

Title: Effects of negative air ions on oxygen uptake kinetics, recovery and performance in exercise: a randomized, double-blinded study

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Abstract

Limited research has suggested that acute exposure to negatively charged ions may enhance cardio-respiratory function, aerobic metabolism and recovery following exercise. To test the physiological effects of negatively charged air ions, 14 trained males (age: 32 ± 7 y; $\dot{V}O_{2\max}$: 57 ± 7 mL \cdot min $^{-1}$ kg $^{-1}$) were exposed for 20 min to either a high-concentration of air ions (ION: $220 \pm 30 \times 10^3$ Ions \cdot cm $^{-3}$) or normal room conditions (PLA: $0.1 \pm 0.06 \times 10^3$ Ions \cdot cm $^{-3}$) in an ionization chamber in a double-blinded, randomized order, prior to performing: 1) A bout of severe-intensity cycling exercise for determining the time constant of the phase II $\dot{V}O_2$ response (τ) and the magnitude of the $\dot{V}O_2$ slow component (SC); and 2) a 30-s Wingate test that was preceded by three 30-s Wingate tests to

measure plasma [adrenaline] (ADR), [nor-adrenaline] (N-ADR) and blood [lactate] (B_{Lac}) over 20 min during recovery in the ionization chamber. There was no difference between ION and PLA for the phase II $\dot{V}O_2 \tau$ (32 ± 14 s vs. 32 ± 14 s; $P = 0.7$) or $\dot{V}O_2$ SC (404 ± 214 mL vs. 482 ± 217 mL; $P = 0.17$). No differences between ION and PLA were observed at any time-point for ADR, N-ADR and B_{Lac} as well as on peak and mean power output during the Wingate tests (all $P > 0.05$). A high-concentration of negatively charged air ions had no effect on aerobic metabolism during severe-intensity exercise or on performance or the recovery of the adrenergic and metabolic responses after repeated-sprint exercise in trained athletes.

Keywords: environmental physiology; central fatigue; catecholamine; ergogenic

Introduction

For more than seventy years there has been an interest in the possible effects of air ions on human performance and behavior (Herrington 1935; Yaglou 1937). Air ions are clusters of molecules and they exist in ambient air as either positively or negatively charged ions in a concentration of approximately $0.5-10 \times 10^3 \text{ Ions cm}^3$ (Krueger and Reed 1976). Naturally existing air ions are produced primarily by the action of cosmic and solar radiation, atmospheric lightning, and from frictional forces on water or sand (e.g. waterfalls and sandstorms) (Sulman et al. 1974). Devices that produce air ions artificially are available and provide sufficient energy via ionizing radiation or high-voltage direct current, leading to the loss or gain of an electron and thereby producing an ion. Although artificially induced air-ionization increases the concentration of air ions up to $2 \times 10^6 \text{ Ions cm}^3$, it should be noted that even such a high-level of ionized molecules is still small compared to non-ionized molecules ($\sim 10^{16}$) (Kröling 1985). It has been suggested that a high-level of negatively charged air ions has positive effects on psychological task performance and mood state (Baron 1987; Hedge and Collis 1987) as well as on cardiovascular responses during rest and exercise that may affect oxidative metabolism (Buckalew and Rizzuto 1984; Hawkins and Barker

1978; Inbar et al. 1982; Ryushi et al. 1998). Finally, enhanced enzymatic activities in the tricarboxylic acid cycle and the electron transport chain in pig hearts after exposure to negatively charged air ions have been reported (Krueger and Reed 1976), which may enhance oxidative metabolism.

During exercise in the heat ($\sim 40^\circ\text{C}$), smaller elevations of rectal temperature and heart rate were reported by Inbar et al. (1982). Others observed significantly lower diastolic blood pressure after 1 h of moderate cycling in untrained subjects during recovery in negative air ion compared to normal ambient conditions (Ryushi et al. 1998). In addition, serotonin and dopamine levels were significantly lower in the presence of negative air ions during the recovery period. Although the underlying mechanisms behind such observations are unknown, it has been suggested that negatively charged air ions reducing serotonin levels by accelerating the enzymatic oxidation of serotonin via the monoamine oxidase enzyme system (Iwama 2004) whereas positive charged air ions inducing the opposite (Diamond et al. 1980; Giannini et al. 1986). Whilst chronically low-levels of serotonin are associated with depression and anxiety, elevated serotonin levels, as induced by physical exercise, may lead to fatigue and symptoms of overtraining (Knicker et al.

2011). Thus, a faster decline of serotonin levels along with other stress-hormones may enhance recovery after strenuous exercise. The ability to quickly recover from previous exercise determines training quality and quantity and thus can positively influence performance (Girard et al. 2011).

We therefore tested the efficacy of a 20-min exposure (as recommended by the manufacturer) to a high-concentration of negatively charged air ions applied in an ionization-chamber in two separate parts: Part one tested the hypothesis that the exposure prior to a square-wave exercise, leads to a faster rise in pulmonary oxygen uptake ($\dot{V}O_2$) at the onset of exercise and reduce the $\dot{V}O_2$ slow component compared to a non-ionized environment. The speed of the fundamental $\dot{V}O_2$ response is indicated by the time constant (τ). Several studies have shown that a faster $\dot{V}O_2$ response (i.e. smaller τ) is related to improved endurance performance (Caputo et al. 2003; Jones and Carter 2000; Marwood et al. 2010). A faster $\dot{V}O_2$ response reduces the O_2 deficit, which leads to a reduction in phosphocreatine and glycogen breakdown, and consequently reduces intracellular perturbations (lower levels of lactic acid and inorganic phosphate) (Barker et al. 2008; Jones et al. 2008).

In the second part of this study we tested the effects of the exposure applied after a series of three 30 s sprint cycling bouts on the decay of plasma catecholamine and blood lactate levels and on performance during a fourth sprint after exposure. We hypothesized that resting for 20 min in the ionization-chamber after exercise would enhance recovery (i.e. faster decline in blood parameters) and thus performance in a final Wingate test.

