EXERCISE, ARTERIAL PRESSURE CONTROL & SYSTEMIC O_2 TENSION: IMPLICATIONS FOR POST EXERCISE HYPOTENSION IN HYPERTENSION

by

Karl James New BSc (Hons) PGCE

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ABSTRACT

This thesis presents four studies investigating the phenomenon of post exercise hypotension in the human condition of pre (borderline)-hypertension. Study one investigated the effects of an acute bout of 30-minutes upright cycling on post-exercise haemodynamics and compared the results to a non-exercise control condition. 9 pre-hypertensive males, mean arterial pressure (MAP) = 106 ± 5 mmHg (50 ± 10 yr), not on medication, were studied for 6 hours following 30-minutes of cycle exercise at 70% maximal oxygen consumption and following 30-minutes of seated rest. Results demonstrate that moderate intensity exercise exerts a modest fall (~6 mmHg) in arterial pressure with the hypotension sustained for 6-hours post-exercise. The fall in arterial pressure equates to a significantly reduced afterload when compared to both pre-exercise baseline and non-exercise control data taken at the same time of day. The arterial pressure responses transcended into a sustained reduction (~20%) in systemic vascular resistance and reciprocal increase in vascular conductance for up to 2-hours post-exercise. Venous atrial natriuretic peptide (ANP) demonstrated an elevation (44%) following exercise and a significant decline (33%) in the post-exercise period mirroring the haemodynamic response. This research reveals that acute exercise is capable of sustained reductions in arterial pressure and vascular resistance beyond the usual labile fluctuations and that the octapeptide ANP may exert a modulatory influence over the post-exercise response.

Increases in O_2 tension beyond the physiological range induces complex effects on the circulatory system with a dominant vasoconstriction following hyperoxia. The purpose of study 2 was to assess the effects of hypoxic (16% O_2) and hyperoxic (50% O_2) exercise on subsequent haemodynamic control when compared with normoxia. 9 pre-hypertensive males, MAP = 106 ± 5 mmHg (50 ± 10 yr), not on medication, performed 30-minutes of cycle exercise at 70% normoxic maximal oxygen consumption in hypoxia (16% O_2), hyperoxia (50% O_2) and normoxia(21% O_2). Hyperoxic exercise blunted post-exercise haemodynamics by significantly attenuating the reductions (from normoxic baseline) in SVR (~45%, $P<0.05$ *vs.* normoxic & hypoxic exercise immediately post-exercise) that persisted throughout 120-minutes recovery in normoxia (~35% *vs.* normoxic & hypoxic exercise, during recovery) and elicited a mildly hypertensive effect, with regards to MAP, whereas normoxic and

hypoxic exercise elicited a hypotension compared to baseline ($P<0.05$). Circulating ANP was decreased in the hyperoxic trial when compared with normoxic and hypoxic exercise [24.3 (13.4) *v.* 31.5 (16.3) and 29.6 (13.9) pg/ml, respectively; $P<0.05$, pooled for state]. Changes in MAP were related to changes in ANP concentration only following hyperoxic exercise ($r = 0.50$, $P<0.01$). These findings indicate that acute modest hyperoxia reflexively induces measurable physiological derangement partly explained by decreased circulating concentrations of ANP.

Study three determined the role of free-radical mediated oxidative stress and redox regulation of circulating $NO^{\cdot}$ metabolism as a primary modulator of vascular tone following exercise in pre-hypertensive humans. Utilising the same cohort and exercise protocol as in study 1 venous blood was sampled from an antecubital vein. Plasma $NO^{\cdot}$ metabolites nitrate (NO^{-}_3) and nitrite (NO^{-}_2) were determined fluorometrically, whilst S-Nitrosothiol (RSNO) concentrations were assayed by the Saville reaction Indirect markers of oxidative stress were determined spectrophotometrically detecting lipid hydroperoxides (LOOH). Exercise led to a delayed increase in LOOH by 60-minutes post-exercise (0.69 ± 0.13 *v.* 0.86 ± 0.18 μmol/l, respectively, $P<0.05$), that remained elevated until termination of the trial 6-hours post-exercise. NO^{-}_3 significantly fell below baseline by 120-minutes post-exercise (10.8 ± 3.3 *v.* 1.1 ± 1.1 μmol/l, respectively, $P<0.05$), remaining attenuated for the remainder of the study. NO^{-}_2 and RSNO were unmodified in the post-exercise period. In parallel to this finding the data also indicates a significant blunting in the hyperaemic response [SVR decreased from a 31% reduction immediately (within 1-minute) post-exercise to ~13 and 8% at 60- and 120-minutes post-exercise, respectively, $P<0.05$] and reversal of the hypotension ($P<0.05$) over the same time frame as the augmented lipid peroxidation and attenuated circulating NO^{-}_3. These results indicate that augmented oxidative stress exerts a deleterious effect on post-exercise haemodynamics and implicates a potential redox regulation pathway of $NO^{\cdot}$ as being a mechanism by which free radical-induced oxidative stress blunts the degree of PEH in the recovery period.

The final study investigated the potential role of a redox-mediated regulation of circulating $NO^{\cdot}$ bioavailability as a modulator of the augmented vasoconstriction

following hyperoxic exercise. The same cohort and exercise protocol were employed as in study 2 and venous blood was assayed for NO^-_3, NO^-_2, RSNO, LOOH, & lipid /water-soluble antioxidant concentrations. Similar adverse haemodynamic effects were noted following hyperoxic exercise as reported previously in study 2. RSNO showed a significant increase following hypoxic exercise only ($P<0.05$, state × time, interaction), whereas NO^-_3, NO^-_2 and LOOH failed to differ between conditions ($P>0.05$, main effect for state [O_2] and state × time, interaction effects). Ascorbic acid was mobilised in response to hyperoxic exercise when compared to normoxia ($P<0.05$, main effect for state [O_2] and state × time, interaction effects) being significantly elevated by 120-minutes post-exercise in hyperoxia compared to normoxia and hypoxia [75.1 (31) *v.* 39.5 (18.3) *v.* 46.7 (14.2) μmol/l, respectively, $P<0.05$]. This data demonstrates an effective endogenous antioxidant response and argues against a redox regulation pathway of $NO^·$ metabolism as a primary mediator of blunted vasodilatation in this scenario. This elucidates a more complex regulation of arterial tone, resulting from a metabolic pathway independent of $NO^·$ in older subjects with pre-hypertension.

This work demonstrates that (1) aerobic exercise exerts a hypotensive effect in humans with pre-hypertension, (2) ANP plays a part in the vasodilatation following exercise, (3) Free-radical mediated oxidative stress & subsequent modulation of $NO^·$ metabolism exerts a deleterious influence on post-exercise haemodynamics (4) Acute hyperoxic exercise induces a sustained vasoconstriction that is mediated via circulating ANP concentration but not by redox regulation of $NO^·$ metabolism.

CERTIFICATE OF RESEARCH

This is to certify that, except where specific reference is made, the work described in this thesis is the result of the candidate. Neither this thesis, nor any part of it, has been presented, or is currently submitted, in candidature for any degree at any other University.

Signed: .....
***Karl J. New** (Candidate)*

Signed: .....
***Professor Bruce Davies** (Director of Studies)*

Date: 19 NOVEMBER 2008

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Without the assistance of the above this thesis would not have been completed. Thank you all.

My office is only a short distance from the once grammar school of the famed Dylan Thomas (1914 – 1935).

Do not go gentle into that good night,
Old age should burn and rave at close of day;
Rage, rage against the dying of the light.

Though wise men at their end know dark is right,
Because their words had forked no lightning they
Do not go gentle into that good night.

Dylan Thomas

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GLOSSARY OF NOMENCLATURE & ABBREVIATIONS

NOMENCLATURE

Antioxidant – Chemical substance which has the ability to prevent and scavenge reactive oxygen species.

Arterial venous oxygen difference [$(a - \bar{v})O_2$diff] – The mathematical subtraction (difference) between arterial and venous oxygen content to determine oxygen utilisation across an isolated or complete vascular bed.

Diastolic – Relaxation phase of the cardiac cycle.

Free Radical – A chemical species in which one or more unpaired electrons occupy an outer orbital.

Heart Rate (HR) – Frequency with which the heart ejects blood from the left (or right) ventricle.

Lipid Peroxidation – Process whereby polyunsaturated fatty acid molecules are subjected to attack and degradation by reactive oxygen species.

Maximal Oxygen Uptake ($\dot{V}O_{2MAX}$) – Maximum rate at which an individual can take up and utilise oxygen whilst breathing at sea level.

Mean Arterial Pressure – Mathematical calculation of mean value for systemic arterial pressure incorporating systolic, diastolic & pulse pressures. Calculated via: Diastolic + 1/3 (Systolic – Diastolic).

Oxidative Stress - Collective name for detrimental free radical reactions.

Oxygen Uptake ($\dot{V}O_2$)– Volume of oxygen consumed per unit of time.

Peak Oxygen Uptake ($\dot{V}O_{2PEAK}$)- The peak rate at which an individual can take up and utilise oxygen whilst breathing at sea level.

Pre-Hypertension – Classification of blood pressure status replacing the pre 2003 "*borderline hypertension*" category. Promulgated by The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure and defined by a mean arterial pressure of 93-106 mmHg.

Pulse Pressure (PP) – The numerical difference between systolic and diastolic arterial pressures that determines the effective pressure driving flow in the circulation.

Cardiac Output (Q̇) - The amount of blood ejected from the left (or right) ventricle per minute.

Radical – Synonymous with the term 'free radical'.

Reactive Oxygen Species (ROS) – Collective term for oxygen derived molecular species.

Redox – The balance between oxidants and antioxidants.

Stroke Volume (SV) – The amount of blood ejected from the left (or right) ventricle per heart beat.

Systolic – Contraction phase of the cardiac cycle

Transit Time (TT) – The time taken for an arterial pulse wave to travel over a defined length of artery.

ABBREVIATIONS

AA – Arachidonic acid

ACE – Angiotensin converting enzyme

Ach - Acetylcholine

ADH – Antidiuretic hormone

ANOVA – Analysis of Variance

ANP – Atrial natriuretic peptide

aPTT - Activated partial thromboplastin time

aPTTr - Activated partial thromboplastin time ratio

APWV – Arterial pulse wave velocity

Ar' - *N*-(1-napthyl)-ethylenediamine

Ar-NH_2 - Sulfanilamide

AT_1 Receptor- Angiotensin I receptor

AT1 Receptor - Angiotensin II type 1 receptor

AT2 Receptor - Angiotensin II type 2 receptor

ATP – Adenosine Triphosphate

AVP – Arginine vasopressin

A1 receptor – Adenosine neural receptor

A2 receptor – Adenosine vascular receptor

BH4 - (*6R*-)5,6,7,8-tetrahydrobiopterin

BHT - Butylated hydroxyl toluene

BMI – Body Mass Index

BNP - b-type natriuretic peptide

BP – Systemic arterial blood pressure

BTPS – Body temperature pressure saturated

BV – Blood volume

$\mathbf{C_{a,O2}}$ – Arterial oxygen content

cGMP - Cyclic guanylate 3', 5'-monophosphate

CHD – Coronary Heart Disease

CHF – Chronic Heart Failure

cm – Centimetre

CNP – c-type natriuretic peptide

CNS – Central nervous system

$\mathbf{CO_2}$ – Carbon dioxide

CV – Coefficient of variation

CVa - Coefficient of analytical variation

CVw - Within subject biological coefficient of variation

CuZnSOD – Copper-zinc superoxide dismutase

°C – Centigrade

dl - Decilitre

DBP – Diastolic blood pressure

DNA – Deoxyribonucleic Acid

Δ - Difference

DAN - 2.3-Diaminonapalene

ECG - Electrocardiography

EDTA – Ethylene-diamine-tetra-acetate

EF – Endothelial function

eNOS – endothelial nitric oxide synthase

ESR – Electron spin resonance spectroscopy

$\mathbf{F_{IO2}}$ - Fraction of inspired oxygen

FOX - Ferrous Oxidation of Xylenol Orange

FXT – Functional exercise stress test

g – grams

GTN – glycerol trinitrate

Hb – Haemoglobin concentration (g/dl)

HCl – Hydrochloric acid

Hct – Haematocrit

Hcy - Homocysteine

HDL-C – High density lipoprotein cholesterol

H_2O – Water

H_2O_2 – Hydrogen Peroxide

H_2SO_4 - Sulphuric acid

HPLC – High performance liquid chromatography

H_1 receptor – Histamine vascular endothelial receptor

H_2 receptor – Histamine smooth muscle receptor

H_3 receptor – Histamine neuronal receptor

IL-6- interleukin-6

iNOS – Inducible nitric oxide synthase

INR - International normalized ratio

Kg – kilograms

l – litre

$[La^-]_B$ - Whole blood lactate

LDL-C – Low density lipoprotein cholesterol

LL – Lower limb

$_L$-NMMA - N^G – monomethyl-$_L$-arginine

LOD - L-lactate:oxygen reductase

LOOH – Lipid hydroperoxide

LVOT - Left ventricular outflow tract

LVVTI - Left ventricular velocity time integral

m. - muscle

m - metre

MANOVA – Multiple Analysis of Variance

MABP – Mean arterial blood pressure

MAP – Mean arterial pressure (blood)

MDA - Malondialdehyde

Min – minute(s)

ml – Millilitre

mmHg – millimetres of mercury

mmol/l – Millimoles per litre

Mn – Manganese

mph – miles per hour

$m.s^{-1}$- meters per second

MSNA – Muscle sympathetic nerve activity

n – Sample number

NADH – Nicotinamide adenine dinucleotide (reduced)

NAD(P)H – Nicotinamide adenine dinucleotide phosphate (reduced)

NaOH - Sodium hydroxide

NA - Noradrenaline

NAT - 2,3-naphthotriazole

NE – Norepinephrine (synonymous with NA)

$NF\text{-}_kB$ - Proinflammatory nuclear transcription complex

N_2 - Nitrogen

$NO^{\cdot}$ – Nitric Oxide

NO_2 - Nitrogen dioxide

N_2O_3 - Dinitrogen trioxide

NO^-_3 - nitrate

NO^-_2 - nitrite

NOS – Nitric oxide Synthase

nNOS – Neuronal nitric oxide synthase

NP - Natriuretic peptide system

NPRA - Natriuretic peptide receptor A

NPRB - Natriuretic peptide receptor B

NPRC - Natriuretic peptide receptor C

O_2 – Oxygen

$O_2^{\cdot-}$ - Superoxide anion

$OH^{\cdot}$ – Hydroxyl radical

$ONOO^-$ - Peroxynitrite

P – Level of significance (Probability)

% - Percentage

P_{O2} – Partial pressure of oxygen

$P_{a,O2}$ – Partial pressure of oxygen in the artery

PCV – Packed cell volume (synonymous with haematocrit)

PEH – Post exercise hypotension

P-$Fe^{2+}O_2$ - Oxyhaemoproteins

PGs - Prostaglandins

pH - Potential of hydrogen (acidity/alkalinity of a solution)

PRA – Plasma renin activity

PKG - cGMP-dependent protein kinase

PT - Prothrombin time

PUFA – Polyunsaturated fatty acid

PV – Plasma volume

PW - Pulsed-wave Doppler

PWV – Pulse wave velocity

RAAS – Renin-angiotensin-aldosterone system

RPE – Rate of perceived exertion

RPM – Revolutions per minute

RPP – Rate pressure product

RSH- Thiols

RSNO - S-Nitrosothiols

RU – Resistance units

SBP – Systolic blood pressure

SD – Standard deviation

s – Second(s)

$S_{a,O2}$ - Arterial haemoglobin oxygen saturation

SOD – Superoxide dismutase

SST – Serum separation tubes

SVC – Systemic vascular conductance

SVR – Systemic vascular resistance

SVRI – Systemic vascular resistance index

TAG - Triacylglycerol

TBA - Thiobarbituric acid

TC – Total cholesterol

Tg - Triglyceride

TNF-α- Tumor necrosis factor α

UL – Upper limb

µl – Microlitre

µmol – Micromol

V_1 receptor - AVP type 1 receptor

V_2 receptors – AVP type 2 receptor

VTI_{AO} - velocity time integral of the aorta

W - Watts

XO- Xanthine oxidase

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RESEARCH COMMUNICATIONS ASSOCIATED WITH THIS THESIS

Peer reviewed scientific presentations:

1. New K, Bailey DM, Hooper J, Davies B. Impact of circulating angiotensin II concentration on post-exercise haemodynamics in pre-hypertension. *British Association of Sport & Exercise Sciences, London, UK,* September, 2008.

2. New K, Bailey DM, James PE, McEneny J, Templeton C, Ellis G, Davies B. Effects of Hyperoxic Exercise on Post-Exercise Haemodynamics & Redox Regulation of Circulating NO Bioavailability in Man. *Physiological Society, Cambridge, UK,* July 2008.

3. New K, Bailey DM, McEneny J, Templeton C, Ellis G, Davies B. Redox regulation of Post-Exercise Hemodynamics in Hypertension. *American College of Sports Medicine, Indianapolis, USA,* May, 2008.

4. New K, Bailey DM, McEneny J, Templeton C, Ellis G, James PE, Davies B. Redox Regulation of Circulating NO Bioavailability & Post-Exercise Hemodynamics in Hypertension. *Experimental Biology, San Diego, USA,* April, 2008.

5. New K, Bailey DM, McEneny J, Davies B. Redox Regulation of systemic arterial pressure: Implication for Post-Exercise Hypotension in Hypertension. *British Association of Sport & Exercise Sciences*, Bath, UK, September, 2007.

