Endothelin-1 and Oxygen Saturation During Exercise in Normoxia and Hypoxia.

by

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Abstract

We tested the hypothesis that decrements in arterial oxyhaemoglobin saturation could be related to elevations in circulating endothelin-1 following 30 minutes of exercise at ventilatory threshold. Eight aerobically trained males (mean ± SEM: age 26.14 ± 1.77 years, height 182.36 ± 1.51 cm, mass 72.89 ± 2.62 kg) completed 2 maximal exercise tests (mean ± SEM: normoxia (n) 68.56 ± 2.06 $mL.kg^{-1}.min^{-1}$; hypoxia (FiO_2 0.14)(h) 53.88 ± 1.35 $mL.kg^{-1}.min^{-1}$), and two 30-minute steady state exercise protocols at the power achieved at threshold during maximal exercise tests (mean ± SEM: power (Watts) 257.14 ± 21.57 (n) 191.25 ± 10.79 (FiO_2 0.14)(h); HR (bpm) 161.7 ± 5.34 (n) 156.6 ± 3.45 (h)). When participants exercised for 30 minutes at ventilatory threshold inspiring 14%O_2, a significant decrease in oxygen saturation (as measured by pulse oximetry) was observed, when compared to values in normoxia (80.2 ± 1.17 % (h) vs 94.12 ± 0.24 % (n); $p<0.001$). This desaturation was not accompanied by significant changes in plasma endothelin-1 (ET-1), big endothelin-1 (BigET-1) or nitric oxide (NO). Both pulmonary artery pressure (PAP) and oscillatory compliance (OC) were significantly greater following exercise ($F_{(1,12)} = 4.74$ $p< 0.05$), compared to pre-exercise values. These outcome variables were not different between normoxia and hypoxia. Plasma ET-1 or BigET-1 levels did not differ significantly over time or across conditions $F_{(1,12)} = 4.74$ $p> 0.05$).

In conclusion, plasma ET-1 levels following 30-minutes of steady state exercise at ventilatory threshold are unrelated to decrements in oxyhaemoglobin saturation.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

$A\text{-}aDO_2$	Alveolar to arterial pressure difference of oxygen
AC	Arterial Compliance
CC	Capacitative Compliance
HR	Heart Rate
L-NAME	N-Nitro-L-Arginine Methyl Ester
NO	Nitric Oxide
OC	Oscillatory Compliance
PAO_2	Partial pressure of alveolar oxygen
PaO_2	Partial pressure of arterial oxygen
PAP	Pulmonary Artery Compliance
RER	Respiratory Exchange Ratio
SaO_2	Oxygen Saturation (measured directly)
SpO_2	Oxygen Saturation (via pulse oximetry)
VE	Minute Ventilation
VO_2	Volume of Oxygen Consumed
VCO_2	Volume of Carbon Dioxide Produced

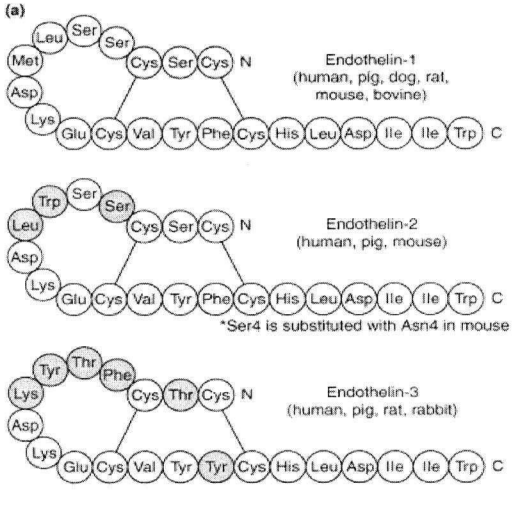
1.0 Literature Review

1.1 Endothelin

1.1.1 Introduction

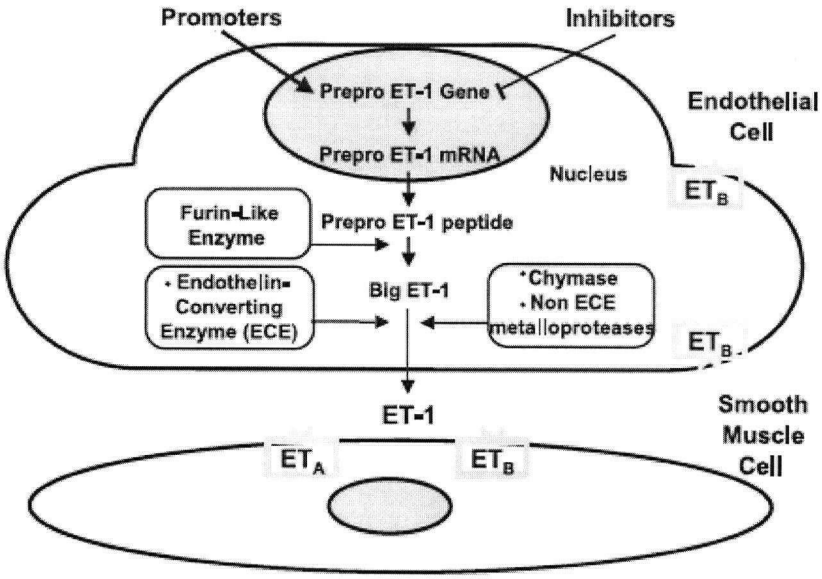
The endothelium is involved in a variety of vasoactive processes; one of the common peptides secreted by these cells is endothelin (ET).[1] ET is a natural amino acid peptide that is present in 3 isoforms; ET-1, ET-2 and ET-3, each possess different genes with a different structure. ET-1 differs from ET-2 by 2 amino acids and ET-3 by 6 amino acids (Figure 1).[2] ET-1 is produced in the endothelial cells from its precursors preproendothelin and big ET.[3] It is synthesized from a 212 amino acid gene product called preproendothelin-1, following the removal of a single peptide it is then processed by intracellular proteases to generate a biologically inactive 38 amino acid peptide called BigET-1. Through the action of a endothelin converting enzyme (ECE), BigET-1 is cleaved to form the biologically active 21 amino acid peptide ET-1 (Figure 2).[4-6] ET-2 and ET-3 are derived from there own precursors, although the detailed mechanisms of such processes have not yet been determined.

Figure 1



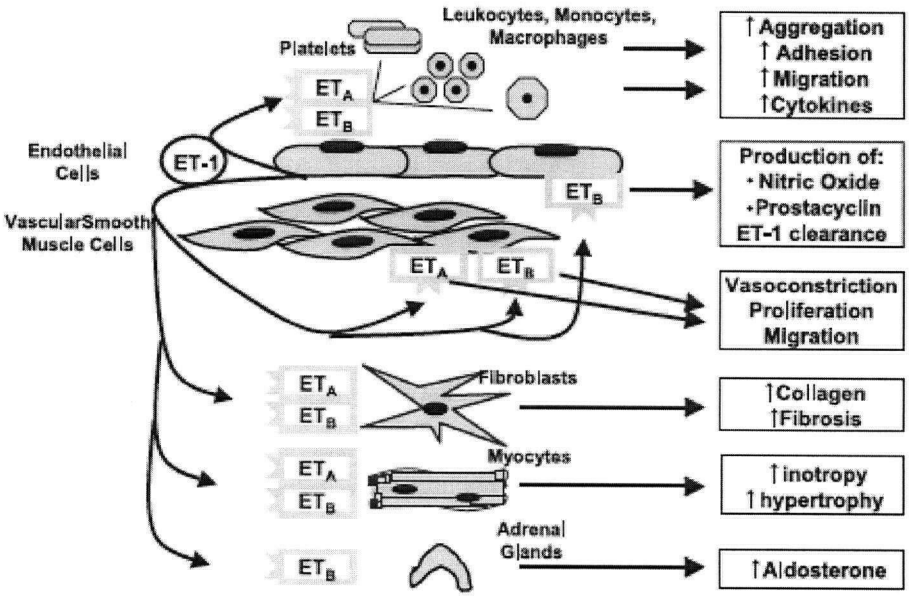
The structure of the three isoforms of endothelin (ET). Grey residues indicate those that are different from ET-1. In mouse ET-2, Ser4 of ET-1 is replaced by Asn4. Reproduced from Sakurai et al.[7]

Figure 2



Schematic representation of the vascular endothelin system. ETa and ETB represent receptors that mediate the actions of endothelin. Reproduced from Galie et al 2004.[8]

Figure 3:



Schematic representation of endothelin-1 effects in different cells types. Reproduced from Galie et al 2004.[8]

Endothelin-1 (ET-1) is produced by the endothelium and it is described as one of the most potent vasoconstrictor peptides that is present in the lung.[1, 9, 10] It is produced in response to a number of factors (Table 1), and acts on neighbouring endothelial and vascular smooth muscle cells (VSMC's) in a autocrine/paracrine manner stimulating the release of relaxing and contracting factors.[11] ET-1 constricts isolated pulmonary arteries and in the isolated perfused lung causes long lasting increases in vascular resistance.[12] It is a major isoform in the cardiovascular system,[13] plays a pivotal role in the maintenance of basal vascular tone in humans and activates the sympathetic nervous system.[2, 4] Due to its ability to increase contractility of VSMC's and cardiomyocytes,[14] it has been identified as a key factor in the development of vascular hypertrophy.[15] Under normal conditions, the effects of the ET-1 are carefully regulated through inhibition or stimulation of ET-1 release from endothelium.

Table 1: Stimulators and Suppressors of ET-1.

Stimulators			Suppressors
Physical	**Hormones**	**Others**	
Shear Stress	Vasopressin	Thrombin	Nitric Oxide
Hypoxia	Angiotensin	TGF	ANF
	Thromboxane A2	LDL	Cyclic GMP
	Epinephrine	Endotixin	
	Insulin	Ca^{2+}	
	Bradykinin	Phorbol esters	
	Glucocorticoids	Interleukin-1	

Stimulators and suppressors of ET-1. Reproduced from Morganti et al 2000.[16]

Two receptor sub-types mediate the actions of ET-1: ET-a and ET-b.[6, 11] In the pulmonary vasculature they cause pressor and depressor responses,[17] that mainly occur in small vessels.[18] ET-a receptors are found on VSMC's and have a higher affinity for ET-1 and ET-2 with a lower affinity for ET-3.[17] ET-b receptors are located on VSMC's and endothelial cells and bind to each of the three isopeptides equally.[17] When bound to ET-b receptors, ET-1 induces positive chronotropic effects, but when bound to ET-a receptors it causes negative chronotropic effects.[19] Both ET-a and ET-b receptors can mediate vasoconstriction in the peripheral vasculature of healthy humans and those with coronary heart failure (HF), but the degree to which ET-b receptors contribute to vasoconstriction is not yet known.

