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Physiological Responses to Acute Airborne Particle Exposure during Maximal Aerobic Power

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ABSTRACT

The effect of the exposure to airborne particles on the physical performances achieved by athletes was investigated. Respiratory and cardiovascular parameters of nine subjects (volunteers, regularly performing physical activity) were measured during their high-intensity exercise performed indoor on a cycle ergometer. A steady-state concentration of airborne particles was generated through incense burning.

Two different particle exposure scenarios were tested: low (no source in operation) and high exposure (particle generation from incense burning phenomena). Alveolar-deposited surface area doses received by subjects during low and high exposure tests were measured equal to 22.7 ± 8.58 and $1.18 \pm 0.22 \times 10^3$ mm^2, respectively.

Oxygen uptake at the peak of the physical activity resulted statistically higher during high exposure tests, whereas lower peak heart rate values were measured for such scenario.

In terms of mechanical efficiency, a higher peak metabolic power was recognized for subjects performing tests in high exposure conditions: results were statistically different (about 1%) with respect to low exposure tests. On the other hand, measurements of exhaled nitric oxide (parameter associated to airway inflammations) performed during the tests showed no significant differences between the two exposure scenarios.

Keywords: Air pollution; Physical exercise; Airborne particle exposure; Oxygen consumption; Exhaled nitric oxide.

INTRODUCTION

According to the World Health Organization, WHO (WHO, 2010), substantial benefits can be gained from regular physical activity. Evidences from scientific and medical studies indicate that participation in regular physical activity leads to additional improvements in health status, as reported by the American College Sport Medicine guidelines (ACSM, 2005), reducing the risk of several diseases (heart disease, stroke, type-2 diabetes, hypertension, cancer and depression) (Warburton *et al.*, 2006; Haskell *et al.*, 2007). The Global Recommendations on Physical Activity for Health (WHO, 2010) provides guidance on the relationship between physical activity and health benefits (i.e., frequency, duration, intensity, type, and total amount of physical activity needed for health enhancement and prevention of non communicable diseases, NCDs) considering three age groups: 5–17 years old; 18–64 years old; > 65 years old. In adults aged 18–64, physical activity includes leisure time physical activity, transportation (e.g., walking or cycling), occupational (i.e., work), household chores, play, games, sports or planned exercise, in the context of daily, family, and community activities.

Most accessible physical exercise, such as walking, cycling and running occur outdoors, typically in urban areas which represent microenvironments with a high exposure to airborne particles (Buonanno *et al.*, 2011; Giles and Koehle, 2014; Nyhan *et al.*, 2014). In fact, air monitoring studies have shown that athletes and people who regularly exercise outdoor (even commuters) are exposed to high short-term particle concentrations typical of the urban environment.

Exposure to airborne particles is associated with various adverse health outcomes including heart and respiratory diseases, lung cancer, asthma, and mortality (Brunekreef and Holgate, 2002; Kreyling *et al.*, 2006; Pope and Dockery 2006; Buonanno *et al.*, 2013). A better understanding of physiological responses to airborne particle exposure during exercising represents a key issue in the evaluation of the

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related health effects. Indeed, risks for athletes could be magnified because of: i) the increased pollutant amount inhaled due to the increased minute ventilation (Carlisle and Sharp, 2001); ii) the larger fraction of air inhaled through the mouth and effectively bypassing the normal nasal filtration mechanisms; iii) the increased velocity of the airflow carrying pollutants deeper and deeper into the respiratory tract. Even though the increase of minute ventilation during exercise affects the mechanism of particle deposition, which can increase even more than 4.5-fold during exercise (Daigle *et al.*, 2003), the scientific literature is not consistent in these findings. Studies on the super-micrometric particle deposition reviewed in Schulz *et al.* (2000) have shown that physical exercise may result either in an unchanged or an enhanced/increased deposition rate per breath. This is reflected by a remarkably increased inter-subject variability in particle deposition during exercise. Despite these individual differences, the increase in minute ventilation from rest to exercise always increases deposition per unit of time. This increase is roughly proportional to the increase in minute ventilation.

