RELATIONSHIP BETWEEN EXHALED AND INHALED NITRIC OXIDE AND EXERCISE-INDUCED HYPOXEMIA

by

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ABSTRACT

The consensus in the literature is that exercise-induced hypoxemia (EIH) occurs secondary to ventilation-perfusion (V_A/Q) inequalities and diffusion limitations resulting from elevated pulmonary pressures causing the development of interstitial pulmonary edema, or decreased pulmonary transit time in the pulmonary vasculature. Endogenously produced pulmonary nitric oxide (NO) has been hypothesized to have several physiological functions including V_A/Q matching and maintenance of low pulmonary vascular resistance. Respiratory derived NO is detectable in exhaled gases. Inhaled NO, a selective pulmonary vasodilator is used in the treatment of diseases characterized by pulmonary hypertension and hypoxemia. Short-term inhalation of NO causes selective pulmonary vasodilation without any systemic effects. Given that athletes with EIH are thought to have altered pulmonary hemodynamics during exercise, the relationship between endogenously produced and exogenously delivered NO and EIH was examined in two separate studies. It was hypothesized that subjects with EIH would have a decreased production rate of NO (V_{NO}) compared to subjects who maintained normal oxyhemoglobin saturation (SaO_2) and that SaO_2 would be correlated with V_{NO}. A group of highly-trained male cyclists (n = 18), some of whom develop EIH performed a maximal cycle test. V_{NO} was determined during the cycle test. No significant differences were observed between those with and those without EIH. There was also no observed linear relationship between delta SaO_2 and delta V_{NO}. It can be concluded that NO present in exhaled air is not related to the etiology of EIH. In a subsequent study, delivery of NO was accomplished using highly trained male cyclists (n = 7) with EIH who performed four 5-min cycle tests at VO_2max under conditions of normoxia (N), normoxia + 20 ppm nitric oxide (N/NO), hypoxia (H), and hypoxia + 20 ppm nitric oxide (H/NO). It was hypothesized that: (i) inhaled NO would reverse EIH during normoxia, and (ii) inhaled NO would improve arterial oxygenation during hypoxia. Inhalation of NO during normoxic or hypoxic conditions did not significantly affect gas

exchange, cardiorespiratory variables, or power output. These findings imply that pulmonary capillary blood volume reaches a maximal morphological limit during exercise and further dilation is not possible.

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DEDICATION

to Judy and Edith, who taught me the value of learning

and

Jen, the most gentle creature

CHAPTER 1. GENERAL INTRODUCTION

Respiratory limitations to exercise and the biology of nitric oxide are contemporary topics of interest to the modern exercise physiologist. This is highlighted by the recent attention these subjects have received in the American College of Sports Medicine series "*Exercise and Sport Sciences Reviews*" [16, 79]. This dissertation examines the relationship between these two areas. Firstly, each topic is briefly introduced along with a description of the research questions that were addressed. Secondly, the details of the two experiments performed are described in separate chapters followed by a concluding chapter.

Exercise-Induced Hypoxemia.

The conventional paradigm held by exercise physiologists is that individuals engaged in strenuous activity at sea-level maintain blood gas homeostasis for both O_2 and CO_2 [8, 9]. Their respiratory system is able to precisely regulate ventilation and gas exchange. It is commonly held that the structural capacity of the normal lung is "over-built" and exceeds the demand for pulmonary O_2 transport in the healthy, exercising human. That is, the upper limits to maximum exercise breathing frequency (fb), tidal volume (V_T), minute ventilation (V_E), and gas exchange are determined structurally by the lung's natural morphology and provide an adequate reserve for exercise demands. It is also believed that the respiratory system remains largely unchanged in response to chronic endurance training [45]. A notable exception to this adequate reserve concept is that in some highly-trained male aerobic athletes decreases in arterial pressure of O_2 (PaO_2), oxyhemoglobin saturation (SaO_2), and a widened alveolar-arterial difference for O_2 ([A-a]DO_2) are observed during heavy exercise [48, 91, 173]. In this population, it appears that demand exceeds the supply limits of the lung. This phenomenon, termed exercise-induced hypoxemia (EIH), occurs in approximately 50% of highly-trained male endurance athletes, while

un-trained and moderately-trained males do not experience EIH [173]. While EIH has been clearly established and well defined in the male athletic population, only recently has it been observed to occur in females (see APPENDIX A) [80]. EIH has been defined as a decrease in PaO_2 of 10-15 mmHg or an SaO_2 less than 92% [174] with negative effects on maximal oxygen consumption (VO_2max) [67, 96, 123, 132, 174, 216] and exercise performance [121].

Decreases in PaO_2 and SaO_2 during heavy exercise represent a physiological failure of the respiratory system. The pulmonary limitation to gas exchange is also manifested by an increase in the $[A\text{-}a]DO_2$, which can reach values as high as 50 mmHg [91]. The cause and significance of EIH have been the topics of a considerable research effort, yet the mechanism(s) responsible remain controversial. Four potential factors have been identified; (i) veno-arterial shunts, (ii) relative alveolar hypoventilation, (iii) ventilation/perfusion (V_A/Q) inequalities, and (iv) diffusion limitations. Veno-arterial shunts [48, 176] and relative alveolar hypoventilation [176, 177] have been identified as minor contributors to the pathophysiology of EIH. In the ideal lung, PA_{O_2} and PaO_2 are essentially identical where $[A\text{-}a]DO_2 \sim 0$. Any cause of inadequate gas exchange such as (i) incomplete diffusive gas mixing in the lung acini, (ii) diffusion limitation of O_2 transfer across the blood-gas barrier, and (iii) intrapulmonary and (iv) extrapulmonary right-to-left shunt will cause an increase in $[A\text{-}a]DO_2$. Consequently, a widened $[A\text{-}a]DO_2$ is a general index of inadequate gas exchange and is not reflective of any one physiological condition [104]. The importance of a widened $[A\text{-}a]DO_2$ is useful in identifying a gas-exchange limitation but is limited by its lack of specificity. More specific identification of gas exchange limitations during exercise have been documented: worsening of V_A/Q relationships [64, 89, 93, 204, 230], decreased red blood cell pulmonary capillary transit time [88, 234], and evidence of pulmonary edema [141, 204, 230].

The presence of interstitial pulmonary edema during exercise is a favored hypothesis to explain the observed V_A/Q inequality and gas exchange limitations [89, 106]. Anecdotal reports [137], depressed post-exercise diffusing capacity of the lung [185, 204], altered lung computerized tomography [39], and magnetic resonance images post-exercise [141] have been identified as possible indicators of pulmonary edema. However, these investigations can be collectively interpreted as indirect and circumstantial. Briefly, post-exercise measures may not correctly assess physiological events that are occurring during exercise. Identification of pulmonary edema during exercise, along with the possible causative factors has been difficult to achieve in exercising humans. The pulmonary blood-gas barrier is extremely thin to allow the least possible resistance to allow diffusion of O_2 and CO_2. At the same time, it must be strong to withstand the high capillary wall stresses that develop during exercise when capillary pressure rises [239]. In some athletic populations, the bioengineering of the pulmonary blood-gas barrier fails, most notably in racehorses where both pulmonary diffusion limitations and exercise-induced pulmonary hemorrhage occur [230, 238]. Exercise-induced pulmonary hemorrhage has also been reported in Shetland ponies [52], and greyhound dogs [114].

Identification of pulmonary stress failure in humans is a substantially more difficult task. Normal mean pulmonary artery pressure (P_{PA}) in humans is ~ 15 mmHg, and any increase is regarded as representing pulmonary hypertension. Three principal mechanisms of pulmonary hypertension are (i) increase in left atrial pressure (P_{LA}), (ii) increase in pulmonary blood flow, and (iii) increase in pulmonary vascular resistance (PVR) [235]. Alterations to systemic arterial pressure, via effects on left ventricular emptying, could alter pulmonary blood flow and P_{LA}. It is well known that mean systemic arterial pressure increases with increasing exercise effort and left atrial and ventricle diastolic pressures also increase. Assuming capillary wedge pressures

(P_{WEDGE}) reflect P_{LA}, it is not surprising that an exercise-related increase in arterial pressure is accompanied by an increase in P_{WEDGE} [190]. At a capillary transmural pressure of 40 mmHg ultrastructural changes to the blood gas barrier are seen using a rabbit model [225], including disruption of the capillary endothelial and alveolar epithelial layers, and in some cases all layers of the wall. Based on available human data, human P_{PA} and P_{WEDGE} can reach values ~ 40 and 27 mmHg respectively [189, 230]. Increased P_{PA} with exercise in humans has been consistently reported [70, 101, 188, 222], while pulmonary capillary pressure must be deduced from the above mentioned pressures. The calculated capillary pressure at the base of the human lung can reach > 35 mmHg during exercise. These pressures observed in humans by Wagner and colleagues [230] are within the range where stress failure of pulmonary capillaries is seen in animal preparations [225, 239]. The net result of stress failure of the pulmonary capillaries at these elevated pressures is altered or disruption of the capillary endothelial and alveolar epithelial layers, collection of red blood cells in the alveolar wall interstitium, proteinaceous fluid and red blood cells in alveolar spaces, interstitial edema and fluid protrusions of the endothelium into the capillary lumen [237]. The cumulative effect of these events is presumably impaired gas-exchange.

It has been demonstrated that high lung volumes increase stress failure in the pulmonary capillaries of an *in situ* vascular perfusion preparation of rabbit lung [61]. Possibly, the distention of lung capillaries during exercise, via pulmonary pressures, could increase permeability and promote fluid shifts into the interstitium [225]. Extrapolation of these data to exercising human athletes is not yet possible, but is suggestive of a putative mechanism for pulmonary edema. Accumulation of interstitial fluid is usually removed by lymph flow, but may be compromised during exercise [61]. Using sheep, it has been shown that 15-min after

prolonged heavy exercise (40-60 min) pulmonary lymph flow is significantly higher than at rest [153]. Possibly, the lymph system is unable to clear the interstitial space during exercise, resulting in mild pulmonary edema. This hypothesis has received support from recent studies [89, 95], but remains to be tested adequately in athletes with EIH.

The pulmonary endothelium is exposed to the entire cardiac output (Q) and is possibly vulnerable to injury. Strenuous exercise can induce an acute inflammatory response marked by leukocytosis and neutrophil activation and release of inflammatory mediators. Inflammatory mediators such as eicosanoids, reactive oxygen species, cytokines all could negatively affect pulmonary vasomotor tone or membrane permeability. The potential for permeability edema and/or stress failure suggests the presence of an inflammatory mediator. One mediator in particular, histamine, has received attention in the EIH literature. Histamine can increase the permeability of the lung vascular membrane [181] and levels of histamine are increased in athletic populations during exercise [7, 83, 147, 149]. A significant correlation ($r = 0.8$) has been observed between the change in plasma histamine and the decrease in PaO_2 in young and masters athletes compared to controls [7]. In addition, inhibition of histamine release via nedocromil sodium reduces the magnitude of decreased PaO_2 and $[A\text{-}a]DO_2$ [180]. In this investigation EIH was not completely reversed, but oxygenation was significantly improved; indicating that histamine was involved but other factors also exist in the development of EIH. Other inflammatory mediators, such as leukotrienes may be involved and have been detected in the bronchoalveolar lavage of highly-trained athletes following maximal exercise [94]. Based on the available data, it is reasonable to suggest that pulmonary hypertension (i.e., increased P_{PA}) during exercise in some highly-trained athletes may be responsible for the development of mild pulmonary edema and the ensuing EIH.

Exhaled Nitric Oxide.

It is now well established that nitric oxide (NO) is produced in a variety of both human and animal cells with numerous biological functions. NO is believed to exert regulatory functions in the circulatory, pulmonary, nervous, and immune systems [244]. NO is produced from one of the nitrogen atoms of the N-guanidino terminals of the common amino acid L-arginine, and molecular oxygen. In addition to NO, citrulline is a product of this reaction. The formation of NO also requires an isoform from the family of enzymes, NO Synthase (NOS), and other co-factors (see APPENDIX B). NO is thought to be important in many regulatory processes because of its potent vasodilatory effect. The production of NO is believed to cause relaxation of smooth muscle cells via a signal transduction system. Here, NO stimulates the formation of 3',5'-cyclic guanosine monophosphate (cGMP), which in turn leads to a decrease in the concentration of free Ca^{++} in the smooth muscle cytosol resulting in relaxation.

Endogenously produced pulmonary NO has been hypothesized to have several physiological functions. Possible roles include V_A/Q matching, maintenance of low PVR, and airway relaxation. The idea that an optimal V_A/Q ratio may be regulated by NO is supported by data showing arterial hypoxia in anesthetized animals upon administration of NOS inhibitors [163]. The importance of NO is evident when inspired NO induces pulmonary vasodilation and improves V_A/Q relationships [60, 168, 182, 197]. Endogenous NO was recently found to be critical to the maintenance of a low PVR in endothelial NOS knockout mice who developed pulmonary hypertension [55]. It is also thought that NO is continuously released to maintain pulmonary arterial tone, systemic pressure, and Q [218] where patients with heart disease have reduced expiratory NO levels, perhaps explaining why they exhibit an increased vasoconstriction of their pulmonary blood vessels [220].

As alluded to above, endogenously produced NO has been detected in the exhaled air of humans [72] and numerous animal species [205]. Expiratory NO concentrations (C_{NO}) and the production rate of NO (V_{NO}) have been examined during physical exercise [130, 167]. It is believed that the formation of NO may play a role in the normal pulmonary response to exercise. Basal release of endogenous NO has been shown to have a role in low pulmonary vascular tone at rest and exercise in sheep [120]. In addition, it has been suggested that a defect in NO synthesis may contribute to high-altitude pulmonary edema (HAPE) [207]. It would seem that endogenously produced NO is necessary for maintenance of V_A/Q matching and a low pulmonary vascular resistance, the extent to which expired concentrations of exhaled NO reflect this process requires clarification.

During exercise, Q increases in proportion to exercise intensity, and the pulmonary circulation is able to accommodate the increase with little rise in pulmonary pressure. This is typically thought to be due to the distension and recruitment of the pulmonary capillaries. It has been traditionally thought that this process is passive in nature where the pulmonary circulation can be fully recruited. It is only possible to speculate at this stage, however it seems likely that NO may play a role in mediating the V_A/Q response during exercise. Preliminary research into this area suggests the possibility that NO production is different between highly-trained athletes and un-trained individuals. It is widely accepted that physical conditioning affects the cardiovascular response to exercise and potentially training-induced alterations to Q, pulmonary blood flow, and V_E may influence the production of NO. Basal C_{NO} has been found to be identical between high aerobic capacity athletes and non-athletes [40, 130] and higher in one subject described as an athlete [19]. However, during exercise, it has been observed that athletes maintain a higher V_{NO} than do moderately trained and sedentary individuals at a given exercise

intensity [130]. In disagreement with these data, no difference in V_{NO} was found between trained men, sedentary women, and sedentary men at rest or during cycle exercise [40]. The dissimilar results may be related to the different levels of physical conditioning and maximal aerobic capacities of the respective athletic groups; 4.4 $l \cdot min^{-1}$ [40] and 5.6 $l \cdot min^{-1}$ [130]. These two groups would likely have different V_E, Q, and pulmonary blood flow, thus they would have different rates of NO production. To date, these are the only data available addressing this issue, leaving the question of the effects of physical conditioning on basal and exercising NO unresolved. Athletes with EIH have impaired gas exchange, possibly related to alterations in pulmonary tone. The relationship between exhaled NO and EIH has yet to be examined.

NO has also been detected in asthmatic patients and is elevated in comparison to matched non-asthmatics [4, 111, 112]. The increase in exhaled NO in asthmatics likely reflects increased activity of inducible NOS that may be mediated by inflammatory cytokines [73]. Exhaled NO may reflect airway inflammation in asthma, and may be used as means of monitoring inflammatory events [18]. The pulmonary endothelium is susceptible to injury given that it is exposed to the entire Q. Various inflammatory mediators may play a role, including NO, in altering pulmonary vascular tone and membrane permeability. Subsequent vasoconstriction or vasodilation may promote overperfusion, increased filtration pressure and edema formation. NO detected in exhaled air, as an indicator of lower airway inflammation, may be representative of these events but has not yet been clearly established.

Inhaled Nitric Oxide.

Inhaled NO is a potent and selective pulmonary vasodilator [59, 60]. Inhaled NO therapy has received much clinical attention in recent years, and has been useful in the treatment of lung diseases characterized by pulmonary hypertension and arterial hypoxemia [2, 25, 194, 195, 198, 245]. Inhalation of NO in low concentrations (5-80 ppm) can cause selective pulmonary vasodilation because of the speed with which NO reacts with hemoglobin. Essentially, free NO never leaves the pulmonary circulation and only dilates vascular beds that are well-ventilated resulting in improved V_A/Q matching [109]. The current consensus is that smooth muscle vasodilation is caused by the NO signal transduction system as described above. The clinical effects and applications of inhaled NO have been described extensively in recent reviews [24, 97, 143, 152]. To summarize, inhalation of NO is used effectively to treat a multitude of disorders including: primary pulmonary hypertension, pulmonary hypertension caused by embolism, pulmonary hypertension associated with cardiopulmonary bypass, adult respiratory distress syndrome, and HAPE [53, 107, 161, 198, 207]

Only recently have the effects of inhaled NO been examined in exercising populations. Roger et al. [197] demonstrated that in chronic obstructive pulmonary disease (COPD) patients who inhaled NO (40 ppm), P_{PA} was reduced both at rest and during submaximal exercise when compared to breathing only room air. During exercise, PaO_2 was reduced while breathing room air (- 5 mmHg) and was slightly improved (+ 2 mmHg) with the addition of NO to the inspirate. Although small, both of these changes were considered statistically significant. It was also observed that when patients exercised while inhaling NO, the degree of V_A/Q mismatch was lessened and likely was causative in improved PaO_2 values. Inhaled NO (40 ppm) has also been shown to improve exercise capacity in patients with congestive heart failure [117]. Patients with

severe heart failure reduced the resistance of the pulmonary vessels leading to augmented maximum exercise workload and VO_2 at the anaerobic threshold. In a separate investigation, Bocchi et al. [26] examined patients with left ventricular dysfunction and chronic heart failure during exercise conditions of control (room air) and NO inhalation (30 ppm). VT and ventilatory equivalent for oxygen (VE/VO_2) were significantly reduced with NO compared to control. Unfortunately, other measures of gas exchange and pulmonary hemodynamics were not measured and the physiological significance of these findings is not clear. Nonetheless, based on these available data, it can be tentatively concluded that inhalation of NO during exercise in clinical populations can improve gas exchange and pulmonary hemodynamics. The effect is likely explained by a preferential distribution of inhaled NO to well-ventilated alveolar units, a reduction in the dispersion of ventilation distribution, and a lowering of pulmonary pressures.

To date, only one study has sought to investigate the effects of NO inhalation on pulmonary gas exchange during exercise in highly trained athletes [49]. Male endurance athletes ($n = 9$: $VO_2max \sim 65$ $ml \cdot kg^{-1} \cdot min^{-1}$) performed a maximal cycle test to exhaustion while inhaling either (i) room air, or (ii) room air combined with 15 ppm NO. No differences between conditions were observed for VO_2max or maximal work. Inhalation of NO caused PaO_2 to decrease at rest, 50%, 75%, and 100% VO_2max (↓ 9.5, 15.0, 14.6, and 5.1 mmHg). Interestingly, delta PaO_2 continuously fell without NO (13.5%). With NO, after a preliminary drop, a stabilization of delta PaO_2 was observed between 75 and 100% VO_2max. This trend was also reflected in $[A\text{-}a]DO_2$. Inhalation of NO also abolished histamine release between 75 and 100% VO_2max but EIH was not reversed. The physiological significance of these results remains to be determined. As concluded by Durand and co-workers [49], the negative effect of NO on gas

exchange at rest was unexpected and continued during exercise; and these preliminary results should be interpreted with caution.

Statement of the Problem.

Firstly, the relationship between endogenous pulmonary NO and EIH is not known. It is currently not clear if athletes with EIH have a different NO response to exercise compared to other highly-trained athletes without EIH. Based on the observation that those more susceptible to HAPE may have a defect in NO synthesis and that untrained, highly-trained athletes, and asthmatics have different levels of exhaled NO, it is reasonable to suggest that athletes with EIH may have an altered exhaled NO response to exercise. Secondly, while the mechanisms of EIH are debatable, the pathology may be related to elevations in pulmonary pressures. Thus inhaled NO, as a known pulmonary vasodilating agent, may reverse or attenuate the mechanisms underlying EIH if elevations in pulmonary pressures are involved.

Hypotheses.

The purpose of these two studies was to determine the relationship between EIH and NO. Specifically, the two fundamental hypotheses tested were:

1. Exhaled NO will be reduced in athletes with EIH compared to matched athletes without EIH.
2. Inhaled NO will improve arterial oxygenation in athletes with EIH during normoxic and hypoxic heavy exercise.

CHAPTER 2. EXHALED NITRIC OXIDE

2.1 INTRODUCTION

During maximal exercise PaO_2 and SaO_2 are decreased significantly in some highly-trained male athletes [48, 91, 173] and an increase in $[A\text{-}a]DO_2$ is associated with the lowered PaO_2. The mechanisms to explain the reduction in blood oxygenation remain controversial. It is generally agreed that V_A/Q inequality and diffusion limitations resulting from elevated pressures in the pulmonary vasculature are a likely explanation [89].

It is now well established that NO is produced in a variety of both human and animal cells and has numerous biological functions. NO is a ubiquitous molecule thought to exert regulatory functions in the circulatory, pulmonary, nervous and immune systems [244]. Production of NO has vasodilatory effects causing relaxation of smooth muscle cells via a signal transduction system. In brief, NO is synthesized from the common amino acid L-arginine by several types of NO synthase (NOS). NO stimulates the conversion of GTP to cGMP leading to relaxation of smooth muscle by decreasing the concentration of free Ca^{2+} in the cytosol. Endogenously produced NO was first detected in the exhaled air of humans by Gustafsson et al. [72] and both the concentration (C_{NO}) and the production rate of NO (V_{NO}) have since been examined during aerobic exercise [19, 40, 100, 130, 135, 167, 169]. Exercise has been shown to have no effect on C_{NO} [19, 100], or cause a slight decrease [40, 130, 167, 169]. In all pulmonary NO-exercise investigations,V_{NO} increases with physical work, however there is considerable variability among studies, possibly related to differences in training status and measurement techniques. Increases in V_{NO} during exercise have been reported as different [19, 130] and the same [40] between athletes and non-athletes. A growing body of evidence suggests that exercise training increases basal NO production in the systemic vasculature [68, 108, 115, 172]. It is well known

that lowered systemic blood pressure and heart rate, increased coronary blood flow and capillary density of skeletal muscle, all characterize the effects of chronic exercise training. Decreased systemic blood pressure may be mediated by augmented basal production of NO and its subsequent vasodilatory effect [172]. An upregulation of NO production in the systemic vasculature caused by training could be mirrored in the pulmonary vasculature.

Endogenously produced pulmonary NO may have several physiological functions including V_A/Q matching and maintenance of low pulmonary vascular resistance [71]. The hypothesis that an optimal V_A/Q ratio may be regulated by NO is supported by data showing the development of arterial hypoxia in anesthetized animals upon administration of NOS inhibitors [163]. The importance of NO is also evident when inspired NO induces pulmonary vasodilation and improves V_A/Q relationships [60, 168, 182, 197]. The consequence of inhaled NO is underscored during submaximal exercise in patients with chronic obstructive pulmonary disease who inhaled 40 ppm NO. Improvements were observed in both V_A/Q relationships and PaO_2 compared to breathing room air [197]. Endogenous NO was recently found to be critical to the maintenance of a low pulmonary vascular resistance in NOS knockout mice who developed pulmonary hypertension [55], and may be continuously released to maintain pulmonary arterial tone, systemic pressure, and Q [218]. In addition, patients with heart disease have reduced expiratory NO levels, perhaps explaining why they exhibit an increased vasoconstriction of their pulmonary blood vessels [220].

The mechanism for increased V_{NO} during exercise has been attributed to elevated shear stress on the pulmonary endothelium via increased pulmonary blood flow [19, 100, 130]. In the peripheral vasculature, an increase in shear stress leads to enhanced endothelial NO release [163] arising from the frictional force of blood flow, although other physical stimuli such as pulsatility

and changes in transmural pressure may be important [170, 201]. Deformation of endothelial cells is the resultant effect of shear stress and initiates the NO-cGMP signal transduction system [142, 242]. Data from an isolated pig lung preparation indicates that the pulmonary vascular endothelium likely contributes to the NO found in exhaled air [44]. Increased pulmonary endothelial NO production may enter the airway lumen via the alveoli. Endurance athletes with EIH are known to have altered V_A/Q relationships during strenuous work [89]. Abnormalities in pulmonary vascular tone, resulting in EIH, may be mediated by a lowered NO response to exercise. Thus, the purpose of this study was to determine the relationship between exhaled NO and SaO_2 in two groups of highly trained male cyclists. We hypothesized that subjects with EIH would have a decreased V_{NO} compared to subjects who maintained normal SaO_2 and EIH would be correlated with V_{NO}.

2.2 METHODOLOGY

Subjects.

Highly-trained male competitive road and mountain cyclists and triathletes were recruited to participate in this study ($n = 18$). All subjects were required to have participated in elite level cycle competition (provincial, national, or international) and have no history of cardio-respiratory disease. Prior to any testing, subjects received a verbal description of the experiment and completed a written informed consent form. This study was approved by the Clinical Screening Committee for Research and other Studies Involving Human Subjects of the University of British Columbia.

Maximal Cycle Ergometer Test.

Prior to all testing, subjects abstained from exhaustive exercise for 24 h, ingestion of food or fluid other than water for 4 h, and alcohol and caffeine consumption for 12 h. VO_2max was

determined using an incremental test on an electronically braked cycle ergometer (Quinton Excalibur). Subjects pedaled at a self-chosen cadence at a progressing workload, which started at 0 watts and increased 30 watts·min^{-1}. Subjects inspired through an air flowmeter (Vacumetrics model 17150, Ventura, CA) using a two-way non-rebreathing valve (Hans-Rudolph, model 2700B, Kansas City, KS) and expired air passed into a 5 litre mixing chamber from which gas samples were analyzed at a rate of 300 ml·min^{-1} for oxygen and carbon dioxide concentrations (S-3A oxygen analyzer and CD-3A carbon dioxide analyzer, Applied Electrochemistry, Pittsburgh, PA). Expired gases and minute ventilation ($\dot{V}_E$) were recorded using a computerized system (Rayfield, Waitsfield, VT). Gas analyzers were calibrated with gases of known concentration prior to each experiment, and the air flowmeter was calibrated by passing 100 litres of air through the system. Heart rate was recorded every 15 s using a portable heart rate monitor (Polar Vantage XL, Kempele, Finland). SaO_2 was measured by a pulse oximeter (Ohmeda Biox 3740, Louisville, CO) with values averaged and recorded every 5 s. This oximeter has previously been shown to be a valid and reliable predictor of SaO_2 during cycling [133] and performs a self-calibration prior to usage. Prior to placement of the oximeter sensor to the pinna of the ear, a topical vasodilator cream (Finalgon, Boehringer/Ingeheim) was applied to increase local perfusion. Attainment of VO_2max was considered when at least three of the four following criteria were met: (i) a plateau in VO_2 with increasing workload, (ii) RER > 1.15, (iii) attainment of 90% of age predicted maximal heart rate, and/or (iv) volitional fatigue.

