

# Blood Oxygen Transport and Prooxidant-Antioxidant Balance in Rats under Hypothermia and Rewarming Combined with a Modification of L-arginine-NO Pathway

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## ABSTRACT---

**Introduction:** The study of processes and functional regulation in body under the changed (including the lower) body temperature is the important medical problem. Increase of body resistance to low environmental temperature is especially important for the patient reanimation after the cold exposure, and the assessment of mechanisms creating the tissue oxygen flux may help the development of ways for reanimation of a cooled body.

**Aim:** Study effect of N-nitro-L-arginine methyl ester, L-arginine, or sodium nitroprusside on the blood oxygen transport and the prooxidant-antioxidant balance during hypothermia and rewarming in rats.

**Methods:** Cold exposure was performed in male rats (body weight 200-250 g, n = 54) for 120 min under the box water temperature of 19°C; rewarming took the next 120 min, with a mean rate of 0.06°C/min. N-nitro-L-arginine methyl ester, L-arginine, or sodium nitroprusside were administered intravenously during the second 60 min of hypothermia in 1 mL of saline. Hemoglobin-oxygen affinity was evaluated by p50 (blood pO<sub>2</sub> at its 50% O<sub>2</sub> saturation) determined by the "mixing" method at 37°C, pH 7.4 and pCO<sub>2</sub> 40 mm Hg and at actual pH, pCO<sub>2</sub> and temperature. We were analyzed the antioxidant protection (catalase activity, α-tocopherol) and lipid peroxidation (conjugated diene, Schiff bases).

**Results:** Infusion of L-arginine promoted the higher cold resistance and oxyhemoglobin dissociation curve shift rightwards, thereby reducing the hypoxic signs. It enhanced the antioxidant defense and reduced the levels of lipid peroxidation products, thereby providing the least prooxidant-antioxidant disbalance during the rewarming. However, other modifiers of L-arginine-NO pathway (N-nitro-L-arginine methyl ester, sodium nitroprusside) had not such effect.

**Conclusion:** The L-arginine effect mediated by hemoglobin-oxygen affinity change may be used for the correction of metabolic disorders and improvement of body resistance to low environmental temperature.

**Keywords**— hypothermia, rewarming, hemoglobin-oxygen affinity, nitric oxide

## 1. INTRODUCTION

The estimation of mechanisms influencing the oxygen flux to tissues is important for the investigations of hypothermia and ways to correct it. Many factors are known to contribute into the hypoxia induced by hypothermia: decrease of pulmonary ventilation, fall of minute blood volume, dramatic shift of the oxyhemoglobin dissociation curve (ODC) leftwards etc. By other hand, hypothermia results in the considerable decrease of tissue energetic needs and therefore in lower oxygen (O<sub>2</sub>) consumption<sup>1, 2, 18</sup>. The pathogenesis of rewarming is less studied, and many aspects of its development are unknown.

Reactive free radicals are constantly generated in body. The antioxidant system reduce their concentrations and effects thereby forming the prooxidant-antioxidant balance<sup>8</sup>. Low body temperature is associated with enhanced generation of reactive oxygen metabolites. This prooxidant shift results from the change in cellular metabolism, with a higher oxygen fraction utilized by oxygenases vs. oxidases (3). Cold stress disrupt the balance in an prooxidant-antioxidant system and cause oxidative damage to several tissues by altering the enzymatic and non-enzymatic antioxidant status, protein oxidation and lipid peroxidation (LPO)<sup>10, 21</sup>. The blood oxygen-binding properties determine the conditions of oxygen diffusion to tissues and the values of tissue oxygen pressure<sup>22</sup> and take a specific part in the complex system of antioxidant defense<sup>29</sup>. The preliminary shift of ODC leftwards during the hypothermia is accompanied by a shift of

prooxidant-antioxidant balance towards activation of the LPO, and the ODC shift rightwards is accompanied by the LPO lowering.

Recently, the nitric oxide (NO) and hemoglobin (Hb) interaction has been actively studied. Hb has evolved to shuttle a triad of gases that are key to life: NO, CO<sub>2</sub>, and O<sub>2</sub><sup>5</sup>. Three main NO derivatives of Hb have been known: nitrosylhemoglobin, nitrosohemoglobin (SNO-Hb) and methemoglobin. In the nitrosylhemoglobin molecule, NO is related to heme Fe<sup>2+</sup> sites, whereas SNO-Hb is the result of the interaction of NO with cysteine (93) on the β-globine chain<sup>12, 26</sup>. The presence of different Hb compounds with NO can affect the hemoglobin-oxygen affinity (HOA) in the whole blood in different ways. Methemoglobin and SNO-Hb increase it, whereas nitrosylhemoglobin decreases. During the hypothermia, a modification of the L-arginine-NO pathway, an enhanced cold resistance, attenuated oxygen deficiency and a weaker ODC shift leftwards were observed only after the administration of L-arginine (the actual P50 decreased by approximately 15 mm Hg and was significantly higher than in control)<sup>28</sup>.

The aim of this study was to investigate the blood oxygen transport and the prooxidant-antioxidant balance in rats during a modification of L-arginine-NO pathway combined with hypothermia and the following rewarming.

## 2. MATERIALS AND METHODS

Male rats (body weight 200-250 g, n = 54) were anesthetized by sodium thiopental (50 mg/kg intraperitoneally). During the hypothermia and rewarming the rats were placed into the special boxes without a direct contact with water. Fall and rise of rat body temperature were achieved by the 120-min hypothermia and 120-min rewarming, respectively, with a mean rate of 0.06°C/min.

