accident

CASE REPORTS

Case 1

A 33-year-old male commercial diver, previously healthy, dived to harvest seafood using surface-supplied compressed-air light dive equipment. He made three dives to 23 meters of sea water (msw) three times for 30 minutes each, with a surface interval of 30 minutes between dives. After completing 30 minutes of his third dive, the diver made an abrupt ascent to the sea surface because he accidentally dropped his dive weights.

After surfacing, he experienced immediate cough and bloody sputum, followed by a two-hour blackout.

Six hours later, the patient was admitted to the hyperbaric medicine department of our hospital for recompression therapy. Physical examination showed his consciousness, cognitive status and cranial nervous reflexes were all normal. Neurological examination revealed motor function of both upper limbs was Grade 2/6, and motor function of both of the lower limbs was Grade 0/6.

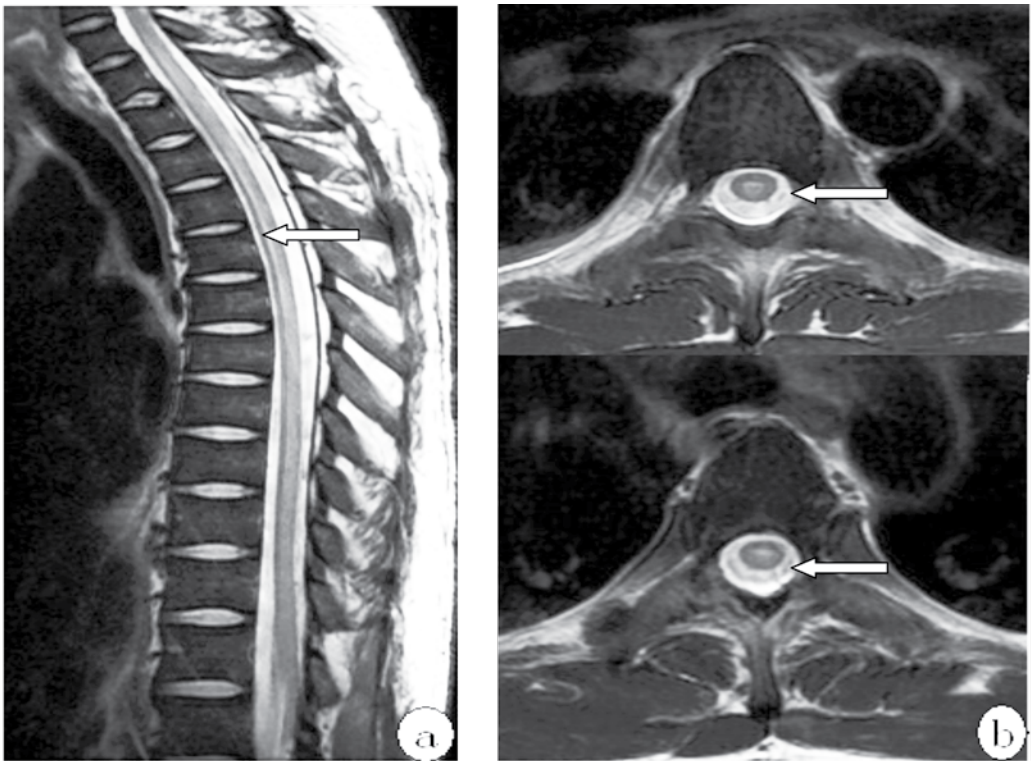
The sensation of the body below the nipples disappeared (T4 level). The urinary catheter was retained in place. Both Babinski signs were negative. After recompression therapy (U.S. Navy Treatment Table 6A), the patient received 2.5 atmospheres absolute (atm abs) of hyperbaric oxygen (HBO_2) therapy for 90 minutes on a daily basis for 16 days and improved neurologically. Motor function of both upper limbs was up to grade 5/6, but motor function of both lower limbs manifested no significant improvement. The sensation of the body below the umbilicus disappeared (T10 level). At 17 days after the accident, the patient finished his HBO_2 therapy and was discharged.

This case has a clarified diagnosis of spinal cord DCS. After the recompression therapy and auxiliary support, an MRI scan of the brain undertaken within 48 hours of the injury showed a return to normal. This patient made a partial recovery (Figure 1).

Case 2

A 45-year-old male, healthy commercial diver was referred to our department with motor weakness on both lower limbs that occurred after an uneventful dive to a maximum depth of 40 meters. Water temperature was approximately 15°C. He was digging for jewels using scuba diving equipment supported by compressed air from the surface. He performed three repetitive dives in total at the maximum depth of 40 meters for 30 minutes

FIGURE 1: MRI scan of Case 1 48 hours after the injury



a) Sagittal T2WI images revealed a high-signal intramedullary lesion in the central gray matter at the T1-12 level.
b) Axial T2WI showed a high-signal intramedullary lesion in the central gray matter.

per dive that day. After the last dive, he ascended to the surface without using a standard decompression table provided by his supporting crew. Fifteen minutes later, the diver began to complain about motor weakness and numbness in his lower legs. He received routine treatment with intravenous fluids in a local clinic, but his symptoms showed no improvement.

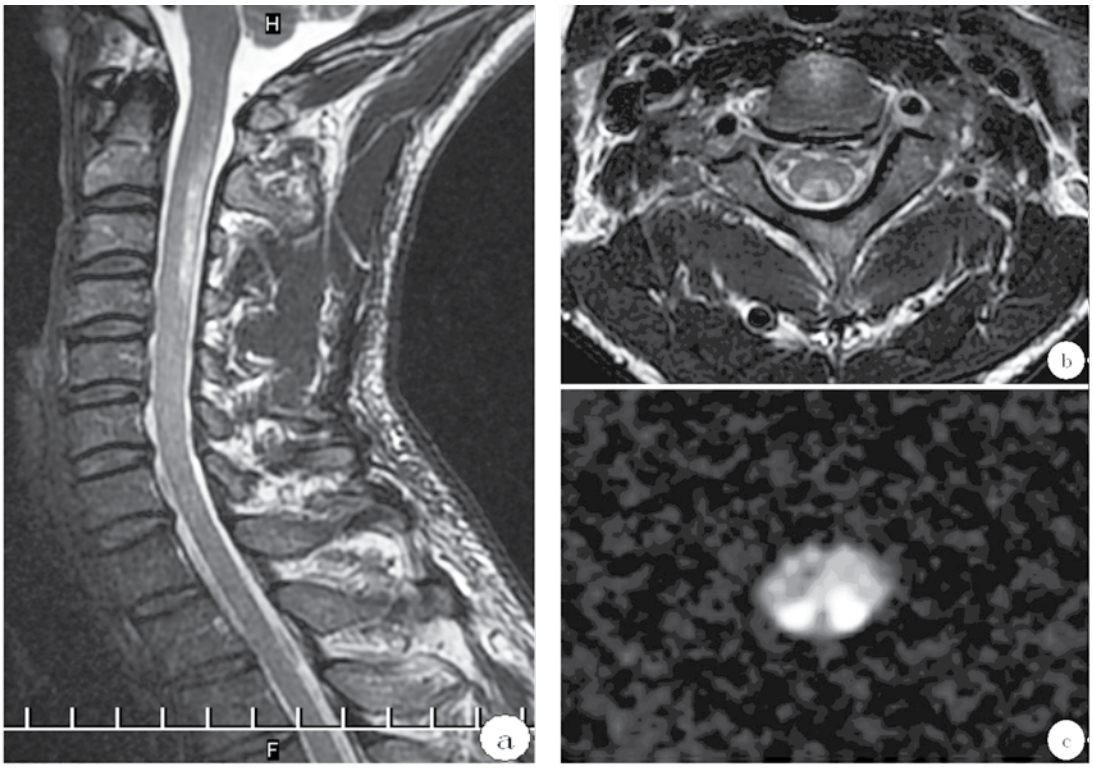
Six days after the accident he was referred to the department of hyperbaric medicine in our hospital. Physical examination showed his level of consciousness, cognitive status and cranial nervous reflex were all normal. Neurological examination revealed both upper limb motor function was grade 5/6 and both lower limb motor function was grade 3/6. The sensation 10 cm below the nipple line had disappeared (T6 level). The patient was received spinal cord MRI scanning (Figure 2) and then was put into in the recompression chamber (United States Navy Treatment Table 6A). After this delayed recompression treatment and hyperbaric oxygen treatment, which lasted for four sessions, the patient recovered upper limb motor function completely. He experienced partial recovery in lower limb motor function (grade 5/6), and he was discharged.

DISCUSSION

Neurological DCS affects the central nervous system. We had reported on the MRI features of seven cases who were diagnosed with of cerebral DCS [6], but MRIs of spinal cord DCS were difficult to obtain even if divers suffered from typical clinical manifestations of spinal cord DCS [3]. We hereby report on two cases of spinal cord DCS, Case 1 affecting gray matter and Case 2 affecting white matter.

The spinal cord is site of greatest concern with DCS, in which the most frequently affected areas include thoracic, lumbar and cervical regions. The white matter of the spinal cord is vulnerable because nitrogen is soluble in myelin [6]. Case 1 showed extensive high-signal lesions within central gray matter. This is very rare to see in our clinical practice or reported literatures. When Warren *et al.* [7] reported 12 cases

FIGURE 2: MRI scan of Case 2 six days after the injury



a) Sagittal T2WI images revealed a high-signal intramedullary lesion at the C1-4 level.
b) Axial T2WI revealed a high-signal intramedullary lesion at the C1-4 level.
c) Axial DWI image revealed a high-signal intramedullary lesion at the C1-4 level.

of spinal cord DCS patients, only three cases showed spinal cord abnormalities in MRI. These lesions on T2-weighted images showed patchy high signals on spinal cord white matter, but not on central gray matter.

Case 2, whose lesions involved the posterior column of spinal cord white matter, had shown high signal on T2-weighted MRI, which was consistent with previous reports [1-3]. Intriguingly, it also showed high signals on diffusion-weighted (DWI) imaging on the same sites, which has never been reported. This means that spinal cord edema and related ischemia may play an important role in the pathogenesis of the disease. Kei *et al.* [8] reported MRI features of spinal cord DCS-related venous infarction caused by dorsal white matter abnormalities, but in more severe cases, central gray matter was constantly involved. In this paper, Case 1 showed significant pathological changes in the gray matter of the spinal cord at the beginning; then after 16 days of recompression therapy, the MRI abnormalities disappeared regardless of his remaining symptoms and signs. This finding is similar to a previous document reported by Yoshiyama *et al.* [5] which confirmed that in early stage of the trauma, MRI abnormalities were much more significant than they were one month later, but the function of patient's central nervous system showed no corresponding improvements. These findings also confirmed the complexity of the pathophysiology of the disease.

In this paper, only two cases in seven total cases of spinal cord DCS patients showed postive readings with MRI imaging. It indicates that MRI's ability to detect spinal cord DCS is quite low, which was consistent with the reported literature [9]. We attributed this finding to the following reasons:

1. for mild DCS cases, MRI cannot clearly display spinal cord ischemic edema;
2. early intervention of hyperbaric oxygen treatment attenuates pathological changes of DCS and lowers the MRI positive rate;

3. for those suspected DCS cases, MRI of spinal cord injury may not be a prompt technique for diagnosis;
4. specific MRI scanning technique and reading skills may be required for experienced diving doctors.

In general, MRI is useful but not sensitive to spinal cord DCS. Doctors can use MRI as a tool to understand the mechanism and confirm the diagnosis of spinal cord DCS if the MRI is positive. Or, doctors can make the diagnosis based on clinical data other than MRI. Decision for hyperbaric oxygen treatment should not be based primarily on MRI findings.

The authors report that no conflict of interest exists with this submission.

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The impact of freediving on psychomotor performance and blood catecholamine concentration

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ABSTRACT

The aim of the study was to investigate the effects of breath-hold diving on divers' psychomotor performance and blood adrenaline, noradrenaline and lactate concentrations. Four male divers took part in the experiment. During the study the divers' choice reaction time as well as plasma concentration of adrenaline, noradrenaline and lactate were measured. The measurements were carried out before immersion (before a warm-up), three minutes after the dive, and 60 minutes after the dive. A reduction in the reaction time to audiovisual stimuli was found in three divers, three minutes after the dive. Diver 4, who broke his personal best record, had a longer choice reaction time at three minutes after the dive. The adrenaline concentration was lowered in Diver 1 and Diver 2, at three minutes after the dive. The adrenaline level in Diver 3 was relatively steady at all test measurements. In Diver 4, who broke his personal best, a twofold increase in adrenaline concentration was noted at three minutes after the dive. All examined divers revealed an increase in noradrenaline blood level at three minutes after the dive. The results of the study are of great practical value since disturbed reactions during freediving can put the diver at the risk of serious barotraumas.

INTRODUCTION

Reaction time and accuracy, appropriate decision-making and choice responses to different stimuli constitute selected characteristics of psychomotor performance. Psychomotor performance is a manifestation of the functioning of the nervous system and peripheral elements of the locomotor system. The majority of research on psychomotor performance has been carried out in laboratory conditions [1,2,3]; only a few studies were conducted during sports competition [4]. Literature on the subject lacks, however, works concerning changes in psychomotor performance and blood catecholamine concentration in extreme conditions of breath-hold diving.

Since the shift of the blood with oxygen to the brain and the heart during diving has been confirmed by researchers [5], we decided to examine the effects of freediving on divers' psychomotor performance. The time and accuracy of reaction to audiovisual stimulation is an important determinant of sport achievement and prevention against barotraumas for divers experiencing high hydrostatic pressure.

The existing knowledge on breath-hold diving indicates two fundamental challenges faced by freedivers: duration of apnea and diving under high hydrostatic pressure [6].

Another reason for undertaking research into freedivers' psychomotor responses is the so-called Taravana syndrome. This phenomenon, observed in professional pearl divers from French Polynesia, was first described in 1965. During one working day the divers made about 30-40 dives each to 30 meters deep. The average diving time was about two minutes, with the resting periods between dives up to six minutes. By the end of the day, 10% to 30% of those divers experienced a syndrome called "taravana," *i.e.*, "falling crazily" in Tuamotu Polynesian. Its mildest symptoms include confusion and visual abnormalities, but it can also lead to loss of consciousness upon immersion, paralysis or even death [7].

A study carried out on athletes during graded exercise revealed a curvilinear relationship between plasma adrenaline and noradrenaline concentration and choice reaction time [8]. These findings inspired us to investigate changes in the concentration of blood catecholamines and psychomotor responses in breath-hold divers.

TABLE 1: Divers' characteristics

Diver	age [years]	body height [cm]	body mass [kg]	years of practice [years]	diving time [seconds]	depth [meters]	personal best [meters]
1	34	184	86	5	80	43	54
2	33	175	80	9	85	28	30
3	27	182	104	8	80	30	34
4	31	174	86	3	58	31	31

Research has also shown that activation of the central nervous system at the plasma catecholamine threshold during incremental exercise does not exceed a level optimal for processing signals during measurement of choice reaction time [8]. It was yet another argument for undertaking research into psychomotor performance and its relation to changes in blood catecholamine concentration during breath-hold diving.

The aim of the study was to investigate the effects of breath-hold diving on divers' psychomotor performance and blood adrenaline, noradrenaline and lactate concentrations.

MATERIAL

Four male divers (body mass 89 ± 10.9 kg; body height 178.8 ± 5.0 cm; age 31.3 ± 3.1 years), who had been practicing freediving three to nine years took part in the experiment (Table 1). The small size of the sample was due to the fact that not many people practice this extreme sports discipline. The subjects were in good health and took no medication or stimulants during the experiment. Each diver was informed about the aim of the study and gave his written consent to participate in the experiment. The study was approved by the ethics committee of the University School of Physical Education, Wrocław, Poland, and conducted according to the Declaration of Helsinki.

METHODS

During the study the divers' choice reaction time as well as plasma concentration of adrenaline, noradrenaline and lactate were measured. The measurements were carried out before immersion (before a warm-up), three minutes after the dive, and 60 minutes after the dive. Plasma lactate was marked before as well as three, five and 60 minutes after the dive (Figure 1). The measurement at three minutes was made after a diver reached the measurement station on a pier. To avoid any extra physical load on emergence, each diver reached the pier with the aid of an underwater propulsion vehicle.

The tests were preceded with a 15-minute warm-up in the water. The water temperature at the depth of 30-43 meters was 6-7°C, and the air temperature 18°C. The experiment was carried out in Germany in a lake 60 meters deep. Divers' choice reaction time was measured with an APR reaction meter (UNI-PAR, Poland). The test protocol involved sending 20 audiovisual stimuli, including 10 positive stimuli (five yellow lights and five sounds), to which each diver was to respond, and 10 negative stimuli (five red lights and five green lights) requiring no response from the divers.

Depending on the type of positive stimuli, each diver was to respond as follows: to a yellow light (five times altogether) by pressing and depressing a switch button with the right thumb as soon as possible; to a sound (five times) by pressing and depressing a switch pedal with the left foot as soon as possible. The signals were sent in a non-rhythmic manner at one- to three-second intervals. Each signal was emitted for one second, and the entire program lasted 60 seconds. The part of the apparatus sending the signals was placed 3 meters opposite the subject's face.

During the psychomotor test each subject was sitting, holding two switch buttons in his hands, with his feet placed on two switch pedals. Before the choice reaction time measurement the divers were given precise verbal instructions and made a few trial responses, according to the specified protocol. Once a subject mastered the response technique, the real measurement of choice reaction time commenced at a signal "Ready, Go." The lactate level in the capillary blood was marked using a Lactate Scout meter (SensLab, Germany) with the measurement range of 0.5-25 mmol/l. The catecholamine concentration in venous blood plasma was measured with the radioenzymatic method.

RESULTS

Reaction time

A reduction in the reaction time to audiovisual stimuli as compared with baseline was found in three divers, three minutes after the dive. Their choice reaction time returned to baseline 60 minutes after the dive (Table 2). The choice reaction time was 110 milliseconds (ms) longer to each positive stimulus (i.e., 1100 ms longer

FIGURE 1: Graphic representation of the experimental session (including psychomotor tests)

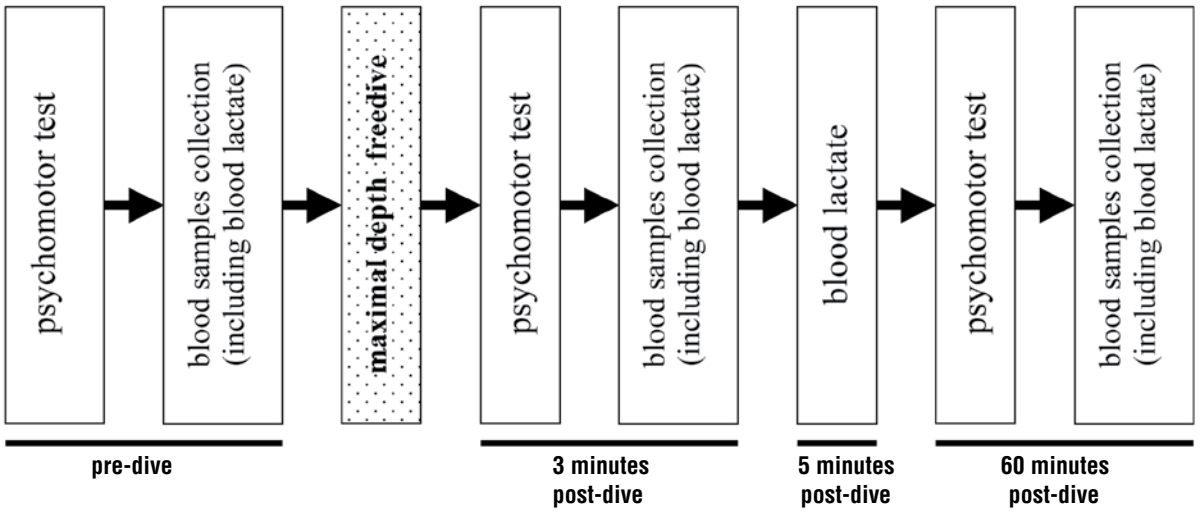


TABLE 2: Blood adrenaline and noradrenaline level and reaction time pre-dive, 3 and 60 minutes post-dive

Diver	measurement	adrenline	noradrenaline	reaction time
1	pre-dive	0.66	3.91	0.4
	3 min post-dive	0.53	4.01	0.34
	60 min post-dive	0.29	3.21	0.402
2	pre-dive	0.64	3.6	0.476
	3 min. post-dive	0.33	4.55	0.423
	60 min post-dive	0.3	3.31	0.48
3	pre-dive	0.24	1.46	0.396
	3 min post-dive	0.21	1.58	0.351
	60 min post-dive	0.27	1.14	0.401
4	pre-dive	0.27	1.45	0.362
	3 min post-dive	0.61	2.7	0.472
	60 min post-dive	0.41	1.43	0.407

Table 2a: Plasma lactate level at rest, at 3, 5 and 60 min post-dive

Diver	pre-dive	3 min post-dive	5 min post-dive	60 min post-dive
1	1.3	4.4	3.7	1
2	1	2.3	2.5	1.2
3	1	3.1	2.2	1.5
4	1.3	4.1	3.9	1.4

for 10 sent signals) in Diver 4, who broke his personal best. His reaction time was close to baseline 60 minutes after the dive but never returned to it. The time of response to each positive stimulus was 45 ms longer than baseline (Figure 3, Table 2).

