

## Acclimatization to neurological decompression sickness in rabbits

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**Su, Chien-Ling, Chin-Pyng Wu, Shao-Yuan Chen, Bor-Hwang Kang, Kun-Lun Huang, and Yu-Chong Lin.** Acclimatization to neurological decompression sickness in rabbits. *Am J Physiol Regul Integr Comp Physiol* 287: R1214–R1218, 2004. First published July 29, 2004; doi:10.1152/ajpregu.00260.2004.—Diving acclimatization refers to a reduced susceptibility to acute decompression sickness (DCS) in individuals undergoing repeated compression-decompression cycles. We demonstrated in a previous study that the mechanism responsible for this acclimatization is similar to that of stress preconditioning. In this study, we investigated the protective effect of prior DCS preconditioning on the severity of neurological DCS in subsequent exposure to high pressure in rabbits. We exposed the rabbits ( $n = 10$ ) to a pressure cycle of 6 absolute atmospheres (ATA) for 90 min, which induced signs of neurological DCS in 60% of the animals. Twenty-four hours after the pressure cycle, rabbits with DCS expressed more heat-shock protein 70 (HSP70) in the lungs, liver, and heart than rabbits without signs of disease or those in the control group ( $n = 6$ ). In another group of rabbits ( $n = 24$ ), 50% of animals presented signs of neurological DCS after exposure to high pressure, with a neurological score of 46.5 (SD 19.5). A course of hyperbaric oxygen therapy alleviated the signs of neurological DCS and ensured the animals' survival for 24 h. Experiencing another pressure cycle of 6 ATA for 90 min, 50% of 12 rabbits with prior DCS preconditioning developed signs of DCS, with a neurological score of 16.3 (SD 28.3), significantly lower than that before hyperbaric oxygen therapy ( $P = 0.002$ ). In summary, our results show that the occurrence of DCS in rabbits after rapid decompression is associated with increased expression of a stress protein, indicating that the stress response is induced by DCS. This phenomenon was defined as "DCS preconditioning." DCS preconditioning attenuated the severity of neurological DCS caused by subsequent exposure to high pressure. These results suggest that bubble formation in tissues activates the stress response and stress preconditioning attenuates tissue injury on subsequent DCS stress, which may be the mechanism responsible for diving acclimatization.

diving acclimatization; decompression stress; heat shock protein; neurological impairment

DECOMPRESSION SICKNESS (DCS) is a disease caused by gas bubble formation during inappropriate pressure reduction in diving and aviation. Acting as foreign material, bubbles may activate offensive cascades (12, 15) and pose a stress to tissues (21). Depending on the number of bubbles and where they form, acute manifestations vary from local pain to severe neurological dysfunction or even death (19, 24). Furthermore, individual susceptibility to bubble formation determines the severity of DCS (5). Divers undergoing repeated compression-decompression cycles are able to reduce their susceptibility to

acute DCS. This phenomenon of acclimatization has been observed for decades (7), but the mechanism remains unknown.

It has been suggested that the formation of silent bubbles, which exist in tissues after decompression but do not lead to acute symptoms of DCS, underlies the etiology of diving acclimatization. Postulated mechanisms for this acclimatization include the "consumption theory," describing the depletion of offensive chemicals (29), and the "induction theory," proposing the accumulation of protective factors (13). The consumption theory hypothesizes that silent bubbles indolently activate the complement cascade in tissues and that repeated exposure to pressure may deplete complement proteins, thus preventing a massive activation of the complement system during decompression with greater bubble formation (28). However, several studies have reported that the complement proteins of human divers remained within normal ranges when they were on a regular diving schedule with repetitive pressure exposure (11, 26). These data weaken the consumption theory. We proposed the induction theory, hypothesizing that silent bubbles may induce a subsymptomatic stress response and induce tissue preconditioning that significantly reduces the severity of acute tissue injury resulting from subsequent exposure to intravascular bubbles (13). We demonstrated in a rat model of acute lung injury that the presence of air bubbles causes tissue injury and induces a stress response. Pretreatment of animals with heat shock reduced the lung injury caused by either venous air infusion or rapid decompression after exposure to high pressure. However, direct evidence is lacking on DCS prevention by thermal preconditioning because of the difficulty of evaluating DCS symptoms in rats. Therefore, the induction theory requires more evidence for its confirmation.

