UNDERSEA MEDICINE
Session E
Mitigation and Prevention of O2 Toxicity

E 1

Ketogenic diet for reduction of CNS oxygen toxicity in working divers (KetOX Study): Year-2 Progress

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Introduction / Background: CNS O2 toxicity (CNSOT) continues to limit Navy diving operations. Previous studies demonstrated an increased latency to seizure in animals with elevated serum ketone levels; nutritional ketosis (NK) has been used to treat epilepsy in children and adults. We seek to compare latency to CNSOT between a conventional diet (CD) versus a ketogenic diet (KD) in divers breathing 100% oxygen.

Materials and Methods: This is a prospective, randomized, blinded crossover study. The primary endpoint was latency to the first manifestation of CNSOT or maximum dive time of 120 minutes on O2 at 2.06 ATA (35 fsw, NAVY limit 25 minutes).

A second aim is to develop an operationally feasible protocol to achieve rapid nutritional ketosis. Physiologic parameters are measured, including invasive blood pressure monitoring, continuous electrocardiogram, standard and quantitative electroencephalogram (qEEG), and cognitive performance.

Results: To date 47 exposures have been completed on 26 volunteer subjects. Two subjects completed the entire 120-minute protocol during both exposures without toxicity. Four subjects completed the protocol without symptoms on one exposure. Average latency to CNS O2 toxicity was 59.8 minutes. Four subjects' dives were aborted due to fatigue or equipment failure. Primary symptoms were headache/facial pressure (7), diaphoresis (6), twitching/tingling (5), concentration difficulty (4), light-headedness (3), nausea (3), auditory disturbance (2), visual disturbance (2), taste disturbance (1), anxiety (1), seizure (1).

Summary / Conclusion: The study is almost 50% complete, and the majority of exposures have resulted in CNS O2 toxicity symptoms. Most symptoms are consistent with known manifestations of toxicity. Facial/forehead pressure has been the most common symptom, which has not been a classic symptom described in previous work. Analysis of physiologic data is ongoing. Investigators are blinded to the subjects' diet for the remainder of the study. Final analysis will occur upon completion of all subjects.

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Ketone ester supplementation does not impair cognitive or motor performance and delays CNS oxygen toxicity in male Sprague-Dawley rats

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Introduction / Background: Ketone metabolic therapy is neuroprotective against CNS oxygen toxicity (CNSOT). Here we test the hypothesis that the dose of ketone ester (KE) that delays CNSOT has no deleterious effects on motor performance or cognition in male Sprague-Dawley (SD) rats.

Materials and Methods: SD rats received either no gavage, vehicle gavage (water), or 7.5 g/kg KE gavage and were subjected to behavioral testing (n=8 rats/group): DigiGait (DG), light/dark (LD), open field (OF), and novel object recognition (NOR). Additional experiments were conducted in which vehicle- or KE-treated rats were implanted with radio telemetry modules, allowing for real-time monitoring of EEG, ECG, and respiratory EMG during exposure to HBO2 (5 ATA; n=9-13 rats/group).

Results: No significant differences were observed between treatment groups in the following tests: DG, NOR and LD tests. This indicates no effect of KE on motor performance (DG), learning and recall (NOR), and fear/anxiety (LD). In the LD test, KE-treated rats exhibited reduced distance traveled in the dark zone, suggesting the KE may be anxiolytic. In OF testing, KE-treated rats exhibited increased overall activity by exploring the OF areas and were less anxious than vehicle controls. Although there were no significant differences between the groups in the NOR test, KE-treated rats exhibited a trend in both exploratory preference and discrimination index suggesting that the animals learned and were able to recall that a novel object was present. Finally, KE-treated animals implanted with telemetry modules showed an increase in latency time to seizure over vehicle (20.27±5.8 (n=14); 6.1±0.4 (n=9)). Furthermore, normalizing the data to vehicle, there is a significant percent increase in latency to seizure with KE-treated animals ($p<0.05$).

Summary / Conclusion: Together, these findings suggest that a single dose of KE after 30 minutes delays onset of CNSOT without hindering cognition and motor performance (ONR Undersea Medicine Program).

