



Effects of Ambient Particles Inhalation on Lung Oxidative Stress Parameters in Exercising Rats

Thiago Gomes Heck^{1,3}, Marcelo Rafael Petry², Alexandre Maslinkiewicz², Pauline Brendler Goettems-Fiorin^{1,2}, Mirna Stela Ludwig¹, Paulo Hilário Nascimento Saldiva³, Pedro Dal Ago² Claudia Ramos Rhoden²

¹Research Physiology Group, Post-graduation Program in Integral Attention to Health, Department of Life Sciences, Regional University of the Northwest of Rio Grande do Sul State, Brazil, ²Post-Graduation Course in Medical Sciences. Federal University of Health Sciences of Porto Alegre, Porto Alegre, RS, Brazil, ³Laboratory of Experimental Air Pollution. Medical School – University of São Paulo, São Paulo, SP, Brazil.

ABSTRACT

Heck TG, Petry MR, Maslinkiewicz A, Goettems-Fiorin PB, Ludwig MS, Saldiva PHN, Dal Ago P, Rhoden CR. Effects of Ambient Particles Inhalation on Lung Oxidative Stress Parameters in Exercising Rats. **JEPonline** 2014;17(3):58-69. This study examined the hypothesis that acute exercise exposed to urban ambient particles (UAP) inhalation could increase oxidative damage in the lung. Wistar rats were submitted to UAP during a 20 or 60 min swimming exercise. Longer periods of exercise (60 min exposure to UAP) showed higher lipid peroxidation (MDA and Chemiluminescence) in the lung and lower catalase activity. The findings indicate that the exposure of rats to urban ambient particles during 60 min of swimming exercise results in higher lipid peroxidation (MDA and Chemiluminescence) and lower CAT activity in the lungs. Short-term exposure to particulate matter during exercise may be a biological risk, while longer periods of an exercise session with exposure to particles can exacerbate oxidative stress in the lungs.

Key Words: Exercise, Oxidative stress, Pollution, Particulate Matter

INTRODUCTION

Inhalation of urban ambient particles (UAP) produces inflammation in the lungs, promotes abnormalities of the epithelial barrier permeability (41), impairs respiratory function (5,28,29,44), increases the secretion of proinflammatory mediators (13,24), and damages the alveolar tissue (14). Additionally, epidemiological studies have consistently demonstrated that exposure to UAP increases cardiorespiratory morbidity, mortality, and the number of hospital readmissions for preexisting respiratory diseases (2,4,9,11,49).

While studies have concluded that the induced oxidative stress from inhaled UAP associates with adverse effects in pulmonary function (16,18,31-33), it appears that individuals with preexisting cardiorespiratory diseases are most susceptible to acute pulmonary responses. Experimental studies have demonstrated that the mechanisms by which UAP inhalation promotes cardiorespiratory injuries might be related to important mechanistic differences. The interaction between pollutants and lung cells may activate pulmonary neural reflexes that initiates modifications in the autonomic nervous system (32) while concomitantly promoting inflammatory response (33) and oxidative stress (16,20).

Numerous studies indicate that the pulmonary damage elicited by particle inhalation is augmented during exercise (6,17,37-39), which may act in synergy with the increase in oxygen transport across the alveolar-capillary surface (35,36). Additionally, high intensity exercise can be associated with an increase in free radical production and often a transient increase in oxidative stress (19). This oxidative imbalance is directly related to fatigue sensation (21) and an “open window” of lowered immunity related to increased upper respiratory symptoms of infection or lung injury (25,26).

In an experimental rat model, we found that UAP showed a significant increase in oxidative stress and adverse respiratory effects in rats maintained at rest (30). More recently, we observed that the intratracheal administration of a high amount of particles before a moderate intensity exercise session promoted oxidative stress in the lungs and heart of rats after a swimming exercise protocol (22). However, the replication of this effect in an urban environment is not known.

Despite the extensive literature on oxidative stress induced by exercise and also oxidative stress induced by UAP, few studies have investigated the effects of UAP inhalation during exercise on lung oxidative parameters. In light of this point, the purpose of this study was to verify the hypothesis that urban ambient particles could induce lung stress during an acute moderate exercise session. We also investigated the effect of the duration of exercise/exposure in the lung oxidative parameters.