Nitric Oxide.

It is known that ambient air contains a variable concentration of NO. During the cycle ergometer test subjects inspired compressed air where NO concentration remained < 2 ppb, indicating that NO in expired air was of endogenous origin. The compressed medical air was

delivered from a large cylinder through water for humidification, then into a large meteorological balloon, which acted as a reservoir for inspired air. Expired air was mixed within a five litre mixing chamber placed distally to the expiratory port of the mouthpiece. Air samples were withdrawn from the chamber, through a column of Drierite to remove water vapor and into a three litre syringe (Hans Rudolph) equipped with a 3-way stop to prevent expired air becoming contaminated with room air. Approximately one litre of gas was then immediately infused into a Mylar collection bag shown to be impermeable to and non-reactive with NO [167]. A resting measurement was performed after an initial period of 10 min, during which the subject was seated and resting quietly on the cycle ergometer breathing compressed air. A five min self-selected cycling warm-up (30-100 watts) was performed prior to commencing the cycle test. Expired NO samples were obtained when subjects reached 100, 200, 250, 300, 350, 400, and 450 watts respectively.

Collected expired air samples were analyzed for C_{NO} using a chemiluminescent analyzer (NOA 280, Sievers Instruments, Boulder, CO). Briefly, chemiluminescence is based on a gas-phase chemiluminescent reaction between NO and ozone resulting in nitrogen dioxide (NO_2). Emission from electronically excited NO_2 is in the red and near-infrared region of the spectrum and is detected by a thermoelectrically cooled red-sensitive photomultiplier tube. The sensitivity of this analyzer for NO is < 1 ppb. The NO analyzer was calibrated prior to each use as per the manufacturer's specifications and is linear over a range of 1 ppb – 500 ppm. The output of the analyzer was connected to a personal computer for recording purposes. V_{NO} was calculated as the product of C_{NO} (ppb) and V_E ($l \cdot min^{-1}$) and was expressed as $nmol \cdot min^{-1}$. The technique employed to examine NO in the present study was similar to those used in other exercise and NO investigations [130, 167].

Statistical Analyses.

Subjects were divided into two groups based on SaO_2 at maximal exercise. Those with $SaO_2 > 92.0\%$ were classified as normal saturation (NOS), those with $SaO_2 \leq 92.0\%$ were assigned to the low saturation group (LOS). Comparisons between NOS and LOS for anthropometric, descriptive, and VO_2max data were made using paired *t*-tests. Expired NO data were examined using a 2 (group) by 8 (time) analysis of variance, with repeated measures across time. When significant *F* values were obtained, Bonferroni's test was applied *post-hoc* to determine where the differences occurred. Pearson's product moment correlation coefficient was utilized to ascertain the relationship between change in percent oxyhemoglobin saturation (delta SaO_2) and change in NO production (delta VNO). The level of significance was set at $P < 0.05$ for all statistical procedures.

2.3 RESULTS

Descriptive and peak exercise values are shown in Table 1. SaO_2 dropped significantly from a resting mean resting value of 97.8% to a peak exercise value of 92.7% ($P < 0.05$). When subjects were divided into NOS and LOS only SaO_2 data were statistically different between groups ($P < 0.05$). Table 2 depicts values for CNO and VNO at rest and during incremental exercise for all subjects. CNO remained unchanged from rest throughout exercise. VNO was significantly greater than rest at 300, 350, 400, and 450 watts. NOS and LOS groups showed no significant differences for CNO (Figure 1) or VNO (Figure 2). The correlation between delta SaO_2 and delta VNO ($r = - 0.12$) was non-significant ($P > 0.05$; Figure 3). All individual subject data is presented in Appendix D.

TABLE 1. Descriptive and peak exercise values in all subjects (ALL), subjects with normal (NOS) and low (LOS) oxyhemoglobin saturation. Definition of abbreviations: VO_2max = maximal oxygen consumption; VE = minute ventilation; RER = respiratory exchange ratio; HRmax = maximal heart rate; SaO_2 = percent oxyhemoglobin saturation. Values are means ± SD. † significantly different than Resting SaO_2, $P < 0.05$. * significantly different than NOS, $P < 0.05$.

	ALL ($n = 18$)	NOS ($n = 12$)	LOS ($n = 6$)
Age (yr)	26.7 ± 4.6	27.8 ± 4.9	24.7 ± 2.6
Height (cm)	179.2 ± 5.2	178.2 ± 5.0	181.3 ± 5.1
Mass (kg)	72.8 ± 7.9	71.0 ± 8.8	76.5 ± 4.0
VO_2max ($l \cdot min^{-1}$)	4.9 ± 0.6	4.8 ± 0.7	5.2 ± 0.3
VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)	67.7 ± 5.2	67.7 ± 4.8	68.7 ± 5.7
VE ($l \cdot min^{-1}$)	174.6 ± 14.0	174.1 ± 14.3	175.6 ± 13.3
RER (VCO_2/VO_2)	1.16 ± 0.04	1.16 ± 0.05	1.16 ± 0.03
HR ($beats \cdot min^{-1}$)	190.6 ± 7.4	190.4 ± 8.2	191.0 ± 5.8
Resting SaO_2 (%)	97.8 ± 0.6	97.9 ± 0.4	97.5 ± 0.8
Lowest SaO_2 (%)	92.7 ± 2.0 †	93.9 ± 0.8 †	90.3 ± 1.0 † *
Power (watts)	459.1 ± 27.6	455.5 ± 30.3	466.2 ± 19.9

TABLE 2. Concentration of nitric oxide (CNO) and production rate of nitric oxide (VNO) at rest and during incremental exercise in all subjects ($n = 18$). Values are means ± SE. * Significantly different than Rest, $P < 0.05$.

WORK (watts)	CNO (ppm)	VNO ($nmol \cdot min^{-1}$)
Rest	8.0 ± 1.4	3.3 ± 0.5
100	6.5 ± 1.1	8.9 ± 1.6
200	6.1 ± 1.1	12.0 ± 2.2
250	6.3 ± 1.3	15.2 ± 2.9
300	6.7 ± 1.2	20.6 ± 3.7 *
350	6.3 ± 1.2	24.1 ± 4.5 *
400	7.0 ± 1.4	32.6 ± 7.2 *
450	7.6 ± 1.5	41.4 ± 8.7 *

FIGURE 1. Concentration of nitric oxide (C_{NO}) at rest and during incremental exercise in subjects with normal (NOS, n =12) and low (LOS, n = 6) oxyhemoglobin saturation. Open circles = NOS, closed squares = LOS. Values are means ± SE.

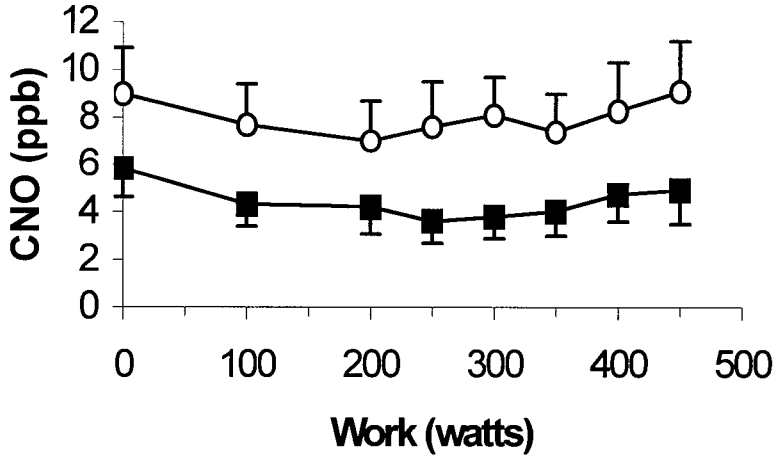


FIGURE 2. Production rate of nitric oxide (V_{NO}) at rest and during incremental exercise in subjects with normal (NOS, n = 12) and low (LOS, n = 6) oxyhemoglobin saturation. Values are means ± SE.

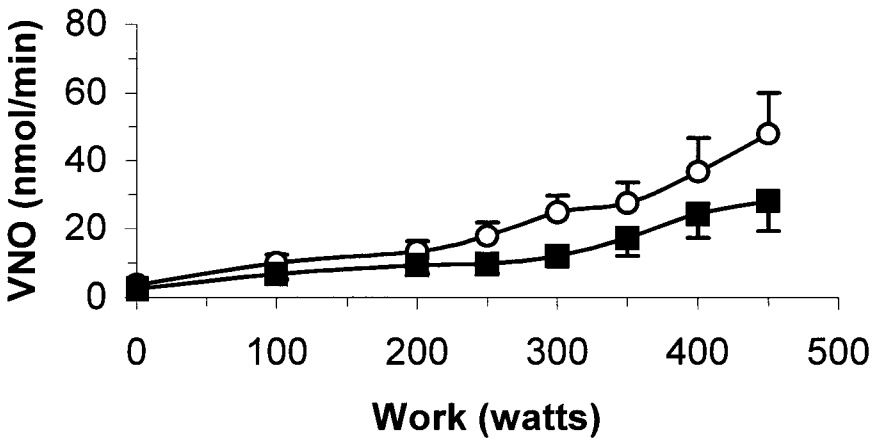
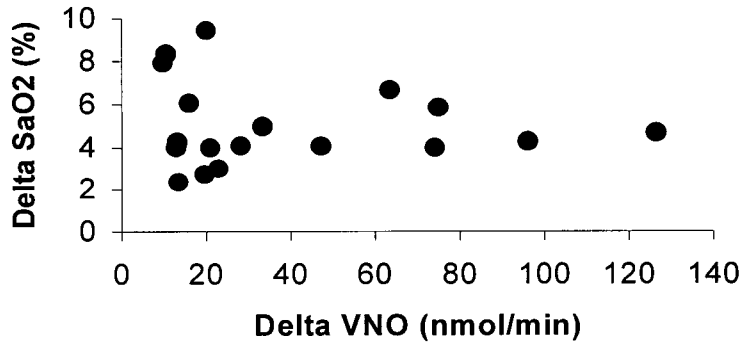


FIGURE 3. Relationship between change in percent oxyhemoglobin saturation (delta SaO_2) and change in nitric oxide production (delta VNO) in all subjects ($n = 18$, $r = -0.12$, $P > 0.05$).



2.4 DISCUSSION

The principal findings of this study were that C_{NO} remained unchanged during incremental exercise to exhaustion, and that V_{NO} increased in proportion to exercise intensity in highly-trained male cyclists. Unique to the findings of this study was the observation that changes to SaO_2 during incremental exercise were unrelated to alterations in exhaled NO. Decreased arterial oxygenation has been widely reported in highly-trained male athletes [48, 91, 173]. Mechanisms to explain EIH remain debatable, however elevated pulmonary pressure resulting in V_A/Q mismatch and diffusion limitations are a likely explanation [48, 89]. Endogenously produced pulmonary NO has been suggested to possibly have several physiological functions including V_A/Q matching and maintenance of low pulmonary vascular resistance [71]. We hypothesized that exhaled NO may be reflective of the processes responsible for EIH. V_{NO} was increased significantly in all subjects during exercise (Table 2), but was not different between NOS and LOS (Figure 2). Delta V_{NO} was not correlated with delta SaO_2 (r = -0.12, $P > 0.05$) (Figure 3). Collectively, these results show that EIH is not related the production rate of NO. We must therefore reject our original hypothesis that subjects with EIH have decreased V_{NO} compared to non-EIH subjects during heavy physical work.

The presence of NO in exhaled air has been widely reported in humans [19, 40, 72, 130, 167] and different animal species [206]. Human values of resting and exercising C_{NO} and V_{NO} are variable. The effect of exercise on C_{NO} has been shown to cause a slight decrease [40, 130, 166, 169], or have no effect [19, 100]. The latter is in agreement with the results of the present study. The physiological importance of maintaining, or slightly reducing, C_{NO} during exercise remains unclear but possibly reflects a shift in the site of NO production. C_{NO} in the nasal passages is predominant in resting humans with the lower airways contributing a smaller fraction

[113, 193, 205]. The contribution of the nasal passages to exhaled NO has recently been observed to decrease during exercise [127, 168, 192], while the proportion from the lower airways increases [168]. Nasal cavity NO levels dropped rapidly (~ 50% resting values) after only one min of exercise compared with rest, and were lowered (~ 76% resting values) during heavy work [127]. This shift may be reflective of a change from nasal to oral breathing at the onset of exercise explaining the immediate decrease in nasal CNO [127], although total exhaled CNO remains unchanged or slightly reduced. This suggests that during exercise a shift occurs where a greater percentage of total CNO comes from a lower airway source rather than the nasal cavity [168]. An increase in pulmonary endothelial production could explain the stability of CNO, in spite of increased ventilation and a dilution effect, since NO derived from the endothelium may enter the airway lumen via the alveoli. Increased NO production may be the result of increased Q and flow through the pulmonary circulation. During exercise, Q increases in proportion to exercise intensity and the pulmonary circulation is able to accommodate the increase with little rise in pulmonary pressure due to the distension and recruitment of the pulmonary capillaries. It has been traditionally thought that this process is passive in nature where the pulmonary circulation is fully recruited. Although not yet fully understood possibly NO aids in regulating this process and is reflected in exhaled NO [71].

The production rate of NO has previously been shown to be elevated during exercise [19, 40, 130, 135, 160, 167, 168, 169, 224]. VNO values obtained in the present investigation are comparable to other studies [40, 130]. Comparison is limited however, as there is currently no consensus on measurement techniques for exhaled NO during exercise. As such, only studies using similar methods can be adequately compared, as it appears that various techniques can alter NO values. It is also unclear what effect training status has on VNO further hindering the

comparison of studies. It is widely accepted that physical conditioning affects the cardiovascular response to exercise. Training-induced alterations to Q, pulmonary blood flow, and V_E all may influence the production rate of NO. Basal C_{NO} has been found to be identical between high aerobic capacity athletes and non-athletes [40, 130] and higher in one subject described as an athlete [19]. During exercise, it has been observed that athletes maintain a higher C_{NO} than do moderately trained and sedentary individuals at a given exercise intensity [130]. In disagreement with these data, no difference in C_{NO} was found between trained men, sedentary women, and sedentary men at rest or during cycle exercise [40]. The dissimilar results may be related to different levels of physical conditioning and maximal aerobic capacities of the respective athletic groups; 4.4 $l \cdot min^{-1}$ [40] and 5.6 $l \cdot min^{-1}$ [130]. These two groups would likely have different V_E, Q, and pulmonary blood flow; thus they would possibly have different rates of NO production. Additionally, disunity in results is possibly related to the method of reporting data. When V_{NO} is expressed per unit of body mass ($pmol \cdot kg^{-1} \cdot min^{-1}$), differences are seen between athletes and non-athletes [130]. However, when V_{NO} is expressed in absolute terms ($nmol \cdot min^{-1}$), no differences between groups are observed [40]. In a preliminary study of unpublished observations Phillips et al [168], describe a statistically significant correlation between exhaled NO output and body surface area ($r = 0.59$). To date, there are few data available addressing this issue, leaving the question of the effects of physical conditioning on basal and exercising C_{NO} and V_{NO} unresolved.

Power analysis was performed to determine the level of power ($1 - \beta$) achieved in the present study. Utilizing a statistical computer program (SPSS), the effect size for V_{NO} was 0.3 and that power for the analysis of variance was ~ 22%. This can be considered low given that a power of 80% represents reasonable protection against type II error. Given the small effect

size (large variability in V_{NO}) in this study that to achieve 80% power, approximately 60 additional subjects would have to participate in the study.

In summary, this study confirmed that V_{NO} increases and that C_{NO} remains unchanged with progressive exercise in highly trained male endurance athletes. This is the first study to show that no differences in V_{NO} and C_{NO} were observed between LOS and NOS. Changes to V_{NO} during exercise were unrelated to exercise-induced alterations to SaO_2. Increased NO production during exercise, determined via exhaled air, was not related to exercise-induced hypoxemia.

CHAPTER 3. INHALED NITRIC OXIDE

3.1 INTRODUCTION

During maximal exercise PaO_2 and SaO_2 are decreased significantly in some highly-trained male athletes [48, 91, 173]. An increase in $[A\text{-}a]DO_2$ is associated with the lowered PaO_2. The mechanisms to explain EIH remain controversial but it is believed that that V_A/Q inequality and diffusion limitations resulting from elevated pressures and pulmonary edema and/or decreased pulmonary transit time with the pulmonary vasculature are likely [74, 89]. Inhaled NO, a selective pulmonary vasodilator, is used in the treatment of diseases characterized by pulmonary hypertension and hypoxemia [116]. If EIH and pulmonary edema is caused by elevations of pulmonary pressures, inhaled NO should diminish the observed hypoxemia during exercise. The observed hypoxemia during hypoxic exercise is more pronounced in athletes with EIH than in normal individuals [123, 132]. Therefore, the aim of this study was to test the effects of inhaled NO (20 ppm) on gas exchange in athletes with EIH during strenuous exercise under conditions of normoxia and hypoxia. We hypothesized that during short duration, high-intensity exercise: (1) inhaled NO would reverse EIH during normoxia, and (2) inhaled NO would improve arterial oxygenation during hypoxia.

3.2 METHODOLOGY

Subjects.

Highly-trained male cyclists were recruited to participate in this study ($n = 8$). One subject was forced to withdraw due to difficulties with placement of the arterial catheter, thus all data will be reported as such ($n = 7$). This investigation was divided into two parts. Subjects who met the inclusion criteria in Part 1 participated in Part 2. Inclusion criteria were (i) normal

spirometry, no history of asthma or cardiorespiratory disease, (ii) $VO_2max \geq 60$ ml·kg^{-1}·min^{-1} and/or 5 l·min^{-1}, (iii) maximal exercise $SaO_2 \leq 91.0\%$, (iv) between the ages of 18-40. Prior to any testing, subjects received a verbal description of the experiment, and completed a written informed consent form. This study was approved by the Clinical Screening Committee for Research and Other Studies Involving Human Subjects of the University of British Columbia.

Preliminary Screening – Part 1.

Subjects reported to the Applied Physiology Laboratory in the Allan McGavin Sports Medicine Center, UBC, having refrained from exhaustive exercise for 24 h, abstained from ingestion of food or fluid for 4 h except for water, and alcohol and caffeine for 12 h. Subjects were weighed and their height was recorded. Both spirometry, and pulmonary diffusion measurements for carbon monoxide (DLCO) were collected using the same commercial apparatus (Collins DS/PLUS II, Braintree, MA). DLCO was determined using the single-breath method [1991], and conformed to the American Thoracic Society standardization of DLCO measurement [215]. Prior to performing DLCO and spirometry measures subjects sat and rested for 30 min to stabilize their pulmonary system.

Maximal oxygen consumption was determined using an incremental test on an electronically braked cycle ergometer (Quinton Excalibur, Lode, Groningen, Netherlands). Subjects pedaled at a self-chosen cadence at a progressing workload, which started at 0 watts and increased 30 watts·min^{-1}. Subjects inspired through an air flowmeter (Vacumetrics model 17150, Ventura, CA) using a two-way non-rebreathing valve (Hans-Rudolph, model 2700B, Kansas City, KS) and expired air passed into a 5 litre mixing chamber from which gas samples were analyzed at a rate of 300 ml·min^{-1} for oxygen and carbon dioxide concentrations (S-3A oxygen analyzer and CD-3A carbon dioxide analyzer, Applied Electrochemistry, Pittsburgh, PA). Expired gases

and VE were recorded using a computerized system (Rayfield, Waitsfield, VT). Gas analyzers were calibrated with gases of known concentration, and the air flowmeter was calibrated by passing 100 litres of air through the system. Heart rate was recorded every 15 s using a portable heart rate monitor (Polar Vantage XL, Kempele, Finland). SaO_2 was measured by a pulse oximeter (Ohmeda Biox 3740, Louisville, CO) with values averaged and recorded every 5 s using a personal computer. This oximeter has previously been shown to be a valid and reliable predictor of SaO_2 during cycling [133]. Prior to placement of the oximeter sensor to the pinna of the ear, a topical vasodilator cream (Finalgon, Boehringer/Ingeheim, Burlington, ON) was applied to increase local perfusion. Attainment of VO_2max was considered when at least three of the four following were met observed: (i) a plateau in VO_2 with increasing workload, (ii) RER > 1.15, (iii) attainment of 90% of age predicted maximal heart rate, and/or (iv) volitional fatigue. During Part 1 cycle ergometry subjects inspired compressed air (FIO_2 = 20.93%). The air was delivered from a large cylinder through a closed container of water for humidification, then into a large meteorological balloon, which acted as a reservoir for inspired air. Those who met the inclusion criteria returned on a separate day at least 72 h later to perform another maximal cycle ergometry test under hypoxic conditions (FIO_2 = 14%). This F_IO_2 has previously been used to accentuate decreases in SaO_2 in exercising trained males [123, 132]. Expired gases, heart rate, and SaO_2 were determined during the hypoxic exercise session as detailed above.

Inhaled Nitric Oxide – Part 2.

Following completion of both VO_2max tests (normoxic and hypoxic) those subjects who met the inclusion criteria returned on a separate day at least 72 h later. Subjects were randomly assigned and blinded to each of the four following conditions: (i) Normoxia (N), (ii) Normoxia/Nitric Oxide (N/NO), (iii) Hypoxia (H), and (iv) Hypoxia/Nitric Oxide (H/NO).

Participants performed a 10-15 min cycling warm-up at a self-selected workload and then sat quietly on the cycle ergometer for 5 min when resting data were obtained (Rest). Cycling intensity was then manually increased to 100% of their respective maximum normoxic or hypoxic work load as determined in Part 1. The duration from rest to maximal workload was 1 min. Subjects cycled at this intensity for 5 min, and cardiorespiratory variables were recorded at each min in the same fashion as during Part 1. Arterial blood samples were drawn at rest and at each min of the 5 min test (see ARTERIAL BLOOD SAMPLING). Following each test condition subjects cycled easily (30-50 watts·min^{-1}) for 10 min and then rested for 50 min prior to commencing the next test condition. An overview of the experimental protocol is depicted in Figure 4.

During N subjects rested for 5 min and cycled while breathing normoxic gas. Condition N/NO consisted of normoxic gas with 20 ppm NO delivered to the inspiratory tubing. During condition H subjects rested for 5 min and cycled while inhaling hypoxic gas (FIO_2 = 14%) and condition H/NO consisted of the same hypoxic gas with 20 ppm NO delivered to the inspiratory tubing. During all conditions the inspired air was delivered from a large cylinder through water for humidification, then into a large meteorological balloon, which acted as a reservoir prior to being inspired by the subject. NO was delivered at the distal end of the tubing, while inspired concentrations of O_2, NO and nitrogen dioxide (NO_2) were monitored continuously during each test condition ~ 5 cm from the subject's mouth using a commercial apparatus (PulmoNOx II, Pulmonox, Tofield, AB). The system was calibrated prior to each experiment as per the manufacturer's specifications. The flow rate of NO was calculated as follows:

NO flow (l·min^{-1}) = (V_E x desired [NO]) / (source tank [NO])

For example, if V_E = 145 l·min^{-1}, desired [NO] = 20 ppm, and source tank [NO] = 2000 ppm

Then ...

$$\text{NO flow } (l \cdot min^{-1}) = (145 \times 20) / 2000$$
$$= 1.45\ l \cdot min^{-1}$$

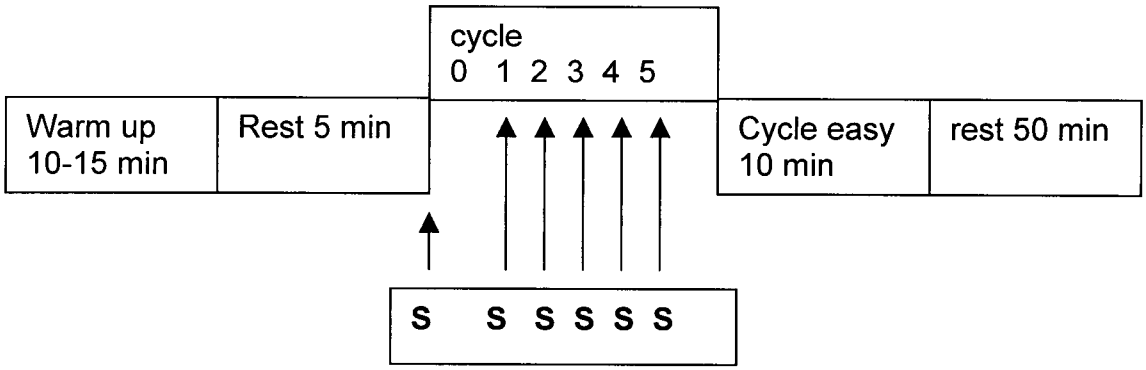
The concentration of NO used in the present study (20 ppm) has previously been shown to improve VA/Q distributions, PaO_2, and PVR in pigs [182], reverse hypoxic pulmonary vasoconstriction, and redistribute blood flow to better ventilated areas of the lung in sheep [168], and is thought to be an appropriate concentration for exercising humans (W. M. Zapol, personal communication). Furthermore, Durand et al. [49] recently utilized a comparable concentration of NO (15 ppm) in exercising athletes to evaluate the effect on gas exchange.

Arterial Blood Sampling.

A 20-gauge arterial catheter was inserted in the radial artery of the non-dominant hand by percutaneous cannulation using 1% local anesthesia (Lidocaine) and sterile technique, and was then secured to the skin. Adequate collateral circulation via the ulnar artery (Allen's test) was estimated before the cannula was inserted. A minimum volume extension tube, connected in series with two, three-way stopcocks arranged at right angles, was flushed with a saline-heparin solution. A rapid response (<0.01 s) thermister (18T, Physitemp Instruments, Clifton, NJ) used to measure peak arterial blood temperature was inserted through a Touhy-Borsch heparin lock (Abbott Hospitals, North Chicago, IL). Catheter patency was maintained with a continuous heparin infusion (1 ml 1:1000 units in 500 ml NS at 3 ml/hr). At the onset of sampling, 12 ml of blood was withdrawn, and the final 3 ml was collected in preheparinized plastic syringes. The remaining 9 ml was then slowly reinfused. Samples were withdrawn at rest and at 1 min intervals for the duration of each test (4 conditions x 6 samples per condition = 24 samples/subject). Blood samples were placed on ice until analyzed for H^+ ion concentration, PO_2, PCO_2, base excess, and HCO_3^- (CIBA-Corning 278 Blood Gas System, CIBA-Corning

Diagnostics Corporation, Medfield, MA). PaO_2 was corrected for temperature and H^+ ion concentration. Temperature increased 0.9 ± 0.1 °C (mean ± SD) from rest to 5 min of exercise across all trials. SaO_2 levels were calculated based on corrected PaO_2. The alveolar gas equation was used to calculated alveolar partial pressure (PAO_2) and [A-a]DO_2 [84].