The purpose of this study was to establish an animal model with assessable indexes of DCS severity and to test the induction theory of diving acclimatization in this model.

### MATERIALS AND METHODS

*Heat shock protein induction by heat shock or DCS.* All the experimental procedures were in accordance with the *Guiding Principles in the Care and Use of Animals* approved by the Institutional Animal Care and Use Committee of the National Defense Medical Center, Taipei, Taiwan. New Zealand White rabbits weighing 2.5–3.0 kg were lightly anesthetized with intraperitoneal injections of ketamine (25 mg/kg). Each animal was placed on the heating pad of a temperature-controlled device (Homeothermic PT-100, DR Instrument, Taipei, Taiwan), and its body temperature was measured with a

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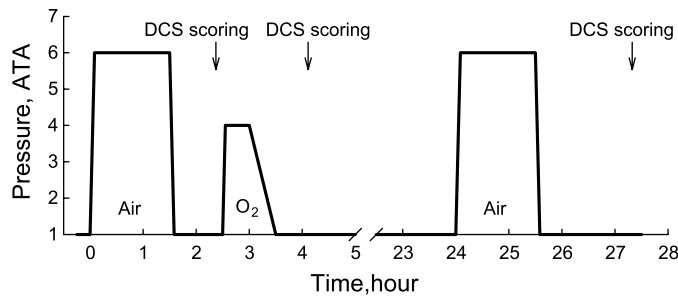


Fig. 1. Pressure profile and experimental protocol of decompression sickness (DCS) preconditioning. ATA, absolute atmosphere.

rectal probe. A light bulb (100 W) was used to accelerate heating and for quick adjustment of body temperature. Heat shock was induced by increasing the core temperature to 41°C for 15 min. The rabbit was then placed on a cooling pad to accelerate temperature restoration. Animals ( $n = 7$ ) were killed 24 h after heat shock treatment for analysis of heat shock protein 70 (HSP70) expression.

Another group of rabbits ( $n = 10$ ) was placed in a steel hyperbaric chamber and pressurized with air to 6 absolute atmospheres (ATA) for 90 min. The chamber temperature was maintained at 27°C. The animals were then decompressed at a rate of 1 ATA/min. After completion of the compression-decompression procedure, the rabbits were observed for 2 h to evaluate signs of DCS and were then killed with an overdose of pentobarbital 24 h after decompression. Liver, heart, and lung tissues were excised for HSP70 analysis.

**DCS preconditioning and DCS.** To ensure the survival of severely sick animals and to allow the induction of stress responses to DCS, 24 rabbits were subjected to recompression and hyperbaric oxygen therapy (HBO<sub>2</sub>) 1 h after the completion of the compression-decompression cycle at 6 ATA for 90 min. In the procedure for recompression and HBO<sub>2</sub>, rabbits were pressurized to 4 ATA with oxygen in the hyperbaric chamber and maintained at this pressure for 30 min before they were slowly decompressed (1 ATA/10 min). We defined this procedure as “DCS preconditioning” if animals presented signs of DCS before HBO<sub>2</sub>. Neurological dysfunction was evaluated before and after HBO<sub>2</sub>. We asked a neurologist to evaluate the severity of DCS using a neurological scoring system initially described by Baker et al. (2) and modified by Ryu et al. (25). This scoring system measures levels of consciousness, respiration, cranial nerves, motor/sensory function, gait, and behavior. The best possible score is zero and the worst is 97. Twenty-four hours after DCS preconditioning, animals were subjected to a second course of pressure exposure to 6 ATA for 90 min. The severity of neurological DCS in each animal was evaluated 2 h after decompression. Figure 1 illustrates the experimental protocol of this set of study. At the end of each experiment, animals were killed for analysis of HSP70 expression. To control the effect of hyperbaric oxygen, another six rabbits were subjected to HBO<sub>2</sub> alone (4 ATA 30 min, HBO<sub>2</sub> group) 24 h before the second course of pressure exposure to 6 ATA for 90 min.