Studies with receptor antagonists (RA's) suggest that both ET-a and ET-b receptors can mediate vasoconstriction. This is self limited by endothelium induced production of NO, and only occurs when nitric oxide synthase (NOS) is deficient.[20, 21] The pressor response of ET-1 is predominantly mediated by ET-a receptors on VSMC, and the depressor response by ET-b receptors on endothelial cells. The binding of ET-1 to ET-a receptors on vascular smooth muscle has mitogenic and inotropic effects, resulting in vasoconstriction and cell proliferation that are greatly enhanced with disease.[2, 17-19, 22] ET-b receptors in the lung are implicated in the clearance of ET-1 from the circulation.[10, 23] They can cause vasodilation through receptors on the endothelial cells via the release of NO and prostacyclin, and vasoconstriction via the receptors located on VSMC's.[13, 17, 18, 24] Deficiency of lung ET-b receptor expression can result in increased lung ET levels, that via ET-a receptors mediate lung vascular endothelin growth factor (VEGF) production that in turn increases vascular permeability and pulmonary oedema.[25]

Circulating levels of ET-1 in healthy adults (0.4-0.8 fmol/mL) are below the pharmalogical threshold and considered to be below a level that produces contractions in humans.[26] Eighty percent of this peptide is secreted toward the vessel wall, suggesting that levels of plasma ET-1 in the blood may not be reflective of local actions.[16] ET-1 has a short plasma half-life of 2–5 min,[27] due to this rapid clearance from the circulation levels of its inactive precursor big ET-1 and the ET-1/big ET-1 ratio may be more effective ways to assess the activation of the ET system in both venous and arterial beds.[28]

1.1.2 Endothelin and Exercise

Plasma ET-1 concentrations increase in parallel with exercise intensity,[29] and at the onset of short-term exercise at maximum capacity they immediately increased by 50%.[30] Endothelial dysfunction can effect exercise capacity and correction of its dysfunction has been associated with an increase in peak VO_2.[31, 32] Individuals with chronic heart failure who exhibited a significant increase in exercise performance (as measured by a 6 minute walk test) following treatment also showed a decrease in BigET-1(2.0 ±0.9 vs 1.5±0.6 fmol/mL), an increase in endothelial function, as measured by reactive hyperaemia, an enhancement of NO dilation and a decrease in brain natriuretic peptide (BNP: a hormone secreted by the heart in response to work).[33] Trained athletes experienced a decreased plasma ET-1 response to exercise, where as this increased in untrained groups.[1] Differing training status and ET-1 responses to exercise were accompanied by training dependent change in plasma volume. Trained athletes experienced a slight reduction in plasma volume where as untrained group experienced a plasma expansion, that could be explained by variances in hydration.

Following exercise until exhaustion at 65 % of maximum aerobic capacity plasma ET-1 significantly decreased by 21 % during the first 30 minutes of exercise but then returned to baseline values by the end of exercise.[34] Thirty minutes of exercise at 90 % ventilatory threshold (T_{VENT}) caused plasma ET-1 levels to significantly increase, with peak levels occurring 30 minutes post exercise. When subjects cycled at 130 % of the power (W) measured at T_{VENT}, plasma ET-1 levels continued to rise and were significantly greater than levels at 90 % T_{VENT} intensity.[29] Following acute exercise plasma ET-1 levels significantly increased in both healthy individuals,[29, 35-37] and those with cardiovascular disease.[38, 39]

During a graded exercise test in horses, plasma ET-1 concentrations remained constant, but increased immediately following exercise and then returned to pre-exercise values following 10 minutes of recovery.[40] This raises the possibility that plasma ET-1 is produced in response to exercise cessation and therefore may not be a factor contributing to its termination.

Performing exercise for 30 minutes at a heart rate of 145 beats per minute on either a cycle ergometer or a treadmill elicited different ET-1 responses. Jogging caused a 61.4% increase in ET-1 where as cycling caused an 11.8% increase. This maybe best explained by an augmented mechanical stimulation of the endothelial lining during jogging, causing the endothelial cells to increase ET-1 release.[41]

In response to exercise the endothelium releases vasodilating factors such as NO into the exercising muscles,[42] that inhibits the production of ET-1 in the vascular endothelium.[43] A rationale for the elevated production of NO in response to exercise

could be that increased shear stress experienced during exercise elicits NO production and therefore prevent the rise in ET-1.

A positive but non-significant correlation was found between systolic and diastolic pressure at rest, and the changes in ET-1 (systolic blood pressure vs. ET-1: r = 0.479; diastolic blood pressure vs. ET-1: r = 0.590) after 3 months of exercise training on a cycle ergometer for 30 min/day, 5 days/wk at 80% of T_{VENT}. [44] This led to the hypothesis that ET-1 plays a role in elevated PAP seen during exercise. Plasma ET-1 levels during exercise have been significantly correlated with mean PAP (MPAP) in patients with interstitial lung disease[45] and associated with an increase in PAP found during exercise in the horse.[46]

In conclusion, plasma ET-1 levels increase with exercise intensity and are subject to the exercise protocol. The variation in conclusions regarding plasma ET-1 levels in response to exercise maybe due to differences in exercise protocol; short term manoeuvres may not be suitable to induce ET-1 release as it is dependent on transcription and translation that requires greater time.[47] With this in mind it is important to interpret the results of studies with different exercise protocols using caution.

1.1.3 Endothelin and Hypoxia

Hypoxia is a potent stimulus of ET-1 synthesis in vivo and in vitro[48] and causes 1 or more substances to be released that are capable of causing constriction.[49] Systemic endothelial cells increase ET-1 synthesis,[50] where as the pulmonary endothelial cells exhibit a decreased or unchanged level of ET-1.[51] However, hypoxia can cause dilation in the systemic arteries but constriction in the pulmonary vasculature.[52]

Plasma ET-1 synthesis can be augmented by alveolar hypoxia,[53] but not always by exercise-induced tissue hypoxia.[54] The lungs have been identified as an important source of plasma ET-1 production in response to hypoxia,[55] and receptor antagonism can attenuate hypoxia induced pulmonary hypertension (PH).[56] The increased ET-1 levels experienced in response to low oxygen tension are reversible on return to a normoxic environment.[18]

Subjects exposed to high altitude exhibited elevated plasma ET-1 levels that were proportional to PAP and inversely related to partial pressure of oxygen (PO_2).[48] Ascension to an altitude of 5050 m from sea level caused a significant oxygen desaturation (98.6 +/- 0.2% at sea level to 80.8 +/- 0.4%) that was negatively correlated to plasma ET-1 levels (from 1.8 +/- 0.1 pg/mL at sea level to 2.7 +/- 0.2 pg/mL).[57] High altitude hypoxia can also result in PH in humans and animals.[58, 59] This suggests that exposure to high altitude and therefore decreases in oxygen saturation result in elevations in plasma ET-1. Hypobaric hypoxia resulted in an over expression of ET-1 mRNA and protein in the rat lung during the development of PH, without alteràtion in plasma ET-1 levels. This suggests that local changes in ET-1 may not be detectable in plasma and thus questions the meaningfulness of measuring circulating ET-1.

During chronic hypoxia, contraction of SMC's are accompanied by an impaired production of pulmonary vascular NO,[60] that could have effects upon NO induced vasodilation. Hypoxic exposure causes an increase in ET-1 and ET-1 mRNA in pulmonary artery cells of patients with primary pulmonary hypertension.[18] In response to hypoxia there is an alteration in ET receptor distribution, ET-a receptor density increases during hypoxia where as ET-b receptors are lost. This suggests that VSMC's could

experience enhanced vasoconstriction through ET-a receptors and reduced vasodilation and ET-1 clearance through ET-b receptors. Based on these and similar findings it could be suggested that a selective increase in the synthesis and release of ET-1 from the pulmonary artery in the lung could be responsible for hypoxic PH.[18]

ET-1 levels during sleep in patients with lung disease have been assessed;[45] arterial ET-1 values at rest are significantly correlated with partial PaO_2 ($r = -0.935$, $p < 0.001$) and PAP ($r = 0.657$, $p < 0.001$). During sleep, both healthy subjects[61] and those with respiratory failure[62] experienced an attenuated ventilation, that was sufficient to account for the hypoxemia observed in patients with respiratory disease.[45] In conjunction with this, a significant increase in plasma ET-1 levels during arterial desaturation while sleeping, and a negative correlation with PaO_2 were found.[45] Following 30 minutes of desaturation to between 75 and 80% (through a variable mixture of oxygen and nitrogen) subjects experienced a 2.6 fold increase in plasma ET-1 levels (0.9 ± 0.11 Vs 2.34 ± 0.34 pmol/l).[63]

High altitude pulmonary oedema (HAPE) is characterized by normal left arterial pressure and elevated PAP,[64] and ET-1 has been implicated in the development of this condition.[25, 65] Elevations in ET-1 may augment capillary hydrostatic pressure,[66] increase vascular permeability,[67] and cause a NO synthesis defect resulting in vasoconstriction in the pulmonary vasculature.[68] In addition to this ET-1 binding to the ET-a receptor stimulates the production of VEGF mRNA, via increases in the expression of hypoxia-inducible factor (HIF) that increases vascular permeability leading to oedema formation in the lung.[25, 69, 70] The elevation in plasma ET-1in response to high altitude has been directly linked to increased systolic pulmonary artery pressure (SPAP) and PAP

measured at high altitude, and decrements in SpO_2.[48, 71] With this in mind it could be suggested that the enhanced release of ET-1 at altitude may represent one of the mechanisms provoking pulmonary vasoconstriction observed in subjects prone to HAPE.[72]

1.1.4 Endothelin and NO

The vasoconstricting actions of ET's only occur when nitric oxide synthase (NOS) is deficient, they have been described as self-regulating by inhibiting their own vasoconstricting effects by generating NO.[20] In response to exercise the endothelium releases vasodilating factors such as NO into the exercising muscles.[42] NO inhibits the production of ET-1 in the vascular endothelium, that implies that it may play a role in the production of ET-1 during exercise.[43] A rationale for the elevated production of NO in response to exercise could be that increased shear stress experienced during exercise elicits NO production and therefore prevents the rise in ET-1. The enhanced vasoconstrictor response to ET-1 during exercise may also be due to an attenuated release of NO.

$_L$-NAME (N-Nitro-L-Arginine Methyl Ester), a competitive inhibitor of NO production, partially inhibited endothelium dependent dilation (EDD) in both trained and sedentary rats, with the inhibition occurring to a greater extent in trained rats.[73] Increases in vascular wall shear stress during exercise hyperaemia may augment EDD.[74] Following 16 weeks of exercise training patients with heart disease experienced greater EDD in coronary conduit arteries that was attributed to training induced production of NO.[75] In addition to this, 16 weeks of exercise training also increased EDD in pulmonary arteries of pigs with disease but not in those without, and was attributed to

elevation in NO production and an attenuation of the constrictor substance prostaniod.[76] With this in mind exercise training may enhance EDD in pulmonary and coronary arteries that may occur through an increase in the production of NO.

1.2 Exercise Induced Arterial Hypoxemia

1.2.1 Introduction

During exercise ventilation and perfusion (V_A/Q) ratios are matched effectively and arterial oxygen saturation (SaO_2) is maintained.[77] This allows the body to preserve homeostasis and meet the metabolic demands encountered during exercise. In healthy individuals SaO_2 is maintained within 2-4% of resting values, when the body is unable to this such as during exercise, individuals experience exercise-induced arterial hypoxemia (EIAH). It is defined as a reduction in SaO_2 below 95%[78], an individual 4% decrease in SaO2 from baseline values[79], a persistent reduction in arterial O_2 pressure (PaO_2) by more than 1 kPa[80] or a reduction in PaO_2 by 10 mm Hg .[81]

Evaluating EIAH directly in the blood reveals that time profiles for PaO_2 and SaO_2 are different. PaO_2 decreases at the onset of exercise, where as reductions in SaO_2 become more pronounced at the end of maximal exercise,[82] suggesting that the intensity of exercise can affect the level of hypoxemia. During incremental exercise at 50-60% VO_{2max} only 2 out of 6 highly trained runners experience a 17-20mmhg decrease in PaO_2, but at 80-100% VO_{2max} all individuals experienced this.[83]

Mild EIAH has been defined as an absolute SaO_2 of 93-95%, moderate EIAH in the range of 88-93%, and severe EIAH corresponds to SaO_2 values <88%[78]. Reductions

in both SaO_2 to 92% and PaO_2 to 7.3 kPa have become the standard for investigating arterial oxygenation during exercise in normoxia.