FIGURE 4. Overview of experimental protocol, repeated for each condition of normoxia (N), normoxia + 20 ppm nitric oxide (N/NO), hypoxia (H), and hypoxia + 20 ppm nitric oxide (H/NO). S = sample for blood and cardiorespiratory variables.



Statistical Analyses.

Mean values and measures of variability were determined for descriptive, anthropometric, and lung function variables obtained during preliminary screening. Maximal cycle ergometry data from Part 1 were compared using *t*-tests for dependent samples (normoxia vs. hypoxia). Experimental data were analyzed using 4 (condition) by 6 (time) two-way factorial analysis of variance with repeated measures on both factors. When sphericity was not assumed, Greenhouse-Geisser *P*-values were utilized. When significant *F*-ratios were observed, Scheffé's test was applied *post-hoc* to determine where the differences occurred. The level of significance was set at $P < 0.05$ for all *t*-tests and $P < 0.01$ for ANOVA procedures. Statistical power calculations were performed *a priori* to estimate an appropriate minimum sample size . A sample size of five was calculated.

3.3 RESULTS

Physical and Maximal Exercise Data. Descriptive data and resting pulmonary function data are presented in Table 3. Data from normoxic and hypoxic maximal cycle ergometry tests are presented in Table 4. Significant differences were observed between normoxic and hypoxic conditions for VO_2max, RER, HRmax, and power output while no significant differences were detected for VE. From rest to maximal exercise SaO_2 dropped significantly under both normoxia (97.7 to 90.2) and hypoxia (97.0 to 75.5). All individual subject data is presented in Appendix E and mean data in Appendix F.

Blood Gases during 5-min Cycling. PaO_2 results are reported in Figure 5. Across all time points there were no significant differences between N and N/NO or between H and H/NO. Both hypoxic conditions were significantly lower than both normoxic conditions. PaO_2 values

were significantly lower at 1, 2, 3, 4, and 5 min of exercise compared to rest for all inspired gas conditions. Similar results were observed for SaO_2, except that values at 1 and 2 min were not significantly different from rest under conditions of N and N/NO (Figure 6). [A-a]DO_2 was significantly different at all time periods when compared to rest for all inspired gas mixtures (Figure 7) and significant differences were detected between N/NO and H at rest and during all exercise measurements. $PaCO_2$ was not significantly different between gas conditions, but was lower compared to rest throughout all exercise for H and H/NO, and at minutes 3, 4, and 5 for both N and N/NO (Figure 8).

Metabolic and Power Output during 5-min Cycling. Oxygen consumption increased significantly from rest to 1-min during all conditions (Figure 9). Across all conditions, VO_2 was also significantly higher at 2, 3, 4, and 5 min compared to rest and 1 min. Significant differences were detected between N/NO and H at 3, 4, and 5 min. Heart rate was significantly higher during exercise compared to rest (Figure 10) while no differences where observed between gas conditions. At minutes 4 and 5 heart rate was higher than minute 1 for all conditions. Power output was significantly lower during both hypoxic conditions compared to normoxic conditions (Table 5).

TABLE 3. Descriptive and resting pulmonary function data. Actual pulmonary function value with % predicted in parentheses. Values are means ± SD. Definition of abbreviations: FVC = forced vital capacity, FEV_1 = forced expired volume in 1 second, $FEF_{25-75\%}$ = forced expiratory flow at 25-75% of FVC, FEF_{max} = maximal forced expiratory flow rate, DLCO = pulmonary diffusion capacity for carbon monoxide, VA = alveolar volume, DLCO/VA = pulmonary diffusion capacity for carbon monoxide/alveolar volume.

Variable	Mean ± SD	
Age (yr)	28.9 ± 3.9	--
Height (cm)	181.4 ± 7.5	--
Mass (kg)	74.7 ± 6.6	--
FVC (L)	5.59 ± 0.81	(102 ± 14)
FEV_1 (L)	4.58 ± 0.73	(102 ± 15)
$FEF_{25-75\%}$ ($l \cdot sec^{-1}$)	4.52± 0.91	(102 ± 18)
FEV_1/FVC (%)	80.54 ± 5.82	(98 ± 7)
FEF_{max} ($l \cdot sec^{-1}$)	9.95 ± 1.08	(102 ± 17)
DLCO ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	36.99 ± 5.04	(117 ± 19)
VA (L)	5.26 ± 1.42	--
DLCO/VA	7.33 ± 1.65	--

TABLE 4. Peak exercise values during maximal cycle ergometer tests. Values are means ± SD. * Significantly different than NORMOXIA, † significantly different than Resting SaO_2 ($P < 0.05$). Definition of abbreviations: VO_2max = maximal oxygen consumption; VEmax = maximal minute ventilation; RER = respiratory exchange ratio; HRmax = maximal heart rate; SaO_2 = percent oxyhemoglobin saturation.

	NORMOXIA FIO_2=21%	HYPOXIA FIO_2=14%
VO_2max ($l \cdot min^{-1}$)	4.88 ± 0.43	4.24 ± 0.49 *
VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)	65.3 ± 1.6	56.6 ± 5.6 *
VEmax ($l \cdot min^{-1}$)	175.9 ± 10.2	168.2 ± 11.5
RER (VCO_2/VO_2)	1.20 ± 0.02	1.12 ± 0.07 *
HRmax ($beats \cdot min^{-1}$)	188 ± 4	179 ± 3 *
Resting SaO_2 (%)	97.7 ± 0.6	97.0 ± 0.8
Lowest SaO_2 (%)	90.2 ± 0.9 †	75.5 ± 4.5 * †
Power (watts)	449 ± 39	371 ± 38 *

FIGURE 5. Arterial pressure of O_2 (PaO_2) during rest and at each min of 5-min cycle ergometry tests under conditions of normoxia (N), normoxia + 20 ppm nitric oxide (N/NO), hypoxia (H), and hypoxia + 20 ppm nitric oxide (H/NO). Values are means ± SD. * indicates N and N/NO significantly different from REST. † indicates H and H/NO significantly different from REST.

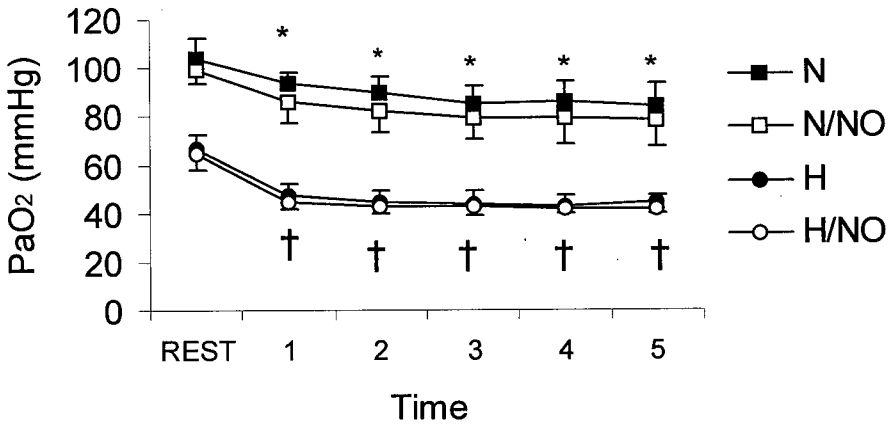


FIGURE 6. Percent oxyhemoglobin saturation (SaO_2) during rest and at each min of 5-min cycle ergometry tests under conditions of normoxia (N), normoxia + 20 ppm nitric oxide (N/NO), hypoxia (H), and hypoxia + 20 ppm nitric oxide (H/NO). Values are means ± SD. * indicates N and N/NO significantly different from REST. † indicates H and H/NO significantly different from REST.

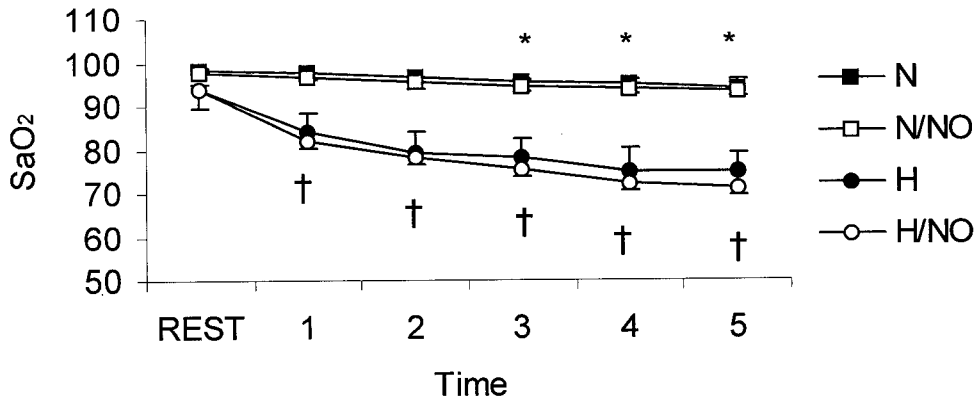


FIGURE 7. Alveolar-arterial difference for O_2 ([A-a]DO_2) during rest and at each min of 5-min cycle ergometry tests under conditions of normoxia (N), normoxia + 20 ppm nitric oxide (N/NO), hypoxia (H), and hypoxia + 20 ppm nitric oxide (H/NO). Values are means ± SD. * indicates N and N/NO significantly different from REST. † indicates H and H/NO significantly different from REST.

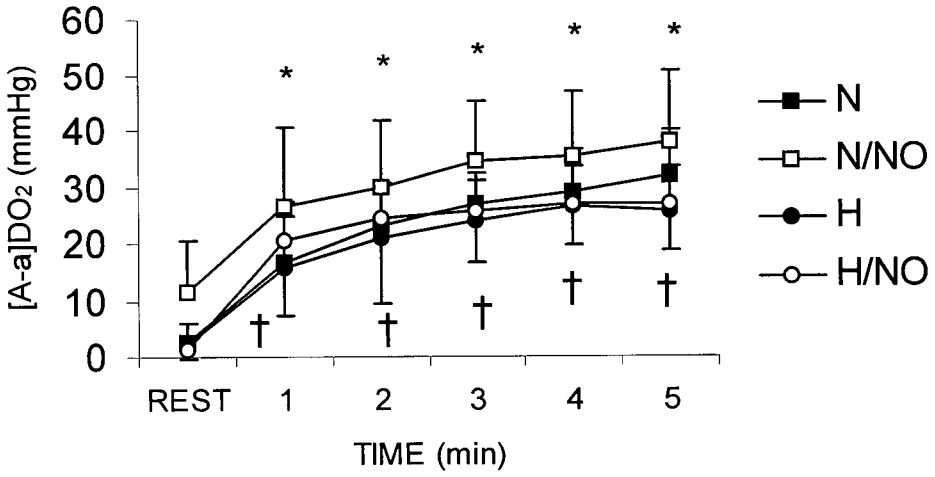


FIGURE 8. Arterial pressure of CO_2 ($PaCO_2$) during rest and at each min of 5-min cycle ergometry tests under conditions of normoxia (N), normoxia + 20 ppm nitric oxide (N/NO), hypoxia (H), and hypoxia + 20 ppm nitric oxide (H/NO). Values are means ± SD. * indicates N and N/NO significantly different from REST. † indicates H and H/NO significantly different from REST.

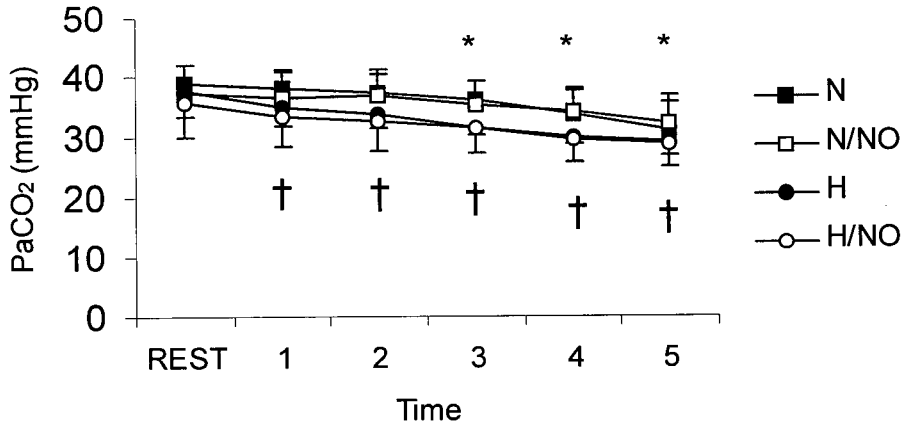


FIGURE 9. Oxygen consumption (VO_2) during rest and at each min of 5-min cycle ergometry tests under conditions of normoxia (N), normoxia + 20 ppm nitric oxide (N/NO), hypoxia (H), and hypoxia + 20 ppm nitric oxide (H/NO). Values are means ± SD. * indicates N and N/NO significantly different from REST. † indicates H and H/NO significantly different from REST.

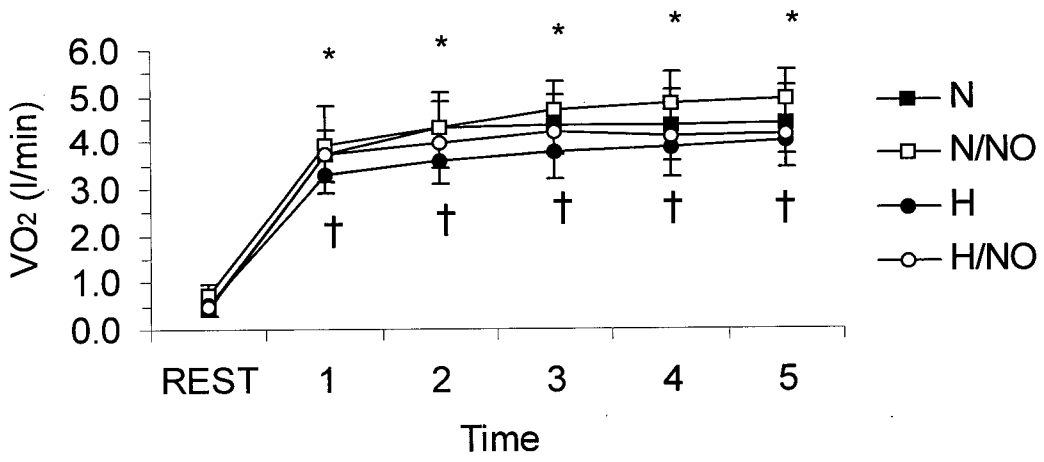


FIGURE 10. Heart rate during rest and at each min of 5-min cycle ergometry tests under conditions of normoxia (N), normoxia + 20 ppm nitric oxide (N/NO), hypoxia (H), and hypoxia + 20 ppm nitric oxide (H/NO). Values are means ± SD. * indicates N and N/NO significantly different from REST. † indicates H and H/NO significantly different from REST.

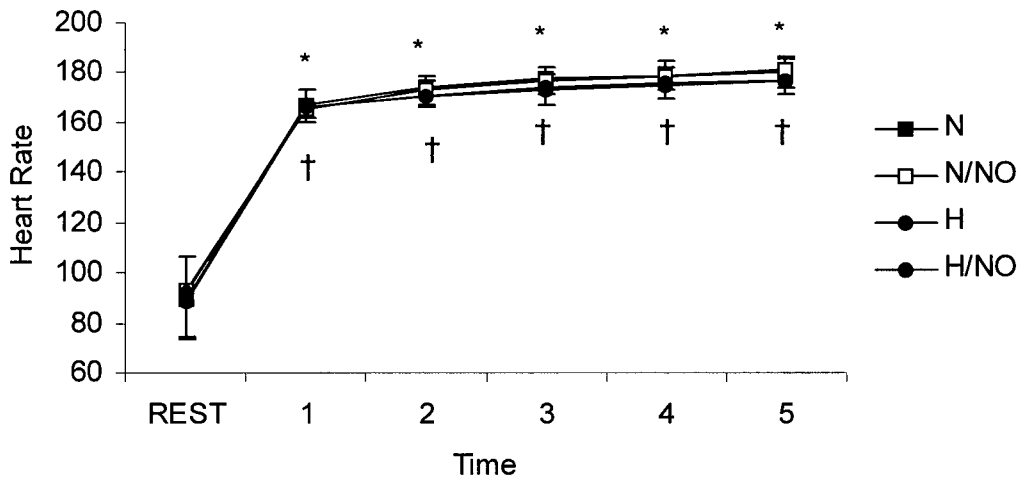


TABLE 5. Power output (watts) during rest and at each min of 5-min cycle ergometry tests under conditions of normoxia (N), normoxia + 20 ppm nitric oxide (N/NO), hypoxia (H), and hypoxia + 20 ppm nitric oxide (H/NO). Values are means (± SD). * indicates significantly different than H and H/NO.

	REST	1 MIN	2 MIN	3 MIN	4 MIN	5 MIN
N	0	390.8 *	368.3 *	353.3 *	336.3 *	333.8 *
	0	(36.1)	(32.5)	(44.6)	(61.7)	(60.9)
N/NO	0	378.3 *	355.7 *	341.7 *	330.0 *	330.0*
	0	(44.8)	(59.0)	(66.2)	(49.0)	(67.2)
H	0	326.3	303.9	296.4	288.6	284.3
	0	(33.4)	(40.7)	(55.4)	(55.0)	(52.3)
H/NO	0	329.3	307.0	278.6	263.3	262.6
	0	(30.3)	(42.7)	(29.3)	(30.8)	(35.6)

3.4 DISCUSSION

The principal finding of this study was that inhalation of 20 ppm NO during normoxic or hypoxic high-intensity, short-duration exercise did not significantly affect gas exchange in highly-trained athletes with exercise-induced hypoxemia.

3.4.1 Rationale for the Study

Exercise-Induced Hypoxemia. The consensus in the EIH literature is that hypoxemia during exercise likely occurs via V_A/Q inequality and diffusion limitations resulting from elevated pulmonary pressures (stress failure) causing the development of interstitial pulmonary edema, or decreased pulmonary red blood cell transit time in the pulmonary vasculature [89]. Identification of pulmonary edema and pulmonary stress failure has been difficult to achieve in exercising humans but has been successfully documented in racehorses [230, 238], Shetland ponies [52], and greyhound dogs [114]. At a capillary transmural pressure of 40 mmHg ultrastructural changes to the blood gas barrier are seen using a rabbit model [225], including disruption of the capillary endothelial and alveolar epithelial layers, and in some cases all layers of the wall. Based on available human data, human pulmonary artery pressure (P_{PA}) and capillary wedge pressures (P_{WEDGE}) can reach values ~ 40 and 27 mmHg respectively [189, 230]. Increased P_{PA} with exercise in humans has been consistently reported [70, 101, 188, 222], while pulmonary capillary pressure must be deduced from the above mentioned pressures. The calculated capillary pressure at the base of the human lung can reach > 35 mmHg during exercise. These pressures observed in humans by Wagner and colleagues [230] are within the range where stress failure of pulmonary capillaries is seen in animal models [225, 239]. The net result of stress failure of the pulmonary capillaries at these elevated pressures is altered or

disruption of the capillary endothelial and alveolar epithelial layers, collection of red blood cells in the alveolar wall interstitium, proteinaceous fluid and red blood cells in alveolar spaces, interstitial edema and fluid protrusions of the endothelium into the capillary lumen [237]. The cumulative effect of these events is presumably impaired gas-exchange. Following this line of reasoning, it is reasonable to hypothesize that a reduction of pulmonary pressures or increased transit time, via pulmonary vasodilation, may reduce or reverse the hypoxemia seen in some highly trained athletes. We attempted to achieve this by using inhaled NO.

Effects of Inhaled NO. Inhaled NO, a selective pulmonary vasodilator, is used in the treatment of diseases characterized by pulmonary hypertension and hypoxemia [116]. Short-term inhalation of gaseous NO causes selective vasodilation without any systemic effects. In patients with chronic pulmonary hypertension the administration of intravenous vasodilators can have beneficial effects (↓ P_{PA}) but these are typically at the expense of systemic hypotension and worsening hypoxemia [183]. Inhalation of NO in adult respiratory distress syndrome (ARDS) patients (2-20 ppm for 2-27 d) has been shown to decrease mean P_{PA} and improve arterial oxygenation [24]. A decrease in pulmonary vascular resistance (PVR), via NO, may also serve to improve right ventricular function [58]. Using normal volunteers, Frostell et al. [59] examined the effects of inhaled NO during normoxia and acute hypoxia (F_{IO_2} = 12%). Normoxic inhalation of NO (40 ppm) had no effect on pulmonary or systemic hemodynamics. Hypoxia induced mild pulmonary hypertension by increasing P_{PA} (+5 mmHg) and PVR (+50 dynes$\cdot s^{-1} \cdot cm^{-5}$) and inhalation of NO decreased P_{PA} and PVR to control values without causing systemic vasodilation. While it seems clear that inhaled NO can improve oxygenation in some patient populations, this effect should not be considered to be a general response but is dependent upon the pathophysiology of the disease/condition. Hopkins et al. [90], utilizing

anesthetized dogs, examined how inhalation of NO (80 ppm) could alter pulmonary gas exchange under conditions of: (i) normal lungs, (ii) shunt, and (iii) V_A/Q inequality. In dogs with V_A/Q inequality, NO variably affected matching which was improved in some dogs and worsened in others. These data are in agreement with other reports of NO inhalation in patients with chronic obstructive pulmonary disease (COPD) where PaO_2 values have been shown to increase slightly [3], have no effect [145], and decrease with V_A/Q inequality [17]. Critical analysis of COPD-NO inhalation studies reveals that grouped data may not be indicative of the NO-inhalation response. Based upon the above mentioned dog data [90], and the variable responses in gas exchange [3, 17, 145], it can be concluded that areas of low V_A/Q matching contribute to the variable clinical effects of inhaled NO. Hopkins et al. [90], surmised that inhaled NO improved gas exchange when the normal areas of the lung increased blood flow, but worsened it when both the normal areas and the areas of low V_A/Q ratio had a fall in PVR. This would likely explain why the response of NO inhalation is variable in COPD patients, and emphasizes the need to understand the pathophysiology of the lung disease/condition.

Studies that have sought to examine the effects of inhaled NO during exercise have been few and have focussed on patient populations. It is well established that COPD patients may develop pulmonary hypertension when exercising [243]. Roger and colleagues [197], demonstrated that in COPD patients who inhaled NO (40 ppm), pulmonary hypertension was reduced and V_A/Q ratios were improved during exercise. However, the effect of NO was different at rest than during exercise. During rest, PaO_2 and V_A/Q ratios were worsened while during exercise they both improved slightly. The authors concluded that during exercise the effect is likely explained by a preferential distribution of inhaled NO to well-ventilated alveolar units with faster time constants and normal V_A/Q ratios. The results are in agreement with the

previous discussion of the variability of NO inhalation at rest in COPD patients. In a different patient population, subjects with left ventricular dysfunction and chronic heart failure were examined during exercise conditions of control (normoxia) and NO inhalation (30 ppm) [26]. V_T and ventilatory equivalent for oxygen (V_E/VO_2) were significantly reduced during exercise with NO inhalation compared to control. There was also a strong, but non-significant, trend for V_E to be reduced ($P = 0.051$). The results of this study are difficult to interpret physiologically. It is also unclear what mechanisms were responsible for alterations in ventilatory parameters. Hemodynamic measurements at rest were determined from a Swan-Ganz catheter placed in the pulmonary artery and Q was determined by the thermodilution technique. No statistical differences were observed for hemodynamic and gas exchange variables. It is also worth noting that the authors of this study utilized in excess of 20 paired t-tests (i.e., large probability for Type I error). In a separate investigation, Koelling et al. [118] examined patients undergoing cardiac transplantation evaluation ($n = 14$). Right heart catheterization was performed and Q was determined by thermodilution. Exercise was performed under normal conditions and with NO (40 ppm). Patients with greater P_{PA}, larger left ventricular end-diastolic volumes, and lower right ventricular ejection fractions were those who benefited the most from NO. Reduction of resistance, via NO, within the pulmonary vessels was the likely cause of augmented maximum workload and VO_2 at the anaerobic threshold. This effect was not observed during treadmill exercise in sheep [120]. With inhalation of NO (30 ppm), pulmonary vascular tone was unchanged at rest, while during exercise the usual reduction in PVR was not observed. The implication of this observation is that the pulmonary vasculature was already maximally dilated during exercise by flow-related pressure effects [120, 187]. These results are consistent with those of Brett et al. [31] who recently characterized the response of the

pulmonary circulation in normal individuals to different doses of inhaled NO (n = 8; NO = 0, 20, and 40 ppm). No significant changes were observed for Q or DLCO implying that the normal pulmonary vascular bed is not amenable to vasodilation by inhaled NO. This is in agreement with the observation that in dogs with normal lungs NO does not alter PaO_2 or V_A/Q matching [90].

The rationale for this study was therefore based upon the following: (i) some athletes experience hypoxemia during exercise as result of abnormal lung function, and (ii) nitric oxide inhalation can reverse hypoxemia in some patient populations.

3.4.2 Effect of Inhaled NO during 5-min Cycling

No differences in PaO_2 were detected between N and N/NO in the present study. These findings do not support our original hypothesis that, if EIH is caused by elevations of pulmonary pressures, inhaled NO (20 ppm) should diminish the observed hypoxemia during exercise. No differences were observed between N and N/NO for other blood gas variables ($[A\text{-}a]DO_2$, SaO_2, $PaCO_2$). Based upon these collective findings we can conclude that inhalation of 20 ppm NO during 5-min heavy normoxic exercise had no effect on gas exchange in subjects who exhibit EIH. Although pulmonary pressures were not measured in this study, we can speculate on two possible scenarios: (1) pulmonary pressures were not altered, or (2) pulmonary pressures were reduced with no effect on gas exchange (or development of pulmonary edema). It is more probable that the first scenario occurred. The pulmonary capillary bed in these athletes may have been already maximally dilated and inhalation of a vasodilator would have no effect. Therefore, no effect on pulmonary pressure would be expected nor would any alteration in gas exchange occur. Our results therefore agree with the hypothesis of Dempsey [45] that pulmonary capillary

blood volume reaches its maximal morphological limit and further dilation is not anatomically possible. This observation also agrees with the observation that when using sheep [120], inhalation of NO (30 ppm) did not change pulmonary vascular tone at rest and during exercise. The implication of this observation, in conjunction with the present study, is that the pulmonary vasculature is already maximally dilated during exercise by flow-related pressure effects [120, 187].