METHODS

Animals

Fourty male Wistar rats aged 90 days from the Animal Breeding Unit of Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA) were subjects in this study. The animals were kept in plastic cages (47 cm x 34 cm x 18 cm) under controlled humidity (75 to 85%) with a temperature of $22 \pm 2^{\circ}\text{C}$, a dark-light cycle of 12 hrs (lights on from 7 am to 7 pm). They were fed with a conventional laboratory diet (Supra-lab, Alisul Alimentos S.A, Brazil) and water ad libitum.

Animal Care

The animals were handled humanely in the performance of this project to minimize the use of animals and to prevent animal distress. All protocols were approved by the Federal University of Health Sciences of Porto Alegre (UFCSPA) Ethical Committee for Research (CPA 017/053).

Adaptation to the Water

All rats were adapted to the water before the beginning of the study. The adaptation protocol consisted of 3 consecutive days of keeping the animals 10 min in shallow water at 30°C to reduce the rat behavior stress related to water contact. Physical training adaptations were not allowed with this intervention (22).

Experimental Design

Lung Oxidative Stress Induced by Urban Ambient Particles Inhalation during the Acute Exercise Session - “Real World” Exposure

After the water adaptation period, 40 rats were divided into 4 groups for UAP inhalation during exercise: (a) the exercise group in filtered air for 20 min (Exerc 20 min, n = 12); (b) the exercise group exposed to urban ambient particles for 20 min (Exerc 20 min + UAP, n = 12); (c) the exercise group in filtered air for 60 min (Exerc 60 min n = 8); and (d) the exercise group in urban ambient particles for 60 min (Exerc 60 min + UAP, n = 8). This study was performed across 5 different days (see Table 1). At the end of the exercise session, blood samples were obtained to determine lactate concentration. Then, the lungs were extracted and stored at -80°C for oxidative stress analysis.

Site of “Real World” Exposure

Inhalation chambers were located on the second floor of the Federal University of Health Sciences of Porto Alegre (UFCSPA) Building, located in Porto Alegre (30°03'45”S, 51°11'15”W), downtown, at a crossroad with heavy traffic, facing an automated air pollution monitoring station of the State Environmental Agency, that provides continuous PM10 measurements (beta monitor). Porto Alegre has a population of approximately 1.5 million inhabitants; it is the central city of a metropolitan industrialized area of 3.2 million inhabitants. In 2007, the vehicle fleet approximated 1 vehicle for every 2.7 individuals, and there is a significant increase of 4.5% vehicles per year. The vehicular traffic is the primary source of air pollution in the large cities of Brazil. They are responsible for approximately 90% of the CO and PM10 emissions, with air pollution representing a serious problem in many regions (39). Porto Alegre has ~500 Km² of total area with 40% of the urban area occupied by the transportation structure. The city presents thermal inversions frequently, and defined climate seasons. On average, the temperature varies from 18 and 25°C. The average of rain days varying from 3 to 6 d·mth⁻¹ (based on 8 yrs of historical weather readings)(30).

Chamber of Exercise and “Real World” Exposure

The swimming exercise protocol and the air pollution exposure were carried through with the animals inside of acrylic chambers (30 cm x 100 cm x 30 cm) filled 45 cm with water at 30°C, hermetically closed with the exception of one entrance (connected to the outdoor environment) and one exit. A vacuum pump was connected to the exit to pumped outdoor air through the exposure chambers at a flow rate of 10 L·min⁻¹. One of the chambers (designated as filtered groups) received a 37 mm Teflon filter (Millipore, Ireland) in the inlet to avoid the admission of ambient particles major than 2.5 µm; whereas, the other received atmospheric air without the filter system (designed as polluted groups). The “Real World” apparatus was adapted from Pereira and colleagues (30). Ambient air condition during exercise in the “Real World exposure” protocol is listed in Table I, which demonstrates a regular condition (moderate levels) of urban air pollution during the experiment days.

Table 1. Particulate Matter Concentration - Day by Day of “Real World” Exposure.

Date	Time of Exposure/Exercise	UAP $\mu\text{g}/\text{m}^3$
Day 1	20 min	70.03
Day 2	20 min	38.15
Day 3	20 min	102.38
Day 4	60 min	28.42
Day 5	60 min	19.99

Measurement of Exercise Intensity by Blood Lactate Concentration

Caudal venous lactate concentrations were determined before and after exercise by using a Lactate Analyzer (Accutrend®Plus System, Roche). The results were expressed as $\text{mmol}\cdot\text{L}^{-1}$.

