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Incidence of middle ear barotrauma in staged versus linear chamber compression during hyperbaric oxygen therapy: a double blinded, randomized controlled trial

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ABSTRACT

Context: Middle ear barotrauma (MEB) is common during chamber compression in hyperbaric oxygen therapy. However, little evidence exists on an optimal compression protocol to minimize the incidence and severity of MEB.

Objective: To compare the incidence of MEB during hyperbaric oxygen therapy using two different chamber compression protocols.

Design: Double-blinded, randomized controlled trial.

Setting: Hyperbaric Medicine Unit, The Townsville Hospital, Queensland, Australia, September 2012 to December 2014.

Patients: 100 participants undergoing their first hyperbaric oxygen therapy session.

Intervention: Random assignment to a staged (n=50) or a linear (n=50) compression protocols. Photographs of tympanic membranes were taken pre- and post-treatment and then graded. Middle ear

barotrauma was defined as an increase of at least one grade on a modified TEED scale.

Results: The observed MEB incidence under the staged protocol was 48% compared to 62% using the linear protocol (P=0.12, exact one-sided binomial test), and thus the staged protocol did not show a significant improvement in MEB. However, the staged protocol resulted in significantly less severe deteriorations in MEB grades when compared to the linear protocol (P=0.028, exact one-sided Mann-Whitney type test).

Conclusion: The use of the assessed staged compression protocol for the first hyperbaric oxygen treatment showed no significant effect on the overall incidence of MEB when compared to the gold standard linear protocol but resulted in a significant improvement in the severity of the experienced MEBs. Further studies are needed to elucidate an optimal compression protocol to minimize middle ear barotrauma.

INTRODUCTION

Middle ear barotrauma (MEB) remains a common complication of hyperbaric oxygen (HBO₂) therapy, with the incidence ranging from 8%-68.7% [1, 2]. Currently, three concurrent practices are used to reduce the incidence of MEB:

- i) assessment to identify patient at risk of MEB;
- ii) teaching patients correct ear equalization techniques;
- iii) slow chamber compression.

Vahidova et al demonstrated that the use of a slow compression technique (1.1 meters/minute vs. 2.8 meters/

minute) significantly reduced the incidence of middle ear barotrauma from 52.3% to 28.5% [3]. Most centers compress their patients to 242 kilopascals/kPa, or 2.4 atmospheres absolute/ATA over 10 to 14 minutes depending on patients' comfort and on their ability to equalize their ears. These compressions are linear at a set rate, with interruptions whenever a patient volunteers difficulty with ear equalization (pain or discomfort). One center (personal communication) routinely uses staged compression in its treatment protocol in an attempt to identify patients with equalization problems, as well as

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to allow patients time to “catch up” with equalization. It is postulated that staged compression will result in a lower incidence of middle ear barotrauma with resulting improvement of patient comfort. Currently there is hardly any literature on this topic. The aim of the study was to evaluate whether a staged chamber compression protocol (trial regimen) during HBO₂ therapy is able to reduce the incidence of middle ear barotrauma to 10% versus an estimated 30% experienced under a linear chamber compression protocol (standard regimen). This study was conducted in a multiplace chamber.

METHODOLOGY

Ethics approval

Ethics approval for this trial was obtained from The Townsville Hospital Human Research Ethics Committee (HREC reference number: HREC/12/QTHS/78). The trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12612000724875).

Study protocol

This was a single-center, double-blinded, balanced randomized, parallel group trial involving 100 new patients older than 18 years old, presenting for their first session of HBO₂ therapy. Informed consent was obtained from all participants prior to the start of the study. Exclusion criteria were previous HBO₂ treatment, contraindications for HBO₂, patients with grommets/active otological conditions requiring grommets, existing tympanic membrane rupture, unconscious patients and any prescribed hyperbaric treatment table other than to 242 kPa (2.4 ATA).

Participants were randomly assigned in a balanced and blocked manner to undergo a staged compression protocol or a linear compression protocol on their first HBO₂ treatment. This study was done in a multiplace chamber with each participant in a sitting position.

Staged compression protocol

1. 101.3 kPa (1 ATA) to 129.7 kPa (1.28 ATA)
@ 1.4 meters/minute for two minutes;
hold pressure for one minute.
2. 129.7 kPa (1.28 ATA) to 172.3 kPa (1.7 ATA)
@ 1.4 meters/minute for three minutes;
hold pressure for one minute.
3. 172.3 kPa (1.7 ATA) to 242.0 kPa (2.4 ATA)
@ 1.4 meters/minute for five minutes.