1.2.2 Prevalence

EAIH has been estimated to occur in around 50% of young highly fit males (VO_{2max} 60-70 $mL.kg^{-1}.min^{-1}$)[78], and is accentuated in those elite trained athletes with the highest VO_{2max}.[79] The development of EIAH only in some elite athletes could be due to variations in the ventilatory response to exercise.[84] More recently, untrained sportsmen and women with a relatively low VO_{2max} (6 subjects with a VO_{2max} < 57 $mL.kg^{-1}.min^{-1}$) also developed EIAH following 8 weeks of supra maximal interval training; the development of EIAH was associated with a decrease in maximal ventilatory equivalent in O_2 in spite of an increase in maximal ventilation during exercise.[85] The significant decrease in the minimum SpO_2 causing the development of EIAH suggests that training status can be accompanied by alterations in the degree of arterial desaturation developed during exercise.

EIAH and pulmonary limitations are more prevalent in the female population.[81, 86-88] In trained females values for PaO_2 and SpO_2 are similar to those reported in males however, the work capacity in females was lower suggesting that EIAH occurred at a much lower VO_2.[82] The presence of EIAH in individuals with normal aerobic capacities is unique to women and has only been reported in men with a VO_{2max} greater than 150% of predicted values.[89] Female subjects who displayed moderate EIAH had a VO_{2max} within 15% of predicted values,[87] where as males with a VO_{2max} within 15% of predicted have rarely exhibited EIAH.[78] The development of EIAH can be attributed to

gender related lung size; generally women have a smaller vital capacity, airway diameter and diffusion surface when compared to men of a similar stature, sitting height and body mass.[90] Individuals with a smaller relative lung size could have smaller airways, that could lead to expiratory flow limitation, constraining the ability to compensate for inadequate alveolar to arterial O_2 exchange causing relative hypoventilation leading to EIAH.[91] Pre pubescent females do not experience EIAH, that suggests that EIAH in women maybe related to maturational factors or exercise training.[92]

1.2.3 Mechanisms

Generally, healthy humans experience an increased A-a DO_2 with exercise and values of 15-25 mm Hg are common in elite athletes.[78] A-a DO_2 increases until 80% of VO_{2max} is attained, above this a further enlargement is developed in those demonstrating EIAH.[93] During moderate and heavy exercise a widening A-aDO_2 was significantly greater than during rest and became accentuated during hypoxia. In addition to this, individuals experiencing EIAH experienced a significant decline in PaO_2 from rest of at least 10 mm Hg larger than controls.[94] The widened A-a DO_2 that occurs during exercise can occur as a result of shunting, diffusion limitation or a V_A /Q mismatch[78, 80]. In humans the severity of EIAH correlates with A-a DO_2, and V_A /Q mismatch maybe responsible for 50% of this difference during rest[80]. The multiple inert gas elimination technique (MIGET) has been used to measure V_A /Q inequality and diffusion limitation in healthy subjects. The severity of V_A /Q mismatch increases during hypoxia and with exercise intensity.[78, 95] During exercise < 65% VO_{2max}, A-a DO_2 can be explained by V_A /Q mismatch, however at greater intensities even though V_A /Q mismatch increases

other factors such as diffusion limitation may also explain A-a DO_2[80] It is unclear why V_A /Q mismatch becomes accentuated with exercise although some mechanisms have been implicated in this process. The increase in V_A /Q mismatch with exercise could be attributable to inflammatory markers released in the lung that subsequently effect small airways and arterioles in the periphery .[96] One possible explanation for V_A /Q mismatch and also a diffusion limitation is the accumulation of interstitial pulmonary oedema.[97, 98]

Relative hypoventilation maybe another mechanism involved in EIAH; it can be seen as insufficient alveolar ventilation to maintain arterial blood gases at normal values. Based on the variance of a single relationship it has been suggested that relative hypoventilation can account for 50% of EIAH.[99] Increasing ventilation in mild hypoxia could improve SaO_2 by raising the gradient for gas exchange in the lung through augmented PAO_2 levels and a rightward shift of the oxyhaemoglobin dissociation curve via a reduction in arterial PCO_2. During heavy exercise, levels of ventilation can explain up to 47% of variance in SaO_2,[94] individuals experiencing the most severe hypoxemia are most likely to have the lowest hyperventilatory response to exercise.[94] Studies that augmented hyperventilation via normoxic helium breathing did not prevent EIAH,[100] furthermore preventing arterial desaturation did not affect V_E at sub maximal VO_2 or at VO_{2max}.[86] These both suggest that relative hypoventilation at maximum exercise may not be a major contributor to the development of EIAH, however, it has been implicated during sub maximal exercise.[80]

During maximal exercise SaO_2 is also affected by pH,[101] a low pH reduces the affinity of O_2 to haemoglobin (Hb) and thus reduces .[82]

1.2.4 Effects on VO_{2max}

EIAH may impair the delivery of O_2 to the working muscles,[102] it could affect the unloading of O_2 from red blood cells and the diffusion into myocytes. Females who showed the most desaturation during normoxia experienced the most improvement in VO_{2max} if desaturation was prevented.[86] Studies preventing EIAH through inspired O_2 suggested that EIAH attenuates VO_{2max} in trained subjects, with a desaturation of 3-4% having measurable effects.[78, 103, 104] Reductions in SaO_2 can have detrimental effects upon VO_{2max}; a 1% decrease in SaO_2, can cause a 1% reduction in VO_{2max} when SaO_2 is below 92%,[105] or a 2% reduction in VO_{2max} when SaO_2 is greater that 95%.[86] Total work output can be impaired when SaO_2 is reduced to 87% but not at 90%.[106] Instances where VO_{2max} is maintained but SaO_2 continues to drop, work capacity tends to decrease.[106]

1.2.5 EIAH and Altitude

Decreases in SaO_2 are accentuated at altitude;[80] in hypoxic environments highly trained athletes usually experiences the greatest decrement in VO_{2max}.[107] At altitude a reduction in SaO_2 has been observed and vasoconstriction in the pulmonary bed can provoke V_A /Q mismatch.[108] Athletes who experience EIAH at sea level (SaO_2 <90%) demonstrated a significant reduction in VO_{2max} when exposed to mild hypoxia (71.1 ± 5.3 Vs 67.2 ±5.0 $mL.kg^{-1}.min^{-1}$) whereas non-EIAH athletes did not (67.2±7.6 Vs 66.2±8.2 $mL.kg^{-1}.min^{-1}$).[109] Based on these findings it could be hypothesized the degree of arterial desaturation during maximum exercise at sea level may be reflective of the ability to maintain VO_{2max} in mild hypoxia.

1.2.6 Exercise modality

When evaluating SaO_2 directly in the blood, it appears that the intensity of exercise determines the levels of EIAH.[82] Differences in cardiovascular responses to various exercise modalities have been identified and the type and duration of exercise can affect EIAH. It is generally accepted that treadmill running results in a higher VO_{2max} (4.83 ± 0.11 Vs 4.61±0.14),[110, 111] a lower SpO_2[111] (88.6 ± 0.6 Vs 92.6± 0.6) and more consistently EIAH when compared to cycle ergometry. Following maximal treadmill running and cycle ergometry; EIAH occurred in all subjects performing exercise on a treadmill, where as this only occurred in five of the thirteen participants during cycle ergometry.[111] This has been attributed to the augmented ventilation experienced during cycling, plus a widened A-a DO_2 in running rather than cycling in athletes with the same VO_2 [78]. In support of this at 90% VO_{2max}, V_E and alveolar ventilation were significantly greater during cycling when compared to running protocols.[112] Generally, PaO_2 was greater during cycling than running (105 ± 2 and 94 ± 2 mm Hg, respectively) and was associated with a smaller A-aDO2 (16 ± 2 vs. 22 ± 2 mm Hg, respectively).[112] This suggests that the difference in between running and cycling could be attributed to a difference in gas exchange and alveolar ventilation. In contrast, variations in SpO_2 have been analyzed in triathletes during running and cycling protocols with no difference being found.[113] This suggests that there were no significant differences in cardiorespiratory measures at maximal exercise between cycle ergometry and treadmill running in a group of subjects well trained in each exercise discipline. Faster ramping protocols can cause a more severe EIAH during cycling.[112] However, these results should

be interpreted with caution as the order of exercise tests was not randomized that may have resulted in an order effect.

Based on the literature it could be suggested that EIAH may occur frequently during both cycling and running modalities depending on the exercise protocol, the type of ergometer and the training background of the subject.

1.2.7 NO and EIAH

NO has been identified as a signalling substance that promotes adequate matching of V_A and Q in the lungs,[114] and has been related to vasodilation through the relaxation of VSMC's.[115, 116] Elevated levels of NO can be attributed to shear stress placed on the endothelium, that serves to attenuate ET-1 induced vasoconstriction.

In response to exercise, the endothelium releases vasodilating factors such as NO into the exercising muscles.[42] The production rate of NO increases with exercise,[117, 118] but has not been associated with alterations in SpO_2,[117] suggesting that it is not related to EIAH. In support of this, measurement of exhaled NO revealed no difference between subjects with and without EIAH.[119] Exercise training increases production of NO in the systemic vasculature;[118] an up regulation of NO production in the systemic vasculature in response to exercise or training could be mirrored in the pulmonary vasculature and may play a role in the matching of V_A and Q and maintenance of low pulmonary vascular resistance.

Inhalation of NO during an exhaustive incremental exercise protocol moderated the drop in PaO_2, implying that it maybe involved in EIAH through V_A and Q regulation.[120] NO concentration in healthy humans have been positively correlated to PaO_2 and negatively correlated to A-aDO_2.[121] Breathing NO at altitude serves to increase

PaO_2 and thus can have implications upon A-a DO_2. This does not occur during exercise at sea level[120] or during simulated hypoxia at sea level.[117] Elevations in PAP have been found in subjects exhibiting low levels of exhaled NO when exposed to hypoxia.[122]

NO is activated and taken up rapidly by haemoglobin, therefore changes that occur in the pulmonary and systemic vasculature may not be detectable in expired air.[123] Furthermore, an increase in systemic/pulmonary vascular endothelial NO release may not be detectable in expired air due to its short half life in physiological systems.[124]

2.1 Statement of the Problem

ET-1 is a known constrictor peptide that has been negatively correlated to SpO_2 and PaO_2. A decline in SpO_2 can have implications for oxygen delivery to the exercising muscles. An increase in ET-1 and therefore its constricting actions may serve to limit exercise performance and reduce SpO_2. To date no studies have investigated the differences in ET-1, BigET-1 (ET-1 precursor) or nitric oxide (NO) levels in healthy individuals during intense exercise in normoxia and hypoxia. The results of this study will determine if ET-1 and BigET-1 levels are increased during exercise in these conditions and will determine whether peptide levels correlate to variations in oxygen saturations. This may provide an insight into the involvement of ET-1 during exercise in healthy humans.

2.2 Objective

The primary purpose of the study was to investigate the differences in ET-1 and BigET-1 production in athletes prior to, during and after exercise in athletes in normoxia and hypoxia. Furthermore, variations in ventilation, SpO_2, arterial compliance (AC), NO, and PAP were measured to determine any relationships to the circulating peptides.

2.3 Hypothesis

Based upon current literature the following hypotheses were derived:

1. Following exercise, plasma ET-1 and BigET-1 will be significantly increased.
2. Plasma ET-1 and BigET-1 will be significantly greater in hypoxia compared to normoxia.
3. Plasma ET-1 will be inversely correlated to SaO_2.

4. Plasma NO, AC and PAP will be significantly different following exercise.

5. Plasma NO, AC and PAP will be significantly different in normoxia and hypoxia.

3.0 Methodology

3.1 Subject Population

Seven aerobically trained male athletes were studied to determine levels of plasma ET-1 and BigET-1, prior to, during and after exercise in normoxia and hypoxia. All subjects were non-smoking, had normal pulmonary function and were free of any history or symptoms of cardiopulmonary disease including exercise-induced asthma.