Methods

Subjects

Fourteen trained male subjects from local cycling and triathlon clubs (means \pm SD; age: 32 ± 7 y; stature: 178.8 ± 5.1 cm; body mass: 75.5 ± 7.7 kg; $\dot{V}O_{2\max}$: 57 ± 7 mL \cdot min $^{-1}\cdot$ kg $^{-1}$) volunteered and gave written informed consent to participate in the study, which was approved by the local ethics committee. To enter the study, a training volume of 8-12 h \cdot week $^{-1}$ was required to recruit a cohort with a $\dot{V}O_{2\max}$ of ~ 55 -60 mL \cdot min $^{-1}\cdot$ kg $^{-1}$. All participants were familiar with the experimental procedures used in the present study. The participants were asked to refrain from strenuous exercise in the 24 h before the tests and having abstained from food, alcohol, caffeine and sports beverages for the preceding 3 h. All tests were separated by at least 48 h.

Study design

All tests were completed in an air-conditioned laboratory at an ambient temperature of 20-22°C and a humidity of 40-45%. The participants visited the laboratory on five occasions within four weeks. During each part of the study, the tests were conducted at approximately the same time of the day (± 2 h). The tests for the first part comprised one incremental graded exercise test (GXT) on a friction loaded cycle ergometer (Monark 839E; Monark Exercise AB, Varberg, Sweden) to calculate the work rate required for the square-wave exercise (70% of the difference between the ventilatory threshold (VT) and $\dot{V}O_{2max}$; i.e. $\Delta 70\%$), which was applied twice in a randomized, double-blinded order after spending 20 min in the ionization-chamber at either ionized (ION) or placebo (PLA) conditions. Part two of the study consisted of four 30 s all-out Wingate tests performed on a conventional bike frame mounted on an electromagnetically braked ergometer (Cyclus 2; Avatronnic, Leipzig, Germany) with the gear fixed at 44 x 11. After three Wingate tests subjects spent 20 min in the ionization-chamber at ION or PLA conditions and blood samples were taken at regular intervals. Immediately after the resting period the fourth Wingate test was performed. Again, the protocol was applied twice in a randomized, double-

blinded order. During all tests the position on the ergometers were replicated for each trial and subjects were allowed to use their own cycling shoe / pedal combination and drink water ad libitum.

Ionization-chamber

A commercially available ionization-chamber was used for this study (Airbutler International, Linz, Austria). The chamber (2.5 m²) is made of foamed aluminium and constructed of four wall- and one top-panel. On one side, a door with a window is incorporated. Inside the chamber a power outlet, a dimmer light and air-ventilation is provided, and the ion-generator is mounted on one wall at a height of 2 m. A dummy switch was mounted in the chamber that allows us to “switch on” the ion-generator without producing air-ions (neutral atmospheric condition; PLA). The ion-generator is a 10 x 20 cm module with ten needle-electrodes on it that produce ions by means of corona discharge. During this process a high current is applied on the electrodes, which creates a strong field surrounding the tip. When air molecules passing through this field, charged particles (ions) are generated and dispersed by the small air-ventilator into the chamber. According to the manufacturer, the module has a capacity to produce more than 14×10^6 Ions·s⁻¹. However, the half-life of the ions is short

as they are attracted to other opposite-charged molecules and therefore the ion concentration in close proximity (10 cm) to the ion-generator is $\sim 1.4\text{-}1.6 \times 10^6 \text{ Ions}\cdot\text{cm}^{-3}$.

Before the study the concentration of air-ions in the chamber was measured with an ionometer (Holbach IM 806; Holbach, Wadern, Germany) for both the ION ($220 \pm 30 \times 10^3 \text{ Ions}\cdot\text{cm}^{-3}$) and PLA ($0.1 \pm 0.06 \times 10^3 \text{ Ions}\cdot\text{cm}^{-3}$) condition. On test days, a laboratory technician not involved in the data acquisition or analysis was instructed to operate the dummy switch and to organize the randomization of the trials. In addition, he measured the concentration of air-ions before the participant or the principal investigator entered the laboratory. The temperature and humidity was $22\text{-}24^\circ\text{C}$ and $45\text{-}50\%$, respectively.

Experimental procedures

Incremental graded exercise test

The participants completed a GXT at a cadence of $90 \pm 2 \text{ rev}\cdot\text{min}^{-1}$ to assess maximal measures of power output (P_{max}), oxygen uptake ($\dot{V}O_{2\text{max}}$) and heart rate (HR_{max}). After 3 min baseline cycling at 40 W, the work rate was increased by $20 \text{ W}\cdot\text{min}^{-1}$ until the limit of tolerance. If the last work rate was not completed, maximal power was calculated according to the method of Kuipers et al. (1985): $P_{\text{max}} = P_{\text{L}} + (t/60 \times P_1)$, where P_{L} is the last completed

work rate (W), t is the time for the incomplete work rate (s) and P_1 the incremental work rate (W). Gas exchange and pulmonary ventilation were collected continuously throughout the test via breath-by-breath open circuit spirometry (Oxycon Mobile; VIASYS Healthcare, Hoechberg, Germany). Before each test, the gas analyzers were calibrated with gases of known concentration ($4.99 \text{ Vol}\% \text{ CO}_2$, $15.99 \text{ Vol}\% \text{ O}_2$, VIASYS Healthcare, Hoechberg, Germany). Flow and volume were calibrated with the integrated system according to the manufacturer. The participants wore a facemask and breathed through a low-resistance impeller turbine (Jaeger Triple V). The $\dot{V}O_{2\text{max}}$ was taken as the highest 30 s value attained before volitional exhaustion. Short-range radio-telemetry was used for the measurement of heart rate (Polar Vantage NV; Polar Electro, Kempele, Finland). The VT was determined using the criteria of an increase of the ventilatory equivalent of O_2 ($\dot{V}E/\dot{V}O_2$) without a concomitant increase of the ventilatory equivalent of CO_2 ($\dot{V}E/\dot{V}CO_2$) and the first loss of linearity in pulmonary ventilation ($\dot{V}E$) and carbon dioxide ventilation ($\dot{V}CO_2$) (Beaver et al. 1986).