A suitable marker in studying the exposure-response relationship in terms of airway inflammation is represented by the exhaled nitric oxide (eNO). Nitric oxide is conveyed by different cell types in the respiratory tract including epithelial cells, macrophages, neutrophils, mast cells and vascular endothelial cells (Ricciardolo, 2003; Jacobs *et al.*, 2010). A number of demographic, anthropometric and biological factors (such as age, gender, height, smoking, infection and allergy), reviewed by Abba (2009), can affect the eNO concentration levels. Previous studies found that eNO can be positively associated with exposure to air pollution both in healthy adults and asthmatic subjects (Jansen *et al.*, 2005; Adar *et al.*, 2007) and also with exposure to indoor cooking aerosol in women (Stabile *et al.*, 2013) as well to electronic and tobacco cigarettes (Marini *et al.*, 2014). As regard the correlation between physical activity and eNO outcomes, mild physical exercise were addressed by Clini *et al.* (2000). They found that nitric oxide concentrations in exhaled air (eNO) significantly decreased during physical exercise; similar results were also found by Persson *et al.* (1994) in healthy subjects during exercise. However further researches are required to increase the limited evidences on the eNO changes related to the physical exercise.

The aim of this work is to investigate respiratory and metabolic effects of low and high particle exposure during high intensity exercise in subjects regularly performing physical activity, in particular intensity exercise close to maximal aerobic capacity. This is the first study aimed to determine the effects of simultaneous particle doses and physical exercise on cardio-respiratory parameters during the exercise itself. To this aim, aerosol characterization, heart rate, and ventilatory parameter measurements were carried out as well as exhaled nitric oxide measurements. Low and high exposure conditions were tested in order to highlight the possible effect of the particle dose on cyclist respiratory, cardiovascular and physiological outcomes.

MATERIALS AND METHODS

Study Design, Study Population and Sampling Site

Tests were carried out in a naturally-ventilated 48-m^3 room of the Sport and Exercise Physiology laboratory "Marco Marchetti" (SEPlab) at the University of Cassino and Southern Lazio (Italy) between January and April 2014. The study involved 9 subjects (men) aged 40.7 ± 6.9 years (height 1.77 ± 0.07 m, body mass 77.1 ± 11.6 kg, Body mass index 24.5 ± 2.5 kg m^{-2}) (Table 1). Selected subjects are amateur athletes, i.e., ordinary people who regularly exercise. They were asked to present recent clinical analyses in order to check their health and allergy status. In particular, non-atopic and non-smoking subjects with no history of respiratory or cardiovascular diseases were considered. The Administrative Board of the University of Cassino and Southern Lazio approved the study, and informed written consent was obtained.

Two particle exposure scenarios were considered during the experimental analysis: low and high exposure. Two tests for each scenario (and each subject) were performed. All exposure tests lasted 90 min; the following procedure was considered: i) 30 min exposure at low/high particle concentration level before physical trial (pre-exercise), ii) about 30 min (29 ± 2 min) of physical trial on a cycle ergometer, including warm-up and recovery (Monark 828E Ergonometer Bike), during which the volunteers were still exposed to the low/high particle concentration level, iii) 30 min exposure at low/high particle concentration level after physical trial (post-exercise). Participants were asked to abstain from: i) exhaustive exercise and alcohol within 24 h before the test, ii) caffeine within 6 h before the test, iii) food or non-water beverages within 2 h before the test. Moreover, cyclists were also asked to have the same meal

Table 1. Summary of the physical characteristics and others generic information of the study subjects.

Subjects	Age (years)	Height (m)	Body mass (kg)	BMI (kg m^{-2})	Job
C_1	49	1.63	55	20.70	employee
C_2	45	1.78	71	22.40	teacher
C_3	49	1.82	97	29.28	teacher
C_4	43	1.80	82	25.30	teacher
C_5	34	1.74	77	25.43	military
C_6	42	1.76	76	24.53	lawyer
C_7	29	1.84	87	25.69	PhD student
C_8	35	1.71	71	24.28	student
C_9	40	1.86	78	22.54	lawyer

before the 4 tests they performed. Each test was carried out at the same time of the day.