Determination of Lung Oxidative Stress

Tissue Preparation

The lungs from each rat were excised, washed in saline solution and quickly frozen in liquid nitrogen. For the determination of oxidative stress parameters, the lung samples were homogenized (1:7 w/v) in 120 mmol KCl, 30 mmol sodium phosphate buffer (pH = 7.4) added with protease inhibitor 0.5 mmol PMSF (Phenylmethanesulfonyl Fluoride) at 0 to 4°C. The suspensions were centrifuged at 600 g for 10 min at 0 to 4°C to remove nuclei and cell debris. The pellets discarded and the supernatants were used as homogenates (22,27,30).

Thiobarbituric Acid Reactive Substances Method (TBARS), Chemiluminescence, and Catalase Enzyme Activity

Homogenates were precipitated with 10% TCA, centrifuged, and incubated with thiobarbituric acid (T5500-Sigma) for 60 min at 100°C. TBARS were extracted using butanol (1:1 V/V). After centrifugation, the absorbance of the butanol layer was measured at 535 nm (6). The amount of TBARS formed was expressed in nanomoles of malondialdehyde per milligram of protein (nmol MDA/mg prot). Malondialdehyde standard was prepared from 1,1,3,3,-tetramethoxypropane (Fluka, USA) (22,27,47,48).

Chemiluminescence (CL) was measured in a liquid scintillation counter in the out-of-coincidence mode (LKB Rack Beta Liquid Scintillation Spectrometer 1215, LKB - Produkter AB, Sweden). Lung homogenates were placed in low-potassium vials at a protein concentration of 0.5 to 1.0 mg of protein·mL⁻¹ in a reaction medium consisting of 120 mmol·L⁻¹ KCl, 30 mmol·L⁻¹ sodium phosphate buffer (pH = 7.4). Measurements were started by the addition of 3 mmol·L⁻¹ tert-butyl hydroperoxide. The data were expressed as counts per sec per milligram of protein. Catalase (CAT) activity was determined by following the decrease at 240 nm-absorbance in a reaction medium containing 50 mmol·L⁻¹ sodium phosphate buffer (pH = 7.2), and 10 mmol·L⁻¹ hydrogen peroxide (H₂O₂) (1). It was expressed as picomole of H₂O₂ reduced per sec per milligram of protein (pmol of H₂O₂/sec/mg prot). Protein was measured by the method of Bradford (3), using bovine serum albumin (1 mg·mL⁻¹ to 0.1 mg·mL⁻¹ curve) as standard. The results were expressed in mg of protein/mL of homogenized tissue (22).

Statistical Analysis

One-way ANOVA followed by Student-Newman-Keuls *post hoc* test were used for the statistical analysis in blood lactate concentration and all oxidative stress parameters (TBARS, CL, and CAT). The results are expressed in mean \pm standard deviation. The level of statistical significance was set at 5%. All statistical analyses were conducted using the SPSS (V.18) Software for Windows.

RESULTS

Exposure to urban ambient particles (UAP) during the 20 min of an exercise session (Exerc 20min group) resulted in no modification in the lung lipid peroxidation (Figure 1A), chemiluminescence (Figure 1B) or catalase enzyme activity (Figure 1C) when compared to 20 min of exercise in filtrated air (Exerc 20min group). However, 60 min of exercise exposed to UAP showed higher levels of lipid peroxidation in the lungs (Figure 1A).

After 60 min of exercise, higher QL levels were observed in the lungs compared to the 20 min exercised groups, with no additional effect of UAP exposure (Figure 1B). After the 60 min exercise session, catalase activity was higher in the lungs from rats in filtrated air, but this effect was not observed in the UAP exposure group (Exerc 60min+UAP) that exercised at the same time and duration (Figure 1C).