**Determination of heat shock protein.** The expression of HSP70 was determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Streagen, Victoria, BC, Canada). The harvested tissue (0.25 g) was homogenized in cold lysis buffer (1 ml) and centrifuged at 12,000  $g$  for 5 min at 4°C. The protein concentration in the supernatant was quantified using a Coomassie protein assay reagent (Rockford, IL). The concentration of HSP70 was determined according to the manufacturer’s instructions. Specimens from rabbits killed for other study purposes served as the control group for HSP70 analysis.

**Statistical analysis.** Data are expressed as means with SD in parenthesis. Differences in the incidence of DCS after decompression were evaluated with a  $\chi^2$  test. Differences in neurological DCS severity of each animal were evaluated by a paired Student’s  $t$ -test,

and by Student’s  $t$ -test between groups. A value of  $P < 0.05$  was deemed significant.

## RESULTS

**Preconditioning.** Prior heat shock induced significant expression of HSP70 in the liver, heart, and lung tissues of rabbits ( $n = 7$ ) compared with the control group ( $n = 6$ ). The liver and heart produced more HSP70 24 h after exposure to heat stress than did the lungs. In the other group of rabbits ( $n = 10$ ), which experienced a pressure cycle of 6 ATA for 90 min, 60% of animals developed signs of severe DCS, and two rabbits died of dyspnea and seizure 2 h after decompression. Animals surviving severe DCS for 24 h produced similar amounts of HSP70 in the liver, heart, and lung tissues as did the rabbits treated with prior heat shock. Without causing signs of DCS, exposure to high pressure alone slightly elevated the expression of HSP70 in the liver, but this increase was not statistically significant (Fig. 2).

**Effects of DCS preconditioning on neurological DCS.** During the induction of DCS preconditioning, 12 of 24 rabbits developed signs of neurological DCS after experiencing the first course of compression-decompression. Neurological scores ranged from 10 to 86. After recompression to 4 ATA and HBO<sub>2</sub>, all animals survived and most signs of neurological impairment disappeared. Neurological scores ranged from 0 to 15. Twenty-four hours later, the second course of exposure to high pressure (6 ATA for 90 min) induced DCS in 50% of these rabbits (6/12), including one rabbit that died of seizure 2 h after decompression. DCS preconditioning significantly reduced the neurological score from 46.5 (SD 19.5) in the first pressure cycle to 16.3 (SD 28.3) in the second pressure cycle ( $P = 0.002$ , Fig. 3). Three of the other 12 rabbits that did not present with DCS in the first cycle of pressure exposure developed signs of neurological impairment after decompression from the last cycle of pressure exposure. Two of these three rabbits with severe DCS died of seizure 10 min after the completion of decompression. In the HBO<sub>2</sub> group, HBO<sub>2</sub> alone did not induce DCS in any rabbit, whereas the high-pressure exposure 24 h later induced DCS in 67% of these rabbits (4/6).