The following L-arginine-NO pathway modifiers were used: N-nitro-L-arginine methyl ester (L-NAME), L-arginine, or sodium nitroprusside (SNP). These substances were administered intravenously during the second 60 min of hypothermia in 1 mL of saline. Rats were subdivided into 6 groups: 1 – control (n=8); 2 – hypothermia (n=8); 3 – hypothermia/rewarming (n=11); 4 - administration of SNP (50 µg/kg) and hypothermia/rewarming (n=9); 5 - administration of L-NAME (40 mg/kg) and hypothermia/rewarming (n=10); 6 - administration of L-arginine (300 mg/kg) and hypothermia/rewarming (n=8). Control, hypothermia, and hypothermia/rewarming groups received 1 mL of saline. Body temperature was measured every 10 min with electric thermometer. Blood samples were taken from right atrium, and then the tissue sampling was performed (lungs, liver, kidneys, heart). All surgical interventions were performed under the adequate analgesia. Experiments using the laboratory animals were approved by of University Ethical Committee on Animal Experiments. During the experimental procedure we followed University guide for the care and use of laboratory animals.

PO<sub>2</sub>, O<sub>2</sub> blood saturation (SO<sub>2</sub>) and acid-base balance (pH, venous carbon dioxide pressure (PCO<sub>2</sub>), concentration of bicarbonate (HCO<sub>3</sub><sup>-</sup>), the concentration of total carbon dioxide (TCO<sub>2</sub>), the actual excess of buffer bases (ABE), the standard excess of buffer bases (SBE) and the standard bicarbonate (SBC)) were measured with micro gas analyzer «Synthesis-15» (Instrumentation Laboratory) at 37°C with the following correction to actual temperature value. HOA was assessed as P50 (blood PO<sub>2</sub> under its 50% saturation by O<sub>2</sub>) as determined by a 'mixing method'<sup>23</sup> at 37°C, pH 7.4 and PCO<sub>2</sub> = 40 mm Hg (P50stand). The values of P50 at actual pH, PCO<sub>2</sub> and temperature (P50act) were calculated from P50stand by Severinghaus' equations (24) using the temperature coefficient  $\Delta \lg P50 / \Delta T = 0.024$ ; the ODCs were calculated according to Hill's equation with n = 2.8 from the measured P50. The amounts of Hb were determined spectrophotometrically. Nitrate/nitrite was determined using the Griess reagent<sup>15</sup>.

Conjugated diene (CD) content was determined by UV absorption at 232-234 nm (indicating the conjugated double bonds of lipid hydroperoxides)<sup>20</sup>. The results were expressed in relative units of optical density in 1 ml ( $\Delta A_{233}/ml$ ). The level of Schiff bases (SB) was determined by fluorescence intensity of chloroform extracts at excitation and emission wavelengths of 344 and 440 nm, respectively, with an F-4010 spectrofluorimeter (Hitachi). The results were expressed in relative units of intensity in 1 ml of plasma or red blood cells (RBCs)<sup>4</sup>.

Antioxidant system: catalase activity was determined by measurement of the rate of decomposition of hydrogen peroxide capable to generate a stable coloured complex with molybdenum salts which was measured with an SF-46 spectrophotometer at 410 nm<sup>4</sup>,  $\alpha$ -tocopherol ( $\alpha$ -T) concentration was evaluated by fluorescence intensity of heptane extraction at excitation and emission wavelengths of 292 and 325 nm with an F-4010 spectrofluorimeter (Hitachi) using  $\alpha$ -tocopherol (Sigma Chemical Co., St. Louis) as reference<sup>20</sup>. Protein content was measured according to Layne<sup>14</sup>.

The results obtained were analyzed statistically using Statistica 8 suite. Data are expressed as mean (M)±standard error mean (SEM). Normal distribution of data was assessed by the Kolmogorov-Smirnov test, Shapiro-Wilk's W test. Since the data were not normally distributed, Mann-Whitney U tests for unrelated results were used to compare differences between the groups. A p value of <0.05 was accepted as statistically significant.

### 3. RESULTS

In rats received 0.9% NaCl, the rectal temperature at the end of cooling was  $28.5 \pm 0.2^\circ\text{C}$  (Figure 1). The least decrease of rectal temperature was noted after injection of L-arginine ( $29.1 \pm 0.1^\circ\text{C}$ ,  $P < 0.05$ ). SNP injection resulted in the lowest rectal temperature ( $27.1 \pm 0.2^\circ\text{C}$ ,  $P < 0.01$ ). In others groups of rats the rectal temperatures were not significantly different from control. At the end of rewarming the control rectal temperature was  $35.4 \pm 0.1^\circ\text{C}$  (Figure 1). The highest rectal temperatures at that time were observed in animals received SNP ( $35.8 \pm 0.1^\circ\text{C}$ ,  $P < 0.05$ ) or L-arginine ( $36.2 \pm 0.05^\circ\text{C}$ ,  $P < 0.001$ ).