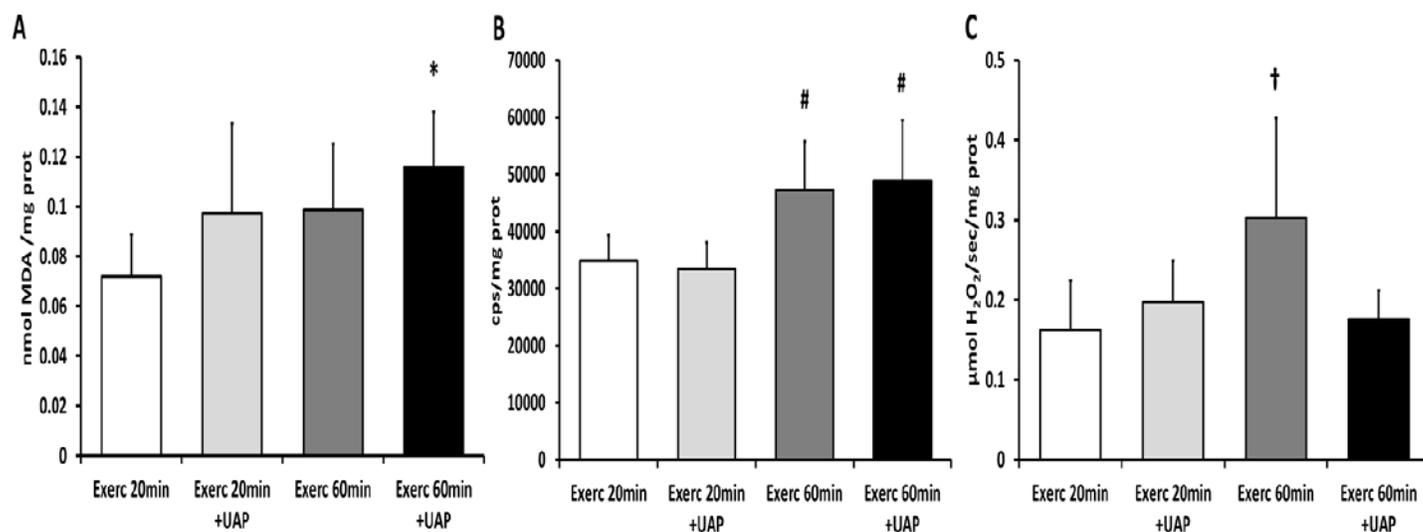


Figure 1. Lung Oxidative Stress of Rats Submitted to Exercise Exposed to Urban Ambient Particles. Lung lipid peroxidation levels measured by (A) MDA concentration and (B) chemiluminescence. (C) Catalase enzyme activity of lung homogenates from rats submitted to 20 or 60 min of swimming exercise exposed to urban ambient particles (+UAP). Results are expressed as mean \pm standard deviation. *Significant difference ($P=0.0013$) vs. Exerc 20min. #Significant differences ($P=0.0007$) vs. Exerc 20min and Exerc 20min+UAP. †Significant differences ($P=0.0025$) vs. all groups. One-Way ANOVA followed by Student-Newman-Keuls *post hoc* test.

Blood lactate concentration ($\text{mmol}\cdot\text{L}^{-1}$) was also measured after the acute exercise session. The results showed that all rats exercised at the same intensity. Blood lactate concentration levels were similar among the groups with no significant effect by exercise duration (20 min vs. 60 min), and it was not influenced by exposure to ambient levels of particles (i.e., filtrated vs. UAP exposure) (refer to Table 1).

Table 1. Blood Lactate Concentration after Exercise Exposed to Urban Ambient Particles.

	Exerc 20 min	Exerc 20 min +UAP	Exerc 60 min	Exerc 60 min +UAP
Blood Lactate Concentration (mmol·L ⁻¹)	4.17 ± 0.59	4.39 ± 0.84	4.08 ± 0.75	4.14 ± 0.71

Blood lactate concentration after swimming exercise in rats exercised in filtered air for 20 min (Exerc 20min, n=12), the exercise group exposed to urban ambient particles for 20 min (Exerc 20min+UAP, n=12), the exercise group in filtered air for 60 min (Exerc 60min, n=8), and the exercise group in urban ambient particles for 60 min (Exerc 60min+UAP, n=8). The results are expressed as mean ± standard deviation. No significant differences were found among the groups (P=0.7223).

DISCUSSION

In the present study, the rats were exposed to urban air pollution (UAP) or clean (filtered) air during an acute short-term (20 min or 60 min) swimming exercise session. Their lungs were evaluated for lipid damage that showed a progressive time-dependent effect of exercise with an increase of lipid peroxidation and lower catalase activity mediated by UAP exposure. Rats exposed to UAP for 60 min presented an almost 2-fold higher lipid peroxidation when compared to the rats that swam in clean ambient air for 20 min and 20% higher than rats that swam in clean ambient air for 60 min. Longer acute exercise (60 min) showed higher catalase enzyme antioxidant activity only in the rats that swam in the filtrated environment. This effect in CAT activity was not observed in the rats exposed to UAP after 60 min of acute exercise. The experimental model used in this study indicates that combining animal studies with “real world” exposure may be useful in understanding the pathogenesis of lung injury promoted by urban particles during exercise. Thus, it is reasonable that the “Real World” Exercise+UAP protocol supports the contention that inhalation of ambient particles air pollution represents a biological hazard (30).

