UNIVERSIDADE FEDERAL DE ALAGOAS

FACULDADE DE MEDICINA

LEONARDO ALVES PASQUA

EXERCISING IN THE URBAN CENTER: INFLAMMATORY AND CARDIOVASCULAR EFFECTS OF PROLONGED EXERCISE UNDER AIR POLLUTION

MACEIÓ

2021

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Trabalho de Conclusão de Curso apresentado à coordenação do curso de Medicina da Universidade Federal de Alagoas.

Orientadora: Profa. Dra. Mayara Vieira Damasceno

MACEIÓ

2021

Chemosphere 254 (2020) 126817

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Exercising in the urban center: Inflammatory and cardiovascular effects of prolonged exercise under air pollution

Leonardo A. Pasqua [a, b, *], Mayara V. Damasceno [a, c], Ramon Cruz [a], Monique Matsuda [d], Marco A.G. Martins [e], Mônica V. Marquezini [d, f], Adriano E. Lima-Silva [g], Paulo H.N. Saldiva [d, h], Romulo Bertuzzi [a]

[a] Endurance Performance Research Group (GEDAE-USP), School of Physical Education and Sport, University of São Paulo, São Paulo, SP, Brazil
[b] Faculty of Medicine, Federal University of Alagoas, Maceió, Brazil
[c] CESMAC University Center, Maceió, Brazil
[d] Laboratory of Investigation in Ophthalmology (LIM-33), Division of Ophthalmology, University of São Paulo Faculty of Medicine, São Paulo, SP, Brazil
[e] Harvard T.H. Chan School of Public Health, Harvard University, Boston, MA, USA
[f] Pro-Sangue Foundation, São Paulo, SP, Brazil
[g] Human Performance Research Group, Academic Department of Physical Education (DAEFI), Technological Federal University of Parana, Curitiba, PR, Brazil
[h] Institute of Advanced Studies, University of São Paulo, São Paulo, SP, Brazil

HIGHLIGHTS

- Effects of air pollution during exercise seem to be dependent on exercise duration.
- Air pollution during long duration exercise might increase post arterial pressure.
- Systemic inflammation occurs after long exercise under air pollution exposure.
- Distance should be considered in active transportation under air pollution exposure.

ARTICLE INFO

Article history:
Received 1 November 2019
Received in revised form
7 April 2020
Accepted 13 April 2020
Available online 18 April 2020

Handling Editor: Hongliang Zhang

Keywords:
Inflammation
Serum cytokines
Arterial pressure
Cycling
Active transport
Health

ABSTRACT

The aim of this study was to investigate, in a well-controlled experimental environment, whether air pollution from an urban center would affect inflammatory and cardiorespiratory responses during prolonged moderate exercise (i.e., 90 min). Ten healthy men performed two experimental trials under filtered and polluted air, inside an environmental chamber located in Sao Paulo downtown, Brazil. Blood samples were obtained at rest, 30, 60, and 90 min of the exercise to determine the serum cytokines concentration, while arterial pressure was recorded immediately after the exercise. The serum cytokines were not altered until 60 min of exercise for both conditions ($P > 0.05$). Otherwise, at 90 min of exercise, the IL-6 ($P = 0.047$) and vascular endothelial growth factor (VEGF) ($P = 0.026$) were significantly higher and IL-10 tended to decrease ($P = 0.061$) in polluted air condition compared to filtered air condition. In addition, both systolic ($P = 0.031$) and diastolic ($P = 0.009$) arterial pressure were higher in polluted air condition than filtered air condition. These findings demonstrate that the exercise of longer duration (i.e., 90 min), but not of shorter duration (i.e., <60 min), performed in vehicular air pollution condition results in pronounced pro-inflammatory and increased arterial pressure responses.

1. Introduction

Outdoor air pollution has been considered a worldwide problem, affecting a large number of people (Lelieveld et al., 2015) by increasing chronic disease risk, exacerbating COPD and other respiratory diseases (*e.g.* asthma) and causing approximately 7 million deaths only in 2012 (World Health Organization WHO Internet,

* Corresponding author. Department of Sport, School of Physical Education and Sport, University of São Paulo, Nise da Silveira Street, 133 – Antares, Maceió, AL, Brazil.
E-mail address: leonardopasqua@gmail.com (L.A. Pasqua).

https://doi.org/10.1016/j.chemosphere.2020.126817
0045-6535/

2014). In urban areas, outdoor air pollution is mainly produced by vehicles and constituted by a mix of gases and solid particles, including nitrogen dioxide (NO_2) and particulate matter (PM). Due to its small size, particulate matter with aerodynamic diameter equals or smaller than 2.5 μm ($PM_{2.5}$) can be deposited in the lung's alveolar region and reach the bloodstream, causing systemic deleterious effects on health (Cutrufello et al., 2012). These adverse effects are often linked to an increase in pro-inflammatory cytokines (*e.g.*, IL-6 and TNFα) and pulmonary bronchi obstruction, due to increased mucus secretion, smooth muscle contraction, and alveolar dysfunction, as well as endothelial damage in blood vessels (Pinkerton et al., 2017).

