# Adaptation to Periodic Hypoxia and Hyperoxia Improves Resistance of Membrane Structures in Heart, Liver, and Brain

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A novel principle of short-term periodic adaptive training by varying the oxygen level from hypo- to hyperoxia is substantiated both theoretically and experimentally. Short-term adaptation to hypoxia-normoxia produced a membrane-protective effect in the heart and cerebral cortex, but increased the sensitivity to free radical oxidation and decreased the level of components of the antioxidant defense system in the liver. Hypo-hyperoxia adaptation produced a membrane-stabilizing effect in the heart, brain, and liver, which was more pronounced compared to the effect of hypoxia-normoxia training. In contrast to hypoxia-normoxia adaptation, in case of hypo-hyperoxia training the adaptive defense developed as early as 15 days after the start of training.

**Key Words:** *adaptation; hypoxia; hyperoxia; free radical oxidation; early response proteins*

Interval normobaric hypoxic training (INHT) increasing resistance to damaging factors is now widely used in the treatment and prophylaxis of various pathological syndromes [13]. The protective effect of INHT is based on activation of mechanisms of adaptation to both hypoxic conditions and reoxygenation during return from hypoxia to normoxia. INHT produces a periodic free radical signal, which increases cell resistance to various damaging factors, including ROS-mediated processes [7,8]. During adaptation, periodic generation of ROS improves organism's resistance to stress [4], physical loads [6,12], hypobaric and normobaric hypoxia [8], and dietary prooxidants [5,9]. Since the above influences trigger tissue-specific responses, it is important to determine the organs, which are most sensitive to the action of these adaptive signals, and to develop the methods preventing possible side effects.

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We propose a new regimen of adaptation to changes in oxygen level based on alternation of hypoxia (HP) and hyperoxia (HO) periods instead of hypoxia-normoxia (HP-NO) protocol, which is characterized by up-regulation of free radical signal compared to classical INHT [3]. This adaptive training resulted in more rapid protective effects in patients with coronary heart disease (CHD) and induced no changes in lipid spectrum or energy metabolism [1].

Here we studied the effect of HP+NO and HP+HO training protocols on defense systems and resistance of membrane structures in the heart, liver, and brain.

## MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 250 g. The animals were subdivided into three groups (10 rats per group). Group 1 rats were controls. Group 2 and 3 rats were subjected to daily 60-min INHT sessions for 15 days: alternation of 5-min inhalation of gas mixture with 10% oxygen and 3-min intervals of NO (group 2) or HO (30% oxygen). Gas

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mixtures with low and high oxygen content were prepared in a setup based on gas membrane separation technique (kindly provided by Hypoxia Medical Academy).

The animals were decapitated on the next day after termination of HP+HO or HP+NO courses. The heart, liver, and cerebral cortex were isolated, washed in physiological saline, and placed into liquid nitrogen. Activities of catalase, SOD, and glutathione reductase were assayed by routine spectrophotometric methods. Specifically, SOD was measured by the rate of superoxide radical generation in the xanthine—xanthine oxidase system before and after addition of the homogenate, glutathione reductase activity was evaluated by NADPH consumption in the presence of reduced glutathione.

The level of immediate early response proteins (HSP70 and heme oxygenase-1, HOx-1) were assessed in cytosol fraction by Western blot immunoassay with monoclonal antibodies against HSP70 and HOx-1 (Stressgen) and second antibody labeled with peroxidase (Jackson Immuno Research). Detection was carried out by chemiluminescence using ELC reagents (Amersham) on Kodak X-ray films.

The rate of accumulation of free radical oxidation products in the heart, liver, and brain during *in vitro* induction of oxidation in the system containing ascorbate (0.75 mM) was evaluated on a Hitachi-557 spectrophotometer by maximum of absorption spectrum of TBA-reactive products [11] with modification [10].

The data were analyzed statistically using Statistica 6.0 software according to biomedical statistical recommendations [2] and presented as medians. Independent groups were compared using Mann—Whitney $U$ nonparametric test.

## RESULTS

The INHT method is a mild adaptive intervention mediated by periodic low-intensity induction of ROS. During long-term INHT course (23 days) we observed an improvement in the resistance of membrane structures of the heart, liver, and cerebral cortex to free radical oxidation or documented absence of its decrease in comparison with the control [13]. For evaluation or comparison of tissue specificity of routine INHT (HP+ NO) and new adaptive training (HP+HO), we used more short training (15 days) corresponding to the end of the rapid adaptation period.