In our study the UAP did not modify the blood lactate concentration, which is a biomarker of exercise intensity in experimental models. Previously, we observed that a high concentration of particles did not modify the blood lactate levels in Residual Oil Fly Ash (ROFA) in an intratracheal instillation model. Interestingly, although the rats received ROFA administration 20 min before the exercise session (22), they showed blood lactate levels similar to this study (ROFA study data = 4.08 ± 0.76 vs. Exerc 20min+UAP= 4.39 ± 0.84). As suggested by the literature, the increase in blood lactate concentration after exercise at values close to 4 mmol·L⁻¹ indicates that the animals reached moderate intensity that was independent of UAP exposure (15,23,45).

A previous study (30) in our laboratory demonstrated that the effect on pulmonary oxidative stress during a continuous short-term UAP exposure is time dependent. We observed higher levels of lipid peroxidation in lung tissue in rats exposed to UAP for 20 continuous hours, but there was no effect in rats exposed to UAP for 6 continuous hours or 20 intermittent hours (average PM₁₀ concentration = 130 ± 10.0 µg/m³) (30). In the present study in Porto Alegre (30°03'45”S, 51°11'15”W), we further explored the possibility of UAP concentrations causing oxidative stress in the lungs by performing a single short-term 20 min or 60 min of exercise. Since a short-term outdoor exercise period close to traffic is quite frequent in the urban scenario, we expected that this study could mimic this situation. In

our experimental exercise conditions (i.e., 20 to 60 min of exposure to an average of about 50 $\mu\text{g}/\text{m}^3$ of urban particles), we found evidence of lipid peroxidation in the lungs only with 60 min of exercise and exposure (Figure 1A) with lower catalase activity induced by UAP. We also observed in a separated experiment that a 24-hr later exercise session had no effect on the groups (filtrated vs. UAP exposure) in MDA concentration, QL or catalase activity (data not shown). Together, the findings in the present study and the previous studies suggest that in the environmental urban air pollution levels and the experimental conditions do not result in a permanent or persistent oxidative stress, although exercise can boost the redox imbalance in the pulmonary tissue induced by PM inhalation.

Since cardiorespiratory demand is considerably increased during exercise, as observed by an increase in oxygen consumption, there is a significant increase in pulmonary ventilation and tissue diffusion capacity. These modifications might be important reasons for augmented risk to particles inhalation damage during exercise (37,39). In fact, it is demonstrated in the literature that the total amount of ultrafine particles deposited in respiratory tract of humans during moderate exercise is approximately five times higher than during resting (7). Based on these findings, it is possible to propose that even short-term exposures to ambient levels of particles influence the capacity of the lungs to respond adequately to an increase in oxidative demand. Healthy rats can manage this situation quite well, as demonstrated by the lack of an increase in MDA in 20 min groups vs. the groups exercised for 60 min. In other words, it is apparent that a healthy organism can repair the oxidative injury.

The question that needs to be answered is this: How would diseased lungs, when submitted to a high oxidative load, respond to the same stimulus? In this context, epidemiological data provide compelling evidence that patients with chronic inflammatory lung conditions (such as chronic bronchitis and asthma) are more susceptible to adverse fluctuations of ambient particles (34). This point is in agreement with both the hospitalizations and out of hospital deaths due to ambient particles (10). Our MDA concentration and catalase activity evaluation indicate that exercising near traffic may lower lung oxidative defense, and might be a risk to the exposition to low levels of air pollution in subjects with pulmonary inflammatory diseases.

The fact that UAP induce lipid peroxidation in the resting condition is not novel. Several studies (8, 12,30-32,47,48) have reported that the pulmonary adverse effects of particles might be mediated by mechanism dependent on oxidant agents, including during exercise session (22). Here, it is important to point out that the physiological adaptations from regular exercise result in a protective effect against lung tissue oxidation and inflammation following the acute exposure of diesel particles (43). This finding may likely be associated with the increase in CAT activity as an defensive effect against lipid peroxidation (42).

Hence, in this way, chronic exercise (i.e., at a moderate intensity and duration of training) may not result in lung damage. This is supported by the epidemiologic study that provided evidence that habitual exercise may prevent premature death attributable to air pollution (46). Thus, the absence of the increase of CAT activity in the Exerc 60min+UAP group might be a marker of lung injury. Since it has been reported that acute exercise can enhance mitochondrial CAT activity by ~360% (40), it is reasonable that the lack of an increase of CAT activity during exercise by UAP exposure requires complementary studies.