Square-wave exercise

The work rate required for the square-wave, severe-intensity exercise ($\Delta 70\%$)

was subsequently calculated after the GXT (work rate at VT plus 70% of the difference between the work rate at VT and $\dot{V}O_{2max}$). After a 10-min warm up at 50 W the subjects rested for 20 min in a semi-recumbent position in the ionization-chamber. Immediately after the rest period subjects cycled for another 5 min at 30 W during which they were attached to the metabolic cart. The square-wave exercise started with a 3-min period of baseline cycling at 30 W followed by an immediate increase to the criterion work rate ($\Delta 70\%$) which the subject cycled at for 6 min, followed by a 3-min cool down at 30 W. The subjects were asked to remain the same trunk position throughout the test, which was replicated during the second test and to pedal at a cadence of 90 ± 2 rev \cdot min $^{-1}$. Gas exchange and pulmonary ventilation were collected continuously as described above.

The breath-by-breath $\dot{V}O_2$ data were examined and errant breaths lying more than three standard deviations from the local mean of 5 data points were removed. The filtered data were linearly interpolated to provide second-by-second values and subsequently time aligned to the onset of the exercise (Whipp and Rossiter 2005). The first 20 s of the transition from baseline to $\Delta 70\%$ was deleted to most likely exclude the cardio-dynamic or phase I response (Murias et al. 2011), and a

single-exponential algorithm was used to model the kinetics of the fundamental (phase II) response of $\dot{V}O_2$ by the following equation:

$$\dot{V}O_2(t) = \dot{V}O_{2baseline} + A(1 - e^{-(t-TD)/\tau})$$

where $\dot{V}O_2(t)$, $\dot{V}O_{2baseline}$, A , TD and τ represent the $\dot{V}O_2$ at any given time (t), the $\dot{V}O_2$ at baseline exercise, the amplitude from baseline to its asymptote, the time delay and the time constant of the response, respectively.

The optimal fitting window to estimate the parameters of the fundamental response was identified with a purpose-designed software (LabView 6.1, National Instruments, Newbury, UK), following the methods of Rossiter et al. (2002). Starting from the initial 60 s of exercise, the fitting window was increased iteratively by 5 s to end-exercise. For each fitting window the estimated τ was plotted against time and the onset of the $\dot{V}O_2$ slow component (SC) was determined through visual inspection as the point at which the estimated τ progressively increased following an initial plateau. The parameter estimates were then resolved by least-squares non-linear regression (Graph Pad Prism 5.0, California, USA). The amplitude of the $\dot{V}O_2$ SC was calculated from the difference between the mean of the final 30 s at end-exercise and the asymptote of the

fundamental response. In addition, the end-exercise and the fundamental $\dot{V}O_2$ gain was calculated by dividing the respective amplitudes by the increment in work rate above baseline ($\Delta\dot{V}O_2/\Delta WR$).

Capillary samples were obtained from the hyperemic ear lobe for the measurement of blood [lactate] after baseline cycling, at 2, 4 and 6 min at the $\Delta 70\%$ work rate and at the end of the cool down period (procedures are described below). Heart rate was monitored continuously as described above.

Wingate test

On the arrival at the laboratory subjects rested for at least 20 min in a supine position on a bed after which a venous blood sample was obtained for determination of resting plasma [ADR] and [N-ADR] (procedures are described below). A standardized warm up of 10 min (5 min at 50 W – two 6 s sprints at minute 6 and 7 – 3 min at 50 W) preceded the first 30 s all-out sprint. For the Wingate tests the ergometer was set in the constant-force mode and a resistance of $0.9 \text{ N}\cdot\text{kg}^{-1}$ was applied at a starting cadence of $80 \text{ rev}\cdot\text{min}^{-1}$. The subjects were asked to slowly increase their cadence from $60 \text{ rev}\cdot\text{min}^{-1}$ and to maximally accelerate at a cadence of $75 \text{ rev}\cdot\text{min}^{-1}$. The three Wingate tests were interspersed by a 4-min rest period at 50 W. All tests were performed in a seated

position and strong verbal encouragement was given. After the third Wingate test subjects rested for 20 min in the ionization-chamber in a semi-recumbent position where further venous blood samples were taken at 1, 3, 6, 9 and 12 min post-exercise to determine plasma [ADR] and [N-ADR] (Vincent et al. 2004). In addition, capillary blood samples for the determination of blood [lactate] were obtained at 1, 3, 6, 9, 12, 15 and 20 min post-exercise. Immediately after the resting period subjects cycled for 5 min at 50 W before the fourth Wingate test was performed. Power output (W) was averaged in 3-s intervals and subsequently analyzed for maximum (P_{\max}) and mean (P_{mean}) power. Minimum power (P_{\min}) was analyzed to calculate the fatigue-index (FI) (Coleman et al. 2005): $\text{FI} (\%) = ([P_{\max} - P_{\min}] / P_{\max}) \times 100$.

Blood samples and biochemical analyzes

To obtain the venous blood samples a heparinized Teflon catheter was inserted into an antecubital vein. The cannula was flushed with 2 ml of 0.9% saline solution after the first sample was taken and immediately after the third Wingate test. Blood samples were collected in 5 ml chilled tubes and immediately mixed with the containing EDTA. Within 30 min the blood samples were centrifuged at $4 g$ for 8 min at 4°C and the plasma was removed

and stored at -20°C for later analysis. Plasma [catecholamine] (pgmL^{-1}) was measured by high-performance liquid chromatography (VWR Hitachi, Tokyo, Japan) using a specific kit (Recipe ClinRep, Munich, Germany). The decay of plasma levels of catecholamine was calculated as the difference between the maximum and the 12-min measure (ΔADR , $\Delta\text{N-ADR}$).

