CLINICAL HYPERBARIC MEDICINE
Session K
Physiology and Mechanisms of Hyperbaric Oxygen Therapy

K 1
Hyperbaric oxygen but not hyperbaric air leads to increased insulin sensitivity in men with Type 2 diabetes mellitus
Wilkinson D, Chapman I, Heilbronn LK
Hyperbaric Medicine Unit, Royal Adelaide Hospital, Adelaide, Australia
Submitting Author: David Wilkinson, MD
david.wilkinson@sa.gov.au

Introduction / Background: People with Type 2 diabetes mellitus (T2DM) undergoing hyperbaric oxygen (HBO2) therapy may experience a fall in blood glucose levels. Using the hyperinsulinemic euglycemic glucose clamp technique under hyperbaric oxygen conditions, our group has previously demonstrated an increase in peripheral insulin sensitivity in men who were obese or overweight, both with and without T2DM. The aim of this study was to test whether this effect is seen during exposure to hyperbaric air (HA).

Materials and Methods: Men who were 40 years or older, had T2DM and were obese or overweight were randomized to two groups – HBO2 (n=13) or HA (n=11). Insulin sensitivity was assessed by hyperinsulinemic euglycemic glucose clamp performed at baseline and two days later during hyperbaric exposure. Both groups were compressed to 2 atmospheres absolute for 90 minutes followed by a linear 30-minute decompression. The HBO2 group breathed oxygen via a hood delivery system while the HA group breathed chamber air. Insulin sensitivity was assessed by the glucose infusion rate (GIR) at two consecutive time periods: the last 30-minutes in the hyperbaric chamber (SS1) and the first 30 minutes after exit (SS2). Data were analyzed by one-way ANOVA to test for between-group effects and paired Student’s t-test for within-group effects.

Results: A between-group difference was evident for the change in GIR at SS1 (p=0.04) in HBO2 vs HA. A trend toward a between-group effect was also evident at SS2 (p=0.09). The HBO2 group increased GIR by an average of 26% (p=0.04) at SS1 and by 23% (p=0.02) at SS2. There was no significant change in GIR for HA.

Summary / Conclusion: Insulin sensitivity was meaningfully increased during a single, two-hour HBO2 exposure in men with T2DM and was still evident 30 minutes after exit from the chamber. In contrast, breathing HA during an equivalent pressure exposure resulted in no change to insulin sensitivity.

K 2
Increases in nitric oxide and hypoxia-inducible factor 1α accelerate skeletal muscle regeneration by stimulating satellite cell proliferation: a mechanism of recovery from skeletal muscle injury with hyperbaric oxygen
Oyaizu T, Yamamoto N, Enomoto M, Horie M, Yagishita K
Tokyo Medical and Dental University, Medical Hospital, Tokyo, Japan
Submitting Author: Takuya Oyaizu, MD
oyaizu.ortho@tmd.ac.jp

Introduction / Background: Hyperbaric oxygen (HBO2) treatment promotes recovery of skeletal muscle from injury. HBO2 increases nitric oxide (NO), which stabilizes hypoxia-inducible factor 1α (HIF1α), a cell activator. The association between NO and HIF1α in the mechanism of muscle repair with HBO2 was examined.

Materials and Methods: The rat calf muscle was contused, and rats received either no treatment, HBO2, HBO2+NAC (a reactive oxygen species (ROS) + nitric oxide synthesis (NOS) inhibitor) or HBO2+L-NAME (a NOS inhibitor). Both NAC and L-NAME were administered as pretreatments one day before contusion. HBO2 treatment consisted of 2.5 ATA 100% oxygen for 120 minutes. Calf muscles were harvested before and three and six hours, as well as one, three, five and nine days after contusion. Tissue levels of NO3− (the final oxidized product of NO) and HIF1α were analyzed by enzyme-linked immunoassay. The number of proliferating satellite cells and regenerating muscle fibers were counted by immunostaining.