Presently, it is a common recommendation to avoid exercise in parks or in recreational areas close to busy roadways or industrial sites (37,39). Also, it is useful to limit the exercise session in terms of hours per day when air pollution is critical. This recommendation is supported by the fact that the rats required to exercise for longer periods (60 min vs 20 min) may represent a risk for lung oxidative

equilibrium between the antioxidant defense and the process of environmental oxidative induction by UAP.

CONCLUSIONS

The findings in this study indicate that the exposure of Wistar rats to urban ambient particles during 60 min of a swimming exercise resulted in high lipid peroxidation (MDA and Chemiluminescence) and lower catalase activity in the lungs. Thus, it is reasonable to conclude that short-term exposure to particulate matter during exercise may represent a risk, and that long periods of an exercise session exposure to particles can exacerbate the oxidative stress in pulmonary tissue.

ACKNOWLEDGMENTS

This work was supported by Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA). T.G. Heck and M. Petry were supported by a fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). Dr C.R. Rhoden, Dr. P. Dal Lago and Dr. P.H.N. Saldiva are supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). The authors declare that they have no conflict of interest. Research Support (PqG-2013 - FAPERGS, process: 002106-2551/13-5 T. G. HECK)

Address for correspondence: Thiago Gomes Heck, PhD, Department of Life Sciences (DCVida). Universidade Regional do Noroeste do Estado do Rio Grande do Sul, Rua do comércio, Bairro Universitário, Ijuí, RS. CEP: 98700-000. Brazil, Email: gomesheck@yahoo.com

REFERENCES

1. Aebi H. Catalase in vitro. *Methods Enzymol.* 1984;105:121-126.
2. Arbex MA, Martins LC, de Oliveira RC, Pereira LA, Arbex FF, Cancado JE, et al. Air pollution from biomass burning and asthma hospital admissions in a sugar cane plantation area in Brazil. *J Epidemiol Community Health.* 2007;61(5):395-400.
3. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* 1976;72:248-254.
4. Braga AL, Saldiva PH, Pereira LA, Menezes JJ, Conceicao GM, Lin CA, et al. Health effects of air pollution exposure on children and adolescents in Sao Paulo, Brazil. *Pediatr Pulmonol.* 2001;31(2):106-113.
5. Brauner EV, Mortensen J, Moller P, Bernard A, Vinzents P, Wahlin P, et al. Effects of ambient air particulate exposure on blood-gas barrier permeability and lung function. *Inhal Toxicol.* 2009;21(1):38-47.
6. Carlisle AJ, Sharp NC. Exercise and outdoor ambient air pollution. *Br J Sports Med.* 2001; 35(4):214-222.

