

## Influence of Acute Ozone Exposure on the Tissue Oxidant/Antioxidant Balance in Physical Exercise

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### Abstract

*Aims:* The influence of acute ozone (O<sub>3</sub>) exposure on the tissue oxidant/antioxidant (O/AO) balance in the brain, myocardium, lungs and striated muscles was studied at rest and during exercise. *Material and Methods:* The research was performed in 3 groups of white male Wistar rats with a weight of 280-300 g: Group I – control group, sedentary rats under normoxia conditions; Group II – sedentary rats, acutely exposed to O<sub>3</sub>; Group III – animals acutely exposed to O<sub>3</sub>, followed by exercise under normoxia conditions. The rats were exposed to ozone for 3 days, 5 min/day. Group III was trained daily for 3 days under normoxia conditions by the swimming test. In order to determine the indicators of the oxidant/antioxidant balance, tissue samples from the brain, myocardium, lungs and quadriceps muscle of the anesthetized animals were taken. On the third day the following were measured: malondialdehyde (MDA), protein carbonyls (PC), hydrogen donor capacity (HD) and total sulfhydryl (thiol) group content (SH). *Results:* Between the indicators of the tissue O/AO balance, significant correlations were evidenced in the brain, myocardium, lungs and muscles. *Conclusions:* Acute combined stress through O<sub>3</sub> pre-exposure and exercise causes OS on account of PC in the brain, myocardium and muscles, a decrease in AO defense capacity on account of HD in the brain, lungs and muscles, and an increase in AO defense capacity in the myocardium.

**Keywords:** Acute exposure; Ozone; Oxidant/antioxidant balance; Exercise.

### Introduction

Ozone (O<sub>3</sub>) is present in atmospheric air in a 0.03% concentration and is an atmospheric air pollutant that occurs in increased amounts in photochemical smog. It is an oxidant agent with predominantly respiratory effects such as the reduction of ventilation, tracheal irritation and cough. After 1984, following the Los Angeles Olympic Games, there has been an increasing interest in the influence of O<sub>3</sub> on physical exercise. The observations in this field evidenced the fact that physical exercise can aggravate the negative effects of O<sub>3</sub> on the respiratory function, through respiratory discomfort (e.g. cough and hypoventilation) [1].

The extent to which physical exercise enhances the negative effects associated with O<sub>3</sub> depends on the concentration of O<sub>3</sub> in environmental air, the intensity of exercise and the time of exposure. In relation to exercise intensity, ventilation is between 60-100 l/min in moderate-intense exercise

and between 30-60 l/min in mild-moderate exercise) [2].

Well trained athletes, capable of maintaining high ventilation for a long time, are more susceptible to the negative effects of O<sub>3</sub> exposure. The response to O<sub>3</sub> exposure during exercise consists of a reduction in the duration of exercise and a decrease in maximal exercise capacity, VO<sub>2</sub> max. (5-15%), heart rate and ventilatory output [3-6].

Our previous experimental researches [7,8] regarding the effect of acute O<sub>3</sub> pre-exposure and the reduction of maximal exercise capacity made us study the acute biochemical changes in the oxidant/antioxidant (O/AO) balance at the level of the main organs that are directly or indirectly involved in exercise.

#### *Aims*

The influence of acute O<sub>3</sub> exposure on the tissue O/AO balance in the brain, myocardium, lungs and striated muscles was studied at rest and during exercise (at the end of the experiment, the animals were euthanized).

## **Material and Method**

The research was performed in the experimental laboratory of the Department of Physiology of the "Iuliu Hațieganu" University of Medicine and Pharmacy Cluj-Napoca, in 3 groups of white male Wistar rats (n=10 animals/group), with a weight of 280-300 g, maintained under adequate vivarium conditions. The animal protection legislation in force was respected during the experimental researches.

#### *Groups*

The groups were divided as follows:

- Group I – control group, sedentary rats under normoxia conditions;
- Group II – sedentary rats, acutely exposed to O<sub>3</sub>;
- Group III – animals acutely exposed to O<sub>3</sub>, followed by exercise under normoxia conditions.