3.2 Procedures and Outcome Measures

Subjects reported to the Cardiac Physiology and Rehabilitation Laboratory at UBC on five occasions; the first day consisted of an explanation of the study, signing of informed consent and familiarization with procedures. All exercise tests were performed a cycle ergometer at least 48 hours apart. The ergometer used was the Velotron Pro cycle ergometer (Racermate Inc, Seattle) controlled by Velotron Coaching Software (Version 1.5.186, RacerMate Inc, Seattle). Prior to the first maximal exercise test, subjects performed a resting pulmonary function test. Subjects breathed through a facemask, attached to a pitot tube pneumotach, with a low resistance, non-rebreathing valve (Hans Rudolph, Kansas City, MO) attached to the distal end of the pneumotach. On the second and third day subjects performed VO_{2max} tests, once in normoxia and once in hypoxia (FiO_2 = 0.14). Day 4 and 5 consisted of a 30-minute steady state exercise protocol at the power achieved at ventilatory threshold in normoxia and hypoxia. The order of steady-state exercise tests was randomized and participants were blinded to the concentration of oxygen that they were inspiring. Throughout all tests SpO_2 was measured continuously and recorded every 30 s and expired gases were measured on a breath-by-breath basis

using a computerized system (Medisoft Hyp'air), and averaged every 15 s. SpO_2 was measured continuously using 2 pulse oximeters (Ohmeda 3740, Louiseville, KY), one at the ear and one on the tip of the index finger. Prior to placing the oximeter on the ear and the finger a topical vasodilator cream was applied to increase local perfusion.

During the hypoxic and normoxic exercise tests blood samples were withdrawn at various times throughout the protocol to measure ET-1, Big ET-1, and NO; PAP and AC were measured immediately before and after exercise.

3.2.1 Resting Pulmonary Function Test

Using an automated ventilatory analysis system (Medisoft Hyp'air), participants performed resting pulmonary function tests, including forced vital capacity (FVC) and forced expiratory volume in the first second (FEV_1).

3.2.2 VO_{2max} Tests

Subjects performed two maximal exercise tests; one in normoxia and one in hypoxia (FIO_2: 0.14). Prior to the test subjects were asked to refrain from exhaustive exercise for 24h, caffeine/alcohol for 12h, and food or drink for 2h prior to testing. After entering the lab subjects had their height and weight measured. Subjects then performed a self-selected cycling warm up (approximately 5 min). Following this, three minutes of resting data were collected prior to the test. The progressive exercise test started at 150 W and increased in a stepwise fashion of 30 W every three minutes until volitional exhaustion. To ensure that VO_{2max} was attained at least 3 of the following criteria were met: 1) a plateau in VO_2 with the last stage increase, 2) attainment of at least 90% of age-predicted maximal heart rate (210 – [0.65 x age]), 3) RER > 1.1, 4) inability to maintain the imposed cycling speed despite maximal effort and verbal encouragement.

During both tests heart rate was recorded every 30 seconds using a portable heart rate monitor (Polar Vantage XL). Minute ventilation (V_E), oxygen consumption (VO_2) and CO_2 output (VCO_2) were measured using a computerized system. Ventilatory threshold was calculated using a combination of ventilatory equivalents and RER.

3.2.3 Steady State Exercise Test in Hypoxia or Normoxia

Subjects were given 20 minutes to perform a self-selected warm up on the cycle ergometer and then asked to perform a 30-minute exercise protocol at the power achieved at ventilaltory threshold. Minute ventilation, VO_2 and VCO_2 were measured at 15-s averages throughout the exercise test and $\%SpO_2$ was measured continuously and recorded every 30 seconds.

Prior to and following each steady state exercise test participants were weighed and then consumed water to replenish any fluids lost during the exercise test in an attempt to maintain plasma volume.

3.2.4 ET-1 and BigET-1

Venous blood samples were taken from right antecubital fossa vein cannulated with a 21 gauge intravenous cannula. During the hypoxic and normoxic exercise tests blood samples were taken at various times throughout the protocol (Time: pre-, 10-, 20-minutes during exercise and immediately following, 10-, 20-, 30- minutes post termination of exercise). Samples were immediately placed on ice and centrifuged at 3000 *g* for 10 min. Plasma was frozen and stored at -80°C until assayed. Following extraction the plasma concentrations of ET-1 and BigET-1 were determined by enzyme immunoassay (Medicorp Inc, Montreal Quebec). The coefficient of variation for ET-1 and BigET-1 was 9.6% and 9.5% respectively.

3.2.5 Nitric Oxide

Plasma NO levels were determined using a ratio of circulating nitrite/nitrate (NOx). Venous blood samples were also taken from right antecubital fossa vein cannulated with a 21 gauge intravenous cannula, immediately before and after both exercise tests. Samples were immediately centrifuged at 2500g for 15 min and stored at −80°C before analysis. NOx concentrations in different dilutions of plasma ultrafiltrate were determined by a colorimetric assay kit (Biovision Inc, Mountain View, Ca) based on a three-step Griess reaction. The sample range of the NO assay was 1-40 uM.

3.2.6 Arterial Compliance

AC was measured immediately before and after the steady state exercise tests. Radial arterial pulse waves were recorded using an arterial tonometer sensor array. The waveform was calibrated by the oscillometric method with a cuff on the opposite arm and a calibration system internal to the device. The tonometer sensor array adjusts itself automatically to obtain the optimal waveform and repeats the calibration until the waveform is stable. When the waveform is calibrated and stable, 30 second long analog tracings of the waveform was digitized at 200 samples per second and stored in a personal computer system for compliance analysis. The diastolic decay of the waveform was mathematically analyzed and two models of arterial compliance were calculated based on a modified Windkessel model of circulation. Capacitative compliance (CC; that which represents compliance of the large vessels) was derived from analysis of diastolic slope of decay of the waveform and oscillatory compliance (OC; that which represents compliance of small vessels) was derived from oscillatory component of diastolic decay.

Intra- and inter- visit measurements of arterial compliance have been known to differ by less than 3 and 4% respectively indicating a good reliability of measure.[125]

3.2.7 Pulmonary Artery Pressure

PAP was measured immediately before and after the normoxic and hypoxic exercise tests. Doppler echocardiography was carried out in the semi-recumbent partial left lateral position. Triplicate estimates of tricuspid regurgitant jet velocity were made and the PAP calculated using the simplified Bernoulli equation and assuming RA pressure of 10 mmHg and a zero gradient across the pulmonary valve. Measures were taken before and immediately after the steady state exercise tests. The standard error of estimate in testing intra- and interobserver reproducibility of Doppler systolic time intervals is less than 5%.[126]

3.2.8 Data Analysis

Data analysis were completed using SPSS software (SPSS Inc, version 11). Tests for normality of distribution and homogeneity of variance were performed prior to further statistical analysis. Standard t-tests were used to determine differences in data between maximal exercise tests. Plasma ET-1 and BigET-1 levels were analyzed using a 2 (Groups: normoxia and hypoxia) x 7 (Time: pre-, 10-, 20-, and immediately following, 10-, 20-, 30- minutes post termination of exercise) analysis of variance (ANOVA) with repeated measures on the second variable.

AC, NO and PAP were analyzed using a 2 (Groups: normoxia and hypoxia) x 2 (Time: pre and post exercise) repeated measures ANOVA. In addition, correlation of the SaO2 to other outcome variables was analyzed using the Pearson product moment calculation. For all tests significance was set at $\alpha = .05$.

3.3 Limitations

The primary limitation of the study that was that ET-1 was measured in plasma. As only 20% of the peptide is circulating in venous blood, it may not be representative of what is happening at a different site of the body.

Measurements of both PAP and AC are time sensitive. Athletes were instructed to remove themselves from the ergometer and lie down as quick as possible while PAP, AC and a blood sample were taken. Due to the number of variables being measured and the possibility of time delay between subjects, this proved to be a confounding variable within the study.

Measurements for PAP were measured once the participants removed themselves from the cycle ergometer and therefore the hypoxic stimulus was removed. This meant that any affects resulting from the hypoxic stimulus might have been normalized.

Any significant differences that were found in the study cannot be applied to other bodies of individuals. Subjects for this study were selected based upon fitness level, that limited the randomization of the study

3.4 Delimitations

In an attempt to reduce the amount of variability within the study subject hydration levels were controlled to ensure that differences in plasma ET-1 are not due to an exercise-induced alteration in plasma volume. Furthermore, time of testing was controlled due to circadian alterations in ET-1.

4.0 Results

Seven male athletes (mean ± SEM: age 26.14 ± 1.77 years, height 182.36 ± 1.51 cm, mass 72.89 ± 2.62 kg) exercised at the Cardiac Physiology and Rehabilitation lab on 4 occasions. Maximal exercise tests (mean ± SEM: normoxia (n) 68.56 ± 2.06 mL.kg^{-1}.min^{-1}; hypoxia (FiO_2 0.14)(h) 53.88 ± 1.35 mL.kg^{-1}.min^{-1}), and two 30-minute steady state exercise protocols at the power achieved at threshold during maximal exercise tests (mean ± SEM: power (Watts) 257.14 ± 21.57 (n) 191.25 ± 10.79 (FiO_2 0.14)(h); HR (bpm) 161.7 ± 5.34 (n) 156.6 ± 3.45 (h)) were completed.

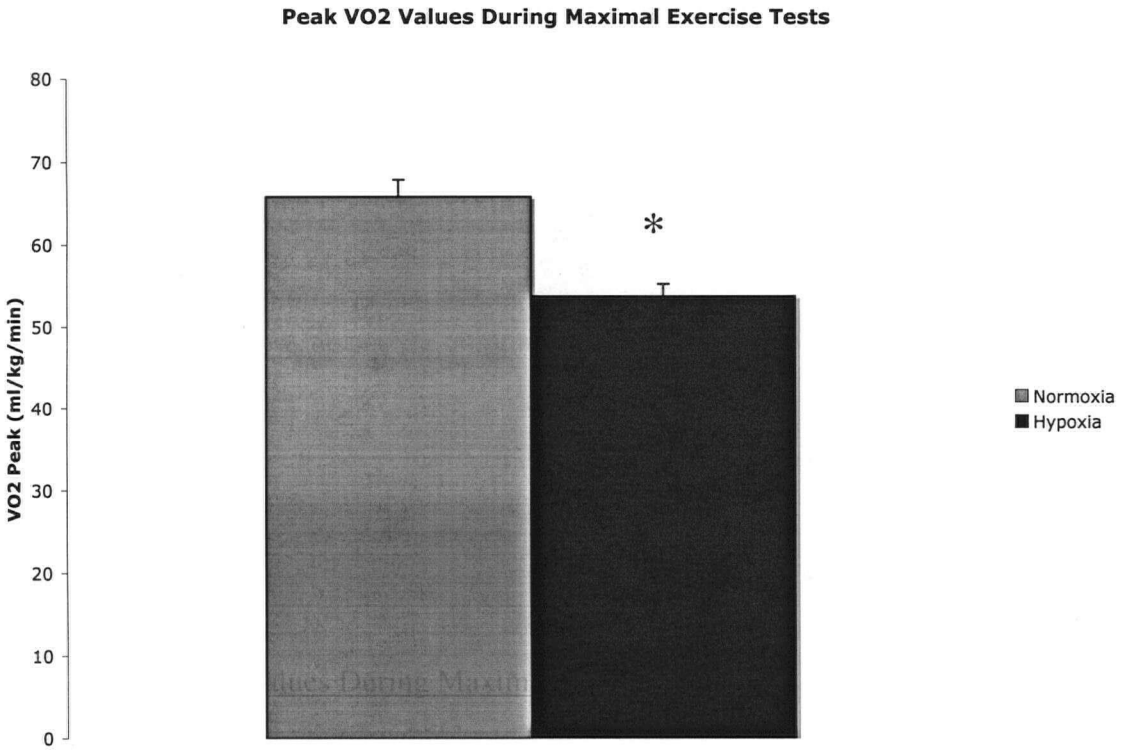
Ventilatory data for maximal exercise tests were analyzed, significant increases in peak VO_2 ($p = 0.001$), VCO_2 ($p = 0.003$) and $\%SpO_2$ ($p = 0.000$) were found when participants exercised in normoxia compared to hypoxia. Mean peak data for maximal exercise tests can be found in Table 2.