6. New K, Bailey DM, James PE., McEneny J, Davies B. Redox Regulation of Circulating NO Bioavailability: Implication for Post-Exercise Hypotension in Hypertension *American College of Sports Medicine, New Orleans, USA,* May, 2007. ***Pollock Scholarship Young Investigator Award.***

7. New K, Hooper J, Bailey DM, Davies B. Dissociation of the Renin-Angiotensin-Aldosterone System to Post-Exercise Hypotension. *American College of Sports Medicine, Denver, USA,* May, 2006.

8. New K, Penney MD, Bailey DM, Davies B. Responses of plasma atrial nautriuretic peptide & anti-diuretic hormone to exercise under varying FiO_2 in subjects with borderline hypertension: Their role in post-exercise hypotension. *All Wales Exercise Science, Sports Medicine & Sports Psychology Symposium, Cardiff, UK,* July, 2005. ***Young Investigator Award Recipient.***

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1. New K, Bailey DM, Hooper J, Davies B. Impact of circulating angiotensin II concentration on post-exercise haemodynamics in pre-hypertension. Abstract, *Journal of Sport Science*, London, UK, September, 2008.

2. New K, Bailey DM, James PE, McEneny J, Templeton C, Ellis G, Davies B. Effects of Hyperoxic Exercise on Post-Exercise Haemodynamics & Redox Regulation of Circulating NO Bioavailability in Man. *Proceedings of the Physiological Society,* July 2008.

3. New K, Bailey DM, McEneny J, Templeton C, Ellis G, Davies B. Redox regulation of Post-Exercise Hemodynamics in Hypertension. *Med. Sci. Sports Exerc.* 2008; 40(5)S.

4. New K, Bailey DM, McEneny J, Davies B. Redox Regulation of systemic arterial pressure: Implication for Post-Exercise Hypotension in Hypertension. Abstract, *Journal of Sport Science*, 2007.

5. New K, Bailey DM, James PE., McEneny J, Davies B. Redox Regulation of Circulating NO Bioavailability: Implication for Post-Exercise Hypotension in Hypertension. *Med. Sci. Sports Exerc.* 2007; 39(5)S.

6. New K, Hooper J, Bailey DM, Davies B. Dissociation of the Renin-Angiotensin-Aldosterone System to Post-Exercise Hypotension. *Med. Sci. Sports Exerc.* 2006; 38(5) S200.

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Chapter 1

General Introduction

1.0 General Introduction

The association between exercise and hypertension has been studied intensively for many years. Most of the interest has focused on two distinct themes. One, the regulation of arterial blood pressure during acute exercise and two, the use of chronic exercise as a non-pharmacological approach to lowering arterial blood pressure (Kenney and Seals 1993). Recent studies have concentrated on a third component of the exercise response. A plethora of animal and human data have demonstrated that immediately after a single bout of exercise there are profound changes in the mechanisms that regulate and determine arterial pressure (Halliwill 2001). The changes result in a sustained reduction of arterial blood pressure, i.e. post-exercise hypotension (PEH), that lasts up to 2-hours in healthy individuals and possibly longer than 12-hours in hypertensive subjects (MacDonald 2002). Whilst the first two aforementioned aspects of the exercise response have received considerable discussion throughout the last century only relatively recently is the interest in PEH growing. Over the past 20-years an exponential growth has been seen in the number of studies investigating PEH. From this, significant contributions to the understanding of PEH have enabled insight into the key features and possible mechanisms of PEH and exercise hyperaemia. The clinical significance of PEH has only, relatively, recently been investigated, but PEH may contribute to some of the long-term adaptations associated with exercise training such as amelioration of hypertension together with homeostatic functions of plasma volume expansion (Halliwill 2001).

Hypoxia, sensed by the peripheral and medullary chemoreceptors (Marshall 1994; Lahiri 2000) is a powerful sympathetic stimulant in healthy (Calbet 2003) and diseased (Fletcher 2000; Heindl, et al. 2001) populations. In support of this, Somers et

al (1988), found enhanced sympathetic activity when borderline hypertensives were exposed acutely at rest to hypoxic gas mixtures (14% and 10% O_2). High arterial blood O_2 tension has also been shown to induce vasoconstriction in humans (Benedict & Higgins, 1911; Eggers et al, 1961; Daly & Bondurant, 1962; Crawford et al, 1997; Milone et al, 1999) that persists for an extended period on return to normal air breathing (Waring et al, 2003; Thompson et al, 2006). The mechanisms of hyperoxia-induced vasoconstriction are poorly defined but findings implicate O_2 tension in modulating one or more endothelial-derived factors that contribute to vascular tone (Messina et al, 1994; Pries et al, 1995; Mouren et al, 1997; Dallinger et al, 2000). With regards to hypertension, Ciarka et al (2005) reduced mean arterial pressure in hypertensive subjects when breathing hyperoxic gas (100% O_2). Furthermore, Izdebska et al (1998) found a potentiation of PEH in hypertensive subjects when inspiring a hyperoxic gas mixture. Indicating that attenuation of the sympathoexcitatory arterial chemoreceptor reflex may play a role in PEH.

There is little further data investigating the effect of systemic oxygen flux on PEH. To date it remains unknown if hypertensive subjects will display PEH following exercise when exposed to augmented or attenuated O_2 tension during exercise.

Likewise, the potential role of reactive oxygen species (ROS) and their interaction with other paracrine or circulating vasoactive factors, as mediators of the post-exercise response remains unexplored to date.

1.1 Experimental aims and objectives

The principal aim of this work is to utilise the acute dynamic cycle exercise paradigm in the prehypertensive (borderline hypertensive) human to investigate the potential role for metabolic parameters to mediate the phenomenon of post-exercise hypotension.

The primary objectives may be outlined as follows:

Study 1 - (a) To establish the existence of post-exercise hypotension (PEH) in a cohort of prehypertensive humans via the effects of an acute bout of dynamic exercise.

- (b) To evaluate the response of systemic arterial blood pressure following a single bout of acute dynamic exercise against the response of systemic arterial blood pressure following a control protocol.

- (c) To measure and quantify central haemodynamic parameters (stroke volume, cardiac output, stroke index) and quantify systemic vascular resistance (SVR)/ systemic vascular conductance (SVC), following an acute bout of dynamic exercise and determine the relationship to the systemic arterial blood pressure response.

- (d) To measure and quantify the effects of an acute bout of dynamic exercise on the systemic venous plasma concentration of adrenaline, noradrenaline, renin, angiotensin II, aldosterone, arginine vasopressin and atrial natriuretic peptide, implicated in the vasodilatory response underlying PEH.

- (e) To analyse the relationship between these key blood-borne metabolites and the magnitude of PEH.

- (f) To measure and quantify both upper limb and lower limb arterial pulse wave velocity (PWV) following a period of acute dynamic exercise and investigate the

relationship between PWV and the magnitude of PEH.

Study 2 - (a) To investigate the results of augmenting or attenuating O_2 tension via normobaric hypoxia or hyperoxia, respectively, during acute dynamic exercise on PEH in a 'PEH-established' cohort of prehypertensives.

- (b) To investigate the effects of systemic oxygen flux during acute dynamic exercise on the concentration of adrenaline, noradrenaline, renin, angiotensin II, aldosterone, arginine vasopressin and atrial natriuretic peptide and the relationship between these metabolites and sustained PEH in a 'PEH-established' cohort of prehypertensives.

- (c) To examine the central haemodynamic results (stroke volume, cardiac output, stroke index) of loading or unloading systemic oxygen flux during acute dynamic exercise and implications for the resultant SVR/SVC on PEH.

Study 3 – (a) To analyse and quantify, indirectly, the free radical-mediated oxidative stress response during a sustained period of PEH in a 'PEH-established' cohort of prehypertensives.

- (b) To measure and quantify venous plasma antioxidant status during a sustained period of PEH in a 'PEH-established' cohort of prehypertensives.

- (c) To measure and quantify venous plasma bio-markers of nitric oxide during a sustained period of PEH in a 'PEH-established' cohort of prehypertensives.

- (d) To investigate the redox regulation of circulating nitric oxide bioavailability following acute dynamic exercise and the implication for PEH in prehypertensives.

- (e) To examine the relationship between oxidative stress, antioxidant status, nitric oxide, arterial compliance and post-exercise haemodynamics during PEH.

- (f) To measure and quantify venous plasma haemostatic factors during a sustained period of PEH in a 'PEH-established' cohort of prehypertensives.

Study 4 – (a) To investigate the effects of O_2 tension during acute dynamic exercise on free radical-mediated oxidative stress in a PEH-established' cohort of prehypertensives.

- (b) To determine the effects of O_2 tension during acute dynamic exercise on venous plasma antioxidant status in a 'PEH-established' cohort of prehypertensives.

- (c) To analyse the effect of O_2 tension during acute dynamic exercise on redox regulation of circulating nitric oxide bioavailability and the resultant implication for PEH.

- (d) To investigate the relationship between oxidative stress, antioxidant status, nitric oxide, arterial compliance and recovery haemodynamics following manipulation of systemic oxygen flux during acute dynamic exercise in prehypertension.

1.2 Overview of thesis

This thesis is presented in 5 main chapters which are briefly outlined in the following paragraphs:

Chapter 2:

The review of literature examines existing knowledge pertaining to systemic arterial blood pressure control and haemodynamic regulatory mechanisms in humans at rest (including the classification of and recent developments into the pathophysiology of essential hypertension), during exercise and following exercise. An overview of post-exercise hypotension is presented with the latter sections focusing on potential mechanisms and haemodynamic characteristics of post-exercise hypotension. The review of literature concludes by discussing purported metabolic and endocrine mediators of the response.

Chapter 3:

The general methodology provides a description of the medical, physiological and statistical testing procedures, in addition to the biochemical analyses undertaken. Details of all equipment used and experimental paradigms are discussed. The chapter includes various preliminary quality control experiments conducted.

Chapter 4:

Study 1 was designed to investigate the effects of an acute bout of 30-minutes dynamic cycle exercise on post-exercise systemic arterial blood pressure (BP), central haemodynamics and systemic vascular resistance (SVR) in prehypertensive human

subjects. The response of systemic arterial blood pressure following the exercise bout was evaluated against the response of systemic arterial blood pressure following a sham protocol. Plasma volume corrected venous blood concentrations of several pertinent biomarkers were evaluated pre and post-exercise over the same time course, to establish any relationship between these specific blood-borne metabolites and PEH.

Study 2 examined the effect of loading or un-loading O_2 tension, via normobaric hypoxic or hyperoxic gas inspiration. Subjects were randomly assigned to three conditions each separated by approximately one week. BP was evaluated for 2-hours post-exercise after each condition to determine any influence on PEH. Plasma volume corrected venous blood was assayed to determine the response of several key blood-borne metabolites and the relationship between these metabolites, systemic oxygen flux and PEH. Physiological data was also collated during exercise in each condition.

Study 3 was designed to investigate the effects of nitric oxide, free radical-mediated oxidative stress and antioxidant status, during PEH in the prehypertensive human. The potential interactive role for these specific metabolic parameters to mediate the enhanced systemic vascular conductance, underlying PEH, was further investigated.

Study 4 investigated the results of O_2 tension during acute dynamic exercise on free radical-mediated oxidative stress, nitric oxide metabolism and antioxidant status, during sustained PEH. Physiological data was also collated during exercise in each condition.

Chapter 5:

The synthesis of findings includes the testing of the *null hypotheses* and the general discussion. This chapter integrates and summarises the research findings of all studies and considers the physiological implications and significance of the results.

Chapter 2

Review of Literature

2.0 Review of Literature

2.1 Introduction to the Area of Interest

During a single bout of dynamic exercise there are profound changes in the mechanisms that regulate and determine arterial pressure, resulting in a post-exercise hypotension that can persist for an extended time period in some individuals (Pescatello et al, 1991). Furthermore, the haemodynamic responses appear to be exaggerated in prehypertensive or hypertensive individuals (Macdonald, 2002). Whereas shorter or less vigorous exercise bouts elicit inconsistent changes in systemic arterial pressure in normotensives, post-exercise hypotension is consistently evoked following longer bouts (30-60-minutes) of moderate-intensity (50-60% $\dot{V}O_{2PEAK}$) dynamic exercise (Macdonald, 2002). In recent years interest has grown in the clinical and exercise science fields into the mechanisms responsible for the sustained reduction of arterial pressure following a single episode of dynamic exercise. It is generally accepted that PEH results from a persistent rise in systemic vascular conductance (SVC) that is not completely corrected for by increases in cardiac output ($\dot{Q}$) although there are some exceptions, notably older individuals (Hagberg and Seals, 1986) and endurance-trained men (Senitko et al, 2002). Although the putative mechanisms underlying the vasodilatation are poorly understood, the sympathetic nervous system (Floras et al, 1989; Hara and Floras, 1992; Halliwill et al, 1996; Kullics et al 1999), baroreflex resetting (Halliwill, 1996a), nitric oxide (Patil et al, 1993; Halliwill et al, 2000) and an unknown vasodilator have all been implicated (Halliwill et al, 2000; 2001).

Neither the physiological nor the pathophysiological consequences of post-exercise hypotension have been studied systematically. Classic and more contemporary

observations, however, point toward the clinical significance of PEH. The mechanisms regulating PEH may be qualitatively and quantitatively similar to those present during orthostatic intolerance and syncope following exercise. Indeed, orthostatic intolerance is common in some individuals during the first hours following exercise (Bjurstedt et al, 1983; Franke et al, 2003), and syncopal episodes immediately after (Kreidiet et al, 2004; Zeilgestein, 2004), or even during exercise (Krediet et al, 2005) are not uncommon. Studies however, have not identified what factors might influence the incidence of orthostatic intolerance after exercise or determined the extent to which PEH would predict orthostatic intolerance after exercise. A secondary arm of the clinical significance of PEH is that of long-term adaptations to exercise training and hypertension. Aerobic exercise has emerged as a powerful adjunctive intervention in the pathogenesis of hypertension (Hamer, 2006). The antihypertensive effects of aerobic exercise are poorly understood but the mechanisms playing a role in mediating PEH are likely to be linked to the long-term adaptations that occur during exercise training. However, the extent to which the pressure-lowering effects of exercise training represent the integration of the acute PEH effects remains unknown. Definitive studies linking PEH to the long-term antihypertensive adaptations associated with chronic exercise training have yet to be conducted. The final arm of the clinical significance of PEH is related to homeostatic fluid balance; Lower arterial pressures post-exercise aid the transcapillary influx of fluid into the capillary from the extravascular space due to the capillary retention of albumin (Hayes et al, 2000). It remains to be seen whether PEH has a similar facilitative effect on the plasma volume expansion that occurs 24-hours after a single bout of exercise.

The first section of the review of literature will discuss the blood pressure and

haemodynamic regulatory mechanisms present in the resting state and during exercise within the human. A brief section is included on abnormalities of blood pressure regulation in the human to provide a short synopsis on the pathophysiology of hypertension including some recent findings on purported contributory factors to the aetiology of essential hypertension. The latter sections are concerned with an overview of PEH, the haemodynamic characteristics, purported mechanisms and potential contributors to the phenomenon in order to draw a basis for the rationale for studies completed in this thesis.

2.2 Arterial Blood Pressure Regulation

The circulatory system is endowed with an extensive network for controlling arterial pressure. The importance of this exquisite pressure control network is that it prevents changes in blood flow in one area of the body from significantly affecting flow elsewhere due to the tight coupling of pressure heads in all areas. Arterial pressure is the product of $\dot{Q}$ [heart rate (HR) × stroke volume (SV)] and systemic vascular resistance (SVR) (O'Leary & Potts, 2006). That is, arterial pressure is the pressure generated by a volume of blood entering the vascular system and the resistance to the flow of that blood throughout the vascular tree. Technically, the term *blood pressure* refers to the force exerted by the blood against any unit area of the vessel wall (Guyton and Hall, 1996). Accordingly, the site of greatest resistance to blood flow in the human vascular tree is determined by vessel diameter. As highlighted by Hagen-Poiseuille's equation:

$$Q = \frac{\pi \Delta P r^4}{8 \eta l}$$

: Q = rate of blood flow

ΔP = the pressure difference between ends of the vessel

r = radius of vessel

l = length of vessel

η = viscosity of the blood

Equation 1: Hagen-Poiseuille's Equation.

Hence from the above equation the rate of conductance through a vessel is directly proportional to the forth power of the radius of that vessel. As resistance is the reciprocal of conductance the reciprocal relationship also holds i.e. resistance is inversely proportional to the forth power of the radius of a vessel. In the systemic circulation approximately 66% of the resistance is located in the small arterioles. This is due in part to their strong vascular walls (Guyton and Hall, 1996). Thus, only small changes in vessel diameter need occur to impart wide-ranging fluctuations in pressure and flow. The mean arterial pressure (MAP) is the average of all the pressures measured millisecond by millisecond over a period of time and lies closer to the diastolic pressure than the systolic pressure (Guyton and Hall, 1996).

2.2.1 Autonomic Nervous System – Sympathetic Innervation of Blood Vessels

Sympathetic vasomotor nerve fibres are the principal regulators of the circulation. They affect two areas of the circulation: firstly, through specific sympathetic nerves

that innervate the vasculature of the internal viscera and heart. Secondly, through spinal nerves that innervate the vasculature of the peripheral areas. Innervation of small arteries and arterioles allows sympathetic stimulation to increase the resistance and therefore to decrease conductance through the vascular tree. Sympathetic vasomotor nerves are under the influence of higher central nervous system (CNS) control emanating from the vasomotor centre within the medulla. The centre regulates almost all blood vessels of the body. The overriding importance of nervous control of the circulation is its capability to induce rapid increases or decreases in arterial pressure (O'Leary & Potts, 2006).