Results: Levels of NO3− and HIF1α increased at three and six hours after injury with HBO2 treatment. In addition, the number of proliferating satellite cells (Pax7+, Ki67+) was increased at three hours and three days and the number of regenerating muscle fibers
(eMHC+ fiber) was increased at five days after injury. No changes in these markers were observed in either the untreated and groups treated with NAC and L-NAME.

**Summary / Conclusion:** HBO2 increased NO3−, stabilized HIF1α and increased proliferating satellite cells early after treatment, which were associated with increased muscle fiber regeneration. HBO2 activation of RNS is likely necessary in increasing muscle fiber regeneration. These results suggest NO and subsequent HIF1α stabilization mediate the therapeutic effect of HBO2-injured skeletal muscle. A pathway involving NO, and cell proliferation likely mediates the therapeutic effect of HBO2 on muscle injury.

**K 3**

Novel biomarkers for dose/effect of hyperbaric oxygen and high-intensity interval training

Kjellberg A1,2, Zheng XW3, Catrina SB3, Lindholm P1,4
1 Dept Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden
2 Perioperative Medicine and Intensive Care/Hyperbaric Medicine, Karolinska University Hospital, Stockholm, Sweden
3 Dept Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden
4 Division of Hyperbaric Medicine, Dept Emergency Medicine, University of California San Diego

Submitting Author: Anders Kjellberg, MD
anders.kjellberg@ki.se

**Introduction / Background:** Oxidative stress is central in the effect of hyperbaric oxygen (HBO2) and high-intensity interval training (HIIT), but the exact mechanism is still unknown. It is also well established that exercise modulates immunity in a dose-dependent manner. Currently there is no clinically available dose marker for HBO2 or HIIT. We hypothesized that HIIT and HBO2 have similar effects and that oxidative stress can be monitored in venous blood, plasma and/or peripheral blood mononuclear cells (PBMCs).

**Materials and Methods:** A prospective controlled study (five male + five female volunteers) performed treadmill HIIT: four intervals of three minutes' total time 28 minutes. Separated by more than two weeks: a 28-minute HBO2 session (250kPa). Venous blood samples were collected at baseline, during, after and up to six hours post intervention. Analysis included superoxide evaluated by electron paramagnetic resonance spectroscopy (EPR) using a benchtop EPR and CP-H probe (via Noxygen, Germany) from whole blood for superoxide and microRNA evaluated by real-time quantitative polymerase chain reaction (RT-qPCR) in plasma and from isolated PBMCs. The Borg scale, lactate, heart rate, were analyzed to evaluate individual exertion.

**Results:** Exercise was considered exhaustive: 19±0 rate of perceived exertion (Borg-20 scale), 98% of estimated heart rate max, and 13.2 ± 3.9 in lactate. Superoxide increased 1.0 ± 0.9 n.s. in HBO2 and 2.7 ± 0.8 p0.01 in HIIT, respectively, n=8. There was a large interindividual variation in superoxide and microRNA in both HIIT (range -3.56 to 6.11µmol/l) and HBO2 (range 1.29-4.71µmol/l), suggesting an individual dose/response. Changes in superoxide correlated HIIT and HBO2 R2=0.67, and 0.65) (15-minute baseline, and one-hour baseline, n=7).

**Summary / Conclusion:** Our preliminary results suggest that there are similarities in the response to both HIIT and HBO2, with large interindividual response that could be measured by sampling of venous blood. More subjects (ongoing) will increase statistical power. This data suggest a dose-response study is warranted and could be feasible in humans.

**K 4**

Cognitive enhancement of healthy older adults with hyperbaric oxygen: A randomized controlled trial

Hadanny A1,2,3, Malka D1, Gil S1, Rahav B-G1, Merav C1, Kobi D1, Hachmo Y5, Ramzia AH1, Efrat S1, Fishlev G1, Lang E1, Polak N1, Keren D1, Friedman M1, Sigal T4, Yonatan Z1, Bechor Y1, Efrati S1,2,3,6
1 The Sagol Center for Hyperbaric Medicine and Research, Shamir (Assaf-Harofeh) Medical Center, Zerifin, Israel
2 Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel
3 Bar Ilan University, Ramat-Gan, Israel
4 Radiology Department, Shamir Medical Center
5 Research and Development Unit, Shamir Medical Center, Zerifin, Israel
6 Sagol School of Neuroscience, Tel-Aviv University