Instrumentation and Quality Assurance

In order to characterize the exposure to airborne particles, in terms of number, surface area and mass concentration the following instruments were considered:

- a butanol-based Condensation Particle Counters (CPC TSI 3775; Shoreview MN, USA) to measure number concentrations up to 1×10^7 part. cm^{-3} of particles down to 4 nm with 1-s time resolution;
- a Nanoscan SMPS spectrometer 3910 (TSI Inc. Shoreview, MN, USA) to measure particle number distributions in the range 10–420 nm with a sampling time equal to 60 s;
- a NanoTracer (Philips Aerasense) to measure the particle number concentration in the range 10–300 nm with a 16-s time resolution that is also able to evaluate the alveolar deposited surface area;
- a DustTrak™ DRX Aerosol Monitors Model 8534 (TSI Incorporated, St. Paul, MN, USA) to measure different fractions of particulate matter concentrations (PM_1, $PM_{2.5}$, PM_{10}) with 30-s time resolution.

The CPC 3775 was calibrated before the experimental campaign by comparison with a TSI 3068B Aerosol Electrometer using NaCl particles generated by a Submicrometer Aerosol Generator (TSI 3940) (Stabile *et al.*, 2013).

The Nanoscan SMPS 3910 is a portable differential mobility particle sizer spectrometer able to size particles according to their electrical mobility. Metrological analysis performed by Stabile *et al.* (2014) showed that the Nanoscan SMPS is accurate in evaluating particle number distributions of different aerosols.

The NanoTracer works by diffusion charging, using an electrometer that measures the number particle concentration by means of the current induced by previously charged particles collected on a filter inside a Faraday cage. The NanoTracer is also able to evaluate the deposited particle surface area per unit volume of inhaled air in some regions of the respiratory tract (we focused on the alveolar one) through a semi-empiric algorithm implemented by Marra *et al.* (2010). Therefore, it can be also used to calculate the dose experienced by people. The NanoTracer was set in advance mode (16 s of sampling time), therefore particle concentrations and average particle sizes were measured (Buonanno *et al.*, 2014). It was calibrated at the beginning of the experimental campaign for data quality assurance by comparison with: i) a Condensation Particle Counter (CPC, TSI Model 3775) for the particle number concentration; ii) a Nanoparticle Surface Area Monitor (NSAM, TSI Model 3550) to assess the human lung-deposited surface area of particles (reported in μm^2 cm^{-3}) corresponding to alveolar (A) region of the lung (Buonanno *et al.*, 2014).

The DustTrak operates on the basis of light scattering technique where the amount of scattered light is proportional to the volume concentration of the aerosol. The particulate matter (PM) values measured through the DustTrak were corrected by comparison with the gravimetric values, as the instrument was calibrated for the specific aerosol studied at the beginning of the experimental campaign.

The CO_2 concentration was determined on the basis of a non-dispersive infrared (NDIR) sensor, the TSI Model 7515 IAQ-CALC^{TM} (TSI Incorporated, Shoreview, MN, USA; CO_2 concentration range: 0–5000 ppm) calibrated by the manufacturer at the beginning of the experimental campaign.

Five measurements were performed in the room under investigation, keeping windows and doors closed during the test. The average AER was measured equal to 0.29 ± 0.09 h^{-1}.

The eNO was measured through an electrochemical analyser NObreath® (Bedfont Scientific Ltd., Rochester, Kent, UK; measurement range: 1–300 ppb, at a constant flow rate of 50 mL s^{-1}) according to the American Thoracic Society and European Respiratory Society guidelines (ATS/ERS, 2005). The NObreath was recently evaluated by Antus *et al.* (2010), who assessed its ability in performing reliable, reproducible eNO measurements: it showed a good agreement with previously calibrated instruments (Pisi *et al.*, 2010).