To date, Durand et al. have been the only group to investigate the effects of NO inhalation on pulmonary gas exchange during exercise in highly trained athletes [49]. Male endurance athletes (n = 9: VO_2max ~ 65 ml·kg^{-1}·min^{-1}) performed a maximal cycle test to exhaustion while inhaling either (i) room air, or (ii) room air combined with 15 ppm NO. In agreement with our results, no differences between conditions were observed for VO_2 (Figure 9) or power output (Table 5). Durand et al., found that inhalation of NO caused PaO_2 to decrease at rest, 50%, 75%, and 100% VO_2max. Interestingly, delta PaO_2 continuously fell without NO (13.5%). With NO, after a preliminary drop, a stabilization of delta PaO_2 was observed between 75 and 100% VO_2max. This trend was also reflected in [A-a]DO_2. These results are seemingly different than ours, but several points are worth noting prior to arriving at such a conclusion. The results of Durand et al. show that at 100% VO_2max, PaO_2 values were not statistically different between exercise without NO (PaO_2 = 84.7 mmHg) and with NO (PaO_2 = 79.6 mmHg). These values are nearly identical to those in the present study during the 5th min of exercise in N (PaO_2 = 84.2) and N/NO (PaO_2 = 78.0) where we also observed no significant difference. What is different between the two studies is that Durand et al., observed a decrease in PaO_2 and [A-a]DO_2 at rest with NO while we did not observe this drop. It is necessary to point out that it is not clear from the Durand manuscript how long subjects inspired NO prior to commencing exercise, whereas in

the present study subjects sat quietly on the cycle ergometer and breathed the gas mixture for 5 min prior to exercise. Possibly, differences in the duration of NO inhalation contributed to the reduced gas exchange at rest.

In contrast to Durand et al., when delta PaO_2 was calculated between time periods in the present study no stabilization was observed. Their conclusion of a "stabilization" must be made with caution as this is based only upon two time points (between 50 and 75% vs. 75 and 100% VO_2max) rather than a longer time course. It is also necessary to note that arterial blood gas measurements were corrected for temperature in the present study, while those of Durand et al. were not. This would likely overestimate the drop in PaO_2 and SaO_2 observed during exercise. Given that both studies utilized relatively small sample sizes, an overestimation of small magnitude can affect the interpretation of results. The apparent difference in delta gas exchange values between the two studies are difficult to reconcile, however another possible explanation is that each study utilized a different exercise protocol. The effect of different exercise protocol has been examined by Lama et al. [122] and no difference was found between three different incremental tests to exhaustion (20, 30, or 40 watts·min^{-1}). However, in the present investigation, we employed a 5-min high intensity test while Durand et al. utilized an incremental test to exhaustion. In both studies PaO_2 declined with exercise. Perhaps the cause of hypoxemia was different with a different mode/intensity of exercise and thus the effect of inhaled NO would not be the same. The result of this would possibly be reflected in a different delta response for PaO_2 and $[A\text{-}a]DO_2$. This highly speculative conclusion requires further study but it remains possible that EIH can occur during short-duration high-intensity exercise via a different mechanism than during an incremental test to exhaustion. Presently, no data exist comparing these two modes of exercise.

Unique to this investigation was the delivery of NO to individuals with EIH during hypoxic exercise. As expected, during H and H/NO, PaO_2 was significantly lower than N and N/NO at all time points (Figure 5). The drop in oxygenation with hypoxia (FIO_2 = 14%) was consistent with that observed in previous EIH studies [123, 132]. The present study showed that inhaled NO did not alter PaO_2, SaO_2, $AaDO_2$, or $PaCO_2$ during hypoxia. Pison et al. [168], showed that addition of 20 ppm NO to a hypoxic (FIO_2 = 12%) gas mixture returned P_{PA} and the pulmonary gas exchange to baseline measures in mechanically ventilated sheep. These are in contrast to our results where no statistical difference was observed for gas exchange variables between H and H/NO. This was an unexpected result as we can assume that hypoxic pulmonary vasoconstriction (HPV) was induced by using a hypoxic inspiratory gas mixture at rest and during exercise. We hypothesized that inhaled NO would have reversed the transient HPV and enhanced pulmonary gas exchange by redistributing blood flow to better ventilated alveoli. Reversal of HPV has been demonstrated in sheep [168], humans with high-altitude pulmonary edema [207], rabbits [164], and in healthy humans breathing hypoxic gas [60]. Potentially, the vasoconstriction at rest in the present study was of a small enough magnitude to have had minimal effect on P_{PA} and no measurable effect on gas exchange. Further speculation is not possible as we did not measure pulmonary hemodynamics in the present study. Ideally, measures of pulmonary artery pressure and cardiac output, along with gas exchange measures would reveal the effect of inhaled NO during hypoxic exercise.

Cardiorespiratory data (VO_2, V_E, heart rate) and power output were not affected by the inhalation of NO. As expected, power output was significantly lower during H and H/NO compared to N and N/NO. A lack of NO effect is consistent with the findings of Durand et al. [49]. These findings confirm that no significant alteration to gas exchange occurred. If gas

exchange was positively affected by NO inhalation (i.e., ↑ PaO_2, ↑ SaO_2, ↓ $[A\text{-}a]DO_2$), the effect would presumably be reflected in whole-body exercise measures such as VO_2, heart rate, V_E, and power output.

3.4.3 Methodology

A debatable point is: how much of the inhaled NO actually reached the lower airways? While it is not possible to measure the amount of inhaled NO that reaches the alveoli, it is assumed that subjects in the present study did inhale 20 ppm NO. We are confident that NO was delivered to the mouth as it was measured only ~ 5 cm from the point of inspiration. The experimental set-up and NO concentration employed in our study were similar to that of Durand et al. [49] (15 ppm). It is possible that the concentration of NO used in this study was not sufficient to induce vasodilation. However, this seems unlikely given that similar concentrations have been used previously to show significant alterations in gas exchange and pulmonary pressures [145, 168, 198]. Our choice of 20 ppm was based upon the above mentioned studies, and in light of the toxicity of higher oxides of nitrogen we chose to use a lower dose than some of the clinical studies, and in addition 20 ppm is thought to be an appropriate and safe concentration for exercising humans (W. M. Zapol, personal communication).

3.4.4 Inter-Subject Mechanisms for EIH.

EIH has been defined as a reduction in PaO_2 of at least 10 mmHg below resting values [48, 80, 175], or 5 mmHg below resting values [6], and when using pulse oximetry an SaO_2 less than 92% [174], 91% [175], or 90% [81, 234]. While the criteria for EIH is debatable, the present study confirms that PaO_2 is decreased and $[A\text{-}a]DO_2$ is widened during strenuous

normoxic exercise in highly-trained male athletes and is significantly worsened with hypoxic gas inhalation. Mean PaO_2 values decreased approximately 20 mmHg from rest during exercise during normoxia and 22 mmHg during hypoxia and are consistent with the EIH literature [7, 48, 91]. Mean values for $PaCO_2$ were lower than rest at each exercise condition. While not statistically feasible to examine this relationship, it is interesting to note that in one subject (NS-subject 5) there appeared to be a definite hypoventilation response to exercise during N and N/NO ($PaCO_2$ ~ 40 mmHg) while during H and H/NO $PaCO_2$ dropped to more "appropriate" levels (~ 30 mmHg). It is also worth noting that this subject had the greatest drop in PaO_2 during hypoxia and normoxia exercise compared to other subjects. In addition, one subject (LB-subject 6) developed EIH during the normoxic cycle test to exhaustion (SaO_2 = 90.92%) but the PaO_2 values decreased only modestly (98 to 92 mmHg) during normoxic 5 min cycling. It is possible that exercise protocol (intensity, duration) can variably affect the drop in arterial oxygenation among individuals. Examination of individual subject data or small groups (i.e., 2-3 subjects, see Dempsey et al. [48]) can provide insight into possible mechanisms of EIH and emphasizes the possibility that EIH may occur via different mechanisms in different individuals. It is conceivable that some individuals are "hypoventilators" while others experience diffusion limitations. The complex interaction between O_2 kinetics, EIH, mode/intensity/duration of exercise, and pulmonary hemodynamics has not been examined. Future research involving single-subject, repeated-measures designs my provide new information regarding the mechanisms of EIH.

3.5.5 Summary

In summary, inhalation of 20 ppm NO during normoxic or hypoxic high-intensity, short-duration cycle exercise did not significantly affect gas exchange in athletes with exercise-induced hypoxemia. Cardiorespiratory variables and power output were unaffected by NO inhalation. The implication of these findings is that pulmonary capillary blood volume reaches its maximal morphological limit during exercise and further dilation is not possible.

CHAPTER 4. GENERAL SUMMARY AND CONCLUSIONS

General Summary

Exercise-induced hypoxemia is a perplexing physiological paradox. Physical training is known to exert many health benefits, improve aerobic capacity and exercise performance. High aerobically powered athletes can be thought to represent the upper state of the health continuum. In contrast to this premise, some highly-trained endurance athletes engaged in strenuous work have an arterial blood gas profile which resembles that of the chronically ill respiratory patient. These two studies sought to determine potential mechanisms for this phenomenon. Specifically investigated was the relationship between endogenously produced exhaled NO and EIH and the effect of inhalation of exogenous NO on EIH.

Exhaled Endogenous Nitric Oxide. A group of highly-trained male cyclists (n = 18, VO_2max = 67.7 ± 5.2 $ml \cdot kg^{-1} \cdot min^{-1}$) some of whom develop EIH, performed a maximal cycle test. CNO and VNO were determined during the cycle test. No significant differences were observed between those with and those without EIH. There was also no observed linear relationship between delta SaO_2 and delta VNO.

Inhaled Nitric Oxide. Highly trained male cyclists (n = 7, VO_2max = 65.3 ± 1.6 $ml \cdot kg^{-1} \cdot min^{-1}$) performed four 5-min cycle tests at VO_2max under conditions of normoxia (N), normoxia + 20 ppm nitric oxide (N/NO), hypoxia (H), and hypoxia + 20 ppm nitric oxide (H/NO). Inhalation of 20 ppm NO during normoxic or hypoxic high-intensity, short-duration exercise did not significantly affect gas exchange in athletes with exercise-induced hypoxemia. Cardiorespiratory variables and power output were also unaffected by NO inhalation.

General Conclusions

Based on the present data it can be concluded that NO present in exhaled air is not related to the etiology of EIH. Inhaled NO had no effect on pulmonary gas exchange and is suggestive that pulmonary capillary blood volume reaches a maximal morphological limit during exercise and further dilation is not possible.

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APPENDIX A

REVIEW OF LITERATURE: EXERCISE-INDUCED HYPOXEMIA

1. INTRODUCTION

Most normal, healthy individuals engaged in strenuous activity at sea-level are capable of maintaining blood gas homeostasis for both O_2 and CO_2 [8, 9]. Their respiratory system is able to precisely regulate ventilation and gas exchange. In contrast, in well-trained athletes, the adequacy of the respiratory system has been challenged when decreases in arterial pressure of O_2 (PaO_2) and oxyhemoglobin saturation (SaO_2) are observed [48, 91, 173]. These changes were first observed in the 50's [87] and 60's [200], however this physiological fact lay dormant until the work of Dempsey et al. [48] stimulated interest. This phenomenon, termed exercise-induced arterial hypoxemia (EIH), occurs in 50% of highly-trained male endurance athletes, while untrained and moderately-trained males do not experience EIH [173]. EIH is defined by a PaO_2 less than 75 mmHg or an SaO_2 less than 92% [174]. Individuals with EIH are also characterized by a widening of the alveolar-arterial difference for oxygen ($[A\text{-}a]DO_2$) which can reach as high as 50 mmHg [91]. A decline of PaO_2 and SaO_2 during heavy exercise represents a physiological failure of the respiratory system. The cause and significance of EIH have thus become topics of considerable interest to physiologists and athletes alike.

Despite a considerable research effort, the mechanism(s) responsible for EIH remain controversial. Four primary factors have been reported in the literature; (i) veno-arterial shunts, (ii) relative hypoventilation, (iii) ventilation/perfusion (V_A/Q) inequalities, and (iv) diffusion limitations. Given the difficult nature of performing truly mechanistic studies in exercising humans, the EIH literature has been slow to evolve. Respiratory factors limiting maximal exercise and EIH have previously been reviewed elsewhere [35, 45, 47, 92, 177], however recent

investigations have forced scientists to reconsider the factors responsible for EIH. In addition, recent studies have sought to examine other athletic populations in relation to EIH, in particular women and older athletes. Insights from animal physiology have also provided information on EIH. Therefore, the purpose of this review is to provide a contemporary review of EIH and the mechanism(s) thought to be responsible.

2. RELEVANCE

Reductions of PaO_2 and SaO_2 during exercise are of interest to the exercise physiologist because they indicate that the pulmonary system may be a limiting factor to maximal oxygen uptake (VO_2max) and exercise performance. Reductions in SaO_2 from resting values have negative effects on VO_2max [67, 96, 123, 132, 174, 216]. To date, only one study has examined the level of arterial desaturation at which maximal performance capacity is impaired [121]. Subjects performed 3 five-min cycle performance tests under normoxia, mild hypoxemia, or moderate hypoxemia. Mean SaO_2 values were 96, 90, and 87% respectively. Mild hypoxemia elicited a detrimental effect but did not reach statistical significance. Moderate hypoxemia caused a significant decline in performance. As was correctly pointed out by the authors, a linear trend was observed between decreasing levels of SaO_2 and diminished work, and in terms of athletic performance, statistical significance holds little importance.

The factors, which provide a limit to VO_2max, have been a source of debate for many years. The traditional view is that oxygen delivery to working muscle represents the primary limiting factor to exercise VO_2 and performance and that the oxygen content of arterial blood is adequate to meet all exercise-imposed metabolic demands [9]. The reader is directed to reviews of cardiovascular [202], and peripheral limitations [228] to VO_2max. It has been postulated that in some elite aerobic athletes the limit to VO_2max, and possibly performance, is the respiratory

system [45, 47]. Accordingly, the first step in the supply of oxygen to working muscle becomes the "weak link". Elite athletes undergo training adaptations in skeletal muscle and the cardiovascular system, which eventually surpasses the capability of the pulmonary system. Dempsey speculates further that this occurs because of the pulmonary system's inability to adapt despite many years of aerobic training. If this supposition holds true then the pulmonary system does in fact pose a constraint to maximal exercise performance because of the inability to match the metabolic requirements of the athlete.

3. MECHANISMS OF EXERCISE INDUCED HYPOXEMIA

3.1 VENO-ARTERIAL SHUNT

A veno-arterial shunt is an anatomical phenomenon that allows mixture of venous and arterial blood causing a decrease in PaO_2. Approximately 50% of the $[A\text{-}a]DO_2$ in resting and exercising humans can be explained by veno-arterial shunts [8, 69, 240]. In subjects with EIH and a widened $[A\text{-}a]DO_2$ during maximal exercise, breathing hyperoxic gas (24-26% O_2) causes PaO_2 to approach normal levels [48, 176]. If veno-arterial shunts were the cause of EIH breathing such an inspirate would have little effect on PaO_2 because of the venous and arterial blood mixture. Based on these findings, veno-arterial shunts have been excluded as an important determinant of EIH.

3.2 HYPOVENTILATION

Minute ventilation ($\dot{V}E$) increases during progressive exercise via elevations in tidal volume (V_T) and respiratory frequency (f_b). At low levels of exercise intensity both V_T and f_b increase, while at higher work loads increases in $\dot{V}E$ are due principally to f_b while V_T remains constant.

V_E increases with exercise intensity raising alveolar PO_2 (P_AO_2) to provide a sufficient pressure gradient for oxygen diffusion. Arterial carbon dioxide tension ($PaCO_2$) is also maintained through increased V_E where alveolar PCO_2 (P_ACO_2) is reduced, facilitating CO_2 diffusion from arterial blood.

Inadequate ventilation, or hypoventilation, during exercise would lead to an inadequate P_AO_2, decreased driving gradient from alveoli to blood, and elevated $PaCO_2$. Hypoventilation is clinically characterized by the retention of CO_2 ($PaCO_2 > 40$ mmHg). It has been suggested that hypoventilation can occur during exercise despite the presence of stimuli to increase ventilation such as elevated $PaCO_2$, temperature, blood catecholamines, metabolic acidosis or a decrease in PaO_2 [45, 48]. Indeed, hypoventilation has been implicated as a causative factor of EIH [38, 45, 47, 48, 162]. Athletes who developed EIH showed minimal compensatory hyperventilation during exercise ($PaCO_2$ was only 1-4 mmHg below resting values) and those who hyperventilated the least showed the greatest hypoxemia [48]. However, given that retention of CO_2 is the cardinal marker of hypoventilation and athletes who exhibit EIH have not retained CO_2, hypoventilation has been ruled out as an important factor in EIH [176, 178]. Alternatively, it may be that hypoventilation during exercise is *relative* and permits a necessary trade-off. Conceivably, arterial blood gas homeostasis is sacrificed to minimize the work of breathing. Dempsey has describe the situation where if the trained male runner at a VO_2 of 5 $l \cdot min^{-1}$ were to achieve the same pressures of O_2 and CO_2 as in the untrained individual exercising at a VO_2 of only 3 $l \cdot min^{-1}$, he would have to sustain a ventilation in excess of 200 $l \cdot min^{-1}$, whereas the untrained individual would need only achieve ~ 120 $l \cdot min^{-1}$ ventilation [45]. Perhaps respiratory muscle pressure development in proportion to, but not out of proportion to, increases VCO_2, and respiratory muscle fatigue is spared at the expense of arterial blood gas homeostasis [45]. The

development of EIH is not limited to the human athlete. Significant arterial hypoxemia has been reported in horses [20-22, 156, 231]. The mechanism for EIH in the horse remains equally elusive but is possibly related to hypoventilation. Horses have been shown to have retain CO_2 with $PaCO_2$ values 5-10 mmHg in excess of resting values during heavy exercise [21].

A critical review of the literature suggests that relative alveolar hypoventilation warrants reexamination and it can not be excluded as was previously concluded [176, 178]. Hypoventilation during exercise would likely be caused by one or a combination of the following (i) respiratory muscle fatigue, (ii) inadequate drive to breathe, or (iii) mechanical limitation.

Respiratory muscle fatigue is defined as the inability of the respiratory muscles (RM) to continue generating a given pleural pressure. RM fatigue , if sufficient in magnitude, may be of physiologic significance to the regulation of ventilation [46]. RM fatigue has been successfully demonstrated following voluntary hyperpnea [15] and marathon running [126]. Bye et al. [35] asked if the fatigue of RM during exercise is a coincidental phenomenon or does it actually set limits to exercise capacity that could be improved if the ventilatory muscles improved? This was addressed by Fairbarn and co-workers [56] who showed 4 weeks of RM training increased RM endurance but had no effect on maximal cycling performance in highly-trained cyclists. The available data is convincing that RM endurance improves with specific RM training [28, 29, 57, 146]. However, the effect of improved RM endurance on whole body exercise and VO_2 is debatable. The two studies of Boutellier [28, 29] are unique because they show large increases in VO_2max of 38% and 50% in non-athletes and athletes respectively. Methodological differences among studies may be reflected in these results, however the large improvements in VO_2max seem incredible and these studies have yet to be replicated.

If the proportion of total VO_2 going to the RM is high, the amount of O_2 available to the locomotor muscles could be reduced. Therefore a stage could be reached when the VO_2 during exercise is insufficient to meet the needs of both the RM and the other exercising muscles. As a result either the exercising muscles or the RM or both would go into oxygen debt and exercise tolerance would be limited [35]. At high levels of exercise the percentage of total blood flow to the RM is 14-16% [82]. Thus the RM are in direct competition with skeletal muscle for oxygen and cardiac output. Dempsey et al. [46] have predicted that RM, if placed under sufficient load during heavy exercise, receive adequate blood flow at the expense of limb locomotor muscles under conditions where total Q is at or near capacity. It appears that at VO_2max, high demand for respiratory muscle blood flow causes locomotor muscle vasoconstriction and compromises limb blood flow [78, 86].

Heavy exercise places substantial demands on the RM to produce and sustain high levels of ventilatory work. While it seems that RM receive adequate blood supply during maximal exercise, the question remains: do respiratory muscles fatigue during strenuous exercise? RM, in particular the diaphragm, can be considered highly resistant to fatigue. The natural recruitment of motor units in the diaphragm optimizes its fatigue resistance (i.e., type I fibers followed by type IIa then type IIb) [46]. The diaphragm, as the primary muscle of inspiration, is required to be fatigue resistant. In humans RM fatigue has been investigated using alterations in pulmonary function, maximal voluntary ventilation, maximal mouth, pleural, or transdiaphragmatic pressure development (Pdi) [34, 41, 98, 126].

Volitional measurements of ventilatory performance after exercise have indicated the presence of RM fatigue [126, 128, 233]. However caution must be take when interpreting these

results. Test conditions in "field" studies are difficult to control and may not be sufficiently objective or independent of whole-body fatigue [46].

More objectively, non-volitional measures can be used to evaluate diaphragm fatigue. Bilateral phrenic nerve stimulation (BPNS) has shown that the human diaphragm can become fatigued [12, 13, 15]. BPNS involves stimulation of the phrenic nerves bilaterally behind the sternocleidomastoid muscles in the neck [10, 11]. Using BPNS, diaphragm fatigue can be divided into high and low frequency fatigue [10, 148]. High-frequency fatigue is characterized by an inability to maintain a given Pdi at high rates of phrenic nerve stimulation (50-100 Hz), whereas low-frequency fatigue is characterized by failure to maintain adequate pressures at low rates of stimulation (1-20 Hz) [46]. Exercise-induced low frequency diaphragm fatigue is seen in normal individuals [12, 13, 102, 125]. Most recently BPNS has been used at low and high frequencies to determine that both low and high frequency fatigue is present following near maximal exercise (95% VO_2max) [14]. What are the consequences of low/high frequency fatigue of the diaphragm? It is unknown if diaphragm fatigue affects V_E during exercise. The relationship between diaphragm fatigue, V_E, and EIH remains to be determined. While speculative, it is possible that if diaphragm fatigue occurs during strenuous exercise, ventilation could be compromised resulting in relative alveolar hypoventilation and EIH.

Pulmonary ventilation is tightly coupled to metabolic demand and is of paramount importance to the maintenance of blood gas homeostasis. The control of ventilation during exercise is a complex and not yet fully understood process. The exactness of pulmonary ventilation is seen during heavy work where the rate and depth of breathing are adjusted quickly and precisely to meet metabolic demands. The stimuli, or drive to breathe, are mediated in large part by peripheral chemoreceptors. Peripheral chemoreceptors are sensitive to changes in PO_2,

PCO_2, temperature, pH, and potassium concentrations during exercise [51, 241].

Endurance performance and chemosensitivity have been linked [131], where endurance athletes have blunted responses to hypoxia and hypercapnia compared to other athletes and sedentary controls [36, 131, 208]. A blunted hypoxic ventilatory response (HVR) and/or hypercapnic ventilatory response (HCVR), results in an inappropriate V_E and a lowered arterial oxygenation. An attractive hypothesis put forth was that athletes who exhibit EIH may have diminished peripheral chemosensitivity resulting in inadequate V_E and consequently EIH [91]. However, resting HVR was not significantly correlated with maximal V_E ($r = 0.08$), V_E/VO_2 ($r = 0.1$), or arterial desaturation ($r = 0.06$), indicating that ventilation was adequate in subjects with EIH. More recently, contrasting data demonstrate that the role of chemosensitivity in EIH is of importance [81]. HVR and HCVR at rest were significantly lower in individuals with EIH. HVR was significantly related to both V_E/VO_2 ($r = 0.43$) and V_E/VCO_2 ($r = 0.61$). In another attempt to examine this relationship significant positive correlations were reported between SaO_2 and $P_{ET}O_2$ ($r = 0.72$), $P_{ET}O_2$ and V_E/VO_2 ($r = 0.91$), SaO_2 and V_E/VO_2 ($r = 0.74$), and HCVR at rest and SaO_2 ($r = 0.45$) [144]. It is worth noting that few subjects met the criteria for EIH; only 3 of 31 subjects had an $SaO_2 \leq 92$, while 28 were ≥ 92. A significant relationship ($r = 0.698$) was also calculated between SaO_2 and V_E/VO_2 during maximal exercise [226]. The discrepancies between these studies and variability among correlation coefficients are difficult to reconcile. The indirect nature of the data is problematic, but more importantly it must be reemphasized that statistically significant correlations do not imply causation [151]. Regardless, what may be of more consequence to understanding EIH is the relationship between HVR and HCVR to EIH *during* exercise rather than at rest. Cooper et al. found that highly trained cyclists who develop EIH have been shown to have a lower HCVR at rest and during exercise at 25%,

50%, and 66% VO_2max than do athletes without EIH [43]. These introductory data suggest a role for altered ventilatory control in the development of EIH. In summary, the relationship between chemosensitivity and EIH remains debatable from the available data and warrants further attention.

A mechanical limitation of the chest wall and lung structures may constrain ventilation during strenuous exercise. In the untrained, expiratory or inspiratory flow limitation is minimal during maximal exercise, end-expiratory lung volumes remain well below resting values and VT operates along the steepest portion of the pressure/volume relationship [46]. Typically, maximal f_b, VT, and VE during exercise are determined by lung structures and an adequate reserve exists. Given the theory that the morphology of the lung does not change with exercise training [45], it is reasonable to suggest that those capable of extremely high VO_2max and VEmax, as a result of exercise training, may exceed the functional limits of the chest wall and lung.

Peak expiratory flow rates have been measured during exercise that approach and exceed maximal expiratory flow measured during maximal expiratory flow-volume (MEFV) curve [103]. During normoxic helium breathing (He-O_2) VE increases and the respiratory system becomes "unloaded" resulting in augmented hyperventilation during exercise [48]. It was hypothesized that He-O_2 breathing increases the MEFV loop and allows for a larger mechanical reserve and a subsequent increase in VE. If hyperventilation with He-O_2 breathing reduces EIH then hypoventilation can be considered an important component of EIH. During a treadmill run of 3-4 min at 75-95% VO_2max subjects breathed either room air or 21% O_2 balance helium [48]. He-O_2 breathing caused hyperventilation, hypocapnia, increased $PETO_2$ (4-11 mmHg), and caused PaO_2 to increase 5-15 mm Hg in four of five runners compared to breathing room air. The effects were quickly reversed by the resumption of room-air breathing. This suggests that

the mechanics of the respiratory system set V_E at a lower level at the cost of arterial oxygenation. Dissimilar results have been reported in subjects breathing a similar He-O_2 mixture during a graded exercise test to exhaustion [32]. Maximal ventilation was increased significantly but SaO_2 values did not differ between He-O_2 and room air breathing. The conflict between these two studies may be related to differences in duration, mode, and intensity of exercise (3-4 min running at 75-95% VO_2max vs. incremental cycle test to exhaustion). The potential importance of a mechanical limitation is demonstrated when He-O_2 breathing was shown to prolong endurance time and reduce VO_2 during heavy work [1], suggesting that high levels of ventilatory work may impact endurance capacity.

Locomotion and breathing are not independent during exercise. Entrainment, or synchronization between locomotor activities and breathing has been described during running, cycling, kayaking, and rowing [23, 66, 119, 138, 139, 157, 221]. Because both exercise and respiration rely on cyclical movements impacting on the thoracic complex, to circumvent any mechanical constraints developing as a result of limb motion, breathing must be made to fit the locomotory cycle [30]. Entrainment is not as clearly described in humans as it has been in other species such as the dog and the horse (for review see [46]). In humans, the degree of entrainment is strongly influenced by the type of exercise. This is reflected in the high variability of entrainment across modes of exercise. It remains possible that at high levels of exercise, ventilation and locomotion are tightly coupled and that ventilation is partially determined by locomotion. To date, only one study has sought to examine the relationship between EIH and entrainment [138]. This preliminary study (n = 5), showed that elite kayakers who involuntarily select a breathing frequency-to-stroke rate of 1:1 had consistently lower SaO_2 values (SaO_2 = 89%) at VO_2max than do those who select a 1:2 ratio (SaO_2 = 91%). It is tempting to speculate

further on the relationship between entrainment, hypoventilation, and EIH but the lack of data makes even this impossible.