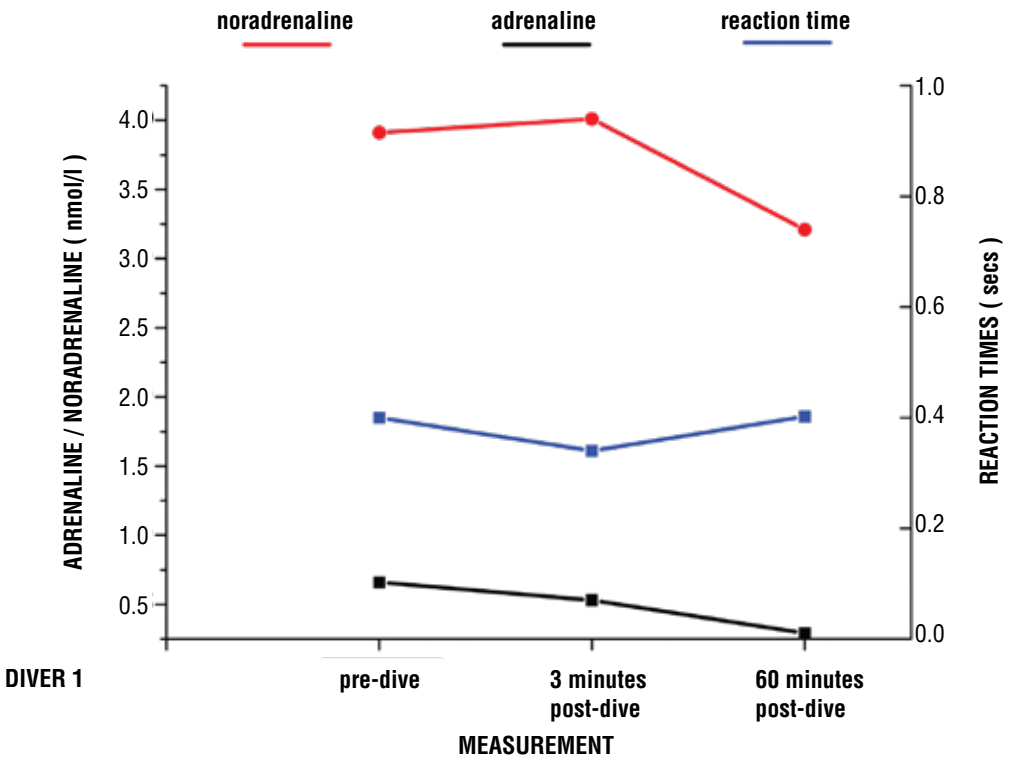
Adrenaline

The adrenaline concentration was lowered in Diver 1 and Diver 2, at three minutes and 60 minutes after the dive, as compared with baseline (Figure 2, Table 2). The adrenaline level in Diver 3 was relatively steady at all test measurements (Table 2). In Diver 4, who broke his personal best (Table 1), a twofold increase in adrenaline concentration was noted at three minutes after the dive, and a decrease at 60 minutes after the dive. After one hour the adrenaline concentration remained, in fact, higher than before the dive (Figure 3, Table 2). Three minutes after the dive a twofold decrease in adrenaline level was found in Diver 2 and a twofold increase in Diver 4. A twofold decrease in adrenaline concentration compared with baseline was noted in Diver 1 and Diver 2, at 60 minutes after the dive (Figure 2, Table 2).

Noradrenaline

All the examined divers revealed an increase in noradrenaline blood level at 3 minutes after the dive, and a return to the baseline level at 60 minutes after the exercise (Table 2). In Diver 4 a nearly twofold increase in the noradrenaline level was found as compared with baseline (Figure 3, Table 2).

FIGURE 2: Changes in choice reaction time and blood adrenaline/noradrenaline concentration pre-dive and 3 and 60 minutes post-dive in diver who reached the greatest depth



Plasma lactate concentration

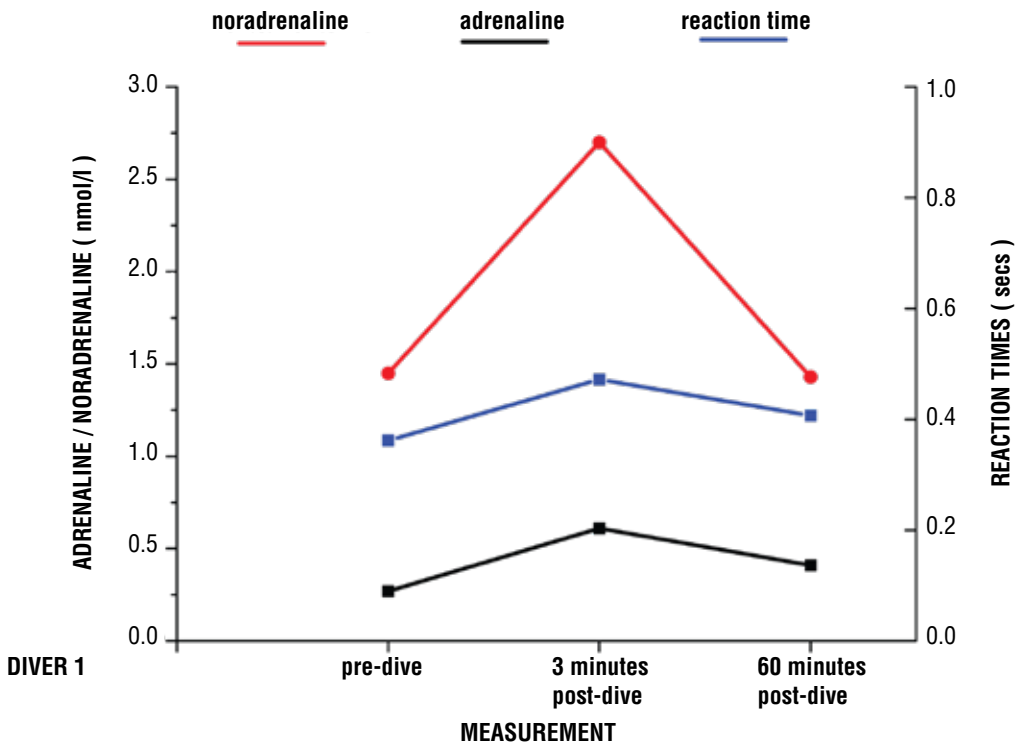
The plasma lactate level (LA) was found to increase from baseline in all the examined divers, at three and five minutes after the dive. The LA concentration was close to the starting level at 60 minutes after the dive. The highest LA was found in Diver 1, who reached the greatest depth (43 meters), followed by Driver 4, who surpassed his personal best (Table 2a).

DISCUSSION

Diver 4, who broke his personal best, had a longer choice reaction time at three minutes after the dive and experienced a twofold increase in blood catecholamine levels. Diver 1, who attained the greatest depth but did not break his personal best, had the greatest reduction in his choice reaction time.

The findings of the study indicate two tendencies of changes in reaction time following freediving. As for the diver who broke his personal best, reaction time, regarded as an index of psychomotor performance, was longer at three minutes after the dive, and at the same time his blood concentration of adrenaline and noradrenaline increased twofold. This could have resulted from the excessive stimulation of the central nervous system during breath-hold diving, especially of those cortical centers responsible for the time and accuracy of responses to audiovisual stimuli. The explanation of this phenomenon is difficult since the blood catecholamine concentration does not directly reflect directly the activation of the sympathetic nervous system [9]. The extreme conditions of high-performance freediving – *e.g.*, long apnetic time, limited visibility, low ambient temperature and hyperbaric stress – can stimulate the central nervous system excessively, leading not only to a prolongation of divers' reaction time but also to disorders of consciousness and the Taravana syndrome, similar to symptoms experienced by Diver 4. During the psychomotor test Diver 4 reported such symptoms as visual abnormalities (*i.e.*, seeing flashes and white spots), pain in the ears, problems with perception of auditory stimuli (sounds), problems with concentration and attention span, shivering and a pins-and-needles sensation all over the body. The diver's lips were also found to turn blue. It should be underlined that presented differences in reaction for freediving between Diver 1 and Diver 4

FIGURE 3: Changes in choice reaction time and blood adrenaline/noradrenaline concentration pre-dive and 3 and 60 minutes post-dive in diver who broke his personal best.



might be attributed to the hyperbaric pressure induced by different water depth achieved by divers.

The remaining divers who reached depths at 80%-93% of their personal best (28-43 meters) revealed shorter choice reaction times (45-60 ms) three minutes after the dive, and longer reaction times (50-62 ms) 60 minutes after the dive, as compared with baseline before diving. These results present an opposite trend to the results attained by the diver who surpassed his personal best. The observation of freedivers' choice reaction time indicates an optimal stimulation of the central nervous system while diving to depths below one's personal best. In the studied sample, the performance of Diver 1, who had the shortest choice reaction time (60 ms to each of the 10 audiovisual stimuli) as compared with baseline, clearly confirms this indication. Moreover, the responses of Diver 1 were the fastest, and he also reached the greatest diving depth (43 meters), which was at 80% of his personal best. The changes in choice reaction time of divers who reached the depth of 80%-93% of their personal best (from 28 to 43 meters) indicate a biphasic psychomotor performance, consisting of reducing (first phase) and prolonging (second phase) of choice reaction time. The fastest choice reactions were noted at three minutes after the dive. It would be interesting to assess the dynamics of psychomotor performance changes by introducing more measurements of reaction time after diving. Similar changes in choice reaction time, however, during incremental exercise, were reported in literature [1]. The shortest choice reaction time was noted at 70%-80% of maximal load, after which it was longer. The load corresponding to the shortest choice reaction time was described as the threshold of psychomotor performance deterioration.

The impact of hyperbaric stress on non-divers was previously discussed by McLellan, *et al.* (10), who noted a deterioration of reaction time in subjects under hyperbaric stress: for 296 ms at 20 minutes post-dive, and for 277 ms at 60 minutes post-dive. Those findings do not correspond to the results of the present study, where, in three out of four examined divers, their reaction time returned to baseline at 60 minutes post-dive. This discrepancy can be explained by different conditions of both experiments. McLellan, *et al.* (2010) carried out their experiment on non-divers

in a hyperbaric chamber, while the present study involved divers during breath-hold diving.

Hyperbaric stress accompanying freediving, especially in terms of the mechanical impact of high pressure on the diver's organ of hearing, seems to be a significant determinant of diver's reaction to auditory stimuli, being a component of psychomotor performance.

In freediving three possible injuries of the ear due to hydrostatic pressure can be distinguished: i) inner ear barotrauma; ii) outer ear barotrauma; and iii) middle ear barotrauma. The most frequent injury is middle ear barotrauma, *i.e.*, damage to the middle ear caused by the direct impact of high pressure on the eardrum during immersion. Its most dominant symptom is increasing pain in the ears, which also persists after diving [11,12].

In the present study the choice reaction time of the diver who broke his personal best was much longer while responding to auditory stimuli than to visual stimuli. This phenomenon has not been described in literature yet but can be caused by high hydrostatic pressure on the eardrum, which at the depth of 31 meters (the diver's personal best) amounted to about 3000 kPa. Such pressure can seriously disturb the hearing function and deteriorate responses to auditory stimuli.

Another issue worth examining is the level of metabolic processes during freediving. The plasma lactate level (LA) has been shown to increase during apnea compared with baseline at rest. The increase becomes more visible during freediving, which is an indication of anaerobic metabolism [13]. Divers with training experience of seven to 10 years display a lower LA level after immersion as compared with non-divers [14]. This is most likely caused by a reduced basal metabolic rate induced by specialist diving training [15]. This finding can serve as an explanation of low LA levels in divers in the present study with training experience between three and nine years.

It should also be noted that extreme divers display blunted ventilatory responses to the increasing carbon dioxide concentration in the blood and cerebrospinal fluid. It is most likely the result of multiple dives and associated apneas of long duration [7,16]. Appropriate adaptation allows divers to immerse with low oxygen partial pressure in arterial blood (< 30 mmHg) and high carbon dioxide partial pressure (> 50 mmHg).

During diving oxygen consumption decreases, while anaerobic glycolysis increases (the rate of blood lactate accumulation during diving is about 0.04 mmol/s) [7]. This observation was not confirmed in our experiment, as the LA level of the examined diver who broke his personal best was 4.1 mmol/l at three minutes post-dive, with the diving time of 58 seconds. The LA level of the diver who reached the greatest depth with the diving time of 80 seconds was 4.4 mmol/l at three minutes post-dive. These findings show that diving deeper is closely associated with a faster lactate accumulation rate.

A methodological shortcoming in the present study is, unfortunately, the small study sample. It is due to the relatively low number of extreme divers and, therefore, rather scarce accessibility of research material.

The findings of the study indicate three fundamental determinants of a diver's psychomotor performance during freediving: i) individual psychomotor characteristics; ii) diving depth; and iii) surpassing one's personal best.

The results of the study are of great practical value since disturbed reactions during freediving can put the diver at the risk of serious barotraumas. A high level of psychomotor performance allows executing appropriate compensatory movements, adjusting the immersion technique in response to specific audiovisual signals and successfully completing a dive during competition.

The authors report that no conflict of interest exists with this submission. ■

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A retrospective cohort study of lidocaine in divers with neurological decompression illness

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ABSTRACT

Lidocaine is the most extensively studied substance for adjuvant therapy in neurological decompression illness (DCI), but results have been conflicting. In this retrospective cohort study, we compared 14 patients who received adjuvant intravenous lidocaine for neurological decompression sickness and cerebral arterial gas embolism between 2001 and 2011 against 21 patients who were treated between 1996 and 2001 and did not receive lidocaine. All patients were treated with hyperbaric oxygen (HBO_2) therapy according to accepted guidelines.

Groups were comparable for all investigated confounding factors, except that significantly more control patients had made an unsafe dive (62% *vs*. 14%, $p = 0.007$). Groups had comparable injury severity as measured by Dick and Massey score (lidocaine 2.7 ± 1.7, control 2.0 ± 1.6), an adapted version of the Dick and Massey score, and the Blatteau score. Number of HBO_2 sessions given was comparable in both groups (lidocaine 2.7 ± 2.3, control 2.0 ± 1.0). There was neither a positive nor a negative effect of lidocaine on outcome (relative risk for objective neurological signs at follow-up in the lidocaine group was 1.8, 95% CI 0.2-16).

This is the first retrospective cohort study of lidocaine in neurological DCI. Since our study is underpowered to draw definitive conclusions, a prospective multicenter study remains the only way to reliably determine the effect of lidocaine in neurological decompression illness.

INTRODUCTION

Neurological decompression illness (DCI) is one of the most serious complications of diving, at times resulting in mortality and permanent morbidity [1]. Neurological DCI encompasses two disease entities, neurological decompression sickness (DCS) and cerebral arterial gas embolism (CAGE). Although pathophysiology and clinical presentation of these two diseases are different, treatment for both conditions is the same and consists of prompt administration of 100% oxygen and intravenous fluids followed by expeditious administration of hyperbaric oxygen (HBO_2) therapy [2].

The search for adjuvant therapies to improve outcome in neurological DCI has led to the investigation of intravenous lidocaine as a neuroprotective agent. This sodium channel blocking and anti-inflammatory agent has shown promising results in several animal studies [3,4], but subsequent animal and human investigations have yielded conflicting results [5-9]. Based on the positive effects of lidocaine reported in the literature, in 2001 the decision was made to apply intravenous lidocaine as adjuvant therapy in all cases of neurological DCI presenting to the Diving Medical Center of the Royal Netherlands Navy. In the present study, we report on the efficacy of lidocaine in our patients from 2001 to 2011, using an historic cohort as the control group.

METHODS

Standardized patient documentation was introduced at our institution in 1996. Adjuvant treatment with lidocaine in all patients with neurological DCI was

TABLE 1: Severity scores used in the study

A. Dick and Massey severity score (total possible score is 10).

Sensory symptoms	1. paresthesia of single limb or area 2. paresthesia of multiple regions 3. numbness of single region or limb 4. numbness of two regions or limbs 5. numbness of three or more limbs
Motor symptoms	1. paresis of single limb or muscle group 2. paresis of multiple limbs or muscle groups 3. paralysis of single limb or muscle group 4. paralysis of two limbs 5. paralysis of three or more limbs

B. Adapted version of Dick and Massey severity score (total possible score is 24).

The following items are scored, giving 0 points for absence, 1 point for mild presence and 2 points for severe presence. This score is added to the Dick and Massey score as calculated in panel A.

Deep boring limb/abdominal pain, headache, vertigo, dyspnea, skinbends, visual disturbances, general malaise.

C. Blatteau severity score (total possible score is 22).

Age ≥ 42	no = 0		yes = 1
Back pain	no = 0		yes = 1
Clinical course before recompression	better = 0	stable = 3	worse = 5
Objective sensory deficit	no = 0		yes = 4
Motor impairment	no = 0	paresis = 4	paralysis = 5
Bladder dysfunction	no = 0		yes = 6

A = Dick and Massey severity score [12]. **B** = adapted version of Dick and Massey severity score. **C** = Blatteau severity score [13].

introduced in June 2001. We reviewed all medical files of patients treated with HBO_2 therapy from 1996 to 2011 to include patients for our study. Included patients were those with a diagnosis of neurological DCI (neurological DCS or CAGE) who received U.S. Navy Treatment Table 6 as their first HBO_2 session at our institution within 72 hours after start of symptoms following a dive. Patients who were comatose on arrival were excluded. Since all patient information was handled anonymously, no informed consent was obtained from the patients.

From the included patient files we extracted sex, date of birth, weight, length, characteristics of the dive – duration, depth, breathing gas, diving in the preceding 18 hours (repetitive dive) – as well as time from end of dive to start of symptoms and time from start of symptoms to start of HBO_2 therapy.

Also, the dive performed was compared to the Canadian Defence and Civil Institute of Environmental Medicine dive tables and their guidelines [10,11] to see whether the required decompression stops were adhered to. If not, or if the diver's dive computer indicated that decompression stops had been missed, the dive was categorized as unsafe. The dive was also classified as unsafe if the diver indicated that a rapid ascent had been made, the water had been cold (< 15°C) or strenuous exercise had been performed during the dive.

Clinical course from start of symptoms to beginning of HBO_2 therapy was noted as improving, stable or worsening. Neurological symptoms were graded according to the Dick and Massey (DM) scoring system [12] (Table 1). We also calculated the severity score as devised by Blatteau [13] (Table 1, an adapted version of the original score introduced by Boussuges [14]). Since both these scores are primarily designed for use in spinal cord DCS, we furthermore calculated an adapted version of the DM scale to include symptoms specific for cerebral DCS, vestibular DCS and CAGE (Table 1). Diagnosis as established based on history and physical and neurological examination, in accordance with the U.S. Navy Diving Manual [15], was recorded.

As for treatment, we noted type and amount of HBO_2 treatments and whether or not lidocaine was given. Neurological symptoms at the end of the last HBO_2 session were noted, from which DM score as well as our adapted version of this score were calculated. Outcome after the last HBO_2 session was also expressed as absence or presence of objective neurological signs. Since follow-up data were available in only 14% of patients, we were not able to determine delayed outcome.

Patients suspected of neurological DCI (including those with only subjective symptoms) who are presented to our institution are immediately treated with 100% oxygen followed by neurological examination and initiation of U.S. Navy Treatment Table 6 as soon as possible. This table is extended if necessary as recommended in the U.S. Navy Diving Manual [16]. Additional treatment tables (U.S. Navy Treatment Table 5 or 6, HBO_2 at 1.9 atmospheres absolute (190 kPa) for 180 minutes or HBO_2 at 1.5 atmospheres absolute (150 kPa) for 90 minutes (at the diving medical officer's discretion) are prescribed when residual symptoms are present. 24-hour intervals are maintained between HBO_2 sessions. Administration of additional HBO_2 sessions is stopped when no further improvement is observed or the patient reports symptoms of pulmonary oxygen toxicity.

Adjuvant treatment with intravenous lidocaine (implemented in June 2001) consists of an initial bolus of 100 mg at the start of the first HBO_2 therapy session followed by continuous administration of 3 mg/minute over eight hours.

Statistical analysis was performed using SPSS version 17.0 (SPSS Inc., Chicago, Ill.). Differences for nominal variables between control and lidocaine groups were tested using Fisher's exact test for $2x2$ tables and Chi-Square test (without continuity correction) for $2x3$ tables. Chi-Square test for trend was used for the ordinal variables (DM score, adapted DM score and Blatteau score). The Mann-Whitney U test was used for scale variables since the values of these variables were not normally distributed (tested using Shapiro-Wilk test). Relative risk was calculated using the Mantel-Haenszel method. All tests were performed two-sided and statistical significance was accepted at $p < 0.05$.