To determine blood [lactate] $20\ \mu\text{l}$ capillary blood samples were obtained from the hyperemic ear lobe and diluted immediately in a $1,000\text{-}\mu\text{l}$ glucose system solution. Blood [lactate] (mmolL^{-1}) was measured using an automated lactate analyzer (Biosen S-line; EKF Diagnostic, Barleben, Germany). As a measure of blood lactate accumulation during the square-wave exercise the difference between blood [lactate] at 6 min and baseline was calculated ($\Delta\text{Lac}_{\text{square}}$). In addition, the decay of blood lactate after the Wingate test was calculated as the difference between the maximum and the 20-min measure ($\Delta\text{Lac}_{\text{Win}}$).

Statistical analyses

Statistical analyses were performed with the software package SPSS Statistics 19 (IBM Corporation, Armonk, NY, USA). Descriptive data are summarized as mean \pm standard deviation (SD). Mean differences between treatments are

provided with 95 % confidence limits (CL). The assumption of normality was verified using Kolmogorov-Smirnov's test and Liliefors probability. A two-factorial mixed ANOVA with treatment (ION vs. PLA) and test order of ionization (1 vs. 2) as model factors was used to compare the parameters of the oxygen uptake response as well as the calculated ΔLac , ΔADR , $\Delta\text{N-ADR}$ and FI. Changes in blood measures and power outputs during the Wingate tests were examined using a three-factorial mixed ANOVA with treatment (ION vs. PLA), test order of ionization (1 vs. 2) and time (1...n) as model factors. Significant main effects were followed up with pairwise comparisons employing the Bonferroni procedure for multiple testing (Bender and Lange 2001). Effect sizes are reported as Cohen's d and considered as small (0.2), moderate (0.5) and large (0.8) effects (Cohen 1988). For all statistical analyses, the level of significance was set at $P < 0.05$.

Results

Square-wave exercise

The results of the GXT are presented in Table 1. The $\dot{V}\text{O}_2$ kinetic responses for the ION and PLA conditions are shown in **Fig.1**. Table 2 presents the mean $\dot{V}\text{O}_2$ kinetic parameters for both the ION and PLA condition. The $\dot{V}\text{O}_2$ at baseline was not significantly different between ION

and PLA ($P = 0.43$, $d = -0.24$). No significant differences between the treatments were observed for the phase II $\dot{V}O_2$ time constant ($P = 0.7$, $d = 0.02$), the time delay ($P = 0.61$, $d = 0.08$), the amplitude ($P = 0.78$, $d = 0.04$) and the gain ($P = 0.76$, $d = 0.06$). The magnitude and onset of the $\dot{V}O_2$ slow component were not significantly affected by the treatment ($P = 0.17$, $d = -0.38$ and $P = 0.66$, $d = -0.13$, respectively). In addition, no significant differences between ION and PLA were found for end-exercise $\dot{V}O_2$ ($P = 0.8$, $d = 0.04$) and end-exercise $\dot{V}O_2$ gain ($P = 0.27$, $d = -0.27$).

Blood [lactate] significantly increased during exercise in both the ION and PLA condition ($P < 0.001$) with no significant differences between the treatments ($P = 0.18 - 0.67$, $d = 0.11 - 0.27$) (**Fig.2**). Blood lactate accumulation ($\Delta\text{Lac}_{\text{square}}$) was 10.9 ± 1.4 and $10.6 \pm 1.4 \text{ mmol}\cdot\text{L}^{-1}$ during ION and PLA, respectively ($P = 0.27$, $d = 0.27$). In addition, no significant main effect on heart rate was found ($P = 0.15 - 0.51$, $d = -0.29 - 0.35$) (**Fig.2**). No significant main effects of test order or interactions between test order and treatment were observed for any measure obtained during the square-wave exercise test ($P > 0.05$).

Wingate test

No significant differences between the ION and PLA condition were found for

maximum ($P = 0.12 - 0.48$, $d = 0.08 - 0.23$) and mean power output ($P = 0.10 - 0.75$, $d = 0.04 - 0.12$), as well as for the fatigue index ($P = 0.21 - 0.95$, $d = -0.29 - 0.15$) (**Fig.3**).

Blood [lactate], plasma [ADR] and [N-ADR] significantly increased in response to exercise (all at $P < 0.001$) but were not significantly affected by the treatment ($P = 0.17 - 0.83$, $d = -0.23 - 0.28$) (**Fig.4**).

The decay of blood [lactate] ($\Delta\text{Lac}_{\text{win}}$) was 5.2 ± 1.2 and $5.2 \pm 1.4 \text{ mmol}\cdot\text{L}^{-1}$ during ION and PLA, respectively ($P = 0.96$, $d = 0$). In addition, no significant treatment effect was observed for ΔADR (ION: 445 ± 332 vs PLA: $449 \pm 364 \text{ pg}\cdot\text{mL}^{-1}$; $P = 0.95$, $d = -0.01$) and $\Delta\text{N-ADR}$ (ION: 1884 ± 939 vs PLA: $1974 \pm 1015 \text{ pg}\cdot\text{mL}^{-1}$; $P = 0.81$, $d = -0.1$). No significant main effects of test order or interactions between test order and treatment were observed for any measure obtained during the Wingate tests ($P > 0.05$).