A temperature control was also performed in the room through a mini data logger Delta ohm HD 206 made up of a NTC (negative temperature coefficient) 10 kΩ thermistor. Data were sampled and recorded every 5 min.

All experimental trials were carried out on a cycloergometer at constant friction (Ergomedic Monark 894E Peak Bike, Vansbro, Sweden™), whereas oxygen consumption and carbon dioxide production were measured by a breath-by-breath metabolimeter with a 1-s time resolution (Cosmed K4 b2, Rome, Italy™). It was also used to acquire Heart Rate (HR) which was continuously measured by a sensor positioned on the chest (Polar, T31, Kempele, Finland™).

Methodology Description

As reported in the previous section, two sets of experimental tests were performed during two exposure scenarios: low and high particle exposure. In the low exposure scenario, subjects were exposed to the concentration of the ambient room air with no indoor sources of particles. In the high exposure scenario, subjects were exposed to the aerosol generated by incense burning phenomena that represents an important source of fine and ultrafine particles (Stabile *et al.*, 2012). Sticks of citronella incense were used to generate particle number concentrations up to 1.5×10^5 part. cm^{-3} in the room. Two sticks every 15 min were burnt in order to maintain a steady-state particle concentration level in the room. The CPC, Nanoscan SMPS, NanoTracer, DustTrak, and IAQ-CALC were placed on a 1-m high table in proximity of the bicycle ergometer in order to measure the exposure received by the subjects at the "individual scale" (i.e., sampling location within 3 m of the cyclist, Cattaneo *et al.*, 2010).

All the experimental tests included about 30 min of exercise trial. The same trial was considered for all the tests and it consisted of: measurements of oxygen uptake ($\dot{V}_{O_2}$, L min^{-1}) at rest level, with the subjects in an orthostatic posture for 5 minutes before physical exercise; a warm-up phase (3 minutes) performed at 60 revolution per minute (RPM) with a load of 1 kg; an exercise phase (about 10 minutes: 9 ± 2 minutes) where the load was increased by 0.1 kg every 20 seconds; and a recovery phase of 10 minutes with the subject in a setting quiet position on the bike. In the exercise phase, the load was increased until to occur almost 2 of the following 3 conditions considered as indicative of exhaustion: i) no further increase of $\dot{V}_{O_2}$ during mechanical power (kg RPM) increase, ii) the respiratory exchange ratio was equal to 1.15, and iii) 95% of theoretical maximal heart rate was reached. The $\dot{V}_{O_2}$ and Heart Rate (HR; b min^{-1}) at exhaustion represent the peak values ($\dot{V}_{O_2\_peak}$, HR_{peak}).

$\dot{V}_{O_2}$, pulmonary ventilation $\dot{V}_E$, and carbon dioxide output $\dot{V}_{CO_2}$ measurements were also adopted to estimate the ventilatory threshold (VT), according to the Wasserman *et al.* (1983) findings. All data of physiological profile were expressed as averaged (sd). The corresponding energy of peak value was estimated by multiplying the O_2 volume (mL kg^{-1}) by 21 (equivalent energy in Joule). These values were taken in account in the result section for each subject and test.

Comparison between ventilatory parameters, obtained by each subject in low and high exposure scenarios, were made through the Student's *t* test in order to verify if there is a difference in physical effort when performing the same test at different particle exposure. A p value < 0.05 was regarded as statistically significant. All the tested data were previously checked for normality in order to realize the pertinence of the Student's *t* test: a Shapiro–Wilk test was applied.