2.2.2 Baroreceptor Reflexes

The baroreceptor reflex is initiated by stretch receptors located in the walls of several of the large systemic arteries (notably the carotid sinus and wall of the aortic arch). The reflex operates via negative feedback control to attempt to maintain arterial pressure at or near optimal operating point (O'Leary & Potts, 2006). A rise in arterial pressure induces a stretch on the baroreceptor producing a reflex generation of signals to the CNS. Feedback signals are then relayed through the autonomic nervous system to provide a corrective reduction in pressure toward the normal operating level. In the normal operating range of arterial pressure at approximately 100 mmHg a slight change in pressure causes a strong change in autonomic reflexes. The reflex responses of the arterial baroreceptors are two fold. The net effects are: *1* – vasodilatation of the peripheral circulatory system and *2* – decreased heart rate and force of contraction. Both effects cause a rapid decrease in arterial pressure due to a profound decrease in SVR and $\dot{Q}$. Therefore, the baroreceptor is extremely important in buffering moment to moment changes in arterial pressure and in acting as 'rapid responders' maintaining arterial blood pressure (O'Leary & Potts, 2006). However, the baroreceptor is un-

important in the long-term regulation of arterial pressure. Due to the fact that the baroreceptors reset in 1 to 2 days to the prevailing pressure to which they are exposed (Raven et al, 2006). Thus, prolonged regulation of arterial blood pressure requires other control systems.

2.2.3 Chemoreceptors

Chemoreceptors are chemosensitive cells sensitive to oxygen lack, carbon dioxide excess, or hydrogen ion excess. Peripheral chemoreceptors are found in two principal locations: the carotid bodies (common carotid artery) and the aortic bodies (adjacent to the aorta) and as such are exposed to arterial blood. Chemoreceptors are highly perfused having an excessive blood flow for their mass (20 times their mass per minute). When arterial pressure falls, chemoreceptors become stimulated due to diminished flow of blood and supply of oxygen coupled with excessive build up of carbon dioxide and hydrogen ions. The reflex response is generated via the CNS causing feedback mechanisms to elevate the arterial pressure back towards normal operating levels. Chemoreceptor discharge is greatest when arterial pressure falls precipitously low so therefore it is more important for regulation at low arterial pressures (O'Leary & Potts, 2006).

2.2.4 Renal System

The previous sections have dealt with the powerful nervous system control of moment-to-moment, rapid changes in arterial blood pressure, highlighting the limitation of the nervous mechanisms in their inability to oppose long-term, weekly or monthly pressure changes. The dominant role of this long-term pressure control is elicited via the renal system. The basis to the renal system control mechanism is that an increase in extracellular fluid levels within the body causes a rise in arterial pressure. The elevation of arterial pressure likewise stimulates the kidneys to excrete

the excess extracellular fluid returning pressure toward baseline. This pressure diuresis is exceptionally sensitive in the human maintaining a fine control over arterial pressure. Concomitantly, a pressure natriuresis maintains a fine control of sodium concentration, which exerts additional regulation of arterial pressure. An overview of the mechanism by which increased extracellular volume increases arterial pressure is presented in Figure 2.1. According to this model an increase in $\dot{Q}$ has both a direct and indirect effect on arterial pressure. The direct effect is via its interaction with the prevailing SVR to increase the arterial pressure. The indirect effect is via tissue autoregulation of blood flow. When increased blood volume induces a rise in $\dot{Q}$ the net effect is an elevation of blood flow in all tissues of the body. These tissues respond by auto regulating their blood flow to maintain perfusion at baseline levels; the response is mediated by vascular constriction. With increased $\dot{Q}$, vascular constriction occurs throughout the body, in turn elevating SVR.

2.2.4.1 Renin-Angiotensin-Aldosterone System (RAAS): Role in Arterial Pressure Control

The RAAS is a bioenzymic cascade that plays an integral role in cardiovascular homeostasis by influencing vascular tone, fluid and electrolyte balance and the sympathetic nervous system. Renin is a small protein enzyme released by the kidneys when the arterial pressure falls too low. An increase in circulating renin concentrations causes arterial pressure to rise in several ways as a counter measure to the initial fall in pressure. Figure 2.2 displays the functional steps in which the RAAS regulates arterial blood pressure.

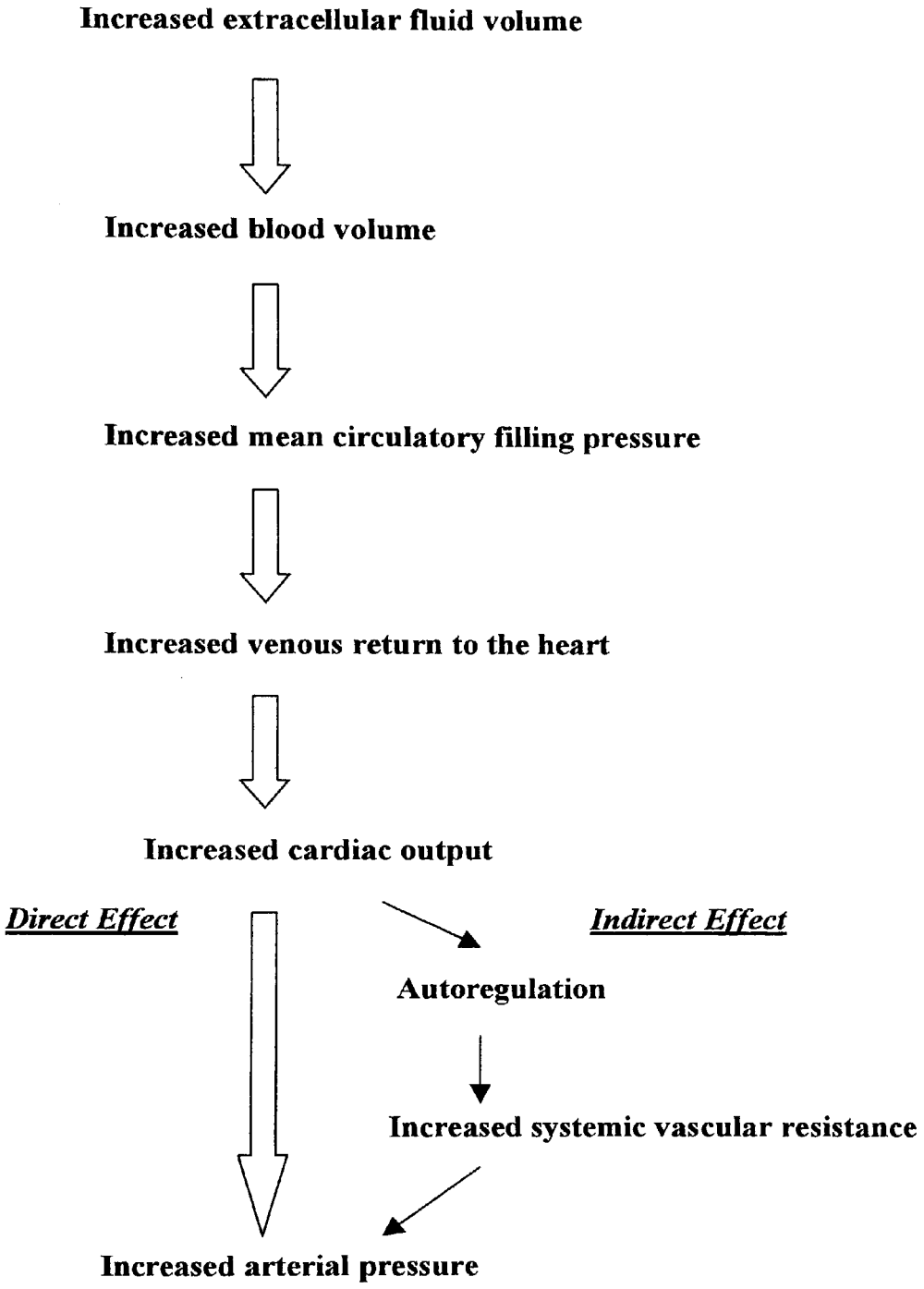


Figure 2.1. Sequential steps by which increased extracellular fluid volume increases arterial pressure (Adapted from Guyton and Hall, 1997).

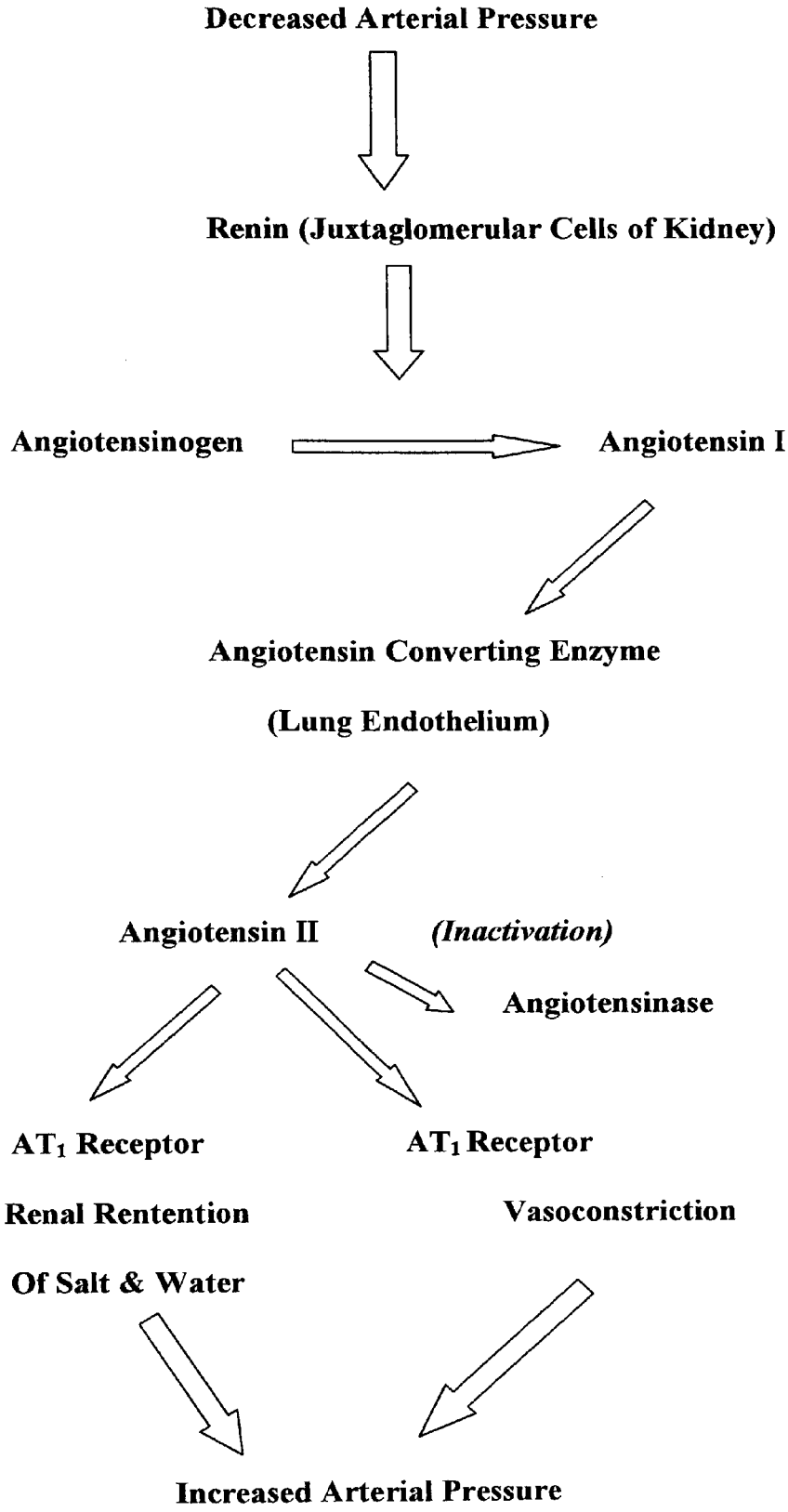


Figure 2.2. Renin-angiotensin system for arterial pressure control (Adapted from Guyton and Hall, 1997).

In response to low arterial pressure renin is released from the kidney where it acts enzymatically on the liver-derived macroglobulin angiotensinogen to produce a 10-amino acid peptide, angiotensin I. After a few seconds angiotensin I is converted by the catalyst angiotensin converting enzyme present in the endothelium of the lung vessels into the 8-amino acid peptide angiotensin II. The activity of angiotensin II persists for only 1 to 2-minutes due to its rapid inactivation by a collective group of enzymes termed angiotensinase.

Angiotensin II is a potent vasoconstrictor. One of its principal effects that elevates arterial pressure is vasoconstriction mediated via the arterioles and to a lesser extent the veins. The second major effect of angiotensin II is to decrease the renal excretion of salt and water. This then increases arterial pressure over a period of hours and days. Angiotensin causes the kidneys to retain both salt and water in two ways: Firstly via a direct action on the kidneys to cause salt and water retention. Secondly, angiotensin causes the adrenal glands to secrete aldosterone that in turn increases salt and water reabsorption by the kidney tubules (Campese and Park, 2006).

2.2.5 Abnormalities of blood pressure regulation – Pathogenesis of Hypertension

Approximately 90-95% of all people who have hypertension are said to have "essential hypertension" – in essence the hypertension is of unknown origin (Chobanian et al, 2003). Of note in most subjects with essential hypertension there is a strong heredity linkage (Chobanian et al, 2003). In essential hypertension the mean arterial pressure is increased in the region of 40-60% with marked reductions in renal blood flow due to increased renal resistance (Chobanian et al, 2003). The latest

guidelines available give precise classifications for determining the extent of blood pressure elevation and the associated health risks as depicted in table 2.0.

Table 2.0 Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure, Classification of Blood Pressure for Adults (Chobanian et al, 2003)

BP Classification	Systolic BP	Diastolic BP	Mean BP
Normal ("normotensive")	<120	and <80	<93
Prehypertension	120-139	or 80-89	93-106
Stage 1 hypertension	140-159	or 90-99	106-119
Stage 2 hypertension	≥160	or ≥100	≥120

BP, systemic arterial blood pressure; values are in mmHg

Inflammation is known to be an important independent contributor to hypertension (Niskanen et al, 2004; Sesso et al, 2003) through causal pathways of endothelial dysfunction (Verma and Yeh, 2003) and the proliferation of vascular smooth muscle (Wang et al, 2004). Furthermore, another important putative factor linked to the development of hypertension is abdominal obesity and insulin resistance that have been associated with several mechanisms including sympathetic overactivity, endothelial dysfunction, vascular stiffening and inflammation (Rahmouni et al, 2005; Hamer, 2006; Förstermann & Münzel, 2006). In support of this, recent evidence has pointed towards an important component dependent on a low-grade inflammatory process. It is purported that Angiotensin II initiates vascular stiffening by inducing oxidative stress, in turn up-regulating pro-inflammatory transcription factors such as

Nuclear factor kappaB (NF-kappaB). The end result is an increase in inflammatory mediators that produce endothelial dysfunction and vascular injury (Savoia and Schiffrin, 2007). Clear independent associations have also been documented between oxidative stress (Banday et al, 2007), the balance of pro- and antioxidant levels (Chaves et al, 2007; Zhou et al, 2006), disruption of endothelial nitric oxide synthesis (Murad, 2006), disruption of the renin-angiotensin-aldosterone axis (Campese and Park, 2006) and the development of hypertension (Hirono et al, 2007). Recent work has also focused on genetic polymorphisms predominantly involved in the development of several hypertension related disorders including the renin-angiotensin-aldosterone axis (Freitas et al, 2007), homocysteine synthesis (Markan et al, 2007), sodium and potassium transporters (Jerrard-Dunne et al, 2007) and the L-arginine transporter (Yang et al, 2007a).

In the aetiology of essential hypertension many candidate genes have been analysed including adducin, beta-adrenoceptors, G protein subunits, regulators of G protein signalling proteins, Rho kinases and G protein receptor kinases (Rosskopf et al, 2007). The individual impact of these common polymorphisms on blood pressure variation and on cardiovascular disease risk is small; however these studies have augmented current understanding on renal function regulation, cellular signal transduction and the integration of both in the pathogenesis of hypertension.

There is an abundance of data that suggests a predisposing factor of essential hypertension is directly related to microvascular dysfunction. The evidence for this is found via: 1, abnormal regulation of vasomotor tone leading to enhanced vasoconstriction or reduced vasodilatation. 2, decreased arteriolar diameters and increased wall-to-lumen ratios of small arteries have been demonstrated in pre-hypertension. 3, a reduction in the rarefaction of arterioles, venules and capillaries

observed in different vascular beds with hypertension (Jonk et al, 2007). Obesity is a designated risk factor in the aetiology of essential hypertension and a host of recent data indicates that obesity is directly associated with microvascular dysfunction (de Jongh et al, 2004; De Filippis et al, 2006).

The first main mechanism of obesity-associated microvascular function centres on the metabolic action and intracellular signalling pathway of insulin. Insulin has both direct vasodilatory characteristics via stimulation of endothelial nitric oxide synthase (eNOS) activity and vasoconstrictor effects mediated to a large degree by insulin-stimulated endothelin-1 production in the vascular endothelium (Jonk et al, 2007). The normal, antagonistic, endothelial-derived vasoconstrictor and vasodilatory effects of insulin are neutral or slightly vasodilatory. Obesity may thus disrupt this delicate balance wherein the vasoreactivity is shifted from vasodilatation to vasoconstriction. Direct support for this is demonstrated in obese, hypertensive individuals who display insulin-induced vasoconstriction and augmented endothelin-1 dependent vasoconstrictor tone with concomitant decreased nitric oxide-dependent vasodilator tone (Cardillo et al, 1998; 2004).