7. Daigle CC, Chalupa DC, Gibb FR, Morrow PE, Oberdorster G, Utell MJ, et al. Ultrafine particle deposition in humans during rest and exercise. *Inhal Toxicol.* 2003;15(6):539-552.
8. Damiani RM, Piva MO, Petry MR, Saldiva PH, Tavares Duarte de Oliveira A, Rhoden CR. Is cardiac tissue more susceptible than lung to oxidative effects induced by chronic nasotropic instillation of residual oil fly ash (ROFA)? *Toxicology Mechanisms and Methods.* 2012; 22(7):533-539.
9. Farhat SC, Paulo RL, Shimoda TM, Conceicao GM, Lin CA, Braga AL, et al. Effect of air pollution on pediatric respiratory emergency room visits and hospital admissions. *Braz J Med Biol Res.* 2005;38(2):227-235.
10. Faustini A, Stafoggia M, Colais P, Berti G, Bisanti L, Cadum E, et al. Air pollution and multiple acute respiratory outcomes. *Eur Respir J.* 2013;42(2):304-313.
11. Freitas C, Bremner SA, Gouveia N, Pereira LA, Saldiva PH. [Hospital admissions and mortality: Association with air pollution in Sao Paulo, Brazil, 1993 to 1997]. *Rev Saude Publica.* 2004;38(6):751-757.
12. Ghelfi E, Rhoden CR, Wellenius GA, Lawrence J, Gonzalez-Flecha B. Cardiac oxidative stress and electrophysiological changes in rats exposed to concentrated ambient particles are mediated by TRP-dependent pulmonary reflexes. *Toxicol Sci.* 2008;102(2):328-336.
13. Ghio AJ, Devlin RB. Inflammatory lung injury after bronchial instillation of air pollution particles. *Am J Respir Crit Care Med.* 2001;164(4):704-708.
14. Ghio AJ, Gilbey JG, Roggli VL, Richards JH, McGee JK, Carson JL, et al. Diffuse alveolar damage after exposure to an oil fly ash. *Am J Respir Crit Care Med.* 2001;164(8):1514-1518.
15. Gobatto CA, de Mello MA, Sibuya CY, de Azevedo JR, dos Santos LA, Kokubun E. Maximal lactate steady state in rats submitted to swimming exercise. *Comp Biochem Physiol A Mol Integr Physiol.* 2001;130(1):21-27.
16. Gonzalez-Flecha B. Oxidant mechanisms in response to ambient air particles. *Mol Aspects Med.* 2004;25(1-2):169-182.
17. Grievink L, Jansen SM, van't Veer P, Brunekreef B. Acute effects of ozone on pulmonary function of cyclists receiving antioxidant supplements. *Occup Environ Med.* 1998;55(1):13-17.
18. Gurgueira SA, Lawrence J, Coull B, Murthy GG, Gonzalez-Flecha B. Rapid increases in the steady-state concentration of reactive oxygen species in the lungs and heart after particulate air pollution inhalation. *Environ Health Perspect.* 2002;110(8):749-755.
19. Hatao H, Oh-ishi S, Itoh M, Leeuwenburgh C, Ohno H, Ookawara T, et al. Effects of acute exercise on lung antioxidant enzymes in young and old rats. *Mech Ageing Dev.* 2006;127(4):384-390.

20. Hatzis C, Godleski JJ, Gonzalez-Flecha B, Wolfson JM, Koutrakis P. Ambient particulate matter exhibits direct inhibitory effects on oxidative stress enzymes. *Environ Sci Technol.* 2006;40(8):2805-2811.
21. Heck TG, Scholer CM, de Bittencourt PI. HSP70 expression: Does it a novel fatigue signalling factor from immune system to the brain? *Cell Biochem Funct.* 2011;29(3):215-226.
22. Heck TG, Nunes RB, Petry MR, Maslinkiewicz A, Saldiva PHN, Dal Lago P, et al. Residual oil fly ash (ROFA) inhalation promotes lung and heart oxidative stress without hemodynamic effects in exercising rats. *JEPonline.* 2014;17(1):78-89.
23. Machado Fde B, Gobatto CA, Voltarelli FA, Rostom de Mello MA. Non-exhaustive test for aerobic capacity determination in swimming rats. *Appl Physiol Nutr Metab.* 2006;31(6):731-736.
24. Nemmar A, Al-Maskari S, Ali BH, Al-Amri IS. Cardiovascular and lung inflammatory effects induced by systemically administered diesel exhaust particles in rats. *Am J Physiol Lung Cell Mol Physiol.* 2007;292(3):L664-670.
25. Nieman DC, Nehlsen-Cannarella SL. The immune response to exercise. *Semin Hematol.* 1994;31(2):166-179.
26. Nieman DC. Upper respiratory tract infections and exercise. *Thorax.* 1995;50(12):1229-1231.
27. Nunes RB, Tonetto M, Machado N, Chazan M, Heck TG, Veiga AB, et al. Physical exercise improves plasmatic levels of IL-10, left ventricular end-diastolic pressure, and muscle lipid peroxidation in chronic heart failure rats. *J Appl Physiol.* 2008;104(6):1641-1647.
28. Penttinen P, Timonen KL, Tiittanen P, Mirme A, Ruuskanen J, Pekkanen J. Number concentration and size of particles in urban air: Effects on spirometric lung function in adult asthmatic subjects. *Environ Health Perspect.* 2001;109(4):319-323.
29. Penttinen P, Timonen KL, Tiittanen P, Mirme A, Ruuskanen J, Pekkanen J. Ultrafine particles in urban air and respiratory health among adult asthmatics. *Eur Respir J.* 2001;17(3):428-435.
30. Pereira CE, Heck TG, Saldiva PH, Rhoden CR. Ambient particulate air pollution from vehicles promotes lipid peroxidation and inflammatory responses in rat lung. *Braz J Med Biol Res.* 2007;40(10):1353-1359.
31. Rhoden CR, Lawrence J, Godleski JJ, Gonzalez-Flecha B. N-acetylcysteine prevents lung inflammation after short-term inhalation exposure to concentrated ambient particles. *Toxicol Sci.* 2004;79(2):296-303.
32. Rhoden CR, Wellenius GA, Ghelfi E, Lawrence J, Gonzalez-Flecha B. PM-induced cardiac oxidative stress and dysfunction are mediated by autonomic stimulation. *Biochim Biophys Acta.* 2005;1725(3):305-313.
33. Rhoden CR, Ghelfi E, Gonzalez-Flecha B. Pulmonary inflammation by ambient air particles is mediated by superoxide anion. *Inhal Toxicol.* 2008;20(1):11-15.