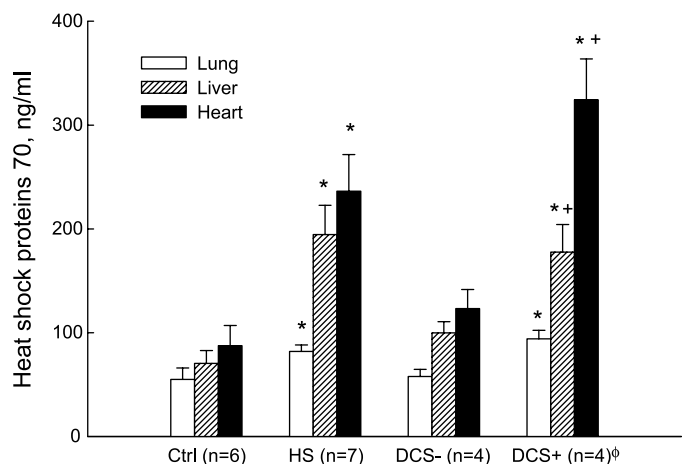


Fig. 2. Expression of heat-shock protein 70 (HSP70) in the lung, liver, and heart of rabbits 24 h after rapid decompression from 6 ATA or after exposure to heat shock (HS). \* $P < 0.05$  compared with the control (Ctrl) group; + $P < 0.05$  compared with rabbits without DCS (DCS-); φ, two rabbits died of severe DCS and were withdrawn from HSP70 analysis. DCS+, with DCS.

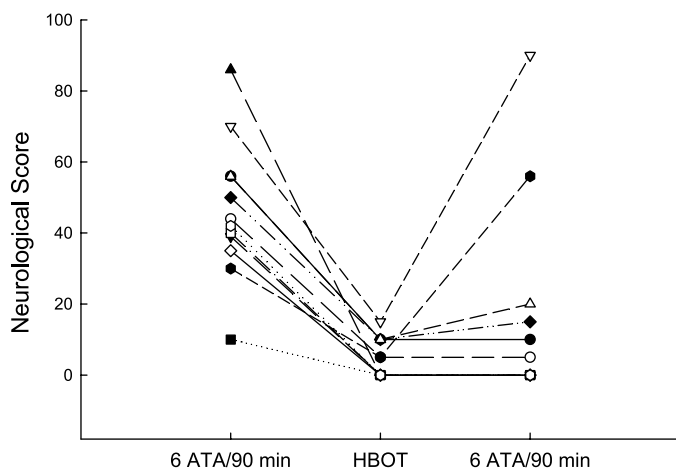


Fig. 3. Effect of DCS preconditioning on the severity of neurological impairment induced by rapid decompression after hyperbaric (6 ATA) exposure. HBOT, recompression and hyperbaric oxygen therapy.

The averaged neurological score was 39.2 (SD 14.4) in the HBO<sub>2</sub> group, which was not different from those in the rabbits without DCS preconditioning but significantly greater than those after DCS preconditioning.

The expression of HSP70 in the lungs, liver, and heart was analyzed at the end of each experiment. HSP70 was significantly higher in rabbits with DCS preconditioning than in those without or in those of the control group (Fig. 4). The extent of increase in HSP70 expression induced by prior DCS was similar to that provoked by heat-shock pretreatment. Pressure exposure by itself, if no DCS occurred, caused an insignificant increase in HSP70 expression.

## DISCUSSION

Diving acclimatization has been described as an adaptive response to decompression stress after repetitive exposure to pressure (7). This adaptation reduces a diver's susceptibility to DCS or the severity of DCS. The mechanism contributing to diving acclimatization, however, remains obscure. We proposed an "induction hypothesis," speculating that repetitive compression-decompression is a form of preconditioning that generates protective factors and reduces the severity of acute tissue injury during subsequent bubble formation. In the present study, our results further demonstrate that DCS induces a stress response and that this DCS preconditioning significantly alleviates the neurological impairment induced by subsequent exposure to high pressure. These results strengthen our induction hypothesis by explaining the mechanism underlying diving acclimatization.