Endurance exercise, such as cycling, is able to reduce traffic-related pollutant emissions and improve the well-being of people living in urban areas (Mueller et al., 2018) by creating an opportunity to provide a substantial increase in physical activity (Pasqua et al., 2018). It is well documented in the literature that increasing physical activity has a considerable capacity to decrease the prevalence of cardiovascular risk factors, such as arterial hypertension (Pescatello et al., 2015; Sanchis-Gomar et al., 2015). Exercise also seems to be able to induce anti-inflammatory effects mainly by controlling the production, release, and activity of TNFα and IL-6 (Ortega, 2016). Through the reduction in TNFα activity, exercise directly reduces pro-inflammatory processes (Akdis et al., 2016). Although IL-6 is considered a systemic pro-inflammatory cytokine, its secretion by the skeletal muscle during exercise is a trigger to increase IL-10 secretion, an important anti-inflammatory circulating cytokine (Akdis et al., 2016).

It is noteworthy that is still on discussion whether exercise-induced health benefits remain when exercising in a polluted environment (Jacobs et al., 2010; Flouris et al., 2012; Giles and Koehle, 2014). For instance, Devlin et al. (1999) observed as higher as 1.8 and 2.0 serum concentration for IL-6 and IL-8 respectively, on intermittent moderate exercise under exposure to 2.0 ppm of NO_2 if compared to filtered air. In contrast, Silva-Renno et al. (2017) observed that under $PM_{2.5}$ and PM_{10} exposures, the exercise was able to reduce plasma IL-1 and TNFα and increase IL-10, suggesting a reduction in systemic inflammation. The reasons for the discordance between the studies are not fully clear, but differences in methodological approaches, such as exposure protocols and exercise duration, may account to part of these inconsistencies. Studies with air pollution in a well-controlled environment has been limited to diesel exhaustion engines only (Ghio et al., 2012; Giles et al., 2014), which produces a smaller diversity of pollutants concentrations when compared with the “real world”. This is particularly relevant because of the air pollution impact on health outcomes appears to be associated to the different pollutants characteristics (Giles et al., 2014). In addition, most of these studies limited the exercise duration to less than 60 min (Flouris et al., 2012; Giles et al., 2014). Taking these into account, experimental approaches that allow the analyses of physiological responses related to exercise for a prolonged period of time, using a well-controlled environment, could provide new insights about the influence of polluted environment on people health outcomes.

Therefore, the aim of this study was to evaluate the inflammatory and cardiovascular effects of prolonged exercise performed under vehicular air pollution. Considering that deleterious effects of air pollution is related to total amount of inhaled air (Nyhan et al., 2014), it was hypothesized that exercise of longer duration (i.e., 90 min) would exacerbate the inflammatory and arterial pressure responses when compared with exercise of shorter duration (≤60 min).

2. Methods

2.1. Experimental design

Participants visited the laboratory on three separate occasions. On the first visit, they underwent to anthropometric assessment and performed an incremental test to determine their ventilatory threshold, respiratory compensation point, and maximal oxygen uptake ($\dot{V}O_2max$). The intensity of the prolonged exercise was determined using the ventilatory threshold and respiratory compensation point. During the second and third visits, participants performed endurance exercise at moderate intensity, i.e., 25% of the difference between the ventilatory threshold and respiratory compensation point (Δ25), in both conditions, filtered and polluted air. The order of the second and third sessions was counterbalanced and separated by 72 h. All of the experimental trials were performed in the same period of the day to avoid some possible impact of circadian variation. The participants were asked to refrain from vigorous physical activities, caffeine and alcohol 48 h before each experimental session.