Submitting Author: Amir Hadanny, MD
amir.had@gmail.com

**Introduction / Background:**
More than half of community-dwelling individuals aged 60 years and older express concern about declining cognitive abilities. To date, no study has examined the effect of hyperbaric oxygen (HBO2) therapy on the cognitive performance in the healthy elderly. The current study aim was to evaluate the effect of HBO2 therapy on cognitive function and brain perfusion in a healthy aging population.

Materials and Methods: This was a randomized, prospective, controlled, assessors-blind clinical trial. Randomization began 02/2016. The trial was completed 01/2020. The final date for of cognitive outcomes was 08/2019.  
• Setting and participants: Seventy participating subjects were assigned either to HBO2 therapy or control (no intervention) arms. Measurement points consisted of baseline tests, post intervention/control .  
• Interventions: The HBO2 group received 60 daily sessions, five sessions per week within a three-month period. Each session included breathing 100% oxygen by mask at 2 ATA for 90 minutes. The control arm received no active intervention as the no-contact group. During the trial neither lifestyle and diet changes, nor medications adjustments were allowed in either group.  
• Main outcome measures: Cognitive function was assessed using two computerized batteries and one traditional paper-based battery by a certified neuropsychologist. Brain perfusion was performed in a 3T MRI system, including dynamic susceptibility contrast sequence analysis.

Results: There was a significant GroupXTime interaction in global cognitive function post HBO2 compared to the control group (p=0.0017). The most striking improvements were in attention (mean change 6.01 ± 7.55, compared to 1.14 ± 5.46, net effect size = 0.745) and information processing speed (mean change 8.30 ± 8.96, compared to -0.25 ± 12.31, net effect size=0.788) Voxel-based analysis showed significant CBF increases in the HBO2 group compared to the control group in the right superior medial frontal gyrus (BA10), right and left supplementary motor area (BA6), right middle frontal gyrus (BA6), left middle frontal gyrus (BA9), left superior frontal gyrus (BA8) and the right superior parietal gyrus (BA7).

Summary / Conclusion: For the first time in humans, HBO2 can induce cognitive enhancement in the healthy aging population. The main improvements include attention, information processing speed and executive functions, which are known to decline with normal aging. In correlation with the cognitive improvement, HBO2 therapy induced significantly increase brain perfusion in regions with high cognitive roles.

K 5

Early or multiple hyperbaric oxygen treatment enhances muscle healing after muscle contusion injury
Naoki Y, Takuya O, Mitsuhiro E, Masaki H, Yasushi K, Kazuyoshi Y
Hyperbaric Medical Center, Tokyo Medical and Dental University, Medical Hospital, Tokyo, Japan
Submitting Author: Naoki Yamamoto, MD
yamamoto.ortho@tmd.ac.jp

Introduction / Background: Contusion injury of muscle causes vascular disruption and subsequent loss of motor function. Thus, promotion of angiogenesis is crucial for early and complete recovery of functioning. Hyperbaric oxygen (HBO2) treatment promotes muscle healing via a nitric oxide-mediated angiogenesis. The timing of HBO2 treatment following muscle injury, for the best recovery, has yet to be determined. Thus, the current study investigated the effect of HBO2 treatment timing on angiogenesis and muscle regeneration on the rat contused muscle.

Materials and Methods: A muscle contusion injury of the calf was performed using the drop mass method. Injured rats were randomized to one of five groups:
• one HBO2 treatment immediately after injury (HBO2 1T D0);  
• one HBO2 treatment three days after injury (HBO2 1T Day 3);  
• three HBO2 treatments immediately, one and two days after injury (HBO2 3T Days 0–2);  
• three HBO2 treatments three, four and five days after injury (HBO2 3T Days 3–5);  
• five daily HBO2 treatments (HBO2 5T); and  
• no treatment.
The HBO2 treatment protocol consisted of 2.5 ATA 100% oxygen for 120 minutes. Tomato-lectin staining was used to quantify the extent of angiogenesis. In addition, regenerating muscle fibers and muscle tensile strength were measured.