RESULTS

A total number of 140 patients was treated with HBO_2 in the investigated period. From this total, 85 were diving accidents that received their first HBO_2 session in our institute. 70 of these patients were treated with U.S Navy Treatment Table 6 as their first treatment. Of this group, 50 were treated within 72 hours after the accident, 35 of whom had neurological signs and/or symptoms on admission and thus met our inclusion criteria. The total patient group consisted of 21 patients who were treated between 1996 and 2001 and did not receive lidocaine and 14 patients who were treated between 2001 and 2011 and did receive lidocaine.

General parameters of the patients are displayed in Table 2; a specification of the individual patients can be found in Tables 3a and 3b. Groups were comparable with regard to gender, age, body mass index, dive depth, dive time, breathing gas, percentage repetitive dives, time until start of symptoms, time until HBO_2, clinical course until start of HBO_2 and percentage of DCS and CAGE. Significantly more patients in the control group made an unsafe dive (62% *vs*. 14%, $p = 0.007$).

With respect to initial injury severity (Table 4), both groups had comparable DM, adapted DM and Blatteau scores. The differences between groups in regard to percentage of patients with objective neurological signs on admission (38% in the control group, 64% in the lidocaine group) was not statistically significant ($p = 0.176$). The number of treatment sessions given was similar in both groups. Treatment reduced DM score from 2.0 ± 1.6 to 0.1 ± 0.5 in the control group and from 2.7 ± 1.7 to 1.4 ± 3.0 in the lidocaine group, differences between groups were not statistically significant.

Percentage of patients with objective neurological signs at the end of the last HBO_2 session was 5% in the control group and 14% in the lidocaine group. DM score, adapted DM score and percentage of patients with objective neurological signs were not significantly different between groups at the end of the last HBO_2 session. The relative risk for unwanted outcome (objective neurological signs) when receiving lidocaine, corrected for objective neurological signs before first therapy, was 1.8 (95% confidence interval 0.2-16).

DISCUSSION

In this small retrospective cohort study, we were not able to demonstrate a positive effect of intravenous lidocaine *vs.* no lidocaine on outcome in patients with neurological DCI.

The use of lidocaine in DCI has been the subject of study for decades. Since the first report of a positive effect of this substance in preventing neurological injury in CAGE-induced in cats [17], multiple animal

TABLE 2: General and diving parameters

		control (*n*=21)	lidocaine (*n*=14)	*p*-value
sex	male female	81% 19%	71% 29%	0.685
age (y)		36 (9.2)	36 (6.8)	0.946
body mass index (kg/m^2)		24 (3.4)	24 (2.8)	0.752
maximum diving depth (m)		24 (12)	30 (15)	0.224
diving time (min)		40 (14)	35 (13)	0.252
breathing gas	air nitrox trimix	76% 24% 0%	79% 7% 14%	0.113
repetitive dive		43%	57%	0.500
unsafe dive		62%	14%	0.007*
time until start of symptoms (h)		3.7 (7.0)	6.5 (11)	0.906
time until HBO_2 (h)		16 (12)	22 (17)	0.418
clinical course until HBO_2	better stable worse	24% 48% 29%	21% 50% 29%	0.985
diagnosis	DCS CAGE	86% 14%	79% 21%	0.664

Values between parentheses are standard deviations. Percentages may not add up to 100% due to rounding errors. * = $p < 0.05$. Nitrox = breathing gas containing oxygen and nitrogen, in which the oxygen content is larger than in air. Trimix = breathing gas containing oxygen, nitrogen and helium.

and human studies have been performed on this matter. Lidocaine is a sodium channel blocker, which accounts for several of its neuroprotective effects, as reviewed by Mitchell [18,19]. Briefly, lidocaine is an anesthetic drug that depresses neuronal metabolism when given intravenously, rendering the brain less vulnerable when it is deprived of oxygen and furthermore lowering intracranial pressure. Secondly, lidocaine stabilizes the neuronal membrane, protecting the cell against damage in the case of ischemia. In the third place, its antiarrhythmic effect attenuates the cardiac arrhythmias that often occur in DCI and contribute to adverse outcome. Furthermore, apart from the effects due to sodium channel blocking, lidocaine has anti-inflammatory properties [20], which attenuate the inflammatory response associated with the endothelial damage that can occur in DCI. Several animal studies on lidocaine in CAGE confirmed the positive results of the first investigation, not only when lidocaine was given as pretreatment, but also when given after induction of CAGE [4,21-23]. Animal studies on lidocaine in DCS were less unequivocal, with one study showing a positive effect [3] and other studies being unable to demonstrate better outcome [9,24]. Human studies on lidocaine in DCI are very scarce and limited to a few case reports [25-28] and a small retrospective study, of which unfortunately only an abstract has been published [29]. The most interesting data, however, come from four human studies on the use of intravenous lidocaine in cardiac surgery. Patients undergoing heart surgery are known to be at risk for postoperative neurocognitive decline, especially in open chamber surgery, and cerebral air embolization has been suggested as an important contributing factor [30]. Therefore, cardiac surgery may have similarities to diving-related CAGE. The first two studies, published

TABLE 3a: Clinical characteristics and diagnosis/Control group

sex	age	symptoms and signs	diagnosis
m	30	pain r shoulder, weakness triceps r	DCS
m	35	malaise, pain r/l knee, hypesthesia r trunk	DCS
m	38	paresthesia and hypesthesia l leg	DCS
m	45	vertigo, nausea, nystagmus	DCS-v
m	37	exhaustion, paresthesia l arm	DCS
f	21	ataxia, hypesthesia r face/arm/leg, weakness r arm/leg	CAGE
f	33	paresthesia and hyperesthesia l arm/leg, hyperreflexia l arm	DCS
f	29	exhaustion, headache, nausea, paresthesia l arm/leg	DCS
m	47	dyspnea, hemoptoe, paresthesia l foot	CAGE
m	32	paresthesia hyperesthesia and weakness r leg	DCS
m	30	paresthesia r/l arm	DCS
m	35	exhaustion, paresthesia back / r/l arm	DCS
m	25	somnolence, ataxia, paresthesia r/l arm/leg, hypesthesia r arm/leg	DCS
m	48	paresthesia and hyperesthesia l leg	DCS
m	25	paresthesia r foot/arm	DCS
f	34	dyspnea, headache, exhaustion, paresthesia neck/shoulders/arms	CAGE
m	44	paresthesia r/l arm	DCS
m	49	vertigo, nausea, nystagmus	DCS-v
m	36	dyspnea, exhaustion, pain r/l arm/leg, dysbasia	DCS
m	27	exhaustion, headache, paresthesia face / r/l leg, weakness l arm	DCS
m	56	paresthesia l arm	DCS

Clinical characteristics and diagnosis of the 21 patients in the control group.
r = right. l = left. DCS = decompression sickness. DCS-v = vestibular decompression sickness.
CAGE = arterial gas embolism.

in 1999 and 2002, showed a positive effect of lidocaine on postoperative neurocognitive decline in patients undergoing open chamber surgery patients [5] and coronary artery bypass grafting with cardiopulmonary bypass [6]. The two other studies, both published in 2009, included mixed groups of patients undergoing open chamber surgery or coronary artery bypass grafting. These studies failed to demonstrate a positive effect [7,8]. In fact, in one of these studies total lidocaine dose was an independent predictor of cognitive decline. All in all, based on these animal and human studies, lidocaine can be regarded as an interesting substance in DCI, but a positive effect has of yet not been proven. The only human studies showing beneficial effects have been performed in cardiac surgical cases, which may have similarities with CAGE but certainly not with other forms of DCI.

For the current study we included all patients who received lidocaine for neurological DCI and compared these patients to an historical cohort. The control group was too small to perform a matched analysis, but the two groups were nevertheless comparable in regard to most confounding factors. Significantly more patients in the control group had made a dive that did not comply with decompression tables and guidelines, and can therefore be said to have suffered an "explainable" injury. This was however not reflected in increased disease severity since none of the injury scores showed statistically significant differences between groups. There was a trend toward increased risk of unwanted outcome in the lidocaine group (relative risk 1.8), even after correction for the larger percentage of objective neurological signs before start of therapy in the lidocaine group, but the large confidence interval (0.2-16) precludes any definitive statements. We must therefore conclude that we observed neither a positive nor a negative effect of lidocaine in our study.

TABLE 3b: Clinical characteristics and diagnosis/Lidocaine group

sex	age	symptoms and signs	diagnosis
m	30	exhaustion, paresthesia r/l leg & l hand, weakness r/l leg	CAGE
f	41	dyspnea, ataxia, paresthesia and hypesthesia r/l arm/leg	DCS
m	37	paresthesia r/l arm	DCS
f	32	paresthesia r/l hand, weakness r hand, areflexia r biceps	DCS
m	33	paresthesia r/l leg, hypesthesia l leg	DCS
m	33	paresthesia and hypesthesia l hand	DCS
m	27	dyspnea, paresthesia l hand, weakness l arm/leg	DCS
m	31	paresthesia and hyperesthesia r/l leg, areflexia l achilles	DCS
m	51	abdominal pain, paresthesia and hypesthesia r/l leg	DCS
f	34	ataxia, paresthesia l face/arm/leg, temporary visual field loss	DCS
m	35	tetraplegia	CAGE
m	29	exhaustion, nausea, vertigo, ataxia, paresthesia r/l arm/leg	DCS
f	34	ataxia, paresthesia r/l hand/foot & r trunk, hypesthesia l arm/ trunk/leg, weakness l arm/leg	CAGE
m	42	pain r arm, paresthesia r arm, subjective weakness r arm	DCS

Clinical characteristics and diagnosis of the 14 patients in the lidocaine group.
r = right. l = left. DCS = decompression sickness. CAGE = arterial gas embolism.

Our study is, of course, limited by its small sample size. Nevertheless, in our opinion this patient population represents the daily practice of the dive physician who faces relatively small numbers of patients with heterogeneous presentations. The heterogeneity is reflected in our study by the varying time until start of symptoms and time until start of HBO_2 therapy (although we limited our study group to patients receiving HBO_2 within 72 after symptom onset). Furthermore, we included all diseases that were eligible for adjuvant treatment with lidocaine: spinal DCS, cerebral DCS, vestibular DCS (together termed neurological DCS); and CAGE. One might argue to analyze these categories separately in order to determine whether lidocaine would have a beneficial effect in any of these subgroups. The small size of our population, however, precluded any meaningful subgroup analysis. Furthermore, it is not always possible to reliably distinguish the various forms of neurological DCI, and a patient may suffer from various types at the same time.

Symptom severity in our patients was relatively low on average, with DM scores of 2.0 ± 1.6 and 2.7 ± 1.7 (maximum possible score 10) in the control and lidocaine groups, respectively. We cannot rule out the possibility that more severely affected patients would have benefited from lidocaine, but in our study subgroup analysis was not possible due to the small sample size. Furthermore, HBO_2 therapy was very effective in our control patients, leaving little room for further improvement due to lidocaine. On the other hand, a positive effect of lidocaine could also have been detectable as a lower number of HBO_2 sessions needed in the lidocaine group, which was not the case.

Our study suffers from possible bias since the control and lidocaine patients were not treated in the same period. The control patients were seen from 1996 to 2001, and the lidocaine patients from 2001 to 2011. Although, except for the addition of lidocaine, we are not aware of any differences in treatment between groups, although we cannot exclude the effect of time as a confounding factor.

The lidocaine dose used in our patients was in line with the doses used in previous investigations and the advice given by the Undersea and Hyperbaric Medical Society [31]. Although we did not obtain plasma levels of lidocaine in our patients, similar infusion strategies used in other studies resulted in lidocaine levels within the desired range [5-8]. We infused lidocaine during eight hours, starting at the beginning of the HBO_2 treatment. The duration of lidocaine administration varies

TABLE 4: Treatment and injury severity before and after HBO_2

	BEFORE first HBO_2 session			AFTER last HBO_2 session		
	control (*n*=21)	lidocaine (*n*=14)	*p*-value	control (*n*=21)	lidocaine (*n*=14)	*p*-value
number of treatment tables	n/a	n/a	n/a	2.0 (1.0)	2.7 (2.3)	0.537
Dick and Massey score	2.0 (1.6)	2.7 (1.7)	0.221	0.1 (0.5)	1.4 (3.0)	0.074
adapted Dick and Massey score	4.2 (2.6)	4.1 (2.6)	0.914	0.5 (1.0)	1.4 (3.0)	0.177
Blatteau score	5.4 (2.8)	7.4 (5.2)	0.152	n/a	n/a	n/a
objective neurological signs	38%	64%	0.176	5%	14%	0.551

Values between parentheses are standard deviations.

between the four published human studies. Two studies used a 48-hour lidocaine infusion [5,8], one study used 12 hours [7] and one study administered lidocaine intraoperatively, without mentioning the exact duration of the infusion [6]. There are currently no data that support any specific duration of lidocaine infusion.

The question remains if there is any future for the use of lidocaine in the treatment of neurological DCI. Our study is presently the most comprehensive investigation available, since larger and/or prospective studies are lacking. Although our study was underpowered to draw definitive conclusions, we have demonstrated that despite data collection over a period of 15 years in a relatively large hyperbaric center, we were not able to demonstrate a positive effect of lidocaine on neurological outcome in DCI. This is mainly caused by the small number of patients and the heterogeneity of the patient population.

Current Undersea and Hyperbaric Medical Society best practice guidelines on DCS and AGE discourage the use of lidocaine in DCS and are impartial on its use in AGE, only giving advice on lidocaine dose for those cases in which the physician chooses to use it [31]. The data on which these recommendations are based, as summarized in the present article, are weak and a prospective study on lidocaine in dive accidents is still lacking. We believe the most rational strategies would be to either abandon the use of lidocaine in neurological DCI altogether, or to perform a prospective study. Given the low prevalence of neurological DCI, the heterogeneous population and the fact that DCS and CAGE should be studied separately, this would call for a large multicenter investigation.

The authors report that no conflict of interest exists with this submission. ■

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Prevalence of cardiomegaly and left ventricular hypertrophy in scuba diving and traffic accident victims

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ABSTRACT

Although frequently asymptomatic, left ventricular hypertrophy (LVH) is an independent predictor of sudden cardiac death (SCD). We hypothesized that diving may increase the propensity for pre-existent LVH to cause a lethal arrhythmia (and SCD) and therefore the prevalence of LVH may be greater among scuba fatalities than among traffic fatalities. We compared autopsy data for 100 scuba fatalities with 178 traffic fatalities. Extracted data contained information on age, sex, height, body mass, heart mass (HM), left ventricular wall thickness (LVWT), interventricular wall thickness (IVWT), and degree of coronary artery stenosis. A case was classified as LVH if the LVWT was > 15 mm. Log risk models were used to compare HM and LVWT in two groups while controlling for body mass, body length, age and sex. The prevalence of LVH was compared using Pearson's test. The mean HM was 428.3 ± 100 for divers and 387 ± 87 for controls. The crude HM ratio for scuba fatalities *vs*. controls was 1.11 (1.05, 1.17), and when controlled for sex, age and body mass the ratio was 1.06 (1.01, 1.09). The mean LVWT was 15 ± 3.5 for divers and 14 ± 2.7 for controls ($p = 0.0017$). HM and LVWT measured at autopsy were greater in scuba than in traffic fatalities.

BACKGROUND AND SIGNIFICANCE

Sudden cardiac death (SCD) is the suspected cause in at least 20% to 30% of all scuba fatalities, most of which occur in older divers [1-4]. SCD is generally defined as unexpected natural death from a cardiac cause within an hour after the onset of acute symptoms in a person without any prior condition that would appear fatal [5]. Although SCD may occur in people without apparent heart disease, it is more often associated with ischemic heart disease and structural heart changes [6,7].

Coronary heart disease (CHD) is responsible for about 75% of SCD and electrical anomalies related to channelopathies for many of the rest, but left ventricular hypertrophy (LVH) is a strong independent predictor of SCD [8-12].

LVH is one of the markers of risk for SCD and can be found in asymptomatic subjects – as most recreational divers are. The prevalence of LVH in the population is 15.5% to 19% in men and up to 21% in women [8-10]. The prevalence of LVH may be similar or greater in women than in men, but the mortality rates of SCD are greater in men than in women [11]. LVH is strongly associated with age, high systolic blood pressure and obesity [12,13]. The prevalence may vary with geography and race [14,15].

Subjects with LVH have a higher prevalence and greater complexity of ventricular premature beats and more serious arrhythmias than patients without LVH [16,17]. Most likely the arrhythmogenecity of LVH is multifactorial in origin. Possible mechanisms include myocardial ischemia (which is also multifactorial and results in reduced cardiac reserve), electrophysiological abnormalities, abnormalities of the hypertrophied myocardial cell, fluctuations in arterial blood pressure, increased sympathetic activity, and electrolyte abnormalities. The mechanisms of arrhythmia in LVH are

also associated with fibrosis of the myocardium, which increases the distance between myocytes and enhances re-entry arrhythmias due to uncoupling [18].

SCD is not strongly associated with any specific type of activity (Reddy *et al.*, 2009) and it may be that the occurrence of SCD in scuba diving is circumstantial. However, diving conditions, especially immersion, which affects preload, afterload and autonomous nervous regulation of the heart and circulation, may increase the risk of LVH-generated arrhythmias. The percentage of scuba deaths due to cardiac causes increases with the age of divers in similar manner as in the general population [2]. In acute cardiac events associated with vigorous exercise on land, myocardial infarction (MI) is a common cause of sudden cardiac arrest [19,20], but a significant number of patients survive.

Unlike that population, the autopsy findings in scuba deaths with suspected cardiac causes rarely include signs of myocardial infarction [21]. In scuba diving the main mechanism of cardiac-related death instead is suspected to be an acute dysrhythmia causing cardiac arrest [22,23].

Prevalence of hypertension and LVH among recreational scuba divers is not known, but since hypertension is not an exclusion for participation in diving, these conditions are probably similar to those in the general population. Our hypothesis was that if diving conditions increase the propensity of LVH for arrhythmia and thus the incidence of SCD, the prevalence of LVH at autopsy would be greater in scuba victims than in traffic accident victims, where SCD is more likely coincidental and occurs independently of LVH. The purpose of this study is to test that hypothesis.

METHODS

Design

This is a cross-sectional study based on existing data. We reviewed the 100 most recent scuba fatalities with complete autopsy reports from the DAN fatalities database (cases) and compared them to a control group of traffic accident fatalities of similar age that occurred in same period.

Selection of cases and controls

The Institutional Review Board at the Divers Alert Network approved all aspects of this investigation. Scuba fatality cases (SF) were selected from the most recent completed reports in the DAN Fatality Database. Only cases with an available autopsy report and a victim between 40 and 70 years of age from the period 2007 to 2011 were included. Controls were traffic fatalities (TF) selected from a database of the San Diego County Medical Examiner's Office in the period from 2007 to 2011.

In order to build an age- and sex-matched group of comparison cases, a number of deaths meeting specific criteria were identified from the database of the San Diego County Medical Examiner's Office (SDMEO). The initial group was selected by choosing motor vehicle accident deaths in the age range of the scuba deaths, with causes of death due to multiple blunt force injuries or blunt force injuries of the head, torso, extremities, or a combination thereof, and for which toxicology testing was performed. Excluded cases were donors of cardiac valves, a history of coronary artery bypass graft surgery or stenting (two of each), lymphoma and breast cancer, paraplegia, alcoholism with cirrhosis, and positive toxicology findings of recreational drug use. None of the cases noted evidence of acute cardiac events. The final list included 178 cases.

That restriction allowed removal of cases of asphyxia, drowning, or thermal injuries occurring in vehicle fires, potentially removing any skewing. Also removed were any cases involving death from complications of injuries (either long- or short-term), such as complications of quadriplegia, where long-term sequelae of injuries may have affected cardiovascular status or where cardiovascular status may have affected survival.

Because of potential long-term cardiovascular sequelae, cases in which alcohol or certain drugs of abuse (methamphetamine, cocaine, cocaine metabolites, morphine/heroin, and methadone) were detected were excluded in order to reduce inclusion of possible cardiovascular effects from chronic alcoholism or drug use. A case involving inhalant abuse (huffing – in this case difluoroethane) was also eliminated. Two cases in which anticonvulsants (carbamazepine or phenytoin) were detected was also eliminated.

It should also be noted that while all cases included received screening for alcohol and common drugs of abuse (to include cocaine/cocaine metabolites, opiates, amphetamines, benzodiazepines, fentanyl, and cannabinoids), some cases also received additional screening. However, the presence of other medications outside common drugs of abuse did not exclude from the study.

TABLE 1: Characteristics of 100 scuba fatalities and 178 traffic fatalities

Variable	Traffic Fatalities		Scuba Fatalities	
	Female	Male	Female	Male
***n* (%)**	66 (37%)	112 (63%)	16 (16%)	84 (84%)
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Age	55.4 ±8.4	54.4 ±8.2	53.2 ± 6.5	54.3 ± 7.2
Body mass (Kg)	80.5 ± 19.1	95.0 ± 20.1	83.1 ± 18.5	94.0 ± 18.0
Body length (cm)	161.2 ± 12.9	175.13 ± 10.0	164.4 ± 8.1	178.4 ± 8.3
BMI	31.5 ± 10.2	30.9 ± 5.7	30.8 ± 7.1	29.6 ± 5.4
HM (g)	334.8 ± 67.7	417.6 ± 82.1	333.9 ± 54.1	446.2 ± 97.7

Indexed heart mass is shown in Table 2.