34. Roberts S. Have the short-term mortality effects of particulate matter air pollution changed in Australia over the period 1993-2007? *Environmental Pollution*. 2013;182C:9-14.
35. Rundell KW, Caviston R. Ultrafine and fine particulate matter inhalation decreases exercise performance in healthy subjects. *J Strength Cond Res*. 2008;22(1):2-5.
36. Rundell KW, Slee JB, Caviston R, Hollenbach AM. Decreased lung function after inhalation of ultrafine and fine particulate matter during exercise is related to decreased total nitrate in exhaled breath condensate. *Inhal Toxicol*. 2008;20(1):1-9.
37. Sharman JE, Cockcroft JR, Coombes JS. Cardiovascular implications of exposure to traffic air pollution during exercise. *QJM: Monthly Journal of the Association of Physicians*. 2004;97(10):637-643.
38. Sharman JE. Clinicians prescribing exercise: Is air pollution a hazard? *Med J Aust*. 2005;182(12):606-607.
39. Sharman JE, Stowasser M. Australian association for exercise and sports science position statement on exercise and hypertension. *J Sci Med Sport*. 2009;12(2):252-257.
40. Somani SM, Frank S, Rybak LP. Responses of antioxidant system to acute and trained exercise in rat heart subcellular fractions. *Pharmacol Biochem Behav*. 1995;51(4):627-634.
41. Timonen KL, Hoek G, Heinrich J, Bernard A, Brunekreef B, de Hartog J, et al. Daily variation in fine and ultrafine particulate air pollution and urinary concentrations of lung Clara cell protein CC16. *Occup Environ Med*. 2004;61(11):908-914.
42. Veloso CF, Dalla Costa C, Rovani BT, Araldi ICC, Freitas RB, Cogo LA, et al. Effects of exercise on cardiac oxidative stress in rats after exposure to cigarette smoke. *J Exerc Physiol online*. 2013;16(5):21-27.
43. Vieira RP, Toledo AC, Silva LB, Almeida FM, Damaceno-Rodrigues NR, Caldini EG, et al. Anti-inflammatory effects of aerobic exercise in mice exposed to air pollution. *Med Sci Sports Exerc*. 2012;44(7):1227-1234.
44. Volpino P, Tomei F, La Valle C, Tomao E, Rosati MV, Ciarrocca M, et al. Respiratory and cardiovascular function at rest and during exercise testing in a healthy working population: Effects of outdoor traffic air pollution. *Occup Med (Lond)*. 2004;54(7):475-482.
45. Voltarelli FA, Gobatto CA, de Mello MA. Determination of anaerobic threshold in rats using the lactate minimum test. *Braz J Med Biol Res*. 2002;35(11):1389-1394.
46. Wong CM, Ou CQ, Thach TQ, Chau YK, Chan KP, Ho SY, et al. Does regular exercise protect against air pollution-associated mortality? *Prev Med*. 2007;44(5):386-392.
47. Zanchi AC, Venturini CD, Saiki M, Nascimento Saldiva PH, Tannhauser Barros HM, Rhoden CR. Chronic nasal instillation of residual-oil fly ash (ROFA) induces brain lipid peroxidation and behavioral changes in rats. *Inhalation Toxicology*. 2008;20(9):795-800.

48. Zanchi AC, Saiki M, Saldiva PH, Barros HM, Rhoden CR. Hippocampus lipid peroxidation induced by residual oil fly ash intranasal instillation versus habituation to the open field. *Inhalation Toxicology*. 2010;22(1):84-88.
49. Zanobetti A, Schwartz J. Air pollution and emergency admissions in Boston, MA. *J Epidemiol Community Health*. 2006;60(10):890-895.

Disclaimer

The opinions expressed in **JEPonline** are those of the authors and are not attributable to **JEPonline**, the editorial staff or the ASEP organization.