Research Article

Effects of Exercise on Oxidative Stress in Rats Induced by Ozone

Catalina Martinez-Campos, 1, 2, 3 Eleazar Lara-Padilla, 1 Rosa Amalia Bobadilla-Lugo, 1 Robert David Kross, 4 and Cleva Villanueva 1

1 Sección de Estudios de Posgrado e Investigación, Escuela Superior de Medicina, IPN, Plan de San Luis y Salvador Díaz Mirón S/N, Colonia Casco de Santo Tomás, 11340 México, DF, México
2 Escuela Médico Militar, Department of Morphology, Boulevard Avila Camacho y Cerrada de Palomas S/N, Colonia Lomas de Sotelo, 11640 México, DF, México
3 Universidad Pablo de Olavide, Facultad del Deporte, Carretera de Utrera Km 1, Edificio 2, Planta Baja, 41013 Sevilla, Spain
4 Kross-Link Laboratories, P.O. Box 374, Bellmore, NY 11710, USA

Correspondence should be addressed to Cleva Villanueva, villanuevacleva3@gmail.com

Received 30 October 2011; Accepted 22 December 2011

Academic Editor: Narisa Futrakul

Copyright © 2012 Catalina Martinez-Campos et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Oxidative stress (OS) induced by acute exercise is reduced by chronic exercise. Ozone (O 3) exposure produces OS. The aim of this study was to determine if aerobic exercise (AE) reduced OS produced by O 3. A pilot experiment was performed with male Wistar rats submitted to AE (trained to swim 90 min/day). Adaptation to exercise was demonstrated three weeks after training by means of changes in reduced nitrates (NOx) in plasma. Therefore, two-week training was chosen for the following experiments. Six of twelve trained rats were exposed to O 3 (0.5 ppm, 4 h/day, one hour before exercise). Two groups of sedentary animals (n = 6 each) were used as controls, one of which was exposed to O 3. At the end of the experiments NOx, 8-isoprostane (8-IP), malondialdehyde (MDA), superoxide dismutase (SOD) activity, and carbonyls (CBs) were measured in plasma. CBs did not change in any group. O 3-induced OS was manifested by reduced NOx and SOD activity, as well as increased 8-IP and MDA. Exercise significantly blocked O 3 effects although SOD was also decreased by exercise (a greater drop occurring in the O 3 group). It is concluded that AE protects against OS produced by O 3 and the effect is independent of SOD.

1. Introduction

Oxidative stress (OS) produced by acute exercise is characterized by an excess of free radicals. It was thought that mitochondria were the main source of free radicals in exercise; however, it is now known that even though mitochondria do contribute, other sources are the main contributors (xanthine oxidase, NADPH oxidase, and phospholipase A2) [1–3]. Chronic exercise reduces OS produced by acute exercise [1, 4, 5]. Adaptation to exercise is due, in part, to an increase in the endogenous antioxidant defense [1, 4–7]. The increase of nitric oxide (NO) availability takes part in the adaptation and the benefits produced by long-term exercise [8–10].

It is thought that the mechanism of adaptation to exercise includes activation of nuclear factor kappa B (NFkB) by free radicals, which upregulates the synthesis of endothelial NO synthase (eNOS) and antioxidant enzymes [8, 9, 11, 12]. Long-lasting moderate exercise has beneficial effects such as prevention of certain cancers, prolonged lifespan in rodents, reduction of cardiovascular effects of aging and menopause, better metabolic control and renal as well as cardiovascular protection in diabetes, and improvement of chronic heart failure [3, 5–7, 11, 13–16].

Ozone (O 3) is a common pollutant in urban areas. The effects of O 3 extend beyond the lung. O 3 exposure produces systemic OS [17]. O 3 exposure has been associated with premature mortality [18], cardiovascular mortality [19], myocardial infarction [20], and cerebrovascular diseases [21]. OS and endothelial dysfunction have been related to the cardiovascular toxic effects of O 3 [22].