Total descent time = 12 minutes.

Linear compression protocol

1. 101.3 kPa (1 ATA) to 242.0 kPa (2.4 ATA)
@ 1.1 meters/minute.

Total descent time = 12 minutes, 43 seconds.

Staged compression protocol is presented in Figure 1.

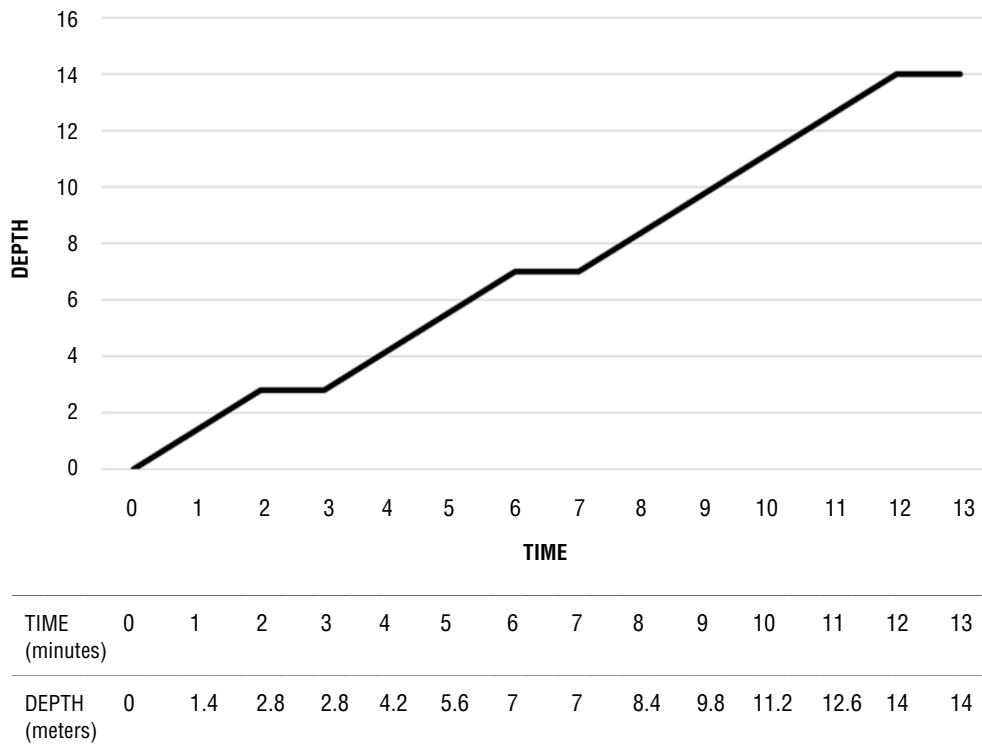
For both protocols, participants were continuously encouraged by the in-chamber hyperbaric nurse to perform ear equalization (any preferred technique: Valsalva, Frenzel, Toynbee, Lowry, Edmonds, swallowing saliva, drinking water, yawning, wiggling of jaw movement) as per usual practice. During the holding stage, continuous inflow and outflow flushing of the chamber was performed to create an illusion of compression, as a method of blinding the participant. The ascent rate at the end of the treatment was 0.95 atm/minute (15 minutes from 14 meters to surface). The study was confined to observation of the participants' first HBO₂ treatment only.

Tympanic membranes (TM) of each participant were photographed pre- and post-hyperbaric treatment with a Welch Allyn Digital Macroview Otoscope by trained staff members using standard mode (no magnification) and auto white balance, producing pictures with a resolution of 1280 x 1024 megapixels in JPEG format. Pre-treatment photographs (left and right ears) were taken immediately prior to entering the hyperbaric chamber and post-treatment photographs taken within 10 minutes of the participant exiting the hyperbaric chamber. Each photograph was assigned a random number. Any earwax impeding the view was removed during the pre-treatment assessment – i.e., before any photograph was taken. All photographs were assessed and graded using modified TEED score by one single experienced otolaryngologist, who was blinded and not involved in the clinical care of the participants.