3.3 VENTILATION-PERFUSION INEQUALITY

The bases of the lung are better perfused while the apices are better ventilated. Consequently, the alveoli at the apex of the lung have less blood flow but are well ventilated, while the base is well perfused but less well ventilated. Regardless, in normal healthy lungs V_A/Q is reasonably well matched and allows for adequate pulmonary gas exchange at rest and during exercise. Dispersion of V_A/Q occurs when ventilation to an area of the lung is compromised and blood passing by does not participate in gas exchange. A mismatching of V_A/Q will lower PaO_2 as blood will be inadequately oxygenated. This concept has been related to EIH where abnormalities of V_A/Q are seen during heavy exercise [74, 93, 204, 223 230], and prolonged exercise [89]. V_A/Q mismatch, as determined by the Multiple Inert Gas Elimination Technique (MIGET), increases with exercise intensity to a $VO_2 \sim 3.5\ l \cdot min^{-1}$, beyond this the ratio does not worsen further while $[A\text{-}a]DO_2$ continues to widen [74, 93]. It seems likely that V_A/Q inequality contributes significantly to EIH, in fact it has been calculated that V_A/Q may contribute > 60% to the $[A\text{-}a]DO_2$ [93].

In a unique investigation, Pederson et al. [159] measured PaO_2 and SaO_2 in highly trained cyclists during upright and supine cycling. A more homogenous V_A/Q distribution during supine cycling would be reflected in a favorable environment for gas exchange compared to upright cycling. No differences between positions were seen in PaO_2 and SaO_2. VO_2max was lower in the supine position, but altered body position (and presumably V_A/Q, although not measured) did not affect arterial O_2 desaturation. This suggests a minimal role for V_A/Q mismatch on PaO_2 and

SaO_2. While these results are intriguing, the lack of a V_A/Q measure makes evaluation of their physiological importance difficult.

While several studies have clearly shown that V_A/Q inequality occurs during exercise the cause remains speculative but may be related to non-uniform pulmonary vasoconstriction, reduced gas mixing in the large airways or the development of interstitial pulmonary edema [203]. The favored hypothesis in the literature for V_A/Q inequality is the formation of interstitial pulmonary edema [89, 106]. The etiology of pulmonary edema during exercise is discussed below.

3.4 REDUCED PULMONARY TRANSIT TIME

During exercise there are several mechanisms which help to maintain adequate pulmonary gas exchange. Pulmonary capillary blood volume (Vc) increases through a 3-fold recruitment of capillaries above resting values. The rise in Vc accommodates exercise related increases in cardiac output and provides adequate transit time of red blood cells for diffusion equilibrium to occur. In some athletes Q can reach over 40 $l \cdot min^{-1}$ [50]. In these elite athletes, Q may continue to increase beyond the point at which Vc has reached its morphological limits; consequently pulmonary transit time (PTT) would be reduced [45]. Under resting conditions red blood cells remain in the pulmonary capillary for ~ 0.75 s [105], while during intense exercise PTT has been estimated to be reduced to ~ 0.25 s [235]. In agreement with Dempsey's model [45], this represents a significant diffusion disequilibrium for O_2 at the end of the pulmonary capillary.

Experimental data examining PTT during exercise have been sparse. It has been observed that Vc does not plateau, nor does mean PTT fall below 0.46 s despite further increases

in exercise intensity and that no relationship exists between mean PTT and a widened [A-a]DO_2 [234]. Oppositely, PTT has been significantly correlated with [A-a]DO_2 (r = - *0.59*) [88]. In this study, estimated mean transit time during maximal exercise was 0.39-0.41 s. This would be sufficient for diffusion disequilibrium to occur [45]. The difference between these two studies has been ascribed to exercise intensity [88]; where subjects exercised at 88% VO_2max [234] and > 90% VO_2max [88]. It is difficult to discern between these two workloads, however subjects exercising at near-maximal intensities would be more likely to have a substantial reduction in PTT. Despite the available theory and data decreased PTT can not be solely responsible for EIH. Athletes capable of high aerobic work (i.e., VO_2max > 65 ml·kg^{-1}·min^{-1}) would have comparable cardiac output during maximal exercise. Yet, only 50% of these athletes develop EIH. Based on the theoretical model [45], and the observation that PTT decreases during heavy work [88, 234], it seems likely that a shortened PTT contributes to EIH in athletes capable of high Q. Further study is required to determine the magnitude of its importance.

3.5 PULMONARY EDEMA

One of the principal factors which limits gas exchange through the alveolar-capillary membrane is the distance between the membrane and the red blood cell. Any increase in this distance hinders gas equilibrium from occurring. Athletes with EIH may possibly enlarge this distance through the formation of interstitial pulmonary edema [33, 129, 185, 204, 230]. The mechanism responsible for the accumulation of extravascular water remains to be determined but is likely caused by increases in capillary hydrostatic pressure, capillary permeability, capillary surface area, or a lymphatic insufficiency [235].

The pulmonary blood-gas barrier is required to be extremely thin for gas exchange but also immensely strong because the capillary wall stresses become very high during exercise. Stress-failure of the blood-gas barrier would cause high-permeability pulmonary edema or hemorrhage [236]. At a capillary transmural pressure of 40 mmHg ultrastructural changes are seen using a rabbit model [225]. Changes include disruption of the capillary endothelial layer, alveolar epithelial layer and in some cases all layers of the wall. In humans mean pulmonary artery pressure (P_{PA}) and capillary wedge pressure (P_{WEDGE}) can reach values ~ 40 and 27 mmHg respectively [189, 230]. Increases in P_{PA} with exercise in humans have been consistently reported [70, 101, 188, 222]. Pulmonary capillary pressure must be deduced from the above mentioned pressures. Unfortunately, accurate measures of P_{PA} and P_{WEDGE} in athletes with high Q and EIH are few. Measures of these pressures are required to confirm existing theories on capillary stress failure during exercise. Nonetheless, the pressures observed by Wagner et al. [230] are within the range where stress failure of pulmonary capillaries have been seen in animals [225, 239]. Stress failure of the pulmonary capillaries at these elevated pressures may cause leakage of fluid and transient pulmonary edema.

Accumulation of interstitial fluid is usually removed by lymph flow, but may be compromised during exercise [42]. Another possibility is that the distention of lung capillaries during exercise, via increased blood volume, could increase permeability and promote fluid shifts [225]. Using sheep, it has been shown that 15 min after prolonged heavy exercise (40-60 min) that pulmonary lymph flow is significantly higher than at rest [153]. Possibly, the lymph system is unable to clear the interstitial space during exercise, resulting in pulmonary edema. This hypothesis remains to be tested.

Mechanical stress failure of the blood-gas barrier during exercise in humans has yet to be proven. However, from the available data on pulmonary pressures and current stress-failure theory it seems possible that pulmonary edema can occur with exercise. Even a mild degree of pulmonary edema could pose an important limit to gas exchange. Coupling decreased PTT and pulmonary edema would impact on diffusion equilibrium. In addition, development of pulmonary edema may compromise V_A/Q relationships.

Anecdotal reports following heavy exercise have reported pulmonary edema [137]. Indeed, much of the evidence for pulmonary edema is indirect and circumstantial. No techniques have yet shown directly the development of a small degree of edema in man. However, exercise has been shown to cause perivascular cuffing in the pig [203]. Pigs exercised for 6-7 min at the highest speed they could sustain. Animals that exercised had a higher percentage of pulmonary arteries with perivascular edema than did non-exercised controls (33.8% vs. 20.0%). These direct data show that edema can occur during short-term heavy exercise in an animal model. Unfortunately, indications of extravascular lung water accumulation in exercising humans have been indirect and inconsistent.

Trans-thoracic electrical impedance (TEI) has been used in an attempt to quantify the accumulation of extravascular water [33]. TEI was decreased for 30 min following exercise and was related to the accumulation lung water. However, training status was not reported nor was a measure of arterial oxygenation. The physiological importance of these findings remains unclear because the measurement of TEI is non-specific and can not explain the exact source of hindered impedance. The decrease may have been due to elevated intravascular volume and not interstitial edema. More recent results [186] demonstrate the opposite where TEI was increased following

maximal exercise. These conflicting studies, and the non-specific nature of TEI highlight the limitation of using TEI as a tool to evaluate pulmonary edema.

Decreases in the diffusing capacity of the lung (D_L) post-exercise have been identified as indicators of pulmonary edema [33, 129, 185, 204]. However, data from these studies have been conflicting and it has been unclear if D_L changes are exclusive to elite athletic populations. Sheel et al. [212] determined that endurance trained, moderately trained and untrained subjects all experience the same degree of change in D_L following exercise. The change in D_L was due primarily to a decrease in Vc rather than pulmonary edema. The observed decrease in Vc post-exercise is supported by the work from several studies [75-77, 140]. The mechanism for decreased Vc is unclear, however the fact that it occurs even after mild exercise makes pulmonary edema seem an unlikely explanation [76]. While pulmonary edema may occur during exercise post-exercise measures of D_L are not a reflection of the processes occurring during exercise.

Using chest radiography, pulmonary edema was not detectable [65]. However, the group of subjects were un-trained therefore EIH nor the development of pulmonary edema would not have been expected. Using well trained triathletes (VO_2max = 68 $ml \cdot kg^{-1} \cdot min^{-1}$) following a triathlon race computerized tomographies (CT) did reveal the possibility of mild edema [39]. CT lung density and mass increased 19% and 21% respectively following the race. Despite this, it is not clear to what degree increased CT density reflects the presence of pulmonary edema and extravascular water rather than an increase in intravascular volume. These results remain indirect and inconclusive. In addition, visual analysis of CT scans did not reveal an obvious image of acute pulmonary edema. In a recent study McKenzie et al. [141] examined changes in extravascular water (EW) using a magnetic resonance scanner pre- and post-45 min of maximum

sustainable cycling. EW increased significantly by 9.4% implying that transient interstitial edema did in fact occur. Unfortunately, no measure of arterial oxygenation (pulse oximeter or blood gases) were made during exercise. Given the above mentioned studies it would seem that pulmonary edema may occur [39, 141]. Alternatively, since neither of these studies measured arterial oxygenation it could be concluded that the mild edema observed is not related to decrements of PaO_2 or SaO_2. The answer to this question awaits further study.

Normal volunteers who performed moderate or heavy exercise over a period of 2-7 h have shown significantly increased concentrations of protein in bronchoalveolar lavage fluid compared to non-exercising controls [54]. Increases in red blood cells protein in bronchoalveolar lavage fluid have been reported following a 7-min cycling race situation compared to sedentary controls [94]. Although no measure of VO_2 was made it was assumed that exercise effort was maximal as there was a cash prize for the fastest time. Unfortunately, no measure of arterial oxygenation was made. These results suggest that the integrity of the blood-gas barrier may be impaired during exercise. Consistent with this theme is the observation that sustained submaximal exercise does not alter the integrity of the lung blood-gas barrier in elite athletes [95].

While the evidence for exercise-induced pulmonary hemorrhage in humans is lacking, this phenomenon occurs frequently in thoroughbred racehorses. Evidence of stress failure of pulmonary capillaries has been described in horses, including disruptions of the capillary endothelial and alveolar epithelial layers, red blood cells in the alveolar wall interstitium, protein-containing fluid and red blood cells in the alveolar spaces, interstitial edema [238]. The authors surmised that the ultrastructural changes were consistent with those changes previously seen in rabbit lungs at high capillary transmural pressure. It becomes attractive to extrapolate the above

mentioned studies back to the human athlete although adequate measures of pulmonary pressures are currently lacking and do not allow for such a comparison.

The pulmonary endothelium is exposed to the entire cardiac output and is possibly vulnerable to injury. Strenuous exercise can induce an acute inflammatory response marked by leukocytosis and neutrophil activation and release of inflammatory mediators. Inflammatory mediators such as eicosanoids, reactive oxygen species, cytokines all could negatively affect pulmonary vasomotor tone or membrane permeability. A significant correlation ($r = 0.8$) between the change in plasma histamine and the decrease in PaO_2 in young and masters athletes compared to controls [7, 149]. Nitric oxide (NO) derived from the pulmonary vascular endothelium may play a role in the regulation of pulmonary vascular tone. Endogenously produced NO has been detected in the exhaled air (V_{NO}) of humans [72] and is altered during exercise [130, 167]. V_{NO} increases to significantly higher levels in highly trained aerobic athletes compared to controls [130]. The role V_{NO} may have in the development of EIH is unknown. Based on these preliminary data the role of inflammatory mediators and NO in EIH remains to be well defined. However, given that the pulmonary microvascular blood flow is know to increase during exercise these are areas that warrant further investigation.

In summary, evidence for pulmonary edema in humans remains unclear. Indications of extravascular lung water accumulation in humans have been indirect and inconsistent. Stress failure of the blood-gas barrier is an attractive theory, which awaits sufficient experimental evidence.

4. GENDER

To date, all investigations except one [80] that have examined the incidence, relevance, or mechanisms of EIH have utilized male subjects. From this one descriptive study it appears that women do indeed develop EIH, and at a lower VO_2max compared to matched males. Males with a VO_2max within 15% of predicted normal (40-50 $ml \cdot kg^{-1} \cdot min^{-1}$) do not demonstrate EIH, while 40% of women with a VO_2max within 15% normal (35-50 $ml \cdot kg^{-1} \cdot min^{-1}$) experienced EIH [80]. The observation of gender-dependent EIH suggests that women are more susceptible to EIH than men. Gender differences were addressed by McClaran et al. [136] in a companion paper where the same women were observed to have significant flow limitations due to their smaller lung volumes and lower maximal flow rates (i.e., mechanical limitation). Possibly, relative hypoventilation was responsible for EIH in these women. Utilizing the same group of women, the effects of prior exercise on EIH was examined [217]. EIH was lessened, not enhanced by prior exercise. This argues for a functionally based cause for EIH that is only present during exercise rather than a persistent mechanism (i.e., pulmonary edema). These observations have recently been corroborated by McKenzie et al. [140] in a group of well-trained male cyclists.

Given the paucity of data examining EIH in women, it is difficult to draw substantial conclusions. However, the pattern of EIH appears to be different between men and women. The incidence of EIH in women needs to be well established before useful gender comparisons can be made. Mechanism(s) responsible for EIH may be different between men and women and gender comparisons may help to elucidate the causative factors of EIH.

5. AGE

The development of EIH has been documented in older athletes [149, 150, 179]. Few studies have been completed on this group of athletes, however significant reductions of PaO_2 and SaO_2 are seen at VO_2max. EIH may occur more frequently in older athletes than young athletes. Seventy percent of older athletes have been shown to experience EIH [134]. Prefaut et al. [179] described a different pattern of EIH between older and younger athletes. For the same absolute work load, the drop in PaO_2 was greater in older athletes for training conditions less rigorous than in young athletes (i.e., training intensity lower in older athletes). The pattern of widening $[A\text{-}a]DO_2$ was similar between young and old athletes, but was greater in magnitude the older group at a given $\%VO_2max$.

It is known that the normal aging process reduces lung elastic recoil and increases the stiffness of the chest wall. This can result in reduced vital capacity and expiratory flow rates, thus the MEFV loop is reduced. Whether or not the older athlete is mechanically constrained during exercise, resulting in EIH, remains to be investigated adequately.

6. SUMMARY

Exercise-induced hypoxemia negatively affects VO_2max and exercise performance capacity in highly trained male endurance athletes. EIH also affects women and older athletes. EIH is likely a multifactorial phenomenon related to diffusion limitations, inappropriate exercise ventilation, and inequality of V_A/Q. The relative contribution of each postulated cause remains debatable. Further study is required to completely examine the role of hypoventilation and the complex ventilatory control mechanisms involved. Stress-failure of the pulmonary capillary and the development of pulmonary edema can not yet be confirmed or refuted from the available

data. Although not yet described in the literature, the mechanism(s) for EIH are possibly varied between individuals. Single-subject design studies may possibly reveal this. To borrow the words of Dr. J. A. Dempsey "...given the long list of our still untested speculations, we could use a bit more data" [45].

APPENDIX B

REVIEW OF LITERATURE: EXHALED NITRIC OXIDE

1. INTRODUCTION

Endogenously produced nitric oxide (NO) has been detected in the exhaled air of humans [72] and the amount of exhaled NO is altered during exercise [130, 167]. It is now well established that NO is produced in a variety of both human and animal cells with numerous biological functions. NO is believed to exert regulatory functions in the circulatory, pulmonary, nervous and immune systems. Expiratory NO concentrations (C_{NO}) and the production rate of NO (V_{NO}) have been examined during physical exercise. It is believed that the formation of NO may play a role in the normal pulmonary response to exercise. This is a contemporary issue of relevance to exercise scientists. However, the effects and relevance of NO to exercise are not yet completely understood. This review summarizes the current literature of the physiology of NO, expiratory NO, and the relationship between exhaled NO and exercise.

2. PHYSIOLOGY OF NITRIC OXIDE

NO is produced from one of the nitrogen atoms of the N-guanidino terminals of the common amino acid L-arginine and molecular oxygen. In addition to NO, citrulline is a product of this reaction. The L-form of arginine is specific to the reaction, as a number of analogs and the D-enantiomer of arginine are inactive. Production of NO occurs via a metabolic pathway requiring an isoform of the enzyme NO synthase (NOS), calcium (Ca^{2+}), nicotinamide adenine dinucleotide phosphate, tetrahydrobiopterin, flavin adenine dinucleotide, flavin mononucleotide, and calmodulin depending on the cellular location and isoform of NOS [85]. Three primary isoforms of NOS exist; (1) constitutive form, dependent on Ca^{2+} and calmodulin found in neural

tissue (nNOS), (2) Ca^{2+}/calmodulin dependent constitutive enzyme found in endothelial cells (eNOS), and (3) Ca^{2+} independent form isolated from macrophages and vascular smooth muscle cells following induction with specific cytokines (iNOS). Despite extensive research, the specific mechanism for the NOS oxidation of L-arginine to NO remains unclear. It is known that the more active forms of NOS are the constitutive forms (nNOS and eNOS). Activation of iNOS requires gene transcription, therefore increased NO production occurs several hours after exposure to stimuli as compared to the constitutive forms which can be activated pharmacologically or physically within seconds or minutes.

The production of NO is believed to cause relaxation of smooth muscle cells via a signal transduction system. NO activates guanylate cyclase (GC), the enzyme which stimulates the conversion of 3', 5'-cyclic guanosine triphosphate (GTP) to 3', 5'-cyclic guanosine monophosphate (cGMP). Two major subtypes of GC exist; particulate guanylate cyclase (pGC) and the more active soluble guanylate cyclase (sGC). The presence of a cGMP-dependent protein kinase also appears obligatory for the activation of cGMP [85]. The NO-cGMP signal transduction system causes relaxation of smooth muscle by decreasing the concentration of free calcium (Ca^{2+}) in smooth muscle cytosol. Three mechanisms have been proposed to account for the smooth muscle relaxant properties of cGMP; (1) cGMP may stimulate Ca^{2+} extrusion by activation of the Ca^{2+}-ATPase on the plasma membrane [171, 211]; (2) cGMP may facilitate uptake of Ca^{2+} into the endoplasmic reticulum by activation of a Ca^{2+}-ATPase [184, 227]; (3) Na^{+}-Ca^{2+} exchange [63]. Nitric oxide-relaxation of smooth muscle is an important physiological process, yet there is no consensus on the mechanism of decreased Ca^{2+}. It is possible that a combination of the aforementioned factors leads to a lowering of Ca^{2+}.

3. EXHALED NITRIC OXIDE

Exhaled endogenous NO was first documented in the expired gas of rabbits, guinea pigs and humans using chemiluminescence [72]. The presence of NO in human breath has been confirmed using gas chromatography-mass spectrometry [124]. Indeed, numerous studies have detected NO in the air of normal, healthy humans (Table 6) and in many different animal species [206]. Reported human values of expired NO are variable, ranging from 2 to 80 ppb. Discrepancy between studies can be attributed to three methodological concerns. Firstly, gas collection has been done using a variety of exhalation maneuvers; direct exhalation, breath-holding, use of nose clip, use of a face mask, fixed expiratory flow rate, direct sampling vs. mixing chamber, and positive end expiratory pressures [71]. Exhaled NO concentrations are sensitive to expiratory flow rates where NO can rise 11-fold as flow increases [214]. To date, there is no standardized measurement technique, however the single-breath measurement is gaining acceptance [86]. Given the range of NO values reported in the literature, it important to be cognizant of the measurement technique used when interpreting and comparing results. Secondly, mixing of nasal and lower airway air gases can significantly elevate NO concentrations. The upper airways, especially the nose, contribute the largest amount of NO to exhaled air [113, 193, 205]. The proportion of exhaled NO derived from the lower airways ranges from 90% [205] to 50% [167]. There is considerable debate surrounding the proportion of NO from lower vs. upper airway, yet it appears reasonable to conclude that some portion of exhaled NO is of a lower airway origin. Lastly, NO is highly reactive with oxygen and various synthetic materials. Studies that do not account for this can have erroneous NO values depending on the reactivity or absorbency of the equipment to NO.

4. EXHALED NITRIC OXIDE DURING EXERCISE

Exercise alters nitric oxide concentration (C_{NO}) and production rate (V_{NO}). Only two investigations have reported no change in C_{NO} during exercise [19, 100], most existing data points to a decrease in C_{NO} during exercise (Table 6). C_{NO} in the nasal passages is predominant in resting humans with the lower airways contributing a smaller fraction [113, 193, 205]. The contribution of the nasal passages to exhaled NO has recently been observed to decrease during exercise [127, 167, 192], while the contribution of lower airways increases [167]. Nasal levels of NO appear to drop rapidly in response to exercise. Nasal cavity NO levels dropped 47% after only one minute of exercise compared with rest with a maximal reduction of 76% during heavy work [127].

The mechanisms underlying the reduction in C_{NO} can only be speculated on at this stage. At first inspection, it would seem that C_{NO} is decreased during exercise due to increased ventilation. However, it has been calculated that the decreased nasal C_{NO} cannot be explained merely by dilution [127]. It is possible that during exercise, a shift occurs where a greater percentage of total C_{NO} comes from a lower airway source rather than the nasal cavity [167]. The shift may be reflective of a change from nasal to oral breathing at the onset of exercise explaining the immediate decrease in C_{NO} [127]. Mucosal blood flow is reduced during exercise in both the sinus [57] and the nose [154]. Regional differences in NO production during exercise likely exist. An increase in endothelial production could, in fact, explain the higher amounts of NO found in orally exhaled air during exercise since NO derived from the pulmonary endothelium may enter the airway lumen via the alveoli.

The production rate of NO (V_{NO}) is the product of expired NO concentration and V_E ($V_{NO} = NO \times V_E$). V_{NO} increases with physical work (Table 6). There is considerable variability

reported in the rate of exercise induced increased V_{NO}. One difficulty in interpreting the data is a lack of consistency in units. Another source of confusion is that the effects of physical conditioning on V_{NO} during rest and exercise have not been adequately examined. It is widely accepted that physical conditioning affects the cardiovascular response to exercise. Training-induced alterations to cardiac output (Q), pulmonary blood flow, and ventilation (V_E) may influence the production of NO. Basal C_{NO} has been found to be identical between high aerobic capacity athletes and non-athletes [40, 130] and higher in one subject described as an athlete [19]. During exercise, it has been observed that athletes maintain a higher C_{NO} than do moderately trained and sedentary individuals at a given exercise intensity [130]. In disagreement with these data, no difference in C_{NO} was found between trained men, sedentary women, and sedentary men at rest or during cycle exercise.[40] The dissimilar results may be related to the different levels of physical conditioning and maximal aerobic capacities of the respective athletic groups; 4.4 $l \cdot min^{-1}$ [40] and 5.6 $l \cdot min^{-1}$ [130]. These two groups would likely have different V_E, Q, and pulmonary blood flow, thus they would have different rates of NO production. To date, these are the only data available addressing this issue, leaving the question of the effects of physical conditioning on basal and exercising C_{NO} unresolved.

Regardless, V_{NO} increases in both athletic and non-athletic populations during dynamic exercise. As mentioned, the rate of increase has been reported to be different between athletes and non-athletes [130] and the same [40]. This disunity is possibly related to the method of reporting data (Table 6). When V_{NO} is expressed per unit of body mass ($pmol \cdot kg^{-1} \cdot min^{-1}$), differences are seen between athletes and non-athletes [130]. However, when V_{NO} is expressed in absolute terms ($nmol \cdot min^{-1}$), no differences between groups are observed [40]. In a preliminary study of unpublished observations, Phillips et al. [167] describe a statistically

significant correlation between exhaled NO output and body surface area ($r = 0.59$). Based on this finding NO output values were expressed per square meter of body surface area. These results require further study to be confirmed or refuted. Given the sparse data and inconsistency between studies, it is difficult to conclude what role physical conditioning and body surface area plays in the determination of V_{NO}.

A growing body of evidence suggests that exercise training increases basal nitric oxide production in the systemic vasculature [5, 68, 108, 115, 210, 213, 232]. A lowering of blood pressure and heart rate, and an increase in coronary blood flow and capillary density of skeletal muscle characterize chronic exercise training. The decrease in blood pressure may be mediated by an augmented basal production of NO and its subsequent vasodilatory effects [172]. The significance of this may be to serve as a protective effect in disease states such as atherosclerosis, cardiac failure and perhaps hypertenstion [108, 115]. It is reasonable to suggest that an upregulation of NO production in the systemic vasculature caused by training could be mirrored in the pulmonary vasculature. This hypothesis remains to be adequately tested.

5. NITRIC OXIDE PRODUCTION DURING EXERCISE

It is widely reported that NO is detected in the exhaled air of humans. However, the origin and stimuli of exhaled NO are poorly understood. A better understanding of where and how exhaled NO originates is necessary to determine its significance. There is contradictory evidence to suggest that NO is primarily released from the upper airways, lower airways or from both areas. Schedin et al. [205], using patients with tracheostomies and a group of anesthetized healthy women, determined that the lower airways and lung are one source of NO, but the quantity is lower compared to the upper airways (10% lower vs. 90% upper). These important

data have been corroborated by several groups suggesting that the major contributor to exhaled NO at rest is the nasal space with a lesser addition from the lower airways [4, 37, 113, 205]. The cellular source of NO from the upper respiratory tract may be airway epithelial cells in addition to a nasal source [211].

In contrast to upper airway data, ~ 50% of NO in mixed expired air has been found to originate from a lower airway source when air from the lung was partitioned from that of the nasopharynx by balloon occlusion [167]. In support of a lower airway origin, fractional alveolar and expired concentration of NO and CO_2 have been measured and it has been predicted that exhaled NO was derived from a similar region of the lung as CO_2 [27]. Conversely, NO levels have been shown to rise to an early peak and plateau, while CO_2 levels continue to rise and peak later during exhalation [37]. It is difficult to reconcile the disparity between these two studies. A lower airway origin of exhaled NO has also been documented in both normal [111, 166] and asthmatic individuals [111]. The cellular source of NO in the lower respiratory tract remains uncertain, however, studies with isolated perfused pig lung preparation suggests an alveolar surface origin, rather than the pulmonary circulation, [44] and may be derived from endothelial NO synthase expressed in the alveolar walls of normal lungs [117]. Based on the available data, it can be concluded that mixed expired air contains both a fraction of lower and upper airway derived NO. More NO is likely derived from the upper airways and nasal passages than the lower airways at rest, yet the respective proportions remain uncertain. During exercise, lower airway sources of NO may become more pronounced.