Variable	Traffic fatalities	Scuba fatalities	T-test *p*-values
HM%BM	0.44±0.08	0.46±0.08	<0.024
rHMBM	4.4±0.9	4.6±0.9	0.136
rHMBSA	193±34	202±30	0.035
rHMBMI	12.8±2.8	14.5±3.1	<0.0001
rHMBL	228±48	239±42	0.046
Severe CAD (%)	18	29	0.015

Indexed heart mass per BSA, BMI and BL were greater in SF. Heart mass indexed per body mas was greater when expressed as a percentage of BM while ratio was similar.

Data

Extracted autopsy data contained information on age, sex, height in centimeters (Hcm), body mass in kilograms (BMkg), heart mass in grams (HMg), and cause of death. When available, left ventricular wall thickness in millimeters (LVWT) and interventricular wall thickness in millimeters (IVWT) were included as well. Medical history data (diabetes, hypertension, smoking, history of heart disease, medications), were grossly incomplete and not included.

A case was classified as left ventricular hypertrophy (LVH) if the LVWT was thicker than 15 mm. Body mass index (BMI) was calculated using the formula:

$$BMI = BMkg/(Hcm)^2$$

Body surface area (BSA) was calculated using the DuBois and DuBois formula:

$$BSA\ (m^2) = 0.007184 \times Hcm^{0.725} \times BMkg^{0.425}$$

Heart mass indices were defined as a percentage of body weight (HM%BM) and as ratios to body weight (body mass) in kilograms (rHMBM), to surface area (rHMBSA), to body length (rHMBL), and BMI (rHMBMI).

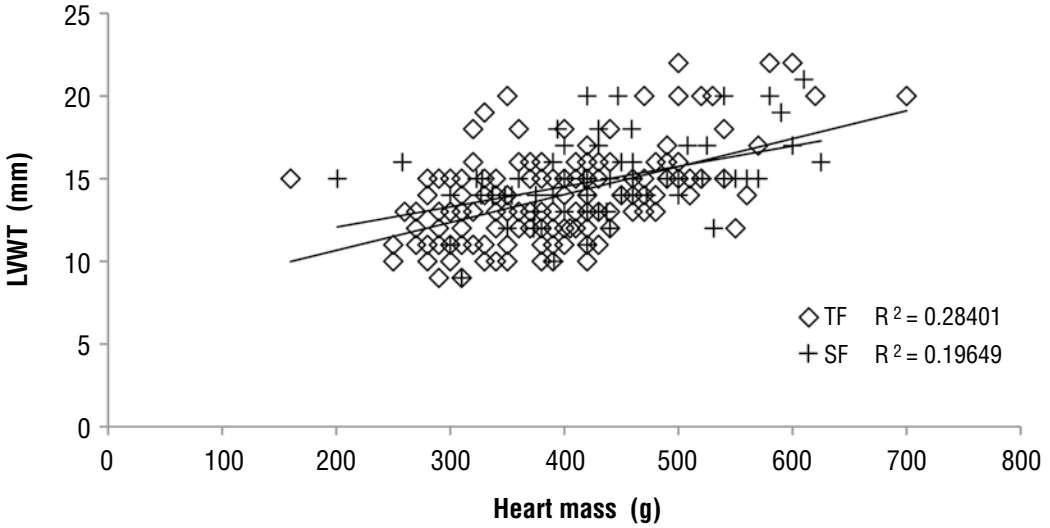
Coronary artery atherosclerotic stenosis was indicated in some cases as a percentage and in other cases as descriptive indicated by mild, moderate or marked (or severe). We addressed this by categorizing cases with reported percent stenosis into none (0), mild (>0-30%), moderate (>30-70%) and severe (>70 %).

Statistical analysis

Differences in age, gender and BMI between the two groups were evaluated by t-test. Linear correlation was used to explore correlation of heart mass to BM, BMI and BSA as well as the correlation of LVWT with indexed HM (rHMBM, HM%BM, rHMBSA and rHMBMI).

Log risk models were used to compare heart mass and left ventricular wall thickness in two study groups while controlling for covariates age, sex, body mass, body length, and BMI. The final selection of these models was done by using backward elimination technique with 10% change in estimate and bias-variance trade-off methods. The prevalence of LVH in two groups was compared using Pearson's test.

FIGURE 1: Correlation of 100 LVWT with heart mass



Correlation of left ventricular wall thickness and heart mass in scuba fatalities (SF) and traffic fatalities (TF).

RESULTS

Subject characteristics

Characteristics of subjects by group and sex are shown in Table 1. The Scuba Fatalities group (SF) consisted of 100 individuals, 16 female and 84 male, with a median age of 55 years. Traffic fatalities consisted of 178 individuals, 66 female and 112 male, with a median age of 55 years. Age was similar across the groups. Body mass was larger in SF females than in TF females while body lengths were larger in SF males and females than in the SF group. The BMI was similar across the groups. The uncorrected heart mass was similar in females of both groups and larger in SF males than in TF males.

Correlation of heart mass with body measures

The mean heart mass was 428.3 ± 100 for divers and 387 ± 87 for controls. The correlation of heart mass with body measures was best with body surface area in both groups ($R^2 = 0.44$). The correlation with the body mass was better in TF ($R^2 = 0.44$) than in SF R^2 = (0.37) group. Correlation of heart mass with body weight was weaker for females regardless of the group.

Heart mass as a percent of body mass (HM%BM), ratio of heart mass *vs.* body surface area (rHMBSA), ratio of heart mass *vs.* BMI (rHMBMI) and ratio of heart mass *vs.* body length (rHMBL) were greater in SF than in TF while ratio of heart mass *vs.* body mass (rHMBM) was similar.

The crude HM ratio for scuba fatalities *vs.* controls was 1.11 (1.05, 1.17). When controlled for sex, age and body mass the final model given by Formula 1 below, provided the best fit and the ratio was 1.06 (1.01, 1.09).

Formula 1

$$Ln(HMg) = \alpha + \beta_1(Group) + \beta_2(sex) + \beta_3(Age) + \beta_4(BMkg) + error$$

Left ventricular wall thickness

The LVWT was available in 67% SF and 88% controls. The mean LVWT was 15 ± 3.5 for divers and 14 ± 2.7 for controls ($p = 0.0017$). The LVWT correlated with the heart mass and was better in TF ($R_2 = 0.2840$) than in SF ($R_2 = 0.1965$) as shown in Figure 1, but did not correlate with indexed heart mass measures (HM%BM, rHMBM, rHMBSA, rHMBMI). The LVWT in females did not correlate with heart mass or with its indexed measures.

The crude LVWT ratio for divers *vs.* controls was 1.10 (1.04, 1.16). When controlled for sex and body mass with a log normal model given in Formula 2 below, the LVWT ratio for divers *vs.* controls was 1.07 (1.01, 1.13).

Formula 2

$$Ln(LVWTmm) = \alpha + \beta_1(Group) + \beta_2(sex) + \beta_3(body\ mass) + error$$

TABLE 2

Group	LVWT > 1.5 cm Yes	No	Missing LVWT
Scuba fatalities	21	46	33
Traffic fatalities	32	124	22

The prevalence of LVH (LVWT > 15 mm) in cases was 31% *vs*. 20% in controls (p=0.042) as shown in Table 2.

DISCUSSION

Comparison of the heart mass in unrelated subgroups of population may be affected by differences in age, sex, body mass, body length and prevalence of diseases. We addressed this by selecting the controls from the same time period and of the same age range while controlling statistically for differences in sex and body composition. Next, we excluded from controls cases with health conditions that would disqualify for scuba diving. Few divers with stent or a coronary artery bypass graft surgery ever get back to diving; most do not feel like it or would not meet current requirements of physical fitness [24]. We also excluded from controls the cases in which there was proof of drug abuse or signs of alcoholism, which may affect the heart mass and could have been disproportionately more present in traffic fatalities than in scuba fatalities.

We could not select cases and controls from the same geographic populations; the scuba fatalities were collected nationwide, while the controls were collected from one particular county. Possible differences in the use of alcohol and illegal drugs we controlled by restricting inclusions.

The study and the control groups ended up unequal in number of cases and gender composition but similar in age and body mass index. We consider that any additional tailoring to achieve same size and characteristics of the group could introduce additional bias. We rather chose to use log risk statistics that, to a certain extent, controls for these differences.

The mean heart mass was larger in the scuba (428 g) fatalities than in the traffic (387 g) fatalities. Comparison by gender shows that mean heart mass in females was similar for both groups and lesser than in males, magnifying the difference at the group level. Scuba fatality males, however, had a significantly greater heart mass than the traffic fatality group males.

The mean heart mass in our sample of scuba fatalities was greater than recently reported in a large series of non-divers without obvious heart or lung disease (388 g males, 328 g females) [25], similar to that in professional divers fatalities (408 g) and larger than in recreational divers fatalities (336 g) reported for a small series of cases 25 years ago [26]. The values used here for healthy general population were established recently with intent to address the issue of increasing BMI [27]. Values for professional and recreational diver fatalities were established in the 1970s and 1980s and thus may be smaller due to general increase in body and heart mass in recent decades [28].

LVH was found more often in recreational scuba fatalities than in traffic fatalities. The difference was significant in males but not in females which is explicable but may not be unexpected regarding sex-related differences in response to hemodynamic overload [29] and lesser probability of women suffering sudden cardiac arrest [16]. In this study, unlike that found in men, LVWT and heart mass in women did not correlate well with body mass or indexes used. The lack of differences in heart mass and LVWT between women in the two groups may indicate that diving does not affect the propensity for dysrhythmia in women.

The effects of diving on left ventricular function were not studied in older divers or divers with chronic diseases who may already have LVH, and it is not known whether diving may increase propensity of LVH for arrhythmia. The differences in heart mass and prevalence of LVH in the SF and TF groups described in this study were statistically significant, and their possible association with increased propensity for arrhythmia while diving makes it worthwhile to look further into these issues.

LIMITATIONS OF THE STUDY

Presentation of anatomic data in this study was limited to what was available in the autopsy reports. Because this was a retrospective study, there was some individual variation in the amount of detail provided in the autopsy reports. As with all forensic cases, there was some variability in the autopsy technique used by the prosecting pathologist, including the examination of the heart. The autopsy data from the TF group originated from a collaborating medical examiner's office, and therefore the number of pathologists performing the autopsies was limited. In such a setting there are typically also some office standards for performing

autopsies. Autopsies of individuals involved in a dive-related death require specific methods to maximize the information gained [35], but the autopsies collected by DAN were performed by pathologists in a number of different offices, and the compliance with current recommendations is not known.

Retrospectively, by interviewing some of the medical examiners who signed some of the included autopsies, we discovered that a couple of them do not always include measurements for ventricular wall and interventricular septum thicknesses, but add these measurements only when they appear grossly thickened to the pathologist performing the examination. Some pathologists include only measurements of the right and left ventricular wall thicknesses, but not the septum.

In our material, LVWT measurements were provided for 67% SF cases and 88% TF controls, but the heart mass was available for all. The mean heart mass was less in those missing LVWT both for SF and TF cases – and this could have affected differences in LVWT – but because strong correlation of LVWT with heart mass, that effect was probably not significant.

CONCLUSIONS

Heart mass and left ventricular wall thickness measured at autopsy were greater in scuba-related deaths than in traffic fatalities. This may indicate that diving increases the propensity for arrhythmia in subjects with left ventricular hypertrophy. Better autopsy data are needed to corroborate or disprove these findings.

The authors report that no conflict of interest exists with this submission. ■

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✦

Multilocus sequencing typing of *Pseudomonas aeruginosa* isolates and analysis of potential pathogenicity of typical genotype strains from occupational oxyhelium saturation divers

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ABSTRACT

Background: *Pseudomonas aeruginosa (P. aeruginosa)* is a common microbe isolated from divers with ear and skin infections. To obtain the epidemic characters of the occurrence of the *P. aeruginosa* infection, multilocus sequence typing (MLST) was used to assess the genetic background of different strains isolated from divers involved in saturation diving.

Methods: A total of 64 *P. aeruginosa* strains from naval divers were sequenced by multilocus sequence typing using seven housekeeping genes *(acsA, aroE, guaA, mutL, nuoD, ppsA* and *trpE)*. The results were analyzed based on the *P. aeruginosa* international MLST database to obtain the allelic profiles and sequence types (STs). MLST data were analyzed by Bionumerics 4.0 (http: // pubmlst.org / mlstanalyse) using LIAN and eBURST. Twenty-eight strains with the typical genotype were selected for further analysis of pathogenic characteristics by *Caenorhabditis elegans (C. elegans)* fast killing model.

Results: Data from MLST revealed a high STs diversity among the strains. Of the 64 strains, 53 strains were assigned to 19 STs, and the remaining 11 clones could not be assigned. ST274 accounted for 18.5% (12/ 64), and ST260 accounted for 15.62% (10/64). *C. elegans* killing assay showed that all the test strains had distinct virulent properties as compared with the negative control group. Clone 503-1 had the highest virulence and clone 54 had the lowest virulence as compared with the positive clinical group.

Conclusion: The *P. aeruginosa* strains carried by the occupational diver groups in Chinese regions have characteristically dominant STs, and have a relatively strong virulence as compared with the standard strain and the clinically isolated positive control strain.

INTRODUCTION

Pseudomonas aeruginosa (P. aeruginosa) is a highly versatile species with pathogenic properties [1]. It can colonize any tissue of individuals with comprised immune systems, causing urinary tract, respiratory system, skin and soft tissue infections. Although *P. aeruginosa* seldom infects healthy hosts, it is the dominant microbe causing skin and ear infections of healthy divers in saturation and commercial diving systems [2-4].

Saturation diving is a widely used technique that employs pressurized chambers where divers may live continuously for several weeks. The atmosphere of a saturation complex is special, usually containing a pressurized mixture of oxygen and helium. Microbial flora within the saturation system is rich both in species and numbers. Otitis externa and other skin disorders have been proven to be major infection problems in saturation diving, and *P. aeruginosa* remains the microbe that is most often isolated from ear and skin infections in divers [2,4].

In their several-year efforts of tracking bacterial isolates from northern Norway sea water, bacterial samples from the diving instruments, and bacterial samples from occupational divers during the same period using the serologic typing method in combination with the PFGE molecular typing method, Ahlén, *et al.* [2] found that there was a high homology between the *P. aeruginosa* strains infecting the divers

and those isolated from the sea water. Therefore, aquagenic *P. aeruginosa* might be an important source of human infection.

Although the PFGE molecular typing technique has an extremely high sensitivity for analysis of bacterial genetic origin, a strain from the same source may be differentiated as a new genotype due to high alteration of the genome, knowing that the genome of *P. aeruginosa* has extremely strong adaptive variation to selective stress of the living environment [5-9]. For this reason, the PFGE molecular typing technique is not suitable for comprehensive investigation and systematic analysis of the genetic origin of *P. aeruginosa*.

In 1998, Maiden, et al. [10] first established the bacterial molecular epidemiological tracing technique by using 7~8 highly conservative housekeeping genes as sequence tags, known as MLST. By combining the high throughout sequencing technique with the mature population genetics technique, the multilocus sequence typing (MLST) technique has proved to be a valuable tool for epidemiological analysis of bacterial infection and structural investigation of infected populations, and can provide accurate clues for the study of occurrence tendency of infection and the pathogenic mechanism of infection at the molecular level [10-13].

MLST is the most successful techniques application for analysis of the population genetic backgrounds of epidemic strains [10-13]. Some studies focused on drug-resistant *P. aeruginosa* in different regions of China and found that some epidemic serotype clones also exist in Chinese hospitals. However, it remains unclear whether the occurrence of *P. aeruginosa* infection is due to some specifically epidemic strains in saturation dive settings. In the present study, we used MLST to analyze the population structure and relationships of *P. aeruginosa* strains isolated from occupational naval divers. The *C. elegans* fast killing assay was used for further analyze the pathogenic phenotype in an attempt to explore and characterize the genotype of epidemic clones in divers.

MATERIALS AND METHODS

Bacterial strains

During August 2006 to November 2010, strains of *P. aeruginosa* were isolated from 64 different occupational naval divers aged 24 to 30 years old and who were working in the North China Sea (n=64). Of the 64 samples, 38 isolates were obtained from the external ears and 26 isolates were isolated from superficial skin infections. Isolates were selected to represent the typical infection population of Chinese occupational divers. Strains included *P. aeruginosa* ATCC 27853 and ATCC15692 as the control sequencing group, and two clones of isolates from clinical patients marked as L1 and L2 in this study as positive virulent property control groups. *Escherichia coli (E. coli)* OP50 was used as the food for *C. elegans*. *P. aeruginosa* isolates were identified by standard microbiologic methods such as colony morphology, oxidase reaction, growth at 42°C, and ability to produce characteristic pigmentations on cetrimide agar.

Multilocus sequence typing

Cells were incubated in a luria broth (LB) mixture overnight and harvested by washing with sterile phosphate-buffered saline (PH = 7.4). Genomic DNA was extracted and purified using a Fast DNA kit (TAKARA). PCR amplification from genomic DNA and subsequent sequencing were performed using primers specific for *acsA, aroE, guaA, mutL, nuoD, ppsA*, and *trpE* (http://pubmlst.org/paeruginosa/) designed by Curran, *et al.* (Table1) [11]. Amplification reaction mixtures were prepared to a final volume of 50 ml consisting of 40 ng genomic DNA, 1×PCR buffer minus MgCl2 (Takara Bio Inc.), 0.2 mM deoxynucleoside triphosphate (dNTP) mix (Takara Bio Inc.), 1 μM each primer (Sangon Bio Inc. China), and 1.25 U La Taq DNA polymerase (Takara Bio Inc.).

The reaction program was as follows: two minutes of initial denaturation at 95°C, then 30 cycles at 95°C for 30 seconds, between 52 to 56°C depending on the locus at 30 seconds, and 68°C for 90 seconds. The nucleotide sequences were determined by using internal nested primers according to the manufacturer's protocol designed by Curran, et al [11].

MLST allele assignments were obtained by BLAST similarity searches against the *P. aeruginosa* MLST database (http://pubmlst.org/paeruginosa/). Based on allelic profiles, MLST data were analyzed by Bionumerics 4.0 (http://pubmlst.org/mlstanalyse).

The eBURST software was used to relate the STs to detect relationships between very closely related genotypes within clonal complexes (CC). Linkage analysis (LIAN) software was used to test the null hypothesis of linkage disequilibrium for the individual loci and for concatenated sequences.

TABLE 1: Primers used for *P. aeruginosa* MLST

Locus and function	PRIMERS SEQUENCE (5'-3') Forward	Reverse	Amplicon size (bp)
acsA			
Application	ACCTGGTGTACGCCTCGCTGAC	GACATAGATGCCCTGCCCCTTGAT	842
Sequencing	GCCACACCTACATCGTCTAT	AGGTTGCCGAGGTTGTCCAC	390
aroE			
Application	TGGGGCTATGACTGGAAACC	TAACCCGGTTTTGTGATTCCTACA	825
Sequencing	ATGTCACCGTGCCGTTCAAG	TGAAGGCAGTCGGTTCCTTG	498
guaA			
Application	CGGCCTCGACGTGTGGATGA	GAACGCCTGGCTGGTCTTGTGGTA	940
Sequencing	AGGTCGGTTCCTCCAAGGTC	GACGTTGTGGTGCGACTTGA	373
mutL			
Application	CCAGATCGCCGCCGGTGAGGTG	CAGGGTGCCATAGAGGAAGTC	940
Sequencing	AGAAGACCGAGTTCGACCAT	GGTGCCATAGAGGAAGTCAT	442
nuoD			
Application	ACCGCCACCCGTACTG	TCTCGCCCATCTTGACCA	1042
Sequencing	ACGGCGAGAACGAGGACTAC	TGGCGGTCGGTGAAGGTGAA	366
ppsA			
Application	GGTCGCTCGGTCAAGGTAGTGG	GGGTTCTCTTCTTCCGGCTCGTAG	989
Sequencing	GGTGACGACGGCAAGCTGTA	GTATCGCCTTCGGCACAGGA	370
trpE			
Application	GCGGCCCAGGGTCGTGAG	CCCGGCGCTTGTTGATGGTT	811
Sequencing	TTCAACTTCGGCGACTTCCA	GGTGTCCATGTTGCCGTTCC	443

Virulence assays

To assess the pathogenic characteristics of *P. aeruginosa* isolates, 28 clones with typical sequence types (STs) were selected for further pathogenic analysis performed by *C. elegans* killing assay as described previously [14]. All *C. elegans* strains were maintained under standard culture conditions on nematode growth medium with *E. coli* OP50 as the food source.

C. elegans killing experiments were performed in a liquid-medium based system using 24-well plates. L4 larvae (35-40) were placed in each well and scored for dead worms. For each strain tested, worms were exposed to a suspension (OD650 = 0.5) of overnight growth cells. Worm mortality was scored over time, and a worm was considered dead when it failed to respond to touch after being co-cultured with cells at 25°C for six to eight hours. The tested strains also included two clinical strains (L1 and L2) and two standard control strains (ATCC27853 and ATCC15692) as positive controls, and *E. coli* OP50 as a negative control for the assay. Each independent assay consisted of three replicates. Killing bars represent the mean of three separate experiments. The percentage of dead worms was used for final comparison. Differences in the worm dead ratios from test and control groups were determined by using the Student's t-test.