Table 2: VO_{2max} Data

Condition	VE (L/min)	VO_2 Peak (mL.kg^{-1}.min^{-1})	VCO_2 Peak (mL.kg^{-1}.min^{-1})	RER	HR (beats/min)	SpO_2 (%)
N	178.01 ± 9.49	65.86 ± 2.06	74.72 ± 2.44	1.13 ± 0.01	190.43 ± 3.19	94.54 ± .24
H	163.67 ± 7.7	53.88 ± 1.35 *	63.4 ± 1.99 *	1.18 ± 0.03	183.1 ± 2.72	85.39 ± 1.17 *

Mean ± SEM
* significant difference between normoxia and hypoxia $p<0.05$
VE p=0.264, VO2* p=0.000, VCO2* p=0.004, HR p=0.108, SpO_2* p= 0.000

Figure 4: Peak VO_2 Values During Maximal Exercise Tests



Mean ± SEM
* significant difference between normoxia and hypoxia $p<0.05$

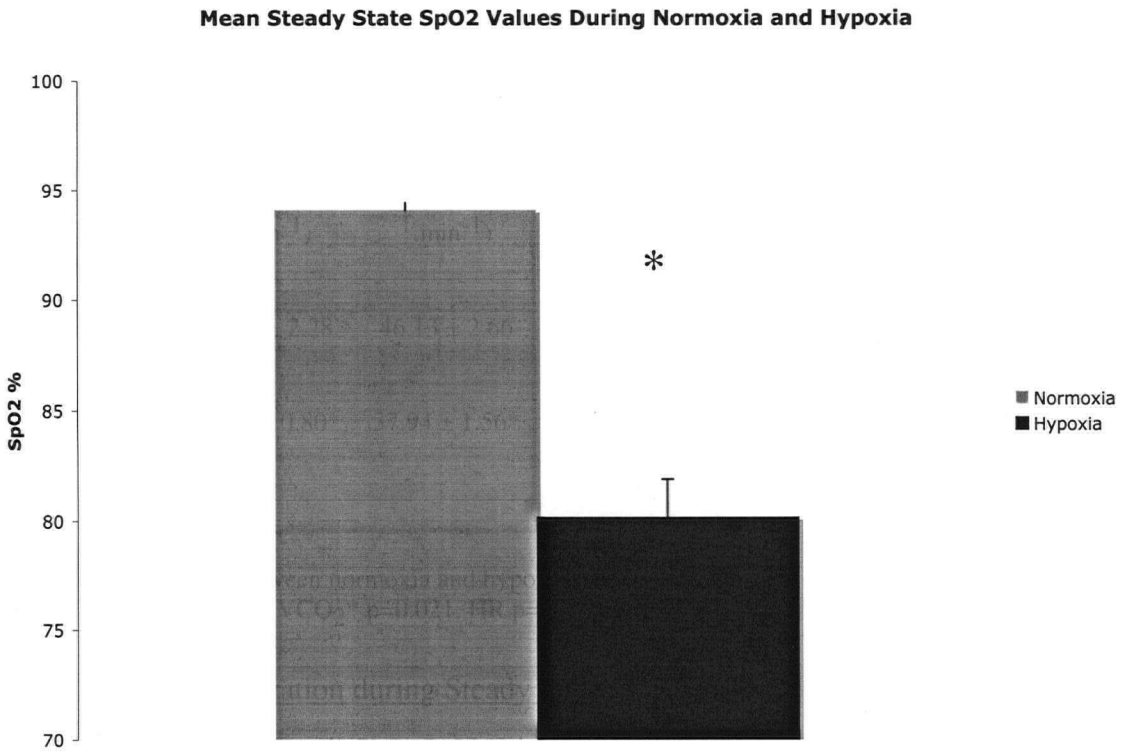
During steady state exercise tests significant increases in mean VO_2 ($p=0.012$), VCO_2 ($p=0.036$) and $\%SpO_2$ ($p= 0.00$) were found when participants exercised in normoxia compared to hypoxia. Mean steady state data can be found in table 3.

Table 3: Mean Steady State Data

Condition	VE (l/min)	VO_2 (mL.kg^{-1}.min^{-1})	VCO_2 (mL.kg^{-1}.min^{-1})	HR (beats/min)	SpO_2 (%)	RER	Power (watts)
N	98.17 ± 9.66	48.77 ± 2.28	46.13 ± 2.66	161.7 ± 5.34	94.12 ± 0.32	0.94 ± 0.01	257.14 ± 21.57
H	85.03 ± 6.03	39.03 ± 0.80*	37.94 ± 1.56*	154.9 ± 3.45	80.2 ± 1.70*	0.97 ± 0.02	188.6 ± 10.79*

Mean ± SEM
* significant difference between normoxia and hypoxia $p<0.05$
VO_2* p=0.04 VE p=0.271, VCO_2* p=0.021, HR p=0.303, Sa O_2* p= 0.00, RER p=0.334, Power p=0.02

Figure 5: Oxygen Saturation during Steady State Exercise



Mean ± SEM
* significant difference between normoxia and hypoxia $p<0.05$

Both PAP and OC were significantly greater following exercise ($F_{(1,12)} = 4.74$ $p< 0.05$), compared to pre exercise values. This difference could not be found when comparing normoxia and hypoxia ($F_{(1,12)} = 4.74$ $p> 0.05$), although oscillatory

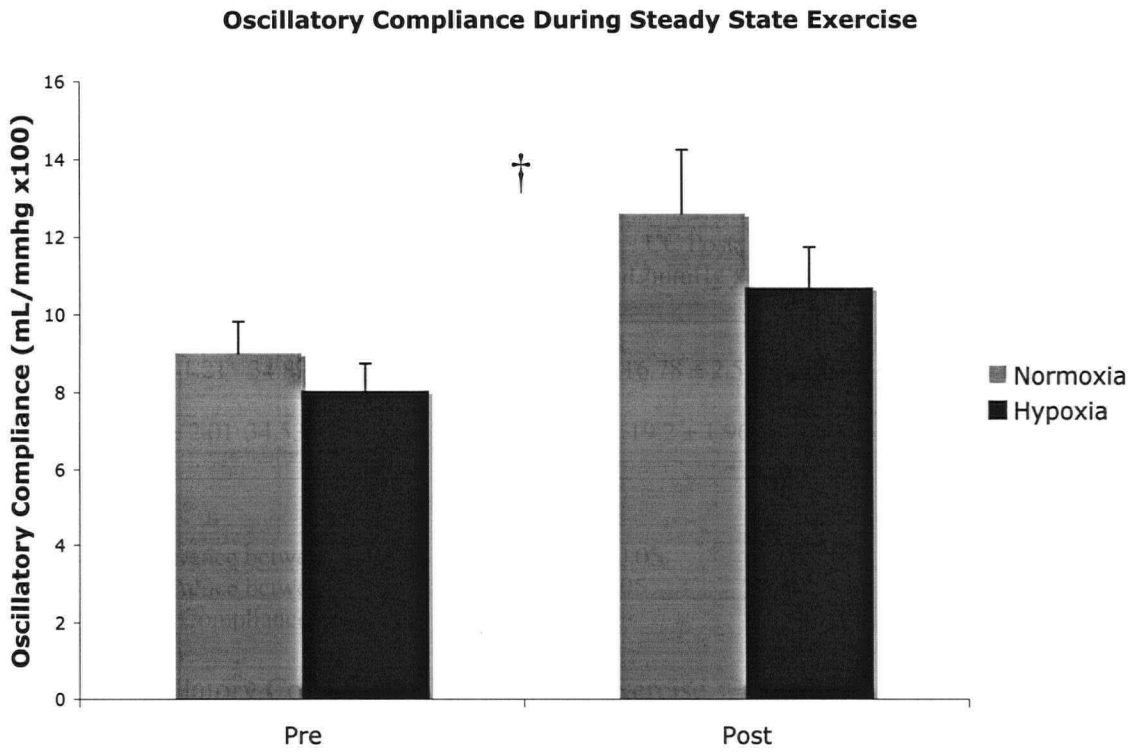
compliance (OC) was on average higher during normoxia. Mean values for PAP, AC and NO can be found in Tables 4 and 5.

Table 4: Mean PAP and Arterial Compliance

Condition	PAP Pre (mmHg)	PAP Post (mmHg)	CC Pre (mL/mmHg x10)	CC Post (mL/mmHg x10)	OC Pre (mL/mmHg x100)	OC Post (mL/mmHg x100)
N	28.7 ± 1.21	34.89 ± 2.07†	20.51 ± 1.85	16.78 ± 2.58	9 ± 0.81	12.6 ± 1.65 †
H	29.29 ± 2.01	34.53 ± 2.70 †	18.03 ± 1.41	19.2 ± 1.96	8.04 ± 0/70	10.69 ± 1.04 †

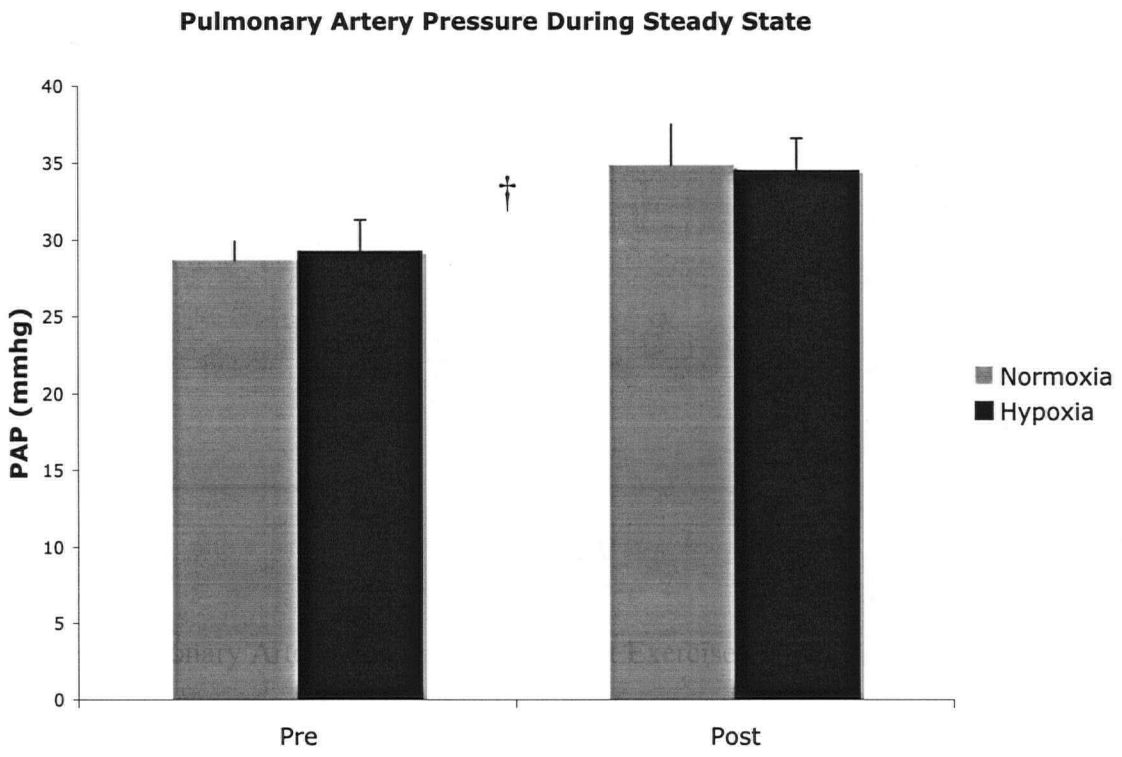
Mean ± SEM
* significant difference between normoxia and hypoxia $p<0.05$
† significant difference between pre and post exercise $p<0.05$
CC: Capacitative Compliance

Figure 6: Oscillatory Compliance Pre and Post Exercise



Mean ± SEM
† significant difference between pre and post exercise

Figure 7: Pulmonary Artery Pressure Pre and Post Exercise



Mean ± SEM
† significant difference between pre and post exercise

Circulating NO did not differ significantly over time or across conditions ($F_{(1,12)} = 4.74$ $p > 0.05$), however were on average higher during normoxia.