The second main mechanism operating in obesity-associated microvascular dysfunction is that of signalling pathways between adipose tissue and the microcirculation. Recent data has indicated that contrary to being regarded as a passive storage depot, adipose tissue is in fact a highly active member of the endocrine system (Jonk et al, 2007). Adipose tissue secretes a plethora of bioactive substances called adipokines. Obesity skews the balance of the normal secretion such that there is an enhanced production of angiotensinogen, leptin, resistin, inflammatory cytokines e.g. tumor necrosis factor (TNF)-α and interleukin-6 (IL-6) (Jonk et al, 2007), whereas the release of adiponectin, an anti-inflammatory mediator is

attenuated (Arita et al, 1999). The increased production and release of the former bioactive molecules is linked to increased blood pressure through mechanisms that are not fully elucidated but that involve the microcirculation and an imbalance in the endothelial vasoconstrictor and vasodilator pathways (Jonk et al, 2007). Moreover, all components of the RAAS necessary to evoke the vasoconstrictor angiotensin II are expressed in human adipose tissue (Karlsson et al, 1998; Schling et al, 2001). Therefore, the mass of adipose tissue may be directly related to the activity of the RAAS such that gains and reductions in body mass (fat mass) leads to parallel changes in RAAS activity. RAAS activity exerts deleterious effects on the microcirculation and hence arterial pressure through a variety of mechanisms mediated via increased angiotensin II, involving inhibited nitric oxide (NO) synthesis (Sowers et al, 2001), reactive oxygen species generation (Zhao et al, 2006), endothelin-1 production in the endothelium (Paul et al, 2006) and stimulation of inflammatory cytokines (Horiuchi et al, 2006). Therefore, it appears that obesity and more specifically adipose tissue, plays a central role in the proliferation of hypertension via its interaction with the microcirculation coupled to a prominent role for stimulating RAAS activation.

2.2.6 Arterial Pulse Wave Velocity (APWV)

Blood pressure measurements elicit information regarding the prevailing blood pressure at an isolated point in the circulatory tree i.e. the cuff sphygmomanometer only measures BP in a specific artery (e.g. the brachial artery). Over the last decade or so, new techniques have evolved that provide direct in vivo assessments of vascular health and arterial stiffness (AS) in humans. Various terms are used to express stiffness, such as distensibility and compliance. Various indirect indices are also used, including pulse wave velocity (PWV), characteristic impedance and augmentation

index (AI^x).

The elastic behaviour of arteries converts pulsatile cardiac ejection into nearly continuous tissue perfusion and reduces systolic pressure relative to flow; this creates a reduced workload relative to perfusion, producing "cardiovascular efficiency". Arteries are characterised by their distensibility (change V/change P) where, V is luminal volume and P is transmural pressure, respectively. Distensibility is therefore determined by the structural components of the arterial wall, smooth muscle tone, and transmural pressure. PWV is inversely related to arterial distensibility (Avolio et al, 1983; Breithaupt et al, 1992; Belz, 1995). Distensibility of large arteries is determined not only by the passive components of the arterial wall but also by changes in vascular tone (Kingwell et al, 1997; Belz, 1995; Ramsey et al, 1995).

Separate from any role as a surrogate marker, Lehmann et al, (1996) showed that AS is an important determinant of pulse pressure, left ventricular function and coronary perfusion pressure. Precise quantification of AS is difficult as stiffness between arteries and even in the same artery varies at different pressures. Proximal elastic arteries and peripheral muscular arteries respond differently to aging and to medication.

The chronic effects of aging and disease on resting arterial distensibility have attracted increasing clinical interest (Avolio et al, 1983; Dart et al, 1991; Lacombe et al, 1992; Cameron et al, 1995; Gatzka et al, 1998). Arterial distensibility and arterial compliance decrease with age and pathological conditions such as atherosclerosis leading to increased SBP, a widening of pulse pressure (PP) and increased left ventricular work, resulting in left ventricular hypertrophy; itself an independent risk factor for cardiovascular morbidity (Laogun and Gosling, 1982; Riley et al, 1986). Studying the changes in vascular properties is of interest because such events precede

the development of more progressive cardiovascular maladies such as coronary artery disease and hypertensive heart disease (Cohn et al, 1995). Moreover, information regarding APWV and compliance alterations both at rest and following exercise provide an insight into systemic vascular responses throughout the arterial tree in vivo. Data indicates that both APWV and compliance is modified early in the course of essential hypertension (Brinton et al, 1996). Moreover, Goodfellow et al (1996) and Ramsey et al (1995) have shown that distensibility, estimated via APWV, is acutely augmented during hyperaemia in normal subjects but not in patients with heart failure or diabetes where shear-related endothelium-mediated vasodilatation is impaired.

A measurement of the physical properties of the vasculature requires determination of the distending force (i.e. arterial blood pressure), preferably at the site of measurement using pressure transducers. The propagation of the pulse wave through the walls of the blood vessels is affected by the mechanical properties of the vessels. The phenomenon of pulse-wave augmentation results from reflections of the pulse wave at distal sites (such as bifurcations), so that the forward wave and reflected wave superimpose (O'Rourke, 1999). This combination of waves produces the typical arterial pulse wave, and due to both progressive reductions in force along the vasculature and differences in wave summation, the arterial pulse wave differs at different sites. The PWV and the amplitude of reflected waves are increased in stiffer arteries and conversely are reduced with increased arterial compliance (O'Rourke et al, 2001). These vasculature features are dependent on a number of haemodynamic variables, including heart rate and PP (Wilkinson et al, 2002a). APWV measurements are also affected by vaso-active drugs (O'Rourke, 1992; Kelly et al, 2001; Wilkinson et al, 2001a) and are sensitive to inhibition of nitric oxide synthase (NOS) (Kinlay et

al, 2001; Stewart et al, 2003). The application of these measurements with concomitant vasodilators has been proposed as an alternative approach for the investigation of vascular function (Wilkinson et al, 2001b).

2.2.7 Measurement of APWV

Measurement of arterial wave propagation as an index of vascular stiffness and vascular health was first investigated in the early part of last century (Bramwell et al, 1922). The arrival of the pulse wave at two different arterial sites is timed and with estimates of the length of blood vessels between these sites (by measuring the distances at overlying skin sites), the velocity of the wave is calculated in $m.s^{-1}$ (O'Rourke, 1999; Lehmann, 1999; Wilkinson et al, 1999). Slight variations occur in correctly determining the intervening distances. This methodological approach provides a reliable measure of arterial stiffness. At a given blood pressure, the stiffer the vessel, the less time it takes for the pulse wave to traverse the length of the vessel, reciprocally, the more compliant the vessel the longer the transit time (TT) of the pulse wave. APWV, especially of the aorta, has emerged as a powerful independent predictor of cardiovascular events (Blacher et al, 1999a).

PWV, which is inversely related to the square root of distensibility, is defined by the Moens-Koeteweg equation:

$PWV = (Eh/2\rho R)$ **Equation 2. Moens-Koeteweg Equation**

Where: E is Young's modulus of the arterial wall

h is wall thickness

R is arterial radius at the end of diastole

ρ is blood density

The time delay between the arrival of a predefined part of the pulse wave, such as the foot, at 2 points is obtained by either simultaneous measurement, or by gating to the

peak of the R-wave of the electrocardiograph (ECG). The foot of the waveform is traditionally measured because wave reflections from the previous cardiac cycle have generally ceased by the time of arrival of a new systolic wave front (Avolio et al, 1983).

Measurement of pulse transit time depends critically on accurate determination of its potentially changing waveform. Using the timing of the foot of the waveform to elicit PWV is inherently imprecise and various techniques have evolved to improve precision, such as identifying the point of the sharp upstroke of the wave (Avolio et al, 1983), taking the intercept between tangents of systolic upstroke and diastole, or its first or second derivative (Kingwell et al, 1997; Liang et al, 1998). In this thesis the pulse wave was characterised and timed by averaging three points along the first 50% of its rising phase during which its upstroke should be relatively uncontaminated by changes in waveform due to reflection of waves and propagation (Naka et al, 2003). Determination of the arterial pulse wave can be achieved through a variety of methods. Arterial pulse waves can be detected by utilising pressure-sensitive transducers (Asmar et al, 1995), Doppler ultrasound (Sutton-Tyrell et al, 2001), or applanation tonometry (Wilkinson et al, 1998b). A novel algorithm contained within the particular apparatus allows the time delay between the foot of two pressure waveforms of similar morphology to be measured with high precision and in real time. The system then measures the TT of the arterial pressure waveforms (Ramsey et al, 1995a).

APWV determinations have been validated against both endothelium-dependent agonists (e.g. Acetylcholine [Ach]) and -independent agonists (e.g. Adenosine) (Ramsey et al, 1995a; 1995b; Doshi et al, 2001). Chronic heart failure (CHF) patients

have unaltered APWV in response to endothelium-dependent agonists whereas normal subjects have a reduction in APWV [increased distensibility] to both endothelium-independent and -dependent agonists (Ramsey et al, 1995b).

Raised APWV occurs with a range of established cardiovascular risk factors (Lehmann, 1998) including age (Bramwell et al, 1923; Vaitkevicius et al, 1993), hypercholesterolaemia (Lehmann et al, 1992a) and sedentary lifestyle (Vaitkevicius et al, 1993). In hypertension, an increased carotid-femoral APWV is an independent predictor of both cardiovascular and all-cause mortality (Laurent et al, 2001).

O'Rourke et al, (2002) reported APWV ranging from 9 to 13 $m.s^{-1}$, whereas recent data report carotid-femoral APWV in healthy individuals between 24 and 62 years of age from 6 to 10 $m.s^{-1}$. In hypertensive subjects without a history of overt cardiovascular disease, APWV also predicts the occurrence of cardiovascular events independently of classic risk factors (Boutouyrie et al, 2002). Moreover, in this regard, aortic APWV $>13 m.s^{-1}$ is a particularly strong predictor of cardiovascular mortality in hypertension (Blacher et al, 1999a).

2.2.8 Physiology & Pathophysiology of Arterial Stiffness

An early tenet of the investigation into the circulatory system was that it is characterised by a Windkessel effect *(Germanic for 'elastic reservoir')*. Windkessel theory states that the circulation is a central elastic reservoir (the large arteries), into which the pulsatile ejection of the heart pumps, and from which blood travels to the tissues through relatively non-elastic conduits (peripheral arteries) (Oliver and Webb, 2003). The compliance and elastic recoil of the large arteries ensures the conversion of pulsatile ejection into steady perfusion throughout the vascular tree. The Windkessel effect also prevents excessive rises in arterial pressure during systole and maintains arterial pressure during diastole. The elasticity of the proximal large

arteries, such as the aorta, is the result of the high elastin to collagen ratio in their walls, which progressively declines toward the periphery. For a simple thin-walled elastic tube, wall tension increases with diameter *[Laplace's Law: **T = (PR)/M.** Where: T is the tension in the walls, P is the pressure difference across the wall, R is the radius of the cylinder, and M is the thickness of the wall]* but the relation is more complex in a muscular conduit artery, such as the brachial and radial arteries. The wall has finite thickness, its individual constituents have different elastic properties, and regional resting arterial diameters vary. The artery shifts from a dominance of elastin to collagen as the artery is distended, these have different stiffnesses and as such elastic change is non-linear with corresponding arterial pressure and diameter changes.

The elasticity of a given arterial segment depends on its distending pressure (Greenfield & Patel, 1962). As distending pressure increases, there is greater recruitment of relatively inelastic collagen fibres (Bank et al, 1996) and, consequently, a reduction in elasticity. MAP determines the prevailing distending pressure in the systemic circulation. Increases in distending pressure increase PWV (Bramwell & Hill, 1922), therefore account should be taken of the level of systemic BP in studies that use PWV as a measure of interventions that reduce BP, so that real differences in the elasticity of the arterial wall can be differentiated from modulations in distending pressure.

The decrease in arterial compliance with age (Vaitkevicius et al, 1993) is the result of progressive elastic fibre degeneration (Avolio et al, 1998) in the arterial wall. PWV in the brachial artery has been reported as increasing with age, indicative of worsening arterial compliance (Avolio et al, 1983). The Windkessel effect is also lost in arterial senescence and in pathological changes to arterial smooth muscle such as

arteriosclerosis and atherosclerosis. Thus, there is augmented SBP in large arteries [isolated systolic hypertension] and decreased DBP, resulting in widening of PP and ineffective peripheral tissue perfusion (Wilkinson et al, 2002a).

In addition to collagen and elastin, the endothelium (Kinlay et al, 2001; Wilkinson et al, 2002b) and arterial smooth muscle bulk and tone (Bank et al, 1996; 1999) also influence elasticity. Genetic influences on arterial stiffness have been elucidated. Polymorphic variation in the fibrillin-1 receptor (Medley et al, 2002), angiotensin II type-1 (V_1) receptor and endothelin receptor (Lajemi et al, 2001a; 2001b) genes are related to arterial compliance. The angiotensin-coverting enzyme (ACE) I/D polymorphism has also been associated with arterial stiffness (Balkestein et al, 2001) but not consistently (Lajemi et al, 2001b).

The ejection of blood from the left ventricle into the systemic circulation initiates an arterial pressure wave that travels toward the periphery. At points of impedance mismatch, principally at the sites of high-resistance arterioles, wave reflection occurs (Nichols & O'Rourke, 1998). As a result of differing elastic qualities and wave reflection, the shape of the arterial waveform varies throughout the arterial tree. The contour and amplitude of the pressure waveform are influenced by large artery PWV. Faster travelling pressure waves arrive at, and are reflected from, the peripheral circulation earlier. In compliant arteries, APWV is relatively slow and thus reflected waves return to the central aorta in diastole; this in turn augments DBP and coronary blood flow, which occurs in diastole. When APWV is increased as a consequence of reduced distensibility, more rapid reflections of pressure waves to the heart will arrive earlier in the cardiac cycle, in turn augmenting central SBP, opposing left ventricular ejection, reducing stroke volume, increasing left ventricular pressure and compromising myocardial oxygen consumption (Bogren et al, 1989; Ohtsuka et al,

1994). Thus, attenuated distensibility is functionally disadvantageous, particularly in the presence of cardiac dysfunction, hypertension or coronary artery disease.

It is important not to consider the heart, large arteries, and small arteries as separate entities with fundamentally different properties. They jointly comprise an integrated circuit. Large arteries affect cardiac performance and cardiac performance will ultimately determine blood flow in small arteries. Microvascular dilatation will reduce peripheral resistance and so increase blood flow throughout the vascular tree, resulting in endothelium-mediated dilatation of large arteries, which thereby become more distensible, unloading the heart and promoting arterial flow. Endothelial dysfunction in large arteries may thus impair the mechanical integration between the heart and resistance vessels.

Vascular tone, modulated via endothelial and neurohumoral influences, together with the arterial radius and inherent elastic wall components mediate arterial distensibility. An increase in intravascular diameter, such as occurs during vasodilatation of an artery, will stretch structural components of the arterial wall along their steepening non-linear compliance curve, thereby increasing arterial distensibility (reducing APWV) to an extent determined by the arc traversed during pulsation. Mean pressure has been regarded as an acceptable measure of such movement (Kingwell et al, 1997). Vasodilatation may therefore increase or decrease APWV. An increase in APWV may occur due to an increase in intra-arterial diameter or an attenuation of APWV may occur during vasodilatation due to the direct action of reducing vascular smooth muscle tone. It appears through investigations with vasoactive drugs that the effect of smooth muscle tone supersedes the passive consequence of stretch (Naka et al, 2000). Some debate exists as to the overriding influence of HR on APWV. Early in vivo pacing studies indicated that HR may have a direct effect on APWV (Mangoni et al,

1996; Liang et al, 1999). Other studies have shown no relationship of HR to APWV (Callaghan et al, 1984; Albaladejo et al, 2001). In the study of Naka et al (2003) a discordant relationship was seen between post-exercise changes in HR and modulations of APWV over the same time-frame. Later studies demonstrate that acute increases in HR markedly attenuate arterial distensibility in both large and middle-size conduit arteries within a HR range of 60-110 beats per minute (Giannattasio et al, 2003). An increase in HR of 40 beats per minute (bpm) increased PWV greater than 1 $m.s^{-1}$ (Lantelme et al, 2002). Thus, it appears prudent to standardise PWV for prevailing HR level to interpret changes in PWV throughout an intervention.

2.2.8.1 Flow mediated changes in APWV as a marker of Endothelial Function

Endothelial function is a hallmark of a healthy vascular response and is altered by numerous pathophysiological insults. Endothelial 'dysfunction' is noted to play a seminal role in the aetiology of many cardiovascular maladies such as atherogenesis (Alexander and Dzau, 2000) and also in attenuation of circulatory 'efficiency' (Henderson, 1997). The key characteristic of endothelial dysfunction is an impairment of flow-mediated arterial dilatation, which can be assessed non-invasively. Despite the wide use of flow-mediated changes in brachial arterial diameter to provide a quantitative evaluation of impaired endothelial function, this technique is critically dependent on operator skill limiting the clinical utility of this approach (Naka et al, 2006). As discussed previously, PWV provides a quantitative assessment of arterial distensibility, which itself is influenced by dynamic changes in vascular tone. Thus, PWV may be used to investigate acute flow-related modulations in vascular tone without pharmacological provocation. Naka et al (2006) recently reported such an approach that appears to be operator independent and relatively robust.

