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Airborne Ultrafine Particle and Acute Physiological Effects during Maximal Aerobic Power Test

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ABSTRACT

BACKGROUND: The practice of physical exercise in polluted areas could lead to adverse health effects that may contribute to the incidence and/or worsening of respiratory and cardiovascular diseases and some types of cancer.

METHODS*:* Male recreational cyclists performed tests in a manner randomized crossover in two environmental conditions: low (environmental noise exposure) and high ultrafine particle concentration. For each trial, oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), respiratory frequency (Rf), tidal volume (Vt), pulmonary ventilation ($\dot{V}E$), and mechanical workload (WL) were measured. Gross efficiency (GE) was determined using the ratio between mechanical power output and metabolic power input. Repeated-measures ANOVA was applied to evaluate differences ($P < 0.05$) between physiological and mechanical parameters and compare oxygen consumption trends in the two scenarios. RESULTS*:* HR, Rf, and VE values do not show any significant difference. On the contrary, $\dot{V}O_{2peak}$ increased ($P < 0.05$) under high exposure (41.6 ± 4.31 mL kg^{-1} min^{-1}), during high-intensity exercise, compared to a low exposure (38.4 ± 4.05 mL kg^{-1} min^{-1}). $\dot{V}O_2$ and GE show differences ($p < 0.05$) between low and high ultrafine particle concentration conditions during exercise above 80% WL_{peak}.

CONCLUSIONS: Present data suggest that high airborne UFPs levels impair recreational cyclists' gross efficiency.

Keywords: Particulate matter, Acute exercise, Oxygen consumption, Mechanical Efficiency, oxygen radicals

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1 INTRODUCTION

The World Health Organization recommends a physically active lifestyle for reducing the risk of several diseases such as cardiovascular, metabolic disease, hypertension, type-2 diabetes, cancer, and depression (Bull *et al.*, 2020). The recommendations on physical activity suggest, for people between 18–64 years, a weekly 150 minutes of moderate-intensity aerobic activity (Piercy *et al.*, 2018). The most common aerobic activities, such as walking, running, or cycling, are typically practiced outdoors. Outdoor exercise improves mental well-being and is psychologically rewarding (Manferdelli *et al.*, 2019). However, large segments of the population live in highly populated metropolitan areas, and physical activity occurs in urban microenvironments characterized by high airborne particle levels. Regarding particulate matter (PM), the European Union has set two limit values for PM_{10} to protect human health. PM_{10} daily mean values should not exceed 50 µg m^{-3} more than 35 times in a year, while the PM_{10} annual mean value should not exceed 40 µg m^{-3} (European Union, 2008). The adverse health effect induced by PM_{10} is related to the ability to penetrate the deepest areas of the human respiratory tract. Inhalation and consequent deposition of these elements are strictly associated with the size of the carrying particles: higher deposition fractions in the lungs are characteristics of submicron and ultrafine particles (UFPs) (Buonanno *et al.*, 2011). For the UFPs daily concentrations, there are not yet limits. Previous studies establish that exposure to high airborne particle concentrations environments correlates with several adverse health outcomes such as heart diseases, respiratory disorders, asthma, cancer, and increased mortality rate (Giles and Koehle, 2014; Buonanno *et al.*, 2013; Pope *et al.*, 2009). The practice of physical exercise in polluted urban areas increases the number of particles inhaled as a result of the increased pulmonary activity (Carlisle and Sharp, 2021; Niinimaa *et al.*, 1981). Previous studies suggest avoiding physical exercise in polluted areas due to increased pollutants inhalation (Carlisle and Sharp, 2021; Buonanno *et al.*, 2013; Pope *et al.*, 2009). Acute adverse effects of PM_{10} (particles with a diameter < 10 µm) were evidenced in predicted maximal oxygen consumption (Gao *et al.*, 2013; Yu *et al.*, 2004) and in female marathon performance (Marr *et al.*, 2010). Furthermore, acute exposure to PM during exercise on a cycle-ergometer impairs maximal accumulated work on a 6-min trial (Marr *et al.*, 2010). This study aimed to evaluate physiological and mechanical parameters, under high UFPs exposure with respect to environmental noise exposure, in healthy and physically active subjects during incremental maximal aerobic tests performed on a cycle ergometer in a fully controlled environmental chamber.

