# Hyperbaric oxygen preconditioning increases tolerance to decompression sickness via HSP70 in rats

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## Introduction

***Decompression sickness (DCS)***

- caused by inert gas bubbles formed in tissues and blood vessels due to inadequate decompression from hyperbaric surrounding to surface (in diving), or from atmospheric pressure to altitude (in aviating or performing “space walk”)

***HBO preconditioning***

- reported to exert protective effects on ischemic-hypoxia injuries
- significantly reduced the incidence of DCS via nitric oxide (NO) in rats
- induce the expression of HSP70 in vivo and in vitro

***HSP70***

- has definite protective effects on the lung and spinal cord, normally through its anti-inflammatory and anti-apoptosis properties
- might be involved in heat stress-induced decrease of bubble-related injuries resulting from decompression
- be involved in diving acclimatization to neurological DCS

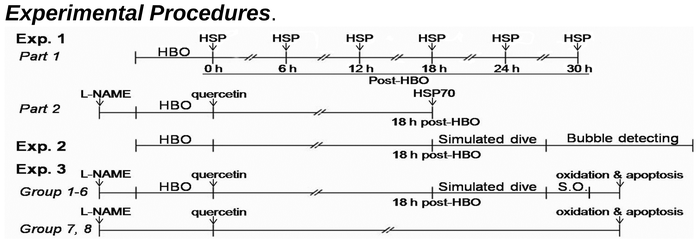
***Purpose of the study***

To explore:

- whether the prophylactic effects of HBO on DCS was relative to HSPs
- whether HBO facilitated acclimatization to DCS by anti-oxidation and anti-apoptosis.

## Materials and Methods

***Experimental Procedures***.




(*Experiment 1.* HSPs expression; *Experiment 2.* DCS bubble formation; *Experiment 3*. The role of HSP70 in HBO preconditioning on DCS)

***HBO exposure*** –The pressure-duration was 280 kPa-60 min.

***Simulated air dive*** – rats were subjected to 700 kPa air for 90 min before decompressing linearly to the ambient pressure at the rate of 200 kPa/min.

***Assessment of Decompression Sickness*** -rats were subjected to walk inside an electrically controlled cylindrical cage rotating at a perimeter speed of 3 m/min for 30 min to standardize the activity level and facilitate DCS assessing, then diagnosed based on observation any of the following symptoms: abnormal breathing patterns, walking difficulties, forelimb and/or hindlimb paralysis, rolling in the rotating wheel, convulsions, or death.

***Determination of HSPs*** -Western Blot and Immunohistochemical Staining.

## Materials and Methods(continued)

***Ultrasound detecting and analysis of DCS bubbles*** –obtained using a Mylab30cv ultrasonic scanner (Esaote, Italy) connected to an ultrahigh frequency (18 MHz) detector. The number of bubbles was scored according to the grading system in the following Table.

**Table 1. Grading of the ultrasonic images of DCS bubbles**

| Grade | Definition |
|---|---|
| 0 | No observable bubbles |
| 1 | Occasional bubbles |
| 2 | At least 1 bubble every 4 heart cycles |
| 3 | At least 1 bubble every heart cycle |
| 4 | At least 1 bubble per cm² in every image |
| 5 | At least 80% of visible lumen obscured by bubble cloud; single bubbles cannot be discriminated |

***Statistical Analysis*** - Incidences of DCS of different groups were compared by means of Chi-square test. One-way ANOVA followed by the Student Newman-Keuls tests were used to compare the ratio of HSP70 to β-actin, bubble scores, MDA, 8-OHdG, $H_2O_2$, caspase 3 and 9 levels among groups. For analyzing the results of cell counting, a non-parametric Kruskal–Wallis ANOVA was used followed by Dunn's test.

## Results

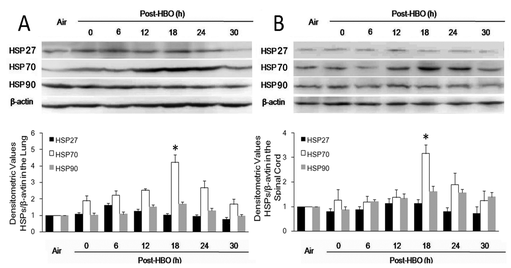



**Fig.1 HSPs expression after HBO exposure.** HSP27 and HSP90 had a slight and non-significant increase after HBO exposure. HSP70 increased and reached at a peak level at 18 h after HBO exposure both in lung and spinal cord.