2.2. Participants

The required sample size was calculated using the software G*Power (version 3.1.9.4). It was based in the clinical significant outcome of the arterial pressure according to the effect size for active individuals (Carpio-Rivera et al., 2016). A sample size of eleven participants was estimated to achieve clinical changes in arterial pressure for a power of 0.8 and a significance level of 0.05. Twelve subjects were recruited to participate in this study, however two subjects dropped out during the experimental period due to personal reasons. Ten young males (age: 25.9 ± 2.2 years, body mass: 72.7 ± 8.9 kg, height: 174.9 ± 9.7 cm, body mass index: 24.0 ± 3.3 kg m^2, and $\dot{V}O_2max$: 42.2 ± 8.3 ml kg^{-1}. min^{-1}), residents in the city of São Paulo and students from the same University, where they spent, on average, about 10 h a day, participated in this study. As an inclusion criterion, participants should be classified as physically active through a previous validated international physical activity questionnaire (IPAQ) (Fogelholm et al., 2006). As exclusion criteria, participants should not present any cardiorespiratory disease, not be engaged in any medical treatment, not using anabolic steroids, and not present recent injuries that could compromise their participation. The participants were informed about the risks associated with the study protocol and signed a consent form agreeing to participate. The Ethics Committee for Human Studies of the University of Sao Paulo approved this investigation, in accordance with Helsinki's Declaration.

2.3. Standardized diet

In the first session, participants received a nutritional recorder to be filled in during the day before the second session and were instructed to repeat the same nutritional intake in the day before the third session. They came to the laboratory after a 6-h fasting and received a standardized snack. The total time between the snack and the experimental session beginning was between 50 and 60 min. Water was provided *ad libitum* during the entire experimental session and was no significant different between sessions two and three.

2.4. Preliminary test

The maximal incremental test was carried out on a cycle ergometer (Biotek 2100, Cefise, Nova Odessa, SP, Brazil). After a 5-

min warm-up at 100 W, the work rate was increased by 30 W every minute until participants had reached volitional fatigue. Participants exercised at a pedal cadence of 70–80 rpm and volitional fatigue was defined as the incapacity to maintain a minimum pedal cadence of 70 rpm. They received strong verbal encouragement to continue as long as possible. Gases exchanges were measured breath-by-breath using a calibrated gas analyzer (Cortex Metalyzer 3B, Cortex Biophysik, Leipzig, Germany). Gases exchanges data were averaged into 30-s intervals for further analysis. The point of a nonlinear increase in the $\dot{V}E/\dot{V}O_2$ relationship determined the ventilatory threshold (Meyer et al., 2005). The point of a concomitant nonlinear increase in $\dot{V}E/\dot{V}CO_2$, a constant increase in the $\dot{V}E/\dot{V}O_2$, and a first decrease in the expiratory fraction of CO_2 determined the respiratory compensation point (Meyer et al., 2005). Two independent investigators determined these thresholds. When the investigators disagreed, a third independent investigator was consulted. $\dot{V}O_2$max was determined when two or more of the following criteria were met: an increase in oxygen uptake of less than 2.1 ml kg^{-1}•min^{-1} between two consecutive stages, a respiratory exchange ratio greater than 1.1 and the attainment of a heart rate ≥90% of the predicted maximal heart rate (i.e., 220-age) (Howley et al., 1995).

2.5. Experimental trials

The exercise consisted of 90 min with constant-load cycling at an exercise intensity corresponding to Δ25. The exercise duration was chosen based on previous findings suggesting that active transportation in a non-planning urban center can exceed 1 h of exercise (Giles-Corti et al., 2016), while the intensity was chosen based on a pilot study that showed it was the maximal intensity that could be maintained throughout 90 min without excessive discomfort. Participants warmed up during 5 min at 50 W and then workload was adjusted to the work rate corresponding to Δ25. Participants were required to maintain 70–80 rpm throughout the exercise. Blood samples were obtained at rest, 30, 60, and 90 min of exercise, and stored for later determination of the serum cytokines concentration. Heart rate was recorded continually throughout the exercise, while arterial pressure was recorded at rest and immediately after the exercise.

2.6. Environmental chamber

The present study was conducted in the Sao Paulo city, Brazil. Sao Paulo is one of the most populous cities in the world with approximately 12 millions of residents (IBGE, 2019a). It has been characterized by increasing traffic of motorized vehicles in consequence to economic growth (IBGE, 2019b). A previous review has discussed that the increase in the air pollution sources and an unorganized urbanization process led to air pollutants concentrations which commonly exceed the air quality standards, with the transport as the main sector for pollutants emissions (Andrade et al., 2017). The experimental trials were performed in an environmental chamber located at 20 m away from the road, and 150 m from a busy traffic intersection in downtown Sao Paulo city, similar to previously reported (Mauad et al., 2008). The last report of Environment Agency of the Sao Paulo State (CETESB, 2018) estimates that approximately 83,941 cars, 9936 diesel vehicles, and 6321 motorcycles circulate daily on the main streets, and 25,590 cars, 5299 diesel vehicles, and 808 motorcycles circulate daily at this intersection. There were no industries or significant biomass burning sources in the surroundings.

