The intranasal AlaxoLito Plus Nasal Stent: Improvement of NO-induced microrheology and oxygen uptake during exercise?

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ABSTRACT

Aim: To investigate the influence of the intranasal AlaxoLito Plus Nasal Stent during exercise on nitric oxide (NO) synthesis, NO exhalation, red blood cell (RBC) deformability and oxygen uptake.
Methods: Parameters were measured before and after acute cycle ergometer test at different intensities. Spirometric, microrheological and NO parameters were determined for oral (OB), nasal (NB) and nasal-stent breathing (SB). RBC deformability was measured and elongation indices for 3.87 Pa and maximal deformability were calculated. RBC/plasma/exhaled NO, oxygen uptake and respiratory rate were determined.
Results: Exhaled NO was higher at rest during OB compared to SB and NB and decreased after exercise with NB and SB. Plasma and RBC NO remained unaltered during intervention. RBC deformability increased at moderate intensity during SB. Deformability decreased at moderate and medium intensity with NB. Respiratory rate for same oxygen uptake did not differ between breathing settings.
Conclusion: The AlaxoLito Plus Nasal Stent may modulate deformability during moderate exercise and increase NO exhalation without major effects on oxygen uptake and performance.

1. Introduction

Professional athletes of every sport aim to improve their physical abilities to their limits. Especially in endurance sports, blood oxygen saturation and oxygen transport capacity are crucial parameters for high endurance performance (Mairbäurl, 2013). During strenuous exercise at the CO_2/O_2 gas exchange threshold, oxygen delivery depends on the effective contribution of both nasal and oral breathing (Recinto et al., 2017). To enhance this skill, legal and illegal substances and devices like ß-agonists, external dilators or nasal sprays were employed (Anderson and Kippelen, 2012). Conflicting data exist in the current literature regarding the usefulness of external or internal dilators on exercise performance (Adams and Peiffer, 2017; Gelardi et al., 2019; Tong et al., 2001). It is known that nasal breathing has the advantage to warm and humidify inspired air before reaching the respiratory system in comparison to oral breathing, thus reducing vocal-cord dehydration. Therefore, nasal breathing is the common breathing mode during rest, whereas oral or oronasal breathing is mostly preferred at the onset of exercise due to the increased work of breathing or alternatively as an indirect effect of hypoventilation, caused by loaded breathing and

hence changes in alveolar air composition (M. Dallam et al., 2018; Morton et al., 1995; Saibene et al., 1978).

Upon stimulation of the parasympathetic nervous system, the concentration of vasodilatory molecules like nitric oxide (NO), which is the main vasodilator in the human body, is increased. Because a disturbed NO metabolism might increase exercise-induced bronchoconstriction (EIH) (Anderson and Kippelen, 2012; Morton et al., 1995; Verges et al., 2005), determination of exhaled NO as well as optimizing NO bioavailability in the lung is of clinical importance. Thus, an improvement of NO bioavailability is one main task of clinical treatment for the reduction of EIH and the subsequently decrease in performance (Jorissen et al., 2001).

Proper oxygen delivery in the microvascular system depends - among others - on red blood cell (RBC) deformability (Simmonds et al., 2014). Various studies suggest that both oxygen and NO can positively influence the mechano-elastic properties of the RBC, and that exercise contributes to better microvascular behaviour and higher deformability (Brun et al., 2010; Connes et al., 2013; Hickok et al., 2013). The functional activity of an NO producing enzyme within RBC – termed RBC-NO-Synthase (RBC-NOS) – was described by Kleinbongard and

colleagues (Kleinbongard et al., 2006). Still, there is some debate about the physiologically effectiveness and target points of RBC-derived NO because of the immediate vicinity of NO-scavenging haemoglobin (Simmonds et al., 2014). But recently, one immediate function in the RBC was shown: RBC-NOS can regulate and improve RBC deformability by posttranslational modification of $\alpha-$ and β-spectrins of the RBC membrane cytoskeleton (Grau et al., 2013). This process facilitates the transition in the cardiovascular and pulmonary system and might contribute to improved performance due to improved oxygen uptake in the lung and oxygen release in the musculature (Connes et al., 2013).

A possible new tool to increase oxygen transport capacity might be the intranasal AlaxoLito Plus Nasal Stent (Alaxo GmbH, Frechen, Germany). The Alaxo stents were originally developed as medical devices to treat decreased nasal breathing and resulting snoring (Traxdorf et al., 2016). But their application during endurance sports with regard to improved nasal breathing and subsequent exercise performance as well as NO metabolism and RBC rheology was not examined yet and was thus aim of the study.