Study outcomes

Primary outcome was incidence of MEB defined as an increase by at least one grade in modified TEED score in one or both ears. Secondary outcomes were severity of MEB, objective experience of pain, number of interrupted treatments, and number of aborted treatments. Interrupted treatment was defined as a pause in the compression protocol due to a participant experiencing ear pain/discomfort but successfully arriving at 242 kPa (2.4 ATA) (any interruption of treatment by another patient during the HBO₂ treatment was recorded as an interrupted treatment). Aborted treatment was defined

Figure 1: Staged compression protocol



as a treatment terminated before reaching 242 kPa (2.4 ATA) after three failed attempts to equalize the ears. Pain score was recorded on a numerical rating scale, rated directly upon reaching treatment depth or when treatment was aborted. A structured self-administered questionnaire was also used prior to HBO₂ treatment to record basic demographic data and potential confounders.

Statistical analysis

Sample size calculations revealed that group sample sizes of 50 in each group will achieve a power in the excess of 80% to detect a reduction in the incidence of middle ear barotrauma on otoscopic examination from 30% in the standard (control) group (linear compression) to 10% in the intervention group (staged compression) at a one-sided significance level of 5% ($P < 0.05$). A one-sided test was chosen since we were exclusively interested in whether the new staged compression was able to achieve a significant improvement when compared to the globally accepted linear compression currently in place.

A balanced, blocked randomization procedure was employed by generating the allocation list based on a random seed using a statistical package and produced by a statistician with no clinical involvement in the trial. The allocation list was blocked ($N=20$) so that after each block of 20 participants the randomization was balanced. From the allocation list, sequentially numbered, opaque, sealed and stapled envelopes containing the random allocation for the single participant were produced. Corresponding envelopes were opened only after the enrolled participant was inside the hyperbaric chamber and the chamber door closed. Envelopes were opened by the chamber operator of the day and the noted regimen applied. Participants, inside nurses, outside nurses and attending hyperbaric doctors were blinded to allocation. Assignment of intervention was known only to the chamber operator, who was not involved in the clinical management or assessment of the participant. The control console of the chamber showing the compression rate and depth was masked by cardboard and could be seen only by the chamber operator. The depth gauge

Table 1: Baseline characteristics

	STAGED (N=50)	LINEAR (N=50)	P-VALUE
age in years (mean (SD))	60.5 (15.8)	59.1 (12.6)	P=0.63
gender (male:female)	40:10	34:16	P=0.25
smoking			
non-smokers	24.0% (N=12)	26.0% (N=13)	P=0.89
ex-smokers	52.0% (N=26)	52.0% (N=26)	
active smokers	24.0% (N=12)	22.0% (N=11)	
scuba divers	20.0% (N=10)	10.0% (N=5)	P=0.26
history of ENT surgery	22.0% (N=11)	32.0% (N=16)	P=0.37
history of recent ENT infection	10.0% (N=5)	18.0% (N=9)	P=0.39
recent URTI*	nil	nil	
history of head/neck radiation (HBO₂ indication for treatment or prevention of ORN)	20.0% (N=10)	12.0% (N=6)	P=0.41
otoscopic confirmation of tympanic membrane movement on Valsalva	77.6% (N=38)	64.0% (N=32)	P=0.19

* URTI = upper respiratory tract infection

inside the chamber was covered with opaque plastic during the treatment. During the hold stage of the staged protocol, continuous inflow and outflow flushing was done to create an illusion of compression. The otolaryngologist assessing the TM photographs was also blinded to allocation.

Statistical data collected was analyzed using SPSS Version 20. Standard univariate and bivariate statistical procedures were employed during the analysis. For descriptive purposes, percentages were used for categorical variables and means (standard deviation) or medians (interquartile ranges) were employed for numerical variables depending on fulfilment of normality assumptions. The incidences of the main outcome measure were compared between the treatment groups by means of exact binomial tests. The same procedure was used to assess the categorical secondary outcome measures. The numerical subjective pain ratings were compared between the groups by non-parametric Mann-Whitney tests since the distribution proved to be highly skewed. Bivariate assessments of potential confounders were assessed by following the same analytical procedures. If adjustment for confounder(s) seemed necessary, binary logistic regression modeling was employed.

RESULTS

From September 2012 to December 2014, a total of 201 new patients were treated in the chamber. Of these patients, 50 were assigned to each staged and linear compression protocols, while 101 patients were excluded for various reasons (Figure 2). There were 74 males and 26 females with a median age of 61 years (interquartile range 50 – 69). Baseline characteristics and confounders proved to be similar for the two groups (Table 1).