2 METHODS

2.1 Participants

The University of Cassino and Southern Lazio's Institutional Review Board-Biomedical Section approved this study. All participants signed informed written consent, as previously reported (Rodio and Fattorini, 2014). Fifteen (age was 40.7 ± 6.9 years, stature 1.77 ± 0.07 m, body mass 77.1 ± 11.6 kg) males, active, non-smoking recreational cyclists (Araújo and Scharhag, 2016), were involved in this study. Enrolled subjects were researchers and Ph.D. students at the University of Cassino and Southern Lazio, practicing cycling as amateurs, i.e., athletes cycling an amount of 7 hours a week, corresponding to about 400 hours per year. Enrolled subjects presented a valid medical certificate obtained after a medical assessment following the Italian Protocol for Sports Medicine Rating System. No subject with asthma was included. Each subject avoided high-intensity exercise and alcohol 24 hours before testing, consumption of caffeine 6 hours before, and food or non-water beverages the 2 hours before the trial. All participants were also required to have the same meal before all the tests.

2.2 Procedures

Acute effects of high indoor airborne ultrafine particle environmental concentration were evaluated during ramp maximal aerobic power tests compared to environmental noise, defined by the authors as low particle condition and without incense. Low ultrafine particle concentration was recorded in standard indoor conditions. High concentration of airborne ultrafine particles was generated by burning incense, as described, and evaluated by Stabile *et al.* (2012). Specifically, two incense sticks were kept burning for 15 minutes to maintain a steady-state ultrafine particles concentration level. To determine the participant's exposure, ultrafine particle concentration levels were monitored using a condensation ultrafine particle counter (CPC TSI 3775, Shoreview MN, USA), assessing concentrations up to $1 \cdot 10^7$ part. cm^{-3} of ultrafine particles under 4 nm with a 1-s time resolution and a Dust Track™ DRX Aerosol Monitors Model 8534 (TSI Inc., MN, USA) assessing concentrations of different particulate matter fractions (PM_1, $PM_{2.5}$, PM_{10}). The CPC TSI 3775 was calibrated using a TSI 3068B Aerosol Electrometer measuring NaCl particles generated by a TSI 3940 Aerosol Generator. The Dust Track was calibrated for the PM fractions studied before experimental tests. To carry out a risk assessment related to the exposure to incense airborne particles of the fifteen subjects in the experimental campaign, we applied a modified risk assessment scheme for airborne particles, whose details are reported in (Buonanno *et al.*, 2015) and based on an existing risk assessment model (Sze-To *et al.*, 2012).

This scheme used particle surface area as the dosimetry for hazardous chemicals in the form of UFPs and mass as the dosimetry for super-micron particles. The lung cancer risk characterization equation for each pollutant is:

$$ELCR_i = \frac{SF_i}{BW} \frac{m_i}{\text{PM}_{10}} \left(c_f \cdot \delta_S + \delta_M \right) \tag{1}$$

where $ELCR_i$ is the excess lifetime cancer risk of the *i-th* pollutant, SF_i is the inhalation slope factor used to describe the cancer potency of the *i-th* pollutant, BW is the bodyweight of the receptor, m_i is the mass concentration of the *i-th* pollutant present on the PM_{10} mass, δ_S (nm^2 day^{-1}) and δ_M (mg day^{-1}) are the daily particle surface area (S) and mass (M) deposited dose. The conversion coefficient (C_f) was obtained based on exposure to heavy-duty vehicle emissions (Sze-To *et al.*, 2012). It represents the equivalent toxicity of the particle surface area metric expressed as particle mass; it is used since the cancer potency data are referred to as particle mass metric (SF). The C_f was defined as a parameter depending on the physical size rather than the chemical constituent of the particulate matter and, therefore, can also be used for different types of particulate matter (Sze-To *et al.*, 2012).

UFPs concentrations, temperature, and humidity rate were constantly monitored during the trials. Each subject performed two-cycle ergometer tests, separated by a gap of seven days, under low and high airborne UFPs conditions. Physiological parameters were measured to investigate metabolic and respiratory modifications induced by high ultrafine particle exposure. For all the subjects, continuous electrocardiogram monitoring has been carried out (data not shown).