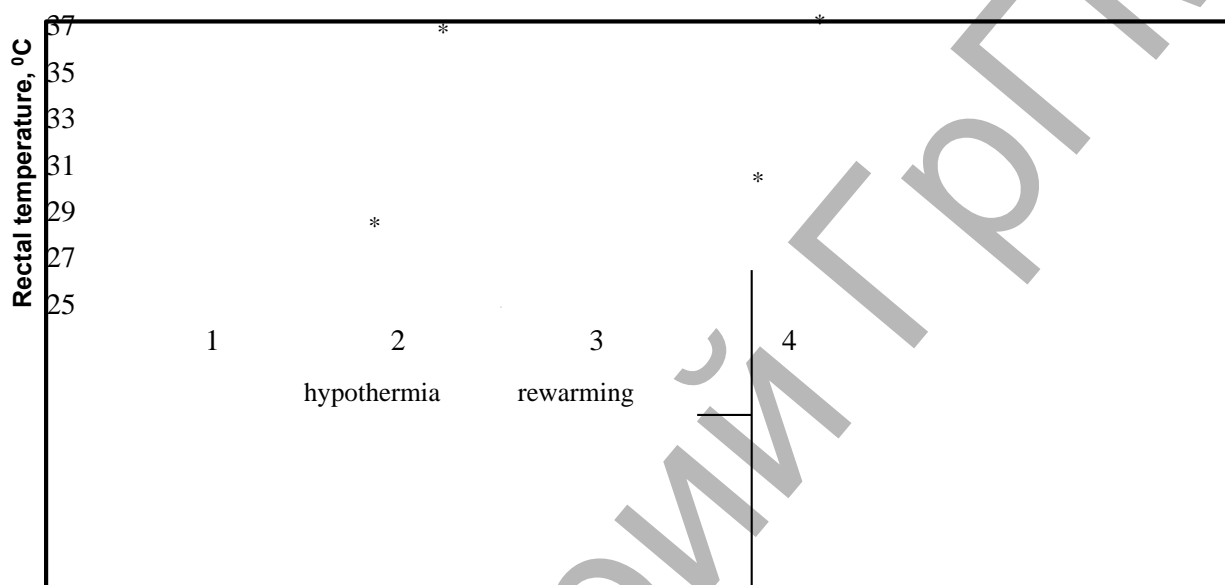


Figure 1: Rectal temperature in rat after the hypothermia and rewarming combined with a modification of L-arginine-NO pathway:

1 – hypothermia/rewarming; 2 - SNP + hypothermia/rewarming; 3 - L-NAME + hypothermia/rewarming; 4 – L-arginine + hypothermia/rewarming. \* - significant difference from hypothermia/rewarming group.

Table 1 presents the data for the changes of blood oxygen transport indices. In hypothermic rats the significant lowering of  $\text{PO}_2$  occurred (12.9%,  $P < 0.001$ ), and  $\text{SO}_2$  increased by 12.3% ( $P < 0.01$ ). Administration of L-arginine resulted into significant increase of  $\text{PO}_2$  and decrease of  $\text{SO}_2$  comparing with group 2 (by 14.4%,  $P < 0.01$  and 10.9%,  $P < 0.001$ , respectively). Injection of SNP or L-NAME did not cause any detectable  $\text{PO}_2$  changes, but significantly decreased oxygen saturation of hemoglobin comparing with group 2 (by 18.8%,  $P < 0.001$ , and 20.4%,  $P < 0.001$ , respectively). Administration of SNP decreased  $\text{PO}_2$  by 7.4%,  $P < 0.01$ , and  $\text{SO}_2$  by 6.2%,  $P < 0.05$ , and also injection of L-NAME decreased  $\text{PO}_2$  by 8.5%,  $P < 0.01$  comparing with group of hypothermia and rewarming. Rats of group 2 had the significantly lower pH ( $7.238 \pm 0.01$  vs.  $7.351 \pm 0.01$  in control,  $P < 0.001$ ).

In rats subjected to cold only,  $\text{P50}_{\text{stand}}$  rose by 7.1% ( $P < 0.01$ ), and  $\text{P50}_{\text{act}}$  fell by 15.4% ( $P < 0.001$ ) from baseline. Cooling with the following rewarming increased  $\text{P50}_{\text{stand}}$  by 4.4% ( $P < 0.05$ ) and  $\text{P50}_{\text{act}}$  by 22.4% ( $P < 0.001$ ) vs. group of hypothermia. L-NAME during the hypothermia and the following rewarming decreased  $\text{P50}_{\text{act}}$  by 7.9% ( $P < 0.01$ ), thereby shifting ODC leftwards (Figure 2), and  $\text{P50}_{\text{stand}}$  - by 10.6% ( $P < 0.001$ ) vs. group 3. Injection of L-arginine during the hypothermia with the following rewarming resulted in the higher  $\text{P50}_{\text{act}}$  (by 8.1%,  $P < 0.01$ ) and ODC shift rightwards (Figure 2), without the changes in  $\text{P50}_{\text{stand}}$  comparing with group 3. SNP did not affect the P50 comparing with group 3.

Table 1: Blood oxygen transport indices in rats under hypothermia and after rewarming combined with a modification of L-arginine-NO pathway ( $M \pm \text{SEM}$ )

Parameter	Control	Hypothermia	Hypothermia /rewarming	SNP + hypothermia /rewarming	L-NAME + hypothermia /rewarming	L-arginine + hypothermia /rewarming
N	8	8	11	9	10	8
$\text{P50}_{\text{stand}}$ , mm Hg	$30.93 \pm 0.42$	$33.13 \pm 0.47^*$	$31.66 \pm 0.54^\#$	$31.38 \pm 0.47^\#$	$28.31 \pm 0.46^* \# \psi$	$33.13 \pm 0.58^*$
$\text{P50}_{\text{act}}$ , mm Hg	$29.20 \pm 0.49$	$24.70 \pm 0.37^*$	$30.23 \pm 0.40^\#$	$30.84 \pm 0.47^* \#$	$27.84 \pm 0.40^\# \psi$	$32.69 \pm 0.29^* \# \psi$