In order to recognize a link between exposure to particle concentrations during exercise and possible respiratory effects, the dose-response correlation was evaluated putting into relation the dose experienced by the subjects, in terms of alveolar-deposited surface area (mm^2), to the eNO data. Particle surface area metric was chosen as it was recently suggested to have a more direct relationship with inhalation toxicity than particle mass concentration (Giechaskiel *et al.*, 2009; Cauda *et al.*, 2012; Oberdörster, 2001; Sze-To *et al.*, 2012). Doses received by each subject were estimated from the alveolar deposited surface area concentration measured through the NanoTracer. In particular, the doses (mm^2) were determined multiplying the alveolar deposited surface area concentration (S_{al}) by the time spent in the room and the inhalation rate (Klepeis, 2006; Buonanno *et al.*, 2012). Comparison between the doses received by each subject in low and high performances were made through the Student's *t* test. A p value < 0.05 was considered as statistically significant. Data were previously tested through a Shapiro–Wilk test evaluating their normality and the pertinence of the Student's *t* test.

Short-term respiratory effects were identified measuring the amount of NO exhaled by the subjects every 30 min. In particular, measurements were performed before and after each of the three steps of the test: a) before the pre-exercise 30-min exposure; b) just before the physical trial, i.e., after the pre-exercise 30-min exposure period; c) after the about 30-min of physical-trial (post-exercise); d) after the complete 90-min long exposure test. Subjects were asked to inhale ambient air to near total lung capacity and, after 3 s, guided by an acoustic signal, to exhale for 10 s through a disposable mouthpiece into the NO breath device. The reproducibility of the measurement was improved with three parallel readings in order to obtain suitable measurements useful in clinical practice (Antus *et al.*, 2010). Therefore, in this study, eNO measurements taken every 30 min were expressed as average on three values.

Furthermore, a zero-test of the NObreath device was performed by sampling indoor air before each test in the room. The instrument was zeroed to the actual indoor concentration before every eNO measurement. This procedure, named ambient NO test, allowed the instrument to settle to zero each time before testing eNO in the subjects as reported in the NObreath manual. Differences in 30-min-spaced eNO data for each exposure scenario were tested through the analysis of variance (ANOVA) and a post-hoc Tukey–Kramer. A p value < 0.05 (95% confidence level) was considered significant. Data previously underwent the normality Shapiro–Wilk test.

RESULTS AND DISCUSSION

Exposure and Dose Measurements

In Table 2 average total particle number concentrations (measured through the CPC 3775) and distribution (measured through the Nanoscan SMPS 3910) obtained during both low and high exposure scenarios are reported as well as alveolar-deposited surface area (measured through the Nanotracer) and PM_{10} (measured through the DustTrak 8534) concentrations. Data represent the mean (and standard deviation) values of the complete 90-min long tests, averaged across the nine subjects involved. Exposure levels experienced by cyclists during high exposure scenario were statistically higher than those measured in low exposure conditions. Average particle number (> 1×10^5 part. cm^{-3}) and alveolar-deposited surface area concentrations (> 1×10^3 μm^2 cm^{-3}) whose subjects were exposed to during the high exposure scenario were similar to those typically occurring in highly polluted urban areas (Kaur *et al.*, 2009; Morawska *et al.*, 2009; Buonanno *et al.*, 2011). Standard deviations of the data collected during high exposure scenario show that tests were performed exposing subjects to steady-state particle concentration levels. Concentration levels obtained for the low exposure scenario were typical of rural areas and sites not directly exposed to particle sources (Buonanno *et al.*, 2010; Buonanno *et al.*, 2011). The particle number distribution modes for low and high exposure scenarios were measured equal to 109 and 159 nm, respectively. The first one represents the typical particle number distribution mode measured for outdoor aged aerosol (Buonanno *et al.*, 2010), whereas the latter is the characteristic mode of the aerosol produced through incense burning phenomena (Stabile *et al.*, 2012). PM_{10} concentrations were measured equal to 25 μg m^{-3} and 2.29 mg m^{-3} during low and high exposure tests, respectively.

Table 2. Average particle concentration levels experienced by subjects during their physical trials in both low and high exposure scenario: particle number (N, measured through the CPC 3775), alveolar deposited surface area (S_{al}, measured through the Nanotracer), PM_{10} (measured through the DustTrak 8534), and particle number distribution parameters (measured through the Nanoscan SMPS 3910) are reported.