Fig. 1 shows a schematic representation of the environmental chamber setup used in the present study. The environmental chamber has 6.06 m in length, 2.44 m in width and 2.59 m in height. The exercise protocols were made in a testing room with 1.63 m in length and the same width and height of the chamber. It was equipped with HEPA (High Efficiency Particulate Air) and biological filters, able to retain a wide range of gaseous and particles pollutants. In the filtered condition, the outside air passed through the filters, while in the polluted condition, the outside air came direct from outdoors, unfiltered. In all the experimental trials, air pollutants concentrations were measured. Two Dust Track analyzers (model 8530-TSI Incorporated, Shoreview, MN, USA) were simultaneously used to measure $PM_{2.5}$ and PM_{TOTAL}. The nitrogen oxides (NO, NO_2, NOx) concentrations were measured using a NOx Analyzer model 42i (Thermo Fisher Scientific, Francklin, MA, USA). Temperature and relative humidity were obtained during all experimental sessions using a digital thermohydrometer (Incoterm, Porto Alegre, Brazil).

2.7. Serum cytokines

Blood samples (~5 ml) were collected from antecubital vein and, after a 15-min clotting time, centrifuged at 2000 rpm and 4 °C for 10 min. The resultant supernatant was extracted and stored at −80 °C until analysis. Serum specimens were prepared for analysis in a 96-well plate using a custom 13-cytokine Milliplex MAP Human Cytokine/Chemokine Magnetic Bead Panel (Millipore Corp., Billerica, MA) following the kit-specific protocols provided by Millipore. Analytes were quantified using a Magpix analytical test instrument, which utilizes xMAP technology (Luminex Corp., Austin, TX), and xPONENT 4.2 software (Luminex). xMAP technology uses fluorescent-coded magnetic microspheres coated with analyte-specific capture antibodies to simultaneously measure multiple analytes in a specimen. After micro-spheres have captured the analytes, a biotinylated detection antibody binds to that complex. Streptavidin PE then attaches as a reporter molecule. Inside the instrument, magnetic beads are held in a monolayer by a magnet, where two LEDs are used to excite the internal microsphere dye and the dye of the reporter molecule, respectively. A CCD camera captures these images, which are then analyzed by Milliplex Analyst software (Millipore). Concentrations of cytokines (pg•ml^{-1}) (IL-1, IL-6, IL-10, TNFα, and VEGF) were determined on the basis of the fit of a standard curve for mean fluorescence intensity versus pg•ml^{-1} (or ng•ml^{-1}). These cytokines were chosen based on their investigation in previous studies analyzing exercise and air pollution effects (Flouris et al., 2012; Giles et al., 2014; Pope et al., 2016; Silva-Renno et al., 2017).

2.8. Cardiovascular parameters

Systolic and diastolic arterial pressure were measured using and automatic equipment (BPA100-Microlife®, Widnau, Switzerland) in a single maneuver to avoid post-exercise arterial pressure kinetics interference. For the baseline measurements, in both conditions, participants rested in a seated position for 5 min. The post exercise measurement, immediately after the exercise, was taken also at seated position in the same chair of the pre-measurement. Participants were asked to not talk and to keep relaxed. The arm used for the arterial pressure measurement was kept resting on a table at heart level. Heart rate was measured continually using a heart rate transmitter (model S810, Polar Electro Oy, Kempele, Finland) and expressed as mean values. Because there are no previous studies analyzing whether the silicone mask, often used to measure the gas exchanges during exercise, could act as a "physical barrier" to pollutants, the mean values of minute ventilation ($\dot{V}E$) during the experimental trials were estimated as previously

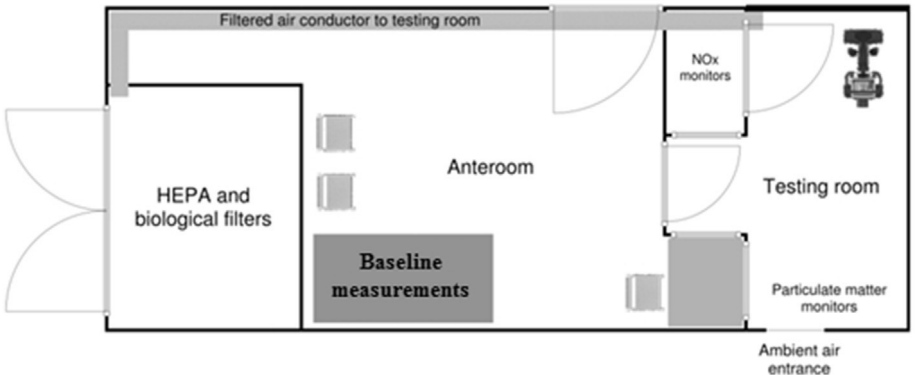

Fig. 1. The environmental chamber schematic representation.