In using the acute response to increased flow, each subject acts as their own control, in contrast to using resting PWV as a measure of arterial stiffness that is dependent on several contributing influences (such as endothelial dysfunction). Naka et al (2006) reported flow-mediated changes in upper and lower limb PWV in 17 healthy control subjects and compared the findings to the same parameter in 8 patients with stable CHF. CHF patients were investigated as they are characterised by impaired endothelial function whereas endothelium-independent dilator responses remain intact. A further corroborative investigation of PWV and brachial artery responses to endothelium-dependent and –independent pharmacological stimuli was included in 8 further, healthy subjects. Flow-mediated reduction of upper and lower-limb PWV (14% with no change in BP) was found in healthy subjects whereas the response was almost completely absent in the CHF patients. The authors concluded that the PWV measurements appear to be inversely related to and relatively greater than brachial artery diameter responses (Naka et al, 2006).

This method of endothelial function assessment provides ongoing assessment of both atherogenic risk and may also provide potentially important information regarding microvascular perfusion, whose control of homogeneity has been shown to be endothelium-dependent (Griffith et al, 1987).

2.2.8.2 Specific APWV Exercise Studies

Following an acute bout of dynamic exercise, Naka et al (2003) showed a 6-10% and 10-23% decline in upper and lower limb APWV, respectively, from 10 to 60-minutes post-exercise. These observed changes in APWV can be attributable to responses in vascular tone to recovery from net systemic vasoconstrictor and local vasodilator signals elicited via exercise, culminating in persistent post-exercise vasodilatation. Furthermore, evidence indicates that aerobic exercise training increases arterial

compliance. Cross-sectional studies indicate that aerobically trained athletes have higher arterial compliance than sedentary individuals (Mohiaddin et al, 1989; Kingwell et al, 1995; Vaitkevicius et al, 1993; Bertovic et al, 1999). Distensibility has also been shown to increase following 4-weeks of exercise training (Cameron and Dart, 1994) and when first measured 30-minutes following an acute bout of sub-maximal cycling exercise (Kingwell et al, 1997). Interestingly, augmented arterial compliance can be isolated to a limb-specific vascular bed via exercise training. Giannattasio et al, (1992) reported increased arterial compliance in the radial artery of the dominant arm, compared to both the contra-lateral arm and an inactive control, in hammer throwers. Miyachi et al, (2003) demonstrated that contrary to the efficacious nature of regular aerobic exercise, resistance training lowered central arterial compliance in healthy middle-aged men due to increased left ventricular hypertrophy. Moreover, several months of resistance training "reduced" central arterial compliance in healthy men in comparison to a control group (Miyachi et al, 2004). Frequent exposure to moderate bouts of sub-maximal exercise is an integral part of life yet there appears to be only a modest amount of data reported for normotensive sedentary or athletically trained individuals. Therefore investigation into the acute effects of exercise on this important physiological variable of large artery distensibility in hypertensive subjects appears warranted.

2.2.9 Blood pressure regulation during exercise

Systolic and arterial pulse pressures increase during conventional forms of large-muscle dynamic exercise and both systolic and diastolic blood pressure increase during small-muscle dynamic and isometric contractions (O'Leary & Potts, 2006). To recall from section 2.2 the increase in systemic arterial pressure during exercise is initiated via the autonomic nervous system eliciting vagally-mediated tachycardic

increases in $\dot{Q}$ and increases in SVR in certain tissue and vascular beds (O'Leary & Potts, 2006). As discussed in section 2.2.2 the arterial baroreceptors play a pivotal role in the moment-to-moment control of arterial pressure. It was initially viewed that arterial baroreflex control of systemic arterial blood pressure was either "switched off" or "overridden" during the transition from rest to static exercise to allow the concomitant rise in heart rate and systemic arterial pressure that occurs during this transition (Raven et al, 2006). Later data emerged elucidating that the arterial baroreflex was functional during exercise and was necessarily complicit in the normal cardiovascular response to exercise. The baroreflex appears to be 'reset' during exercise allowing the baroreflex to operate at the prevailing arterial pressure evoked by the exercise bout (Raven et al, 2006). Moreover, these recent investigations have confirmed that resetting of the baroreflex is in relation to the intensity of dynamic exercise without a change in sensitivity of the reflex. The original mechanism proposed to be responsible for baroreceptor resetting during exercise was that central command (heart rate, oxygen consumption and ratings of perceived exertion) and the muscle metaboreflex (the chemical component of the exercise pressor reflex) mediate the observed resetting of the reflex (Rowell & O'leary, 1990; Raven et al, 2006). In addition, more recent studies have indicated that activation of central command or the exercise pressor reflex, in isolation or combination, is required for the baroreflex to be reset with exercise. Although, it has been suggested that input from all three neural mechanisms (central command, the exercise pressor reflex and the arterial baroreflex) is required for the normal physiological response of arterial blood pressure to exercise (Figure 2.3) (Raven et al, 2006).

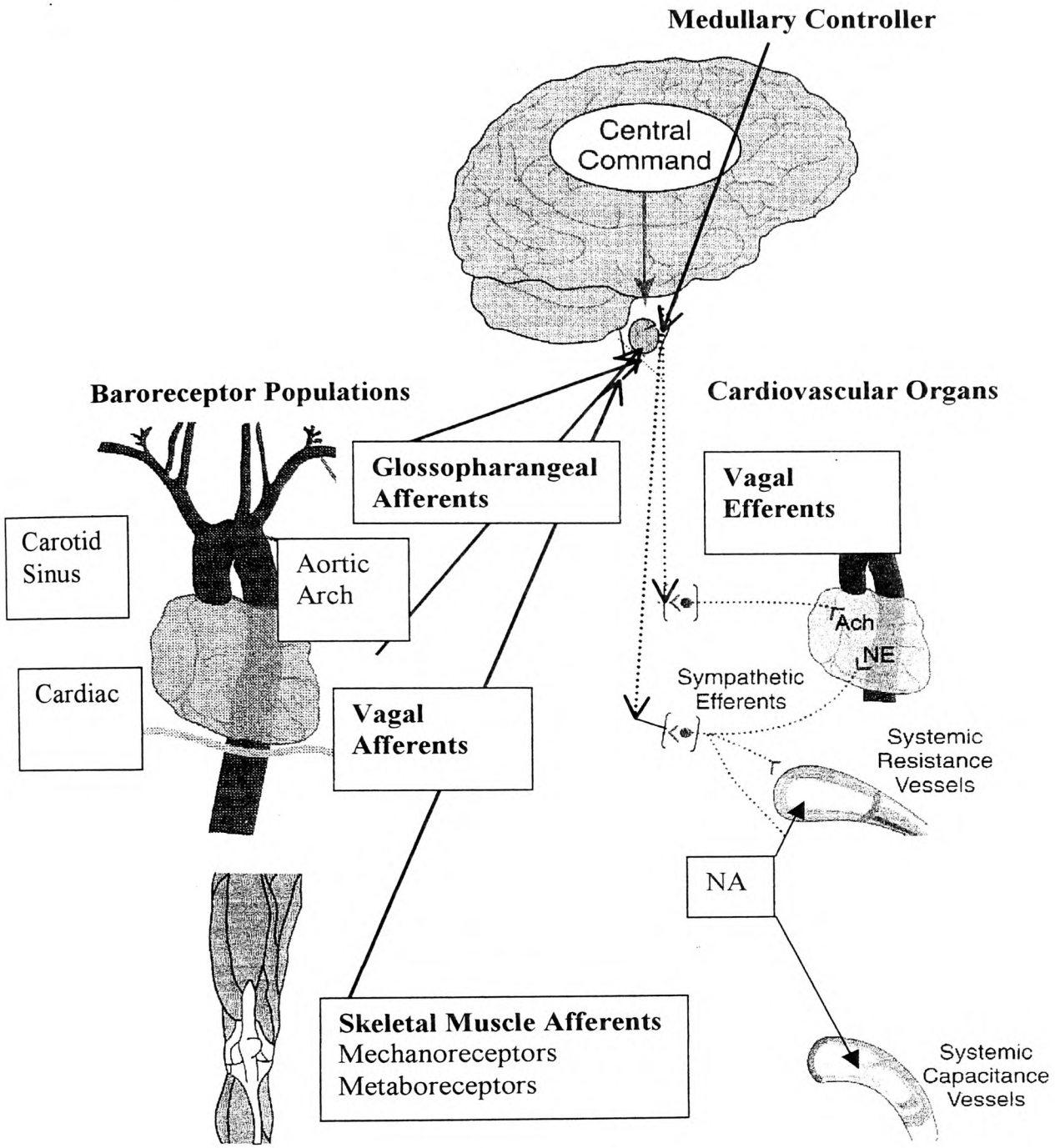


Figure 2.3. Mechanisms mediating the large cardiovascular responses to exercise (O'Leary & Potts, 2006). NA/NE, noradrenaline/norepinephrine; Ach, acetylcholine. Activation of central command, skeletal muscle afferents and resetting of the arterial/cardiopulmonary baroreflexes together affect areas that control sympathetic and parasympathetic activity to the heart and peripheral blood vessels.

2.3 Blood pressure regulation following exercise: Post Exercise Hypotension

Post exercise hypotension (PEH) is defined as a reduction in systolic and/or diastolic arterial blood pressure below control levels after a single bout of exercise (Kenney & Seals, 1993). The phenomenon of PEH was first noted in the literature some 111 years ago (Hill, 1897). Hill observed the decline in his own arterial blood pressure for 90-min following the 400-yard dash. In 1922, Schneider and Trunesdell reported that standing blood pressures were lower 2-minutes after a step exercise protocol (Schneider & Trunesdell, 1922). A further investigation also documented orthostatic intolerance following exercise (Eichna, 1947). The first report of PEH stood in isolation for 84 years until a seminal anecdotal report (Fitzgerald, 1981) upon the effects of jogging on Fitzgerald's own labile hypertension emerged. Fitzgerald noted that jogging 3 miles in approximately 25 minutes (~ 8 minute mile / 7.5 mph) lowered his resting arterial blood pressure (ABP) below pre-exercise levels (~ 12 mmHg) and that the decrease persisted for several hours (4-10 hours). Fitzgerald concluded that the effect induced by the dynamic exercise was valuable in lowering ABP. Around the same time an investigation by Bjurstedt et al, (1983) documented an increased incidence of orthostatic collapse immediately following a single bout of strenuous leg exercise. Since then the medical and scientific community have systematically examined PEH. Several investigators have reported a statistically and clinically significant reduction in ABP following a single bout of dynamic exercise in individuals with mild hypertension (MacDonald, 2002). Wilcox et al, (1982) investigated both normotensive and hypertensive subjects following an acute bout of dynamic exercise (treadmill walking). The investigators found a significant decline in systolic blood pressure (SBP), following the exercise bout, in both the normotensive (-22%) and hypertensive (-25%) individuals. This decline persisted throughout the

entire 30-minute recovery period. Kaufman et al, (1987) also investigated the hypotensive effects of a single bout of dynamic exercise in normotensive and hypertensive individuals. The results elucidated that SBP was significantly decreased (~12 mmHg) for the 60-minute recovery period. There have been a plethora of further studies demonstrating that the blood pressure lowering effect of exercise is sustained for several hours following exercise (Hannum & Katch 1981; Hagberg et al, 1987). Hagberg et al, (1987) reported that SBP was significantly reduced in older hypertensive men for up to 3-hours following moderate dynamic exercise. Hannum and Kasch, (1981) highlighted a reduction (~12 mmHg) in SBP for 2-hours following moderate intensity dynamic exercise in hypertensives.

The first study to note a reduction in BP over an extended period of time was that of (Pescatello et al, 1991). The investigators noted a fall in mean arterial pressure (MAP) (~8 mmHg) for up to 12.7 hours following moderate dynamic exercise, evaluated via ambulatory monitoring. However this investigation has been challenged by Somers et al, (1991) who reported that following exhaustive dynamic exercise BP was not different from baseline values 2-24 hours post-exercise. Further data on the magnitude and purported mechanisms of PEH have been collected (MacDonald, 2002) as well as the relationship between exercise and PEH, elucidating the benefits of different exercise modes and durations upon PEH (DiCarlo, 1994).

2.3.1 Human model of PEH

PEH has clearly been demonstrated in human subjects. PEH appears to have a variable response in differing populations with the critical factor appearing to be the current state of blood pressure prior to taking part in the exercise bout. PEH is well documented in humans with both borderline hypertension (MacDonald, 2002) and established hypertension (MacDonald, 2002), with the greatest response occurring in

those individuals with the highest blood pressure (Kenney & Seals 1993). The occurrence of PEH in normotensive humans is inconsistent (MacDonald et al, 1999a). PEH appears to be unaffected by gender as evidenced by both gender specific (Pescatello et al, 1999; Pescatello, 2004; Guidry, 2006) and mixed gender studies finding similar degrees of hypotension (Raglin, 1993; Brown, 1994; Reuckert, 1996; Halliwill et al, 2000; Senitko, 2002; Lockwood et al, 2005b; Williams et al, 2005; McCord et al, 2006). Lynn et al, (2007) directly investigated the effects of both gender and menstrual cycle on post-exercise haemodynamics, finding no differences between men or women. It also appears that PEH is independent of age being reported across the human age span of young adults (Kaufman et al, 1987) to older adults aged up to 67 years (Hagberg et al, 1987).

2.3.2 Animal model of PEH

Along with data produced from the human population concomitant data has been promulgated from studies utilising the rodent model. Because exercise is associated with activation of somatic afferents (Kenney and Seals, 1993), electrical stimulation of the sciatic nerve and of hind limb skeletal muscles in the rat has been used to study post-stimulation hypotension (PSH). For the purpose of this thesis PSH and PEH will be used synonymously. PEH has been documented in Wistar-Kyoto normotensive (Yao 1982) and spontaneously hypertensive rats (SHR) (Chandler and DiCarlo 1997; Kulics 1999). PEH is also observed in conscious (Yao 1982; Hoffmann 1988) and anaesthetised SHR (Shyu et al 1984). Data also indicate that the Dahl salt-sensitive rat exhibits PEH (Kenney et al 1991) whereas the Dahl salt-resistant (Kenney et al 1991) and renal hypertensive rat (Hoffmann, 1986) do not display PEH. Taking into consideration the variable response of PEH in normotensive humans, the lack of hypotension in these particular animal studies is consistent with the human data.

Kenney et al (1991) suggest that the degree of PEH may be related to the genetic pre-disposition of the animal to hypertension.

2.3.3 Magnitude of blood pressure decline during PEH in humans

From the published literature the average reduction in blood pressure following exercise is approximately 8/9 mmHg (systolic/diastolic blood pressure) in normotensive subjects 14/9 mmHg in borderline hypertensive subjects and 10-20/7 mmHg in hypertensive subjects (MacDonald 2002). The most recent studies investigating PEH have reported reductions of mean arterial blood pressure in the range of 4-9 mmHg predominantly in normotensive individuals (Lynn et al, 2007; McCord and Halliwill 2006; McCord et al, 2006; Lockwood et al, 2005; Williams et al, 2005; Lockwood et al, 2005a; 2005b; Wilkins et al, 2004; Halliwill et al 2003; Senitko et al, 2002).

2.3.4 Exercise characteristics and PEH

PEH has been documented following a variety of aerobic exercise modalities, with the most common being cycle ergometry (MacDonald 2002). Limited evidence also supports the use of resistance exercise to produce a sustained PEH. In a direct comparison of the haemodynamic responses to aerobic and resistance exercise MacDonald et al (1999b) found no difference in the magnitude or duration of the observed hypotension between exercise modalities.

2.3.4.1 Intensity of Exercise

The investigation of the effects of exercise intensity on PEH has been one of the most intensely studied areas of PEH research. The majority of studies have utilised submaximal cycle ergometry protocols between 40-100% of maximal exercise capacity. A number of physiological markers have been used to signify attainment of sub-maximal exercise level (MacDonald 2002). The weight of evidence suggests that

PEH persists independently of exercise intensity. One study (Piepoli et al, 1994) has reported that PEH may be exercise intensity dependent. The study of Piepoli et al, (1994) used normotensive subjects and only found PEH following maximal exercise. A possible explanation is that maximal exercise induces a greater effect on the haemodynamic response in sedentary, normotensive individuals.

2.3.4.2 Duration of Exercise

PEH has been observed following a vast array of exercise durations from 10-minutes (Bennet et al, 1984) up to 170-minutes (Seals et al, 1988). The common time frame for most studies however appears to be between 20 and 60 minutes. Although data on humans is inconclusive (MacDonald et al, 1999a; 2000) the body of literature (taking into consideration animal data) indicates that the duration of hypotension may be influenced by the exercise duration (Hoffmann 1988; Overton 1988; Halliwill, 2001; MacDonald, 2002).

2.3.5 Duration of PEH

Some studies have found the onset of hypotension to occur within the initial 5 minutes following exercise (Piepoli et al, 1993; 1994). The vast majority of studies indicate that hypotension occurs between 10-minutes and 1-hour following exercise (MacDonald 2002). The common time frame for most studies is a post-exercise measurement period of 1-2 hours, with the majority of studies finding a nadir in blood pressure during that time with a return or trend towards baseline pressure at the cessation of recording. A select few studies have attempted to determine if an acute bout of exercise can produce sustained reductions in blood pressure during activities of daily living via ambulatory monitoring (Pescatello et al, 1991; Somers et al, 1991; Hara & Floras, 1994; Brownley et al, 1996; Wallace et al, 1997; 1999; MacDonald, et al. 2001; Pescatello & Kulikowich 2001). Results from these studies are inconclusive

and could be confounded by a lack of control over post-exercise activity. One well controlled study utilising continuous, indwelling blood pressure monitoring has found a significant decrease in blood pressure to the end of a 70-minute post-exercise monitoring period following a bout of cycle exercise (MacDonald et al, 2001). The divergence between studies reporting a rapid return to control levels or a pronounced hypotension following exercise may be explained by a long duration blood pressure oscillation occurring post-exercise (Pescatello et al, 1991). Studies only tracking the blood pressure response during the first 2-hours following exercise may not fully represent the longer-term changes in blood pressure.