Given that the site of NO production is debatable, the stimulus for production remains equally obscure. However, two schools of thought have emerged in the literature; increased pulmonary blood flow [19, 100, 130] and increased ventilation [166, 167, 169, 219]. In the

peripheral vasculature, an increase in shear stress leads to enhanced endothelial NO release [163]. Shear stress on the blood vessel wall arises from the frictional force caused by blood flow, although other physical stimuli such as pulsatility and changes in transmural pressure may be important [170, 201]. It is recognized that an acute increase in blood flow will lead to an increase in NO production and a relaxation of underlying smooth muscle. It is believed that shear stress causes deformation of endothelial cells and initiates the NO-cGMP signal transduction system causing changes in smooth muscle tone [142, 242]. However, the shear stress-vasodilation response within the pulmonary architecture is not as clearly defined as in the peripheral vasculature.

Much evidence for a shear stress/pulmonary endothelium source of NO in expired air is indirect; statistically significant positive correlations are seen between V_{NO} and heart rate, VO_2, VCO_2, V_E, and cardiac index [40, 100, 130, 167, 169]. While these data provide some insight, it is difficult to extrapolate from correlative evidence as to the causative factors involved with increased V_{NO} during exercise. Several studies have attempted to manipulate the variables that could alter V_{NO}. To determine the effects of altered pulmonary blood flow, Pogliaghi et al. [169] measured V_{NO} with head-out water immersion (↑ pulmonary blood flow) or increased gravity (↓ pulmonary blood flow) at rest and during cycling. No differences were observed between conditions at similar levels of ventilation and it was concluded that altering pulmonary vascular endothelial stress does not alter lung output of NO. Despite the uniqueness of these data, they remain difficult to interpret as no pulmonary measures were made and it is uncertain to what extent a shear stress response occurred. Other studies have attempted to manipulate pulmonary blood flow with dobutamine infusion [167]. Dobutamine infusion did not increase NO production as compared to exercise. However, caution must be taken for ruling out a vascular

origin of NO, as the level of exercise was only moderate. Possibly, an effect of increased blood flow on increased V_{NO} output would be evident at a higher exercise intensity. The most physiological method of increasing cardiac output and skeletal and coronary blood flow is through exercise. Moreover, it was also observed that mean V_{NO} was increased during dobutamine infusion compared with rest control V_{NO}. Interestingly, patients with heart disease have decreased basal production of expired NO [220]. While not conclusive, these data imply that impaired cardiac function reduces the shear stress response of pulmonary endothelial cells and is reflected in basal NO levels.

The stimulus for NO production during rest or exercise will likely need to be resolved using an animal model. Indeed, evidence from an isolated pig lung preparation indicates that the pulmonary vascular endothelium likely contributes to the NO found in exhaled air [44]. From these data, it can be concluded that the lung itself can synthesize adequate NO to account for C_{NO} seen in exhaled air. However, it is not clear if the cells within the lung are in fact the source of exhaled NO in an intact animal model or human. Presently, a non-endothelial source of NO can not be completely ruled out.

Voluntary increases in ventilation without exercise have been used in an attempt to determine the site of release of exhaled NO [19, 100, 166, 167]. By increasing voluntary ventilation V_{NO} increases. Stretching of the lung parenchyma or airway epithelia by positive end expiratory pressure increases V_{NO} in rabbits [165, 219]. It is therefore possible that changing ventilation alters V_{NO}. The observations of lung distention effects on exhaled NO suggest the possibility of stretch receptors coupled to NO formation within the lung. Further study is required to fully examine this hypothesis.

6. SIGNIFICANCE OF EXHALED NITRIC OXIDE

Endogenously produced pulmonary NO has been found to have several physiological functions. Possible roles include V_A/Q matching, maintenance of low pulmonary vascular resistance, and airway relaxation. The idea that an optimal V_A/Q ratio may be regulated by NO is supported by data showing hypoxia in anesthetized animals upon administration of NOS inhibitors [163]. The importance of NO is evident when inspired NO induces pulmonary vasodilation and improves V_A/Q relationships [60, 168, 182, 197]. Endogenous NO was recently found to be critical to the maintenance of a low pulmonary vascular resistance in eNOS knockout mice who developed pulmonary hypertension [55]. It is thought that NO is continuously released to maintain pulmonary arterial tone, systemic pressure, and cardiac output [218]. Patients with heart disease have reduced NO levels, perhaps explaining why they exhibit an increased vasoconstriction of their pulmonary blood vessels [220]. It would seem that endogenously produced NO is necessary for maintenance of V_A/Q matching and a low pulmonary vascular resistance, the extent to which expired concentrations of exhaled NO reflect this process requires clarification.

NO has also been detected in asthmatic patients and is elevated in comparison to non asthmatics [4, 111, 112]. The increase in exhaled NO in asthmatics likely reflects increased activity of iNOS that may be induced by inflammatory cytokines [73]. Exhaled NO may reflect airway inflammation in asthma, and may be used as means of monitoring inflammatory events [18].

During exercise Q increases in proportion to exercise intensity and the pulmonary circulation is able to accommodate the increase with little rise in pulmonary pressure. This is due to the distension and recruitment of the pulmonary capillaries. It has been traditionally thought

that this process is passive in nature where the pulmonary circulation is fully recruited. It is only possible to speculate at this stage, however it seems likely that NO may aid in mediating the V_A/Q response during exercise. Further research is required to determine the relationship between exercise, NO, pulmonary pressures and V_A/Q relationships. Patients with chronic obstructive pulmonary disease (COPD) who inhaled 40 parts per million of NO during submaximal exercise improved V_A/Q relationships and PaO_2 compared to breathing room air [196, 197]. Interestingly, some highly trained athletes experience hypoxemia during heavy exercise [48, 91]. Approximately 2/3 of their hypoxemia can be explained by a V_A/Q mismatch [93]. The possible role of NO in this phenomenon has yet to be examined.

7. SUMMARY

Nitric oxide is present in varying concentrations in the exhaled air of exercising humans. The concentration decreases with exercise intensity, while the production rate of NO increases. With exercise, the lower airways increase their production rate while nasal production decreases. NO may be partly responsible for the normal pulmonary response to exercise and exhaled NO may reflect this process. At the present time, there is considerable debate in the nitric oxide literature. Further research is warranted to determine the role of NO in the pulmonary response to exercise in normal, athletic, and clinical populations.

TABLE 6. Published values for exhaled nitric oxide during rest and exercise.

Author	Exercise	Subjects	Basal C_{NO}	Exercise C_{NO}	Basal V_{NO}	Exercise V_{NO}
Persson [166]	cycling submaxial	NR n = 12 male ? female ?	10 ppb	6 ppb	10 nl/min	30 nl/min
Bauer [19]	cycling submaximal	NR n = 4 male	12.9 ppb	14.3 ppb	72.1 pmol/min/kg	135.6 pmol/min/kg
Iwamoto [100]	running maximal	S n = 8 2 female 6 male	26.3 ppb	26 ppb	12.3 nmol/min	NR
Matsumoto [135]	cycling maximal	NR n = 5 male	10 ppb	6 ppb	121 nl/min	398 nl/min
Trolin[224]	cycling submaximal	NR n = 8 4 female 4 male	11.9 ng/l	5.31 ng/l	111ng/min	209 ng/min
Maroun [130]	cycling moderate	S I A male n = 8/group	12 ppb 11 12	5 ppb 5 9	85 pM/kg/min 86 85	110 pM/kg/min 110 450
Phillips [167]	cycling supine submaximal	NR n = 8 male ? female ?	NR	NR	53 nl/min/m^2	160 nl/min/m^2
Chirpaz-Oddou [40]	cycling maximal	S male, n = 7 S female, n = 7 A male, n = 8	14.8 ppb 16.2 15.9	9 ppb 10.5 10.5	5 nmol/min 5 6	18 nmol/min 20 37
Pogliaghi [169]	cycling maximal	S n = 10 male	16.3 ppb	6.1 ppb	137.7 ppb/min	544.7 ppb/min

Definition of abbreviations: NR = training status not reported; C_{NO} = nitric oxide concentration; V_{NO} = exhaled nitric oxide output; S = sedentary; I = intermediate; A = athlete; NR = not reported.

APPENDIX C

REVIEW OF LITERATURE: INHALED NITRIC OXIDE DURING EXERCISE

1. INTRODUCTION

Until recently, nitric oxide (NO) was regarded as a toxic gas responsible for a portion of the morbidity related to air pollution [158]. Currently NO is recognized as a major endogenous mediator of multiple physiological processes, and the use of inhaled NO is prevalent in anesthesia and critical care. Although many findings are preliminary, inhaled NO has found a place in clinical medicine. The magnitude and importance of its use awaits the results of further study. This review is not intended to be a comprehensive resource of inhaled NO literature, rather it briefly summarizes the physiology of inhaled NO, the clinical effects of inhaled NO, and describes the available literature examining inhaled NO during exercise.

2. PHYSIOLOGY OF INHALED NITRIC OXIDE

NO was reported in 1987 to be an important endothelium-derived relaxing factor [99, 155]. Furchgott and Zawadzki [62] first demonstrated that endothelial cells must be present for acetylcholine to induce relaxation of isolated rabbit aorta. Subsequent to these studies, NO has been identified as an important biological second messenger where NO is found endogenously or is provided exogenously during therapy. The mechanisms of action are thought to be the same.

Inhalation of NO is believed to cause relaxation of smooth muscle cells via a signal transduction system. NO activates guanylate cyclase (GC), the enzyme which stimulates the conversion of 3', 5'-cyclic guanosine triphosphate (GTP) to 3', 5'-cyclic guanosine monophosphate (cGMP). Two major subtypes of GC exist; particulate guanylate cyclase (pGC) and the more active soluble guanylate cyclase (sGC). The presence of a cGMP-dependent

protein kinase also appears obligatory for the activation of cGMP [85]. The NO-cGMP signal transduction system causes relaxation of smooth muscle by decreasing the concentration of free Ca^{2+} in smooth muscle cytosol. Three mechanisms have been proposed to account for the smooth muscle relaxant properties of cGMP; (i) cGMP may stimulate Ca^{2+} extrusion by activation of the Ca^{2+}-ATPase on the plasma membrane [171, 209], (ii) cGMP may facilitate uptake of Ca^{2+} into the endoplasmic reticulum by activation of a Ca^{2+}-ATPase [184, 227], (iii) Na^{+}-Ca^{2+} exchange [63]. NO-relaxation of smooth muscle is an important physiological process, yet there is no consensus on the mechanism responsible for the decrease in Ca^{2+}. It is possible that a combination of the aforementioned factors leads to a lowering of Ca^{2+}. Nonetheless, selective relaxation of pulmonary vascular smooth muscle has numerous clinical applications.

3. CLINICAL EFFECTS OF INHALED NITRIC OXIDE

Short-term inhalation of gaseous NO causes selective vasodilation without systemic effects. When inhaled, NO diffuses across the alveoli to the pulmonary vasculature where it exerts a vasodilating effect. NO, *in vivo*, has a biological half-life between 111-130 ms, depending on the ambient oxygen concentration [110], and is quickly inactivated by hemoglobin to form methemoglobin and nitrate and nitrite ions [191]. The actual and potential uses of NO are summarized in Table 7. Indeed, NO inhalation (5-80 ppm) has been shown to have positive effects on arterial oxygenation in respiratory-compromised patients. Excellent reviews of these effects are described elsewhere in detail [24, 97, 144, 153]. A synopsis of the clinical effects of inhaled NO is presented below.

Under normal conditions, the blood vessels surrounding underventilated areas of the lung will constrict resulting in a decreased perfusion of these areas and a preservation of V_A/Q

matching. This occurrence, termed hypoxic vasoconstriction (HPV), serves to limit arterial hypoxemia but can lead to increased pulmonary artery pressure (P_{PA}). In patients with chronic pulmonary hypertension [i.e., adult respiratory distress syndrome (ARDS)] the administration of intravenous vasodilators (i.e., nitroprusside, nitroglycerin) can have beneficial effects (↓ P_{PA}) but these are typically at the expense of systemic hypotension and worsening hypoxemia [183]. Inhalation of NO in ARDS patients (2-20 ppm for 2-27 d) has been shown to decrease mean P_{PA} and improve arterial oxygenation [25]. In such a case as ARDS, when hypoxia and increased P_{PA} are chronic it is routinely observed that inhaled NO improves PaO_2 and P_{PA}. A decrease in pulmonary vascular resistance (PVR), via NO, may also serve to improve right ventricular function [58]. Using normal volunteers, Frostell et al. [59] examined the effects of inhaled NO during normoxia and acute hypoxia (FiO_2 = 12%). Normoxic inhalation of NO (40 ppm) had no effect on pulmonary or systemic hemodynamics. Hypoxia induced mild pulmonary hypertension by increasing P_{PA} (+5 mmHg) and PVR (+50 dynes·s^{-1}·cm^{-5}) and inhalation of NO decreased P_{PA} and PVR to control values without causing systemic vasodilation.

Inhaled NO therapy may have also found a place in high-altitude medicine. Mountaineers who had previously documented HAPE ascended to 4559m (barometric pressure = 440 mmHg) [207]. Ten of 18 subjects exhibited radiographic evidence of HAPE. Inhalation of NO (40 ppm for 15 min) decreased systolic pulmonary-artery pressure (from 66 to 40 mmHg), improved SaO_2 (from 67 to 73%) and PaO_2 (from 36 to 41 mmHg), and lessened the [A-a]DO_2 (from 15 to 11 mmHg). While it seems clear that inhaled NO can improve oxygenation in some patient populations, this effect should not be considered to be a general response but is dependent upon the pathophysiology of the disease/condition.

Hopkins et al. [90], utilizing anesthetized dogs, examined how inhalation of NO (80 ppm) can alter pulmonary gas exchange under conditions of: (i) normal lungs, (ii) shunt, and (iii) V_A/Q inequality. In dogs with V_A/Q inequality, NO variably affected matching which was improved in some dogs and worsened in others. These data are in agreement with other reports of NO inhalation in patients with COPD where PaO_2 values have been shown to increase slightly [3], have no effect [145], and decrease with V_A/Q inequality [17]. V_A/Q inequality is the most common gas exchange abnormality and marked intrapulmonary shunt is absent in COPD [229]. Critical analysis of COPD-NO inhalation studies reveals that grouped data may not be indicative of the NO-inhalation response. Based upon the above mentioned dog data [90], and the variable responses in gas exchange [3, 17, 145], it can be concluded that areas of low V_A/Q matching contribute to the variable clinical effects of inhaled NO. Hopkins et al. [90], surmised that inhaled NO improved gas exchange when the normal areas of the lung increased blood flow, but worsened it when both the normal areas and the areas of low V_A/Q ratio had a fall in PVR. This would likely explain why the response of NO inhalation is variable in COPD patients, and emphasizes the need to understand the pathophysiology of the lung disease/condition.

While the effects of NO inhalation are widely described in the clinical literature, few data exist concerning the response of normal individuals. It is useful to succinctly recount the existing data here. Brett et al. [31], recently characterized the response of the pulmonary circulation in normal individuals to inhaled NO (n = 8; NO = 0, 20, and 40 ppm). No significant changes were observed for Q or DLCO implying that the normal pulmonary vascular bed is not amenable to vasodilation by inhaled NO. This is in agreement with normal lungs in dogs where NO did not alter PaO_2 or V_A/Q matching [90].

4. INHALED NITRIC OXIDE DURING EXERCISE

Studies that have sought to examine the effects of inhaled NO during exercise have been few. Nonetheless, these investigations have provided valuable insight into the effects of NO therapy. It is well established that COPD patients may develop pulmonary hypertension when exercising [243]. Roger and colleagues [197], demonstrated that in COPD patients, inhaled NO (40 ppm) reduced pulmonary hypertension and improved V_A/Q ratios during exercise. However, the effect of NO on PaO_2 was different at rest than during exercise. During rest, PaO_2 and V_A/Q ratios were worsened while during exercise they both improved slightly. The authors concluded that during exercise the effect is likely explained by a preferential distribution of inhaled NO to well-ventilated alveolar units with faster time constants and normal V_A/Q ratios. The results are in agreement with the previous discussion of the variability of NO inhalation at rest in COPD patients. Patients with left ventricular dysfunction and chronic heart failure were examined during exercise conditions of control and NO inhalation (30 ppm) [26]. V_T and ventilatory equivalent for oxygen (V_E/VO_2) were significantly reduced during exercise with NO inhalation compared to control. There was also a strong, but non-significant, trend for V_E to be reduced ($P = 0.051$). The results of this study are difficult to interpret physiologically. It is unclear what mechanisms are responsible for alterations in ventilatory parameters. Hemodynamic measurements at rest were determined from a Swan-Ganz catheter placed in the pulmonary artery and Q was determined by the thermodilution technique. No statistical differences were observed for hemodynamic and gas exchange variables. It is also worth noting that the authors of this study utilized in excess of 20 paired t-tests (i.e., large probability for Type I error).

In a separate investigation, Koelling et al. [118], examined patients undergoing cardiac transplantation evaluation ($n = 14$). Right heart catheterization was performed and Q was

determined by thermodilution. Exercise was performed under normal conditions and with NO (40 ppm). Patients with greater PPA, larger LV end-diastolic volumes, and lower RV ejection fractions were those benefited the most from NO. Reduction of resistance, via NO, within the pulmonary vessels likely leads to augmented maximum workload and VO_2 at the anaerobic threshold. This effect was not observed during treadmill exercise in sheep [120]. With inhalation of NO (30 ppm), pulmonary vascular tone was unchanged at rest, while during exercise the usual reduction in PVR was not observed. The implication of this observation is that the pulmonary vasculature is already maximally dilated during exercise by flow-related pressure effects [120, 187].

To date, only one study has sought to investigate the effects of NO inhalation on pulmonary gas exchange during exercise in highly trained athletes [49]. Male endurance athletes (n = 9: VO_2max ~ 65 $ml \cdot kg^{-1} \cdot min^{-1}$) performed a maximal cycle test to exhaustion while inhaling either (i) room air, or (ii) room air combined with 15 ppm NO. No differences between conditions were observed for VO_2max or maximal work. Inhalation of NO caused PaO_2 to decrease at rest, 50%, 75%, and 100% VO_2max (↓ 9.5, 15.0, 14.6, and 5.1 mmHg). Interestingly, delta PaO_2 continuously fell without NO (13.5%). With NO, after a preliminary drop, a stabilization of delta PaO_2 was observed between 75 and 100% VO_2max. This trend was also reflected in [A-a]DO_2. Inhalation of NO also abolished histamine release between 75 and 100% VO_2max but EIH was not reversed. The physiological significance of these results remains to be determined. As concluded by Durand and co-workers [49], the negative effect of NO on gas exchange at rest was unexpected and continued during exercise; and these preliminary results should be interpreted with caution.

5. SUMMARY

Nitric oxide is a potent and selective pulmonary vasodilator. In patients with lung disease, inhalation of NO can improve V_A/Q matching, reduced pulmonary vascular resistance, and improve arterial oxygenation. These effects appear to continue during exercise. In normal individuals the effect of NO inhalation during exercise is not clearly established. From the available preliminary data (one study), it appears that athletes with exercise-induced hypoxemia have worsened gas exchange at rest with NO inhalation and a stabilization occurs as exercise progresses.

TABLE 7. Actual and potential uses of inhaled nitric oxide. Adapted from Kacmarek [109].

Persistent pulmonary hypertension of the newborn
Congenital cardiac anomalies
Adult respiratory distress syndrome
Chronic pulmonary hypertension
Cardiac disease
Pulmonary disease
Asthma
Cardiac surgery
Lung transplantation
Pulmonary embolism
Acute myocardial infarction

APPENDIX D

INDIVIDUAL DATA: EXHALED NITRIC OXIDE

TABLE 8. Individual subject data for age, height, mass and sport. Definition of abbreviations: Tri. = triathlete, Road = road cyclist, Mtn. = off-road, mountain cyclist.

SUBJECT	AGE (yr)	HEIGHT (cm)	MASS (kg)	SPORT
1 (MB)	30	179.6	75.1	Road
2 (GA)	29	184.0	81.0	Tri.
3 (TD)	29	173.0	62.7	Mtn.
4 (PH)	26	179.0	72.6	Tri.
5 (JM)	27	171.0	61.6	Road
6 (KS)	22	172.5	67.3	Mtn.
7 (MM)	22	185.5	73.4	Tri.
8 (OU)	24	180.0	62.7	Road
9 (SD)	28	173.1	66.9	Road
10 (MM)	24	187.0	79.4	Tri.
11 (MK)	27	183.0	71.3	Road
12 (JJ)	25	182.7	73.7	Road
13 (MP)	27	175.9	68.2	Mtn.
14 (RR)	26	182.3	80.5	Mtn.
15 (MS)	20	181.0	73.2	Mtn.
16 (TB)	30	181.3	76.3	Road
17 (JB)	36	184.5	94.1	Road
18 (DM)	24	170.7	71.0	Mtn.

TABLE 9. Individual subject data for respiratory variables during maximal cycle ergometer exercise. Definition of abbreviations: VO_2max = maximal oxygen consumption, $\dot{V}E$ = minute ventilation, RER = respiratory exchange ratio.

SUBJECT	VO_2max ($l \cdot min^{-1}$)	VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)	$\dot{V}E$ (BTPS) ($l \cdot min^{-1}$)	RER (VCO_2/VO_2)
1 (MB)	5.18	72.96	179.42	1.15
2 (GA)	5.47	66.95	199.47	1.22
3 (TD)	4.63	74.44	173.16	1.21
4 (PH)	5.08	69.97	168.1	1.2
5 (JM)	3.9	63.31	154.92	1.13
6 (KS)	4.22	62.7	188.37	1.17
7 (MM)	4.38	59.67	152.3	1.14
8 (OU)	4.09	61.04	185.84	1.17
9 (SD)	4.72	68.51	163.91	1.15
10 (MM)	4.61	58.06	178.76	1.19
11 (MK)	4.63	64.94	173.1	1.24
12 (JJ)	5.11	69.34	170.12	1.14
13 (MP)	4.92	72.14	169.77	1.18
14 (RR)	5.46	67.82	176.89	1.15
15 (MS)	5.5	75.14	173.84	1.14
16 (TB)	5.16	67.63	176.02	1.14
17 (JB)	6.42	68.2	203.69	1.06
18 (DM)	5.31	74.89	154.54	1.14

TABLE 10. Individual subject data for heart rate, saturation, and power during maximal cycle ergometer exercise. Definition of abbreviations: HRmax = maximal heart rate, SaO_2 = percent oxyhemoglobin saturation.

SUBJECT	SaO_2 – Rest (%)	SaO_2 – Lowest (%)	HRmax (beats·min^{-1})	Power (watts)
1 (MB)	97.69	93.34	187	449
2 (GA)	97.66	91.01	197	450
3 (TD)	98.14	92.32	193	458
4 (PH)	97.66	93.65	198	439
5 (JM)	97.49	95.12	183	372
6 (KS)	97.69	93.62	192	462
7 (MM)	98.14	95.12	182	444
8 (OU)	98.14	93.85	184	472
9 (SD)	98.66	94.64	180	469
10 (MM)	96.75	91.13	187	449
11 (MK)	98.82	94.56	187	462
12 (JJ)	98.61	88.57	192	456
13 (MP)	98.14	93.88	194	467
14 (RR)	98.02	90.11	189	484
15 (MS)	98.11	89.65	199	502
16 (TB)	98.08	93.91	197	478
17 (JB)	97.72	94.24	208	494
18 (DM)	97.47	91.41	182	456

TABLE 11. Individual subject data for concentration of nitric oxide (C_{NO}) at rest and during incremental exercise (watts). C_{NO} values are in ppm.

SUBJECT	Rest	100	200	250	300	350	400	450
1 (MB)	22.7	18.8	16.5	20.0	17.7	17.1	21.1	22.5
2 (GA)	11.5	8.2	8.2	7.9	7.7	8.7	9.5	11.1
3 (TD)	10.4	10.6	10	11.8	11.7	13	14.1	14.3
4 (PH)	4.6	3.1	1.8	2.1	2.0	1.5	2.1	2.3
5 (JM)	6.4	5.3	4.0	4.1	3.3	3.3	-	-
6 (KS)	6.4	2.8	2.3	3.4	5.2	3.7	3.3	7.9
7 (MM)	5.3	5.7	4.7	4.9	4.5	4.9	4.2	4.9
8 (OU)	22.1	18.0	18.0	18.4	17.5	16.2	17.0	18.5
9 (SD)	4.0	4.9	5.0	4.2	2.9	4.6	3.2	4.3
10 (MM)	6.7	4.9	3.9	4.1	3.6	4.1	4.9	6.6
11 (MK)	11.8	12.3	12.1	13.0	11.7	12.8	13.2	14.0
12 (JJ)	5.0	3.3	2.7	2.7	2.0	3.0	3.1	3.9
13 (MP)	2.5	2.6	2.0	3.3	2.5	2.6	2.0	2.5
14 (RR)	4.7	2.3	2.2	2.0	2.4	2.2	2.0	2.0
15 (MS)	3.0	2.1	1.5	2.1	5.1	3.4	4.9	2.3
16 (TB)	8.6	4.7	4.0	4.0	10.1	6.2	6.4	5.5
17 (JB)	3.5	3.2	3.4	2.3	8.5	3.0	5.1	3.4
18 (DM)	3.9	4.7	6.9	2.8	2.0	3.1	3.6	3.6

TABLE 12. Individual subject data for production rate of nitric oxide (V_{NO}) at rest and during incremental exercise (watts). V_{NO} values are in $nmol \cdot min^{-1}$.

SUBJECT	Rest	100	200	250	300	350	400	450
1 (MB)	7.6	21.1	28.8	31.0	53.5	59.1	112.1	134.1
2 (GA)	4.3	13.2	19.5	21.7	26.2	43.4	57.5	67.8
3 (TD)	3.7	15.5	20.8	34.3	39.0	55.5	73.3	78.6
4 (PH)	1.7	3.4	2.9	4.7	5.9	5.8	10.8	14.6
5 (JM)	2.3	6.3	8.5	12.7	11.5	15.7	0.0	0.0
6 (KS)	2.3	3.8	4.9	9.1	17.6	14.2	16.1	49.4
7 (MM)	2.0	6.1	9.5	13.0	14.0	17.3	20.4	24.8
8 (OU)	8.4	27.4	35.3	48.7	53.4	59.8	80.2	104.6
9 (SD)	2.4	7.2	8.0	10.6	9.1	15.9	14.5	23.4
10 (MM)	3.8	8.5	9.8	11.9	12.5	18.6	27.6	37.2
11 (MK)	6.4	15.4	22.8	26.8	33.4	45.1	55.3	80.5
12 (JJ)	2.0	5.8	6.4	8.0	6.5	12.1	16.5	22.1
13 (MP)	1.0	3.4	3.3	8.1	7.6	9.3	9.0	14.1
14 (RR)	2.0	4.0	5.0	5.7	8.2	8.7	9.5	11.8
15 (MS)	1.0	2.6	2.8	4.9	13.5	10.7	19.0	11.5
16 (TB)	3.3	5.5	7.6	10.2	30.3	21.0	28.2	31.4
17 (JB)	1.3	4.5	7.4	6.1	22.7	10.7	21.0	20.9
18 (DM)	2.2	6.1	12.5	5.6	5.5	10.7	16.2	18.2

TABLE 13. Concentration of nitric oxide (C_{NO}) (ppm) in subjects with normal (NOS) and low (LOS) oxyhemoglobin saturation at rest and during incremental exercise. Values are means ± SE. * significantly different than Rest, $P < 0.05$.