RESULTS

MLST analysis of related and unrelated *P. aeruginosa* isolates

The data from MLST analyses indicated that 53 of the 64 strains were assigned to 19 STs, and the remaining 11 strains could not be assigned by the previously known *P. aeruginosa* database. eBURST analysis showed that of the 53 strain, 42 isolates with 10 STs were clustered into four different CC, named as CC1~CC4, while the remaining 11 strains with nine STs were classified as singletons. CC1 included 15 isolates containing three

TABLE 2

E-BURST analysis of *P. aeruginosa* isolates(54/64)

ST	E-BURST indices						
FREQ[a]	SLV[b]	DLV[c]	TLV[d]	SAT[e]	Mean distance	Percentage (%)	
CC1	NO. Isolates = 15, NO. STs = 3, Predicted Founder = 260						
ST260	10	2	0	0	0	1.0	15.62
ST503	4	1	1	0	0	1.5	6.25
ST264	1	1	1	0	0	1.5	1.56
CC2	NO. Isolates = 14, NO. STs = 3, Predicted Founder = 274						
ST274	12	2	0	0	0	1.0	18.75
ST936	1	1	1	0	0	1.5	1.56
ST268	1	1	1	0	0	1.5	1.56
CC3	NO. Isolates = 8, NO. STs = 2, Predicted Founder= None						
ST549	6	1	0	0	0	1.0	9.38
ST699	2	1	0	0	0	1.0	3.13
CC4	NO. Isolates = 5, NO. STs = 2, Predicted Founder= None						
ST729	3	1	0	0	0	1.0	4.68
ST671	2	1	0	0	0	1.0	3.13
Singletons	NO. Isolates = 11, NO. STs = 9, Predicted Founder = None						

a: the frequency of the ST; **b**: single-locus variants; **c**: double-locus variants; **d**: three-locus variants; **e**: satellite strains.

STs (ST260, ST503 and ST264). CC2 included 14 isolates also containing three STs (ST274, ST936 and ST268). CC3 included eight isolates containing two STs (ST549 and ST699). CC4 included five isolates containing two STs (ST729 and ST671). The nine singletons were known as ST54, ST58, ST63, ST164, ST234, ST324, ST795, ST816 and ST975. Among CC1 and CC2, ST260 and ST274 were assigned as the most likely ancestral genotype, with both STs defining the number of two SLVs. ST260 and ST274 were also STs corresponding to the highest frequency of all STs, the percentage being 15.62% (10/64) for ST260, and 18.75% (12/64) for ST274 (Table 2).

The independence of alleles at different loci was analyzed by LIAN to characterize the null hypothesis of linkage disequilibrium for multilocus data. Among the 53 isolates investigated, the number of housekeeping gene alleles ranged from 11 for *guaA* to five for *aroE* and *nouD* (Table3). The mean gene diversity of the entire dataset over seven loci was estimated to be H = 0.7728, where, to the genetic diversity at individual alleles loci, the maximum was 0.9298 in *guaA* and the minimum was 0.3860 in *aroE* (Table 3). The rate of recombination at different allele loci was calculated by standardized I^s_A. The result showed a slightly lower but still significant non-zero value, with $I^s_A = 0.1501$ of the 53 isolates (Table 3).

Potential virulence of selected typical genotype *P. aeruginosa* isolates

To get an overview of possible differences in virulence, *C. elegans* was used as the model organism to test a selected subset of *P. aeruginosa* isolates. A total of 28 isolates representative of the typical genetic groups determined via MLST sequencing were chosen as the test groups; two clinical strains (L1 and L2) and two control strains (ATCC27853 and ATCC15692) were chosen as positive control groups, while *E. coli* OP50 was chosen as the non-virulent control group. Compared with the OP50 group (100%

survival at eight hours), all the test strains showed different virulent properties, with dead values ranging from 17.5% (Clone54) to 87.5% (Clone503-1) (Figure 1).

Compared with positive control groups, the majority of the virulent strains were clustered in group CC1, with clone 503-1 showing the highest virulence potential in all the strains (87.5% dead at eight hours). Group CC2, CC3 and CC4 showed moderate virulent properties as compared with the positive control groups, except that clone 671 of CC4 group showed a much higher virulence (85% dead at eight hours). Different virulence properties were observed in the test singletons strains, and the mean virulence was much lower than that of group L1 and L2. The virulence of clone 54 was the lowest of all test strains (Figure1).

TABLE 3

Genetic diversity at individual loci of *Pseudomonas aeruginosa*

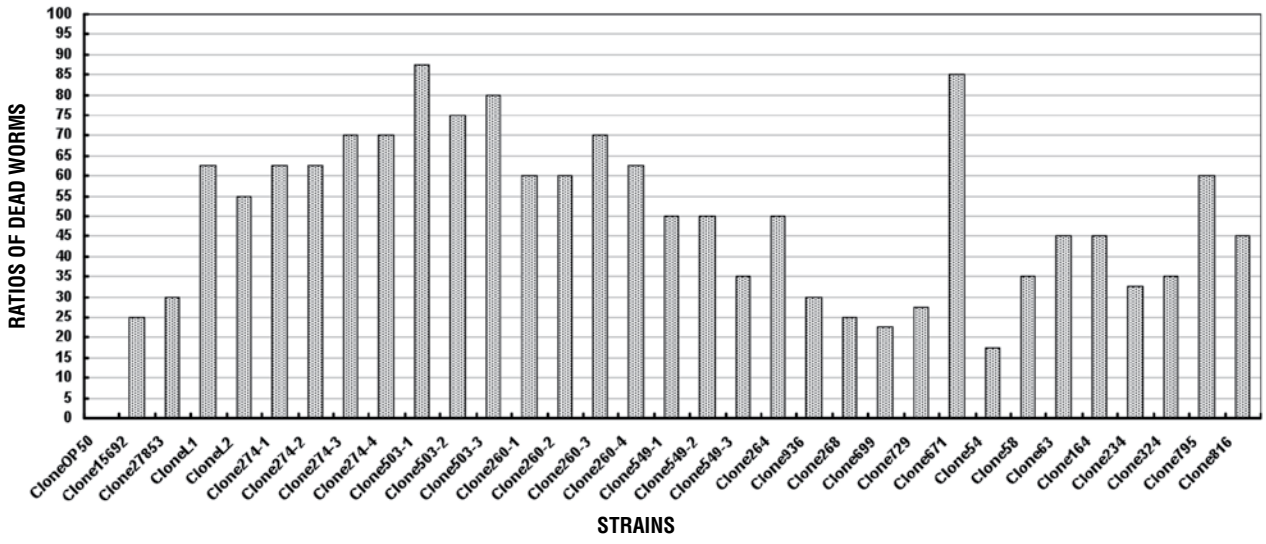
Locus	Fragment size (pp)	No. of alleles	genetic diversity (h)
acsA	390	5,1,23,4,7,14,2	0.8713±0.091
aroE	498	5,11,63,1,4	0.3860±0.092
guaA	373	57,11,3,16,7,12,70,10,1,65,21	0.9298±0.063
mutL	442	13,3,7,30,11,5	0.8012±0.081
nuoD	366	24,4,1,20,10	0.6667±0.061
ppsA	370	4,10,74,2,1,12,13,6	0.9240±0.093
trpE	443	3,4,7,10,16,23,74,80	0.8304±0.064

Mean genetic diversity *(H)*:0.7728±0.0727; Standardized I_A: 0.1501.

DISCUSSION

Previous data have indicated that the characterization of pathogenic isolates plays a pivotal role in the epidemiology of infectious diseases, generating the information necessary for identifying, tracking and intervening against disease outbreaks [8,13]. Molecular typing techniques seem to suggest that some genotypes of *P. aeruginosa* strains are responsible for most infections through transmission between cystic fibrosis (CF) patients in clinical settings, causing high morbidity and mortality [5-8]. However, it remains unclear whether the incidence of *P. aeruginosa* infection is due to some specifically epidemic strains in saturation dive settings. To gain a more systematic understanding

FIGURE 1



P. aeruginosa isolates pathogenic characteristics analyzed by *C. elegans* fast killing model. 28 isolates representative of the typical genetic groups determined via MLST sequencing were chosen as the test groups; two clinical strains (L1 and L2) and two control strains (ATCC27853 and ATCC15692) were chosen as positive control groups, *E. coli* OP50 was chosen as the non-virulent control group. Bars are representing the dead ratios of L4 *C. elegans* co-cultured with tested strains after eight hours. The mean of at least three experiments was determined for each strain.

about the molecular epidemiological characteristics of *P. aeruginosa* in occupational divers, the present study used the MLST technique to analyze the genetic type of *P. aeruginosa* strains carried by occupational divers in North China Sea and investigate the virulence phenotype of the representative typical STs.

According to previous studies, many STs of *P. aeruginosa* strains have been found frequently in many clinical settings and, among those, ST235, ST111, ST395 and ST175 are more often associated with worldwide outbreaks [13,15]. Ji, *et al.* [16] also report the MLST data of 896 *P. aeruginosa* strains collected from 65 hospitals in 22 regions of China and find that ST244, ST357, ST235, ST292, ST316, ST699, ST357, ST270 and ST313 are the main sequence types of the clinically isolated *P. aeruginosa* strains in those Chinese regions, and the other strains are defined as new sequence types. In the same data, Ji, *et al.* maintain that ST244, ST277 and ST235 are the dominant gene sequence types of clinically isolated strains epidemic in the Chinese regions [16].

In the present study, e-BURST analysis showed that the prevalence STs of *P. aeruginosa* strains isolated from the occupational divers of North China Sea were ST274 (18.75%), followed by ST260 (15.62%), ST549 (9.38%), ST503 (6.25%) and ST729 (4.68%). The two typical dominant CCs, as represented by ST260 and ST274 sequence types, accounted for 45.3% of the total isolates. About 17 percent of the total isolates were non-typable (Table2). The notable worldwide epidemic STs, known as ST235 and ST111, were not detected in this investigation. But, with further comparison by E-burst analysis, data showed that the ST58 and ST324 reported in this study and ST235 epidemic in the worldwide dataset belonged to same clonal complex. Although the frequency of isolates and predominance of ST235 have been reported by Ji, *et al.* in different regions of China [16], ST58 and ST324 were assigned as singletons and the accounted occurrence rate was less than 3.12% in current study (Table2).

In another study, Ahlén, *et al.* [17] reported that serotype O:11 and O:6 were the dominant serotypes among the *P. aeruginosa* isolates from the diving environment and divers from Norwegian Sea. These two serotypes also are reported to be the predominant worldwide serotypes and corresponded to two notable sequences as ST235 (Serotype O:11) and ST175 (Serotype O:6) respectively [15,18]. The above findings and reports indicated that, although the STs of *P. aeruginosa* strains reported here are to some extent correlated with those epidemics in the Chinese regions as well as other areas of the world, the genotypes of the *P. aeruginosa* dominant strains carried by the divers in Chinese regions are still distributed characteristically by occupational groups.

Data obtained from the virulence phenotype study showed that the mean virulence of group CC1 was stronger than that of the clinical positive control strains of the same period ($p = 0.03$). The mean virulence of group CC2 was equivalent to that of the clinical positive control strain ($p = 0.37$), but was higher than that of the standard control ATCC15692 and ATCC27853 ($p = 0.009$). These results suggest that all dominant epidemiological bacterial strains isolated in this study were virulent, but their natural virulence did not seem to be directly correlated with their epidemicity.

Using the LIAN technique, we further analyzed linkage disequilibrium of the amplified alleles and found that *guaA* had the highest number of the seven allele groups of bacterial genomes, followed by *ppsA, acsA, mutL* and *trpE,* while *nuoD* had the smallest number of alleles. The mean genetic diversity of the seven allele groups was 0.7728, closer to 1 than 0. As the coefficient of variation is positively correlated with genetic diversity, there was certain diversity in the bacterial genomes investigated in this study. The standardized index of association (I^s_A) is an index reflecting the efficiency of spontaneous recombination of different bacterial alleles, and this figure usually fluctuates between 0 and 1: the smaller the figure, the lower the efficiency of spontaneous recombination of alleles [19,20]. The I^s_A value of the 54 strains was relatively small ($I^s_A = 0.1501$), indicating that the probability of spontaneous gene recombination of the bacterial strains isolated from the occupational divers in this study is small under natural conditions.

In summary, we used the MLST technique to analyze 64 bacterial strains, of which 54 strains belonged to the known STs. We were unable to categorize the remaining 10 strains. Further experiments are needed to confirm whether they could be labeled as new sequence types. Our typing data show that there are dominant epidemiological CCs in diving populations, and they have relatively strong virulence.

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Case Report

Skin-sparing mastectomy flap ischemia salvage using urgent hyperbaric chamber oxygen therapy: A case report

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ABSTRACT

Since its introduction in 1991, skin-sparing mastectomy has emerged as an acceptable surgical technique in the management of breast cancer patients, providing optimal oncological safety and efficacy with favorable aesthetic results. Rates of native skin flap ischemia and necrosis after skin-sparing mastectomy are 2%-30% and result in a decreased aesthetic outcome and delay of necessary adjuvant treatment. Hyperbaric oxygen therapy has been advocated for the management of various compromised flaps, and when instituted immediately postoperatively, may prevent progression of ischemia into necrosis.

We report the case of a 41-year-old female who developed skin flap ischemia after undergoing skin-sparing mastectomy and was immediately treated with hyperbaric oxygen. The patient received a total of five hyperbaric oxygen therapy sessions, achieving full resolution of the ischemia without any complications. Further research is essential to determine the role of hyperbaric oxygen therapy in managing skin flap ischemia post skin-sparing mastectomy. Until such studies exist, hyperbaric oxygen therapy may be considered a preferred option in the management of native skin flap ischemia after skin-sparing mastectomy.

CASE REPORT

A 41-year-old Caucasian female non-smoker with a positive family history of breast cancer presented to our clinic. In 2010, she underwent lumpectomy with sentinel lymph node biopsy (SLNB) as well as radiation therapy for treatment of a 1.9 cm grade 3 infiltrating ductal carcinoma located in the inferomedial quadrant of her right breast. Two years later, the patient was diagnosed with a 10 mm infiltrating mucinous carcinoma in the right breast proximal to the inframammary fold. Imaging studies showed no signs of metastasis, and she was scheduled for skin-sparing mastectomy (SSM) with SLNB and immediate breast reconstruction.

A standard SSM with SLNB was performed using a periareolar elliptical incision to allow for removal of the breast tissue and a subaxillary incision for the SLNB. During the reconstructive portion of the procedure, a 2 *x* 3 cm poorly demarcated area of purple skin color and decreased capillary refill was observed between the periareolar incision and the subaxillary incision, indicating possible ischemia of the native skin flap. A 400 cc expander was placed submuscularly; however, no saline was injected into the expander in order to reduce possible tension on the skin flap. After skin closure, the area of ischemia remained poorly demarcated with slight improvement in its color; therefore, it was decided not to excise the ischemic area. In order to improve oxygen delivery to the skin flap, the patient was sent to the hyperbaric oxygen center for treatment six hours postoperatively after being cleared by the anesthesia and hyperbaric oxygen center teams.

The patient began hyperbaric oxygen (HBO_2) therapy the day of surgery and continued until postoperative day (POD) 3. Initial transcutaneous oximetry ($TcPO_2$) measured was 26 mmHg. In total, she underwent five sessions of HBO_2 therapy, each 90 minutes in length, under 2 atmospheres absolute with $FiO_2 = 1.0$ via face mask according to the Undersea and Hyperbaric Medical Society protocol for the treatment of compromised skin flaps [1]. By POD 3, the previously compromised area showed marked improvement, with no signs of potential ischemia. She was discharged home without any complications on POD 4. Upon follow-up at one and two weeks postoperatively, the mastectomy skin flaps appeared well perfused.

DISCUSSION

Native skin flap necrosis is an important complication of SSM and has been reported to occur in 2%-30% of operations [2]. Skin flap necrosis is difficult to manage and has a major impact because it can delay necessary adjuvant treatment and affect cosmetic outcome, in some cases requiring removal of the tissue expander or implant. If left untreated, skin flap necrosis can lead to edema, fibrosis, and wound contraction which can distort breast shape, retract the skin envelope, decrease ptosis, and cause asymmetry. Risk factors of skin flap necrosis include previous surgical procedures on the breast, preoperative radiation, tobacco smoking, increased BMI, and diabetes [3]. Our patient was previously operated upon and irradiated in the operated breast, which apparently increased the risk of skin ischemia.

In SSM, thickness of the native skin flap varies with the location on the breast, the thickness of subcutaneous tissue of each patient, as well as the ablative techniques of the surgeon [4]. Care must be taken to avoid excessive thinning of the flap, which can limit the skin's blood supply and increase the risk of necrosis. Meticulous surgical technique and gentle handling of tissues is essential to prevent skin flap ischemia.

Assessment of skin perfusion after mastectomy is crucial in order to identify areas of ischemia that have the potential for necrosis. Skin perfusion is easily assessed intraoperatively on the basis of flap thickness, color, temperature, turgor, capillary refill and dermal edge bleeding [5]. Recently, more advanced techniques have emerged, including laser-assisted indocyanine green angiography using the SPY Imaging System (Novadaq, Bonita Springs, Fla.), illumination with Wood's lamp after fluorescein dye administration, near-infrared spectroscopy, and laser-Doppler velocimetry [5]. Assessment of the skin flap with advanced techniques is not regularly undertaken at our facility and the area of ischemia in our patient was adequately determined by clinical observation alone.

HBO_2 therapy has been shown to provide an efficacious treatment for a variety of soft tissue injuries and has been advocated for the management of skin grafts and the treatment of ischemic flaps [6]. The Undersea and Hyperbaric Medical Society supports the use of HBO_2 for flaps and grafts that are compromised by decreased perfusion or hypoxia [1]. HBO_2 exerts favorable effects on wound healing in several systemic and local mechanisms. First, hyperoxia leads to increased production of radical oxygen and radical nitrous molecules. These molecules, such as nitrous oxide, oxygen-derived free radicals, hypochlorous acid and hydrogen peroxide, increase mobilization of stem cells from the bone marrow and augment their growth factor synthesis and secretion. The increased level of growth factors such as vascular endothelial growth factor (VEGF), angiopoietin, basic fibroblast growth factor (bFGF) and transforming growth factor β1 accelerate collagen synthesis and cross linking, cell migration and differentiation, as well as angiogenesis and vasculogenesis leading to neovascularization of the wound bed [7].

Most complications of HBO_2 therapy involve the middle ear and have been reported to occur in 1.9% of hyperbaric oxygen exposures [8]. A majority of these complications can be easily managed and rarely necessitate termination of treatment. Rare complications such as seizures, progressive myopia, and cataract formation have been reported after prolonged HBO_2 treatment [8]. Our patient did not develop any complications during her course of five hyperbaric oxygen sessions.

Although significant animal data as well as historic, non-randomized cohort or case control studies exist to support the use of HBO_2 in the treatment of ischemic flaps, there are no well-controlled randomized clinical studies evaluating its efficacy. Bowersox, *et al.* retrospectively reviewed 65 patients with compromised skin flaps treated with hyperbaric oxygen [9]. Of these flaps, 55% healed completely, and 34% showed marked improvement. It was noted that the patients who failed HBO_2 therapy had begun treatment an average of 15.2 days later than the successful group. Ueda, *et al.* reported on 26 patients who developed flap ischemia immediately after surgery [10]. The authors noted an average improvement of 92.1% while emphasizing increased success when hyperbaric oxygen was administered immediately after creation of the flap. Without prospective randomized clinical trials, it is impossible to assess the efficacy of HBO_2 in the prevention of necrosis in an ischemic skin flap post-SSM.

Hyperbaric oxygen therapy appears to be a promising modality in managing ischemia post skin-sparing mastectomy that warrants further investigation. The potential to easily prevent the progression of skin flap

ischemia into necrosis without altering aesthetic outcome is compelling. Further controlled studies will be required to firmly establish the safety and efficacy of this treatment for skin flap salvage after SSM.

The authors report that no conflict of interest exists with this submission.

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Hyperbaric Oxygen Therapy Indications

GAS-RELATED ISSUES IN HBO_2

HBO_2 Treatment for Decompression Sickness

AND

HBO_2 Treatment for Air or Gas Embolism

Hyperbaric oxygen treatment for decompression sickness

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ABSTRACT

Decompression sickness (DCS) is a clinical syndrome occurring usually within 24 hours of a reduction in ambient pressure. DCS occurs most commonly in divers ascending from a minimum depth of 20 feet (6 meters) of sea water, but can also occur during rapid decompression from sea level to altitude (typically >17,000 feet / 5,200 meters). Manifesta-tions are one or more of the following: most commonly, joint pain, hypesthesia, generalized fatigue or rash; less common but more serious, motor weakness, ataxia, pulmonary edema, shock and death. The cause of DCS is *in situ* bubble formation in tissues, causing mechanical disruption of tissue, occlusion of blood flow, platelet activation, endothelial dysfunction and capillary leakage. High inspired concentration of oxygen (O_2) is recommended as first aid for all cases and can be definitive treatment for most altitude DCS. For most other cases, hyperbaric oxygen is recommended, most commonly 100% O_2 breathing at 2.82 atmospheres absolute (U.S. Navy Treatment Table 6 or equivalent). Additional treatments (generally no more than one to two) are used for residual manifestations until clinical stability; some severe cases may require more treatments. Isotonic, glucose-free fluids are recommended for prevention and treatment of hypovolemia. An evidence-based review of adjunctive therapies is presented.