The HP+NO course increased SOD activity in liver, brain, and heart by 19, 21, and 20%, respectively (Table 1). Changes in catalase activity were less pronounced, but also significant: in the liver it decreased by 15%, while in the heart it increased by 30%. Activity of glutathione reductase in the liver and brain decreased by 28 and 34%, respectively. Hence, HP+NO training activated antioxidant defense enzymes in the heart, while in the brain this activation was poor or absent; in the liver this training inhibited some enzyme systems. These findings suggest that short-term HP+NO produces a more potent protective effect on the heart, and less pronounced effect on the liver and brain.

These assumptions were corroborated in the study of the resistance of intracellular membrane structures

**TABLE 1**. Effect of HP+HO Adaptation on Activity of Antioxidant Protective Enzymes (mg protein) and Level of Immediate Early Response Proteins in Rat Heart

| Enzyme | | Control | HP+NO | HP+HO |
|---|---|---|---|---|
| Liver | SOD, rel. units/mg protein | 7.8 | 9.3** | 7.7 |
| | catalase, μmol $H_2O_2$/min | 75.7 | 64.0** | 70.9 |
| | glutathione reductase, μmol NADPH/min | 3.6 | 2.6* | 3.7 |
| | HOx-1, RDU | 2.49 | 3.1 | 2.85 |
| | HSP70, RDU | 5.7 | 8.6 | 7.4 |
| Brain | SOD, rel. units/mg protein | 4.0 | 4.82** | 3.91 |
| | catalase, μmol $H_2O_2$/min | 0.28 | 0.29 | 0.24 |
| | glutathione reductase, μmol NADPH/min | 0.95 | 0.63** | 0.62** |
| | HOx-1, RDU | 5.8 | 7.4 | 6.9 |
| Heart | SOD, rel. units/mg protein | 2.53 | 3.04* | 2.72 |
| | catalase, μmol $H_2O_2$/min | 1.74 | 2.27** | 1.93 |
| | glutathione reductase, μmol NADPH/min | 2.49 | 2.15 | 2.36 |
| | HOx-1, RDU | 5.9 | 6.1 | 5.7 |
| | HSP70, RDU | 8.8 | 15.9 | 12.8 |

**Note.** RDU, relative densitometric units. *$p \le 0.01$, **$p \le 0.05$ compared to the control.

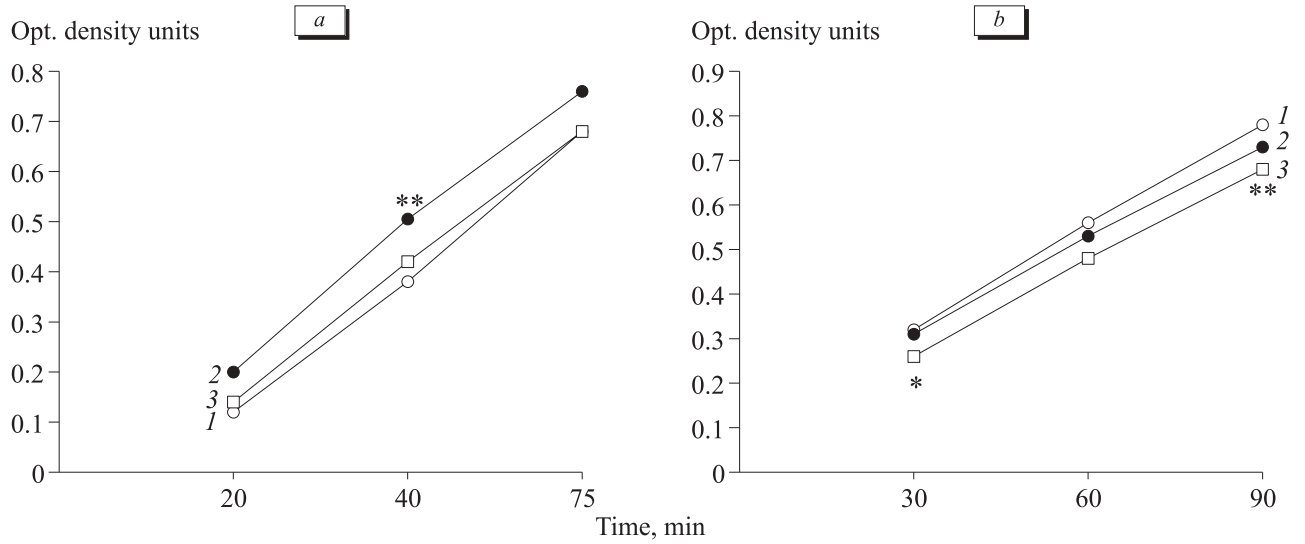


**Fig. 1**. Effect of HP+NO and HP+HO on accumulation of TBA-reactive products of free radical oxidation in the liver (*a*) and brain (*b*) during *in vitro* induction of oxidation. *1*) Control; *2*) HP+NO; *3*) HP+HO. Here and in Fig. 2: **p*<0.01, ** *p*<0.05 compared to the control.