WORK (watts)	NOS ($n = 12$)	LOS ($n = 6$)
Rest	9.0 ± 1.9	5.8 ± 1.2
100	7.7 ± 1.7	4.3 ± 0.9
200	7.0 ± 1.7	4.2 ±1.1
250	7.6 ± 1.9	3.6 ± 0.9
300	8.1 ± 1.6	3.8 ± 0.9
350	7.4 ± 1.6	4.0 ± 1.0
400	8.3 ± 2.0	4.7 ± 1.1
450	9.1 ± 2.1	4.9 ± 1.4

TABLE 14. Production rate of nitric oxide (V_{NO}) ($nmol \cdot min^{-1}$) in subjects with normal (NOS) and low (LOS) oxyhemoglobin saturation at rest and during incremental exercise. Values are means ± SE. * significantly different than Rest, $P < 0.05$.

WORK (watts)	NOS (n = 12)	LOS (n = 6)
Rest	3.5 ± 0.5	2.6 ± 0.7
100	10.0 ± 1.6	6.7 ± 2.3
200	13.3 ± 2.2	9.3 ± 3.1
250	17.9 ± 2.9	9.6 ± 4.0
300	24.8 ± 3.7	12.0 ± 4.9
350	27.5 ± 4.5	17.4 ± 6.0
400	36.7 ± 7.2	24.4 ± 10.1
450	48.0 ± 8.7	28.1 ± 12.1

APPENDIX E

INDIVIDUAL DATA: INHALED NITRIC OXIDE

TABLE 15. Individual subject data for age, height, mass, sport, level of competition, and amount of training per week. Definition of abbreviations: Tri. = triathlete, Road = road cyclist, Mtn. = off-road, mountain cyclist.

SUBJECT	AGE (yr)	HEIGHT (cm)	MASS (kg)	SPORT	LEVEL OF COMPETITION	TRAINING (h/wk)
1 (RR)	27	183.4	80.4	Mtn.	National	10-15
2 (SF)	27	181.7	80	Mtn.	National	10-15
3 (AP)	27	195.3	71.7	Road	Provincial	10-15
4 (TD)	31	173.1	64.1	Mtn.	National	10-15
5 (NS)	24	180.0	78.7	Road	National	10-15
6 (LB)	30	183.4	80.0	Tri.	National	10-15
7 (BJ)	35	173.0	68.5	Tri.	Provincial	5-10

TABLE 16. Individual subject data for pulmonary function. Actual value with % predicted value below in parentheses. Definition of abbreviations: FVC = forced vital capacity, FEV_1 = forced expired volume in 1 second, $FEF_{25-75\%}$ = forced expiratory flow at 25-75% of FVC, FEFmax = maximal forced expiratory flow rate, DLCO = diffusion capacity of the lung for carbon monoxide, VA = alveolar volume, DLCO/VA = diffusion capacity of the lung for carbon monoxide/alveolar volume.

SUBJECT	FVC (L)	FEV_1 (L)	$FEF_{25-75\%}$ ($l \cdot sec^{-1}$)	FEV_1/FVC (%)	FEF_{max} ($l \cdot sec^{-1}$)	DLCO	VA (L)	DLCO/ VA
1 (RR)	5.73	4.54	4.26	79.2	7.57	38.19	7.01	5.45
	(93)	(97)	(83)	(94)	(73)	(115)	-	-
2 (SF)	5.75	4.93	5.02	75.85	10.62	36.53	7.06	5.17
	(100)	(103)	(100)	(102)	(104)	(110)	-	-
3 (AP)	6.69	5.52	5.14	82.85	10.31	36.56	9.20	3.98
	(97)	(96)	(88)	(98)	(90)	(106)	-	-
4 (TD)	4.93	3.84	3.41	77.84	9.87	36.38	5.92	6.15
	(131)	(129)	(114)	(97)	(126)	(148)	-	-
5 (NS)	5.39	3.93	3.20	72.87	10.46	43.69	7.88	5.54
	(95)	(83)	(80)	(86)	(104)	(131)	-	-
6 (LB)	6.32	5.42	5.31	85.77	10.51	40.31	9.96	4.05
	(108)	(112)	(105)	(102)	(103)	(123)	-	-
7 (BJ)	4.31	3.86	5.33	89.59	10.31	27.28	6.59	4.14
	(90)	(98)	(127)	(108)	(114)	(89)	-	-

TABLE 17. Individual subject data for respiratory variables during normoxic (FIO_2 = 21%) maximal cycle ergometer exercise. Definition of abbreviations: VO_2max = maximal oxygen consumption, VE = minute ventilation, RER = respiratory exchange ratio.

SUBJECT	VO_2max ($l \cdot min^{-1}$)	VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)	VE (BTPS) ($l \cdot min^{-1}$)	RER (VCO_2/VO_2)
1 (RR)	5.28	65.67	176.15	1.22
2 (SF)	5.11	63.88	187.15	1.23
3 (AP)	4.57	63.74	168.70	1.22
4 (TD)	4.37	68.17	162.23	1.20
5 (NS)	5.21	65.45	169.60	1.16
6 (LB)	5.28	66.00	190.54	1.19
7 (BJ)	4.38	63.92	177.11	1.22

TABLE 18. Individual subject data for heart rate, saturation, and power during normoxic (FIO_2 = 21%) maximal cycle ergometer exercise. Definition of abbreviations: HRmax = maximal heart rate, SaO_2 = percent oxyhemoglobin saturation.

SUBJECT	HRmax ($beats \cdot min^{-1}$)	SaO_2 – Rest (%)	SaO_2 – Lowest (%)	Power (watts)
1 (RR)	187	96.84	89.82	487
2 (SF)	189	98.14	90.61	485
3 (AP)	183	97.66	90.89	460
4 (TD)	190	97.66	90.92	417
5 (NS)	192	97.66	88.28	466
6 (LB)	194	97.68	90.92	452
7 (BJ)	183	98.73	90.21	377

TABLE 19. Individual subject data for respiratory variables during hypoxic (FIO_2 = 14%) maximal cycle ergometer exercise. Definition of abbreviations: VO_2max = maximal oxygen consumption, VE = minute ventilation, RER = respiratory exchange ratio.

SUBJECT	VO_2max ($l \cdot min^{-1}$)	VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)	VE (BTPS) ($l \cdot min^{-1}$)	RER (VCO_2/VO_2)
1 (RR)	3.91	47.74	165.60	1.15
2 (SF)	4.66	57.96	188.67	1.14
3 (AP)	4.10	56.26	166.63	1.10
4 (TD)	3.78	59.06	156.34	1.14
5 (NS)	4.05	51.46	164.87	1.21
6 (LB)	5.18	64.45	159.32	0.98
7 (BJ)	4.06	59.27	178.82	1.13

TABLE 20. Individual subject data for heart rate, saturation, and power during hypoxic (FIO_2 = 14%) maximal cycle ergometer exercise. Definition of abbreviations: HRmax = maximal heart rate, SaO_2 = percent oxyhemoglobin saturation.

SUBJECT	HRmax (beats·min^{-1})	SaO_2 – Rest (%)	SaO_2 – Lowest (%)	Power (watts)
1 (RR)	178	95.70	78.86	431
2 (SF)	183	97.17	74.02	400
3 (AP)	177	96.55	76.27	363
4 (TD)	186	97.66	77.28	351
5 (NS)	176	96.68	98.07	371
6 (LB)	181	98.14	82.81	374
7 (BJ)	178	97.17	73.11	310

TABLE 21 to TABLE 46 Individucal subject data for blood gas and cardiorespiratory measurements during rest and at each min of 5-min cycle ergometry tests under conditons of normoxia (N), normoxia + 20 ppm nitric oxide (N/NO), hypoxia (H), and hypoxia + 20 ppm nitric oxide (H/NO). Definition of abbreviations: PaO_2 = arterial pressure of O_2, $[A\text{-}a]DO_2$ = alveolar-arterial difference for O_2, SaO_2 = oxyhemoglobin saturation, $PaCO_2$ = arterial pressure of CO_2, HCO_3^- = bicarbonate, VO_2 = oxygen consumption; V_E = minute ventilation; RER = respiratory exchange ratio; HR = heart rate.

TABLE 21. Individual subject data for SUBJECT 1 (RR) during five-minute exercise under condition H.

	TIME					
VARIABLE	REST	1 min	2 min	3 min	4 min	5 min
pH	7.42	7.39	7.34	7.33	7.31	7.30
PaO_2 (mm Hg)	59	51	50	49	48	48
SaO_2 (%)	90.9	85.4	83.4	82.4	80.7	79.7
[A-a]DO_2 (mm Hg)	5.1	9.7	15.2	17.6	21.4	20.9
$PaCO_2$ (mm Hg)	41	39	36	34	32	33
HCO_3^{-1} ($mmol \cdot l^{-1}$)	27	24	19	18	16	16
Base Excess ($mmol \cdot l^{-1}$)	2.6	-0.7	-5.1	-6.5	-8.4	-8.9
VO_2 ($l \cdot min^{-1}$)	0.27	2.99	2.88	2.94	2.95	3.12
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	3.86	37.24	35.87	36.61	36.74	38.85
VE ($l \cdot min^{-1}$)	10.0	105.56	127.56	124.0	133.06	139.26
RER (VCO_2/VO_2)	.91	1.04	1.10	1.08	1.12	1.14
HR ($beats \cdot min^{-1}$)	89	167	167	171	173	174
Power (watts)	0	375	315	320	315	315

TABLE 22. Individual subject data for SUBJECT 1(RR) during five-minute exercise under condition H/NO.

	TIME					
VARIABLE	REST	1 min	2 min	3 min	4 min	5 min
pH	7.41	7.37	7.32	7.29	7.27	7.25
PaO_2 (mm Hg)	63	46	46	44	43	44
SaO_2 (%)	97.3	81.1	77.9	74.7	73.1	72.5
[A-a]DO_2 (mm Hg)	0.4	7.1	14.0	15.3	16.7	16.6
$PaCO_2$ (mm Hg)	43	41	41	39	36	35
HCO_3^{-1} ($mmol \cdot l^{-1}$)	27	24	21	18	16	15
Base Excess ($mmol \cdot l^{-1}$)	2.7	-0.9	-4.7	-7.6	-9.5	-11.0
VO_2 ($l \cdot min^{-1}$)	0.86	3.84	3.78	4.21	4.03	4.16
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	10.71	47.82	47.07	52.43	50.19	51.81
VE ($l \cdot min^{-1}$)	28.59	100.66	109.19	118.67	112.47	117.87
RER (VCO_2/VO_2)	1.12	1.02	1.23	1.12	1.08	1.04
HR ($beats \cdot min^{-1}$)	70	158	163	164	166	166
Power (watts)	0	375	330	300	300	300

TABLE 23. Individual subject data for SUBJECT 2 (SF) during five-minute exercise under condition N.

	TIME					
VARIABLE	REST	1 min	2 min	3 min	4 min	5 min
pH	7.42	--	--	7.24	--	--
PaO_2 (mm Hg)	105	--	--	83	--	--
SaO_2 (%)	98.0	--	--	94.5	--	--
[A-a]DO_2 (mm Hg)	0.6	--	--	29.6	--	--
$PaCO_2$ (mm Hg)	37	--	--	35	--	--
HCO_3^{-1} ($mmol \cdot l^{-1}$)	24	--	--	15	--	--
Base Excess ($mmol \cdot l^{-1}$)	0.3	--	--	-11.5	--	--
VO_2 ($l \cdot min^{-1}$)	0.70	3.59	5.18	5.23	5.39	5.50
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	8.70	44.60	68.32	68.70	68.70	71.68
V_E ($l \cdot min^{-1}$)	17.48	74.83	155.93	170.62	175.57	180.07
RER (VCO_2/VO_2)	0.74	0.71	1.00	0.95	1.01	0.94
HR ($beats \cdot min^{-1}$)	105	158	173	181	185	190
Power (watts)	0	445	390	420	420	420

TABLE 24. Individual subject data for SUBJECT 2 (SF) during five-minute exercise under condition N/NO.

	TIME					
VARIABLE	REST	1 min	2 min	3 min	4 min	5 min
pH	7.41	7.39	7.32	7.26	7.20	7.17
PaO_2 (mmHg)	105	78	80	76	73	74
SaO_2 (%)	98.0	96.0	95.0	94.0	92.0	92.0
[A-a]DO_2 (mm Hg)	1.9	30.1	28.5	35.8	41.4	43.6
$PaCO_2$ (mm Hg)	36	38	39	37	35	31
HCO_3^{-1} ($mmol \cdot l^{-1}$)	24	23	20	17	15	13
Base Excess ($mmol \cdot l^{-1}$)	0.3	-1.4	-4.6	-8.7	-10.8	-13.3
VO_2 ($l \cdot min^{-1}$)	0.75	3.57	4.78	5.07	5.10	5.12
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	8.45	52.67	69.32	74.03	77.71	77.64
V_E ($l \cdot min^{-1}$)	16.85	88.83	142.65	169.63	169.59	177.97
RER (VCO_2/VO_2)	0.82	0.91	0.95	0.99	1.01	0.98
HR ($beats \cdot min^{-1}$)	106	165	178	184	187	190
Power (watts)	0	445	445	450	400	450

TABLE 25. Individual subject data for SUBJECT 2 (SF) during five-minute exercise under condition H.

	TIME					
VARIABLE	REST	1 min	2 min	3 min	4 min	5 min
pH	7.44	7.41	7.35	--	7.29	--
PaO_2 (mm Hg)	69	42	40	--	39	--
SaO_2 (%)	94.0	78.0	74.0	--	69.0	--
[A-a]DO_2 (mm Hg)	0.0	20.9	29.7	--	31.8	--
$PaCO_2$ (mm Hg)	36	33	32	--	31	--
HCO_3^{-1} ($mmol \cdot l^{-1}$)	24	23	20	--	15	--
Base Excess ($mmol \cdot l^{-1}$)	1.5	-2.7	-6.3	--	-10.2	--
VO_2 ($l \cdot min^{-1}$)	0.79	3.11	4.23	4.32	4.76	4.92
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	9.81	38.63	53.66	55.65	59.13	59.25
V_E ($l \cdot min^{-1}$)	15.6	107.83	141.70	159.65	185.34	181.01
RER (VCO_2/VO_2)	0.87	0.89	1.09	1.11	1.10	1.10
HR ($beats \cdot min^{-1}$)	79	158	177	177	182	182
Power (watts)	0	350	375	400	380	360

TABLE 26. Individual subject data for SUBJECT 2 (SF) during five-minute exercise under condition H/NO.

	TIME					
VARIABLE	REST	1 min	2 min	3 min	4 min	5 min
pH	7.43	7.45	7.40	7.36	7.32	7.26
PaO_2 (mm Hg)	73	42	43	42	42	42
SaO_2 (%)	95.0	80.0	79.0	77.0	74.0	71.0
[A-a]DO_2 (mm Hg)	0.2	20.1	21.7	24.5	24.1	25.2
$PaCO_2$ (mm Hg)	38	36	35	32	31	30
HCO_3^{-1} ($mmol \cdot l^{-1}$)	25	21	18	--	15	--
Base Excess ($mmol \cdot l^{-1}$)	1.19	2.2	-2.0	-5.5	-8.4	-12.2
VO_2 ($l \cdot min^{-1}$)	0.39	4.11	4.52	4.53	4.50	4.22
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	4.84	51.06	56.15	56.27	56.02	51.18
V_E ($l \cdot min^{-1}$)	21.15	136.64	166.43	169.14	161.25	158.07
RER (VCO_2/VO_2)	0.89	0.96	1.01	0.97	0.92	0.92
HR ($beats \cdot min^{-1}$)	116	175	179	184	184	186
Power (watts)	0	350	360	300	280	290

TABLE 27. Individual subject data for SUBJECT 3 (AP) during five-minute exercise under condition N.

	TIME					
VARIABLE	REST	1 min	2 min	3 min	4 min	5 min
pH	7.38	7.38	7.32	7.27	7.22	7.17
PaO_2 (mm Hg)	91	94	92	88	94	93
SaO_2 (%)	96.8	97.7	96.4	95.6	95.8	95.2
[A-a]DO_2 (mm Hg)	5.2	5.7	12.3	17.4	15.8	17.9
$PaCO_2$ (mm Hg)	44	41	40	39	35	34
HCO_3^{-1} ($mmol \cdot l^{-1}$)	26	24	21	18	14	13
Base Excess ($mmol \cdot l^{-1}$)	0.6	-0.1	-4.9	-8.4	-12.7	-15.7
VO_2 ($l \cdot min^{-1}$)	0.23	2.9	3.52	3.41	3.34	3.33
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	10.60	49.09	47.56	48.95	46.95	46.58
V_E ($l \cdot min^{-1}$)	14.03	114.62	133.71	133.71	133.23	133.67
RER (VCO_2/VO_2)	0.83	0.70	0.74	0.78	0.80	0.81
HR ($beats \cdot min^{-1}$)	91	168	176	179	180	181
Power (watts)	0	410	410	390	390	380

TABLE 28. Individual subject data for SUBJECT 3 (AP) during five-minute exercise under condition N/NO.

	TIME					
VARIABLE	REST	1 min	2 min	3 min	4 min	5 min
pH	7.41	7.39	7.32	7.26	7.20	7.17
PaO_2 (mmHg)	105	94	93	90	83	84
SaO_2 (%)	97.29	97.1	96.5	95.8	94.0	93.6
[A-a]DO_2 (mm Hg)	1.4	3.4	10.2	17.6	25.7	25.8
$PaCO_2$ (mm Hg)	40	43	41	37	36	35
HCO_3^{-1} ($mmol \cdot l^{-1}$)	25	26	21	17	14	13
Base Excess ($mmol \cdot l^{-1}$)	1.4	1.3	-4.6	-9.5	-12.9	-15.4
VO_2 ($l \cdot min^{-1}$)	1.00	4.17	3.88	4.70	4.87	5.19
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	13.95	58.16	54.11	65.55	67.92	73.92
V_E ($l \cdot min^{-1}$)	17.34	105.92	133.92	133.54	134.58	133.57
RER (VCO_2/VO_2)	0.83	0.84	1.03	0.98	0.92	0.88
HR ($beats \cdot min^{-1}$)	80	165	174	177	178	179
Power (watts)	0	410	400	380	370	350

TABLE 29. Individual subject data for SUBJECT 3 (AP) during five-minute exercise under condition H.

	TIME					
VARIABLE	REST	1 min	2 min	3 min	4 min	5 min
pH	7.39	7.41	--	7.33	7.28	7.24
PaO_2 (mm Hg)	68	53	--	48	47	47
SaO_2 (%)	93.3	87.7	--	81.5	78.6	76.4
[A-a]DO_2 (mm Hg)	1.1	14.1	--	20.3	20.8	20.4
$PaCO_2$ (mm Hg)	43	37	--	31	30	29
HCO_3^{-1} (mmol·l^{-1})	26	23	--	17	14	12
Base Excess (mmol·l^{-1})	1.4	-0.2	--	-7.8	-10.9	-13.6
VO_2 (l·min^{-1})	0.67	3.49	3.60	3.62	3.68	4.00
VO_2 (ml·kg^{-1}·min^{-1})	9.34	48.68	50.21	50.49	51.32	55.79
V_E (l·min^{-1})	16.63	130.93	132.31	130.59	131.88	131.29
RER (VCO_2/VO_2)	1.15	1.21	1.05	1.03	0.97	0.92
HR (beats·min^{-1})	98	164	170	174	176	178
Power (watts)	0	330	330	310	310	310

TABLE 30. Individual subject data for SUBJECT 3 (AP) during five-minute exercise under condition H/NO.

	TIME					
VARIABLE	REST	1 min	2 min	3 min	4 min	5 min
pH	7.40	7.43	7.37	7.31	7.27	7.23
PaO_2 (mm Hg)	54	47	46	45	45	45
SaO_2 (%)	88.1	84.4	80.5	77.7	75.9	73.9
[A-a]DO_2 (mm Hg)	3.5	17.0	20.1	22.0	22.3	20.4
$PaCO_2$ (mm Hg)	40	36	33	31	30	30
HCO_3^{-1} (mmol·l^{-1})	25	24	19	16	14	13
Base Excess (mmol·l^{-1})	0.9	0.5	-5.1	-8.8	-11.5	-13.6
VO_2 (l·min^{-1})	0.46	4.04	4.21	4.25	4.29	4.42
VO_2 (ml·kg^{-1}·min^{-1})	6.42	56.35	58.72	59.27	59.83	61.65
V_E (l·min^{-1})	12.50	125.50	141.15	141.17	146.07	150.83
RER (VCO_2/VO_2)	0.97	1.05	1.02	0.98	0.92	0.89
HR (beats·min^{-1})	102	164	171	173	176	177
Power (watts)	0	330	330	300	280	280

TABLE 31. Individual subject data for SUBJECT 4 (TD) during five-minute exercise under condition N.

	TIME					
VARIABLE	REST	1 min	2 min	3 min	4 min	5 min
pH	7.40	7.39	7.33	7.25	7.32	7.19
PaO_2 (mm Hg)	109	95	85	81	81	77
SaO_2 (%)	98.0	97.2	95	94.4	93.9	92.6
[A-a]DO_2 (mm Hg)	0.3	18.5	30.3	33.3	33.6	37.8
$PaCO_2$ (mm Hg)	39	35	34	34	32	31
HCO_3^{-1} ($mmol \cdot l^{-1}$)	24	21	18	15	13	12
Base Excess ($mmol \cdot l^{-1}$)	0.0	-2.9	-7.0	-11.1	-13.4	-15
VO_2 ($l \cdot min^{-1}$)	0.64	4.41	4.55	4.79	4.79	4.83
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	9.98	68.80	70.98	74.73	74.73	75.35
V_E ($l \cdot min^{-1}$)	23.74	153.90	154.36	155.28	155.09	155.97
RER (VCO_2/VO_2)	0.91	1.01	1.04	1.00	0.94	0.91
HR ($beats \cdot min^{-1}$)	99	174	178	178	178	177
Power (watts)	0	380	330	320	290	285

TABLE 32. Individual subject data for SUBJECT 4 (TD) during five-minute exercise under condition N/NO.

	TIME					
VARIABLE	REST	1 min	2 min	3 min	4 min	5 min
pH	7.41	7.37	7.28	7.25	7.23	7.21
PaO_2 (mmHg)	95	85	77	77	75	74
SaO_2 (%)	97.4	96.3	94	93.5	92.7	91.9
[A-a]DO_2 (mm Hg)	13.7	28.0	34.2	34.3	36.5	38.3
$PaCO_2$ (mm Hg)	36	32	34	33	31	30
HCO_3^{-1} ($mmol \cdot l^{-1}$)	23	19	16	15	13	12
Base Excess ($mmol \cdot l^{-1}$)	-0.7	-4.8	-9.5	-11.1	-13.5	-14.7
VO_2 ($l \cdot min^{-1}$)	0.8	4.46	4.84	4.97	5.24	5.20
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	12.35	68.83	74.68	76.70	80.86	80.25
V_E ($l \cdot min^{-1}$)	19.0	152.27	157.66	156.63	156.86	156.72
RER (VCO_2/VO_2)	0.90	0.90	0.91	0.88	0.82	0.81
HR ($beats \cdot min^{-1}$)	100	164	175	176	177	177
Power (watts)	0	360	300	280	280	280

TABLE 33. Individual subject data for SUBJECT 4 (TD) during five-minute exercise under condition H.

	TIME					
VARIABLE	REST	1 min	2 min	3 min	4 min	5 min
pH	7.44	7.41	7.31	7.31	7.27	7.25
PaO_2 (mm Hg)	64	45	42	42	42	42
SaO_2 (%)	93.5	81.7	74.2	74.2	71.5	71.1
[A-a]DO_2 (mm Hg)	1.4	23.9	26.6	26.6	27.1	26.7
$PaCO_2$ (mm Hg)	32	31	31	31	29	28
HCO_3^{-1} ($mmol \cdot l^{-1}$)	22	20	15	15	14	13
Base Excess ($mmol \cdot l^{-1}$)	-0.7	-3.1	-9.4	-9.4	-11.8	-13.0
VO_2 ($l \cdot min^{-1}$)	0.31	3.57	3.60	3.60	3.59	3.71
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	4.78	55.09	55.56	55.56	55.40	57.25
V_E ($l \cdot min^{-1}$)	15.73	136.38	133.43	133.43	135.04	139.13
RER (VCO_2/VO_2)	0.95	1.04	1.03	1.03	0.97	0.92
HR ($beats \cdot min^{-1}$)	102	171	175	175	175	175
Power (watts)	0	289	230	230	210	200

TABLE 34. Individual subject data for SUBJECT 4 (TD) during five-minute exercise under condition H/NO.

	TIME					
VARIABLE	REST	1 min	2 min	3 min	4 min	5 min
pH	7.42	7.40	7.35	7.30	7.26	7.24
PaO_2 (mm Hg)	62	46	43	42	42	42
SaO_2 (%)	92.2	82.8	76.8	73.9	71.4	69.5
[A-a]DO_2 (mm Hg)	0.1	23.1	25.4	25.3	25.7	25.9
$PaCO_2$ (mm Hg)	36	31	31	31	29	28
HCO_3^{-1} ($mmol \cdot l^{-1}$)	23	19	17	15	13	12
Base Excess ($mmol \cdot l^{-1}$)	-0.2	-4.1	-6.8	-9.7	-12.5	-14.0
VO_2 ($l \cdot min^{-1}$)	0.31	3.59	3.82	3.90	3.80	3.90
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	4.78	55.40	58.95	60.19	58.64	60.19
V_E ($l \cdot min^{-1}$)	16.68	140.83	143.95	144.13	143.84	142.34
RER (VCO_2/VO_2)	0.97	1.05	1.02	0.98	0.92	0.89
HR ($beats \cdot min^{-1}$)	98	171	175	175	177	179
Power (watts)	0	310	239	230	210	200

TABLE 35. Individual subject data for SUBJECT 5 (NS) during five-minute exercise under condition N.