Table 5: Mean Nitric Oxide Values (μM)

Condition	Pre-Exercise	Post-Exercise
N	1.48 ± 0.55	1.50 ± 0.53
H	0.95 ± 0.17	0.95 ±0.16

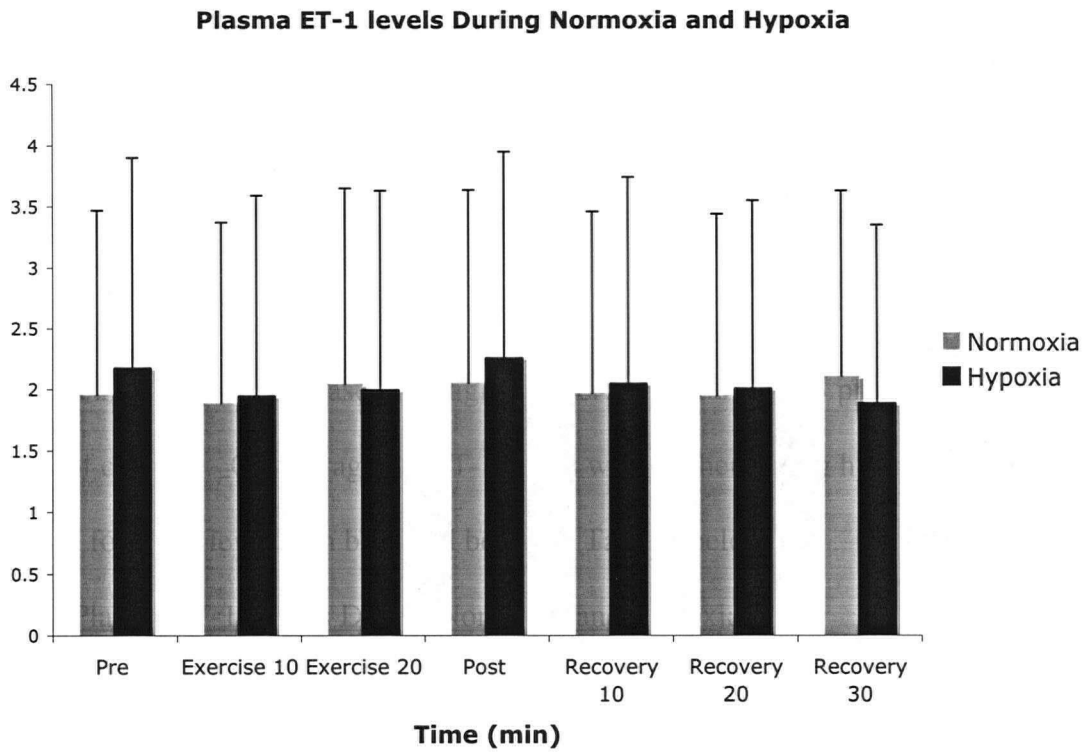
Mean ± SEM

Plasma ET-1 or BigET-1 levels did not differ significantly over time or across conditions $F_{(1,12)} = 4.74$ $p > 0.05$), and were not significantly correlated to alterations in oxygen saturation. In addition, both peptides were not significantly different when ΔET-

1/BigET-1 was analysed ($F_{(1,12)}$ = 4.74 p> 0.05). However BigET-1 decreased 10 minutes into exercise and then increased throughout exercise, returning to pre- exercise levels at the end of exercise. On average BigET-1 levels were higher during hypoxia.

Mean data for ET-1 levels can be found below in Table 6 below.

Figure 8: Plasma ET-1 Levels During Normoxia and Hypoxia



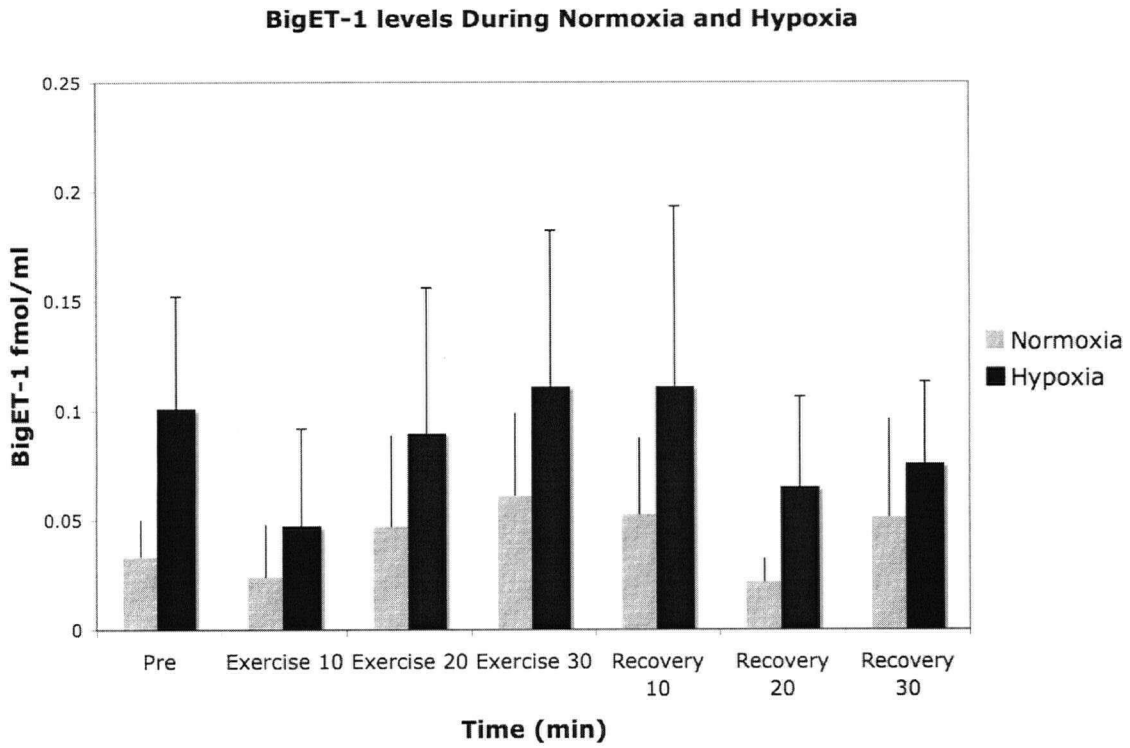
Mean ± SEM

Table 6: Mean Plasma ET-1 (fmol/mL)

Condition	Pre	Exercise 10	Exercise 20	Exercise 30	Recovery 10	Recovery 20	Recovery 30
N	1.96 ± 1.51	1.89 ± 1.48	2.05 ± 1.60	2.06 ± 1.57	1.97 ± 1.49	1.95 ± 1.50	2.11 ± 1.52
H	2.18 ± 1.73	1.94 ±1.65	1.99 ± 1.64	2.25 ± 1.70	2.04 ± 1.70	2.0 ± 1.56	1.88 ± 1.47

Mean ± SEM

Figure 9: BigET-1 Levels During Normoxia and Hypoxia



Mean ± SEM

5.0 Discussion

5.1 Introduction

Seven aerobically trained males underwent 30 minutes of exercise at ventilatory threshold. Following exercise we found that PAP and OC significantly increased compared to pre-exercise values. The administration of 14 % O_2 during exercise failed to elicit changes in these parameters when compared to normoxia, but resulted in a significant reduction in oxygen saturation. In addition, NO, plasma ET-1 and BigET-1 levels did not differ significantly over time or following hypoxic exposure. Circulating levels of BigET-1 dropped 10 minutes into exercise and then returned almost to pre-exercise levels by the end of the protocol. Furthermore, on average BigET-1 trended towards higher values during hypoxia, where as NO tended to be lower. Correlation analysis suggests that outcome variables were not related to alterations in oxygen saturation following exercise in normoxia and hypoxia.

5.2 ET-1 and BigET-1

5.2.1 ET-1/BigET-1 and Exercise

ET-1 and its pre-cursor BigET-1 are part of a vasoconstrictive pathway that may serve to reduce blood flow and increase pressure in vessels through the constriction of smooth muscle. Much of ET-1 is secreted by vascular smooth muscle cells towards the vessel wall, resulting in the remaining 20% distributed into circulation.[16] Consequently, plasma levels are the result of spill over from the vascular endothelium into the bloodstream.

ET-1 is secreted by a number of cells including vascular smooth muscle cells in response to exercise. Matsakas et al.[1] found that plasma ET-1 increased 2-fold following at 20 minutes of exercise in a 60 minutes exercise protocol. Mean plasma ET-1 levels measured prior to, during and following exercise within the current study ranged from 1.89 - 2.11 fmol/mL(n) and 1.81 - 2.25 fmol/mL(h). These levels are considerably higher when compared to normal circulating levels for healthy adults (0.4 -0.8 fmol.ml).[26] It should be noted that one participant exhibited plasma ET-1 levels 10 – 12 times higher than all other participants within the group that served to elevate the overall mean. When these values were omitted from the results ET-1 levels resembled values similar to those found by Matsaki et al.[26] The participant who experienced elevated ET-1 levels comparable to patients with cardiovascular disease had similar AC, PAP, BigET-1 and NO levels to the remainder of the group. One possible explanation for this elevation in ET-1 without variation in other parameters could be a desensitization of ET-1 receptors, resulting in an elevated amount of circulating plasma ET-1 required to initiate a similar response.

Circulating levels of BigET-1 (0.02 – 0.06 fmol/mL (n) and 0.05 – 0.11 fmol/mL (h)) within this study were considerably lower than other values in healthy humans (0.6-1.8 fmol/mL).[127] This could have occurred due to a number of reasons such as assay sensitivity or an attenuated action of ECE.