Discussion

The aim of this study was to test the effects of negatively charged air ions on aerobic metabolism and repeated-sprint performance in trained athletes. The principle novel findings of this study are that the application of a high-concentration of negatively charged air ions prior to severe-intensity exercise had no significant effects on any of the parameters describing

the oxygen uptake kinetic response. In addition, no significant effects on recovery of exercise-induced plasma [catecholamine], blood [lactate] and performance were observed when the treatment was applied after a series of all-out maximal exercise. These results therefore do not support the notion that the exposure to negatively charged air ions may be of an ergogenic benefit to athletes. Despite decades of research on whether or not air ions are biologically active only few studies investigated possible effects during exercise (Inbar et al. 1982; Iwama 2004; Ryushi et al. 1998). Iwama (2004) found significantly lower blood [lactate], higher blood pH and improved erythrocyte deformability during 1-h exposure to negative air ions created by water shearing compared to normal ambient air under resting conditions. No such effects were found when negative air ions were created by corona discharge, as used in the present study. It was concluded that a potential mechanism could be that negative air ions bound to water enter the organism via the lungs whereas the lifetime of air ions created by corona discharge might be too short to reach the alveoli sufficiently. Whether or not the respiratory tract is the main location of entry for air ions into the organism remains a topic of discussion (Kröling 1985; Krueger and Reed 1976). It has been argued that the concentration of

artificially created air ions is too small compared to non-ionized molecules in ambient air and there must be a specific receptor in the respiratory tract to be sensitive to changes in air ion concentration to cause any effect (Kröling 1985). Small differences under resting conditions however, do not necessarily indicate positive effects during exercise. Inbar et al. (1982) reported smaller elevations of rectal temperature, rating of perceived exertion and heart rate when their subjects cycled for 3 x 30 min at moderate intensity in the heat (~40°C) under negative air ion or neutral exposure. The control group (both trials under non-ionized conditions) in that study showed no such effects and no significant differences between the groups were reported for oxygen uptake, minute ventilation, oxygen pulse or sweat rate. Another study observed significantly lower diastolic blood pressure after 1 h of moderate cycling exercise in untrained subjects (~40 mL·min⁻¹·kg⁻¹) during 1 h recovery in negative air ion or normal ambient conditions (Ryushi et al. 1998). In addition, serotonin and dopamine levels were significantly lower in the presence of negative air ions during the recovery period. As serotonin is a potent neurohormone acting as a vasoconstrictor, reduced serotonin levels might be associated with reduced blood pressure. In

accordance with the present study, no effects of negative air ions were observed for plasma [ADR] and [N-ADR] or oxygen uptake during exercise and recovery.

Square-wave exercise

In the present study a controlled and double-blinded design was used to measure oxygen uptake kinetics as a non-invasive insight into muscle oxidative metabolism (Krustrup et al. 2009). Training-induced improvements in endurance performance have been reported to result in a faster $\dot{V}O_2$ response to a sudden increase in work rate (Jones and Carter 2000) and thus it is important to evaluate the ability to “switch-on” the oxidative metabolism. It has been shown that muscle phosphocreatine kinetics are closely coupled to the phase II $\dot{V}O_2$ response at the onset and offset of exercise (Barker et al. 2008) and also that a faster $\dot{V}O_2$ response reduce glycogen breakdown, and thus alleviate the accumulation of lactic acid and inorganic phosphate (Jones et al. 2008). In addition, trained endurance athletes have been shown to have superior on-kinetics compared to untrained subjects (Caputo et al. 2003; Koppo et al. 2004).

The phase II time constant in our subjects was assumed to be ~ 30 s and based on previous studies (Berger et al. 2006) the smallest worthwhile effect ($d = 0.8$) was estimated to be 5 s. Based on these

assumptions we were confident to detect such an effect with a required statistical power of 80% with our sample size. Our results show that the mean difference between ION and PLA for the phase II time constant was 0.3 ± 3.2 s ($P = 0.7$; $d = 0.02$) and thus, the statistical power was only 5%. It should be noted however, that firstly such a small difference as observed in the present study is most unlikely to have any meaningful physiological effect; secondly the 95% confidence interval of the estimated time constant vary by $\sim 8\%$ (i.e. 2.5 s at a τ of 30 s) and thirdly the required sample size would increase to $\sim 20,000$ (Lamarra et al. 1987). Although the end-exercise $\dot{V}O_2$ was similar in both conditions ($P = 0.8$; $d = 0.04$), the magnitude of the $\dot{V}O_2$ slow component was small to moderately affected by the treatment, with ION being ~ 80 mL lower compared to PLA ($P = 0.17$; $d = -0.38$) and the statistical power achieved was 26%. As the development of the slow component is related to muscle fatigue and during severe-intensity exercise drives $\dot{V}O_2$ to $\dot{V}O_{2\max}$ this small difference could possibly improve exercise tolerance. As previously shown, a six week continuous or intermittent training intervention is sufficient to reduce the $\dot{V}O_2$ slow component during severe exercise from 0.38 ± 0.10 to 0.29 ± 0.09 L \cdot min $^{-1}$ and from 0.41 ± 0.28 to 0.30 ± 0.28 L \cdot min $^{-1}$,

respectively (Berger et al. 2006). As shown in Table 2, none of the $\dot{V}O_2$ kinetic measures were significantly affected by the treatment. These results indicate that negative air-ions had no meaningful effects on $\dot{V}O_2$ on-kinetics during severe-intensity exercise.

In contrast to previous studies we used trained athletes and investigated the responses to severe-intensity exercise (i.e. Δ 70%), which more closely reflects competitive performance in athletes.

Wingate test

Part two of the present study examined the responses to supra-maximal exercise. The ability to repetitively produce high power outputs is a key variable in many sports. It has been shown that beside the power-producing phase the ability to recover between the bouts is crucial (Girard et al. 2011). Our study was designed to induce a high metabolic and adrenergic response to three 30-s Wingate tests before blood parameters were measured during recovery under both experimental conditions and a subsequent Wingate test was performed. In accordance with previous studies (Jacob et al. 2004; Vincent et al. 2004) our results confirm that a series of three 30-s Wingate tests is sufficient to stimulate anaerobic glycolysis and the release of catecholamines. Blood [lactate] increased from a resting level of \sim 1.1 to \sim 18.5

mmol·L⁻¹ within 6 min after the third Wingate test and decreased to \sim 13.5 mmol·L⁻¹ at the end of the 20-min resting period in the ionization chamber. Likewise, plasma [ADR] and [N-ADR] increased from \sim 80 to \sim 750 pg·mL⁻¹ and from \sim 300 to \sim 3000 pg·mL⁻¹, respectively (**Fig.4**). However, no significant differences between ION and PLA were observed for blood [lactate], plasma [ADR] and [N-ADR] as well as on the respective rates of disappearance. The small effect sizes (ranging from -0.01 to -0.29; statistical power 5-17%) indicate no meaningful effects of air ions on adrenergic or metabolic responses to supra-maximal exercise. In addition, no significant effects of air ions were found on performance during the final Wingate test (P_{\max} : 919 ± 132 vs 909 ± 124 W; $P = 0.48$; $d = 0.08$; P_{mean} : 707 ± 79 vs 699 ± 69 W; $P = 0.27$; $d = 0.11$, for ION and PLA, respectively).