proposed by Zuurbier et al. (2009) (equation (1)).

$$\dot{V}E = \exp(c + m * HR) \quad (1)$$

where $\dot{V}E$ is the estimated minute ventilation ($L \bullet min^{-1}$), HR is the heart rate, c is the intercept of equation (1.03 for men) and m is the slope of equation (0.021 for men).

2.9. Statistical analysis

Data normality was confirmed through Shapiro-Wilk test. The air pollution concentration, temperature, relative air humidity, and cardiovascular parameters were compared between filtered and polluted conditions through a paired *t*-test. Arterial pressure and serum cytokines were, respectively, transformed in delta values (post − pre) and fold changes ($\bullet pre^{-1}$) in order to mitigate the impact of inter-individual data variability and to better compare the behavior between the different cytokines, as reported in a previous study (Almada et al., 2013). A two-way ANOVA with repeated measures (time and condition as factors) was used to compare the serum cytokines response during the exercise between the conditions. When a significant *F* value was detected, *Bonferroni* correction was used to identify significant differences. Significance level was set at $\alpha = 0.05$. Statistical analyses were performed using a statistical software package (Statistica 8.0, Stat Soft, Tulsa, OK, USA).

3. Results

3.1. Environmental chamber

The concentrations of $PM_{2.5}$, PM_{TOTAL}, NO and NO_2 measured in the environmental chamber are presented in Fig. 2. As expected, the concentrations of all air pollutants were significantly higher in the polluted air compared to the filtered condition ($P < 0.05$). There was no significant difference between the experimental trials for temperature (filtered: 20.8 ± 0.5 °C; polluted: 20.3 ± 0.6 °C; $P = 0.86$) and relative air humidity (filtered: $71 \pm 3\%$; polluted: $70 \pm 3\%$; $P = 0.79$).

3.2. Serum cytokines

Serum cytokines concentrations measured at rest and 30, 60 and 90 min of exercise are presented in Fig. 3. All serum cytokines did not change until 60 min of exercise ($P > 0.05$). However, they increased at 90 min if compared to previous time points ($P > 0.05$). This increase was larger in polluted air condition for IL-6 ($P = 0.047$) and VEGF ($P = 0.026$), compared to the filtered condition. There was also a tendency ($P = 0.061$) for lower values of IL-10 at 90 min of exercise in polluted condition when compared to filtered. No statistical differences were observed between filtered and polluted conditions for IL-1 and TNFα ($P > 0.05$).

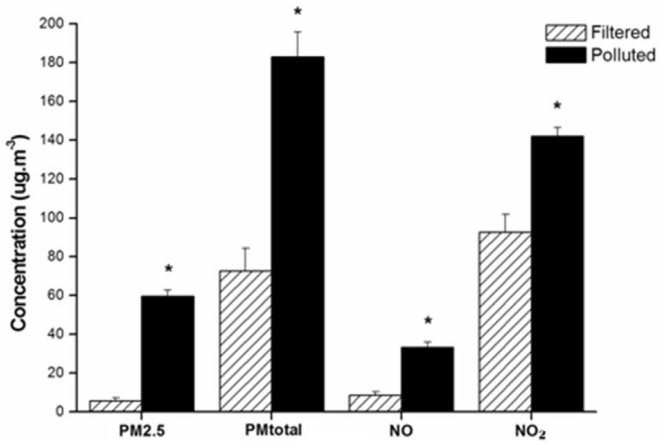

Fig. 2. Air pollutants concentrations measured in the environmental chamber. Data are the mean ± standard deviation. $PM_{2.5}$: particulate matter with 2.5 μm diameter or less. PM_{TOTAL}: total particulate matter in the air. NO: nitrous oxide; NO_2: nitrous dioxide. * Significantly higher than filtered condition ($P < 0.05$).