Hb, g/L	106.3±2.66	113.4±6.19	106±3.39	107.9±2.67	118.2±3.83 *	119.9±3.83 * ψ
PO <sub>2</sub> , mm Hg	28.00±0.42	24.38±0.50 *	27.00±0.47 #	25.00±0.29 * ψ	24.70±0.40 * ψ	27.88±0.52 #
PO <sub>2</sub> act, mm Hg	24.76±0.85	13.19±0.19*	24.03±0.50 #	22.8±0.35 * #	21.71±0.28 * # ψ	26.23±0.50 # ψ
PCO <sub>2</sub> , mm Hg	54.31±0.68	56.51±0.86	50.45±0.87 * #	51.22±0.54 * #	51.73±0.91* #	51.73±1.10 #
PCO <sub>2</sub> act, mm Hg	50.09±0.97	38.14±0.83 *	47.02±0.60 * #	48.26±0.66 #	47.62±0.92 #	49.74±1.02 # ψ
SO <sub>2</sub> , %	28.80±0.45	32.34±0.81 *	27.99±0.38 #	26.27±0.47 *# ψ	25.76±0.94* #	28.83±0.19 #
metHb, %	0.11±0.06	0.14±0.07	0.06±0.03	0.11±0.07	0.05±0.02	0.11±0.05
pH, units	7.351±0.01	7.238±0.01 *	7.347±0.002 #	7.348±0.01 #	7.324±0.01 #	7.358±0.01 #
pHact, units	7.378±0.01	7.361±0.01	7.37±0.003	7.367±0.01	7.351±0.01	7.37±0.01
HCO <sub>3</sub> <sup>-</sup> , mM	31.35±0.89	28.98±0.66	30.18±0.92	28.64±0.56 *	28.18±0.86 *	31.20±0.99
TCO <sub>2</sub> , mM	32.94±0.94	31.40±0.72	31.72±0.98	30.27±0.62 *	29.88±0.95 *	32.30±0.94
ABE, mM	4.78±0.63	0.07±1.13 *	4.79±0.68 #	2.92±0.38 *	1.70±0.53 * ψ	5.61±0.43 #
SBE, mM	5.18±0.76	0.23±1.35 *	4.85±0.85 #	2.80±0.45 *	1.74±0.70* ψ	5.55±0.97 #
SBC, mM	27.25±0.49	24.10±0.71 *	26.95±0.65 #	25.84±0.30 * #	24.80±0.40 * ψ	27.48±0.54 #

Abbreviations used: P50stand, blood PO<sub>2</sub> under its 50% saturation by O<sub>2</sub> as determined at 37°C, pH 7.4 and PCO<sub>2</sub> = 40 mm Hg; P50act, P50 at actual pH, PCO<sub>2</sub> and temperature; Hb, hemoglobin; PO<sub>2</sub>, venous oxygen pressure; PCO<sub>2</sub>, venous carbon dioxide pressure; SO<sub>2</sub>, O<sub>2</sub> blood saturation; metHb, methemoglobin; HCO<sub>3</sub><sup>-</sup>, plasma concentration of hydrocarbonates; TCO<sub>2</sub>, concentration of total carbon dioxide; ABE, the actual excess of buffer bases; SBE standard excess of buffer bases; SBC, standard hydrocarbonate.

\* - Significant difference from control group;

# - Significant difference from hypothermic group;

ψ - Significant difference from hypothermia/rewarming group.