Although catecholamine release is important to immediately provide energy for a short period of time, its chronic elevation has been reported as a factor for central fatigue by increasing serotonin and dopamine levels in the central nervous system (Meeusen et al. 2006). It has been shown that the administration of bupropion, a re-uptake inhibitor for dopamine and nor-adrenaline, improve time-trial performance in a warm environment (30°C) by 9% compared to a

placebo treatment (Watson et al. 2005). Further studies that have elucidated the specific roles of dopamine and nor-adrenaline indicate that the re-uptake inhibition of dopamine might be mainly responsible for this performance improvement (Roelands et al. 2008). Although it was thought that central fatigue and serotonin is involved in the action of negative air ions (Diamond et al. 1980; Giannini et al. 1986), this remains unproved.

One possible explanation for the lack of effect of negative air ions is, that during electrical air ionization reactive oxygen species (ROS), like superoxide, peroxide or ozone are formed and thereby could induce “oxidative stress”. Superoxide is constantly produced in human cells through mitochondrial respiration and together with its reactive molecule (hydrogen) peroxide, acts protectively against micro-organisms (Krueger and Reed 1976) and modulates several factors of mitochondrial biogenesis (Radak et al. 2013). However, excessive levels of ROS in vivo are associated with adverse health effects like cardiovascular disease, DNA damage and cell death (Radak et al. 2013). In addition, the participants in this study were trained athletes, which could possibly alleviate any treatment effect. It is well known that performance changes in response to training interventions or

treatments are much smaller in trained athletes compared to untrained subjects as used in most previous studies. It is therefore possible, that populations with a lower training status might benefit from a treatment as used in the current work. This study has investigated acute effects of air ions immediately after a 20-min application. We cannot exclude however, positive effects on the parameters used in our study, in response to multiple and/or longer exposure to negatively charged air ions. It remains to be shown, whether or not there is a dose-response that affects performance measures. Finally, the present study tested physiological responses to sub-maximal and supra-maximal exercise. In many sports, psychological task performances and cognitive functions are as much important as physiological measures. For example, a positive effect on these skills can enhance the overall performance in team-sports. Further studies are required to address these questions.

In conclusion, the results of the present study suggest that exposure to a high-concentration of negatively charged air ions has no acute effects on parameters of oxygen uptake kinetics, recovery of metabolic- and stress-related blood measures and on performance in trained athletes and therefore is not considered as

an ergogenic intervention in that population.

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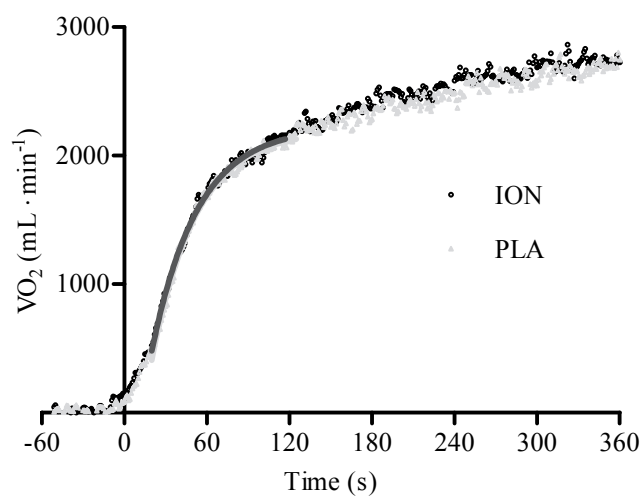
Figure captions

Fig.1 Oxygen uptake response to square-wave exercise transitions for ION and PLA experimental conditions. Error bars are omitted for clarity. Solid lines represent the modelled responses. No significant treatment effects ($P > 0.05$)