3.3. Cardiovascular parameters

At rest, there were not significant differences for systolic (filtered = 116.5 ± 8.5 mmHg, polluted = 117.1 ± 9.9 mmHg; $P = 0.78$) and diastolic (filtered = 77.1 ± 6.3 mmHg, polluted = 77.4 ± 5.1 mmHg; $P = 0.81$) arterial pressure when compared between experimental conditions. Fig. 4 demonstrates the arterial pressure measured immediately at the end of the prolonged exercise. There was a decrease in both systolic and diastolic arterial pressure in the filtered condition, while there was an increase in these variables during polluted condition. These changes in systolic ($P = 0.031$) and diastolic ($P = 0.009$) arterial pressure were significantly different between the experimental conditions. Heart Rate (HR) did not show significant difference between filtered and polluted air conditions (filtered = 138 ± 20 bpm, polluted = 136 ± 17 bpm) and VE (filtered = 47.3 ± 12.1 L min^{-1}, polluted = 47.8 ± 11.9 bpm $L \bullet min^{-1}$).

4. Discussion

It has been well described in the literature that air pollution

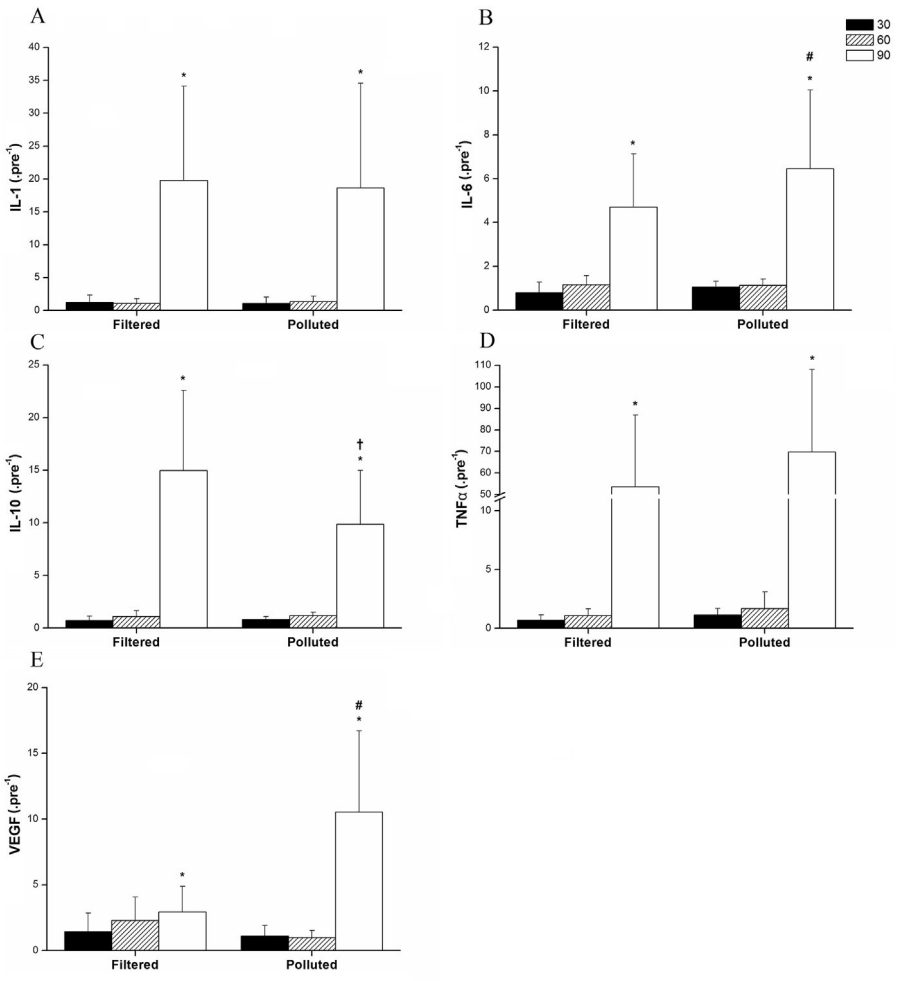

Fig. 3. Serum cytokines concentrations were expressed as fold changes of pre-exercise values during prolonged exercise. Data are the mean ± standard deviation. * Significantly higher compared to previous moments at the same condition. # Significantly higher compared to the same moment at filtered condition. † A tendency of being significantly lower compared to the same moment at the filtered condition.