2. Methods

2.1. Study population

12 healthy male participants (non-smokers, west European background, no blood donor) without extensive exercise training were recruited. Participants were not specifically endurance trained or professional cyclers, but were mostly sport students and in good physical condition. Basal anthropometric parameters of participants were as follows (mean ± SD): age [years]: 25.4 ± 5.0, height [cm]: 181.2 ± 6.6, weight [kg]: 82.0 ± 10.1.

The protocols used in this study were approved by the ethics committee of the German Sport University Cologne. These protocols align with the Declaration of Helsinki and all participants gave written informed consent to participate in this study.

2.2. Stent familiarisation

All participants received a medical examination of their health status and their nasal airways. Familiarisation with the AlaxoLito Plus Nasal Stent application (Fig. 1) started at least one week prior to the exercise test to avoid nasal secretion and to get used to increased wearing time progressively.

2.3. Exercise settings and spirometric parameters

2.3.1. Performance test to determine individual lactate thresholds

A Hollmann/Venrath step test (Rost and Appell, 2002) was conducted on a cycle ergometer to determine individual lactate thresholds for following exercise test (Ergo-metrics 900, Ergoline, Bitz, Germany). Briefly, all participants started with 30 W. Resistance was increased every three minutes by 40 W until subjective exhaustion. At the end of each step, capillary blood was sampled from the earlobe and lactate concentration was determined with the permanently installed lactate
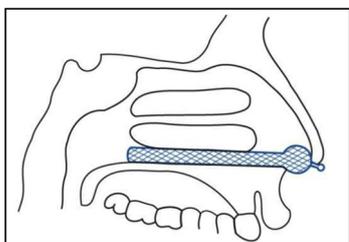

Fig. 1. Schematic application of the AlaxoLito Plus Nasal Stent (© with permission by Alaxo GmbH).

measurement device Biosen S-Line (Biosen S-Line, EKF-diagnostic, Barleben, Germany). This device uses an enzymatic-amperometric measurement principle with chip sensors and exhibits high accuracy with maximum measurement error of 1.5%. Watt power was calculated corresponding to the individual 2 mmol (light), 3 mmol (moderate) and 4 mmol (intense) lactate threshold, respectively, to be applied in the following exercise test. These lactate thresholds were chosen to ensure comparable intensities between participants and to cover light and submaximal to intense and high intensities. Spirometry data were recorded and analysed by the software MetaSoft 3 (Cortex Biophysik GmbH, Leipzig, Germany). To guarantee validity and reliability of spirometry measurements, the volume flow sensor of the spirometry system was calibrated with a volume of 3 litres and an O_2/CO_2-sensor calibration with the ambient air was conducted. In addition, the O_2/CO_2-sensor was at least once a month calibrated with a standardised gaseous mixture (Hodges et al., 2005). The accuracy and stability of this O_2/CO_2-sensor was confirmed by the several studies (Macfarlane and Wong, 2012; Vogler et al., 2010).

2.3.2. Exercise tests

Exercise tests were scheduled two weeks after the performance test. Three different exercise tests were conducted with one of the following breathing settings: oral breathing (OB), nasal breathing (NB) or nasal breathing with the AlaxoLito Plus Nasal Stent (SB). A one-week rest period was scheduled between the three tests. The protocol followed a random cross-over design to avoid adaptations to the exercise protocol. To avoid nasal contribution during OB, a nose-clip was placed over the nose. Due to the tightness of the spirometer mask and to avoid uneasiness, it was impossible to seal the mouth with assistive devices during NB and SB. Thus, all participants were strongly advised to avoid oral breathing and to signal any switch to oronasal breathing.

Participants were told to avoid a nitrate-rich diet at least 24 h prior to each test to minimize the acute effect of nutrition on NO-concentrations in blood and exhaled air.

During all exercise tests, spirometry data were recorded and analysed by the same software MetaSoft 3 as described above. Participants conducted an incremental step test on the cycle ergometer at their individual 2 mmol, 3 mmol and 4 mmol lactate threshold, respectively. Duration of each step was 15 min with 2.5 min between each step for capillary and venous blood collection. Blood samples were taken before (T0), after 2 mmol (T1), 3 mmol (T2) and 4 mmol lactate threshold (T3), respectively (Fig. 2).