MEB was detected in 24 of 50 participants (48.0%) in the staged compression group compared with 31 of 50 participants (62.0%) in the linear compression group when an increase by at least one grade was used as the definition of MEB. This observed difference did not reach statistical significance (P=0.12). A more specific post-hoc analysis taking the degree of deterioration in TEED grades into consideration showed that the staged protocol resulted in significantly fewer severe grades when compared to the linear protocol (P=0.028, exact one-sided Mann-Whitney type test, Table 2). It especially revealed that not a single participant in the intervention arm experienced a deterioration by more than two grades, compared to 12% (N=6) in the control arm.

The secondary outcomes (interrupted treatment

Figure 2: CONSORT participants' flow through the trial

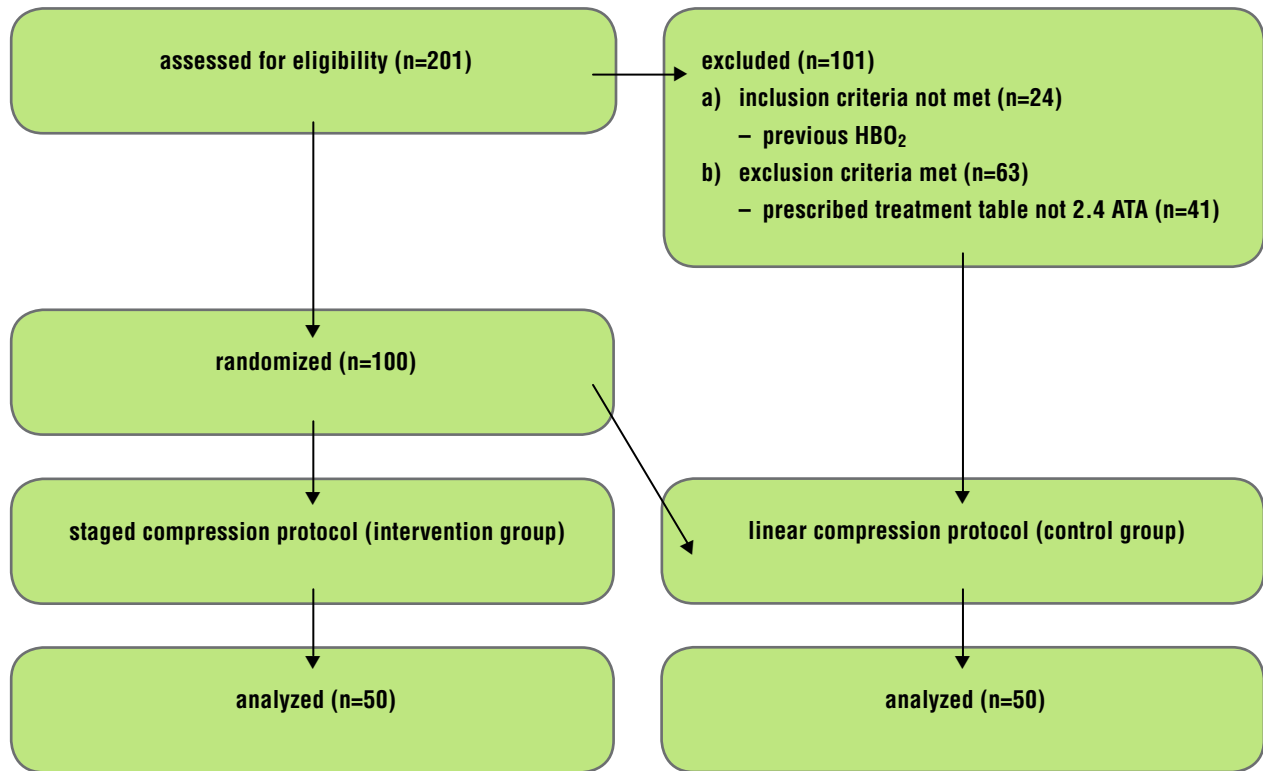


Table 2: MEB by protocol and absolute deterioration in grades

protocol	MEB grade deterioration				total
	no MEB (no deterioration)	MEB deterioration by 1 grade	MEB deterioration by 2 grades	MEB deterioration by 3 grades	
linear	38.0% (N=19)	34.0% (N=17)	16.0% (N=8)	12.0% (N=6)	100.0% (N=50)
staged	52.0% (N=26)	32.0% (N=16)	16.0% (N=8)	0.0%	100.0% (N=50)

and aborted treatment) were not affected by the use of staged compression. Eight participants (16.0%) in the staged group versus 12 (24.0%) in the linear group experienced interrupted treatment ($P=0.317$), while one participant (2.0%) in the staged group and three participants (6.0%) in the linear group had their treatment aborted ($P=0.307$). Pain-free hyperbaric treatment was reported in 84% ($N=42$) under the staged protocol and 88% ($N=44$) under the linear protocol ($P=0.77$).