Exposure to high pressure exerts significant stress on the human body. Hirayanagi et al. (10) reported that in a human diver experiencing a hyperbaric saturation dive, sympathetic nervous activity was enhanced and stress hormones were elevated. Matsuo et al. (18) reported another hyperbaric saturation dive that induced increased expression of heat shock proteins in lymphocytes after exposure to high pressure. These studies suggest that simulation dives, with hyperbaric exposure in a dry environment, induce a stress response. Our results show that pressure exposure by itself, with no DCS, did not cause a significant increase in HSP70 expression. The discrepan-

cy between Matsuo's finding and our results may be due to a different pressure profile. In Matsuo's study, divers underwent a 39-day saturation dive to a pressure of 40 ATA. In contrast, our rabbits were pressurized to 6 ATA for 90 min. Therefore, the hyperbaric protocol in this study induced only an insignificant increase in HSP70 expression. However, the occurrence of DCS increased HSP70 expression to a level similar to that induced by exposure to heat shock, indicating that DCS significantly enhances the stress response after exposure to high pressure.

The HSP70 is one of the main stress proteins induced by heat shock in mammals (30). Detection of HSP70 expression has become a standard to evaluate stress response and thermal preconditioning (20). HSP70 expression may be induced by a variety of stresses, including heat, ischemia, hypoglycemia, and drugs (3). The increased HSP70 expression in tissues of lung, liver, and heart in this study indicated a systemic stress response after heat exposure. DCS is also a systemic disease and may induce a whole body preconditioning to the stress of pressure exposure. Similar to heat or hypoglycemia, high pressure produces its effects evenly to all organs and cells in the same body. We have previously demonstrated that DCS induced HSP70 expression in the lung of rats (13). In the present study, our results further proved that occurrence of DCS significantly increased expression of HSP70 in the liver and heart, indicating that DCS induces a systemic stress response.

The stress response is a self-repairing mechanism that protects living beings from repetitive insult (20). It has been shown that prior heat stress significantly attenuates tissue injuries induced by a variety of insults, such as cardiac surgery (4, 16), sepsis (9, 27), and ischemia-reperfusion (14). In a previous study, we demonstrated that prior heat shock induced a stress response in rats and protected the animals from acute lung injury caused by pulmonary air embolism (13). However, prior induction of the stress response did not reduce the occurrence of DCS in rats after exposure to high pressure. This insignificant protection against DCS may be due to the impossibility of grading the severity of DCS in small animals. In the

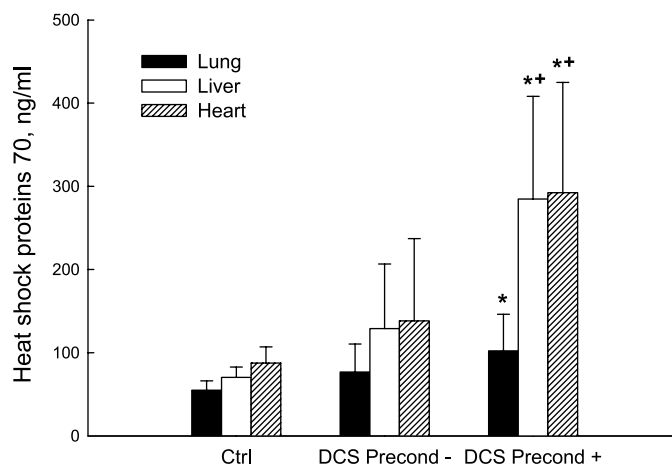


Fig. 4. Expression of HSP70 in the lung, liver, and heart of rabbits after rapid decompression from a second course of exposure to 6 ATA pressure for 90 min. \* $P < 0.05$  compared with the control group; + $P < 0.05$  compared with rabbits without DCS preconditioning (DCS Precond-). DCS Precond+, with DCS preconditioning.

previous study using a rat model, the protocol used to expose the rats to pressure induced symptoms of DCS, such as dyspnea, seizure, and death, which are too severe to be evaluated for the protective effects of stress preconditioning. Therefore, we used a rabbit model in the present study and a published neurological scoring system (25) to evaluate DCS severity. Our results show that 50% of rabbits developed signs of neurological DCS after experiencing the first course of compression-decompression. These rabbits survived severe DCS after treatment with hyperbaric oxygen and developed into animals with DCS preconditioning within 24 h. Although 50% of these 12 rabbits presented signs of DCS in the next cycle of high pressure, the severity of neurological impairment was significantly reduced (Fig. 3). This result indicates that DCS preconditioning reduces the neurological impairment caused by subsequent rapid decompression after exposure to high pressure.