Results in normoxia are in accordance with Lenz et al.[54] and Letizia et al.[128] who found that exercise did not alter plasma ET-1 and BigET-1. One possible explanation for these findings could be the similarities between rate of uptake and release of ET-1, resulting in unchanged peptide levels. It could be suggested that this rate of uptake and

release in healthy humans maybe more efficient than their less healthy counterparts, resulting in elevated levels of ET-1. Furthermore, tissue hypoxia induced solely by maximal exercise may not be enough of a stimulus to induce the production of this peptide.[54]

In contrast to this study, Maeda et al.[29] found a difference between plasma ET-1 levels following steady state exercise at 130% of ventilatory threshold on a cycle ergometer. This difference could have occurred for a number of reasons; the exercise intensity was less in our study, therefore was not enough to induce changes in ET-1 levels. Moreover, when comparing the power outputs from participants in both studies (257.14 ± 57.07 vs. 164.8 ± 11.2 watts, for the present study and Maeda et al.[29], respectively); athletes within the current study were on average cycling at a higher resistance, suggesting that they were stringer cyclists. Supporting the notion that fitter cyclists have a lower endothelin response to exercise, Matsakas et al.[1] found that trained athletes experienced a decreased plasma ET-1 response to exercise, where as the ET-1 response to exercise increased in untrained groups.[1] In addition, 3 months of aerobic training decreased resting plasma ET-1 levels [44] and can prevent the abnormal rise of this peptide in response to acute exercise in normotensive subjects.[129] Consequently, the differing ET-1 responses between the current study and others with similar stimuli may be due to a difference in training status. This higher training status may have resulted in an alteration in vasomotor function in the vascular smooth due to adaptations in the endothelium and smooth muscle cells.[73] Furthermore, as our athletes were aerobically trained, they could have experienced an attenuated ET-1 response to exercise, or the stimulus required to induce its production is greater or takes longer than in untrained

individuals. Another possible contributor might be that experienced cyclists are more efficient, with less unnecessary movement resulting in decreased mechanical stimulations of the endothelium and less production of the peptide during exercise.

If ET-1 production is dependent upon a stimulus greater than a particular threshold, one might speculate that aerobically trained athletes have increased the threshold at which production occurs.

To establish if exercise intensity caused discrepancies between studies, a protocol whereby athletes exercise above threshold for 30 minutes or at threshold for greater than 30 minutes maybe useful to determine this. However, after observing athletes within this study cycle for 30 minutes at threshold it is questionable whether many would be able to sustain exercise above ventilatory threshold for greater than 30 minutes. Consequently, the duration of exercise may not be sufficient to induce changes in ET-1. The endothelin peptide is not stored in vesicles, therefore secretion is dependent upon transcription and translation.[47] This pathway supports the hypothesis that short term maneuvers might not result in ET-1 release as protein synthesis is required.

Although both peptides did not differ significantly over time or across conditions BigET-1 followed a similar pattern to that demonstrated in work by, Richter et al.[34] Ten minutes after the onset of exercise, plasma BigET-1 levels dropped but then returned to baseline levels by the end of exercise. BigET-1 tended to be higher during hypoxia when compared to normoxia, where as NO tended to be lower.

5.2.2 ET-1/ BigET-1 and Hypoxia

ET-1 is synthesized in the lung in response to hypoxia.[55, 57] Following systemic hypoxia Smith et al.[55] found that ET-1 levels increased, which were associated with an

increase in lung weight and pulmonary perfusion pressure. In this study a comparison of plasma ET-1 and BigET-1 levels in normoxia and hypoxia found no alterations in their serum concentration, suggesting that the duration of hypoxic exposure was not long enough to elicit an adequate ET-1 response.

Analysis of plasma samples revealed that BigET-1 was greater (NS) during hypoxia compared to normoxia. The tendency for BigET-1 to be higher in hypoxia would lead to the hypothesis that ET-1 would also be higher. As plasma ET-1 did not alter across conditions this suggests that the assay may not have been sensitive enough to detect the production of BigET-1 or the stability of the precursor in venous blood was inadequate in this group of participants. As vascular reactivity to bigET-1 requires a conversion to ET-1 to elicit full haemodynamic effects, it could be hypothesized that highly trained athletes experience a reduction in the actions of ECE in an attempt to reduce the likelihood of the vasoconstrictive actions of ET-1.

Many studies have found that hypoxia is a potent stimulus of ET-1 production, which has been associated to decrements in oxygen saturation. Morganti et al.[57] found that ascension to an altitude of 5050m from sea level over a period of 8 hours caused a significant oxygen desaturation (98.6 ± 0.2% at sea level to 80.8 ± 0.4%) that was negatively correlated to plasma ET-1 levels (from 0.72 ± 0.04 fmol/mL at sea level to 1.08 ± 0.08 fmol/mL). In the current study oxygen saturation was significantly lower in hypoxia when compared to normoxia. These reductions in oxygen saturation were similar to those experienced by participants in the study by Morganti et al.[57]; however, they were not accompanied by alterations in ET-1 or BigET-1. The discrepancy could have occurred for a number of reasons. For example, participants in the study by Morganti et

al.[57] were at exposed to altitude over a long period of hours, where as participants within this study experienced hypoxia for only 30 minutes. In addition, some participants within this study have had previous exposure to altitudes of 2500m and greater. It is not known how this previous exposure to altitude could affect ET-1 and BigET-1 response to hypoxia. To the authors knowledge there is no literature that documents the duration of hypoxic exposure required to induce ET-1 production. However, as the duration of exposure in this study is considerably less than others, this suggests that durations of greater than 30 minutes while breathing 14% O_2 are required.

Battistini et al.[28] suggested that due to the rapid clearance of ET-1 from the circulation levels of its inactive precursor BigET-1 and the ET-1/big ET-1 ratio may be a more effective way to assess the activation of the ET system in both venous and arterial beds. [28] However, as plasma BigET-1 levels within this study were so small the ratios were not an effective way to measure this.

5.3 Pulmonary Artery Pressure

Plasma ET-1 has been implicated in the pathophysiology of conditions associated with vasoconstriction such as pulmonary hypertension and coronary heart disease [18, 130-132]. ET-1 causes mitogenesis of human pulmonary artery smooth muscle cells (PASMC's), via the ETa receptor, which could be associated with vascular remodelling and therefore alter pressures in VSMC's.[133] Selective increase in the synthesis and release of ET-1 from pulmonary endothelial cells could account for hypoxic PH and vascular remodelling. Overproduction of this peptide may contribute to smooth muscle hypertrophy and remodelling of the pulmonary vasculature associated with chronic hypoxic PH [18].

ET-1 has been associated with an increase in PAP found during exercise in the horse.[46] A positive correlation was found between systolic/diastolic pressure and the changes in ET-1 after 3 months of exercise training on a cycle ergometer for 30 min/day, 5 days/wk at 80% of ventilatory threshold (T_{VENT}) in humans. This highlights the role of ET-1 in BP and led to the hypothesis that ET-1 plays a role in elevated PAP seen during exercise. Elevations in PAP through ET-1 could cause uneven vasoconstriction resulting in a ventilation perfusion mismatch (V_A/Q), leading to exercise induced arterial hypoxemia (EIAH).

With this in mind it was hypothesized that the elevated PAP observed following exercise could be correlated to alterations ET-1. Resting values for systolic pulmonary artery pressure in our study were similar to the normal values found by Chemla et al.[134] (28.7 ± 1.21 vs 23 ± 3 mmhg). Following 30 minutes of exercise at ventilatory threshold participants in the current study experienced significantly greater PAP compared to pre-exercise values. As plasma ET-1 and BigET-1 levels did not alter significantly over time or across conditions it could be concluded that ET-1 and its pre-cursor BigET-1 were not involved in exercise induced changes in PAP. The elevation in PAP is likely due to elevations in cardiac output accompanying exercise.

Ascent to high altitude is associated with increases in pulmonary artery pressure, which occurs through hypoxic pulmonary vasoconstriction.[135] A number of vasoactive substances such as ET-1 are involved in the pressor response to hypoxia.[136] Modesti et al. further highlighted the involvement of ET-1 in high altitude pulmonary hypertension through the use of endothelin receptor antagonists. Administration of Bosentan an ETa and ETb receptor antagonist (62.5 mg for 1 day and 125 mg for the following 2 days;

n=10), attenuated the pressor response in participants following a rapid ascent to 4559m.[137] Following hypoxic exposure participants in this study did not experience either an alteration in PAP or plasma ET-1. This data further supports the notion that endothelin plays a role in augmentation of PAP during hypoxia of several hours duration. Shorter exposures, such as in the present study are likely not sustained enough to elicit vasoconstriction and production to ET-1.

McEniery et al.[138] found that when using a receptor antagonist (BQ-123) in normotensive individuals there was little effect on the peripheral vasdilator response to forearm exercise, but normalised the exercise-induced vasodilatation in hypertensive patients. This suggests that the endothelium maybe actively involved in exercise induced vasodilatation in the hypertensive patients but not in healthy humans. At altitude, there is a transient hypertension that resolves on return to sea level. Perhaps exposures to hypoxia of at least several hours duration induce a hypertensive response which can be altered by plasma ET-1.

5.4 Nitric Oxide

Normal vascular tone is maintained through a balance of dilating and constricting factors. Nitric Oxide is a potent vasodilator that causes the relaxation of vascular smooth muscle.[139] The production rate of NO increases with exercise due to increased shear stress,[117, 118] which may serve to attenuate ET-1 induced vasoconstriction. It has been hypothesized that elevations in NO could be associated with arterial hypoxemia through ventilation-perfusion V_A/Q mismatch.[140] However, measurements of exhaled NO have not been associated with alterations in SpO_2, or in athletes with and without EIAH[117, 119]

The current study found that plasma NO did not significantly change following normoxic or hypoxic exercise. Oxygen saturation was significantly reduced during exercise in hypoxia, without a concomitant change in NO levels, which supports the notion that there is little relationship between NO and SaO_2. Furthermore, an increase in OC following exercise suggests that the increase in arterial stiffness following exercise occurs independent of NO production. In support of this finding, Otsuki et al.[141] recently found that NO could not be associated with arterial pulse wave velocities when comparing strength-trained, endurance-trained and healthy controls.

The absence of variation in both ET-1 and NO further suggests that they are not involved in exercise-induced alterations in vascular smooth muscle, Furthermore the hypoxic stimulus within this study was not great enough to alter levels of each substance.

5.5 Arterial Compliance

ET-1 has been hypothesized to play a role in maintaining basal vascular tone, and perhaps arterial compliance. Arterial compliance is increased in endurance-trained athletes and decreased in strength-trained athletes.[141] Plasma ET-1 was decreased in nine endurance trained males athletes following 30 minutes of cycling at 60% of maximum aerobic power (1.73 ± 0.44 to 0.8 ± 0.24 fmoL/ml), whereas untrained athletes experience an increase in ET-1 (1.04 ± 0.24 to 1.33 ± 0.44 fmoL/ml).[1] Arterial stiffness has also been increased by arterial infusion of ET-1.[142] Moreover, ET-1 has been identified as being involved in the maintenance of basal vascular tone in humans that has led to the hypothesis that there is a relationship between arterial stiffness and the endothelium.

Following 30-minutes exercise at ventilatory threshold we found that OC was greater compared to pre-exercise values. It did not significantly change when participants

exercised in normoxia compared to hypoxia; however, there was a trend towards OC being higher during normoxia indicating more compliant arteries. This increased compliance was not accompanied by changes in either plasma ET-1/BigET-1 or NO, suggesting that the exercise induced alterations in compliance of arteries are not related to these peptides. In support of this, Otsuki et al.[141] found that the relationship between pulse wave velocity(PWV) and AC to ET-1 was linear (i.e. as PWV increases so does ET-1 where as when AC increases ET-1 decreases) and the relationship between these factors were independent of blood pressure. They did not find a relationship between mean blood pressure and ET-1 but did find that endurance-trained athletes exhibited less ET-1 than their strength-trained counterparts and this was associated with a reduction in arterial compliance.

Hypertensive subjects exhibit abnormalities and reductions in the oscillatory component of the diastolic waveform following pulse contour analysis when compared to healthy controls (0.075 versus 0.052 mL/mm Hg, $P<.05$). [143] This difference suggests that there could be an association between increased pressures in the pulmonary artery and decreased compliance of peripheral vessels. In contrast we found that as the pressure in the pulmonary artery increased following exercise the compliance in the peripheral vessels also increased. This finding suggests that stiffness in the peripheral arteries is independent of larger vessels such as the pulmonary artery in healthy athletes.