Exposure scenario	N (part. cm^{-3})	mode (nm)	Geometric standard deviation (nm)	S_{al} (μm^2 cm^{-3})	PM_{10} (mg m^{-3})
Low exposure	$4.22 \pm 0.92 \times 10^3$	109 ± 17	1.92 ± 0.03	20.1 ± 8.1	0.025 ± 0.013
High exposure	$1.42 \pm 0.10 \times 10^5$	159 ± 10	1.55 ± 0.02	$1.07 \pm 0.07 \times 10^3$	$2.29 \pm 0.28 \times 10^3$

Average alveolar-deposited surface area doses for low and high exposure tests were measured equal to 22.7 ± 8.58 and $1.18 \pm 0.22 \times 10^3$ mm^2, respectively. The doses experienced during high exposure scenario were similar for all the subjects under investigation, since a standard deviation lower than 20% was detected. Such dose corresponds to about half of the average daily alveolar-deposited surface area dose of an Italian adult (> 2×10^3 mm^2 day^{-1}; Buonanno *et al.*, 2012b).

Physiological Parameters: Heart Rate, Oxygen Uptake and Workload Peak Values

In Fig. 1 the oxygen uptake change measured for the subject C_4 as a function of the increase of mechanical workload is reported: it can be considered as representative for all subjects. In particular, the $\dot{V}_{O_2}$ increases proportionally to the mechanical power growth and its increase was comparable for both low and high exposure up to moderate work load condition (up to the ventilatory threshold, VT). As the work load exceed the ventilatory threshold, the $\dot{V}_{O_2}$ onset becomes greater in high than in low exposure. Since the VT is the point during exercise training at which the correlation between the pulmonary ventilation and the oxygen consumption is no more linear, it defines the upper limit of exercise intensity that can be supported uniquely by the aerobic metabolism. In trained and sedentary subjects, the ventilatory threshold is on average equal to 60–75% of peak oxygen consumption.

Table 3 shows average values for $\dot{V}_{O_2 peak}$, HR_{peak} and mechanical work load (WL_{peak}), obtained during the ramp exercise test, performed on the bicycle ergometer under low and high exposure test conditions. $\dot{V}_{O_2 peak}$, measured during high exposure, was significantly higher than that found for low exposure conditions, both in terms of absolute value and normalized by body mass. On the contrary, HR_{peak} was significantly lower under high than under low exposure. Low and high exposure WL_{peak} values were equal according to the experimental design.

In Table 4 average values of Peak Metabolic Power ($MetP_{peak}$), calculated as energy expenditure ($\dot{V}_{O_2 peak}$) by equivalent energy (about 21 J) per time unit, Peak Mechanical Power (MP_{peak}), and Mechanical Efficiency (ME) are shown for both high and low particulate exposure scenarios. The $MetP_{peak}$ resulted statistically greater in high