2.4. Performance data

Spirometry data were continuously collected during all trials. To express oxygen uptake at the respective time point adjusted to body weight, relative VO_2 (relVO_2) – the mean value of at last 10 min of each step – was calculated to ensure a steady-state condition and thus to minimize breathing alterations. The respiratory rate (RER) was calculated from the CO_2/O_2 ratio.

2.5. Blood sample analysis

Venous blood samples were taken at all time points for RBC and plasma analyses. Blood was sampled from the *Vena mediana cubiti* and anticoagulated in heparin vacutainers (BD, Franklin Lakes, New Jersey, USA). For preparation of RBC and plasma analysis, blood samples were centrifuged at 3500 g for 1 min and the buffy coat removed.

2.5.1. NO concentrations

RBC nitrite and plasma nitrite levels, the primary oxidation products of the main vasodilator NO, were measured using an ozone-based chemiluminescence NO detector (CLD 88e, EcoPhysics, Switzerland) according to Hendgen-Cotta et al (Hendgen-Cotta et al., 2008). Briefly, plasma supernatant was collected in Eppendorf tubes for plasma nitrite
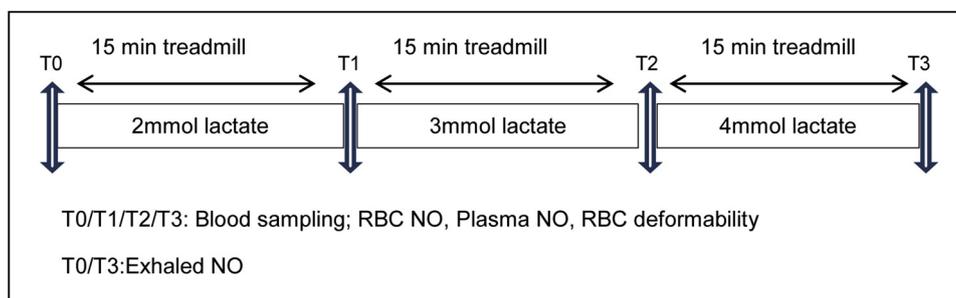
Fig. 2. Time frame of the acute exercise test on a cycle ergometer with sampling time points and the respective analyses.

analysis and immediately stored at −80 °C until measurement. For RBC nitrite measurement, RBC were immediately mixed with preservation solution (800 mM $K_3[Fe(CN)_6]$, 100 mM NEM, 10 V-% Igepal, 90 V-% aqua dest.) in a 5:1 ratio, snap frozen and stored at −80 °C until measurement. Analysis was performed with the Chart FIA software (EcoPhysics, Switzerland). Samples for RBC and plasma nitrite at all time points were measured in triplicate and RBC nitrite concentration of the sample was corrected for nitrite levels of methanol and preservation solution according to the protocol of Pelletier et al. (Pelletier et al., 2006).

Exhaled NO was measured at T0 and T3 using the CLD 88e. Participants had to breathe evenly into a gas-tight breathing bag (CareFusion Germany 234 GmbH, Hoechberg, Germany) holding up to five litres. The bags contained a non-return valve to avoid exchange with the ambient air. For measurement of exhaled NO, which was determined T0 and T4, the respective breathing bag was connected directly to the CLD and the content was measured at constant evacuation flow for a defined time interval of 45 min for comparability of NO content. The NO baseline concentration of ambient air was subtracted from total NO determination.

2.5.2. RBC deformability

RBC deformability was directly measured at each time point by ektacytometry using the Laser-assisted-optical-rotational cell analyzer (LORCA; RR Mechatronics, The Netherlands). The protocol was described in detail by Hardeman et al (Hardeman et al., 2001). Briefly, whole blood samples were mixed with a viscous polyvinylpyrrolidone solution (viscosity 28 cP) and filled in a Couette system. Nine shear stresses between 0.3 and 50 Pa were applied to the RBC, and width (W) and length (L) of the laser diffraction pattern was analysed by the LORCA software to calculate an Elongation Index (EI): $EI = (L-W)/(L + W)$. The EI at 3.87 Pa ($EI_{3.87}$), representing average physiological shear stress in the cardiovascular system, and EI_{max} as the theoretical maximal deformability at infinite shear stress were calculated from the curves according to Baskurt 2009 (Baskurt et al., 2009).