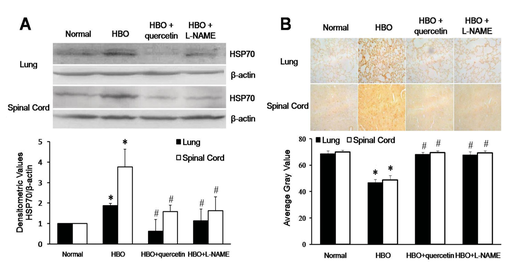
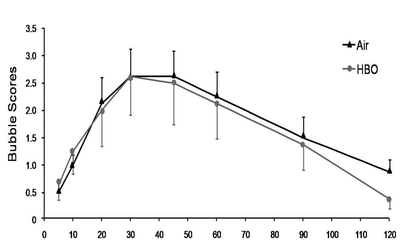



**Fig.2 the effects of quercetin or L-NAME.** Quercetin (an inhibitor of HSPs) or L-NAME [$N^G$-nitro-L-arginine methyl ester, a non-selective nitric oxide synthase (NOS) inhibitor] significantly inhibited the effects of HBO

**Fig.3 Bubble formation in DCS rat models with different pre-treatments.** The relative bubble count increased gradually after decompression, to reach a maximum at around 31 min (95% CI 22 – 40 min). There’s no difference of the bubble scores at each time point or the total scores, or of the time to reach maximum value between the two groups




## Results (continued)

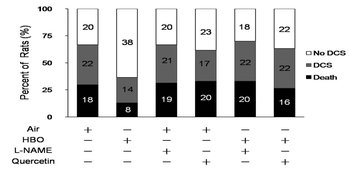



**Fig.4 Effects of HBO preconditioning on the incidence of DCS.** HBO significantly decreased the incidence and mortality of DCS ($P$<0.05). L-NAME or quercetin nullified the protective effects of HBO. They didn’t affect DCS incidence when used alone.

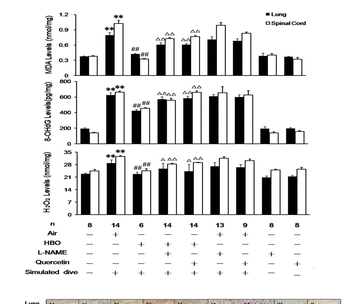



**Fig.5 Changes of oxidation in DCS rats with different pre-treatments.** Compared with the normal controls, MDA, 8-OHdG, $H_2O_2$ in the lung and spinal cord increased significantly ($P$<0.01) in DCS rats (Air Group). HBO preconditioning significantly lowered the levels ($P$<0.01), which could be partially counteracted by L-NAME or quercetin ($P$<0.05 or $P$<0.01). L-NAME or quercetin didn’t affect the oxidation parameters both in normal or DCS rats when used around 20 h before.

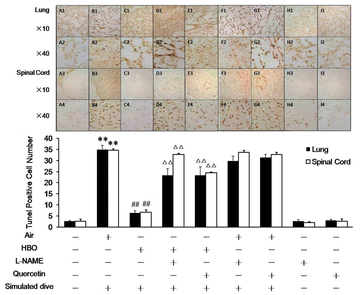



**Fig.6 Changes of apoptosis in DCS rats with different pre-treatments.** TUNEL-positive cells were markedly increased in DCS rats. HBO preconditioning dramatically reduced the number of positive cells, the effects were inhibited by L-NAME or quercetin.

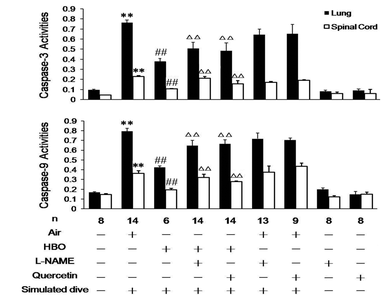



**Fig.7 Changes of Caspases in DCS rats with different pre-treatments.** The activities of caspase 3 and 9 increased significantly in DCS rats, and were decreased in rats pretreated with HBO ($P$<0.05). L-NAME or quercetin inhibited the effects of HBO.

## Conclusion

- HBO preconditioning significantly reduced the morbidity of DCS, the increased levels of oxidation and apoptosis, but had no effects on bubble formation.
- HSP70 was involved in the beneficial effects of HBO on DCS, mainly by the anti-oxidation and anti-apoptosis effects.

HBO exposure is a promising modality to induce HSP70, and could be feasibly applied in diving practice in the prevention of DCS.

## Acknowledgement

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