RATIONALE FOR TREATMENT OF DECOMPRESSION SICKNESS

Decompression sickness (DCS, "bends") is due to the formation of inert gas bubbles in tissues and/or blood due to supersaturation, where either the mechanical stresses caused by bubbles or their secondary cellular effects cause organ dysfunction [8,14,16,21,22]. DCS can be caused by a reduction in ambient pressure during ascent from a dive or rapid altitude excursion [25] either in space or a hyperbaric/hypobaric chamber.

In diving, compressed-gas breathing is usually necessary, although rarely DCS has occurred after either repetitive or deep breath-hold dives [30,51]. Bubble formation occurs when decompression occurs sufficiently fast that tissue inert gas partial pressure exceeds ambient pressure, causing supersaturation and bubble formation. The resulting clinical manifestations include joint pains (limb bends), cutaneous eruptions or rashes (skin bends), neurological dysfunction (peripheral or central nervous system bends), cardiorespiratory symptoms and pulmonary edema (chokes), shock and death [15].

Several mechanisms have been hypothesized by which bubbles may exert their deleterious effects. These include direct mechanical disruption of tissue, occlusion of blood flow, platelet deposition and activation of the coagulation cascade [46], endothelial dysfunction [44,45], capillary leakage [2,5,6,32,34] endothelial cell death, complement activation [58,59], inflammation [33] and leukocyte-endothelial interaction [23]. Recent evidence suggests that microparticles may play a role in DCS pathophysiology [53,65].

The diagnosis of DCS is made on the basis of signs and/or symptoms after a dive or altitude exposure [15]. Although arterial gas embolism can occur after a dive as shallow as 1 meter, the threshold depth for DCS is around 20 feet of sea water (fsw) [54]. DCS can occur after rapid decompression to altitude, *e.g.*, in a military jet, hypobaric chamber or during flight in a commercial aircraft with accidental loss of pressure. DCS after a dive can be provoked by mild altitude exposure, such as a commercial aircraft flight [17,57], but without a preceding dive the threshold altitude for DCS occurrence is approximately 20,000 feet [62,63].

DCS manifestations most commonly include paresthesias, hypesthesia, joint pain, skin rash and malaise [56]. More serious signs and symptoms include motor weakness, ataxia, dyspnea, urethral and anal sphincter dysfunction, shock and death [10,12,15]. Severe DCS may be accompanied by hemoconcentration and hypotension [2,5,6,34]. Severe symptoms usually occur within one to three hours of decompression; the vast majority of all symptoms manifest within 24 hours, unless there is an additional decompression (*e.g.*, altitude exposure).56 Altitude DCS has similar manifestations, although cerebral manifestations seem to occur more frequently [56].

Chest radiography prior to hyperbaric oxygen (HBO_2) treatment in selected cases may be useful to exclude pneumothorax (which may require tube thoracostomy placement before recompression). If the clinical presentation is ambiguous, neural imaging is occasionally useful to exclude causes unrelated to diving for which treatment other than HBO_2 would be appropriate (*e.g.*, herniated disc or spinal hemorrhage). However, imaging studies are rarely helpful for the evaluation or management of DCS [48,60]. MRI is not sufficiently sensitive to detect anatomic correlates of neurological DCI [19]. Bubbles causing limb pain cannot be detected radiographically. Neither imaging nor neurophysiological studies should be relied upon to confirm the diagnosis of DCS or be used in deciding whether a patient with suspected DCS needs HBO_2.

Improvement of decompression sickness symptoms as a result of compression was first noted in the 19th century [47]. Recompression with air was first implemented as a specific treatment for that purpose in 1896 [38]. Oxygen breathing was observed by Bert in 1878 to improve the signs of decompression sickness in animals [3]. The use of oxygen with pressure to accelerate gas diffusion and bubble resolution in humans was first suggested in 1897 [68] and eventually tested in human DCS and recommended for the treat-ment of divers in the 1930s [66]. The rationale for treatment with HBO_2 includes immediate reduction in bubble volume, increasing the diffusion gradient for inert gas from the bubble into the surrounding tissue, oxygenation of ischemic tissue and reduction of CNS edema. It is also likely that HBO_2 has other beneficial pharmacological effects, such as a reduction in neutrophil adhesion to the capillary endothelium [35,67]. The efficacy of administration of oxygen at increased ambient pressure (hyperbaric oxygen, HBO_2) is widely accepted, and HBO_2 is the mainstay of treatment for this disease [28,41,42,52,56].

PATIENT SELECTION CRITERIA

Treatment is recommended for patients with a history of a decompression and whose manifestations are consistent with DCS. HBO_2 treatment is recommended for all patients with symptoms of DCS whenever feasible, although oxygen administration at ground level may be sufficient for the treatment of altitude DCS when neurological manifestations are adequate. For definitive treatment of altitude-induced cases that do not respond to ground-level O_2, and for DCS after diving, HBO_2 remains the standard of care [39,41,43].

At a consensus workshop on remote treatment of mild DCS (limb pain, constitutional symptoms, subjective sensory symptoms or rash, with clinical stability for 24 hours or more and a normal neurological exam), it was concluded that if recompression therapy is not possible or may entail risk, some patients with mild symptoms and signs after diving can be treated adequately without recompression. However, in the absence of recompression, patients with DCS may recover more slowly [37].

CLINICAL MANAGEMENT

In addition to general supportive measures, including fluid resuscitation, airway protection, and blood pressure maintenance, administration of 100% oxygen at ground level (1 atmosphere absolute) is recommended as first aid for all cases of DCS. Normobaric oxygen can be definitive treatment for altitude-induced DCS [26,31].

Recommended treatment of DCS is administration of oxygen at suitable pressures greater than sea level (hyperbaric oxygen). A wide variety of initial hyperbaric regimens have been described, differing in treatment pressure and time, partial pressure of oxygen and diluent gas. Although there are no human outcome data obtained in prospective, randomized studies for the treatment of diving-related decompression sickness, broad principles that are generally agreed upon include: (a) complete resolution is most likely to result from early hyperbaric treatment [41,42]; (b) the U.S. Navy (USN) oxygen treatment tables [43] (and the similar Royal Navy and Comex tables), with initial recompression to

60 fsw (18 msw, 2.82 atm abs) have been the most widely used recompression procedures for DCS treatment beginning at the surface, and have achieved a high degree of success in resolving symptoms [1,10, 49,52,56].

For the majority of cases of DCS, superiority of treatments at pressure exceeding 2.82 atm abs, using helium as the diluent gas, or using saturation treatments has not been demonstrated. The use of treatment schedules that deviate from the USN oxygen treatment tables or published monoplace tables are best reserved for facilities and personnel with the experience, expertise and hardware necessary to deal with untoward responses. The choice of treatment table and the number of treatments required will depend upon: (a) the clinical severity of the illness; (b) the clinical response to treatment; and (c) residual symptoms after the initial recompression.

The great majority of cases of DCS respond satisfactorily to a single hyperbaric treatment, although repetitive treatments may be required depending on the patient's initial response. For patients with residual defects following the initial recompression, repetitive treatments are recommended until clinical stability has been achieved. HBO_2 should be administered repetitively as long as step-wise improvement occurs, based upon clearly documented symptoms and physical findings. The need for such follow up ("tailing" treatments) should be supported by documentation of the clinical evaluation before and after each treatment. Complete resolution of symptoms or lack of improvement on two consecutive treatments establishes the endpoint of treatment, typically no more than one to two treatments [10,56]. Although a small minority of divers with severe neurological injury may not reach a clinical plateau until 15-20 repetitive treatments have been administered, formal statistical analysis of approximately 3,000 DCI cases supports the efficacy of no more than five to 10 repetitive treatments for most individuals [55]. In 83 cases of DCS in recreational divers reported by the Divers Alert Network in 2006, the median number of hyperbaric treatments was 1 [10].

Although few data to support an outcome effect of rapid *vs.* delayed treatment [18,49], timely treatment is preferred. However, the data currently available have not established a maximum time (hours or days) after which recompression is ineffective [9,13,24,29,36,50,64]. Monoplace chambers were originally designed for the continuous administration of 100% oxygen and were not equipped to administer air for "air breaks," which are incorporated in USN treatment tables. For monoplace chambers of this type, tables are available for treatment of DCS that are shorter than standard USN treatment tables [11,20,27]. Retrospective evidence, using telephone follow-up, suggests that such tables may be as effective as standard USN tables for the treatment of mildly or moderately affected patients [4,7,28]. However, many monoplace chambers are now fitted with the means to deliver air to the patient, and thus can be used to administer standard 2.82 atm abs USN treatment tables [61].

Altitude DCS. The following algorithm has been used effectively by the U.S. Air Force [42]:

Symptoms that clear on descent to ground level with normal neurological exam:

- 100% oxygen by tightly fitted mask for two hours minimum; aggressive oral hydration; observe 24 hours.

Symptoms that persist after return to ground level or occur at ground level:

- 100% oxygen; aggressive hydration; hyperbaric treatment using U.S. Navy Treatment Tables 5 or 6, as appropriate.

For individuals with symptoms consisting of limb pain only, which resolves during oxygen breathing while preparing for hyperbaric treatment:

- a 24-hour period of observation should be initiated; hyperbaric therapy may not be required.

For severe symptoms of DCS, including neurological symptoms, "chokes," hypotension, or symptoms that progress in intensity despite oxygen therapy:

- continue 100% oxygen; administer intravenous hydration; initiate immediate hyperbaric therapy using USN Treatment Table 6.

Adjunctive therapy. Adjunctive treatment such as first-aid oxygen administration, fluid resuscitation and, for patients with leg immobility and venous thrombo-embolism prophylaxis are indicated. These are discussed in detail in a separate monograph [40]. A summary of current recommendations for adjunctive therapy is available on the Undersea and Hyperbaric Society web site (*http://www.uhms.org*; also see the following text).

TABLE 1: Evidencebased review of adjunctive therapies for DCS (from Moon [40]. Summaries can be accessed at www.uhms.org)

Condition	Surface O_2 (1 atm abs) Class	Level	Intravenous Fluid Therapy Class	Level	Aspirin Class	Level	NSAIDs Class	Level	Anticoagulants* Class	Level	Corticosteroids Class	Level	Lidocaine Class	Level
AGE (no significant inert gas load)	I	C	D5W†	III	IIb	C	2B	C	IIb	C	III	C	IIa	B
			LR/crystalloid‡	IIb										
			Colloid#	IIb										
DCS pain only/ mild	I	C	D5W†	III	IIb	C	2B	B	III	C	III	C	III	C
			LR/crystalloid‡	I										
			Colloid#	I										
DCS neurological symptoms	I	C	D5W†	III	IIb	C	2B	B	IIb§	C	III	C	IIb	C
			LR/crystalloid‡	I										
			Colloid#	I										
DCS chokes (cardiorespiratory)	I	C	D5W†	III	IIb	C	2B	C	IIb	C	III	C	III	C
			LR/crystalloid‡	IIb										
			Colloid#	IIb										

§ For decompression illness with leg immobility, low molecular weight heparin is recommended as soon as possible after injury (enoxaparin 30 mg or equivalent, subcutaneously every 12 hours.
† 5% dextrose in water.
‡ Lactated Ringer's solution, normal saline or other isotonic intravenous fluid not containing glucose.
# Starch, gelatin or protein fraction with isotonic electrolyte concentration.
* Full dose heparin, warfarin, thrombin inhibitors, thrombolytics, IIB/IIIA antiplatelet agents.

EVIDENCE-BASED REVIEW

The use of HBO_2 for decompression sickness is an AHA Level I recommendation (level of evidence C). A number of adjunctive therapies have been used for the treatment of DCS (Table 1) and discussed in the *Report of the Decompression Illness Adjunctive Therapy Committee* of the Undersea and Hyperbaric Medical Society [40]. These guidelines can be accessed via the internet at www.uhms.org.

UTILIZATION REVIEW

Utilization review should occur after 10 treatments.

COST IMPACT

Only those people exposed to increased ambient pressure (divers or compressed air workers) or who suffer decompression sickness at altitude are affected. Because there are relatively few individuals who develop this condition, the application of HBO_2 will be limited. HBO_2 is a treatment that usually provides resolution or significant improvement of this disorder that can otherwise result in permanent spinal cord, brain or peripheral nerve damage or death, and is therefore an exceptionally cost effective treatment.

The author reports that no conflict of interest exists with this submission. ■

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Hyperbaric oxygen treatment for air or gas embolism

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ABSTRACT

Gas can enter arteries (arterial gas embolism) due to alveolar-capillary disruption (caused by pulmonary overpressurization, *e.g.*, breath-hold ascent by divers) or veins (venous gas embolism, VGE) as a result of tissue bubble formation due to decompression (diving, altitude exposure) or during certain surgical procedures where capillary hydrostatic pressure at the incision site is sub-atmospheric. Both AGE and VGE can be caused by iatrogenic gas injection. AGE usually produces strokelike manifestations, such as impaired consciousness, confusion, seizures and focal neurological deficits. Small amounts of VGE are often tolerated due to filtration by pulmonary capillaries. However, VGE can cause pulmonary edema, cardiac "vapor lock" and AGE due to transpulmonary passage or right-to-left shunt through a patent foramen ovale. Intravascular gas can cause arterial obstruction or endothelial damage and secondary vasospasm and capillary leak. Vascular gas is frequently not visible with radiographic imaging, which should not be used to exclude the diagnosis of AGE. Isolated VGE usually requires no treatment; AGE treatment is similar to decompression sickness (DCS), with first aid oxygen then hyperbaric oxygen. Although cerebral AGE (CAGE) often causes intracranial hypertension, animal studies have failed to demonstrate a benefit of induced hypocapnia. An evidence-based review of adjunctive therapies is presented.

RATIONALE FOR TREATMENT OF AIR OR GAS EMBOLISM

Gas embolism occurs when gas bubbles enter arteries or veins. Arterial gas embolism (AGE) was classically described during submarine escape training, in which pulmonary barotrauma occurred during free ascent after breathing compressed gas at depth. Pulmonary barotrauma and gas embolism due to breath-holding can occur after an ascent of as little as one meter [10]. AGE has been attributed to normal ascent in divers with lung pathology such as bullous disease and asthma [70,114]. Pulmonary barotrauma can also occur as a result of blast injury in or out of water [36,67], mechanical ventilation [76], penetrating chest trauma [41], chest tube placement14 and bronchoscopy [115].

Venous gas embolism (VGE) occurs commonly after compressed-gas diving [38,98]. Normally, VGE bubbles are trapped by the pulmonary capillaries and do not cause clinical symptoms. However, in large volumes, VGE can cause cough, dyspnea and pulmonary edema [30,37] and may overwhelm the capacity of the pulmonary capillary network, allowing bubbles to enter the arterial circulation [16,110]. VGE can also enter the left heart directly via an atrial septal defect or patent foramen ovale [71,93,111,112]. Another cause of VGE is rapid exposure to altitude [5]. However, this occurs only at rapid rates of decompression such as may occur during flight in a military jet, in a hypobaric chamber or with accidental loss of pressure during flight in commercial aircraft.

Causes of gas embolism other than diving include accidental intravenous air injection [1,53], cardiopulmonary bypass accidents [88], needle biopsy of the lung [59], hemodialysis [6], central venous catheter placement or disconnection [86,109], gastrointestinal endoscopy [90], hydrogen peroxide irrigation [7,51,100] or ingestion [19,79,89], arthroscopy [32,40], cardiopulmonary resuscitation [47], percutaneous hepatic puncture [43], blowing air into the vagina during orogenital sex [11,13,52] and sexual intercourse after childbirth [8]. Air embolism can occur during procedures in which the surgical site is under pressure (*e.g.*, laparoscopy [20,22,39,58,78], transurethral surgery [101,104], vitrectomy [60], endoscopic vein harvesting

[64] and hysteroscopy48,95). Massive VGE can occur due to passive entry of air into surgical wounds that are elevated above the level of the heart (such that the pressure in adjacent veins is subatmospheric) [66]. This has classically been described in sitting craniotomy [72], but has also occurred during cesarean section [34], prostatectomy using the radical perineal [50] and retropubic [3,91] approaches, spine surgery [57,116], hip replacement [4], liver resection [61], liver transplantation [85] and insertion of dental implants [15,23].

Clinical deficits can occur after intra-arterial injection of only small volumes of air. Intravenous injection is often asymptomatic. Injection of up to 0.5-1 mL/kg has been tolerated in experimental animals [75]. In humans, continuous IV infusion of oxygen at 10 mL/minute has been reported as well tolerated, while 20 mL/minute has been reported to cause symptoms [102]. Compared with constant infusions, bolus injections are more likely to cause clinical abnormalities [117].

There are several possible mechanisms of injury, including intracardiac "vapor lock," with resulting hypotension or acute circulatory arrest, and direct arterial occlusion. Animal studies using a cranial window have demonstrated that bubbles can cause a progressive decline in cerebral blood flow [45,46] even without vessel occlusion, an effect that requires neutrophils [44] and can be initiated by bubble-induced stripping of the endothelium from the underlying basement membrane [63,83,84]. Even without direct mechanical damage, bubble contact with endothelial cells can initiate opening of transient receptor potential vanilloid (TRPV) ion channels, calcium entry, mitochondrial dysfunction and cell death [54,96,97]. In some cases of cerebral AGE there is clinical improvement followed by delayed deterioration a few hours later [87]. Proposed mechanisms for this include edema, bubble regrowth and secondary thrombotic occlusion.

Manifestations of arterial gas embolism include loss of consciousness, confusion, focal neurological deficits, cardiac arrhythmias or ischemia, while venous gas embolism may include hypotension, tachypnea, hypocapnia, pulmonary edema or cardiac arrest [12, 28,29,33,35,56]. AGE in divers with a pre-existing inert gas load (due to a dive) can precipitate neurological manifestations that are more commonly seen with DCS, such as paraplegia due to spinal cord damage [82]. While imaging studies sometimes reveal intravascular air, brain imaging is often normal even in the presence of severe neurological abnormalities [9, 18,92,94,113]. Imaging is therefore not recommended to make the diagnosis (see below). Findings that support the diagnosis of AGE include evidence of pulmonary barotrauma, and evidence of intravascular gas using ultrasound or direct observation (*e.g.*, aspiration of gas from a central venous line).

PATIENT SELECTION CRITERIA

Hyperbaric oxygen HBO_2 is recommended in cases of AGE with neurological manifestation. A short interval between embolism and recompression treatment is associated with a higher probability of good outcome. However, a response to treatment has been observed after 24 or more hours [68]. Some patients spontaneously resolve shortly after symptoms develop, particularly after administration of first aid oxygen. However, because secondary deterioration can later occur, HBO_2 is recommended even in the absence of symptoms. HBO_2 is rarely indicated for VGE unless AGE is also present. An isolated case report suggests that HBO_2 may be effective for the treatment of pulmonary edema due to VGE [119].

CLINICAL MANAGEMENT

The presumptive diagnosis of AGE is made on the basis of clinical criteria. Diagnostic imaging is unnecessary, has low diagnostic sensitivity [9] and does not affect management. Absence of intravascular gas should not prevent treatment. Performing brain imaging when there is a high degree of suspicion of AGE usually delays the initiation of appropriate HBO_2 treatment and only serves a useful clinical purpose if other pathology is detected that requires different treatment. The only rational reason to perform diagnostic imaging is to exclude other pathology that might have similar manifestations as AGE but require different management (*e.g.*, intracranial hemorrhage). Immediate treatment of gas embolism should consist of airway management, maintenance of blood pressure and administration of as high an oxygen concentration as is feasible. Hypotension can augment the injury, and should be actively treated [107]. Supplemental oxygen is recommended not only to maintain arterial oxygenation, but also to facilitate bubble resorption. Nitrous oxide (N_2O) administration causes bubbles to grow, and if gas embolism is suspected in an anesthetized patient N_2O should be discontinued in favor of 100% oxygen.

Head-down position was formerly recommended for the initial treatment of patients with AGE, in order to

minimize the risk of additional cerebral embolization because of buoyancy, and shrinkage of bubbles due to increased hydrostatic pressure, and some anecdotal cases support its use [55]. Lateral decubitus position has been recommended for VGE. However, buoyancy has little if any effect upon arterial [17] or venous [69] distribution of intravascular air, and head-down position can worsen cerebral edema [25]. Therefore, except for a brief period (less than 10 minutes) of head-down position, which might conceivably result in enhanced clearance of bubbles from the cerebral circulation after a large volume of gas entry, the supine position is preferable.