#### *Methods*

##### *a) The exposure to ozone*

The rats were exposed to ozone for 3 days, 5 min/day at values of 0.5 ppm, according to EU norms, using an AIR O<sub>3</sub>NE Labor apparatus. The AIR O<sub>3</sub>NE device is part of the equipment of the Experimental Research Laboratory of the Department of Physiology of the "Iuliu Hațieganu" University of Medicine and Pharmacy Cluj-Napoca and is provided by the SC Triox SRL company, which is specialized in air ozonation. The device allows to regulate air ozone concentration from 50 mg/m to 500 mg/m. The air flow is 5 liters/min ( $\pm 20\%$ ). Duration may vary between 1 minute and 9 hours. The device aspirates an air volume, ensures its physical processing, then eliminates it at the programmed ozone concentration.

##### *b) Exercise test*

Group III was trained daily for 3 days under normoxia conditions by the swimming test. The test was performed in a pool with thermostatic water at 32°C.

##### *c) Exploration of the oxidant-antioxidant balance*

Biochemical determinations were performed in the Laboratory for the Study of Oxidative Stress of the Department of Physiology of the "Iuliu Hațieganu" University of Medicine and Pharmacy Cluj-Napoca.

In order to determine the indicators of the oxidant/antioxidant balance, tissue samples from the brain, myocardium, lungs and quadriceps muscle of the anesthetized animals were taken. The analyzed time moment was day 3. The following oxidative stress indicators were measured:

- malondialdehyde (MDA) (the fluorescence dosage method, according to Conti) [9]; the concentration values are expressed in *nmol/ml*.
- protein carbonyls (PC) (determination of protein carbonyls according to Reznick) [10]; the

concentration values are expressed in *nmol/mg protein*.

The following antioxidant defense indicators were determined:

- hydrogen donor capacity (HD) (dosage method according to Janaszewska) [11]; the values were expressed as per cent of free radical inhibition (%);
- total sulfhydryl (thiol) group content (SH) (determination of SH groups according to Hu) [12]; the values are expressed in  $\mu\text{mol/ml}$ .

*Statistical analysis* was performed using SPSS 19.0 and Microsoft Excel. The data were introduced in a SPSS v.19 database and analyzed with adequate statistical methods. A univariate statistical analysis was used for the description of the studied groups. Quantitative variables were summarized using mean  $\pm$  standard deviation, 95% confidence interval for means. According to the laboratory values, the values for the control group were normal. A bivariate statistical analysis (Pearson correlation, One-Way Anova and LSD post-hoc test) was used to identify the significant association between the groups and between the indicators of the tissue O/AO balance (MDA, PC, HD and SH) was set at  $p \leq 0.05$  for analyses.

## Results

### *Comparative statistical analysis of the indicators of the O/AO balance*

The indicators of the tissue O/AO balance were compared between sedentary animals and animals performing physical exercise, under normoxia conditions after O<sub>3</sub> exposure. The majority of the comparisons were significant (Tables 1, 2, 3 and 4).

**Table 1.** Comparative statistical analysis of the indicators of the O/AO balance in the brain in the studied groups

Group A	Group B	MDA	PC	HD	SH
Group I	Group II	1.000	1.000	1.000	1.000
Group I	Group III	0.000	0.028	0.000	0.281
Group II	Group III	0.000	0.028	0.000	0.281

MDA = malondialdehyde; PC = protein carbonyls;

HD = hydrogen donor capacity; SH = total sulfhydryl group content.

### *Analysis by groups*

Acute ozone exposure (group II) compared to normoxia exposure (group I) determines:

- insignificant changes in the O/AO balance in the brain;
- a significant increase in MDA and a significant increase in SH groups in the myocardium;
- a significant increase in MDA and PC and a significant decrease in HD and SH groups in the lungs;
- a significant increase in MDA and a significant decrease in PC in the muscles.

Acute ozone exposure followed by physical exercise under normoxia conditions (group III) compared to normoxia exposure (group I) determines:

- a significant decrease in MDA and HD and a significant increase in PC in the brain;
- a significant increase in MDA, PC and HD in the myocardium;
- a significant decrease in HD in the lungs;
- insignificant changes in the O/AO balance in the muscles.

Acute ozone exposure followed by physical exercise under normoxia conditions (group III) compared to acute ozone exposure (group II) determines:

- a significant decrease in MDA and HD and a significant increase in PC in the brain;
- a significant increase in PC and HD and a significant decrease in SH groups in the myocardium;
- a significant decrease in MDA and PC in the lungs;

- a significant increase in PC and a significant decrease in HD in the muscles.