The goal of this study was to determine if moderate aerobic exercise affected OS produced by O 3.
2. Material and Methods

2.1. Animals. Male Wistar rats, 10 weeks old (230–250 g), were supplied by Harlan Mexico. Animals were fed with Purina chow and water ad libitum and submitted to light/dark periods of 12/12 h. Animals were kept in a room fed with filtered air to maintain O₃ within normal concentrations (<0.05 ppm) according to the USA Environmental Protection Agency (http://www.epa.gov/air/ozo-nepollution/standards.html). The local Institutional Animal Committee approved all the procedures.

2.2. Training Protocol. Rats were trained to swim 90 min a day, 7 days a week. Animals swam in water at 35–37°C. The training protocol was as follows: One hour after O₃ exposure the animals swam continuously for 90 min per day. The exercise was performed everyday at noon. After exercise the animals were carefully dried and maintained at room temperature (25°C).

2.3. Ozone Exposure. O₃ exposure was made in groups of six animals in an OTC-1 chamber (In USA, Inc.). The Chamber had a servomechanism to maintain O₃ concentrations at 0.5 ± 0.05 ppm. The chamber was programmed to destroy O₃ in such a way that it was impossible to open the chamber if O₃ concentration was above normal (≥0.05 ppm). Animals were exposed to O₃ 4 hours a day, every day (07:00–11:00 h).

2.4. Groups. Groups of 6 animals were formed as follows.

1. Pilot groups: eight pilot groups were formed in order to analyze adaptation to exercise. Half of those groups were sedentary (kept in their cages, which allowed for free movement) and half were submitted to aerobic exercise, as mentioned above. One, two, four, or eight weeks after training, two groups (sedentary and trained) were anesthetized (sodium pentobarbital 45 mg/Kg, ip). The left carotid was cannulated with a PE50 catheter, and a blood sample (3 mL) was taken and treated with EDTA. The animals where then sacrificed by anesthesia overdose. Adaptation to exercise was evaluated by measuring reduced nitrates (NOₓ) in plasma using the Griess method (Cayman Chemical Co. kit).

2. With the results of the pilot groups (see below), a two-week period (just before adaptation) was chosen for the following experiments.

(a) Sedentary group. This group remained sedentary and it was kept in the O₃ chamber at normal concentrations (<0.05 ppm), for 4 hours a day for 2 weeks, in order to have the same confinement stress as that the rats exposed to O₃.

(b) Sedentary group exposed to O₃: this group remained sedentary and was exposed to O₃ (0.5 ppm, 4 h a day) daily for 2 weeks.

(c) Trained group this was kept in the O₃ chamber at normal concentrations (see group (a)), and one hour later they were trained as explained.

(d) Trained group exposed to O₃ this group was exposed to O₃ (0.5, 4 h a day) daily for 2 weeks. One hour after O₃ exposure the animals swam as described.

At the end of the two-week experiment, all animals were anesthetized and 5 mL arterial blood samples were taken. The animals were then sacrificed by anesthesia overdose.

2.5. Oxidative Stress Evaluation. Arterial blood samples were heparinized and centrifuged at 1200×g, 15 min at 4°C. Plasma was separated and divided into 5 aliquots of 200 μL to measure

(a) reduced nitrates (NOₓ, modified Griess method, Cayman Chemical Co. Kit),

(b) 8-isoprostane (8-IP, Cayman Chemical Co. ELISA kit),

(c) Malondialdehyde (MDA, Cayman Chemical Co. TBARS kit),

(d) Protein carbonyls (Cayman Chemical Co. kit),

(e) Total activity of superoxide dismutase (SOD, Cayman Chemical Co. kit).

2.6. Statistical Analysis. Data are presented as mean ± standard error of the mean (SEM) of n experiments. Data were analyzed using the one way ANOVA test and Tukey’s multiple comparison test post hoc or the two-way ANOVA test and the Bonferroni test post hoc.