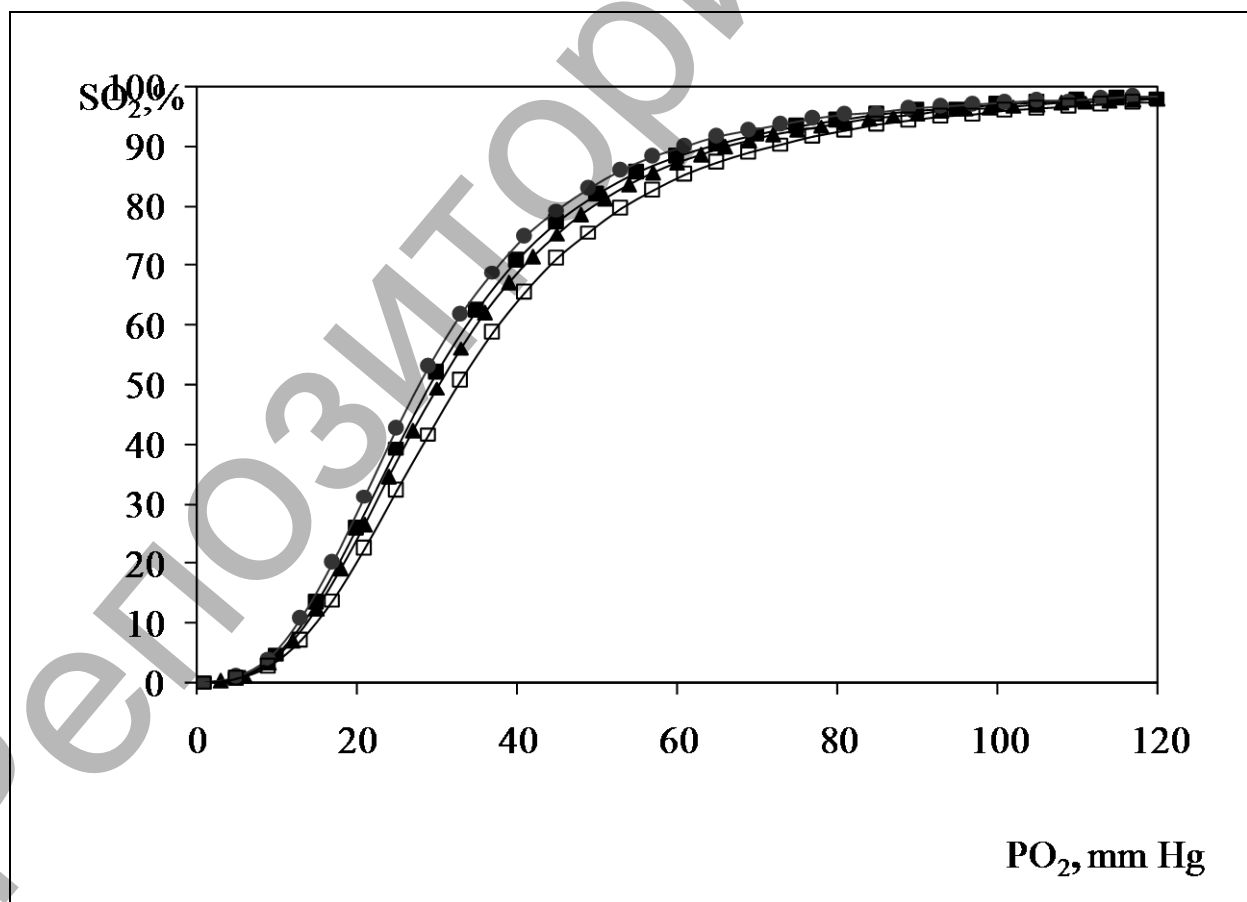


Figure 2: Actual oxyhemoglobin dissociation curves in rats of the following groups:

Control (■), hypothermia/rewarming (▲), L-arginine + hypothermia/rewarming (□), L-NAME + hypothermia/rewarming (●).

Hypothermia increased the nitrate/nitrite content by  $34.7 \pm 0.43\%$  ( $P < 0.001$ ) vs. control (Fig. 3). The rewarming only partially restored it ( $9.8 \pm 0.37\%$ ,  $P < 0.05$  vs. control). SNP and L-arginine rose the nitrate/nitrite level by  $31.1 \pm 0.54\%$  ( $P < 0.001$ ) and  $10.9 \pm 0.35\%$  ( $P < 0.05$ ), respectively, comparing with a group 3, whereas the injection of L-NAME decreased it by  $21.7 \pm 0.22\%$  ( $P < 0.001$ ) (Fig. 3).

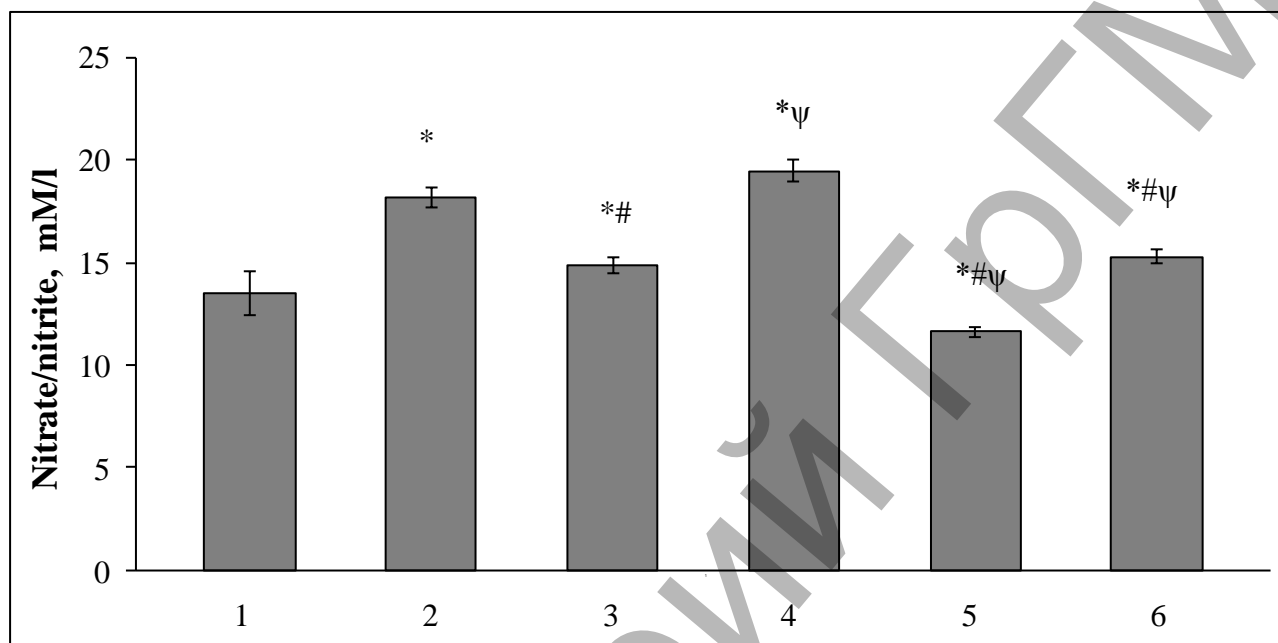


Figure 3: The content of nitrate/nitrite in plasma of rats after the hypothermia and rewarming combined with a modification of L-arginine-NO pathway:

1 - control; 2 - hypothermia; 3 - hypothermia/rewarming; 4 - SNP + hypothermia/rewarming; 5 - L-NAME + hypothermia/rewarming; 6 - L-arginine + hypothermia/rewarming. \* - significant difference from control group; # - significant difference from hypothermic group; ψ - significant difference from hypothermia/rewarming group.