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Fluorescence imaging of reactive species production in real time in rat brain slices during exposure to normobaric hyperoxia, hyperbaric oxygen and ketone salts

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Introduction / Background: We tested the hypothesis that ketone salts (KS) decrease superoxide production in the caudodorsal medulla oblongata during exposure to normobaric hyperoxia (NBO2) and hyperbaric oxygen (HBO2). Ketone metabolic therapy delays onset of CNS oxygen toxicity seizures (CNSOT) in animals.

Materials and Methods: New methods for measuring superoxide production in brain slices during exposure to HBO2 were developed that complement our ongoing experiments using NBO2. Superoxide production was measured using the fluorescent probe dihydroethidium (DHE; 2.5uM). Brain slices (300-400um) were harvested from the caudal medulla oblongata of Sprague-Dawley rats (P12-40). All experiments were run at ~35°C in control O2 (0.4 ATA) and CO2 (0.05 ATA). Hyperoxia was tested using NBO2 (0.95 ATA) and HBO2 (1.95 and 4.95 ATA). The rate of superoxide production was measured in arbitrary fluorescence intensity units per minute (FIU/min) and analyzed using Nikon NIS Elements. KS were used in a 1:1 ratio of beta-Hydroxybutyrate:acetoacetate. The chamber atmosphere was filled with 100% helium (1-5 ATA).

Results: Exposure to NBO2/HBO2 increases the rate of superoxide production in medullary neurons, but not all cells produce superoxide at the same rate. Most DHE-loaded neurons exhibited varying FIU initially in 0.4 ATA O2 that we labeled as either "dim cells" (FIU < average FIU at t=0) or "bright cells" (FIU ≥ average FIU at t=0). O2-

sensitive cells significantly increased superoxide production (≥25% ΔFIU/min) in both dim and bright cells after exposure to hyperoxia, whereas other bright and dim cells were O2-insensitive cells (<25% ΔFIU/min). 5mM of KS significantly decreased superoxide production in O2-sensitive bright cells at 0.95/1.95 ATA O2.

Summary / Conclusion: These are the first measurements of superoxide production during exposure to HBO2. Our findings suggest that cells in the caudo-dorsal medulla are heterogeneous in their capacity for superoxide production during exposure to hyperoxia and that O2-sensitive neurons are protected by ketone bodies during exposure to hyperoxia (ONR Undersea Medicine Program).

E 4

Hyperbaric oxygen treatment related oxygen toxicity: Our experience as a single center of three years

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Introduction / Background: Oxygen toxicity seen during hyperbaric oxygen therapy depends on reactions between free oxygen radicals and cell components and the effect of enzymes. To prevent oxygen toxicity at pressures higher than 2 ATA, a five- to 10-minute air break is recommended between the oxygen periods of 20-30 minutes. In our study we aimed to present the cases and their features in individuals who developed oxygen toxicity within a three-year period in our center.

Materials and Methods: We retrospectively analyzed 1,362 HBO2 therapy sessions applied to 785 patients treated in our clinic. We investigated the treatment protocols applied, the diagnosis of patient condition and how many sessions they encountered with this condition and their comorbidities.

Results: In total, 13,498 sessions were performed due to 10 different diagnoses; 188 sessions were planned as an emergency. Forty patients received U.S. Navy Treatment Table 5 (TT5) for Type I decompression sickness. U.S. Navy TT6 was applied to 36 patients for Type II decompression sickness and to two patients for arterial gas embolism. TT9 was applied to all other routine and emergency patients. We observed symptoms of oxygen toxicity in five patients (0.0037%). Although the toxicity observed in only one patient was during TT5 (2.5%), other patients were routinely administered TT9.

Summary / Conclusion: Although the rate of risk in patients with U.S. Navy TT5 was higher, all other sessions were the same as in the literature. Intermittent oxygen breathing periods at pressures higher than 2 ATA are considered to be protective. Larger series of studies are needed for oxygen toxicities that canbe observed in different treatment tables.

End Session E