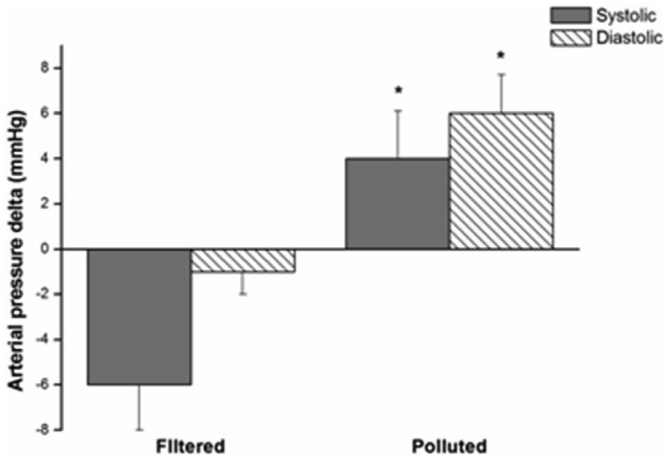

Fig. 4. Arterial pressure response during exercise at the filtered and polluted conditions. Data are the delta mean ± standard deviation of changes in systolic and diastolic arterial pressure. *Significantly higher compared to filtered condition ($P < 0.05$).

leads to increase inflammatory processes and produces deleterious effects on arterial pressure (World Health Organization WHO Internet, 2014), while endurance exercise has a positive effect in these health outcomes (Pescatello et al., 2015). However, while the possible isolate effects of air pollution (Lelieveld et al., 2015) or exercise (Sanchis-Gomar et al., 2015) have been well explored, much less has been investigated concerning the interaction between these factors (i.e., exercise and vehicular air pollution), especially during prolonged exercise performed under real-world conditions. Our results showed an increase in pro-inflammatory (i.e., IL-6 and VEGF) and a tendency to reduce anti-inflammatory (i.e., IL-10) cytokines after 60 min of exercise performed in the vehicular air pollution condition. These changes in inflammatory profile in polluted air condition were accompanied by a larger increase in systolic and diastolic arterial pressure immediately after 90 min of exercise. The novel findings of the current study indicate that exposure to air pollution often presented in urban centers could exacerbate inflammatory and arterial pressure responses, mainly after 60 min of endurance exercise.

The beneficial effects of endurance exercise on health outcomes are being confronted since the end of the 20th century due to the presence of air pollution in urban areas (Drinkwater et al., 1974; Pasqua et al., 2018). Some studies have analyzed the impact of the exercise performed in a polluted environment and demonstrated positive (Vieira et al., 2012) or negative (Jacobs et al., 2010; Kubesch et al., 2015; Pasqua et al., 2018) effects. The reasons for these

inconsistences are not fully clear, however differences in methodological approaches may have contributed for these contradictory findings. Most of these well-controlled laboratory studies were conducted simulating the air pollution with chambers connected to diesel exhaust engines (Ghio et al., 2012; Giles et al., 2014), which could not reproduce all diversity of pollutant often found in real-world environment. This is particularly relevant because the variety in air pollutant components and concentrations might lead to distinct adverse biological effects (World Health Organization Internet, 2005). Studies conducted at outdoor environment, in turn, are able to represent the real-world pollutants diversity in big cities (Int Panis et al., 2010; Nyhan et al., 2014), but they were often conducted in public areas and could not have fine control of other variables (i.e., temperature and relative humidity) able to influence health outcomes (Jacobs et al., 2010). Therefore, the methodological approach used in this study (i.e., environmental chamber) represents a unique opportunity to combine a realistic scenario and well-controlled condition in air polluted environments. Our results revealed that the filtered air condition presented all pollutants concentration lower than the polluted condition ($P < 0.05$). These pollutants concentration, except for NO_2, were also above the WHO standards ($PM_{2.5}$: 560%; PM_{TOTAL}: 163%; NO: 275%; NO_2: 81%). These findings indicate that, besides the environmental chamber has produced well distinct experimental conditions, the polluted condition was composed by a considerable mix of pollutants and their concentrations represented a possible threshold to worse health consequences according to existent scientific data (World Health Organization Internet, 2005).