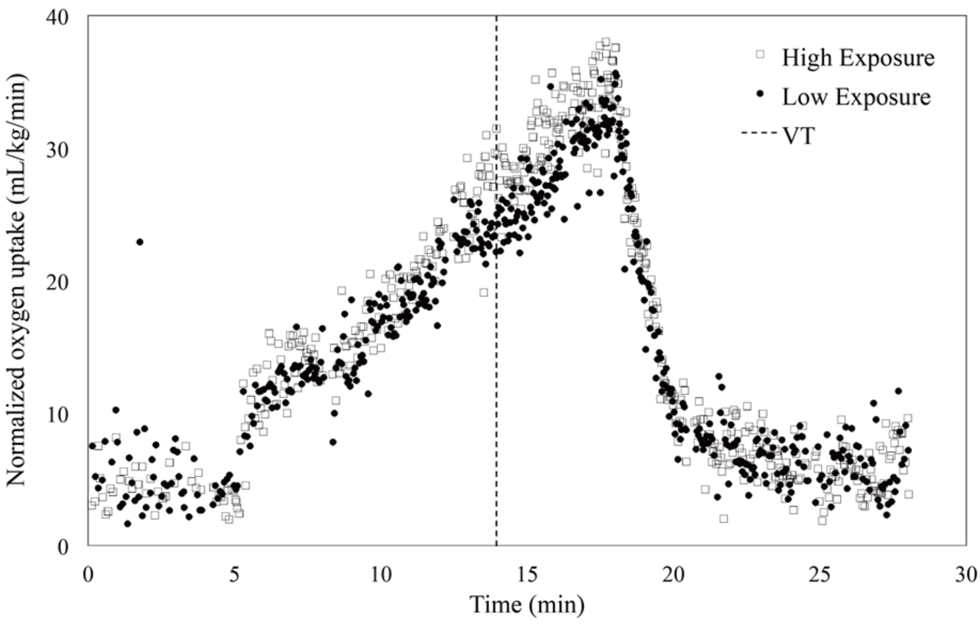

Fig. 1. The oxygen uptake at exercise onset obtained during maximal aerobic power test. The behaviors of oxygen uptake is shown during the exhaustive incremental test performed during low and high exposure to airborne particles. The vertical line indicates the ventilatory threshold.

Table 3. Metabolic, cardiac and mechanical parameters obtained by maximal aerobic power test

	Oxygen uptake, $\dot{V}_{O_2 peak}$ (L min^{-1})	Oxygen uptake normalized by body mass (mL kg^{-1} min^{-1})	HR_{peak} (b min^{-1})	Load (kg)
Low Exposure	3.0 ± 0.12	38.4 ± 4.18	175 ± 5.9	4.1 ± 0.1
High Exposure	3.2 ± 0.20*	40.1 ± 4.57*	173 ± 6.4*	4.1 ± 0.1nd

* $p < 0.05$
nd no differences.

Table 4. Peak Metabolic and Mechanical Power and relative Efficiency

	Metabolic Power, $MetP_{peak}$ (kW)	Mechanical Power, MP_{peak} (kW)	Mechanical Efficiency, ME (%)
Low Exposure	1.05 ± 0.068	0.23 ± 0.028	22.4 ± 1.88
High Exposure	1.09 ± 0.048*	0.23 ± 0.028nd	21.5 ± 2.08*

* $p < 0.05$
nd no differences.

exposure scenario compared to the low one, whereas ME was lower to that measured under low exposure condition (about 1%). As expected, MP_{peak} did not differ significantly because the work load was the same for the two exposure tests due to experimental design.

In a recently research, Giles *et al.* (2014) studied both respiratory and cardiac responses to low and high-intensity during cycling in two different exposure scenarios: exposure in a room to diesel exhausts, exposure in a room to filtered air. Authors demonstrated an increase of energy expenditure per work load with resulting decrease of ME during low intensity exercise at 48% of $\dot{V}_{O_2 peak}$, whereas no difference was found during high intensity exercise at about 77% of $\dot{V}_{O_2 peak}$. Moreover, no main effects of exposure on heart rate were recorded (Giles *et al.*, 2014). In the present research results show that performing high-intensity exercises when exposed to high particle concentrations may require a major energy expenditure. This could be probably due to a greater involvement of respiratory muscles and the cardiovascular system causing a lower ME and a lower endurance to high workload. In particular, this finding may be useful for those who practice outdoor competitive exercise since a reduction of the ME could affect the performance outcome.

Differently from previous studies, HR_{peak} value was found to decrease during high exposure, this could be due to a decrease in cardiac efficiency specifically at high exercise intensity due to altered compensatory mechanisms aiming to maintain the cardiac output in response to an increase in vascular peripheral resistance induced by the particular matter. Nelin *et al.* (2011) report the effects of particulate matter on the cardiovascular system directly by entering into the systemic circulation. This process causes myocardial dysfunction through mechanisms of reactive oxygen species production, calcium ion interference, and vascular dysfunction.

eNO Results

In Table 5 eNO data measured during complete 90-min long test, averaged across the nine subject, are reported. Average data measured before the pre-exercise exposure (initial value), just before starting the physical trial (i.e., at the end of the pre-exercise session), at the end of the about 30-min physical-trial (i.e., post-exercise), and at the end of the complete 90-min long exposure test (final value). Statistically similar eNO initial values were measured for both low and high exposure tests (17.1 ± 6.3 and 15.8 ± 7.9, respectively). Such values were typical of non-atopic, non-smoking adults, whereas higher and lower values are expected for allergic and smoking subjects, respectively, as measured by Travers *et al.* (2007), Dressel *et al.* (2008).