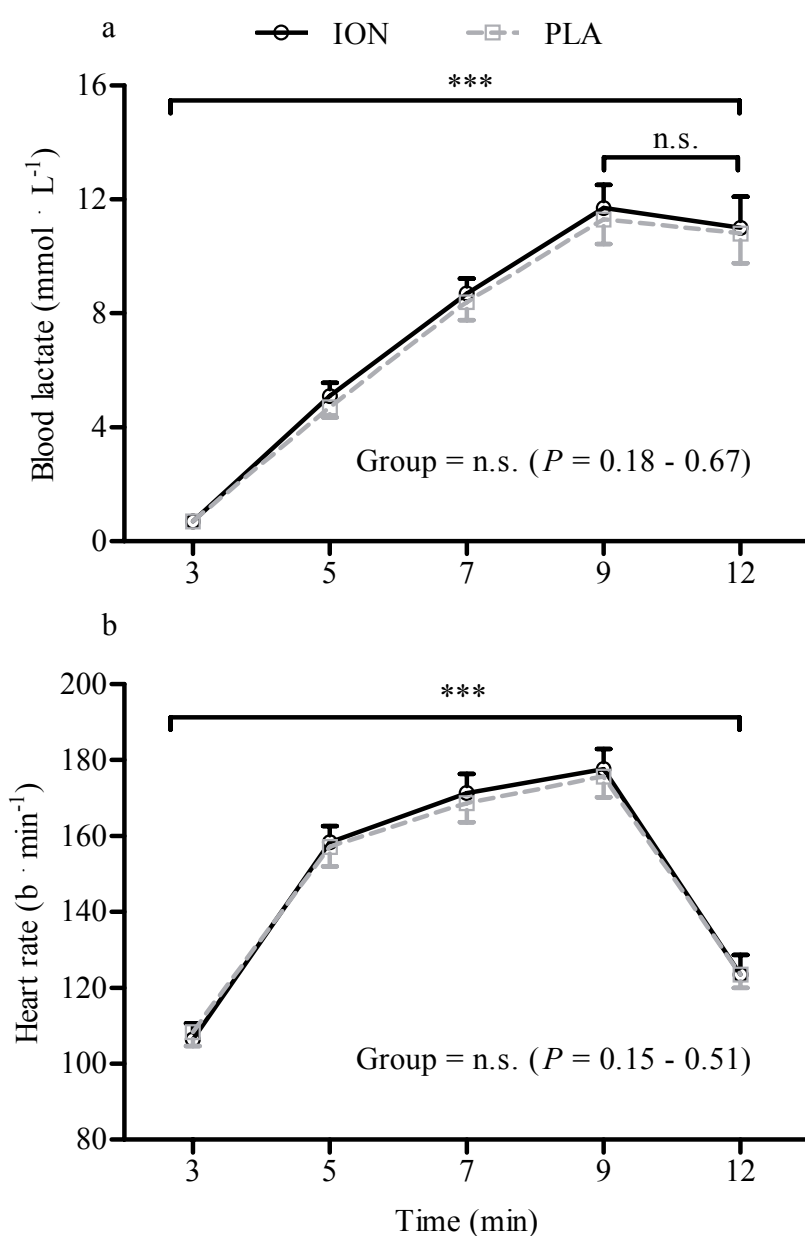


Fig.2 Blood lactate (a) and heart rate (b) response to square-wave exercise transitions for ION and PLA experimental conditions. Error bars represent 95% CL. No significant treatment effects ($P > 0.05$). *** = significant effect of time ($P < 0.001$)

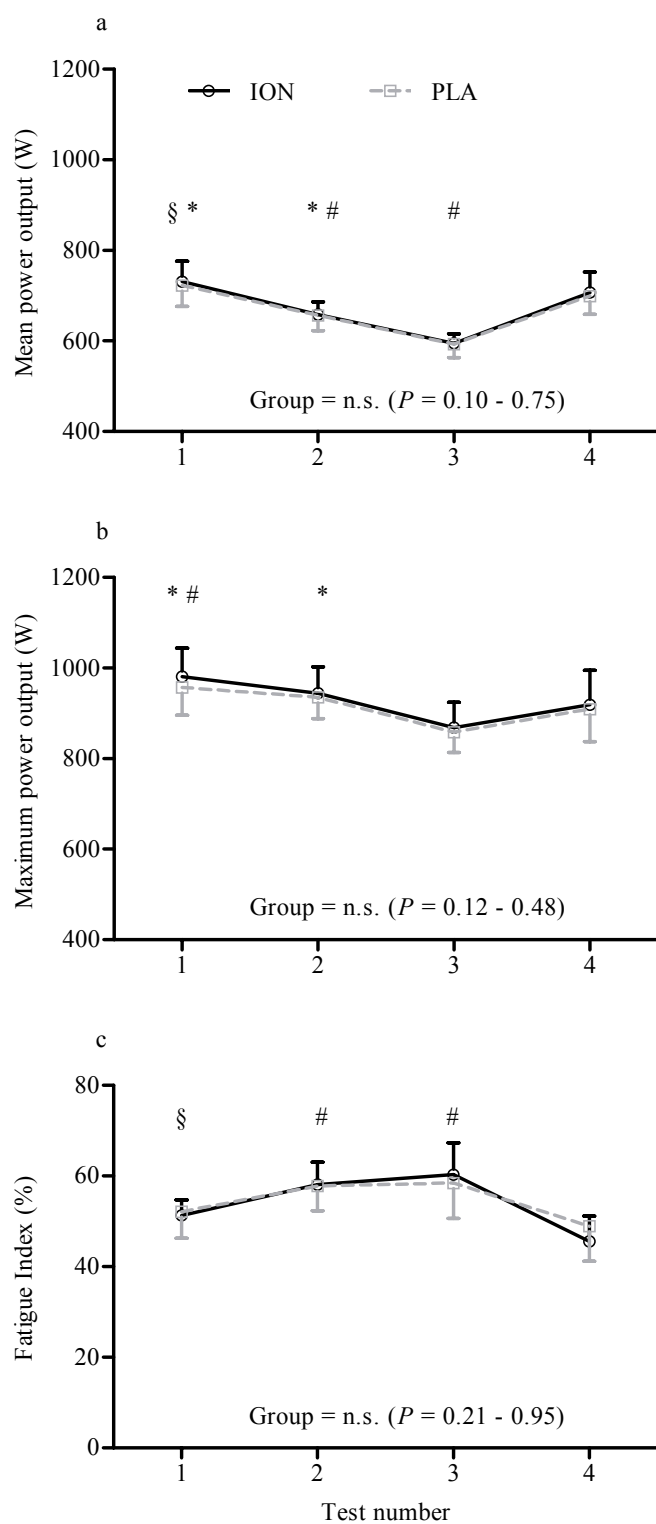


Fig.3 Mean power output (a), maximum power output (b) and fatigue index (c) obtained from the four Wingate tests for ION and PLA experimental conditions. Error bars represent 95% CL. No significant treatment effects ($P > 0.05$). §, *, # = significantly different at $P < 0.05$ from test 2, 3 and 4, respectively