3. Results

3.1. Adaptation to Exercise. Results are shown in Figure 1. Adaptation to exercise, measured through NOₓ plasma concentration, was reached after two weeks of training. Therefore, a 2-week training was chosen for the experiments where rats were or were not submitted to O₃.

3.2. Oxidative Stress Measurement. Protein carbonyls were similar in all the groups (data not shown). O₃ exposure significantly decreased NOₓ levels (P < 0.05) (Figure 2), whereas it increased both 8-IP (Figure 3) and MDA levels (Figure 4) (P < 0.5). Exercise prevented those changes although the effect was partial on 8-IP. SOD activity (Figure 5) significantly decreased with O₃ and independently with exercise (P < 0.05). However, the combination of O₃ and exercise resulted significantly increased values of SOD activity.
Figure 1: Adaptation to exercise. Plasma reduced nitrate (NO\textsubscript{x}) levels decreased significantly one week after training, whereas they significantly increased two weeks after training and returned to normal levels 4 weeks after training (with no changes thereafter). The return to normal is considered adaptation to exercise. Data were analyzed using the two-way ANOVA test and the Bonferroni test post hoc. Data are shown as the mean ± standard error of mean (n = 6 per group).

Figure 2: Plasma NO\textsubscript{x} concentrations at week two. Ozone (O\textsubscript{3}) exposure (0.5 ppm 4 hours/day) significantly decreased whereas exercise (E, 90 min per day) significantly increased plasma NO\textsubscript{x} concentrations. The effect of O\textsubscript{3} exposure was completely blocked by exercise. Data were analyzed using the one-way ANOVA test and Tukey’s multiple comparison test post hoc. Data are shown as the mean ± standard error of mean (n = 6 per group).

Figure 3: Plasma 8-isoprostane (8-IP) concentrations at week two. Ozone (O\textsubscript{3}, 0.5 ppm 4 hours/day) exposure significantly increased 8-IP levels. Even though exercise (90 min per day) did not change 8-IP, it partially but significantly blocked the O\textsubscript{3} effect. Data were analyzed using the one-way ANOVA test and Tukey’s multiple comparison test post hoc. Data are shown as the mean ± standard error of mean (n = 6 per group).

Figure 4: Plasma malondialdehyde (MDA) concentrations at week 2. Ozone (O\textsubscript{3}, 0.5 ppm 4 hours/day) exposure significantly increased MDA levels. Even though exercise (90 min per day) did not change MDA, it blocked completely O\textsubscript{3} effect. Data were analyzed using the one-way ANOVA test and Tukey’s multiple comparison test post hoc. Data are shown as the mean ± standard error of mean (n = 6 per group).

4. Discussion

Acute exercise produces OS mainly through superoxide production [1, 2]. Chronic exercise reduces OS generated by acute exercise [1, 4, 5]. The mechanism of such adaptation seems to be through activation of NFkB by free radicals, which in turn increases the synthesis of antioxidant enzymes and NO synthases [4, 9, 12, 23]. Moreover, benefits produced by exercise seem to be given, at least in part, precisely by the induction of antioxidant enzymes and NO [1, 4, 9]. In the present study adaptation to exercise, evaluated through NO production, was reached after three weeks of training. Adaptation to exercise, measured through other biomarkers, was reported previously in the same period using a similar training model [24].

Since O\textsubscript{3} exposure produces OS, we wanted to know if exercise could affect such OS just before adaptation to exercise was reached. Therefore, evaluation of OS in the presence or absence of O\textsubscript{3} was made with or without two
weeks of training. We chose a two-week training period because it was the time when NO\textsubscript{x} significantly increased, with no changes in concentrations thereafter.

Measurements were made in plasma in order to evaluate the systemic effects of exercise. Other authors report changes produced by exercise in skeletal muscle [24]. However, beneficial effects of exercise are probably systemic. OS produced by O\textsubscript{3} was confirmed through the increase of 8-IP and MDA as well as the reduction of NO\textsubscript{x} (group). One-way ANOVA test and Tukey’s multiple comparison test were used. Data were analyzed using the one-way ANOVA test and Tukey’s multiple comparison test post hoc. Data are shown as the mean ± standard error of mean (n = 6 per group).