Similar eNO trends for high and low exposure scenarios were measured. In particular, ANOVA and Tukey–Kramer tests applied to the data clearly showed that during the two exposure scenarios eNO data measured every 30 min were statistically similar: i.e., no increase in eNO values was detected for cyclists when exposed to both high and low particle concentrations. Therefore, even if during high exposure scenario cyclists received a great particle dose ($> 1 \times 10^3$ mm^2), no particle dose-response effects were recognized in terms of eNO production. Since the tests were performed on the basis of a rigorous procedure (e.g., no food within 2 h before the tests as described in the methodology section), then making negligible the effect of possible confounders on eNO data, the eNO values are a function of two parameters: i) the physical activity (30-min cycling test), and ii) the exposure to particles. As regards the exposure effect, in previous papers, Marini (2014), Vardavas (2012), Malinovschi (2006) and Min (2014) measured slight eNO reductions during their investigations on eNO outcomes in people smoking cigarettes and electronic cigarettes with/without nicotine. They speculated that a possible pathway in eNO reduction could be represented by the inhibition of alveolar macrophages from producing nitric oxide (Miles, 1999; Therriault, 2003) due to the high particle dose in the alveolar region. In fact, alveolar macrophages, located within the alveolar regions and the small airways of the lungs, are responsible of the main clearance mechanism for

Table 5. eNO average value measured before the pre-exercise 30-min exposure (initial value), just before the physical trial (pre-exercise), after the about 30-min of physical-trial (post-exercise), and after the complete 90-min long exposure test (final value).

Exposure scenario	Initial value (ppb)	Pre-exercise (ppb)	Post-exercise (ppb)	Final value (ppb)
Low exposure	17.1 ± 6.3	18.0 ± 6.5	18.9 ± 6.2	17.6 ± 5.3
High exposure	15.8 ± 7.9	18.8 ± 8.2	17.0 ± 9.1	16.9 ± 7.7

sub-micrometric particles (Stöber, 1994), therefore, when they have to process high amount of particles, they cannot produce NO at their typical rate. Nonetheless, the authors point out that in the present study a synergic effect of physical activity and particle exposure on eNO could be present: then the negligible variation in eNO data cannot be immediately referred to the particle exposure, therefore, eNO can be considered a proper indicator only when no physical activity is performed or, on the contrary, if the effect of the physical activity is properly taken into account as a variable in the experiment set up.

CONCLUSIONS

In the present paper the respiratory and metabolic effects of low and high particle exposure during high-intensity exercise in subjects regularly performing physical activity was investigated.

Aerosol exposure characterization as well as physiological, heart rate and ventilatory parameters were measured. In high exposure scenario, particles were generated through incense-burning phenomena reaching particle number concentration typical of highly polluted urban environments (1.5×10^5 part. cm^{-3}). The alveolar-deposited surface area particle doses received by volunteers during low and high exposure tests were of 22.7 and 1.18×10^3 mm^2.

During high exposure tests, statistically significant differences in oxygen uptake, peak heart rate, and required metabolic power with respect to the low exposure scenario were recognized then leading to a reduction in subject mechanical efficiency of about 1%. Otherwise, no differences were measured in terms of exhaled NO.

In conclusion, the present research highlights new information relative to metabolic and cardiac responses at high exposure to airborne particles during high intensity exercise. In particular, the results, regarding the behavior of the cardiovascular system, play a crucial role in health of the individual, because whilst an oxygen consumption increase per power output could affect athletic performance, the alterations of the cardiovascular system, both peripheral and central, could put the individual's life at risk.

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