DCS is a disease caused by gas bubble formation in tissues. Air bubbles produce their effects by mechanical obstruction, by altering the biochemical environment, or both. Bubble formation interrupts blood flow and compresses or disrupts tissues (22). Air bubbles can also initiate an air-liquid interface reaction in tissues, which activates plasma proteins, including clotting factors, enzymes, and immunoglobulins (15). The complement system, polymorphonuclear leukocytes, and oxygen metabolites are proven factors that mediate air-bubble-induced tissue injury (22). Protection from air-bubble-induced tissue injury may result from a smaller number of bubbles or from less tissue reaction to air bubbles. Wisloff and Brubakk (31) reported that endurance exercise reduces bubble formation and increases survival in rats exposed to hyperbaric pressure. It is not known whether DCS preconditioning reduces bubble formation after the next episode of decompression from a hyperbaric environment. Nevertheless, endurance exercise is a stressor that increases the expression of HSP70 and may represent a powerful preventative agent against tissue injury in several models (8, 23). These reports suggest that stresses such as endurance exercise can activate bioprotective mechanisms. Compatible with these reports, our results show that prior DCS is also a stress inducer, which may activate a bioprotective mechanism similar to that induced by endurance exercise. This suggests that this protection involves mechanisms more complex than a reduction in bubble formation.

Hyperbaric oxygen plays an important role in DCS treatment as an adjunct to the recompression therapy. In addition to enhancement of washing out dissolved tissue nitrogen, HBO<sub>2</sub> may produce its protective effect by tissue preconditioning. In the present study, HBO<sub>2</sub> was applied to ensure the survival of rabbits after the first section of pressure exposure (Fig. 1). This protocol of experiment raised a concern that the protective effect of reducing DCS severity might be derived from HBO<sub>2</sub> but not from the DCS preconditioning. To control the effect of HBO<sub>2</sub>, rabbits were subjected to HBO<sub>2</sub> 24 h before the pressure exposure to 6 ATA for 90 min. Our results showed that prior HBO<sub>2</sub> alone did not reduce the incidence or neurological severity of DCS induced on the second course of high-pressure exposure. These results indicated that prior HBO<sub>2</sub> at 4 ATA for 30 min may not be able to induce tissue preconditioning to decompression stress. Nevertheless, our results did not preclude the possibility that HBO<sub>2</sub> of various experimental protocols may induce tolerance against DCS. It has been reported that daily HBO<sub>2</sub> pretreatment for 3–5 days induced significant

tolerance against ischemic injury in the spinal cord of rabbits (6). Martin and Thom (17) also demonstrated that prior HBO<sub>2</sub> of 3 ATA for 90 min reduced vascular leukocyte sequestration in the brain of rapidly decompressed rats. Prior HBO<sub>2</sub> may also reduce DCS by enhancement of gas nuclei elimination and by reduction of the number of bubble formation after explosive decompression (1). HBO<sub>2</sub> pretreatment indeed may induce significant tissue preconditioning. However, in the present study, a short HBO<sub>2</sub> treatment is unlikely the mechanism responsible for inducing tolerance against decompression stress and reducing DCS severity on the second course of high-pressure exposure.

In summary, our results show that DCS induces a stress response, as confirmed by the expression of heat-shock protein in lung, liver, and heart tissue. DCS preconditioning reduced the neurological impairment caused by subsequent rapid decompression from exposure to high pressure. We conclude that bubble formation in tissues after decompression can activate a stress response and that the protective effects derived from this stress response may be the mechanism responsible for the phenomenon of diving acclimatization.

#### GRANTS

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