2.3 Experimental Test

Tests were carried out in a 48 m^3 room at the "Marco Marchetti" Sport and Exercise Physiology laboratory of the University of Cassino and Southern Lazio in spring. Although the exercise performance is reproducible in recreational cyclists on the contrary of less trained participants that exhibit greater variability in performance and pacing. All subjects, to reduce variability, have prior performed a familiarization with the exercise protocol, during which was also identified the mechanical peak workload. Each subject carried out in a manner randomized crossover the low and high exposure condition, interspersed each by 7 days; each trial comprised: i) 30 min environmental exposure at rest ii) physical exercise, including warm-up and recovery for about 30 minutes. All physical trials were performed on a cycle ergometer at constant friction (Monark 894E peak Bike, Vansbro, Sweden™), and respiratory and metabolic parameters measurements were carried out by a breath-by-breath metabolimeter (Cosmed K4 b2, Rome, Italy™) (Rodio *et al.*, 2008). According to the legislation, cardiac activity was monitored all along with the trial by using an electrocardiogram, to detect possible signs of health risk in the act (Cosmed Quark 12cpet, Rome, Italy) (Buonanno *et al.*, 2016). Each progressive maximal aerobic power test was structured as follows: 5 minutes of oxygen uptake ($\dot{V}O_2$) measurement in an orthostatic posture before physical exercise; 3 minutes of warm-up executed at 60 RPM with a 1 kg workload (~60 watt); exertion phase conducted at 60 RPM and workload (WL) increase of 0.1 kg every 20 seconds (~18 watts for 1 min) up to exhaustion; recovery phase sitting on the stationary bike for 10 minutes. The workload was increased until to occur two of the following three conditions were considered indicative of exhaustion obtained: i) $\dot{V}O_2$ value not higher than 150 mL^{-1} kg^{-1} min^{-1}, ii) respiratory exchange ratio (RER) equal to 1.15 or above, iii) achieving 95% of theoretical maximal heart rate. Peak oxygen consumption values were used to characterize the fitness level of the subjects, as previously reported (Piercy *et al.*, 2018). Each subject performed trial in low ultrafine particle concentration first, then a second trial was carried out in the other condition after one week. Maximum WL value (WL_{peak}) achieved in Low conditions was set as the WL_{peak} also for the second one (High conditions), to evaluate physiological differences (i.e., oxygen kinetic) in both conditions at the same values of WL_{peak} and its fractions (100%, 90%, 80%, 60%, and 30%). Moreover, gross efficiency (GE) was determined through the ratio between mechanical power output (W) and metabolic input (W). To calculate this parameter, $\dot{V}O_2$ was converted to Joule (J) by using caloric equivalents for oxygen, in the function of relative RER, and a conversion factor of 4.184 kJ per kcal.

2.4 Statistical Analysis

Metabolic, cardiovascular, respiratory, and mechanical parameters measured throughout the trials were compared to investigate physiological modifications induced by the two different environmental conditions. A repeated-measures ANOVA was used to compare values of physiological and mechanical parameters measured during tests and to compare oxygen consumption ($\dot{V}O_2$) trends in the two different scenarios. Data were previously tested through a Shapiro-Wilk test evaluating their normality and the pertinence of the repeated-measures ANOVA. The results were significant at $P < 0.05$. Physiological parameters values mean and SD in each different atmosphere were calculated. Statistical analyses were realized using Stat View version 5.01 (Sas Institute, Inc., USA).

3 RESULTS AND DISCUSSION

Based on the concentrations of the major pollutants on the incense particles (See *et al.*, 2007; Yang *et al.*, 2013) and of the inhalation slope factors obtained from the Office of Environmental Health Hazard Assessment, a lung cancer risk for each participant for all the tests equal to 10^{-8} was estimated. This value is three orders of magnitude less than the accepted threshold value from the Environmental Protection Agency US: therefore, the overall risk of participants can be considered as negligible. In Table 1, UFPs number concentrations and PM_{10} concentrations obtained during experimental trials were summarized. Ultrafine particles number (expressed in part. cm^{-3}) was 33.2 times higher in high concentration conditions than low (environmental noise). As well as for particle number, PM_{10} concentrations (mg m^{-3}) were about 92 times higher in the high than low scenario. Respiratory parameters (VE; Vt; Rf) values obtained during tests in low and high UFPs exposure conditions are reported in Table 2. No significant differences in Rf, Vt, and VE have been detected at different percentages of WL_{peak} in two conditions. Table 3 shows $\dot{V}O_2$, $\dot{V}CO_2$, and RER mean values. By splitting exercise into two phases, light-moderate intensity, below 80% of WL peak, and moderate-high intensity, above it, we could observe different physiological responses. In the first phase, from 0% to 80% of WL_{peak}, no differences between the two environmental conditions were found. In the second phase, while exercise

Table 1. Average (sd) ultrafine particle number concentrations.