2.4 Potential Mechanisms of Post-exercise Hypotension

2.4.1 Overview of Central and Peripheral Haemodynamics

Compared with rest (pre-exercise), PEH is characterised by a persistent drop in SVR that is not completely offset by increases in $\dot{Q}$ (Halliwill 1996a; Halliwill et al, 2000). At the cessation of exercise $\dot{Q}$ decreases from high exercising values more rapidly than SVR recovers. This imbalance in the two determinants of arterial pressure results in a pronounced hypotension that can persist for several hours. This supports early investigations that noted orthostatic collapse following exercise was due to declines in SVR and not cardiac filling pressure (Bjurstedt et al, 1983).

Data indicates that the vasodilatation that underlies PEH is not restricted to the sites of active skeletal muscles but also involves inactive regions (Halliwill et al 1996a; 2000). Furthermore, the associated rise in arterial blood inflow through the vasodilated regions contributes to an increase in venous pooling of the blood (Halliwill 2001). During exercise the rhythmically contracting skeletal muscles in the leg reduce the amount of venous pooling by squeezing veins, in essence this 'milking'

effect aids venous return to the heart. This purported 'muscle pump' activity is absent during passive recovery from exercise. The increase in venous pooling coupled with the loss of plasma volume (Haskel et al 1997) associated with exercise leads to a reduction in central venous pressure and cardiac filling pressure [preload] (Halliwill et al, 2000). Halliwill et al (1996a; 1996b) has shown that in the face of a fall in cardiac preload, stroke volume (SV) is maintained due to the reduction in cardiac afterload [i.e. SVR decreases throughout active and inactive muscle beds] and a probable increase in cardiac contractility. The net effect of these influences on the blood vessels and heart is that $\dot{Q}$ is elevated due to an increase in heart rate (HR) and unchanged SV. Figure 2.4 displays the proposed integration of post-exercise haemodynamics operating during a period of sustained PEH.

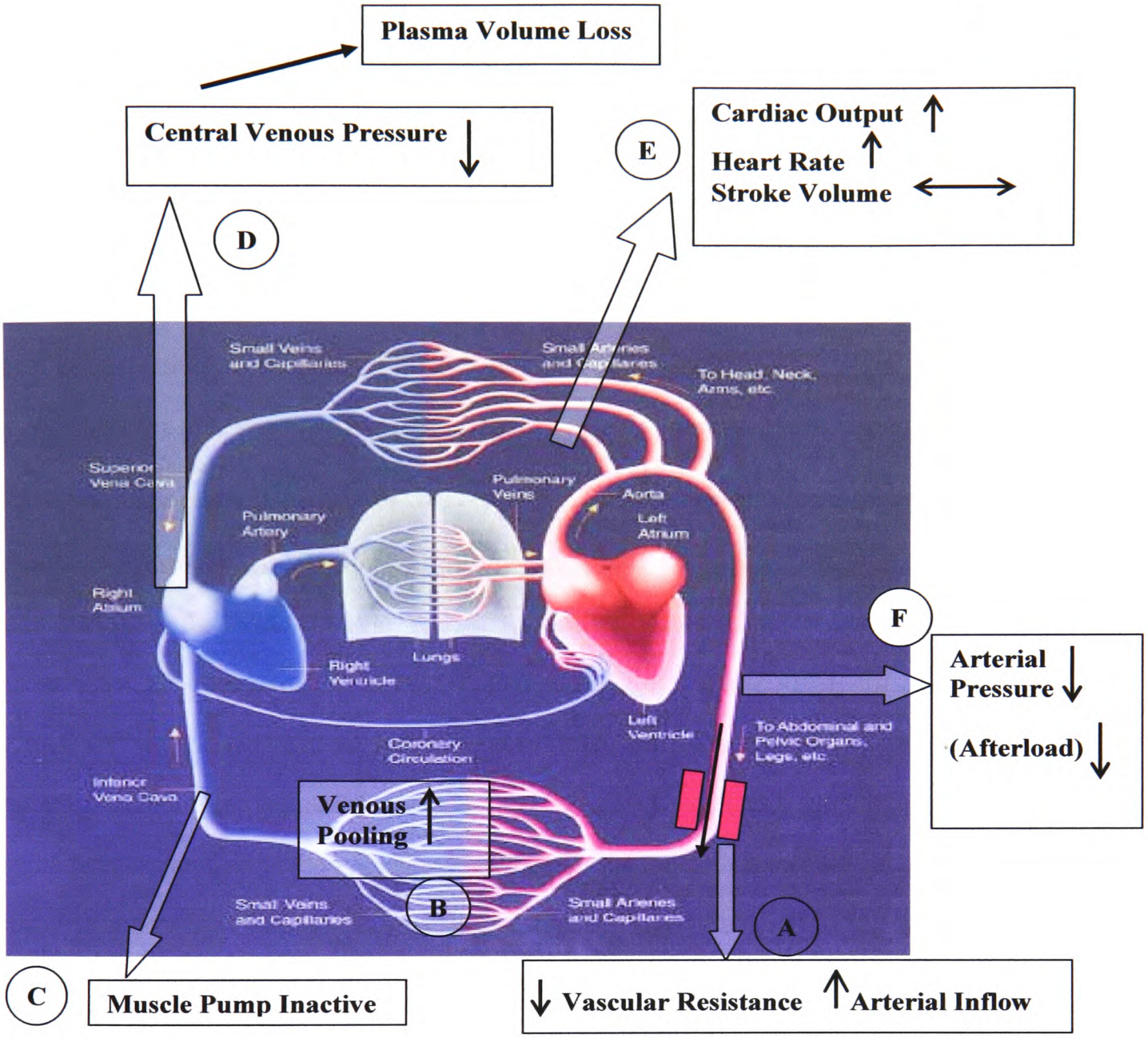


Figure 2.4. Schematic representation of haemodynamic changes during post-exercise hypotension compared to the resting state (Halliwill, 2001). **A.** SVR is decreased by approximately 30% (Halliwill 1996a, 2000). **B.** The consequent rise in blood flow through the vasodilated regions contributes to an increase in venous pooling of the blood. **C.** 'Muscle pump' is absent during passive recovery from exercise. **D.** Increase in venous pooling with the concomitant plasma volume loss associated with exercise leads to a reduction in central venous pressure and cardiac filling pressure. **E.** Despite the fall in cardiac preload, SV is maintained due to the reduction in cardiac afterload/ increase in cardiac contractility. The net result is an elevated $\dot{Q}$. **F.** PEH is resultant from a sustained drop in vascular resistance that is not completely offset by a rise in $\dot{Q}$.

2.4.2 Population Differences in the Systemic Vascular Resistance Response

Across most of the populations studied the mechanism believed to be operating during PEH is quantitatively similar to figure 2.4. However, in older (>60 years old) hypertensive subjects (Hagberg et al, 1987) and endurance-exercise trained males (Senitko et al, 2002; McCord & Halliwill, 2006), the haemodynamics underlying PEH may be qualitatively divergent from that of the other populations studied. Hagberg and co-workers (1987) found increases in SVR and hence a decrease in $\dot{Q}$ during PEH. The authors speculate that the decreased $\dot{Q}$ resulted from either reduced venous return or reduced myocardial contractility. Of note is that these subjects were studied post-exercise in the seated position. The discrepancy in results may be related to aging, state of hypertension and/or the superimposed orthostatic stress from the seated recovery. Notwithstanding, it may also suggest that the mechanisms governing PEH differ between subject populations. In direct support of this, Senitko et al, (2002) highlighted differences in the systemic vasodilatory (and hence vascular resistance) response underlying PEH between endurance trained and sedentary individuals. In sedentary subjects, regardless of gender, and in endurance-trained females the hypotension resulted from a reduction in SVR of similar magnitude, whereas in endurance-trained males hypotension was mediated via a reduction in $\dot{Q}$ with no change in vascular resistance. A later study by McCord and Halliwill (2006) however, identified that post-exercise skeletal muscle hyperaemia occured in endurance exercise-trained men and women. Thus, post-exercise skeletal muscle hyperaemias exhibited in endurance-trained men even though net peripheral vasodilatation was absent (i.e. unchanged SVR post-exercise). This indicates that vasoconstriction in other areas, perhaps the splanchnic or renal vascular beds, may offset the vasodilatation in skeletal muscle in this population.

2.4.3 Sympathetic Vascular Regulation

As interest has grown into the clinical implications of PEH most research has focused on putative mechanisms responsible for the sustained decrease in regional and systemic vascular resistances following a single bout of exercise. Analysis of the data reveals the sustained vasodilatation is associated with two principle findings. Halliwill (1996b) elucidated a 'neural' and a 'vascular' component to the alteration in sympathetic vascular regulation. Studies have indicated that the neural component of the vasodilatation is a reduction in the order of ~30% in the outflow of sympathetic vasoconstrictor nerve activity to skeletal muscle vascular beds (Hara & Floras, 1992; Kenney and Seals, 1993; Halliwill et al, 1996a). The vascular component has been postulated as being the attenuation of vascular responses to sympathetic vasoconstriction together with the potential influence of local and circulating vasodilator substances (Halliwill, 2001). For the purpose of this thesis the 'vascular' component will be considered as 'metabolic' factors.

2.4.4 Sympathoinhibition Following Exercise - Efferent Sympathetic Nerve Activity

Several studies have documented a quantitative decrease in sympathetic nerve traffic, that mediates vasoconstriction, in the leg during PEH in humans (Hara and Floras 1992; Kenney and Seals, 1993; Halliwill et al, 1996a). As highlighted in section 2.2.2, at rest muscle sympathetic nerve activity (MSNA) is tightly controlled via the arterial baroreflexes and cardiopulmonary receptor reflexes (Raven et al, 2006). Exercise produces a powerful perturbation to the baroreflex whereby it is 'reset' to a higher operating point and sympathetic activity is increased. Conversely, following exercise these reflexes are reset to lower operating points so that sympathetic outflow from the central nervous system (CNS) is reduced below pre-exercise levels (DiCarlo et al,

1994; Raven et al, 2006) (Figure 2.5). Previously, Halliwill et al, (1996a) have shown in humans that the baroreflex is reset to a lower pressure after exercise creating a reduction in sympathetic vasoconstrictor outflow.

Contradictory findings have been illuminated by VanNess et al, (1996), which highlight that the role of sympathoinhibition in predicting PEH may be limited in normotensive humans. This indicates that the role of sympathoinhibition is more pronounced in subjects with elevated sympathetic activity. An early tenet, utilising animal data, postulated that the sympathoinhibition may result from activation of endogenous opioid receptor pathways in the CNS (Thoren, et al. 1990). Data has directly challenged this hypothesis by finding no alteration in post-exercise arterial pressure or sympathetic nerve activity with opioid receptor blockade (Hara and Floras, 1992). Later work by MacDonald et al, (2002) showed that PEH was no different between trials when subjects were on placebo or a selective serotonin re-uptake inhibitor, indicating that the central serotonergic system was not responsible for PEH in their borderline hypertensive cohort.

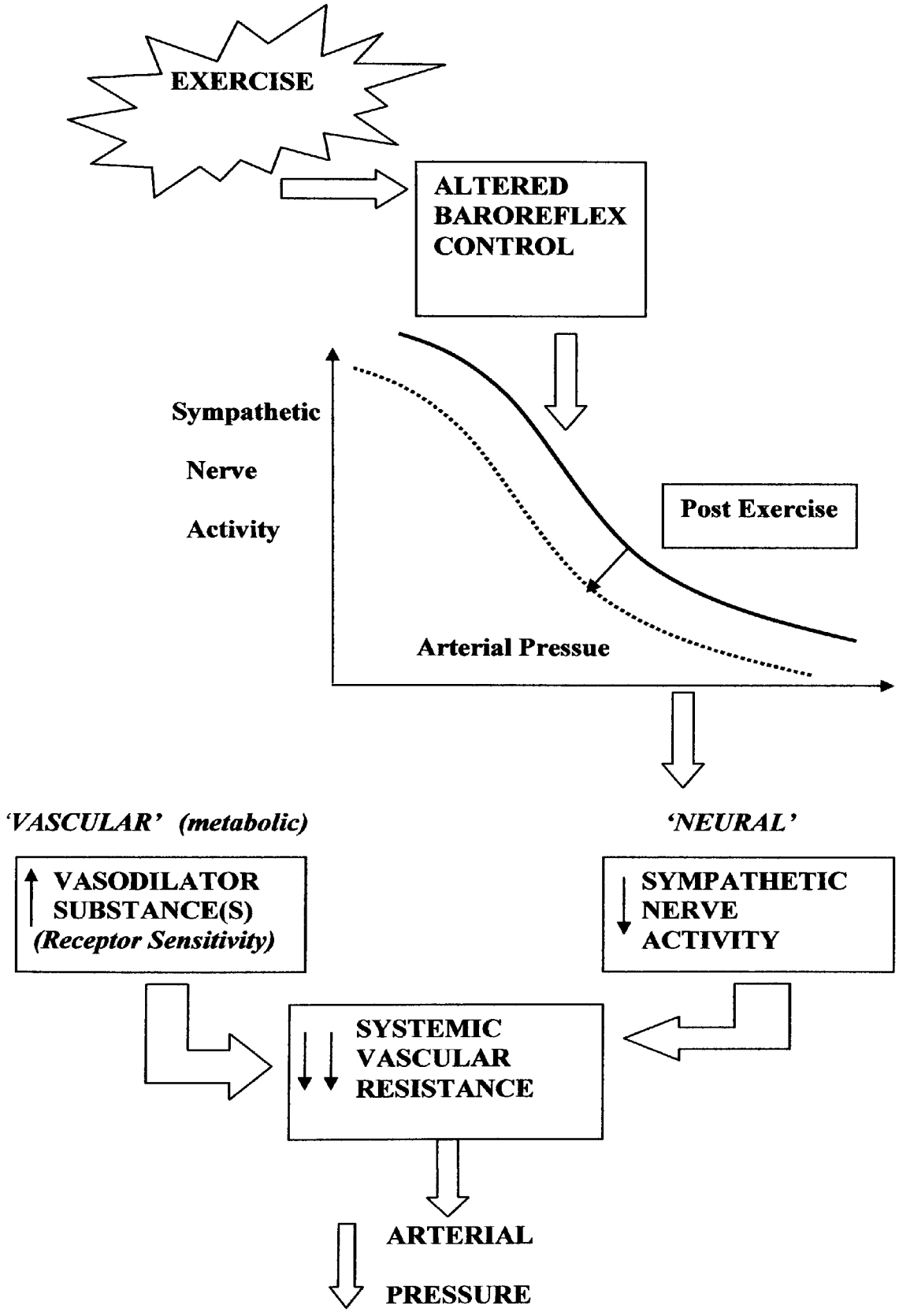


Figure 2.5. Schematic overview of changes in neural control of the circulation in relation to PEH (Halliwill, 2001).

2.4.5 Vascular Endothelial Sensitivity

Coupled with augmented sympathoinhibition post-exercise, vascular responsiveness to sympathetic vasoconstrictor outflow is impaired after exercise. In effect, vascular resistance is attenuated for a given level of sympathetic nerve activity following exercise (Patil et al, 1993; DiCarlo et al, 1994; Halliwill et al, 1996a), and is independent of changes in α–adrenergic receptor responsiveness (Halliwill et al, 2003a). The augmented vascular conductance that underlies PEH is largely in skeletal muscle vascular beds with little or no contribution from skin, splanchnic or renal circulations (Pricher et al, 2004; Wilkins et al, 2004). The mechanism of this vascular component has not been isolated but several possibilities exist. Halliwill (2001) suggests that ineffective transduction of sympathetic outflow into SVR could result from competing influences at the vascular endothelium pertaining to the release of local vasodilator agents or by modulation of the α–adrenergic pathway; such as pre-synaptic inhibition of noradrenaline release from sympathetic vasoconstrictor nerves post-exercise. Despite the clear evidence of reduced sympathetic outflow to skeletal muscle vascular beds in humans (Floras et al, 1989; Halliwill et al, 1996a) and rats (Kulics et al, 1999), blockade of α–adrenergic receptors is unable to reproduce the magnitude of post-exercise vasodilatation in skeletal muscle (Halliwill et al, 2000). Furthermore, contrary to evidence of vascular α–adrenergic hyporesponsiveness in the animal model (Rao et al, 2002), α1 and α2–adrenergic vascular responsiveness is intact in humans (Halliwill et al, 2003a).

2.4.5.1 Nitric Oxide ($NO^{\cdot}$)

The perturbation of acute exercise is associated with several factors at the level of the systemic vascular endothelium. Of note, acute exercise produces increases in blood flow, cyclic wall stress due to pulsatile blood flow and catecholamines. These factors

produce an up-regulation and release of nitric oxide ($NO^{\cdot}$) from the vascular endothelium (Busse and Fleming 1998; 2006) via the enzyme nitric oxide synthase (NOS) (Figure 2.6). NOS exists in three isoforms: neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS), in part related to its anatomical location (Murad, 2006). $NO^{\cdot}$ released toward the vascular lumen is a powerful vasodilator responsible for mediating basal vascular tone (Stamler et al, 1994). Furthermore, $NO^{\cdot}$ is a potent inhibitor of platelet aggregation and adhesion, an inhibitor of leukocyte adhesion to the vessel wall and prevents fibrous plaque formation (Förstermann & Münzel, 2006). Recent data has reported an attenuation of vasodilatation in hypertensive subjects due to decreased $NO^{\cdot}$-dependent vasodilatation (Holowatz & Kenney, 2007) mediated via increased oxidative stress (see section 2.4.5.2) or decreased L-arginine availability via upregulated arginase (Holowatz et al, 2006). Some human data indicate that $NO^{\cdot}$ production may be augmented following acute exercise (Jungersten et al, 1997; Yang et al, 2007b).