**Table 2.** Comparative statistical analysis of the indicators of the O/AO balance in the myocardium in the studied groups

Group A	Group B	MDA	PC	HD	SH
Group I	Group II	0.000	0.123	0.921	0.009
Group I	Group III	0.000	0.000	0.000	0.952
Group II	Group III	0.647	0.000	0.000	0.034

MDA =malondialdehyde; PC = protein carbonyls;  
HD = hydrogen donor capacity; SH = total sulfhydryl group content.

**Table 3.** Comparative statistical analysis of the indicators of the O/AO balance in the lungs in the studied groups

Group A	Group B	MDA	PC	HD	SH
Group I	Group II	0.000	0.002	0.003	0.024
Group I	Group III	0.154	0.975	0.000	0.053
Group II	Group III	0.000	0.001	0.173	0.992

MDA =malondialdehyde; PC = protein carbonyls;  
HD = hydrogen donor capacity; SH = total sulfhydryl group content.

**Table 4.** Comparative statistical analysis of the indicators of the O/AO balance in the muscles in the studied groups

Group A	Group B	MDA	PC	HD	SH
Group I	Group II	0.045	0.000	0.998	0.999
Group I	Group III	0.406	1.000	0.080	0.642
Group II	Group III	0.671	0.000	0.045	0.499

MDA =malondialdehyde; PC = protein carbonyls;  
HD = hydrogen donor capacity; SH = total sulfhydryl group content;

#### *Analysis by tissues*

In the brain of animals exposed to ozone and submitted to physical exercise (group III), a significant increase in OS on account of PC and a significant decrease in AO defense on account of HD were found compared to sedentary groups (group I and group II).

In the myocardium of animals exposed to ozone and submitted to physical exercise (group III), a significant increase in OS on account of PC and a significant increase in AO defense on account of HD were found compared to sedentary groups (group I and group II).

In the lungs of animals exposed to ozone and submitted to physical exercise (group III), there was a significant decrease in HD compared to sedentary controls (group I) and a significant decrease in OS on account of MDA and PC compared to the group exposed to ozone (group II).

In the muscles of animals exposed to ozone and submitted to physical exercise (group III), OS was maintained on account of PC, which significantly increased, and AO defense decreased on account of HD, which significantly decreased compared to the group acutely exposed to ozone (group II).

#### *Analysis by indicators*

MDA showed significant increases in the myocardium, lungs and muscles of animals acutely exposed to ozone (group II) compared to sedentary control animals (group I) and significant decreases in the brain and lungs of animals acutely exposed to ozone and submitted to physical exercise (group III) compared to the group acutely exposed to ozone (group II).

PC were significantly increased in the lungs and significantly decreased in the muscles of the group acutely exposed to ozone (group II) compared to the control group (group I) and significantly increased in the brain, myocardium and muscles of animals acutely exposed to O<sub>3</sub> and

submitted to physical exercise (group III), compared to the group acutely exposed to O<sub>3</sub> (group II).

HD was significantly decreased in the lungs of animals acutely exposed to ozone (group II) compared to sedentary control animals (group I), significantly increased in the myocardium and significantly decreased in the muscles of animals acutely exposed to ozone and submitted to physical exercise (group III) compared to the group acutely exposed to ozone (group II).

SH groups were significantly increased in the myocardium and significantly decreased in the lungs of animals acutely exposed to ozone (group II) compared to sedentary control animals (group I) and significantly decreased in the myocardium of animals acutely exposed to ozone and submitted to physical exercise (group III) compared to the group acutely exposed to ozone (group II).

*Correlations for the indicators of the O/AO balance by groups*

Pearson r correlation coefficients between the indicators of the O/AO balance in each group of animals are shown in Tables 5, 6, 7 and 8. Significance was considered at \*p≤0.05 and \*\*p≤0.01.