Condition	Ultrafine particles number (part. cm^{-3})	PM_{10} (mg m^{-3})
Low exposure	$4.22 \pm 0.92 \times 10^3$	0.025 ± 0.013
High exposure	$142 \pm 0.10 \times 10^3$	2.29 ± 0.28

Table 2. Respiratory indices mean values and sd at different workloads (%WL_{peak}).

WL	WL	Low	High	Low	High	Low	High
(% WL peak)	(watt)	Rf (breaths min^{-1})		Vt (L $breath^{-1}$)		VE (L min^{-1})	
30	71.37 ± 5.83	20.22 ± 3.76	20.63 ± 4.99	1.72 ± 0.36	1.69 ± 0.33	33.37 ± 2.84	33.23 ± 5.69
60	143.25 ± 17.69	23.47 ± 3.12	22.93 ± 3.33	2.42 ± 0.51	2.44 ± 0.46	55.33 ± 7.56	54.31 ± 4.05
80	189.75 ± 21.74	29.79 ± 3.23	29.14 ± 3.81	2.61 ± 0.38	2.73 ± 0.43	77.05 ± 9.10	77.86 ± 9.60
90	216 ± 26.25	36.13 ± 9.06	34.18 ± 6.35	2.89 ± 0.53	2.95 ± 0.48	101.37 ± 19.07	98.82 ± 14.26
100	239.25 ± 28.22	39.27 ± 11.01	36.99 ± 8.89	2.91 ± 0.53	3.02 ± 0.57	111.13 ± 19.07	108.42 ± 22.09

Table 3. Metabolic indices mean values and sd at different workloads (%WL_{peak}).

WL	WL	Low	High	Low	High	Low	High
(% WL peak)	(watt)	$\dot{V}O_2$ (mL kg^{-1} min^{-1})		$\dot{V}CO_2$ (mL kg^{-1} min^{-1})		RER (a.u.)	
30	71.37 ± 5.83	16.54 ± 3.38	17.52 ± 5.41	15.47 ± 3.52	15.41 ± 5.29	0.94 ± 0.07	0.88 ± 0.05
60	143.25 ± 17.69	27.79 ± 2.78	28.63 ± 4.23	27.84 ± 2.52	27.42 ± 3.66	1.00 ± 0.04	0.96 ± 0.05
80	189.75 ± 21.74	34.28 ± 4.4	35.97 ± 4.48	37.27 ± 4.43	37.27 ± 4.43	1.08 ± 0.05	1.04 ± 0.04
90	216 ± 26.25	37.44 ± 3.98	40.74 ± 3.97*	44.13 ± 5.73	44.64 ± 5.75	1.18 ± 0.07	1.11 ± 0.06*
100	239.25 ± 28.22	38.38 ± 4.05	41.63 ± 4.31*	45.62 ± 5.27	45.30 ± 7.04	1.20 ± 0.07	1.12 ± 0.07*

* = in high exposure condition, $\dot{V}O_2$ resulted significantly ($p < 0.05$) higher, while **RER** resulted lower ($p < 0.05$).

increased in intensity and definitively over the ventilatory threshold, a diverging trend, at 80%, 90%, and 100% of WL_{peak}, was detected for $\dot{V}O_2$ and RER statistically differences ($p < 0.05$); the $\dot{V}O_2$ and RER trend values are reported in Table 3 and resulted increasing in high UFPs condition. No differences were found for $\dot{V}CO_2$ all over the ramp tests, even during high-intensity exercise. Table 4 summarize slope and intercepts values representing the oxygen consumption kinetics that have been obtained in two experimental conditions. For both, slope and intercept, significant differences ($P < 0.05$) were found in polluted conditions meaning that high particles concentrations induce an increment in oxygen consumption. Table 5 reports mean values for gross efficiency (GE) at different workloads ($\%WL_{peak}$). The results obtained show that the GE decreased ($P < 0.05$) at workloads above 80% of WL_{peak} in high compared to low ultrafine particle conditions. This result is due to the different behavior of the $\dot{V}O_2$, which significantly increased in high conditions above 80% of WL_{peak} at the same mechanical output (WL), that was fixed experimentally. We could observe that above 80% of WL_{peak}, GE reduced about of 7 and 8% at 90 and 100% of WL_{peak} respectively in the high UFPs condition.