HBO_2 to treat gas embolism remains the definitive treatment for arterial gas embolism [21,81]. A review of 597 published cases of arterial gas embolism reveals superior outcomes with the use of HBO_2 compared to non-recompression treatment [2,12,13, 26,28,31,42,49,62,77,80,105,118]. HBO_2 treatment is not required for asymptomatic VGE, however it has been associated with clinical improvement in patients with secondary pulmonary edema [119]. Gas bubbles have been known to persist for several days and there are many reports noting success when HBO_2 treatments were begun after delays of hours to days [13,65,99,118]. While early HBO_2 has the highest likelihood of success, hyperbaric treatment is indicated even after a significant delay following the embolic event [12]. Because of the tendency for patients with AGE to deteriorate after apparent recovery, early HBO_2 is recommended even for patients who appear to have spontaneously recovered. One author has suggested that the presence or absence of air detectable by brain computed tomography should be used as a criterion for HBO_2 therapy [24]. However, timely administration of HBO_2 usually causes clinical improvement even in the absence of demonstrable air.

In patients with AGE caused by pulmonary barotrauma there may be a coexisting pneumothorax, which could develop into tension pneumothorax during chamber decompression. Therefore, if the patient will be treated in a monoplace chamber, placement of a chest tube in patients with pneumothorax prior to HBO_2 is recommended. For multiplace chamber treatment careful monitoring is a feasible option. Coexisting pneumomediastinum does not generally require any specific therapy, and will usually resolve during HBO_2.

Administration of repetitive treatments is recommended until there is no further stepwise improvement, typically after no more than one to two hyperbaric treatments, but occasionally up to five to 10 [74,103,108].

Immediate recompression to 6 atmospheres absolute (atm abs) pressure was recommended in the past. However, there is no conclusive evidence that pressures higher than 2.82 atm abs (18 msw, 60 fsw) offer any advantage. If possible, an initial compression to 2.82 atm abs (60 fsw or 18 msw equivalent depth) breathing 100% oxygen is recommended, using U.S. Navy Treatment Table 6 or equivalent. If the clinical response to treatment is judged to be suboptimal, options including deeper recompression or extension of the treatment table can be instituted according to the expertise and resources available.

The standards against which other treatment schedules ("tables") should be compared are those of the U.S. Navy (USN Diving Manual, available at http://www.supsalv.org/) and similar procedures used by other navies and commercial diving operations [74,81].

Adjunctive therapies are discussed in separate publications [73,108]. A summary of adjunctive therapy can be obtained on the Undersea and Hyperbaric Society web site *(http://www.uhms.org)*. Specific adjunctive therapies and their recommendations are listed below.

EVIDENCE-BASED REVIEW

The use of HBO2 for arterial gas embolism and symptomatic venous gas embolism is an AHA Class I recommendation (level of evidence C).

Adjunctive therapies for isolated AGE include the following:

- oxygen administered as a first aid measure (class I, level C);
- lidocaine (class IIa, level B);
- aspirin, NSAIDs (class IIb, level C);
- anticoagulants (class IIb, level C);
- corticosteroids (class III, level C);
- intravenous fluids (D5W class III, level C;
- isotonic crystalloid, colloid class IIb, level C).

Although isolated AGE does not require specific fluid therapy, patients with accompanying decompression sickness may have significant hemoconcentration, and require aggressive fluid resuscitation (see Decompression Sickness). For patients who are immobilized for 24 hours or greater due to neurological injury, low molecular weight heparin is recommended for prophylaxis against venous thromboembolism (class I, level A). In addition, since hyperthermia can adversely affect neurological outcome, aggressive treatment of fever is recommended.

For critically ill patients with AGE, no systematic human studies are available. However, systematic large-animal studies support the use of normotension and isocapnia [27,106,107], along with HBO_2.

UTILIZATION REVIEW

Utilization review is recommended after 10 treatments.

COST IMPACT

The primary treatment of choice for air embolism from any cause is HBO_2 therapy. Decreased high mortality rates and prevention or moderation of permanent neuro-logical damage make this modality cost-effective.

The author reports that no conflict of interest exists with this submission. ■

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Letters to the editors

High-fat diets and decompression stress revisited

Dear editors:

The authors Kaczerska, *et al.* conclude in their paper "The influence of high-fat diets on the occurrence of decompression stress after air dives" [1], that a high-fat diet significantly increases the severity of DCS stress. Unfortunately, the term "symptoms of DCS stress," as used in their paper, is confusing, as they apparently mean the risk of DCS. Since the paper considers bubble grades, not occurrence of DCS itself, it would have been more clear to label the horizontal axis of the legendless Figures 3-9 with "bubble grade" and with "low" and "high."

Despite this, it is disputable whether the diet can be seen as a causal factor as they suggest. To date, several demographic characteristics are considered as DCS stressors, such as age, and VO_{2max} (see Ref 2, also for references). A stressor has an underlying mechanism enhancing the amount of venous gas bubbles (VGB) and consequently increasing the DCS risk. For a century, body fat (BF) and BMI have often been considered as a stressor. In the introduction the authors adopt the classical assumed mechanism, the five-time higher solubility of N_2 in fat compared to water. Then with increasing BF, total body N_2-load increases. However, in a recent paper this load was modeled for a lean diver and fat diver; the difference appeared to be so small that a notable difference in VGB can be excluded [2].

Although some studies found a (cor)relation between DCS risk or VGB with BF or BMI, this does not at all imply causality. The problem with BMI and BF is that they are strongly mutually correlated with age and VO_{2max}; the problem of multicollinearity. In two studies the BF (or BMI) correlation with VGB was significant [3,4], but when the correlation was corrected for the correlation with age and VO_{2max} by calculating partial correlation coefficients, any correlation with VGB vanishes.

Also, another study with very small ranges of age and VO_{2max} showed no correlation between BF and VGB [2]. Studies claiming BMI or BF as a stressor, according to our definition, have in fact a disputable or inappropriate study design since multicollinearity was not examined.

In the study discussed, the multicollinearity (*i.e.*, calculating the partial correlations) between BMI, age, recommended daily fat intake (RDI), total cholesterol (CHOL) and triglycerides (TG) were not examined. It seems quite likely that daily RDI, TG and CHOL are mutually highly correlated and that these variables are also correlated with BMI. BMI is in turn correlated with VGB (via its negative correlation with the stressor VO_{2max}).

Surprisingly, this study examines BMI, not BF, whereas the correlation between BF and VO_{2max} is much higher than between BMI and VO_{2max}. Although BMI and BF are well correlated, many, especially young subjects with high BMIs, have unexpected low BFs and high VO_{2max} values (e.g., rowers, weight-lifters).

In addition to statistical concerns, there is also a physiological one.

In the discussion the authors implicitly indicate that the difference in dissolved N_2 in serum due to the diet would be the cause for the difference in VGB. From the authors' data it can be calculated directly that the difference in volume% of fatty molecules in the serum between the low bubblers (KM<2) and the high bubblers is 0.8 mL/L. This is a serum load difference of only 3%. It is highly unlikely that this difference will result in the large observed differences in KM. (Unfortunately, detailed information about the distribution of KM grades and scoring methods are lacking.)

In conclusion, from experimental findings and theoretical considerations it seems likely that RDI, TG, and CHOL are indicators for bubble stress, but with the present study design and statistical analysis the question cannot be answered whether they are stressors.

To date, BMI is an indicator, poorer than BF; according to new insights they have lost their status as stressor.

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Response: High-fat diets and decompression stress revisited

Dear editors:

According to the theory, decompression stress without symptoms always carries the risk of decompression sickness (DCS). But even if it does not lead to the onset of DCS, it can lead to the so-called late sequelae of diving. In our study decompression stress or lack thereof was observed. Results were divided into two groups and the signatures of the charts indicate the group: without stress and with stress. Changing of signatures into "low stress" and "high stress" could suggest that stress was observed in both groups, but in varying degrees.

For years, the research on the impact of age, body weight, fat mass, maximal oxygen uptake went on, but no one paid attention to the factor as obvious as nutrition. This is why in our research we focus our attention on diet and its influence on the level of cholesterol and triglycerides and presence of decompression stress. Many times it has been proven that a high-fat diet with a predominance of animal products causes an increase in cholesterol and triglyceride levels in the blood. It should also be stressed in the mechanism of fat digestion and absorption and a long half-life in the blood, from a few hours to a few days depending on the type of the fat fraction. The results indicate a strong causal relationship between the studied parameters. Just highlighting the effects of diet was the aim of our research

The influence of BMI on the risk of decompression stress has been investigated in the past as "by the way," with researchers having results, analyzing them and drawing conclusions. Even statistically BMI influences on stress are not reliable indicators of risk. This is evident particularly among young people who are well trained with highly developed muscle tissue: A relatively high body weight has nothing to do with being overweight.

In such cases, in fact it is better to examine the body fat mass or perform measurements of the thickness of skin folds.

The question arises in reference to research conducted by the authors of the letter as to whether it would not be worthwhile to do your research including evaluation of food preferences and maximal oxygen absorption.

As we know, a high-fat diet with a majority of products of animal origin is an acidic diet. During metabolic acidosis an increase in oxygen uptake caused by hyperventilation is observed. There is a high probability that a high-fat diet increases the maximal oxygen uptake, not the mass of adipose tissue.

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(410) 677-6605

Wound Care & Hyperbaric Medicine Center
† **Carroll Hospital Center**
Westminster, Maryland
(410) 871-6334

Wound Care & Hyperbaric Medicine Center
† **Northwest Hospital Center**
Randallstown, Maryland
(410) 496-7191

MASSACHUSETTS

Wound Healing Center
† **Anna Jaques Hospital**
Newburyport, Massachsetts
(978) 463-1303

The Wound & Hyperbaric Medicine Center
† **Beverly Hospital**
Beverly, Massachsetts
(978) 921-1210

MICHIGAN

The Center for Wound Healing & Hyperbaric Medicine
† **Oakwood Hospital-Taylor**
Taylor, MI
(313) 295-5343

The Hyperbaric Medicine Program
† **Spectrum Health**
Grand Rapids, Michigan
(616) 391-1269

Metro Health Wound Healing Center
Metro Health Hospital
Wyoming, Michigan
(616) 252-7494

Wound Healing & Hyperbaric Medicine Center
† **Detroit Receiving Hospital**
Detroit, Michigan
(313) 745-8450

MINNESOTA

* **Hyperbaric Medicine**
† **Hennepin County Medical Center**
Minneapolis, Minnesota
(612) 873-4050

* **Hyperbaric & Altitude Medicine Program**
† **Mayo Clinic**
Rochester, Minnesota
(507) 266-4602

MISSISSIPPI

Anderson Wound Healing Center
† **Anderson Regional Medical Center**
Meridian, Mississippi
(601) 484-3432

Department of Hyperbaric Medicine
Rush Foundation Hospital
Meridian, Mississippi
(601) 703-4200

MISSOURI

Center for Wound Care & Hyperbaric Medicine
† **St. Mary's Hospital of Blue Springs**
Blue Springs, Missouri
(816) 655-5780

* **Cox Hyperbaric Medicine & Wound Care Center**
† **Cox Healthcare**
Springfield, Missouri
(417) 269-9950

* **Hyperbaric Medicine**
† **Kindred Hospital**
Kansas City, Missouri
(816) 995-2115

* **Hyperbaric Oxygen of Kansas City**
† **Saint Joseph Health Center**
Kansas City, Missouri
(816) 943-4600

The Wound Care Center
† **North Kansas City Hospital**
North Kansas City, Missouri
(816) 691-5055

Wound Care & Hyperbaric Medicine Center
Freeman Hospital
Joplin, Missouri
(413) 347-4800

NEW HAMPSHIRE

Wound Healing Center
Concord Hospital
Concord, New Hampshire
(603) 230-1970

NEW JERSEY

Central Jersey Wound & Hyperbaric Treatment Center
† **CentraState Healthcare System**
Freehold, New Jersey
(866) 659-6863

* **Hyperbaric Medicine Unit**
† **Morristown Memorial Hospital**
Morristown, New Jersey
(973) 971-6015

The Hyperbaric Medicine Program
† **Englewood Hospital & Medical Center**
Englewood, New Jersey
(201) 894-3898

* **Hyperbaric Program**
† **Overlook Medical Center**
Summit, New Jersey
(908) 522-5900

Wound Healing Center
† **Warren Hospital**
Phillipsburg, New Jersey
(908) 213-6653

NEW MEXICO

Christus St. Vincent Regional Wound & Hyperbaric Ctr
† **Christus St. Vincent**
Santa Fe, New Mexico
(505) 946-3180

NEW YORK

Center for Wound Care & Hyperbaric Medicine
† **Olean General Hospital**
Olean, New York
(716) 375-7577

Center for Wound Care & Hyperbaric Medicine
St. Joseph's Hospital Health Center
Fayetteville, New York
(315) 329-7770

Hyperbaric Medicine & Wound Care
† **Plainview Hospital**
Plainview, New York
(516) 796-1313

* **Hyperbaric Medicine and Wound Care Center**
Upstate University Hospital
Syracuse, New York
(315) 464-4910

Hyperbaric and Wound Healing Center
† **St. Joseph Hospital**
Bethpage, New York
(516) 520-2788

Institute for Wound Care & Hyperbaric Medicine
† **Hudson Valley Hospital Center**
Cortlandt Manor, New York
(914) 734-3030

Westchester Hyperbaric Center
† **Westchester Medical Center**
Valhalla, New York
(914) 493-1500

Wound Care Center
† **Vassar Brothers Medical Center**
Poughkeepsie, New York
(845) 431-2400

Wound & Hyperbaric Institute at Good Samaritan
† **Good Samaritan Hospital**
Suffern, New York
(845) 368-5590

The Wound Healing Center at Kingston Hospital
† **The Kingston Hospital**
Kingston, New York
(845) 334-4325

Wound Healing Center & Hyperbaric Medicine Program
† **Winthrop-University Hospital**
Mineola, New York
(516) 663-8498

Wound Healing Center
† **Catskill Regional Medical Center**
Harris, New York
(845) 794-3300

Wound Healing Center
† **Orange Regional Medical Center**
Middletown, New York
(845) 333-7700

Wound Healing Center
† **Rochester General Hospital**
Rochester, New York
(585) 922-4325

NORTH CAROLINA

* **Center for Hyperbaric Medicine & Environmental Physiology**
† **Duke University Medical Center**
Durham, North Carolina
(919) 684-6726

Comprehensive Wound Care
Kinston, North Carolina
(252) 527-9928

Park Ridge Wound Center & Hyperbaric Medicine
† **Park Ridge Health**
Hendersonville, North Carolina
(855) 774-5433

OKLAHOMA

Midwest Wound Care
† **Midwest Regional Medical Center**
Midwest City, Oklahoma
(405) 610-8056

OHIO

Wound Care & Hyperbaric Medicine Center
† **Kettering Medical Center**
Wright-Patterson AFB, Ohio
(937) 257-8600

OREGON

Bend Memorial Clinic
Bend, Oregon
(541) 317-4378

Hyperbaric Center
Sacred Heart Medical Center at Riverbend
Springfield, Oregon
(541) 222-4500

Hyperbaric Medicine & Advanced Wound Care
† **Providence Portland Medical Center**
Portland, Oregon
(503) 215-6061

PENNSYLVANIA

Advance Wound Healing Center
† **Carlisle Regional Medical center**
Carlisle, Pennsylvania
(717) 243-1900

Guthrie Center for Wound Care / Hyperbaric Medicine
† **Robert Packer Hospital**
Sayre, Pennsylvania
(570) 882-6724

Wound Healing & Hyperbaric Medicine Center
† **Reading Hospital**
Wyomissing, Pennsylvania
(610) 568-3931

Wound & Hyperbaric Center-East
† **Pinnacle Health**
Harrisburg, Pennsylvania
(717) 671-2050

Wound & Hyperbaric Center-West
† **Pinnacle Health**
Harrisburg, Pennsylvania
(717) 791-2440

Wound Care & Hyperbaric Services
† **Lancaster General Health**
Lancaster, Pennsylvania
(717) 544-3216

RHODE ISLAND

* **Wound Recovery Center**
† **Kent County Hospital**
Warwick, Rhode Island
(401) 736-4646

SOUTH CAROLINA

* **The Department of Hyperbaric Medicine**
† **Roper Hospital**
Charleston, South Carolina
(843) 324-3395

* **The Hyperbaric Medicine Service**
† **Palmetto Richland Memorial Hospital**
Columbia, South Carolina
(803) 434-7101

Wound Healing Center
† **Spartanburg Regional Healthcare System**
Spartanburg, SC
(864) 560-6000

TENNESSEE

Erlanger Wound Care & Hyperbaric Oxygen
† **Erlanger Hospital**
Chattanooga, Tennessee
(423) 778-4027

Wound Care & Hyperbaric Center
† **Regional Medical Center at Memphis**
Memphis, Tennessee
(901) 545-8999

TEXAS

* **Institute for Exercise & Environmental Medicine**
† **Presbyterian Hospital**
Dallas, Texas
(214) 345-4651

Institute for Wound Healing
† **Central Texas Medical Center**
San Marcos, Texas
(512) 353-6430

Memorial Hermann Center for Hyperbaric Medicine
† **Memorial Hermann Hospital**
Houston, Texas
(713) 704-4268

* **Nix Hyperbaric & Wound Care Center**
† **Nix Hospital**
San Antonio, Texas
(210) 223-1145

Northwest Wound Care Center & Hyperbaric Oxygen Therapy
† **Northwest Hospital**
Amarillo, Texas
(806) 351-4155

* **Southwest Center for Wound Care**
Southwest General Hospital
San Antonio, Texas
(210) 690-2424

* **USAF Hyperbaric Medicine Center**
59 MDTS/SGVDH
Lackland AFB, San Antonio, Texas
(210) 292-3483

Wound Care & Hyperbaric Center
† **Methodist Charlton Medical Center**
Dallas, Texas
(214) 947-0752

Wound Care & Hyperbaric Medicine Center
† **Methodist Dallas Medical Center**
Dallas, Texas
(214) 447-5000

Wound Care & Hyperbaric Program
San Jacinto Methodist Hospital
Baytown, Texas
(281) 425-2160

* **Wound Care Center**
† **Trinity Mother Frances Hospital**
Tyler, Texas
(903) 531-5788

The Wound Center at Knapp
† **Knapp Medical Center**
Weslaco, Texas
(956) 969-5185

* **Wound Healing Center**
† **East Texas Medical Center**
Tyler, Texas
(903) 526-4325

UTAH

* **Department of Hyperbaric Medicine**
† **Dixie Regional Medical Center**
St. George, Utah
(435) 688-4293

Hyperbaric and Wound Center
Davis Hospital and Medical Center
Layton, Utah
(801) 807-7900

Hyperbaric and Wound Center
Jordan Valley Medical Center
West Jordan, Utah
(801) 601-2322

* **Hyperbaric Medicine**
† **Intermountain Medical Center**
Murray, Utah
(801) 408-3623

* **Hyperbaric Medicine Department**
† **LDS Hospital**
Salt Lake City, Utah
(801) 408-3623

* **Hyperbaric Medicine Department**
† **Utah Valley Regional Medical Center**
Provo, Utah
(801) 357-8156

Hyperbaric Medicine & Wound Treatment Ctr. of Utah
Salt Lake Regional Medical Center
Salt Lake City, Utah
(801) 582-4268

Logan Regional Wound & Hyperbaric Center
† **Intermountain Logan Regional Hospital**
Logan, Utah
(435) 716-2834

McKay-Dee Wound and Hyperbaric Center
† **McKay-Dee Hospital**
Ogden, Utah
(801) 387-4870

Wound Care and Hyperbaric Center
† **Cache Valley Specialty Hospital**
North Logan, Utah
(435) 713-1355

VIRGINIA

Center for Wound Care & Hyperbaric Medicine
† **Virginia Baptist Hospital**
Lynchburg, Virginia
(434) 200-1800

* **Department of Hyperbaric Medicine**
† **Retreat Hospital**
Richmond, Virginia
(804) 254-5372

* **The Hyperbaric Medicine Service**
† **Sentara Leigh Hospital**
Norfolk, Virginia
(757) 466-2325

* **The Hyperbaric Medicine Service**
† **Sentara Port Warwick**
Newport News, Virginia
(757) 594-1060

* **Hyperbaric Medicine Unit**
† **Inova Mount Vernon Hospital**
Alexandria, Virginia
(703) 664-7218

The Wound Healing & Hyperbaric center
† **Virginia Hospital Center**
Arlington, Virginia
(703) 558-6600

WASHINGTON

* **Center for Hyperbaric Medicine**
† **Virginia Mason Medical Center**
Seattle, Washington
(206) 583-6543

Center for Wound Healing & Hyperbaric
† **Swedish Edmonds Hospital**
Edmonds, Washington
(425) 673-3380

WISCONSIN

Center for Comprehensive Wound Care & Hyperbaric Oxygen Therapy
† **Aurora Health Center-Oshkosh**
Oshkosh, Wisconsin
(920) 456-7407

Wound Care Center
† **Aurora Medical Center in Summit**
Summit, Wisconsin
(262) 434-1000

* **Wound Care & Hyperbaric Medicine Center**
† **Aurora Medical Center**
Hartford, Wisconsin
(414) 649-6609

Wound Care & Hyperbaric Medicine Center
† **St. Luke's Medical Center**
Milwaukee, Wisconsin
(414) 649-6609

* **Wound Care & Hyperbaric Medicine Center**
† **West Allis Memorial Hospital**
West Allis, Wisconsin
(414) 649-6609

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Instructions for Authors

UNDERSEA and HYPERBARIC MEDICINE

The Journal of the Undersea & Hyperbaric Medical Society, Inc.