Table 2 shows the LPO parameters. Hypothermia increased CD content comparing with control in all the tissues studied: in lungs by 46% ( $P < 0.001$ ), in liver by 34% ( $P < 0.01$ ), in kidneys by 33% ( $P < 0.001$ ), and in myocardium by 41% ( $P < 0.001$ ). CD content in group 3 also was higher than in control: by 42% in lungs ( $P < 0.001$ ), by 26% in liver ( $P < 0.01$ ), by 27% in kidneys ( $P < 0.01$ ), and by 32% in myocardium ( $P < 0.001$ ). In group 2 SB content rose comparing with control: by 18% in lungs ( $P < 0.001$ ), by 25% in liver ( $P < 0.001$ ), by 29% in kidneys ( $P < 0.001$ ), and by 24% in myocardium ( $P < 0.001$ ). Group of hypothermia and rewarming displayed the similar SB increase vs. control: by 16% in lungs ( $P < 0.001$ ), by 29% in liver ( $P < 0.001$ ), by 21% in kidneys ( $P < 0.001$ ), and by 22% in myocardium ( $P < 0.001$ ). L-arginine administration during the hypothermia resulted in the lower CD content during the rewarming in all the tissues studied: in lungs by 20% ( $P < 0.01$ ), in liver by 14% ( $P < 0.01$ ), in kidneys by 10% ( $P < 0.05$ ), and in myocardium by 14% ( $P < 0.01$ ); SB content also fell comparing with group 3: by 8% in lungs ( $P < 0.01$ ), by 19% in liver ( $P < 0.001$ ), by 10% in kidneys ( $P < 0.01$ ), and by 17% in myocardium ( $P < 0.001$ ). SNP or L-NAME did not change the LPO activity after the hypothermia and rewarming.

Table 2: Lipid peroxidation indices in rat blood and tissues under hypothermia and rewarming combined with modified L-arginine-NO pathway ( $M \pm SEM$ )

Parameter	Control	Hypothermia	Hypothermia /rewarming	SNP hypothermia + /rewarming	L-NAME hypothermia + /rewarming	L-arginine hypothermia + /rewarming	
CD, Δ	Lungs	7,35±0,24	10,73±0,34 *	10,41±0,39 *	10,36±0,91 *	10,96±0,31 *	8,34±0,42 # ψ
	Liver	9,23±0,48	12,34±0,65 *	11,59±0,32 *	12,94±0,42 *ψ	14,97±0,47 *#ψ	10,01±0,34 #ψ

A <sub>233</sub> /g	Kidneys	7,85±0,36	10,41±0,27 *	9,98±0,32 *	12,08±0,38 *#Ψ	10,09±0,43 *	8,96±0,25 #Ψ
	Heart	7,86±0,38	11,09±0,37 *	10,35±0,27 *	10,99±0,37 *	10,96±0,46 *	8,88±0,33 #Ψ
SB, units/g	Lungs	213,8±2,57	252,7±2,73 *	248,5±3,81 *	244,4±2,62 *	252,1±3,03 *	228,1±1,76 *#Ψ
	Liver	199,7±3,08	249,0±2,61 *	257,4±2,87 *	264,9±3,11 **	262,7±3,19 **	208,1±2,17 #Ψ
	Kidneys	114,1±2,69	146,9±2,06 *	137,8±2,57 **	149,3±2,54 *Ψ	135,6±2,80 **	124,3±2,54 *#Ψ
	Heart	208,6±2,42	259,3±2,10 *	248,3±2,54 **	245,9±2,87 **	245,1±3,61 **	205,8±2,87 #Ψ

Abbreviation used: CD - conjugated diene content; SB - Schiff base content.

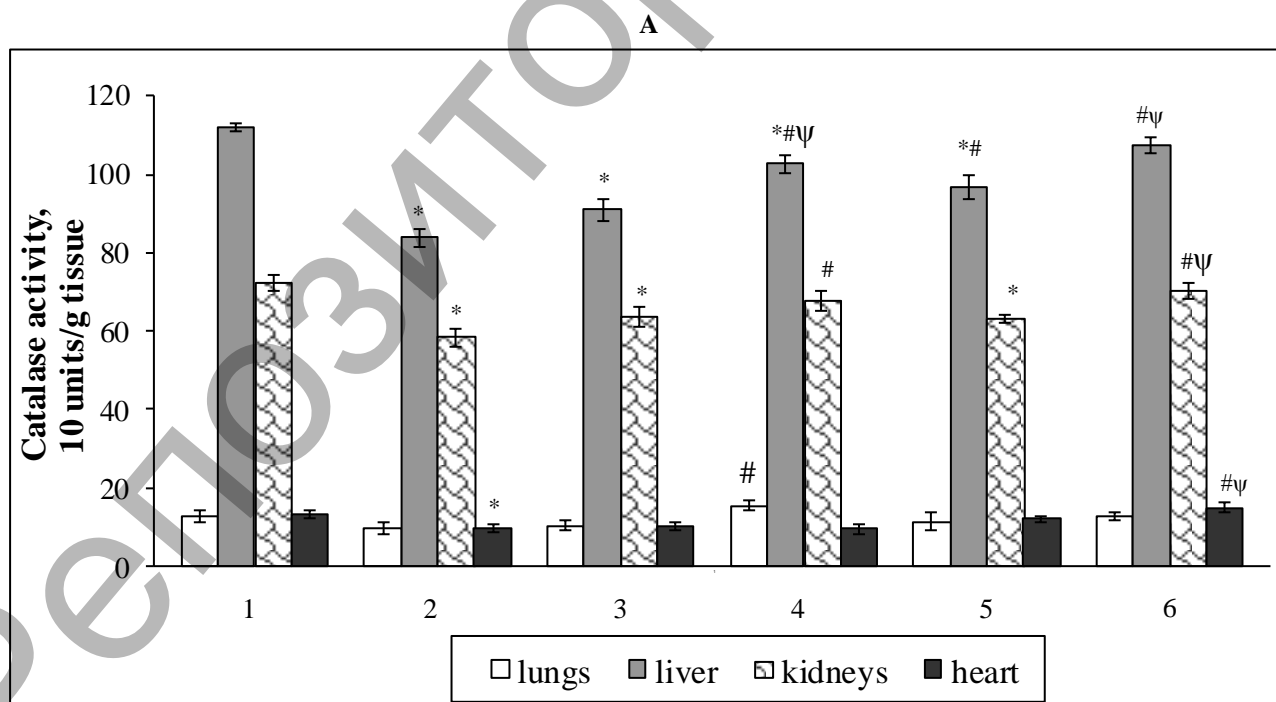
\* - Significant difference from control group;