Results: Comparing to the untreated contused muscle, rapid muscle healing and angiogenesis were observed with either a single immediate HBO2 treatment or several daily treatments (HBO2 1T D0, HBO2 3T Days 0–2 and HBO2 5T). Late several daily treatments resulted in rapid muscle healing (HBO2 3T Days 3–5), not in angiogenesis.
**Summary / Conclusion:** Early HBO2 treatment appears to be most effective for rapid recovery from injury. Even long after injury, several treatments led to significant recovery in muscle healing in our study. Whether the same mechanism is observed with early or late HBO2 treatment on injury remains to be examined.

**K 6**

**Hyperbaric oxygen therapy to inhibit Acanthamoeba keratitis infections**

Lorenzo-Morales J1, Weaver LK2,3, Sifaoui I1, Capote-Yanes E4, Piñero JE1, Deru K2
1 Instituto Universitario de Enfermedades Tropiclas y Salud Pública de Canarias (IUETSPC), Universidad de la Laguna, Santa Cruz de Tenerife, Canary Islands, Spain
2 Division of Hyperbaric Medicine Intermountain Medical Center, Murray, Utah, and Intermountain LDS Hospital, Salt Lake City, Utah
3 University of Utah School of Medicine, Salt Lake City, Utah
4 Ophthalmology Unit, Hospital Universitario Nuestra Señora de la Candelaria, Tenerife, Spain

Submissions Author: Lindell Weaver, MD
lindell.weaver@imail.org

**Introduction / Background:** Acanthamoeba keratitis is a rare but debilitating infection that causes severe pain, visual loss, requires therapy for months, and frequently, corneal transplantation. Resistance is due to cyst formation and lack of effective therapeutic agents against the different Acanthamoeba genotypes. Hyperbaric oxygen (HBO2) therapy suppresses some bacterial growth and enhances antibiotic effects and can reduce inflammation. In a recent clinical case we considered HBO2 as adjunct but declined since there is no information about HBO2 and Acanthamoeba keratitis, worried that HBO2 might worsen infection since this organism uses oxidative burst as one method of killing. Therefore we studied the evolution of Acanthamoeba in vitro when exposed to HBO2.

**Materials and Methods:** *Acanthamoeba castellanii* Neff cells were exposed to 100% oxygen at 2.0 ATA for 20 and 90 minutes using a small hyperbaric chamber.

**Results:** In this study, amoebae were eliminated after exposure to a single dose of HBO2 that is commonly used clinically. Since Acanthamoeba keratitis involves the cornea, the partial pressure of oxygen we used could be effective topically (e.g., monoplace chamber or by hood in the multiplace chamber).

**Summary / Conclusion:** The next steps in this research endeavor is to try different doses of HBO2 in vitro and to determine if Acanthamoeba keratitis in animals could be suppressed by HBO2. If such research shows benefit, offering HBO2 to patients with Acanthamoeba keratitis may offer advantage.

**K 7**

**Evaluation of hyperbaric oxygen effects on coagulation using thromboelastography**

Tanaka HL1, Le D2
1 Division of Hyperbaric Medicine, Dept of Emergency Medicine, University of California San Diego
2 Division of Coagulation, Dept of Pathology, University of California San Diego.

Submissions Author: Hideaki Leo Tanaka, MD
htanaka@health.ucsd.edu

**Introduction / Background:** Hypercoagulability is associated with hypobaric environments, but sparse studies have investigated hyperbaric exposures. Lab research on experimental models show hyperbaric oxygen preventing coagulation disorders. However, human experiments simulating compressed-air diving in a hyperbaric chamber suggest hypercoagulable effects.