2.6. Statistical analysis

Statistical analysis was performed with the Graph-PadPrism 6 software package (La Jolla, USA). Gaussian distribution was tested using the Kolmogorov Smirnov normality test. Differences between the three trials at the respective time points were analysed with a two-way analysis of variance (ANOVA) with repeated measures to detect time or breathing setting interactions. Main effects or interactions were analysed using a multiple comparison Bonferroni post-hoc test. Differences within the respective breathing setting from step to step were analysed using a paired sample t-test for normal distributed data. Otherwise a Wilcoxon signed rank test was used. Differences between the breathing settings at only one time point were assessed using a one-way ANOVA followed by multiple comparison Bonferroni post-hoc test for significant differences. If normality was violated, Friedman test was used followed by Dunn's Multiple Comparison Test. Descriptive statistics of the data was presented as mean ± standard deviation unless otherwise described. Statistical differences were considered to be significant for values of $p < 0.05$.

3. Results

3.1. Performance data

RER and $relVO_2$ increased at all breathing settings with increasing workload. Two-way ANOVA revealed possible interactions depending on time point at RER ($p = 0.0044$) and $relVO_2$ ($p < 0.001$) but no further significance was detected with post-hoc test (Fig. 3).

3.2. NO concentrations

RBC nitrite remained unaltered during the tests and no difference was detected between the groups (Fig. 4A). Plasma nitrite analysis with two-way ANOVA revealed probable interactions depending on breathing setting ($p = 0.0021$) but no further significance was detected with post-hoc test (Fig. 4B).

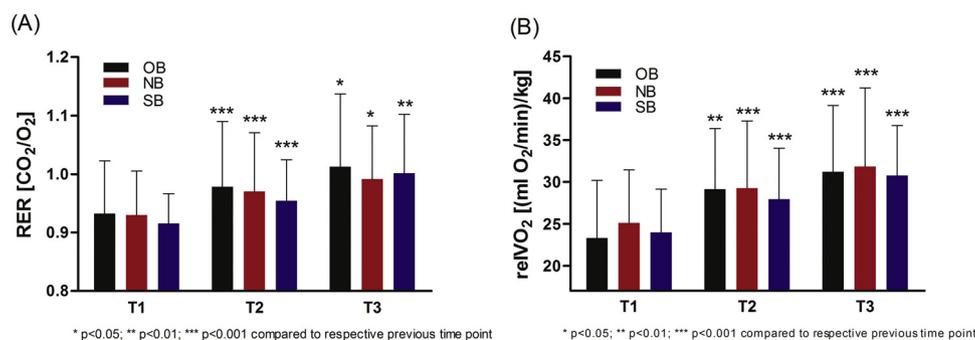Exhaled NO concentrations were significantly increased with NB

Fig. 3. Analysis of respiratory quotient (RER) and oxygen uptake at the respective time point adjusted to body weight ($relVO_2$) revealed a progressive increase of RER and $relVO_2$ with increasing workload, but no significant differences between settings were detectable.
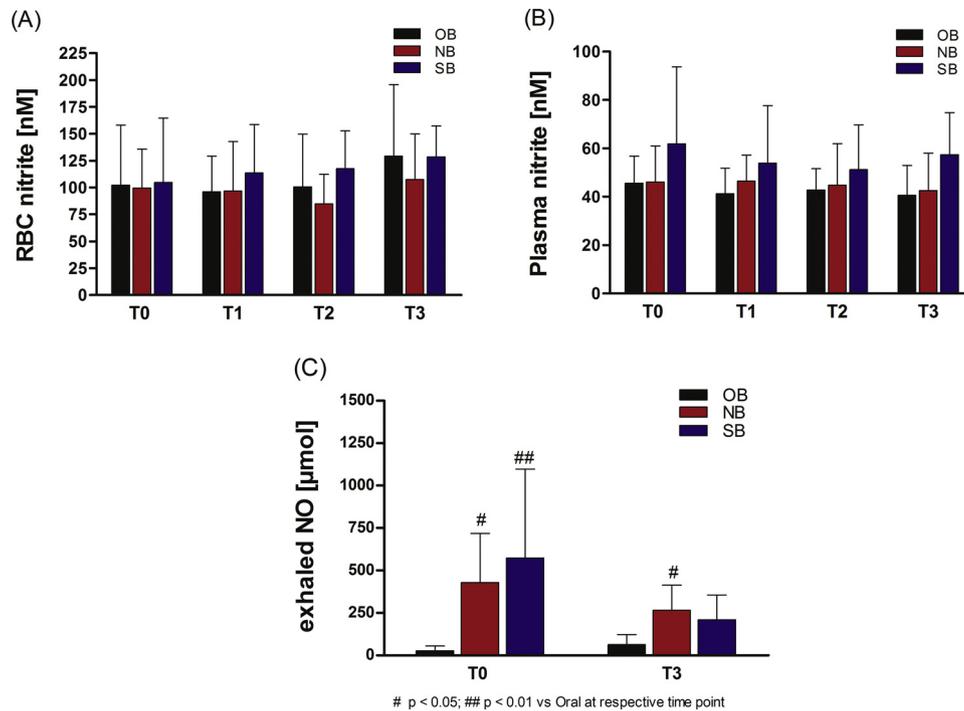
Fig. 4. Analysis of NO metabolism in blood revealed no changes in RBC and plasma nitrite levels during the test (Fig. 4A + B), whereas the exhaled NO content was significantly higher for NB and SB at T0 and T3 compared to OB, but decreased at T3 by 41% for NB and 62% for SB, respectively (Fig. 4C).