# - Significant difference from hypothermic group;

Ψ - Significant difference from hypothermia/rewarming group.

Hypothermia resulted in lower catalase activity (Figure 4A): by 25% in liver (P<0.001), by 19% in kidneys (P<0.01), and by 27% in myocardium (P<0.05). Rewarming also decreased the catalase activity vs. control: by 19% in liver (P<0.001), and by 12% in kidneys (P<0.05). SNP injection increased its activity comparing with group 3: by 49% in lungs (P<0.05), and by 13% in liver (P<0.05). L-arginine rose it in liver by 18% (P<0.001); by 11% in kidneys (P<0.05), and in myocardium by 47% (P<0.05). However L-NAME did not affect this enzymatic activity comparing with a group 3.

During the hypothermia α-T contents were lower than control in all the tissues studied (Figure 4B): in lungs by 26% (P<0.001), in liver by 17% (P<0.001), in kidneys by 13% (P<0.001), and in myocardium by 16% (P<0.001). Similar α-T decrease was observed in a group of hypothermia and rewarming: by 17% in lungs (P<0.001), by 16% in liver (P<0.001), by 11% in kidneys (P<0.001), and by 17% in myocardium (P<0.001). L-arginine increased α-T content during the rewarming comparing with group 3: by 13% in lungs (P<0.001), by 32% in liver (P<0.001), by 24% in kidneys (P<0.001), and by 16% in myocardium (P<0.001). SNP or L-NAME enhanced this content relatively to group 3 in liver, kidneys and myocardium.



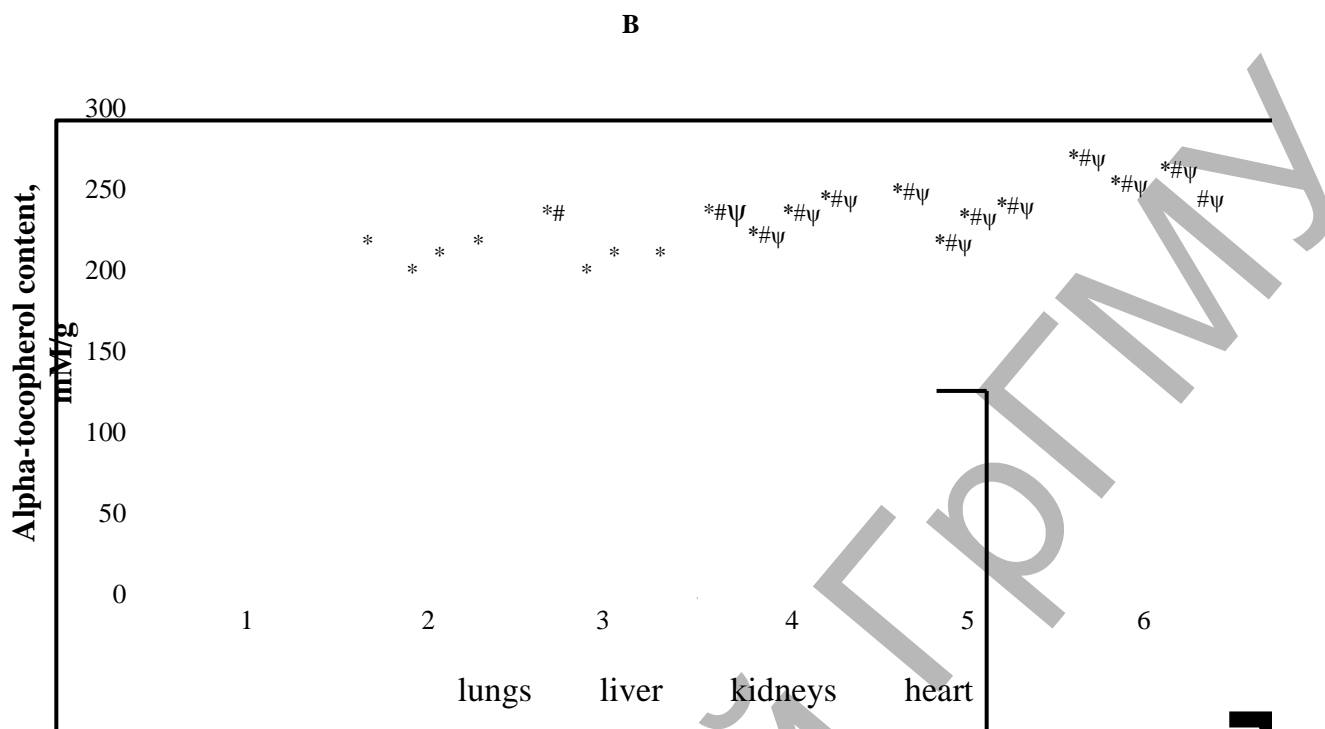


Figure 4: Changes in antioxidant defense indices (A - catalase activity, and B -  $\alpha$ -tocopherol content) in rat tissues during hypothermia and rewarming combined with the modified L-arginine-NO pathway:

1 - control; 2 - hypothermia; 3 - hypothermia/rewarming; 4 - SNP + hypothermia/rewarming; 5 - L-NAME + hypothermia/rewarming; 6 - L-arginine + hypothermia/rewarming. \* - significant difference from control group; # - significant difference from hypothermic group;  $\psi$  - significant difference from hypothermia/rewarming group.