It is concluded that AE protects against OS produced by O\textsubscript{3}, and the effect is independent of SOD.

Acknowledgments

The CONACYT, Grant no. 083090, supported this work. The study was presented as a poster at the meeting Experimental Biology 2011 in Washington, DC. C. Martinez-Campos is a Ph.D. student at the Universidad Pablo de Olavide, Spain. The authors are grateful for the technical help of Mr. Jorge Campos, who gently trained and took care of the animals.

References

[1] M. C. Gomez-Cabrera, E. Domenech, and J. Víña, “Moderate exercise is an antioxidant: upregulation of antioxidant genes by training,” Free Radical Biology and Medicine, vol. 44, no. 2, pp. 126–131, 2008.
[2] M. J. Jackson, “Free radicals generated by contracting muscle: by-products of metabolism or key regulators of muscle function?” Free Radical Biology and Medicine, vol. 44, no. 2, pp. 132–141, 2008.
[3] M. Ristow and S. Schmeisser, “Extending life span by increasing oxidative stress,” Free Radical Biology and Medicine, vol. 51, no. 2, pp. 327–336, 2011.
[4] L. L. Ji, M. C. Gomez-Cabrera, and J. Vina, “Exercise and hormesis: activation of cellular antioxidant signaling pathway,” Annals of the New York Academy of Sciences, vol. 1067, no. 1, pp. 425–435, 2006.
[5] A. Boveris and A. Navarro, “Systemic and mitochondrial adaptive responses to moderate exercise in rodents,” Free Radical Biology and Medicine, vol. 44, no. 2, pp. 224–229, 2008.
[6] V. Pialoux, A. D. Brown, R. Leigh, C. M. Friedenreich, and M. J. Poulin, “Effect of cardiorespiratory fitness on vascular regulation and oxidative stress in postmenopausal women,” Hypertension, vol. 54, no. 5, pp. 1014–1020, 2009.
[7] A. Linke, V. Adams, P. C. Schulze et al., “Antioxidative effects of eccentric exercise training in patients with chronic heart failure: increase in radical scavenger enzyme activity in skeletal muscle,” Circulation, vol. 111, no. 14, pp. 1763–1770, 2005.
[8] F. P. Leung, L. M. Yung, I. Laher, X. Yao, Z. Y. Chen, and Y. Huang, “Exercise, vascular wall and cardiovascular diseases: an update (part 1),” Sports Medicine, vol. 38, no. 12, pp. 1009–1024, 2008.
[9] E. Lima-Cabello, M. J. Cueva, N. Garatachea, M. Baldini, M. Almar, and J. Gonzalez-Gallego, “Eccentric exercise induces...
nitric oxide synthase expression through nuclear factor-
KB modulation in rat skeletal muscle,” *Journal of Applied
Physiology*, vol. 108, no. 3, pp. 575–583, 2010.

[10] Q. J. Zhang, S. L. Mcmillin, J. M. Tanner, M. Palionyte, E.
D. Abel, and J. D. Symons, “Endothelial nitric oxide synthase
phosphorylation in treadmill-running mice: role of vascular
signalling kinases,” *Journal of Physiology*, vol. 587, no. 15, pp.
3911–3920, 2009.

[11] J. M. Lawler, H. B. Kwak, J. H. Kim, and M. H. Suk, “Exercise
training inducibility of MnSOD protein expression and activ-
ity is retained while reducing prooxidant signaling in the heart
of senescent rats,” *American Journal of Physiology*, vol. 296, no.
5, pp. R1496–R1502, 2009.

[12] L. L. Ji, M. C. Gomez-Cabrera, N. Steinhafel, and J. Vina,
“Acute exercise activates nuclear factor (NF)-κB signaling
pathway in rat skeletal muscle,” *The FASEB Journal*, vol. 18,
no. 13, pp. 1499–1506, 2004.