Overall, 14 participants reported pain scores reaching from 3 to 9 (with a total of three participants in each protocol reporting a score above 5 on a scale of 1 to 10). Reported pain scores did not differ significantly between the compression regimen; exact Mann-Whitney type test $P=0.491$. There was a slightly lower mean rank (lower pain score) under the staged protocol (mean rank 6.8) than under the linear protocol (mean rank 8.4).

Overall, 51.7% of those with no observable TM movement developed MEB compared with 55.7% (P=0.83) in those with TM movement. A higher percentage (62.5% vs. 53.6%) of participants with a history of head and neck radiation developed MEB in our study, though this was not statistically significant (P=0.591). There was, however, a higher percentage of interrupted treatments in this subgroup, 37.5% vs. 16.7%, though again no statistical difference was found (P=0.084).

DISCUSSION

MEB during compression is caused by a reduction in middle ear volume based on Boyle's law. Active maneuvers (e.g., Valsalva) to open the Eustachian tubes are usually required to equalize middle ear pressure. Thus, not surprisingly a slower compression will allow time for patients to perform the active maneuvers and reduce the rate of MEB as demonstrated by Vahidova, et al. [3]. As the pressure gradient increases, these active maneuvers become increasingly difficult to perform [4, 5]. In a linear compression, a patient may be too slow in performing these active maneuvers, and MEB may have occurred before any intervention such as interrupting the compression. This is more likely to happen in a new patient undergoing the first hyperbaric compression due to unfamiliarity. By having stopping stages during the compression, the patient may be able to have more time to equalize the middle ear pressure before the Eustachian tube becomes blocked.

We designed our staged compression protocol based on the following:

- i) both protocols to have similar total compression time (12 minutes vs. 12 minutes 43 seconds, which should have insignificant effect on MEB incidence);
- ii) uncomplicated compression protocol with practical stopping stages for our chamber operators (time and number of stops); and
- iii) pressures where new patients usually start having trouble with ear equalization.

At our center, the majority of new patients develop ear equalization problems at around 121.6-136.8 kPa (1.2-1.35 ATA). This was calculated as 16%-25% middle ear volume change assuming no active ear equalization was being done. We thus choose 129.7 kPa (1.28 ATA) and 172.3 kPa (1.7 ATA) as our stopping stages, corresponding to a maximum 22% and 25% volume change respectively.

In this trial, the use of a staged compression proto-

col, as compared with a standard linear compression protocol, did not reduce the incidence of MEB, when defined as any deterioration (i.e., an increase in modified TEED score by a single grade – e.g., from 0 to 1), in a participant's first hyperbaric oxygen treatment. The rates of secondary outcomes (pain, interrupted treatments and aborted treatments) were also similar. However, in a more specific analysis – i.e., taking the degree of deterioration into account, the staged compression protocol resulted in significantly less severe deteriorations in grades when compared to the linear compression protocol (P=0.028; exact one-sided Mann-Whitney type test). In this context it seems noteworthy that not a single participant in the staged compression group suffered a severe deterioration (exceeding 2 MEB grades), compared with 12% (N=6) in the linear compression group.

We offer two possible explanations for these findings. First, our chosen depth and assumed middle ear volume change for the holding stage may be inappropriate. Change in pressure and volume during chamber compression is a continuous process. Furthermore, the percentage of change in volume is greater at lower pressures. Thus, a different staged compression protocol may be more appropriate – i.e., an earlier, longer initial holding period; and more than two stages. However, this may result in longer total compression time and make comparison more challenging. Alternatively, an exponential compression protocol with an initial slow compression and increasing rate of compression over time could be considered. Calculation of these types of protocols, however, seems difficult, especially for the exponential compression protocol. An exponential compression protocol would also be very challenging to implement manually by a chamber operator and would require modern computer controlled compression.