Furthermore, it has been shown in the animal model that $NO^{\cdot}$ lessens the vasoconstrictor response to α–adrenergic receptor stimulation (Rao et al, 2002; DiCarlo et al, 1994; Patil et al, 1993) and as such $NO^{\cdot}$ is a key contributor to PEH by blunting vasoconstrictor responses. Work involving the human model of PEH has shown contrary results. Halliwill et al (2000), found that PEH ensues during systemic $NO^{\cdot}$- synthase inhibition with N^{G} – monomethyl-$_{L}$-arginine ($_{L}$-NMMA). Thus, it appears that in normotensive humans PEH is independent of increased $NO^{\cdot}$ production. Although due to the methodology employed these findings cannot exclude a role for $NO^{\cdot}$ in modifying α–adrenergic responses after exercise.

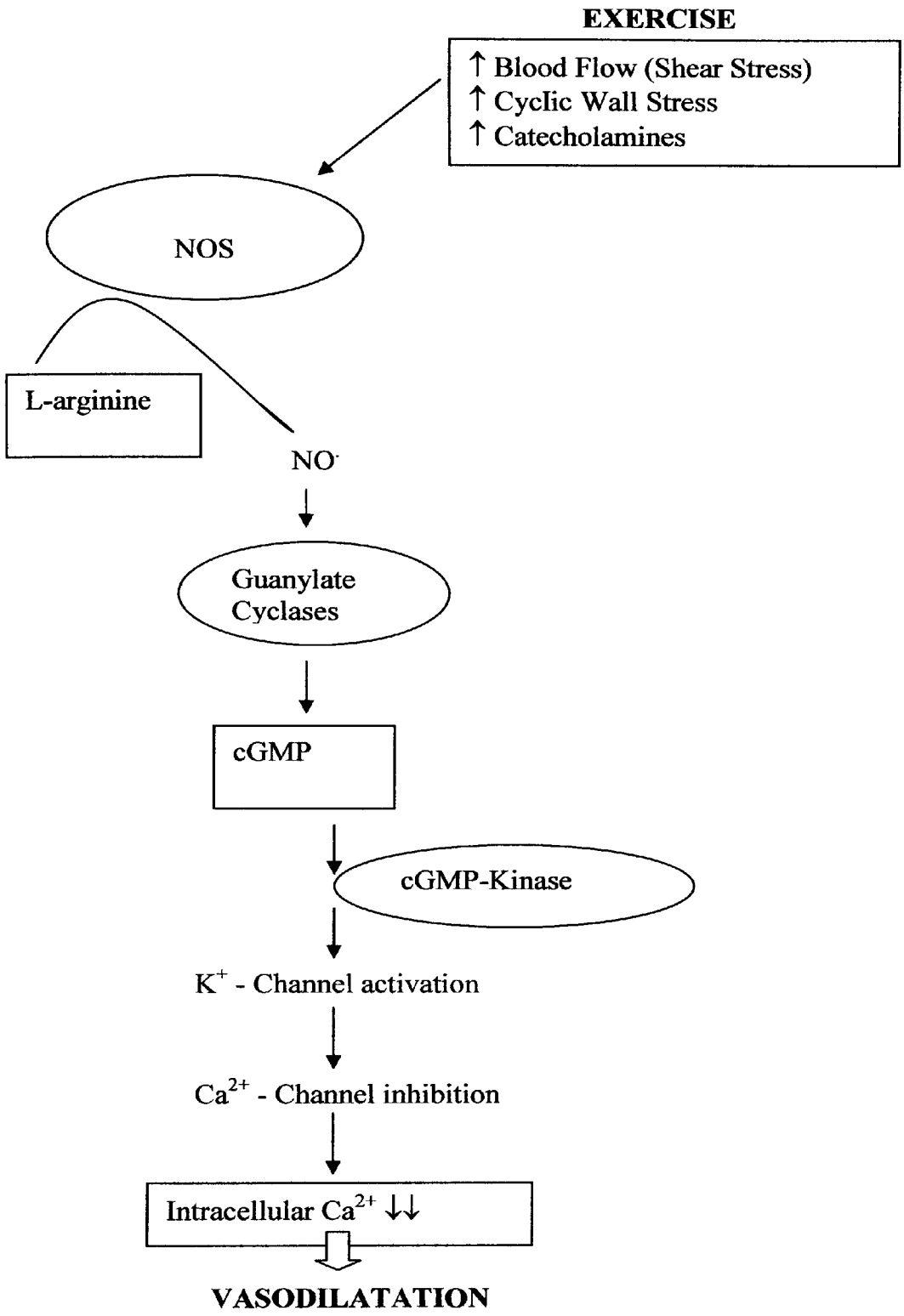


Figure 2.6. Nitric oxide (NO·) metabolism pathway (Adapted from Ghofrani et al, 2004). In the presence of increased blood flow, cyclic wall stress and catecholamines, NO synthases (NOS) are activated and produce NO· from L-arginine via L-citrulline. The NO· activates soluble and membrane-bound guanylate cyclases, which synthesise cyclic guanylate monophosphate (cGMP), which subsequently activates cGMP-kinase. This enzyme evokes a reduction of intracellular calcium (Ca^{2+}) concentration resulting in vasodilatation.

Furthermore, systemic infusion of NOS inhibitors increases blood pressure and probably evokes potentially confounding cardiovascular reflexes such as baroreflex-mediated reductions in sympathetic vasoconstrictor tone (Rådegran and Saltin, 1999; Boushel et al, 2002). This withdrawal of sympathetic outflow and subsequent vasodilatation could 'mask' the physiological contribution of $NO^{\cdot}$ to augmented vascular conductance post-exercise, under these conditions.

Several other potential vasodilators however, have been postulated as possible modifiers of α–adrenergic responsiveness and as such are principal regulators of the vascular endothelial response (Figure 2.7).

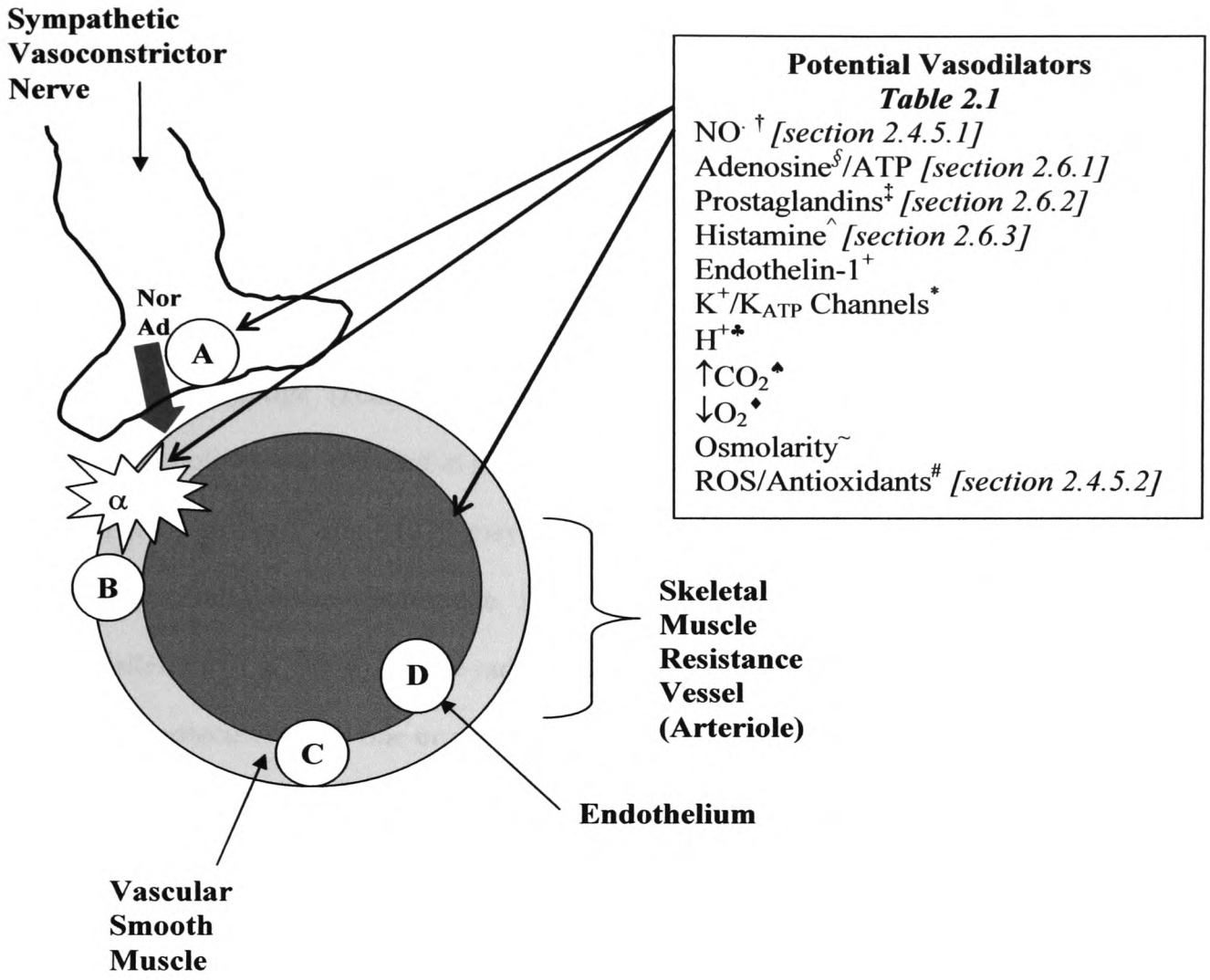


Figure 2.7. Overview of neural and local control of vascular tone in relation to PEH. (Adapted from Halliwill, 2001). Attenuated vasoconstriction and an up-regulation in vasodilation may be produced by any one of the potential vasodilator substances acting via presynaptic (A), post-synaptic (B) modulation of the α–adrenergic pathway, by direct effects on smooth muscle relaxation (C) or by endothelial derived vasoactive factors (D). Nor Ad, Noradrenaline; $NO^{\cdot}$, Nitric Oxide; ATP, Adenosine Tri-phosphate; K_{ATP}, ATP-sensitive potassium channels; ROS, reactive oxygen species.

2.4.5.2 Reactive Oxygen Species

Reactive oxygen species (ROS) are highly reactive substances also called *'free radicals'* that are present *in vivo* and produce the state of oxidative stress. Oxidative stress is defined as:

'a disturbance in the proxidant-antioxidant balance in the favour of the former, leading to potential damage' (Halliwell and Gutteridge, 1999).

Free radical metabolism is implicated in over 100 human disease states (Halliwell and Gutteridge, 1984); however, some ROS may contribute beneficially to disease pathophysiology (Halliwell and Gutteridge, 1999) and be implicated in cell signalling (Palacious-Callender et al, 2004). A free radical may simply be defined as any species (atoms, ions or molecules) with one or more unpaired electrons in their outer atomic or molecular orbital, which are capable of existing independently (Halliwell and Gutteridge, 1999). Any reactive molecule with an unpaired electron is conventionally represented by the application of a superscript dot (·) (Halliwell et al, 1992). ROS is a collective term for oxygen-derived molecular species. Thus, this term includes free radical species in addition to species that have paired electrons but are capable of becoming involved in harmful reactions that cause damage to other biomolecules (Halliwell and Gutteridge, 1999).

Free radicals are believed to be formed *in vivo* at rest and during exercise as by-products of normal energy metabolism (Jackson, 1995). During the process of complete tetravalent reduction of molecular oxygen to water, free radical intermediates and other toxic products are univalently formed (Davison et al, 2008) (Figure 2.8).

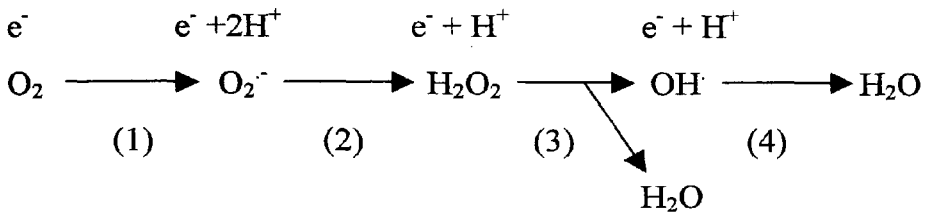


Figure 2.8. Univalent reduction pathway of molecular oxygen (Adapted from Fridovich, 1978). The first one-electron reduction of molecular oxygen generates the superoxide anion radical ($O_2^{\cdot -}$). Addition of a second electron and two protons to superoxide will form hydrogen peroxide (H_2O_2). A third electron and one proton formulates the highly reactive hydroxyl radical ($OH^{\cdot}$) while adding another electron and proton to hydroxyl will produce inert water (H_2O) (Davies, 1995).

Several mechanisms *in vivo* produce ROS. $O_2^{\cdot -}$ results from mitochondrial electron transport chain leakage, ischemia/reperfusion, auto-oxidation reactions, respiratory burst involving phagocytic cells and continuous production of $O_2^{\cdot -}$ by the vascular endothelium to neutralise $NO^{\cdot}$ (Young and Woodside, 2001). The primary mechanism of $O_2^{\cdot -}$ production during exercise appears to be from the mitochondria. H_2O_2 is produced by a variety of intracellular reactions, although the predominant pathway is by dismutation of superoxide by the enzyme superoxide dismutase (SOD) (Halliwell and Gutteridge, 1999). By far the most widely known mechanism of formation *in vivo,* of the extremely pernicious $OH^{\cdot}$, is the transition metal catalysed (e.g. copper and iron fenton chemistry) decomposition of $O_2^{\cdot -}$ and H_2O_2 (Young, 1994). Within vascular endothelial cells the primary site of ROS generation emanates from the electron transport chain located within the mitochondria. Although the majority of molecular oxygen is reduced at complex IV to water, 1-4% of the oxygen is incompletely reduced to $O_2^{\cdot -}$, which can yield other ROS via numerous enzymatic

or nonenzymatic reactions (Figure 2.9) (Zhang and Gutterman, 2007).

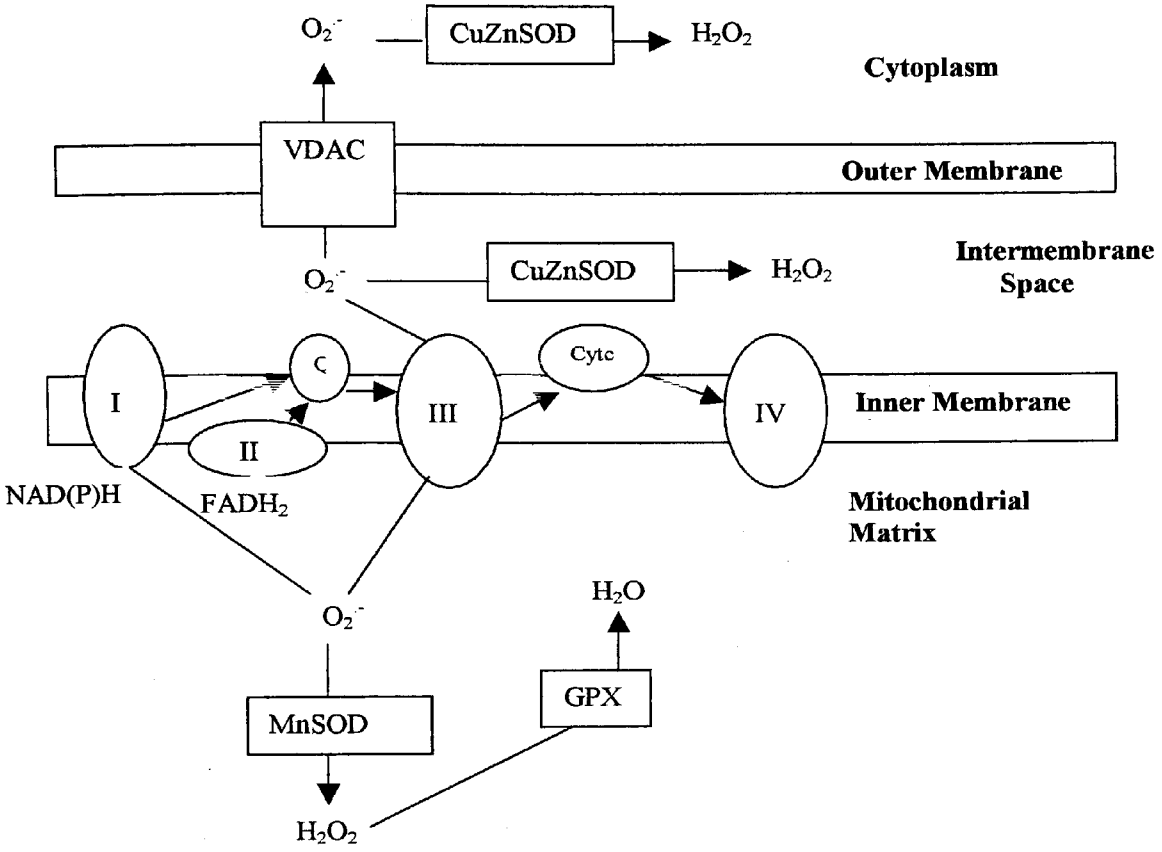


Figure 2.9 Mitochondrial ROS in vascular endothelial cells (Zhang & Gutterman, 2007). Superoxide ($O_2^{\cdot-}$) is mainly generated at complexes I and III of the electron transport chain located in the mitochondrial inner membrane. $O_2^{\cdot-}$ is released into both the matrix and the inter-membrane space, and some diffuses into the cytosol via the voltage-dependent mitochondrial anion channel (VDAC). Several isoforms of superoxide dismutase (SOD) degrade $O_2^{\cdot-}$ to hydrogen peroxide (H_2O_2), including manganese SOD (Mn-SOD) in the matrix, copper/zinc SOD (CuZn-SOD) in the inter-membrane space and cytosol. In the matrix, H_2O_2 is further detoxified to water primarily by glutathione peroxidase (GPX).