[13] S. Ghosh, M. Khazaei, F. Moien-Afshari et al., “Moderate
exercise attenuates caspase-3 activity, oxidative stress, and
inhibits progression of diabetic renal disease in db/db mice,”
*American Journal of Physiology*, vol. 296, no. 4, pp. F700–F708,
2009.

[14] G. K. McConnell, S. J. Bradley, T. J. Stephens, B. J. Canns, B.
A. Kingwell, and R. S. Lee-Young, “Skeletal muscle nNOS
protein content is increased by exercise training in humans,”
*American Journal of Physiology*, vol. 293, no. 2, pp. R821–R828,
2007.

[15] F. Moien-Afshari, S. Ghosh, S. Elmi et al., “Exercise restores
coronary vascular function independent of myogenic tone or
hyperglycemic status in db/db mice,” *American Journal of Physi-
ology*, vol. 295, no. 4, pp. H1470–H1480, 2008.

[16] H.-K. Na and S. Oliynyk, “Effects of physical activity on cancer
prevention,” *Annals of the New Academy of Sciences*, vol.
1229, no. 1, pp. 176–183, 2011.

[17] G. Valacchi, A. Van der Vliet, B. C. Schock et al., “Ozone
exposure activates oxidative stress responses in murine skin,”
*Toxicology*, vol. 179, no. 1–2, pp. 163–170, 2002.

[18] M. L. Bell, R. D. Peng, and F. Dominici, “The exposure-
response curve for ozone and risk of mortality and the adequacy of current ozone regulations,” *Environmental Health
Perspectives*, vol. 114, no. 4, pp. 532–536, 2006.

[19] Y. Zhang, W. Huang, S. J. London et al., “Ozone and daily mortality in Shanghai, China,” *Environmental Health
Perspectives*, vol. 114, no. 8, pp. 1227–1232, 2006.

[20] J. B. Ruidavets, M. Cournot, S. Cassadou, M. Giroux, M.
Meybeck, and J. Ferri`eres, “Ozone air pollution is associated
with acute myocardial infarction,” *Circulation*, vol. 111, no. 5,
pp. 563–569, 2005.

[21] C. C. Chan, K. J. Chuang, L. C. Chien, W. J. Chen, and W. T.
Chang, “Urban air pollution and emergency admissions for
cerebrovascular diseases in Taipei, Taiwan,” *European Heart
Journal*, vol. 27, no. 10, pp. 1238–1244, 2006.

[22] D. J. Sánchez-González, M. A. Moro, C. Castillo-Henkel et al.,
“Ozone exposure induces iNOS expression and tyrosine nitra-
tion in rat aorta,” *Environmental Toxicology and Pharmacology*,
vol. 17, no. 1, pp. 1–7, 2004.

[23] L. L. Ji, M. C. Gomez-Cabrera, and J. Vina, “Role of nuclear
factor kappaB and mitogen-activated protein kinase signaling
in exercise-induced antioxidant enzyme adaptation,” *Applied
Physiology, Nutrition, and Metabolism*, vol. 32, no. 5, pp. 930–
935, 2007.

[24] K. Higashida, S. H. Kim, M. Higuchi, J. O. Holloszy, and D.-
H. Han, “Normal adaptations to exercise despite protection
against oxidative stress,” *American Journal of Physiology*, vol.
301, no. 5, pp. E779–E784, 2011.

[25] R. J. Gryglewski, R. M. Palmer, and S. Moncada, “Superoxide
anion is involved in the breakdown of endothelium-derived
vascular relaxing factor,” *Nature*, vol. 320, no. 6061, pp. 454–
456, 1986.

[26] C. M. Wong, C. Q. Ou, T. Q. Thach et al., “Does regular
exercise protect against air pollution-associated mortality?”
*Preventive Medicine*, vol. 44, no. 5, pp. 386–392, 2007.