and SB compared to OB at rest (T0) and after exercise (T3) for NB, respectively. Two-way ANOVA indicated possible interactions depending on time point (p = 0.0356) and breathing setting (p < 0.001). Post-hoc test showed significant differences between NB and OB (p < 0.005) as well as between SB and OB (p < 0.001) at T0. Exhaled NO decreased (n.s.) after exercise with NB and SB, whereas OB increased (p = 0.07) (Fig.4C).

3.3. RBC deformability

RBC deformability exhibited a decrease for NB at medium intensity T2 at 3.87 Pa (p = 0.012) as well as at maximal shear stress (p = 0.017) compared to T0, whereas EI_{max} for SB increased at moderate intensity T1 (p = 0.04). No difference was detected with OB (Fig. 5).

4. Discussion

In the last decades, a lot of studies tried to examine the still unresolved issues if 1) nasal breathing is more economic and energetic compared to oral breathing, if 2) breathing can be further improved by the application of external and internal nasal dilators and if so, 3) which

breathing pattern and application might contribute to enhance oxygen transport capacity and subsequently physical performance. The performed studies provide conflicting data by showing both unchanged or slightly improved performance parameters, either objective or subjective (Adams and Peiffer, 2017; Dinardi et al., 2016; Gelardi et al., 2019; Peltonen et al., 2004; Tong et al., 2001). Therefore, the present study aimed to determine the distinct contribution of either oral, nasal or nasal breathing with an internal stent not only on physical performance, but in addition on NO production in the exhaled air and blood as well their possible influence on haemorheological behaviour.

With regard to the described different breathing settings, neither an increased oxygen uptake nor an improved CO_2/O_2-ratio, expressed as the respiratory rate, could be observed in our study. Although the active dilatation by the stent theoretical increases nasal airflow and facilitates breathing at rest, there seems no beneficial impact on these parameters during cycling exercise. This observation was partly confirmed by our spirometry data, including ventilatory equivalents of VO_2, tidal volume and breathing frequency (data in supplemental material). These breathing parameters can indirectly be used to show possible beneficial effects in breathing mechanics. Although breathing with SB was more economical with regard to breathing frequency and tidal volume than
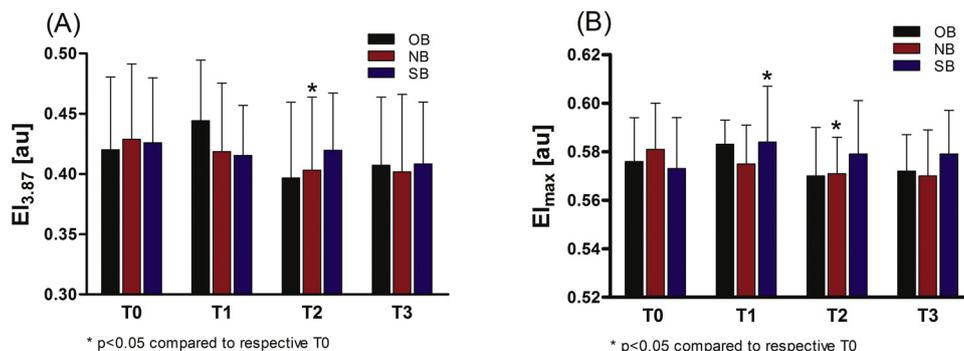

Fig. 5. $EI_{3.87}$ showed a decrease with NB at T1 (p = 0.052) and T2 (p = 0.012) compared to T0, whereas it decreased with OB form T1 to T2, but only by trend (p = 0.065) (Fig. 5A). The maximum deformability EI_{max} was significantly increased at T1 for SB and decreased at T2 for NB compared to T0 (Fig. 5B).