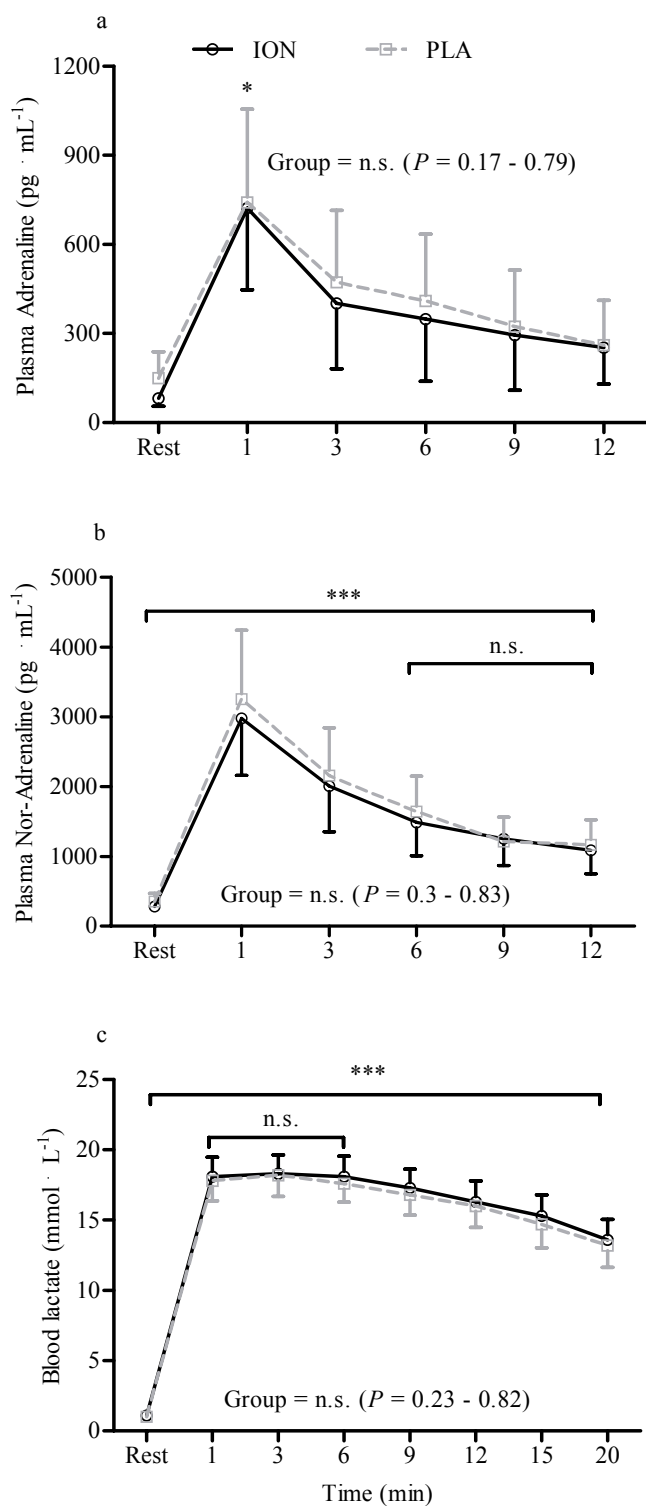


Fig.4 Adrenaline (a), Nor-Adrenaline (b) and blood lactate (c) response to the Wingate test for ION and PLA experimental conditions. Error bars represent 95% CL. No significant treatment effects ($P > 0.05$). * = significantly different from all other measures ($P < 0.005$); *** = significant effect of time ($P < 0.001$)

Table captions

Table 1 Performance measures obtained from the incremental graded exercise test (mean \pm SD)

Measure	Group (N=14)
P_{\max} (W)	330 \pm 31
$\dot{V}O_{2\max}$ (mL \cdot min $^{-1}$)	4252 \pm 368
HR $_{\max}$ (b \cdot min $^{-1}$)	188 \pm 8
VT (W)	148 \pm 23
$\Delta 70\%$ (W)	275 \pm 27

P = power output; $\dot{V}O_2$ = oxygen uptake; HR = heart rate; VT = ventilatory threshold;

$\Delta 70\%$ = work rate at 70% between VT and P_{\max}

Table 2 Oxygen uptake kinetic responses to square-wave exercise transitions during severe-intensity exercise at $\Delta 70\%$ (mean \pm SD)

Measure	Treatment		Difference 95% CL	ANOVA Effect size
	ION	PLA		
Baseline $\dot{V}O_2$ (mL min ⁻¹)	1326 \pm 126	1361 \pm 169	-35 \pm 160 -127 – 58	$P = 0.43$ $d = -0.24$
Phase II $\dot{V}O_2$ time constant (s)	32 \pm 14	32 \pm 14	0.3 \pm 3.2 -1.5 – 2.2	$P = 0.7$ $d = 0.02$
Phase II $\dot{V}O_2$ time delay (s)	16 \pm 7	15 \pm 6	0.5 \pm 3.7 -1.6 – 2.7	$P = 0.61$ $d = 0.08$
Phase II $\dot{V}O_2$ amplitude (mL min ⁻¹)	2274 \pm 383	2261 \pm 410	14 \pm 183 -92 – 119	$P = 0.78$ $d = 0.04$
Phase II $\dot{V}O_2$ gain (mL min ⁻¹ ·W ⁻¹)	9.3 \pm 1.1	9.2 \pm 1.1	0.06 \pm 0.7 -0.4 – 0.5	$P = 0.76$ $d = 0.06$
$\dot{V}O_2$ slow component (mL)	404 \pm 214	482 \pm 217	-78 \pm 199 -194 – 37	$P = 0.17$ $d = -0.38$
$\dot{V}O_2$ slow component time delay (s)	161 \pm 37	166 \pm 43	-4.9 \pm 40.2 -28.4 – 18.5	$P = 0.66$ $d = -0.13$
End-exercise $\dot{V}O_2$ (mL min ⁻¹)	4055 \pm 352	4041 \pm 365	14 \pm 183 -100 – 127	$P = 0.8$ $d = 0.04$
End-exercise $\dot{V}O_2$ gain (mL min ⁻¹ ·W ⁻¹)	10.9 \pm 1.3	11.2 \pm 1.0	-0.3 \pm 0.9 -0.8 – 0.3	$P = 0.27$ $d = -0.27$

$\dot{V}O_2$ = oxygen uptake; CL = confidence limit; d = effect size; ANOVA represent the main effect of condition