It has been proposed that endurance exercise could provide substantial health benefits for the urban area residents due to increasing physical activity (Giles-Corti et al., 2016). Among the health benefits, previous studies demonstrated that endurance exercise increases mitochondrial respiration rate, increasing reactive oxygen species production, and leading to a moderate proinflammatory stimulus, which induces an anti-inflammatory effect (Scheele et al., 2009; Peake et al., 2015; Ortega, 2016). Muscle contraction induces IL-6 production, resulting in an increase of anti-inflammatory cytokines (*e.g.*, IL-10) circulation and decreases the TNF-α synthesis, a pro-inflammatory cytokine (Peake et al., 2015). In the present study, there were no significant changes in serum cytokines until 60 min of exercise, which corroborate with previous studies that used durations until 60 min of exercise (Flouris et al., 2012; Giles et al., 2014). Interestingly, at 90 min of exercise in the polluted air condition, a significant increase in IL-6 and VEGF, and a decrease in IL-10 were found. The mechanisms by which the air pollution could lead to a pro-inflammatory state are not fully elicited, although it seems related to epithelial and endothelial damage with consequent activation of immune cells, the release of growth factors, and inflammatory mediators (Ovrevik et al. 2016). Flouris et al. (2012) found no significant increase in IL-6 at 1-h exposure to secondhand smoke followed by a maximal exercise lasting ~10 min. Jacobs et al. (2010) also did not find a significant difference between clean room and a near-road in serum IL-6 after 20 min of moderate cycling. In contrast, Devlin et al. (1999) observed a significant increase in IL-6 after 4 h of NO_2 exposure alternating 15 min of rest and moderate treadmill exercise. With a main role in angiogenesis induction, VEGF also plays a role in inflammation mechanism, and may even stimulate IL-6 synthesis (Angelo and Kurzrock, 2007). These findings suggest that exercise duration has a role in inflammatory responses when performed under vehicular air pollution. Our results indicate that the pro-inflammatory response of exercising under polluted environment presents a time-dependent behavior, with 60 min of exposure being a potential threshold for the beginning of elevations of the inflammatory responses.

The acute hypotensive effect is another positive health adaptation often observed after endurance exercise. Previous findings have clearly demonstrated that exercise produces vasorelaxation which leads to a temporary reduction in arterial pressure, which is a very important adaptation for individuals presenting cardiovascular diseases, particularly, arterial hypertension (Pescatello et al., 2015). However, our results demonstrated significant elevations in systolic (+4.1 mmHg) and diastolic (+6.1 mmHg) arterial pressure after 90 min of exercise in air polluted condition, while in filtered air condition exercise-induced systolic hypotension (-6.2 mmHg) was observed. This health outcome agrees with a previous finding showing a lowered exercise-induced hypotension at air polluted (- 1.6 mmHg) compared to filtered condition (−2.3 mmHg) (Kubesch et al., 2015). Although the present study does not allow determination of the mechanism by which air pollution annuls the exercise-induced hypotensive effect, this may be related to the reduction in NO synthesis often observed in a polluted environment (Brucker et al., 2013). As a powerful vasodilator, NO reduction might decrease arterial smooth muscle relaxation, increasing arterial stiffness and peripheral arterial resistance (Virdis and Taddei, 2016). From a practical perspective, these findings suggest that endurance exercise exceeding 1 h in an air polluted environment can produce undesirable effects in the arterial pressure regulation.

The present study has some limitations that need to be considered. Firstly, the reduced sample size ($n = 10$) can be a limiting factor to extrapolate our data to general population. In addition, the participants of the current study were considered healthy. This could be especially relevant because previous findings have suggested that individuals with inflammatory disorders and cardiovascular diseases could be more susceptible to the negative effects of air pollution (Franklin et al., 2015; Brook et al., 2017). Finally, the interval between experimental sessions (minimum of 72 h) might be considered short when compared to previous studies that adopted 4 (Kubesch et al., 2015) or 7 (Giles et al. (2014) days of interval between experimental sessions. However, it is important to note that we used a counterbalanced design that might mitigate some possible order effect in the experimental sessions.

In conclusion, the present study yields novel findings regarding air pollution exposure consequences of inflammatory and cardiovascular responses during endurance exercise performed in an urban area. Our results reveal that pro-inflammatory response to exercise in a polluted environment is exacerbated in a time-dependent manner, where 60 min of endurance exercise is a potential ceiling after which there are no further positive health effects. Furthermore, it demonstrates that 90 min of exercise under air pollution annuls the exercise-induced hypotensive arterial pressure effect.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Leonardo A. Pasqua: Conceptualization, Methodology, Writing - original draft, Writing - review & editing, Funding acquisition, Resources. **Mayara V. Damasceno:** Methodology, Writing - review & editing. **Ramon Cruz:** Methodology, Writing - review & editing. **Monique Matsuda:** Methodology, Writing - review & editing. **Marco A.G. Martins:** Conceptualization, Methodology, Writing -

review & editing. **Mônica V. Marquezini:** Formal analysis. **Adriano E. Lima-Silva:** Formal analysis. **Paulo H.N. Saldiva:** Writing - review & editing, Funding acquisition, Resources. **Romulo Bertuzzi:** Conceptualization, Methodology, Formal analysis.

Acknowledgements

L.A.P. was supported by Coordination for the Improvement of Higher Education Personnel (CAPES) with a PhD scholarship.

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