OB, no distinction between NB and SB was measurable. Examination of the tidal volume showed the highest values with SB, followed by NB and OB, but without significant statistical differences. The reversed order was seen for breathing frequency, which might indicate improved breathing with SB, but interpretation remains controversial. Regarding other available studies with stent applications, the inconsistency of current literature is underlined by the study of Adams et al which tested the effectiveness of an external and internal nasal dilator on cycling trial performance and did not find any benefit on performance parameters with both applications (Adams and Peiffer, 2017). This observation was partly contradicted by a recent study where fatigue perception was reduced with an internal dilator (Gelardi et al., 2019), whereas Dinardi et al (Dinardi et al., 2016) used an internal stent in adolescent athletes and determined improved nasal patency without major effects on performance parameters. In contrast, another study examined the influence an external nasal dilator strip on energy parameters in adult men and women and showed beneficial effects for nasal ventilation during exercise by stiffening the lateral nasal vestibule walls, but only in responders (˜ 75%) (Gehring et al., 2000).

In our male participants, we did not measure objective differences in performance or blood parameters, but observed distinct subjective toleration towards the application of the AlaxoLito Plus Nasal Stent, e.g feelings of a foreign body in the nose which might limit exercise performance. In general, active internal dilatation seems to exceed the capability to reduce nasal resistance compared to external nasal strips (Peltonen et al., 2004) and it was shown that a combination of internal stenting and topical mucosal vasoconstriction might lead to nasal flow capacities comparable to breathing via the mouth (Pertuze et al., 1991).

As stated before, breathing through the nose has the advantage to warm and humidify inspired air (M. Dallam et al., 2018; Morton et al., 1995), but the inhaled volume is limited by increased flow resistance and decreased ventilation compared to oral breathing (Hall, 2005; Pertuze et al., 1991). This is especially important for endurance or Nordic sport athletes which have a higher incidence for EIH (Anderson and Kippelen, 2012). A possible cause is the cooling and drying of the airways during exercise because of increased ventilation at higher intensities (Morton et al., 1995). In addition, decreased pulmonary NO in EIH has been found in hypoxic conditions which might contribute to performance impairment during high intensity exercise due to impaired oxygen supply and dilation in the respiratory system (Verges et al., 2005).

Physical activity per se increases NO bioavailability and thus improves the cardiovascular and cardiopulmonary system (Nosarev et al., 2014), but the origin of exhaled NO and the contribution of the respective airways systems are still under debate. Kimberly et al concluded that at rest the majority of exhaled NO originates in the nasopharynx (Kimberly et al., 1996), whereas in general NO is produced in the nasal mucosa and in the paranasal sinuses (Lundberg and Weitzberg, 1999; Olin et al., 1998) with local as well as distal effects in the pulmonary system. NO in the exhaled air is reflected and can be analysed by the stable storage forms nitrite or nitrate (Nadziakiewicz et al., 2006; Olin et al., 1998). A study by St Croix et al. (1999) examined plasma nitrate during exercise, hyperventilation and hypoxia. They did not find any changes during low, moderate and high intensity cycling exercise, which can be confirmed by our results where no changes in plasma or RBC nitrite concentration with regard to the breathing mode or exercise intensity could be detected. It was suggested that the reduction of luminal NO leads to a decrease in the concentration gradient between the alveolar space and pulmonary capillary blood, which results in the fraction of NO taken up by the blood and an increase in the volume of NO recovered in the exhaled air (St Croix et al., 1999).