Hyperbaric technicians are ideal subjects, as they are healthy and work regularly in a hyperbaric environment. They also breathe 100% oxygen at depth to reduce risk of decompression syndrome. Hyperbaric oxygen effects on coagulation can thus be evaluated. Thromboelastography (TEG) gives a simple pictograph of coagulation. This profile displays clot kinetics, giving a holistic picture as compared to standard lab values. No prior study has looked at TEG profiles after hyperbaric oxygen exposure.

**Materials/Methods:** A prospective cohort study was performed with five hyperbaric technicians. Informed consent was obtained for this IRB-approved study. Peripheral venipuncture was accomplished, intended to be within 30 minutes of hyperbaric chamber exposure, both pre- and post-. Hyperbaric exposure was to 137.89 kPa (2.4 ATA) for 90 minutes using air, except for the final 15 minutes at depth using 100% oxygen. Descent and ascent were on air.

All blood was analyzed via TEG within two hours of being drawn. TEG before and after were compared and interpreted. Additionally, CBC and clotting studies, to include PT/PTT/Factor VIII and D-dimer, were analyzed.
**Results:** There were no clinical differences of coagulation markers pre- and post-chamber.

**Summary/Conclusions:** No significant coagulation changes occur in hyperbaric technicians working with U.S. Navy Treatment Table 9. This was not a study of hyperbaric oxygen therapy; therapeutic dosages may elicit a measurable change in coagulation. Hyperbaric pressure in this study also did not approximate the 6.5 ATA reported in a previously published diver study that suggested hypercoagulable effects, although the conclusions they reached are debatable. Studies involving coagulation profile changes after therapeutic hyperbaric oxygen may show different results.

**K 8**

**Traumatic pneumothorax exposed to reduced pressure**

Majercik S1, Weaver LK1,2, Churchill S1, Deru K1

1 Division of Trauma Services and Critical Care Medicine, Intermountain Medical Center, Murray, Utah 2 Division of Hyperbaric Medicine Intermountain Medical Center, Murray, Utah, and Intermountain LDS Hospital, Salt Lake City, Utah

3 University of Utah School of Medicine, Salt Lake City, Utah

Submitting Author: Lindell Weaver, MD

*lindell.weaver@imail.org*

**Introduction / Background:** Traumatic pneumothoraces (tPTX) are contraindicated for hyperbaric exposure. We conducted a study of patients with tPTX to determine if they could fly commercially safely (Majercik S, et al. Cleared for takeoff: the effects of hypobaric conditions on traumatic pneumothoraces. J Trauma Acute Care Surg. 2014;77:729Y733). This study has implications for patients with tPTX for hyperbaric pressurization.

**Materials and Methods:** Twenty patients (Phase 1, 10; Phase 2, 10) with tPTX were enrolled. They were treated by chest tube (CT) or oxygen therapy. CT was removed 48 hours of enrollment. Participants began at altitude of 4,700 feet (640 mmHg), then Phase 1 members were exposed in a multipurpose hypobaric chamber to two hours of hypobaria (554 mm Hg, altitude = 8,400 feet). In Phase 2, they were exposed to 471 mmHg (altitude = 12,640 feet, to mimic the volume change from sea level to 8,400 feet, but starting at 4,700 feet). Vital signs and symptoms were recorded during the exposure. After two hours, a portable chest radiograph (CXR) was obtained at altitude. tPTX sizes at baseline, at reduced pressure, and afterward were compared.

**Results:** Sixteen participants (80%) were male. Mean age was 49 ± 5 years and Injury Severity Score was 10.5 ± 4.6. Fourteen (70%) had a CT to treat tPTX, which had been removed 19 hours (range four to 43 hours) before the study. No participant complained of cardiorespiratory symptoms while at increased altitude. Radiographic increase in tPTX size at altitude was 5.6 ± 0.61 mm from baseline. No participant developed a tension tPTX or needed procedural intervention during the altitude exposure. Four hours afterward, all tPTXs had returned to baseline size.

**Summary / Conclusion:** Participants with recently treated tPTX have a well-tolerated, small increase tPTX size when exposed to simulated altitude, with implications for air travel after tPTX. These results suggest that some patients with tPTX may not need thoracostomy before hyperbaric pressurization.

End Session K