**Table 5.** Correlation between the indicators of the O/AO balance in the brain in the studied groups and significance

Indicators	Group I - r (p)	Group II - r (p)	Group III - r (p)
MDA – PC	-0.683 (0.317)	-0.683 (0.317)	0.235 (0.765)
MDA - HD	0.953* (0.047)	0.953* (0.047)	-0.048 (0.952)
MDA – SH	-0.333 (0.667)	-0.333 (0.667)	0.406 (0.594)
PC – HD	-0.527 (0.473)	-0.527 (0.473)	0.820 (0.180)
PC – SH	0.914 (0.086)	0.914 (0.086)	-0.555 (0.445)
HD – SH	-0.140 (0.860)	-0.140 (0.860)	-0.913 (0.087)

MDA =malondialdehyde; PC = protein carbonyls;  
 HD = hydrogen donor capacity; SH = total sulfhydryl group content;  
 r = Pearson correlation coefficients; p = statistical significance; \*Significance for p≤0.05

**Table 6.** Correlation between the indicators of the O/AO balance in the myocardium in the studied groups and significance

Indicators	Group I - r (p)	Group II - r (p)	Group III - r (p)
MDA – PC	0.140 (0.860)	-0.649 (0.351)	0.872 (0.128)
MDA - HD	0.561 (0.439)	0.493 (0.507)	0.577 (0.423)
MDA – SH	-0.196 (0.804)	-0.426 (0.574)	0.991** (0.009)
PC – HD	0.851 (0.149)	0.237 (0.763)	0.835 (0.165)
PC – SH	-0.988* (0.012)	0.534 (0.466)	0.811 (0.189)
HD – SH	-0.826 (0.174)	-0.358 (0.642)	0.465 (0.535)

r = Pearson correlation coefficients; p = statistical significance;  
 \* Significance for p≤0.05; \*\*Significance for p ≤ 0.01

**Table 7.** Correlation between the indicators of the O/AO balance in the lungs in the studied groups and significance

Indicators	Group I - r (p)	Group II - r (p)	Group III - r (p)
MDA – PC	0.371 (0.629)	0.406 (0.594)	-0.996** (0.004)
MDA - HD	0.105 (0.895)	-0.685 (0.315)	-0.442 (0.558)
MDA – SH	-0.660 (0.340)	0.450 (0.550)	0.248 (0.752)
PC – HD	-0.836 (0.164)	0.042 (0.958)	0.376 (0.624)
PC – SH	-0.835 (0.165)	-0.324 (0.676)	-0.160 (0.840)
HD – SH	0.657 (0.343)	-0.055 (0.945)	-0.859 (0.141)

r = Pearson correlation coefficients;  
 p = statistical significance; \*\*- Significance for p≤0.01

**Table 8.** Correlation between the indicators of the O/AO balance in the muscles in the studied

groups and significance

Indicators	Group I - r (p)	Group II - r (p)	Group III - r (p)
MDA – PC	0.111 (0.889)	0.856 (0.144)	-0.802 (0.198)
MDA - HD	0.819 (0.181)	0.666 (0.334)	0.860 (0.140)
MDA – SH	0.264 (0.736)	-0.499 (0.501)	0.366 (0.634)
PC – HD	-0.372 (0.628)	0.953* (0.047)	-0.991** (0.009)
PC – SH	-0.842 (0.158)	-0.315 (0.685)	-0.777 (0.223)
HD – SH	0.490 (0.510)	-0.092 (0.908)	0.686 (0.314)

r = Pearson correlation coefficients; p = statistical significance;

\* Significance for  $p \leq 0.05$ ; \*\*Significance for  $p \leq 0.01$

#### *Analysis of correlations between the indicators of the tissue O/AO balance*

Between the indicators of the tissue O/AO balance, significant correlations are evidenced: in the brain: in sedentary animals between MDA and HD; in the myocardium: in sedentary animals between PC and SH in group I, as well as in animals trained after O<sub>3</sub> exposure, between MDA and SH in group III; in the lungs: in animals trained after O<sub>3</sub> exposure, between MDA and PC in group III and in the muscles: between PC and HD in sedentary animals exposed to O<sub>3</sub> in group II, as well as in animals trained after O<sub>3</sub> exposure in group III.

## Discussion

We found no literatures studies on serum and tissue redox homeostasis under the influence of acute ozone exposure and its implication in exercise.