Not unexpected, the NO determined in the exhaled air in our study was comparable with previous findings, ranging between 4–160 ppb with OB and 200–2000 ppb with NB (Jorissen et al., 2001). But the concentrations for SB even exceeded the concentrations found for NB in

other studies which might have a beneficial dilatory effect in the respiratory system, at least at rest, and may reduce symptoms of EIH. During the moderate to high intensity cycling exercise exhaled NO dropped by 41% for NB and 62% for SB and increased by 68% for OB, which confirmed previous findings that exercise leads to an decrease of exhaled NO in healthy individuals and coronary patients (Mantione et al., 2007; Nadziakiewicz et al., 2006) and can be attributed to normalization of the total amount to the increased airflow rate and ventilation during exercise (Sheel et al., 1999; St Croix et al., 1999). Accordingly, higher exhaled NO with NB at different exercise intensities compared to OB was found in a study from Yasuda et al (Yasuda et al., 1997).

But although the breathing mode or the dilation by SB did not seem to have any major influence on blood NO metabolism, acute changes in RBC deformability could be observed. As one prerequisite for a smooth transition through the cardiovascular and especially the microvascular system, RBC deformability contributes to sufficient blood flow and oxygen supply to the respective tissues (Simmonds et al., 2014). Endurance sport leads to higher deformability and thus an increased performance capability (Connes et al., 2013). Interestingly, the application of the AlaxoLitoStent showed increased maximal deformability at moderate intensities (T1), and that increase seemed unrelated to NO metabolism. Interestingly, both moderate and medium intensity exercise followed a decrease of EI_{max} and $EI_{3.87}$ with NB, which indicates that addition of a stent to support nasal breathing might alter oxygen and NO utilization with a direct influence on haemorheology and microcirculation. A possible explanation may be that the increased nasal dilation and reduced flow resistance might facilitate oxygen uptake in the RBC under aerobic conditions which in turn might improve RBC rheological behaviour and economize oxygen kinetics. A consequence may be that, especially at moderate intensities, the internal AlaxoLitoStent further economizes breathing and oxygen supply. This is in line with different previous studies which concluded that nasal only breathing might be comparable or even energetically favourable to oral breathing at aerobic intensities (LaComb et al., 2017; M. Dallam et al., 2018; Morton et al., 1995; Tong et al., 2001), whereas an advantage of oral breathing was proposed at higher intensities (LaComb et al., 2017). In contrast, Recinto et al found no difference in power output between nasal and oral breathing in an anaerobic test and thus stated that each individual might choose the breathing method which suits them best (Recinto et al., 2017).

All in all, previous examined dilators had in common that they only stabilized or dilated the nasal wings. The intranasal AlaxoLito Plus Nasal Stent used in our study supports and dilates not only the nasal atrium, but in addition the nasal passage which might have an additional effect to reduce flow resistance and facilitate oxygen uptake and NO synthesis.

5. Conclusions and limitations

The examination of the novel AlaxoLitoStent suggests that it may improve endurance capacity at moderate intensities. This is based on the increase of RBC deformability at moderate intensities which may improve perfusion of the microcirculation, without major changes in plasma nitrite and RBC nitrite. This might hint to economized oxygen uptake and release due to improved blood lung perfusion by RBC at aerobic exercise and improved blood oxygen utilization with same oxygen uptake compared to OB and NB alone. The observation of decreased, but still higher exhaled NB-NO and SB-NO after the intervention compared to OB can partly by attributed to dilution effects due to increased respiratory rate, lung perfusion and oxygen uptake, but indicates possible beneficial function by NO-mediated dilatory effects.

But the definite usefulness of nasal dilators in sport remains a disputable issue. Differences in exercise protocols, study population and dilator devices reduce the comparability between the different available studies. Hence it has to be stated that one limitation of the

presented study is that the exercise intensities based on individual lactate thresholds, which showed a participants' correspondence of around 50%.in comparison with ventilation data. This method was chosen due to manipulation examination of the ventilation by different breathing techniques, but is not routinely used in training studies and thus limits the comparability to other studies. Additionally, lactate accumulation at the described thresholds can be differently exhausting for the respective participant, depending on sport background and fitness. It is further to mention that the participant number of 12 male and not specifically endurance-trained participants reflects only a small study population, especially in the case of stent application with possible incidence of responders and non-responders. Nevertheless, the presented results might contribute to understand the complex interactions between artificial nasal dilatation, physiological respiration and performance parameters, regarding the clinical and performance related importance of facilitated breathing during exercise.

Therefore, further combined studies of respiratory and blood rheological examinations are needed to resolve the contribution of internal stents on oxygen uptake, breathing mechanics, blood flow improvements and subsequent increased performance capability and reduced fatigue.

Declaration of Competing Interest

None.

Acknowledgements

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.resp.2019.103260.

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