The aim of this study was to determine metabolic and respiratory acute effects, in environmental controlled, induced by UFPs concentrations during high-intensity exercise. Subjects were all physically active and healthy; their fitness level, corrected for age, ranged from average to good, following Physical Activity Guidelines for Americans (Piercy *et al.*, 2018). Their average $\dot{V}O_{2max}$ was about 40 mL kg^{-1} min^{-1}. In this study, UFPs mean concentration in low exposure scenarios was 34 times lower than other conditions. To the best of our knowledge, this is the first time that physiological parameters have been assessed throughout maximal aerobic ramp test, starting from low to reach maximal intensity exercise, fixing the same maximum workload, in a fully controlled environmental chamber and with negligible risk for the subjects' health. Splitting exercise in two phases, no significant differences for $\dot{V}CO_2$ were found, showing that the anaerobic contribution was similar during light-moderate and moderate-high exercise intensity. As for oxygen consumption, a paradoxical situation emerged. Where exercise intensity increased from light to moderate, $\dot{V}O_2$ and mechanical power increased proportionally, the $\dot{V}O_2$ behaviors were comparable for the two scenarios. On the contrary, while exercise intensified, above 80% of WL_{peak}, a higher oxygen consumption under high UFPs exposure compared to low was observed. A higher $\dot{V}O_2$ in high UFPs conditions indicates better aerobic power and, consequently, a positive effect. But considering the ratio between mechanical and metabolic work (GE), the ultrafine particle-rich environment proved to be detrimental. Higher oxygen consumption at the same workload means a negative effect induced by UFPs on gross efficiency, i.e., on performance. Considering that GE is defined as one of the most important functional abilities of athletes (Coyle, 1995), these results suggest that physical performance is impaired by high ultrafine particles concentration levels, especially if the physical activity is carried out mostly at low particulate environments. As mentioned earlier, a higher oxygen consumption under high ultrafine particle concentrations was assessed, but only during sustained exercise above the ventilatory threshold, the performance suffers a remarkable

Table 4. Slope and intercepts values representing the oxygen consumption kinetics in high and low experimental conditions.

	Low		High	
	Slope	Intercept	Slope	Intercept
mean	0.0380	–2.4330	0.0450*	–6.4300*
sd	0.0055	4.6283	0.0047	5.0228

***** = in high exposure conditions, slope and intercept resulted significantly ($p < 0.05$) higher.

Table 5. Mean and sd values for gross efficiency (GE) at different workloads ($\%WL_{peak}$).

WL ($\%WL_{max}$)	GROSS EFFICIENCY (GE)				
	30	60	80	90	100
Low (%)	16.87 ± 2.31	19.24 ± 1.82	20.70 ± 1.26	21.49 ± 1.04	23.24 ± 1.35
High (%)	16.39 ± 3.66	18.88 ± 2.79	19.94 ± 2.2	20.03 ± 1.95*	21.47 ± 1.75*

*****= in High exposure condition GE resulted significantly lower ($p < 0.05$).

decrease in efficiency of 5% higher. It is well known that in mitochondria a small quote of electrons escapes from the mitochondrial oxidative chain generating reactive oxidant species (ROS). At rest conditions, ~2% of electrons generate ROS (Barja *et al.*, 2004; Powers Jackson, 2008). Furthermore, increasing oxygen consumption at several increasing workloads can result in elevated ROS production and oxidative stress (Allen and Tresini, 2000). Previous studies suggest that physical exercise under airborne UFPs exposure increases ROS production and oxidative stress (Kelly, 2003; Brunekreef and Holgate, 2002). So, the vertiginous/accelerated/extremely rapid increment in $\dot{V}O_2$ emerging under high exposure conditions may be justified by considering the synergy of airborne UFPs exposure and high-intensity exercise (above 80% of WL_{peak}) could have amplified ROS production. In this study, tests have been performed in a controlled environmental chamber, where concentrations of UFPs were constant.

4 CONCLUSIONS

Present data suggest that athletes' gross efficiency is impaired in polluted areas and support the notion that results achieved in sports competitions carried out in urban environments (i.e., marathons, cycling, 20- and 50-km walk) could be affected by high airborne UFPs levels (El Helou *et al.*, 2012; Kargarfard *et al.*, 2011; Marr *et al.*, 2010) Furthermore, recreational athletes should train in a low pollution environment since ultrafine particles (mainly UFPs) boost ROS production during high-intensity exercise, which could in turn have an impact on health (Marr and Ely, 2010; Jacobs *et al.*, 2010; Vinzents *et al.*, 2005; Tauler *et al.*, 2002). It should be also noted that the UFPs concentrations level, achieved in this study could be the basis for future studies to identify the safe environmental level for UFPs as for PM_{10}.

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