#### 4. DISCUSSION

L-arginine resulted in the slower rectal temperature fall in rats during the cold exposure and its faster return to baseline during the rewarming. However, other modifiers of L-arginine-NO pathway (SNP, L-NAME) had not such effect, revealing the complex nature of NO system contribution into the temperature homeostasis. NO may affect the temperature homeostasis by directly, modulating the thermoregulatory mechanisms<sup>25</sup>, or indirectly, through its influence on the blood oxygen transport and prooxidant-antioxidant balance<sup>28</sup>. The pattern of changes in PO<sub>2</sub> and acid-base parameters during the hypothermia reflects the development of metabolic acidosis and hypoxia, most weak in animals received L-arginine only. Lower temperature shifts ODC leftwards resulting in the reduction of oxygen flux to tissues<sup>29</sup>.

The higher CD and SB and lower  $\alpha$ -T levels in tissue homogenates indicated the prooxidant-antioxidant disbalance both after the cooling and after the rewarming. Acute cold exposure of homoiothermic body resulted in LPO activation in various organs and tissues<sup>3</sup>. Rewarming did not restore the prooxidant-antioxidant balance revealing an incompensability of its changes, at least, in a period of 2 hrs. L-arginine reduced the prooxidant-antioxidant disbalance. Our previous experiments indicated the various changes of the blood oxygen transport indices in rats receiving the various L-arginine-NO pathway modifiers. For example, L-arginine resulted in enhanced cold resistance, milder oxygen deficiency and weaker ODC shift, whereas SNP had not the protective effect<sup>28</sup>. The present results showed the similar difference in modifier effect also during the rewarming. In our experiments, L-arginine displayed the antioxidant properties. NO is an endogenous free radical scavenger; its presence markedly reduced the cytotoxicity of O<sub>2</sub><sup>-</sup> or H<sub>2</sub>O<sub>2</sub><sup>7</sup>. The observed rise of  $\alpha$ -tocopherol content in rats treated by L-arginine also would facilitate the free radical utilization and changed the activities of antioxidant enzymes through its membrane-stabilizing effect and limiting of reactive oxygen species permeation into the internal hydrophobic membrane part.

The changes in L-arginine-NO pathway activity on the whole affect the blood oxygen transport indices<sup>19</sup>. NO is made possible through an allosterically controlled nitrite reductase reaction with the heme moiety of Hb<sup>12</sup>. Hb is capable of fulfilling the function of NO storage in the microcirculatory network. In the vascular network, the nitrosothiols produced in NO-mediated thiol nitrosylation play an important role in NO transport, storage and metabolism<sup>9</sup>. RBCs express a functional endothelial NO synthase. An endothelial isoform nitric oxide synthase-like protein and activity in RBCs serving regulatory functions in RBCs and platelets<sup>13</sup>. The effect of SNO-Hb population on NO supply to tissues can be very pronounced since the mechanism of its release from this Hb compound is very sensitive to deoxygenation and is especially manifested in considerable PO<sub>2</sub> changing (in hypoxic area)<sup>16</sup>. The dependence of NO on the allosteric state of Hb suggests that the NO-donor activity of SNO-Hb may enhance the O<sub>2</sub> delivery properties of blood in a fashion

dependent on local hypoxia<sup>17</sup>. The rate at which NO dissociates from Hb is cooperative so that, similar to the case of O<sub>2</sub>, the cooperativity in equilibrium ligand binding is manifested in the dissociation rate rather than the association rate<sup>11</sup>. Recent studies reveal a novel role for Hb as an allosterically regulated nitrite reductase that may mediate NO-dependent signaling along the physiological oxygen gradient<sup>6</sup>. NO can be the allosteric effector of Hb, increasing or decreasing its HOA – possibly, through the generation of different NO-Hb derivatives.<sup>27</sup> The importance of NO binding with Hb hemes is not only in its immediate effect on the functional behavior of NO-carrying molecules, but also on the Hb population at this site of the vascular system. As for microcirculation, this can be extremely important for modifying NO oxygen-binding properties and, in the long run, for tissue oxygenation<sup>30</sup>. HOA decrease is favorable during the deep hypothermia due to the optimized oxygen flux to tissues and its lower fraction spent for the free radical reactions that is associated with the less marked distresses of prooxidant-antioxidant balance<sup>29</sup>.

The present data showed that L-arginine administration improved the oxygen transport – probably, through Hb interaction with NO. Infusion of L-arginine promoted the higher cold resistance and ODC shift rightwards, thereby reducing the hypoxic signs. In addition, it enhanced the antioxidant defense and reduced the levels of LPO products, thereby providing the least prooxidant-antioxidant disbalance during the rewarming.

## 5. CONCLUSION

The L-arginine effect mediated by hemoglobin-oxygen affinity change may be used for the correction of metabolic disorders and improvement of body resistance to low environmental temperature.

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