5.6 Hypothesis Revisited

5.6.1 Hypothesis # 1

Based of previous literature a number of hypothesis were derived; below is a summary of how our data supports or does not support the original hypothesis and why this may have occurred.

The first hypothesis that plasma ET-1 and BigET-1 would be significantly increased following exercise. Data within this study did not support this hypothesis; which may have occurred for the following reasons:

1. Training Status
2. Exercise Intensity
3. Exercise Duration

The notion that as individuals become more aerobically trained the ET-1 production in response to exercise is attenuated has been supported by Maeda et al.[44] They found that following 3 months of aerobic exercise participants produced less endothelin in response to exercise. As athletes in this study were aerobically trained the possibility that they experienced an attenuated ET-1 response to exercise exists.

The intensity at which individuals were exercising may not have been enough to elicit a significant ET-1 response. Maeda et al.[29] found a significant increase in plasma ET-1 in response to exercise at 130% Tvent; athletes within the current study were exercising at ventilatory threshold. The rationale for this intensity was based upon the fitness level of the athletes; as they were highly trained their ventilatory threshold may have been considerably elevated compared to untrained individuals. For these athletes to exercise above threshold for 30 minutes may not have been possible. Furthermore, as

athletes become more trained they may increase the threshold that is required to elicit production of ET-1.

In addition the duration of exercise may not have been long enough to elicit the production of ET-1. Previous literature highlighted ET-1 may not be produced in response to short term exercise as it requires time for transcription and translation,[47] suggesting that ET-1 may be involved in a more long term response to exercise

5.6.2 Hypothesis # 2

The second hypothesis that plasma ET-1 and BigET-1 would be significantly greater following exercise in hypoxia compared to normoxia, was also not supported. However, BigET-1 tended to be higher during hypoxia compared to normoxia, without an accompanying rise in ET-1. This may have occurred as the conversion of BigET-1 to its active precursor could have required a longer duration. In addition, we hypothesized that individuals may have experienced a down regulation of ECE as a mechanism to limit the vasoconstrictive action of ET-1; which also may be a product of training status.

When comparing the oxygen saturation of individuals with this study to those in other studies that found a difference in ET-1, it appeared that our hypoxic stimulus was considerably shorter. Due to the nature of the formation of the peptide, it could be possible that the duration of hypoxia was not long enough to elicit ET-1 production.

5.6.3 Hypothesis # 3

Levels of ET-1 did not alter over time or across condition, with this in mind the data did not support hypothesis # 3 ; furthermore analysis also revealed no significant correlation between ET-1 and SaO_2.

5.6.4 Hypothesis # 4

The hypothesis that plasma NO, AC and PAP would be significantly different following exercise was somewhat supported. Oscillatory compliance, which represents peripheral vessels, and PAP were significantly greater following exercise when compared to pre-exercise values. The elevations in PAP could be due to the exercise-induced rise in cardiac output.

5.6.5 Hypothesis # 5

The fifth hypothesis that plasma NO, AC and PAP would be significantly different in normoxia or hypoxia was not supported; however, NO tended to be higher in normoxia. This data suggests a down regulation of vasodilators in hypoxia.

6.0 Conclusion

No associations were found between plasma ET-1/BigET-1 and any other outcome variables that were measured throughout the study. The response of this peptide is slow as it requires time for transcription and translation. This suggests that the endothelin pathway may not be involved in the physiological responses to steady state exercise or to short term hypoxic exposure during exercise. Exercise for 30-minutes at ventilatory threshold was a sufficient stimulus to increase both PAP and OC. As both outcome variables significantly increased following exercise this suggests that stiffness of the peripheral vessels cannot be related to increased pressures in the pulmonary artery.

7.0 Future Studies

Future studies could attempt to investigate ET-1 production in response to exercise training by measuring plasma ET-1 levels prior to, during and after following an exercise training protocol. It would also be interesting to document if alterations in plasma ET-1occur how long it takes for then to return to pre-training levels.

In addition attempting to determine the level and duration of hypoxia that is required to stimulate ET-1 production would be valuable Furthermore, the exercise intensity and duration needed to induce ET-1 production would enable us to further understand the involvement of the endothelin system during short term hypoxic exposure.

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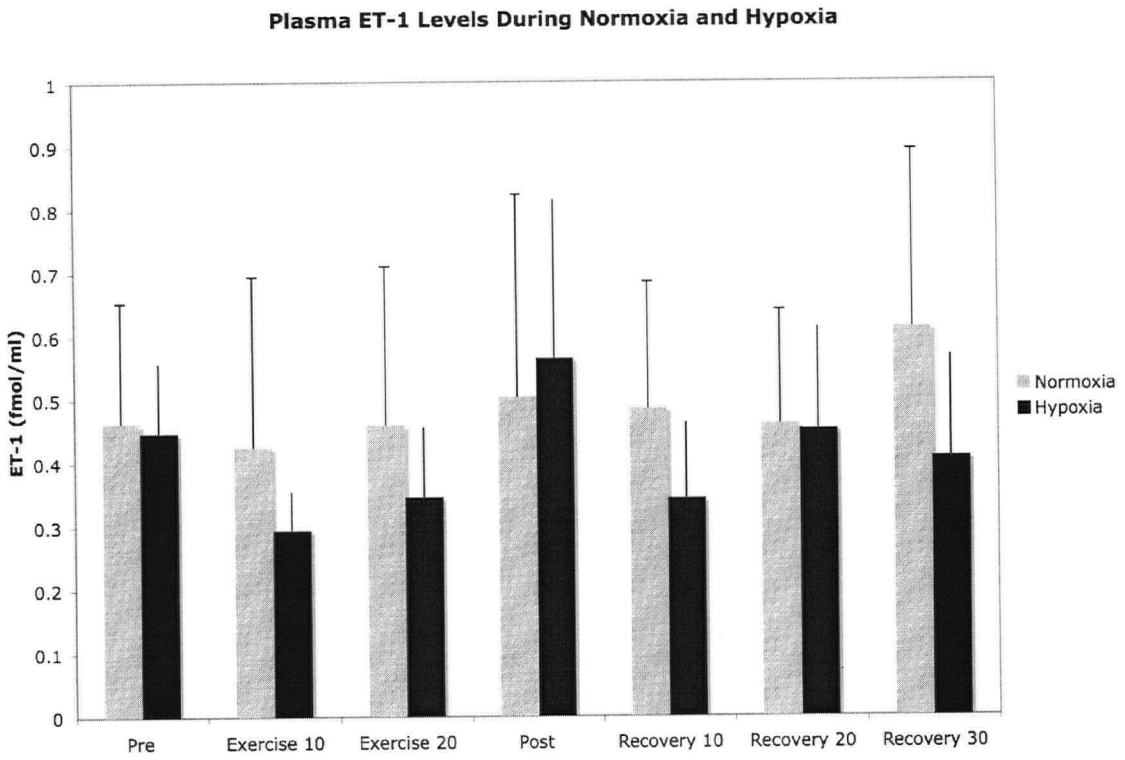
9.0 Appendix

Figure 10: Chart displaying plasma ET-1 levels after removal of the participant with values 10-12 times greater than other participants



Raw Data

Individual Peak $VO2_{max}$ Data

	VO_2 Peak (ml/kg/min)		VE (l/min)		VCO_2 (ml/kg/min)		HR (beats/min)		SpO_2 (%)	
	N	H	N	H	N	H	N	H	N	H
1	66.2	50.1	196.6	197.5	76	59.6	190	172	94.6	79.2
2	60.2	55.4	132	137.1	71	65.1	183	180	93.2	86.1
3	66.8	55.8	181.4	171.6	74.1	64.5	179	178	94.4	84.3
4	62.8	49.9	181.2	157,5	68.3	57.7	198	188	95	88.3
5	61.2	51.4	157.9	149.8	68.1	60	197	190	95.2	87.2
6	76.4	55	204	153.1	85.9	63,3	185	182	94.7	84.9
7	67.4	59.6	193	179.1	79.7	73,6	201	192	94.7	87.7

Individual Mean Steady State Data

	VO_2 (ml/kg/min)		VE (l/min)		VCO_2 (ml/kg/min)		HR (beats/min)		SpO_2 (%)		RER	
	N	H	N	H	N	H	N	H	N	H	N	H
1	46.96	40.28	95.35	109.7	41.55	37.56	153.3	156,2	93.47	73.53	.88	.93
2	42.97	39.77	67.03	79.03	41.45	40.55	152.19	162.02	95.3	87.3	.96	1.02
3	57.67	38.83	131.25	79.28	55.96	37.99	162.87	141.56	93.03	77.03	.97	.98
4	55.24	37.03	133.23	73.22	54.76	33.22	179.25	153.41	93.87	84.07	.99	.9
5	47.32	35.44	79.04	64.66	44.36	32.38	167.61	152.2	95.13	78.9	.94	.91
6	41.29	41.31	82.39	87.28	37.27	39.96	140.1	148.9	94.3	80.17	.9	.97
7	49.97	40.57	98.92	102.09	47.57	43.99	176.77	169.9	93.77	80.5	.95	1.08

Individual PAP and Arterial Compliance

	PAP (mmhg) Normoxia		PAP (mmhg) Hypoxia		CC Normoxia (mL/mmhg x10)		CC Hypoxia (mL/mmhg x10)		OC Normoxia (mL/mmhg x100)		OC Hypoxia (mL/mmhg x100)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	28	30	28.9	37.9	19.95	18.65	13.2	22.1	10.05	16.2	7.1	.10.4
2	35	43	40.8	46.3	18.7	7.6	15.1	25.4	9.45	11.5	6.2	15.4
3	27.4	.31	27.4	30.2	23.7	23	23.7	20	13.2	10.6	6.9	8.3
4	26.4	33.8	28.7	25.5	23.3	12.6	21.2	14.8	7.75	6.3	9.8	11.4

5	29	36	28.6	34	11.7	26.1	19.8	18,1	7.9	19.7	10.4	12.4
6	30	41.2	26	38.8	27	19.2	17.6	23.5	7.7	13.5	9.7	9.8
7	25.1	29.2	24.6	29	19.2	10.3	15.6	10.55	7	10.4	6.2	7.1

Individual Plasma ET-1 (fmol/ml)

	-1		10		20		30		40		50		60	
	n	h	n	h	n	h	n	h	n	H	n	h	N	h
1	0.168	0.541	0.1254	0.318	0.1706	0.41	0.1186	0.313	0.564	0.157	0.568	0.236	0.72	0.2181
2	0.173	0.141	0.159	0.101	0.295	0.173	0.1186	0.373	0.166	0.159	0.092	0.155	0.161	0.1344
3	0.337	0.22	0.065	0.263	0.169	0.279	0.272	0.22	0.105	0.086	0.135	0.237	0.19	0.27
4	0.216	0.484	0	0.33	0.076	0.151	0	0.2843	0.258	0.37	0.219	0.446	0.088	0.216
5	0.486	0.412	0.434	0.195	0.33	0.225	0.339	0.386	0.378	0.435	0.484	0.439	0.588	0.449
6	1.397	0.889	1.764	0.559	1.72	0.838	2.109	1.812	1.436	0.847	1.262	1.198	1.924	1.162
7	10.954	12.541	10.69	11.83	11.59	11.83	15.6	12.374	10.86	12.24	10.9	11.3	11.12	10.681

Individual Nitric Oxide Values (μM)

	Normoxia		Hypoxia	
	Pre	Post	Pre	Post
1	3.390	3.045	0.417	0.473
2	0.383	0.394	0.340	0.455
3	1.173	0.965	0.976	0.982
4	0.476	0.527	0.640	0.687
5	0.778	1.084	1.435	1.348
6	0.442	0.525	1.091	1.244
7	3.724	3.934	1.446	1.435