OVERVIEW

- Manuscripts must be submitted in MS WORD via electronic transmittal.
- Only manuscripts in the English language will be considered.
- Address email to: *renee@uhms.org*.

Please be sure to include:

- correct attributions;
- mailing address;
- telephone numbers;
- email address of corresponding author(s); and
- suggestions for three independent reviewers, with email addresses.

Note: Before manuscript acceptance, UHM will ask authors to sign an authorship/conflict-of-interest form. Specific information and forms will be provided at the onset of the review process by the editorial office.

LANGUAGE

The language of the journal is standard American English. Please write in a clear and concise style: Well-written papers have the best chance of acceptance. UHM does not provide translation or writing services; authors who are not fluent in the language should have the manuscript edited before submission by a native English speaker or professional language editor.

NOTE: The journal will decline to review manuscripts that are not written clearly enough for an informed reader to follow the line of arguments.

MANUSCRIPT GUIDELINES

Membership in the Undersea and Hyperbaric Medical Society (UHMS) is not a prerequisite for publication in the journal. Manuscripts are accepted for publication on the condition that they are contributed solely to this journal. Authors submitting a manuscript do so with the understanding that if it is accepted for publication, copyright for the article is assigned exclusively to the UHMS. On request, permission will be given to quote from papers or to use tables and illustrations in other publications, provided credit is given to the original source.

Acceptance of a manuscript is based on originality and quality of the work as well as the clarity of presentation. Two or more members of the Editorial Board or guest referees will evaluate all manuscripts for significance, soundness and conformance to journal format.

Authors should recommend three qualified individuals to act as independent referees for their papers; the Editor-in-Chief welcomes these suggestions but is not obliged to follow such recommendations.

After papers have been accepted, authors are asked to submit the final version of the paper electronically.

FEES

Authors of accepted papers will be assessed a flat publication fee of $250 U.S. dollars. Additional fees will be incurred for color reproduction.

The Editor-in-Chief may consider waiving the fee on a case-by-case basis for undo hardship. Editorial consideration of a paper is in no way related to the payment of page charges.

PROOFS

Proofs are sent to authors to be checked carefully. Necessary changes must be clearly indicated on the galley, with corrections typed in a color text or highlighting. Proofs must be sent back within the time specified by the managing editor. Authors can find reprint instructions on the final two pages of the journal or at *http://www.uhms.org/?page=Journal* under "Reprint Order Form."

TREATMENT OF SUBJECTS

The UHMS endorses the principles of the Declaration of Helsinki on the treatment of human subjects and the guiding principles in the care and use of animals approved by the Council of the American Physiological Society. For more on these topics see the sections entitled "Scope of the Journal" and "Recommendations from the Declaration of Helsinki " in the following pages.

TYPES OF ARTICLES IN THIS PUBLICATION

To meet its responsibilities to its readers and to the public at large, the *Undersea and Hyperbaric Medicine Journal* strives to provide unbiased scientific information and fair analyses through its publication of the following types of papers.

1. **Research Reports:** Results of experimental, theoretical and clinical investigations on topics important to the understanding of undersea, submarine and hyperbaric medicine.
   Short reports that make a substantial scientific contribution as well as extensive studies will be considered.
2. **Clinical communications and clinical case reports:** Observations of an exceptionally revealing nature.
3. **Review articles:** May cover scientific and practical subjects and may express personal opinions of the author.
4. **Current issues:** Well-reasoned essays on topics of interest to the journal's readers; may draw on new or published experimental data and may be controversial in nature.
5. **Technical communications:** Descriptions of new methods or equipment; must include data to support contentions.
6. **Proceedings of symposiums or workshops:** Usually a group of short communications that have the flavor of reviews.
7. **Letters to the editor:** Discussion of scientific papers that have appeared in the journal or scientific issues of interest to the journal's readers; should include an informative title and be as short as possible. References may be used if necessary, but tables and figures are discouraged.

PREPARATION OF MANUSCRIPTS

The overriding principles are that the composition is correct and unambiguous, clear and concise. When writing, the active voice is usually preferable to the passive voice.

Parallel construction of groups of like items and/or concepts aids in comprehension. Figures should be uncomplicated and legible. Abbreviations and acronyms should not be overused, be clearly defined at first appearance in the abstract as well as in the text and avoided in the title.

Specific items of information should appear only once in the manuscript. There should not be verbatim repetition of *Copyright©2012 Undersea and Hyperbaric Medical Society, Inc.* in the text of material that appears in a table or figure, duplication of data in graphs and tables; neither should there be repetition in Discussion of information that appears in Results.

Authors are encouraged to use papers that have appeared in recent issues of *Undersea & Hyperbaric Medicine* as models for their manuscript preparation. All accepted manuscripts are subject to final editing by the editors to improve readability and conserve space.

MANUSCRIPT REQUIREMENTS:

1. Manuscripts must be submitted electronically, formatted on an 8½-by-11-inch letter-size document with 1-inch margins with double-spacing between lines (this facilitates reading by reviewers).
2. References and legends for illustrations must be adjacent to the graphics. Graphics can be embedded in the text or placed at the end of the paper with their placement clearly marked at the spot in the text where they are to appear.
3. A cover sheet must accompany the manuscript. It should give the title of the paper, the names and affiliations of the authors, a short title (referred to as the *running head*) and the name, address, telephone and fax numbers – as well as the e-mail address – of the corresponding author.
   Please note: Both reviewers and authors for *UHM* are blinded to one another's identities; authors' names should appear only on the cover sheet.
4. An accompanying letter must include a statement that all authors have read and approved the manuscript, that the material in the paper has not been published elsewhere (except as an abstract), and that the paper is not currently being considered for publication by another journal.
5. Conflict of interest forms must be submitted. All submissions should be accompanied by clear disclosures from all authors, noting any affiliations, funding sources or financial holdings that could raise questions about possible sources of bias.
   In the event of no conflict in the viewpoint of the authors a statement to that effect should accompany the manuscript.
   Before manuscript acceptance, UHM will ask authors to sign an authorship/conflict-of-interest form. Specific information will be provided at the onset of the review process.
6. **Author responsibility:** If a submission is the work of a group within one center or at multiple centers, that group should select one individual who accepts direct responsibility for the manuscript's content as well as the agreed sequence of contributing authors. This person will serve as corresponding author or guarantor, and this designation must be clearly stated on the title page of the manuscript, with the following contact information: mailing address, email address, telephone number and fax number.
7. **Text requirements:** *Undersea & Hyperbaric Medicine* participates in the agreement established by the international Committee of Medical Journal Editors as set forth in Uniform Requirements for Manuscripts Submitted to Biomedical Journals: *Ann Intern Med* 1988; 108:258–265 and *Br Med J* 1988; 296:401–405.

8. **Title page:** Should include the following.
   a. title of no more than 85 characters, including spaces;
   b. authors' names;
   c. laboratory or institution of origin, with city and state or country;
   d. a running head, not to exceed 50 characters, including spaces;
   e. a complete address for mailing proofs; *plus*
   f. telephone and fax numbers and email address.

   Titles should be informative; the implication that a manuscript is one of a series of related papers is discouraged (*e.g.*, *Decompression sickness studies I*).
9. **Abstracts:** An informative abstract of 200 words or fewer, suitable for abstracting agencies without rewording, should state the purpose of the research, what was done, what was found, and what was concluded. Titles should contain indexable words.
10. **Text:** Except in unusual situations, the manuscript should be divided into Introduction, Methods, Results and Discussion. Long stretches of text should be broken by suitable subheadings, but subheadings should not be overused.

    Unusual symbols should be avoided.

    Statistical methods should be described in Methods; information about presentation of statistical material can be found in Bailar J, Mosteller F. 'Guidelines for statistical reporting in articles for medical journals: amplifications and explanations,' *Adv Intern Med* 1988; 108:268–273.
11. **References:** Authors are responsible for supplying complete references and verifying them against the original documents. References must be numbered consecutively in the order in which they first appear in the text, and identified in the text by Arabic numerals in parentheses or brackets.

    References cited only in tables or legends should be numbered in accordance with a sequence corresponding to the first mention of the table or figure in the text.
12. **Authors:** List names and initials of all authors when six or fewer; when seven or more, list only the first three authors and add, *et al*. Citations in the reference list are to be in the form used by the U.S. National Library of Medicine and *Index Medicus*.

    Thorsen E, Risberg J, Segadal K, Hope A. Effects of venous gas microemboli on pulmonary gas transfer function. Undersea Hyperbaric Med 1995; 22:347–353.

    Hempleman HV. History of decompression procedures. In: Bennett PB, Elliott EH, eds. The physiology and medicine of diving. London: WB Saunders, 1993:324–375.

    Kindwall EP, Goldmann RW. Hyperbaric medicine procedures. Milwaukee, WI: St. Luke's Medical Center, 1970.

    Manuscripts that have been accepted should be cited in the reference list as regular references, with 'in press' in place of journal pages. Citations such as unpublished observations, personal communication, manuscript in preparation or to be published are not to appear in the reference list, although reference to such a communication, if it exists in written form, may be cited in the text in parentheses. References to government reports should not be cited unless such reports are easily available to all readers.
13. **Equations:** Equations should appear in the text in an appropriate type style (*italics*, **bold** type, etc.). Authors should carefully distinguish between capital and lower-case letters, Roman and Greek characters and letters and numerals.

    Number equations sequentially, in parentheses on the left edge of the text. All constituent terms should be defined when they initially appear. Authors are responsible for correct formatting of each term in the equation and, because of potential conversion problems, they must be sent using the Times New Roman font in a TIFF file. Equations should be considered camera-ready when they are submitted.
14. **Tables:** Tables should be limited to material needed to make the point of the paper and should be nearly self-explanatory. They should be numbered consecutively in Arabic numerals and bear a short title.

    Explanatory matter, excluding definitions of abbreviations, should appear in table footnotes. Statistical measures of variation (SD, SE, *etc*.) should be stated.

    Tables should be in one- or two-column widths and no more than eight rows by eight columns of data, with one row for the column headings. Headings should use only horizontal text – no vertical text. Preferred font is Times New Roman.
15. **Acknowledgments:** Acknowledgments of persons who aided in the work and of funding agencies, along with any other special considerations about the manuscript, should appear at the end of the text, before the references.

16. **Footnotes:** Footnotes to material in the text are discouraged. Footnotes to tables are acceptable and should be identified in sequence by lowercase letters of the alphabet in italic superscript.

17. **Units of measure:** The Systéme Internationale d'Unités (SI units) format will be used to express measurements of pressure, depth, length, weight, time, temperature, energy, power, force and concentration [*Standard Practice for Use of the International System of Units* (SI) Document E380-89a, American Society for Testing and Materials, Philadelphia, Pa. 1989.

    If the subject matter makes it appropriate to use non-SI units such as fsw, msw, atm or bar, a parenthetical conversion to pascals, kilopascals or megapascals should accompany the first mention of a pressure value in the abstract and in the text.

    Units of *fsw* and *msw* should not be used to express partial pressure or when the nature of the subject matter requires precise evaluation of pressure. The proper method for the expression of other units or appreciations may be found in *Br Med J* 1978; 1:1334–1336 and *Aviat Space Environ Med* 1984; 55: 93–100.

    Authors must include after all units a small parenthetical (a) or a small parenthetical (g) to indicate whether units are in absolute or gauge terms.

18. **GRAPHICS**

    General: All graphics, which includes anything other than text, should be numbered in Arabic numerals, in sequence as they appear in the text and ***must conform to one-column (3.125") width or two-column width (6")***. Each is to be accompanied by a suitable legend not exceeding 40 words.

    Please note that text within graphics should be in the Times New Roman font.

    Symbols used should be defined in the legend.

    Diagrams, charts and other line drawings should be sharp and clear.

    Freehand or typewritten lettering on figures is not acceptable. Lettering must be proportional to the size of the illustration to ensure that it is legible after reduction. Size to fit the journal page should be considered.

    An internal scale marker (a bar of defined length) should be drawn directly on all micrographs, and the length specified in the legend.
    Good line drawings of equipment are usually more effective than photographs.

    Upon acceptance of the manuscript, authors must be prepared to submit graphics in TIFF format, 300 dpi or better. Grayscale is preferable to color, both for simplicity and because the author will be assessed a substantial charge for color printing.

    If color is to be used, however, graphics must be in CMYK, 300 dpi or better. Authors are responsible for visual clarity.

    Depiction of animals: ***Animals must be depicted only by line drawing or other form of animation.*** It is the journal's policy not to publish photographs that might be perceived as raising animal welfare concerns.

    Depiction of patients: *Undersea and Hyperbaric Medicine* publishes only photos of subjects who have provided express, written permission to the author to do so. The terms of the subject/patient consent determine whether a de-identified photo (*i.e.*, with a black box obscuring the identity of the subject) would need to be used. *UHM* will insert an editorial comment in articles in which such photos are included specifically documenting that consent was obtained.

AUXILIARY PUBLICATIONS

Detailed tables, appendices, mathematical derivatives, extra figures and other supplementary matter may be deemed too voluminous to be included in the journal article. Such material may be submitted for deposition with the American Society for Information Sciences (ASIS), National Auxiliary Publication Service, at no charge. The information is deposited by the editorial office with the consent of the author, and a footnote will appear in the published article to the effect that photoprint or microfiche copies are available at a moderate cost.

Revised September 2012 ■

SCOPE OF THE JOURNAL

Undersea & Hyperbaric Medicine accepts manuscripts for publication related to the areas of diving research and physiology, hyperbaric medicine and oxygen therapy, submarine medicine, naval medicine and clinical research related to the above topics. Scientific papers must deal with significant and new research in an area related to biological, physical and clinical phenomena related to the above environments.

The following types of papers are published: Original Research (theoretical and experimental); Clinical Communications (which may include case reports if they include control observations of a revealing nature); Current Issues; Technical and Preliminary Notes; Letters to the Editor; and Book Reviews.

Reports of major contributions or symposiums will be considered and may be published as supplements to regular issues. Authors are referred to "Instructions for Authors" for more details on the categories of papers.

Undersea & Hyperbaric Medicine is abstracted and/or indexed in Chemical Abstract Service, Excerpta Medica, Oceanic Abstracts, Bioscience Information Service of Biological Abstracts, Current Contents, Index Medicus and Current Awareness in Biological Sciences. *Undersea & Hyperbaric Medicine* is available on 16-, 35- and 105-mm microfiche from University Microfilms International, 300 North Zeeb Road, Ann Arbor, MI 48106.

On file in the administrative offices of the Society are two documents pertaining to Institutional Review Board regulations CFR50 and21cfr56. The UHMS, as publisher of the *UHM* journal, acknowledges that all human research requires informed consent and IRB approval in accordance with the laws of the country in which the work was performed. This includes abstracts as well since they are published in *UHM.*

The Society endorses the principles embodied in the Declaration of Helsinki *(see below)* and expects that all investigations involving man reported in its journal will have been conducted in conformity with these principles.

The Society expects that the Guiding Principles in the Care and Use of Animals *(see below)* will have been observed in all animal experimentation reported in its journal. n

Recommendations from the

DECLARATION OF HELSINKI

BASIC PRINCIPLES

1. Clinical research must conform to the moral and scientific principles that justify medical research and should be based on laboratory and animal experiments or other scientifically established facts.
2. Clinical research should be conducted only by scientifically qualified persons and under the supervision of a qualified medical man.
3. Clinical research cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.
4. Every clinical research project should be preceded by careful assessment of inherent risks in comparison to foreseeable benefits to the subject or to others.
5. Special caution should be exercised by the doctor in performing clinical research to which the personality of the subject is liable to be altered by drugs or experimental procedures.

CLINICAL RESEARCH COMBINED WITH PROFESSIONAL CARE

1. In the treatment of the sick person, the doctor must be free to use a new therapeutic measure, if, in his judgment it offers hope of saving life, re-establishing health, or alleviating suffering.

   If at all possible, consistent with patient psychology, the doctor should obtain the patient's freely given consent after the patient has been given a full explanation.

   In case of legal incapacity, consent should also be procured from the legal guardian; in case of physical incapacity the permission of the legal guardian replaces that of the patient.
2. The doctor can combine clinical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that clinical research is justified by its therapeutic value for the patient.

NON-THERAPEUTIC CLINICAL RESEARCH

1. In the purely scientific application of clinical research carried out on human beings, it is the duty of the doctor to remain the protector of the life and health of that person on whom clinical research is being carried out.

2. The nature, the purpose and risk of clinical research must be explained to the subject by the doctor.

3a. Clinical research on a human being cannot be undertaken without his free consent after he has been informed; if he is legally incompetent, the consent of the legal guardian should be procured.

3b. The subject of clinical research should be in such a mental, physical, and legal state as to be able to exercise fully his power of choice.

3c. Consent should, as a rule, be obtained in writing. However, the responsibility for clinical research always remains with the research worker; it never falls on the subject even after consent is obtained.

4a. The investigator must respect the right of each individual to safeguard his personal integrity, especially if the subject is in a dependent relationship to the investigator.

4b. At any time during the course of clinical research the subject or his guardian should be free to withdraw permission for research to be continued. The investigator or the investigating team should discontinue research if in his or their judgment, it may, if continued, be harmful to the individual.

GUIDING PRINCIPLES IN THE CARE AND USE OF ANIMALS

Only animals that are lawfully acquired shall be used in this laboratory, and their retention and use shall be in every case in strict compliance with state and local laws and regulations.

Animals in the laboratory must receive every consideration for their bodily comfort; they must be kindly treated, properly fed and their surroundings kept in a sanitary condition.

Appropriate anesthetics must be used to eliminate sensibility to pain during operative procedures. Where recovery from anesthetics is necessary during the study, acceptable technique to minimize pain must be followed. Curarizing agents are not anesthetics. Where the study does not require recovery from the anesthesia, the animal must be killed in a humane manner at the conclusion of the observation.

The postoperative care of animals shall be such as to minimize discomfort and pain, and in any case shall be equivalent to accepted practices in schools of Veterinary Medicine.

When animals are used by students for their education or the advancement of science, such work shall be under the direct supervision of an experienced teacher or investigator. The rules for the care of such animals must be the same as for animals used for research.

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UNDERSEA AND HYPERBARIC MEDICINE

The Journal of the Undersea & Hyperbaric Medical Society, Inc.

PRESSURE CONVERSION TABLE

The units of pressure preferred for manuscripts submitted to *Undersea & Hyperbaric Medicine* are the pascal (Pa = Newton / m^2), kilopascal (kPa), or megapascal (MPa), defined by the International System of Units (SI). If the nature of the subject matter makes it appropriate to use non-SI units, such as fsw, msw, atm or bar, a parenthetical conversion to pascals, kilopascals, or megapascals should accompany the first mention of a pressure value in the abstract and in the text.

Atmospheres absolute is a modified unit of pressure due to the appendage "absolute"; the use of "atm abs" is an acceptable abbreviation; "ATA" is also recognized.

1 atm	=	1.013250 bar	1 atm	=	33.08 fsw	1 atm	=	10.13 msw
1 atm	=	101.3250 kPa	1 bar	=	32.646 fsw[a,b]	1 bar	=	10.00 msw
1 atm	=	14.6959 psi	1 fsw	=	3.063 kPa	1 msw	=	10.00 kPa
1 atm	=	760.00 torr[d]	1 fsw	=	22.98 torr	1 msw	=	1.450 psi
1 bar	=	100.000 kPa	1 psi	=	2.251 fsw	1 msw	=	75.01 torr
1 bar	=	100,000 Pa[d]						
1 bar	=	14.50377 psi						
1 bar	=	750.064 torr						
1 MPa	=	10.000 bar						
1 psi	=	6,894.76 Pa[d]						
1 psi	=	51.7151 torr						
1 torr	=	133.322 Pa[d]						

[a]Primary definition for fsw; assumes a density for seawater of 1.02480 at 4°C (the value often used for depth gauge calibration).

[b]These primary definitions for fsw and msw are arbitrary since the pressure below a column of seawater depends on the density of the water, which varies from point to point in the ocean. These two definitions are consistent with each other if a density correction is applied. Units of fsw and msw should not be used to express partial pressures and should not be used when the nature of the subject matter requires precise evaluation of pressure; in these cases investigators should carefully ascertain how their pressure-measuring devices are calibrated in terms of a reliable standard, and pressures should be reported in pascals, kilopascals, or megapascals.

[c]Primary definition for msw; assumes a density for seawater of 1.01972 at 4°C.

[d]Signifies a primary definition (1) from which the other equalines were derived.

1. Standard Practice for Use of the International System of Units (SI). Doc. E380-89a. Phila., PA: Am. Soc. for Testing and Materials, 1989.

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