Our results regarding the effect of acute O<sub>3</sub> exposure in sedentary animals show that the OS sources are the myocardium and the muscles, where significant MDA increases occur, and the lungs, where significant MDA and PC increases are found. After acute O<sub>3</sub> exposure followed by exercise, there is a significant increase in PC in the brain, myocardium and muscles. The increase in AO defense and exercise endurance is significant in the myocardium, where HD is increased. The diminution of AO and exercise endurance is significant in the brain, lungs and muscles, where HD is decreased.

The reduction of maximal exercise capacity evidenced by us [7] could be attributed to redox changes at muscular level, with the increase in OS and the occurrence of muscle fatigue and diminished AO defense.

Our results regarding the influence of acute ozone exposure on the tissue oxidant/antioxidant balance in physical exercise are in accordance with the results obtained for maximal exercise capacity [8].

The reduction of maximal exercise capacity following acute ozone exposure evidenced by us can be attributed to the negative effect of ozone on ventilation, which has also been shown in well trained athletes [4].

## Conclusions

1. Acute stress by O<sub>3</sub> exposure and combined stress by O<sub>3</sub> pre-exposure and exercise influence the tissue O/AO balance in a different way.
2. O<sub>3</sub> exposure in sedentary animals induces an increase in OS on account of MDA in the myocardium, lungs and muscles, and on account of PC in the lungs. AO defense decreases in the lungs on account of HD and SH and increases in the myocardium on account of SH.
3. Acute combined stress through O<sub>3</sub> pre-exposure and exercise causes OS on account of PC in the brain, myocardium and muscles, a decrease in AO defense capacity on account of HD in the brain, lungs and muscles, and an increase in AO defense capacity in the myocardium.
4. At tissue level, acute stress by O<sub>3</sub> exposure and acute combined stress induce an increase in

- AO defense capacity in the myocardium and a decrease in AO defense capacity in the lungs.
5. The decrease in the AO defense capacity of the lungs might explain the negative effects of acute stress through ozone exposure and of acute combined stress in the case of athletes.

### **Ethical Issues**

The Ethics Commission from the "Iuliu Hațieganu" University of Medicine and Pharmacy, Cluj-Napoca approved the study.

### **Conflict of Interest**

The authors declare that they have no conflict of interest.

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### **References**

1. Olinescu R. Radicalii liberi în fiziopatologia umană. București: Ed. Tehnică; 1994. pp106-107.
2. Țăran V. Ozonoterapia în tratamentul sindromului dureros lombar (II). *Săptămâna Medicală* 2010;VI:109-125.
3. Adams WC. Effects of ozone exposure at ambient air pollution episode levels on exercise performance. *Sports Med* 1987;4:395-424.
4. Foss MI, Keteyian SJ. Fox's Physiological Basis for Exercise and Sport. Sixth ed. New York: Mc.Graw-Hill; 1998. p188.
5. Dejica, D.(sub red). Stresul oxidativ în bolile interne. Cluj-Napoca: Ed. Casa Cărții de Știință; 2000. Stresul oxidativ pp. 77-130.
6. Dejica D. Antioxidanți și terapie antioxidantă. Cluj-Napoca: Ed. Casa Cărții de Știință; 2001. Stresul oxidativ și antioxidanții în efortul fizic. pp. 198-237
7. Ugron Á, Tache S, Moldovan R. The effect of acute moderate hypoxia and acute ozone exposure on exercise performance in rats. *Studia Educatio Artis Gymnasticae* 2011;LVI(4):47-54.
8. Ugron Á, Bidian C, Moldovan R, Decea N, Boroș-Balint I, Tache, S. Influence of acute ozone and hypobaric hypoxia exposure on the serum oxidant/antioxidant balance in physical exercise. *Studia Educatio Artis Gymnasticae* 2012;LVII:101-108.
9. Conti M, Morand PC, Levillain P, Lemonnier A. Improved fluorimetric determination of malondialdehyde. *Clin Chem* 1991;37:1273-1275.
10. Reznick AZ, Packer L. Oxidative damage to proteins: spectrophotometric method for carbonyl assay. In: *Methods in enzymology* 1994;233:357-363.
11. Janzsewska A, Bartosz G. Assay of antioxidant capacity: comparison of four methods as applied to human blood plasma. *Scand J Lab Invest* 2002;62:231-236.
12. Hu ML. Measurement of protein thiol groups and glutathione in plasma. In: *Methods in Enzymology*, Academic Press, Inc. 1994; 233:380-384.