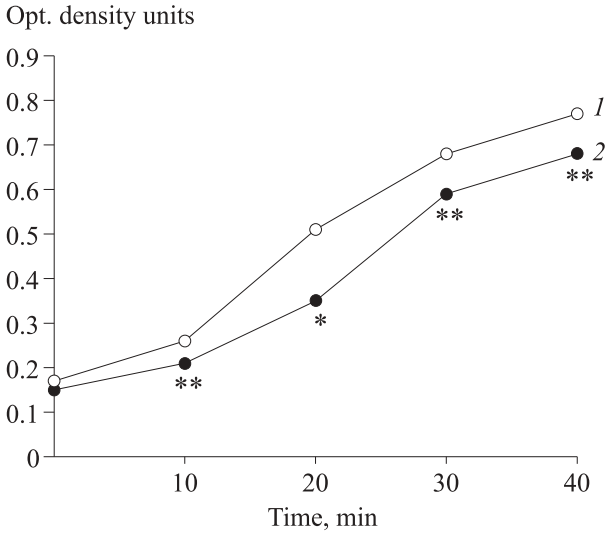


**Fig. 2**. Effect of HP+HO on the level of free radical oxidation in the heart. *1*) control; *2*) HP+HO.

in these organs. In the liver, HP+NO increased sensitivity to induction of ROS-mediated processes (Fig. 1, *a*). After 40-min induction ROS generation increased by 33% in comparison with the control ($p \leq 0.05$). In contrast, HP+NO training did not stimulate free radical oxidation.

At the next stage of the study, we used adaptive training consisting in HP and HO periods (HP+HO).

In contrast to previous training, which elevated sensitivity of the liver tissue to induction of free radical oxidation by 30-40%, HP+HO course did not decrease the resistance of membrane structures in the liver to ROS (Fig. 1, *a*). Moderation of free radical oxidation in the HP+HO group occurred against the background of preserved activity of antioxidant defense enzymes, but in the HP+NO group activity of these enzymes greatly varied. The level of immediate early response proteins after 15-day training indicated the absence of both stressor component to HP+HO adaptation and excessive ROS signal (judging from the absence of pronounced synthesis of HSP70 and HOx-1, Table 1). These data indicate protective effect of HP+HO on the liver characterized by high sensitivity to ROS.

In the brain, changes of antioxidant defense enzymes were similar in HP+NO and HP+HO groups. Both training courses decreased glutathione reductase activity by 34% compared to the control. Changes in HOx-1 were also similar in both groups (Table 1). However, when oxidation was induced *in vitro* accumulation of free radical oxidation products differed in these groups (Fig. 1, *b*)., The resistance of brain tissue to ROS in the HP+NO group did not differ from the control, but increased in the HP+HO group. The level of ROS-active products decreased after 30 and 90 min induction by 19 ($p \leq 0.01$) and 13% ($p \leq 0.05$), respectively, in comparison with the control. Thus, HP+HO adaptation more effectively improved the resistance of the brain tissue to induction of free radical oxidation.

In the heart, 15-day HP+HO adaptation did not increase activities of SOD, catalase, and glutathione reductase and did not provoke expression of HSP70 or HOx-1 (Table 1). The latter proteins are markers of injury and protect the cells against ROS. These findings suggest that after 15-day HP+HO training, the level of free radical signal decreased, *i.e.* long-term adaptation was achieved despite shortened period of training. The resistance of membrane structures in the heart to induction of free radical oxidation increased after HP+HO training (Fig. 2). After *in vitro* induction accu-

mulation of free radical oxidation products after HP+HO decreased by 20-30% compared to the control ($p \leq 0.01$).

Thus, short-term adaptation to HP+HO improves the resistance of membrane structures in the heart to induction of free radical oxidation and to the action of other damaging factors. Moreover, this protective effect was induced without excessive activation of cellular protective systems. Introduction of the HO component ensures more rapid attaining of the positive effect of routine INHT and prevents side effects, which is of vital importance when this type of training is used in the complex therapy and prophylaxis.

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