Secondly, as both the participants and in-chamber nurses were blinded to allocation, the benefit of extra attention by the in-chamber nurse to encourage the participant to perform active maneuvers during the holding stage may have been lost.

The incidence of MEB in our participants who underwent the linear compression protocol was 62%. This was within range of the incidences reported in the literature [1, 2] and is reflective of different patient population, criteria for grading MEB and inter-observer factors. However, compared with Vahidova's study, our MEB incidence was relatively high. This can be explained

by the fact that we photographed all ears irrespective of whether they develop any problems with ear equalization during compression. We were thus actively looking for MEB, and our result was similar to the 43% incidence of MEB in Lehm and Bennett's study [6]. If we were to use the incidence of pain or interrupted treatments as surrogate for MEB, not dissimilar to usual clinical practice, our incidence of 12% participants with pain and 24% with interrupted treatment would be more in line with other reports. For example, Commons, et al. performed a prospective analysis of independent patient risk factors for middle ear barotrauma at our center prior to our study and found 22.6% incidence of MEB after the first treatment [7].

Predictors of middle ear barotrauma include tympanic membrane movement on otoscopy with Valsalva, and results of dynamic tympanometry [6]. These predictors are thought to demonstrate the ability of the subject to equalize the middle ear in the face of changes in pressure and volume, based on Boyle's law. Techniques to equalize the middle ear are routinely taught to patients undergoing HBO₂ therapy to help reduce the incidence of middle ear barotrauma (Valsalva, Frenzel and Toynbee maneuvers; swallowing saliva; drinking water; yawning and wriggling jaw movements) [8]. Beuerlein, et al. used movement of the TM with Valsalva maneuver on otoscopic examination prior to HBO₂ as a predictor of MEB. In that study, 91% "non-inflaters" vs. 37% "auto-inflaters" developed MEB [9]. In contrast, our

study did not show any significant difference of MEB in those with no observable TM movement compared with those with observable TM movement (51.7% vs. 55.7%; P=0.83). History of head and neck radiation is another accepted predictor of difficulty in ear equalizing and developing MEB [2]. In this subgroup, once again we could not demonstrate any difference in incidence of MEB.

CONCLUSION

The use of a staged compression protocol for the first hyperbaric oxygen treatment was not superior to a linear compression protocol in terms of incidence of MEB, pain and interrupted/aborted treatments but resulted in less severe MEB. Further studies are needed to identify the best compression protocol to minimize MEB, pain and treatment interruptions. ■

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Conflict of interest statement

Authors declare no conflicts of interest exist with this submission.

REFERENCES

1. Yoshikazu et al. Middle ear barotrauma associated with hyperbaric oxygenation treatment. *Acta Otolaryngol.* 1993;113 No s504: 143-145.
2. Blanshad J, Toma A, Bryson P, Williamson P. Middle ear barotrauma in patients undergoing hyperbaric oxygen therapy. *Clin Otolaryngol Allied Sci* 1996; 21:400-403.
3. Vahidova D, Sen P, Papesch M, Zein-Sanchez MP, Mueller PHJ. Does the slow compression technique of hyperbaric oxygen therapy decrease the incidence of middle-ear barotraumas? *J Laryngol Otol* 2006; 120(6): 446-449.
4. Carlson S, Jones J, Brown M, Hess C. Prevention of hyperbaric-associated middle ear barotrauma. *Ann Emerg Med* 1992; 21: 1468-1471.
5. Marco L, Luciano F, Maria C, Fayez B. Update on middle ear barotraumas after hyperbaric oxygen therapy – insights on pathophysiology. *Int. Arch. Otorhinolaryngol*; DOI: 10.1055/s-0034-1366974.
6. Lehm JP, Bennett MH. Predictors of middle ear barotrauma associated with hyperbaric oxygen therapy. *SPUMS J* 2003; 33:127-133.
7. Commons KH, Blake DE, Brown LH. A prospective analysis of independent patient risk factors for middle ear barotrauma in a multiplace hyperbaric chamber. *Diving Hyperb Med.* 2013; 43(3):143-147.
8. Kay, E. Prevention of middle ear barotrauma. <http://staff.washington.edu/ekay/MEbaro.html>
9. Beuerlein M, Nelson RN, Welling DB. Inner and middle ear hyperbaric oxygen-induced barotrauma. *Laryngoscope* 1997; 107: 1350-1356. ◆