	TIME					
VARIABLE	REST	1 min	2 min	3 min	4 min	5 min
pH	7.40	7.35	7.28	7.22	7.18	7.17
PaO_2 (mm Hg)	106	85	80	74	73	72
SaO_2 (%)	99.0	97.2	95.2	93.8	92.4	91.1
[A-a]DO_2 (mm Hg)	6.8	25.2	28.3	27.0	31.9	34.0
$PaCO_2$ (mm Hg)	35	40	41	41	40	38
HCO_3^{-1} ($mmol \cdot l^{-1}$)	21	21	19	16	15	13
Base Excess ($mmol \cdot l^{-1}$)	-31	-4.1	-7.3	-10.9	-13.2	-14.4
VO_2 ($l \cdot min^{-1}$)	0.27	4.08	4.34	4.49	4.59	4.67
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	3.39	51.26	54.52	56.53	57.66	58.67
V_E ($l \cdot min^{-1}$)	10.39	132.87	146.56	154.39	155.85	166.12
RER (VCO_2/VO_2)	1.07	1.12	1.21	1.24	1.24	1.21
HR ($beats \cdot min^{-1}$)	104	172	175	177	178	182
Power (watts)	0	400	380	350	350	350

TABLE 36. Individual subject data for SUBJECT 5 (NS) during five-minute exercise under condition N/NO.

	TIME					
VARIABLE	REST	1 min	2 min	3 min	4 min	5 min
pH	7.41	7.39	7.31	7.26	7.22	7.19
PaO_2 (mmHg)	96	74	72	68	67	63
SaO_2 (%)	98.3	95.8	94.3	92.5	91.0	89.7
[A-a]DO_2 (mm Hg)	21.4	42.6	42.2	46.8	48.6	55.5
$PaCO_2$ (mm Hg)	33	35	40	40	39	35
HCO_3^{-1} ($mmol \cdot l^{-1}$)	21	21	20	18	16	13
Base Excess ($mmol \cdot l^{-1}$)	-3.2	-3.5	-6.0	-8.7	-11.6	-14.2
VO_2 ($l \cdot min^{-1}$)	0.69	4.79	4.90	5.09	5.02	5.00
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	8.67	60.18	61.56	63.94	63.07	62.81
V_E ($l \cdot min^{-1}$)	14.81	114.38	142.20	156.18	156.77	158.73
RER (VCO_2/VO_2)	0.81	0.89	1.03	1.09	1.11	1.10
HR ($beats \cdot min^{-1}$)	106	171	178	181	185	186
Power (watts)	0	385	369	350	340	330

TABLE 37. Individual subject data for SUBJECT 5 (NS) during five-minute exercise under condition H.

	TIME					
VARIABLE	REST	1 min	2 min	3 min	4 min	5 min
pH	7.42	7.39	7.33	7.29	7.22	7.17
PaO_2 (mm Hg)	62	42	40	37	40	42
SaO_2 (%)	94.2	80.7	75.8	71.4	69.8	69.2
[A-a]DO_2 (mm Hg)	0.3	18.4	25.0	33.3	32.6	30.6
$PaCO_2$ (mm Hg)	37	35	35	31	29	29
HCO_3^{-1} ($mmol \cdot l^{-1}$)	24	20	18	14	12	11
Base Excess ($mmol \cdot l^{-1}$)	-0.1	-3.7	-6.8	-11.0	-14.2	-16.4
VO_2 ($l \cdot min^{-1}$)	0.54	3.70	4.02	3.96	4.04	4.12
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	6.78	46.48	50.50	49.75	50.75	51.76
V_E ($l \cdot min^{-1}$)	20.05	135.87	149.18	152.89	164.10	165.15
RER (VCO_2/VO_2)	1.04	1.08	1.15	1.14	1.15	1.14
HR ($beats \cdot min^{-1}$)	106	169	172	174	177	177
Power (watts)	0	320	297	285	285	285

TABLE 38. Individual subject data for SUBJECT 5 (NS) during five-minute exercise under condition H/NO.

	TIME					
VARIABLE	REST	1 min	2 min	3 min	4 min	5 min
pH	7.41	7.40	7.34	7.29	7.24	7.19
PaO_2 (mm Hg)	67	42	38	37	38	39
SaO_2 (%)	95.5	81.4	75.1	70.9	63.2	66.9
[A-a]DO_2 (mm Hg)	0.6	25.3	30.0	31.7	32.7	32.4
$PaCO_2$ (mm Hg)	38	34	35	34	32	31
HCO_3^{-1} ($mmol \cdot l^{-1}$)	23	21	18	16	14	11
Base Excess ($mmol \cdot l^{-1}$)	-0.9	-3.3	-6.6	-9.5	-12.5	-15.4
VO_2 ($l \cdot min^{-1}$)	0.62	3.61	3.65	4.10	3.98	4.05
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	7.79	45.35	45.85	51.51	50.00	50.88
V_E ($l \cdot min^{-1}$)	38.57	136.70	132.02	147.25	149.22	160.32
RER (VCO_2/VO_2)	1.07	1.09	1.14	1.12	1.09	1.09
HR ($beats \cdot min^{-1}$)	106	169	173	175	177	180
Power (watts)	0	320	295	295	275	275

TABLE 39. Individual subject data for SUBJECT 6 (LB) during five-minute exercise under condition N.

	TIME					
VARIABLE	REST	1 min	2 min	3 min	4 min	5 min
pH	7.41	7.42	7.38	7.35	7.33	7.33
PaO_2 (mm Hg)	98	95	95	94	93	92
SaO_2 (%)	98.0	98.0	97.2	97.2	96.8	97.0
[A-a]DO_2 (mm Hg)	--	--	22.1	24.5	28.7	35.8
$PaCO_2$ (mm Hg)	40	37	35	34	31	23
HCO_3^{-1} ($mmol \cdot l^{-1}$)	25	23	20	18	16	12
Base Excess ($mmol \cdot l^{-1}$)	0.4	-0.8	-3.9	-6.3	-8.3	-12.0
VO_2 ($l \cdot min^{-1}$)	--	--	4.45	4.55	4.50	4.57
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	--	--	58.14	56.38	55.76	56.63
V_E ($l \cdot min^{-1}$)	--	--	155.39	161.23	156.98	161.21
RER (VCO_2/VO_2)	--	--	1.16	1.19	1.22	1.16
HR ($beats \cdot min^{-1}$)	71	167	171	174	195	178
Power (watts)	0	370	320	340	310	310

TABLE 40. Individual subject data for SUBJECT 6 (LB) during five-minute exercise under condition N/NO.

	TIME					
VARIABLE	REST	1 min	2 min	3 min	4 min	5 min
pH	7.39	7.42	--	--	7.38	7.38
PaO_2 (mmHg)	98	97	--	--	95	93
SaO_2 (%)	97.6	98.0	--	--	98.2	98.5
[A-a]DO_2 (mm Hg)	--	--	--	--	17.5	20.4
$PaCO_2$ (mm Hg)	45	40	--	--	36	36
HCO_3^{-1} ($mmol \cdot l^{-1}$)	27	26	--	--	21	20
Base Excess ($mmol \cdot l^{-1}$)	1.2	1.1	--	--	-3.3	-4.0
VO_2 ($l \cdot min^{-1}$)	--	--	--	4.82	5.24	5.48
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	--	--	--	59.73	64.93	67.91
V_E ($l \cdot min^{-1}$)	7.29	141.80	162.31	162.95	172.39	175.95
RER (VCO_2/VO_2)	--	--	--	1.03	1.01	1.04
HR ($beats \cdot min^{-1}$)	75	163	166	168	172	178
Power (watts)	0	350	310	310	310	310

TABLE 41. Individual subject data for SUBJECT 6 (LB) during five-minute exercise under condition H.

	TIME					
VARIABLE	REST	1 min	2 min	3 min	4 min	5 min
pH	7.40	7.45	7.45	7.41	7.39	7.38
PaO_2 (mm Hg)	95	93	70	53	52	49
SaO_2 (%)	94.8	90.8	88.7	88.0	86.2	84.2
[A-a]DO_2 (mm Hg)	0.7	0.2	0.1	15.4	16.4	18.8
$PaCO_2$ (mm Hg)	40	37	36	32	31	31
HCO_3^{-1} ($mmol \cdot l^{-1}$)	24	25	22	19	18	17
Base Excess ($mmol \cdot l^{-1}$)	-0.4	-1.3	-1.6	-5.0	-5.8	-7.1
VO_2 ($l \cdot min^{-1}$)	0.34	3.71	3.85	4.54	4.59	4.65
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	4.21	45.97	52.28	56.26	56.88	57.62
V_E ($l \cdot min^{-1}$)	8.05	150.53	150.53	170.46	181.63	178.62
RER (VCO_2/VO_2)	0.82	0.94	1.15	1.06	1.02	1.00
HR ($beats \cdot min^{-1}$)	71	167	167	171	174	176
Power (watts)	0	340	290	280	280	280

TABLE 42. Individual subject data for SUBJECT 6 (LB) during five-minute exercise under condition H/NO.

	TIME					
VARIABLE	REST	1 min	2 min	3 min	4 min	5 min
pH	7.36	7.41	7.41	7.38	7.34	7.33
PaO_2 (mm Hg)	71	50	47	48	44	44
SaO_2 (%)	94.8	86.6	83.1	83.3	79.0	78.2
[A-a]DO_2 (mm Hg)	0.2	21.9	27.1	26.7	31.8	31.0
$PaCO_2$ (mm Hg)	25	26	25	25	23	23
HCO_3^{-1} ($mmol \cdot l^{-1}$)	15	16	15	15	12	12
Base Excess ($mmol \cdot l^{-1}$)	-7.2	-6.3	-8.3	-10.0	-11.3	-12.1
VO_2 ($l \cdot min^{-1}$)	0.40	4.27	4.78	4.88	4.97	4.92
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	4.96	52.91	59.23	60.47	61.59	60.97
V_E ($l \cdot min^{-1}$)	15.73	156.78	187.71	182.10	178.07	180.97
RER (VCO_2/VO_2)	0.81	0.96	1.01	1.04	1.00	0.96
HR ($beats \cdot min^{-1}$)	69	161	167	169	170	174
Power (watts)	0	340	330	280	263	263

TABLE 43. Individual subject data for SUBJECT 7 (BJ) during five-minute exercise under condition N.

	TIME					
VARIABLE	REST	1 min	2 min	3 min	4 min	5 min
pH	7.39	7.34	7.29	7.22	7.20	7.17
PaO_2 (mm Hg)	114	97	95	90	87	87
SaO_2 (%)	98.1	97.0	96.0	95.4	94.7	94.2
[A-a]DO_2 (mm Hg)	0.9	16.7	22.4	29.3	34.2	34.8
$PaCO_2$ (mm Hg)	38	38	36	34	31	30
HCO_3^{-1} ($mmol \cdot l^{-1}$)	23	21	18	14	12	11
Base Excess ($mmol \cdot l^{-1}$)	-0.9	-4.3	-8.1	-12.7	-14.9	-16.7
VO_2 ($l \cdot min^{-1}$)	0.38	3.53	3.78	3.73	3.58	3.59
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	5.54	51.46	55.10	55.37	52.19	52.33
V_E ($l \cdot min^{-1}$)	14.69	125.12	154.54	166.37	169.34	163.04
RER (VCO_2/VO_2)	0.95	1.11	1.20	1.21	1.17	1.15
HR ($beats \cdot min^{-1}$)	65	163	172	175	175	174
Power (watts)	0	370	370	340	310	310

TABLE 44. Individual subject data for SUBJECT 7 (BJ) during five-minute exercise under condition N/NO.

	TIME					
VARIABLE	REST	1 min	2 min	3 min	4 min	5 min
pH	7.39	7.39	7.34	7.31	7.28	7.26
PaO_2 (mmHg)	93	86	86	83	79	80
SaO_2 (%)	97.1	96.6	96.1	95.5	94.4	94.4
[A-a]DO_2 (mm Hg)	18.1	27.4	33.1	38.4	42.6	42.9
$PaCO_2$ (mm Hg)	33	31	31	30	29	27
HCO_3^{-1} ($mmol \cdot l^{-1}$)	20	19	17	15	13	12
Base Excess ($mmol \cdot l^{-1}$)	-3.2	-4.4	-7.4	-9.6	-11.6	-13.1
VO_2 ($l \cdot min^{-1}$)	0.29	2.64	3.04	3.47	3.45	3.72
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	10.64	38.05	44.17	48.25	50.00	54.23
V_E ($l \cdot min^{-1}$)	9.12	113.11	138.59	152.85	157.53	158.29
RER (VCO_2/VO_2)	0.85	0.85	1.06	1.13	1.09	1.06
HR ($beats \cdot min^{-1}$)	89	162	167	173	175	178
Power (watts)	0	350	310	310	310	310

TABLE 45. Individual subject data for SUBJECT 7 (BJ) during five-minute exercise under condition H.

	TIME					
VARIABLE	REST	1 min	2 min	3 min	4 min	5 min
pH	7.40	7.43	7.36	7.32	7.28	7.24
PaO_2 (mm Hg)	75	46	41	40	39	40
SaO_2 (%)	95.2	83.6	75.6	71.7	68.5	67.3
[A-a]DO_2 (mm Hg)	5.2	23.7	29.1	29.9	34.2	36.2
$PaCO_2$ (mm Hg)	34	32	32	31	29	26
HCO_3^{-1} ($mmol \cdot l^{-1}$)	21	21	21	18	16	13
Base Excess ($mmol \cdot l^{-1}$)	-2.1	-1.9	-5.6	-8.5	-11.6	-14.6
VO_2 ($l \cdot min^{-1}$)	0.73	2.61	3.03	3.31	3.43	3.72
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	4.21	45.97	38.05	50.58	50.29	54.23
V_E ($l \cdot min^{-1}$)	12.52	119.26	134.60	159.69	161.08	168.10
RER (VCO_2/VO_2)	0.85	1.11	1.13	1.12	1.15	1.17
HR ($beats \cdot min^{-1}$)	71	167	167	171	174	176
Power (watts)	0	340	260	250	240	240

TABLE 46. Individual subject data for SUBJECT 7 (BJ) during five-minute exercise under condition H/NO.

	TIME					
VARIABLE	REST	1 min	2 min	3 min	4 min	5 min
pH	7.43	7.43	7.38	7.32	7.27	7.24
PaO_2 (mm Hg)	60	41	40	40	40	40
SaO_2 (%)	91.9	78.5	74.5	71.6	68.7	66.6
[A-a]DO_2 (mm Hg)	4.7	28.7	31.9	31.8	35.0	35.6
$PaCO_2$ (mm Hg)	31	30	30	29	27	26
HCO_3^{-1} ($mmol \cdot l^{-1}$)	20	20	17	15	12	11
Base Excess ($mmol \cdot l^{-1}$)	-2.0	-2.8	-5.8	-9.1	-12.8	-14.6
VO_2 ($l \cdot min^{-1}$)	0.29	2.60	3.12	3.47	3.33	3.50
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	4.23	37.90	45.48	50.58	48.54	51.02
V_E ($l \cdot min^{-1}$)	15.19	142.37	168.01	178.50	184.66	178.98
RER (VCO_2/VO_2)	0.89	1.03	1.13	1.08	1.15	1.13
HR ($beats \cdot min^{-1}$)	69	161	167	169	170	174
Power (watts)	0	280	265	245	235	230

APPENDIX F

GROUP DATA: INHALED NITRIC OXIDE

TABLE 47. Oxygen consumption (VO_2, $l \cdot min^{-1}$) during rest and at each min of 5-min cycle ergometry tests under conditions of normoxia (N), normoxia + 20 ppm nitric oxide (N/NO), hypoxia (H), and hypoxia + 20 ppm nitric oxide (H/NO). Values are means (± SD).

	REST	1 MIN	2 MIN	3 MIN	4 MIN	5 MIN
N	0.44	3.70	4.30	4.37	4.37	4.42
	(0.21)	(0.58)	(0.59)	(0.68)	(0.77)	(0.81)
N/NO	0.70	3.93	4.29	4.69	4.82	4.95
	(0.26)	(0.85)	(0.81)	(0.61)	(0.69)	(0.62)
H	0.52	3.31	3.60	3.76	3.86	4.03
	(0.22)	(0.42)	(0.50)	(0.56)	(0.64)	(0.61)
H/NO	0.48	3.72	3.98	4.19	4.13	4.17
	(0.20)	(0.56)	(0.56)	(0.45)	(0.52)	(0.44)

TABLE 48. Oxygen consumption (VO_2, $ml \cdot kg^{-1} \cdot min^{-1}$) during rest and at each min of 5-min cycle ergometry tests under conditions of normoxia (N), normoxia + 20 ppm nitric oxide (N/NO), hypoxia (H), and hypoxia + 20 ppm nitric oxide (H/NO). Values are means (± SD).

	REST	1 MIN	2 MIN	3 MIN	4 MIN	5 MIN
N	7.64	53.04	58.67	60.05	59.33	60.21
	(3.08)	(9.23)	(9.14)	(9.54)	(10.44)	(11.17)
N/NO	10.81	55.58	60.77	64.70	67.42	69.37
	(2.37)	(11.39)	(12.12)	(10.27)	(11.09)	(9.77)
H	6.53	45.48	50.13	53.05	53.96	55.98
	(2.55)	(6.42)	(6.21)	(3.06)	(3.69)	(2.68)
H/NO	5.50	49.83	54.06	56.38	55.77	55.93
	(1.34	(7.03)	(6.60)	(4.40)	(5.37)	(5.51)

TABLE 49. Minute ventilation (V_E, $l \cdot min^{-1}$) during rest and at each min of 5-min cycle ergometry tests under conditions of normoxia (N), normoxia + 20 ppm nitric oxide (N/NO), hypoxia (H), and hypoxia + 20 ppm nitric oxide (H/NO). Values are means (± SD).

	REST	1 MIN	2 MIN	3 MIN	4 MIN	5 MIN
N	16.07	120.27	150.08	156.93	157.68	160.01
	(4.98)	(29.20)	(8.73)	(12.98)	(14.59)	(15.23)
N/NO	14.07	119.39	146.22	155.30	157.86	160.21
	(4.77)	(23.51)	(11.21)	(12.21)	(13.37)	(16.07)
H	14.10	126.62	138.62	147.24	156.02	(157.51)
	(4.15)	(16.44)	(8.74)	(17.74)	(22.94)	20.52
H/NO	21.22	134.21	149.78	153.71	153.65	155.63
	(9.29)	(17.48)	(26.20)	(23.33)	(24.10)	(21.76)

TABLE 50. Respiratory exchange ratio (RER, VCO_2/VO_2) during rest and at each min of 5-min cycle ergometry tests under conditions of normoxia (N), normoxia + 20 ppm nitric oxide (N/NO), hypoxia (H), and hypoxia + 20 ppm nitric oxide (H/NO). Values are means (± SD).

	REST	1 MIN	2 MIN	3 MIN	4 MIN	5 MIN
N	0.87	0.93	1.06	1.06	1.06	1.03
	(0.16)	(0.21)	(0.18)	(0.18)	(0.18)	(0.16)
N/NO	0.84	0.88	1.00	1.02	0.99	0.98
	(0.04)	(0.03)	(0.06)	(0.09)	(0.11)	(0.11)
H	0.94	1.04	1.12	1.09	1.08	1.06
	(0.12)	(0.11)	(0.04)	(0.04)	(0.07)	(0.08)
H/NO	0.95	1.01	1.07	1.05	1.02	0.99
	(0.11)	(0.06)	(0.09)	(0.08)	(0.09)	(0.09)

TABLE 51. Heart rate (beats·min^{-1}) during rest and at each min of 5-min cycle ergometry tests under conditions of normoxia (N), normoxia + 20 ppm nitric oxide (N/NO), hypoxia (H), and hypoxia + 20 ppm nitric oxide (H/NO). Values are means (± SD).

	REST	1 MIN	2 MIN	3 MIN	4 MIN	5 MIN
N	89	167	174	177	179	180
	(17)	(6)	(3)	(3)	(4)	(6)
N/NO	93	165	173	177	179	181
	(13)	(3)	(5)	(6)	(6)	(5)
H	89	166	171	174	176	177
	(14)	(4)	(4)	(2)	(3)	(3)
H/NO	92	166	171	173	175	177
	(18)	(6)	(5)	(6)	(6)	(6)

TABLE 52. Arterial pressure of O_2 (PaO_2, mmHg) during rest and at each min of 5-min cycle ergometry tests under conditions of normoxia (N), normoxia + 20 ppm nitric oxide (N/NO), hypoxia (H), and hypoxia + 20 ppm nitric oxide (H/NO). Values are means (± SD).

	REST	1 MIN	2 MIN	3 MIN	4 MIN	5 MIN
N	103.8	93.2	89.4	85.0	85.6	84.2
	(8.2)	(4.7)	(6.7)	(7.2)	(8.8)	(9.3)
N/NO	98.7	85.7	81.6	78.8	78.7	78.0
	(5.2)	(8.9)	(8.1)	(8.2)	(9.7)	(10.2)
H	66.7	47.4	44.3	44.2	43.3	44.3
	(5.4)	(4.9)	(5.3)	(5.2)	(4.2)	(3.4)
H/NO	64.3	44.9	43.3	42.6	42.0	42.3
	(6.6)	(3.3)	(3.4)	(3.6)	(2.4)	(2.2)

TABLE 53. Alveolar-arterial difference for O_2 ([A-a]DO_2, mmHg) during rest and at each min of 5-min cycle ergometry tests under conditions of normoxia (N), normoxia + 20 ppm nitric oxide (N/NO), hypoxia (H), and hypoxia + 20 ppm nitric oxide (H/NO). Values are means (± SD).

	REST	1 MIN	2 MIN	3 MIN	4 MIN	5 MIN
N	2.8	16.5	23.1	26.9	28.9	32.0
	(3.0)	(8.1)	(7.0)	(5.5)	(7.6)	(8.1)
N/NO	11.3	26.3	29.6	34.6	35.4	37.7
	(9.2)	(14.2)	(11.9)	(10.7)	(11.6)	(12.8)
H	2.0	15.8	21.0	23.9	26.3	25.6
	(2.2)	(8.6)	(11.5)	(7.2)	(6.9)	(6.8)
H/NO	1.4	20.5	24.3	25.3	26.9	26.7
	(1.9)	(7.0)	(6.2)	(5.7)	(6.6)	(6.8)

TABLE 54. Oxyhemoglobin saturation (SaO_2, %) during rest and at each min of 5-min cycle ergometry tests under conditions of normoxia (N), normoxia + 20 ppm nitric oxide (N/NO), hypoxia (H), and hypoxia + 20 ppm nitric oxide (H/NO). Values are means (± SD).

	REST	1 MIN	2 MIN	3 MIN	4 MIN	5 MIN
N	98.0	97.4	96.3	95.2	94.7	94.0
	(0.7)	(0.4)	(0.4)	(0.7)	(1.3)	(1.8)
N/NO	97.7	96.6	95.2	94.3	93.7	93.4
	(0.3)	(0.6)	(1.3)	(1.2)	(1.0)	(1.0)
H	93.7	84.0	79.0	78.2	74.9	74.7
	(1.4)	(4.3)	(4.9)	(4.5)	(5.6)	(4.3)
H/NO	93.5	82.1	78.1	75.6	72.2	71.2
	(4.0)	(1.9)	(1.6)	(1.8)	(1.9)	(1.9)

TABLE 55. Arterial pressure of CO_2 ($PaCO_2$, mmHg) during rest and at each min of 5-min cycle ergometry tests under conditions of normoxia (N), normoxia + 20 ppm nitric oxide (N/NO), hypoxia (H), and hypoxia + 20 ppm nitric oxide (H/NO). Values are means (± SD).

	REST	1 MIN	2 MIN	3 MIN	4 MIN	5 MIN
N	38.8	38.2	37.2	36.2	33.8	31.2
	(3.1)	(2.4)	(3.1)	(3.1)	(3.8)	(5.5)
N/NO	37.2	36.5	37.0	35.4	34.3	32.3
	(4.6)	(4.7)	(4.3)	(3.9)	(3.7)	(3.6)
H	37.6	34.9	33.7	31.7	30.1	29.3
	(4.0)	(3.0)	(2.3)	(1.2)	(1.2)	(2.4)
H/NO	35.9	33.4	32.9	31.6	29.7	29.0
	(6.0)	(4.9)	(5.0)	(4.3)	(4.1)	(3.8)

TABLE 56. Values for pH during rest and at each min of 5-min cycle ergometry tests under conditions of normoxia (N), normoxia + 20 ppm nitric oxide (N/NO), hypoxia (H), and hypoxia + 20 ppm nitric oxide (H/NO). Values are means (± SD).

	REST	1 MIN	2 MIN	3 MIN	4 MIN	5 MIN
N	7.40	7.38	7.32	7.26	7.23	7.21
	(0.01)	(0.03)	(0.04)	(0.05)	(0.06)	(0.07)
N/NO	7.41	7.39	7.32	7.27	7.26	7.24
	(0.01)	(0.02)	(0.02)	(0.02)	(0.06)	(0.08)
H	7.42	7.41	7.36	7.33	7.29	7.26
	(0.02)	(0.02)	(0.04)	(0.04)	(0.05)	(0.07)
H/NO	7.41	7.41	7.37	7.32	7.28	7.25
	(0.02)	(0.03)	(0.03)	(0.04)	(0.04)	(0.04)

TABLE 57. (HCO_3^-, $mmol \cdot l^{-1}$) during rest and at each min of 5-min cycle ergometry tests under conditions of normoxia (N), normoxia + 20 ppm nitric oxide (N/NO), hypoxia (H), and hypoxia + 20 ppm nitric oxide (H/NO). Values are means (± SD).

	REST	1 MIN	2 MIN	3 MIN	4 MIN	5 MIN
N	23.8	22.0	19.2	16.0	14.0	12.2
	(1.7)	(1.4)	(1.3)	(1.7)	(1.6)	(0.8)
N/NO	23.3	22.3	18.8	16.4	15.3	13.8
	(2.6)	(3.2)	(2.2)	(1.3)	(3.0)	(3.1)
H	24.1	22.0	19.2	16.8	15.0	13.7
	(2.1)	(2.0)	(1.9)	(1.9)	(1.9)	(2.3)
H/NO	22.6	21.3	18.4	15.9	13.9	12.4
	(4.0)	(3.3)	(2.4)	(1.8)	(1.7)	(1.4)

TABLE 58. Base excess ($mmol \cdot l^{-1}$) during rest and at each min of 5-min cycle ergometry tests under conditions of normoxia (N), normoxia + 20 ppm nitric oxide (N/NO), hypoxia (H), and hypoxia + 20 ppm nitric oxide (H/NO). Values are means (± SD).

	REST	1 MIN	2 MIN	3 MIN	4 MIN	5 MIN
N	-0.5	-2.4	-6.2	-10.2	-12.5	-14.8
	(1.4)	(1.9)	(1.8)	(2.4)	(2.5)	(1.8)
N/NO	-0.7	-2.0	-6.4	-9.5	-10.6	-12.5
	(2.1)	(2.7)	(2.1)	(1.0)	(3.7)	(4.2)
H	0.3	-1.9	-5.4	-8.0	-10.4	-12.2
	(1.6)	(1.3)	(2.0)	(2.1)	(2.7)	(3.5)
H/NO	-0.7	-1.4	-5.6	-8.6	-11.2	-13.3
	(3.3)	(2.2)	(2.0)	(1.6)	(1.7)	(1.6)