14. Trygg J, Holmes E, Lundstedt T. Chemometrics in metabolomics. *J Proteome Res.* 2007; 6(2):469-479.
15. Huang Q, Tan Y, Yin P, Ye G, Gao P, Lu X, Wang H, et al. Metabolic characterization of hepatocellular carcinoma using nontargeted tissue metabolomics. *Cancer Res.* 2013; 73(16):4992-5002.
16. Wishart DS, Jewison T, Guo AC, Wilson M, Knox C, Liu Y, Djombou Y et al. HMDB 3.0--The human metabolome database in 2013. *Nucleic Acids Res.* 2013; 41(Database issue):D801-807.
17. Horai H, Arita M, Kanaya S, Nihei Y, Ikeda T, Suwa K, Ojima Y, et al. MassBank: a public repository for sharing mass spectral data for life sciences. *J Mass Spectrom.* 2010; 45(7):703-714.
18. Haitao Wang YF, Pu You, Xiaochen Bao, Hengrong Yuan, Jun Ma, Fangfang Wang, Kaicheng Li. Expression changes of inflammatory factors in the rat lung of decompression sickness by fast buoyancy ascent escape. *Undersea Hyperb Med.* 2015; 42(1):15-22.
19. Kaczerska D, Siermontowski P, Olszanski R, Krefft K, Malgorzewicz S, Van Damme-Ostapowicz K. The influence of high-fat diets on the occurrence of decompression stress after air dives. *Undersea Hyperb Med.* 2013; 40(6):487-497.
20. Wurtz P, Havulinna AS, Soininen P, Tynkkynen T, Prieto-Merino D, Tillin T, Ghorbani A et al. Metabolite profiling and cardiovascular event risk: a prospective study of 3 population-based cohorts. *Circulation.* 2015; 131(9):774-785.
21. Bishop WR, Bell RM. Assembly of phospholipids into cellular membranes: biosynthesis, transmembrane movement and intracellular translocation. *Annu Rev Cell Biol.* 1988; 4: 579-610.
22. Zarringhalam K, Zhang L, Kiebish MA, Yang K, Han X, Gross RW, Chuang J. Statistical analysis of the processes controlling choline and ethanolamine glycerophospholipid molecular species composition. *PLoS One.* 2012; 7(5):e37293.
23. Han X, Cheng H, Mancuso DJ, Gross RW. Caloric restriction results in phospholipid depletion, membrane remodeling, and triacylglycerol accumulation in murine myocardium. *Biochemistry.* 2004; 43(49):15584-94.
24. Kiechl S, Willeit J, Mayr M, Viehweider B, Oberhollenzer M, Kronenberg F, Wiedermann CJ et al. Oxidized phospholipids, lipoprotein(a), lipoprotein-associated phospholipase A2 activity, and 10-year cardiovascular outcomes: prospective results from the Bruneck study. *Arterioscler Thromb Vasc Biol.* 2007; 27(8):1788-1795.
25. Williams SD, Gottlieb RA. Inhibition of mitochondrial calcium-independent phospholipase A2 (iPLA2) attenuates mitochondrial phospholipid loss and is cardioprotective. *Biochem J.* 2002; 362(Pt 1):23-32.
26. Shahrestanifar M, Fan X, Manning DR. Lysophosphatidic acid activates NF-kappaB in fibroblasts. A requirement for multiple inputs. *J Biol Chem.* 1999; 274(6):3828-3833.
27. Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, Benjamin EJ et al. Vitamin D deficiency and risk of cardiovascular disease. *Circulation.* 2008; 117(4):503-511.
28. Donneyong MM, Hornung CA, Taylor KC, Baumgartner RN, Myers JA, Eaton CB, Gorodeski EZ et al. Risk of heart failure among postmenopausal women: a secondary analysis of the randomized trial of vitamin D plus calcium of the women's health initiative. *Circ Heart Fail.* 2015; 8(1):49-56.
29. Perwaiz S, Tuchweber B, Mignault D, Gilat T, Yousef IM. Determination of bile acids in biological fluids by liquid chromatography-electrospray tandem mass spectrometry. *J Lipid Res.* 2001; 42(1):114-119.
30. Ganitkevich Ia V. [An increase in the resistance of animals to decompression sickness with bile acid preparations]. *Biull Eksp Biol Med.* 1970; 69(6):33-36.