By definition NO (nitrogen monoxide) is a free radical ($NO^{\cdot}$) due to the presence of an unpaired electron. As previously discussed in section 2.4.5.1, $NO^{\cdot}$ exerts its effects on the vascular endothelium to mediate, amongst other events, vascular tone and blood flow. However, it also has the ability to rapidly bind to the powerful oxidant superoxide ($O_2^{\cdot -}$) to produce the peroxynitrite anion ($ONOO^-$), (Hsiai et al, 2007; Pacher et al 2007). The second order rate constant for the reaction of $NO^{\cdot}$ with $O_2^{\cdot -}$ is $6.7 \times 10^9\ M^{-1}\ S^{-1}$ (Huie & Padmaja, 1993). This rate is approximately 3 times greater than the superoxide dismutase-catalysed dismutation of $O_2^{\cdot -}$. Therefore $NO^{\cdot}$ is capable of outcompeting SOD for $O_2^{\cdot -}$ in conditions such as atherosclerosis, ischemia-reperfusion, increased shear stress and exercise in which production of $NO^{\cdot}$ and $O_2^{\cdot -}$ is concomitantly increased. Figure 2.10 displays the biochemical reaction between $NO^{\cdot}$ and $O_2^{\cdot -}$ to yield $ONOO^-$ and various other downstream metabolites.

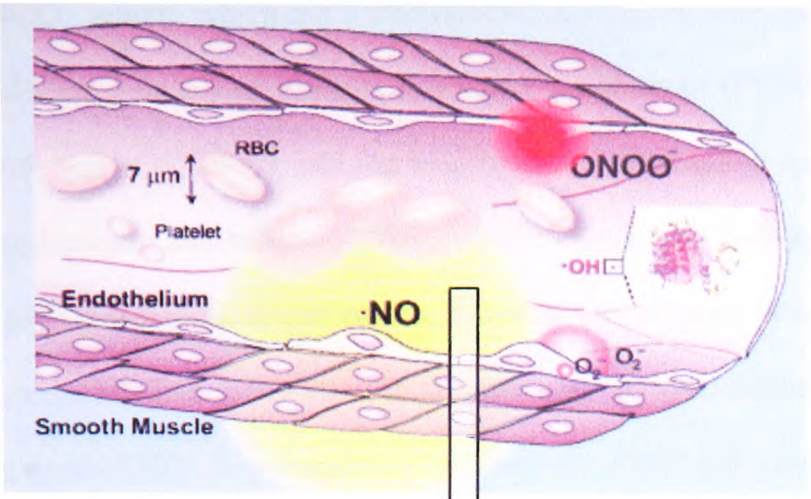


Figure 2.10 A

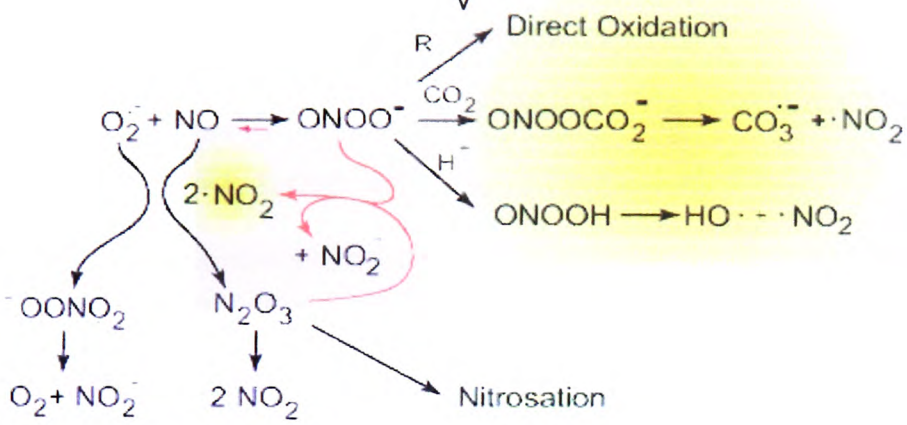


Figure 2.10 B

Figure 2.10 A: Cellular diffusion of nitric oxide, peroxynitrite and hydroxyl radicals within their estimated first half-lives at the vascular endothelium (Pacher et al, 2007). Shading around species indicates the extent to where each species from a point source would decrease by 50%. Diffusion distances based on half-life of nitric oxide ($NO^{\cdot}$) ~ 1s (Pacher et al, 2007); peroxynitrite ($ONOO^{-}$) ~ 10-20ms (Pacher et al, 2007); hydroxyl radical ($^{\cdot}OH$), ~1×10^{-9}s (Pryor, 1986); Superoxide ($O_2^{\cdot-}$) ~ 1×10^{-6}s (Rimbach et al, 1999).

B: Interplay of nitric oxide, superoxide, peroxynitrite and nitrogen dioxide (Pacher et al, 2007). When nitric oxide and superoxide are present they may also combine to form N_2O_3 and peroxynitrate ($^{-}OONO_2$). Peroxynitrate decomposes to yield nitrite (NO_2^{-}) and oxygen while N_2O_3 can react with thiols to give nitrosothiols or with hydroxyl anion to give nitrite. ONOOH, peroxynitrous acid, HO^{-}, hydroxyl anions; $^{\cdot}NO_2$, nitrogen dioxide; $ONOOCO_2^{-}$, nitrosoperoxycarbonate; $CO_3^{\cdot-}$, carbonate radical.

$ONOO^-$ which, whilst not a free radical, is a highly toxic molecule capable of attacking and causing damage to cellular membranes (Pacher et al 2007; Suzuki et al, 2007; Sen et al, 2000), and can lead to eNOS uncoupling and enzyme dysfunction (Förstermann and Münzel, 2006). This is exquisitely demonstrated in several pathologies such as hypertension, diabetes and atherosclerosis, which are associated with endothelial dysfunction in that the vascular endothelium produces insufficient amounts of $NO^{\cdot}$. The purported mechanism whereby the reduced cofactors nicotinamide adenine dinucleotide phosphate (NADPH) and (*6R*-)5,6,7,8-tetrahydrobiopterin (BH4), which are required by the nitric oxide synthases to produce $NO^{\cdot}$, are oxidised by ROS resulting in the nitric oxide synthases producing $O_2^{\cdot-}$ instead of $NO^{\cdot}$, further inhibiting NOS. (Murad, 2006). Thus, the net effect is decreased $NO^{\cdot}$ formation, accelerated $NO^{\cdot}$ removal and production of the highly toxic $ONOO^-$.

In turn, $ONOO^-$ is a powerful oxidant capable of mediating vascular tone (Faraci, 2006). In some vascular beds, such as the cerebral circulation, $ONOO^-$ has been reported as eliciting both vasoconstrictor and vasodilator properties (Faraci, 2006). *In vitro* studies of other vascular beds have elucidated $NO^{\cdot-}$ like properties for $ONOO^-$ inducing pulmonary (Wu et al, 1994), mesenteric (Benkusky et al, 1998) and coronary (Liu et al, 1994; Villa et al, 1994) artery dilatation and inhibiting platelet aggregation (Moro et al, 1994). Moreover, systemic administration of $ONOO^-$ elicits pronounced hypotensive and vasodilator responses in the animal model (Kooy & Lewis, 1996; Benkusky et al, 1998; Nossaman et al, 2004; Ohashi et al, 2005). The mechanism of this vasodilatation has been linked to activation of K_{ATP} channels (Ohashi et al, 2005). Further data exists from the human model. *In vitro* studies on human coronary arteries suggests that $ONOO^-$ inhibits Ca^{2+} -activated potassium channel activity possibly

impairing endothelium-derived hyperpolarizing factor-mediated dilatation (Liu et al, 2002).

Data also indicate that at the level of the vascular endothelium the interaction between $NO^{\cdot}$ and O_2 or ROS generated from the mitochondria may serve as a cellular signalling process (Quintero et al 2006; Pacher et al, 2007). Whilst it has additionally been shown that H_2O_2 can increase eNOS expression through transcriptional and posttranscriptional mechanisms (Drummond et al, 2000). Thus, there appears to be an intimate relationship *in vivo* between $NO^{\cdot}$ and ROS especially at the level of the vascular endothelium.

As discussed below (Section 2.4.5.3) recent work by Richardson and co-workers (2007) suggests that there is an exercise-induced reliance upon pro-oxidant-stimulated vasodilatation revealing an important and beneficial role of free radicals at the vascular endothelium. In support of this, there is an abundance of data suggesting that ROS, particularly H_2O_2, play an essential role in eliciting vasodilatation in several vascular beds (Zhang & Gutterman, 2007) including human coronary arteries (Liu et al, 2003; Miura et al, 2003; Sato et al, 2003), human mesenteric arteries (Matoba et al, 2002), mouse mesenteric arteries (Matoba et al, 2000; Yan et al, 2005) and cat cerebral arterioles (Wei et al, 1996; 1998). Figure 2.11 presents a schematic illustration of the interrelationships between ROS that may affect vascular tone.

At present there appears to be no data published on the role of free radicals in mediating vascular conductance during a period of sustained PEH.

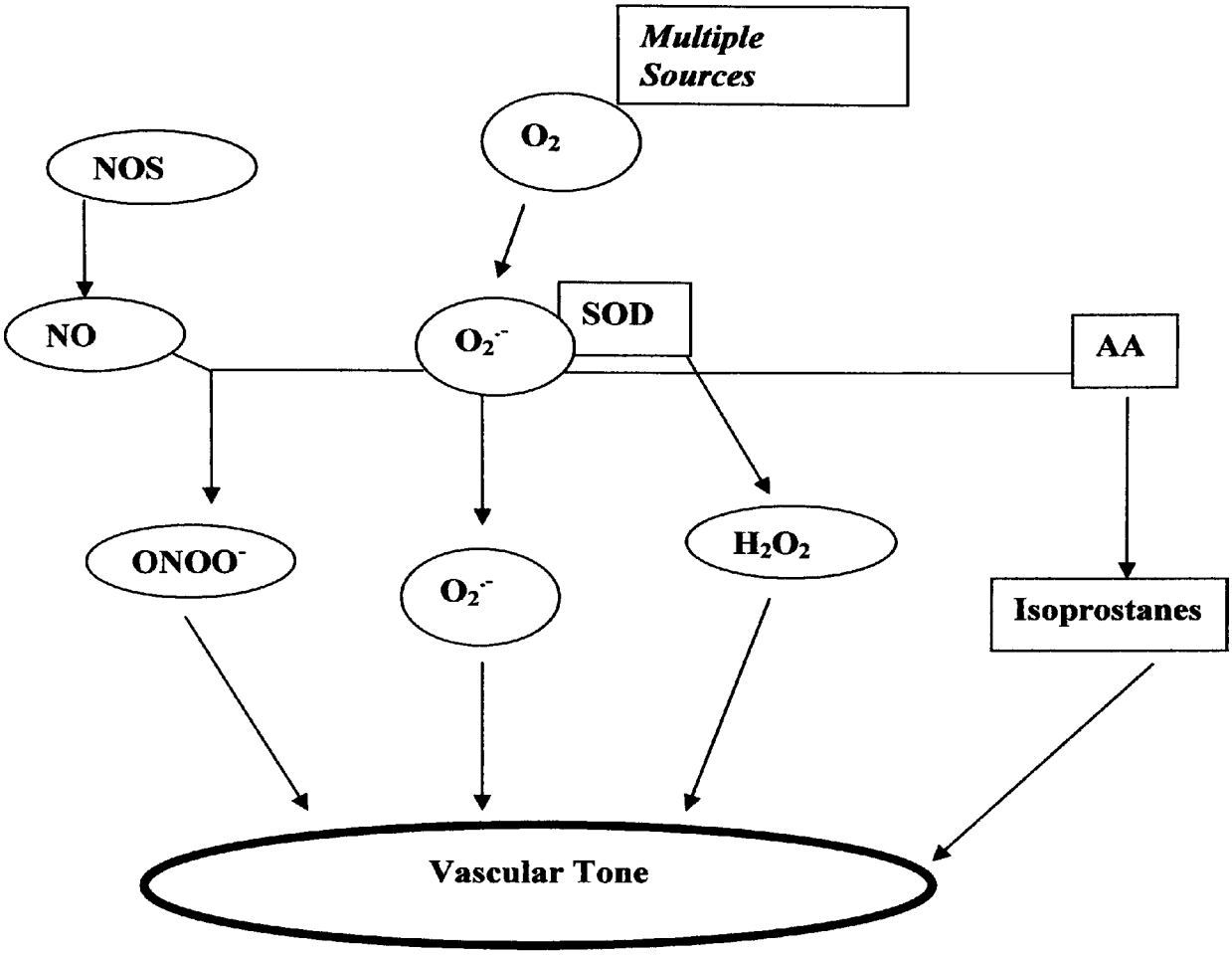


Figure 2.11. Schematic illustration of interrelationships of various reactive oxygen species that may affect vascular tone (Adapted from Faraci, 2006). Superoxide ($O_2^{\cdot-}$) is produced from a variety of molecular sources and can have a direct effect on vascular muscle. $O_2^{\cdot-}$ may also be dismutated, via superoxide dismutase (SOD), to hydrogen peroxide (H_2O_2). H_2O_2 can also be degraded by other enzymatic antioxidant systems such as catalase and glutathione peroxidase (pathway not shown). H_2O_2 elicits vasodilatation via direct actions on vascular muscle. $O_2^{\cdot-}$ reacts with nitric oxide (NO) formed via nitric oxide synthases (NOS) to produce the volatile peroxynitrite ($ONOO^-$) which evokes vasodilatation in several vascular beds. $O_2^{\cdot-}$ may also react with arachadonic acid (AA) to form isoprostanes which modulate vascular tone.

2.4.5.3 Antioxidants

Whilst the formation and propagation of ubiquitous free radicals is inevitable during rest and exercise in aerobic mammals due to the shared reliance on O_2. Fortunately, the organism is well placed to defend against the generation of ROS. All mammalian aerobes have a series of antioxidant defence systems in an attempt to protect against ROS.

An antioxidant is

"any substance which, when present at much lower concentrations than an oxidisable substrate, significantly delays or prevents oxidation of that substrate" (Halliwell and Gutteridge, 1999).

Many antioxidants exist *in vivo* some of which are enzymatic and some of which are non-enzymatic. Enzymatic antioxidants range from intra/extra cellular SOD and its isoforms (to reduce $O_2^{\cdot-}$), to catalase, and glutathione peroxidase (to detoxify H_2O_2). Non-enzymatic antioxidants include ascorbic acid (Vitamin C) [inhibits lipid oxidation], α-tocopherol (Vitamin E) [prevents lipid peroxidation] and the carotenoids (precursors of Vitamin A) [scavenge several ROS] (Powers and Hamilton, 1999). Zhou et al (2006) recently showed that hypertensive individuals have reduced plasma extracellular-superoxide dismutase activity and hence increased oxidative stress. In an animal model Peluso et al (2004) highlighted that exogenous supplementation with antioxidants reversed peroxyl-induced hypotension, implicating a redox regulation pathway for blood pressure control. Tian et al (2005) displayed decreased arterial pressure and improved renal function by supplementing both Vitamins A and E in salt sensitive hypertension. Davis Manning Jr et al (2005) presented a concise review of oxidative stress and antioxidant treatment in

hypertension. In the human relatively high dose supplementation of Vitamin E has reduced several cardiovascular risk factors (Stephens et al 1996; Boaz et al,2000) whereas several studies have shown an inverse relationship between serum Vitamin C and blood pressure, with some studies showing reduction of blood pressure with specific Vitamin C treatment (Duffy et al, 1999a). With regards to investigations into the potential for redox regulation of systemic vascular conductance, Richardson et al, (2007) found that supplementing a cohort of young healthy subjects with an antioxidant cocktail (Vitamins A, E & alpha-lipoic acid) significantly attenuated exercise-induced vasodilatation of the brachial artery following sub-maximal forearm handgrip exercise. Suggesting that the disruption of the delicate redox regulation in healthy subjects can negatively affect vascular function. To this authors' knowledge no study has investigated redox regulation i.e. the balance between pro-oxidant and antioxidant levels during a sustained period of PEH in the animal or human model.

2.5 Local Tissue Responses

Exercise stress leads to production of a host of local tissue vasodilator substances such as adenosine, prostaglandins, histamine and Adenosine Triphosphate (ATP), capable of moderating PEH independently or in concert with other metabolites (MacDonald, 2002; Halliwill, 2001; Halliwill et al, 1996a).

2.5.1 Adenosine

Adenosine accumulates in the interstitial space during exercise (Hellsten et al, 1998), where its primary, direct action is potent vasodilatation of vascular beds with direct actions via the vascular A2 receptor. It also produces a secondary indirect action by inhibiting noradrenaline release presynaptically through the neural A1 receptor. These

two actions result in adenosine being a powerful antagonist of the vasoconstrictor effects of sympathetic discharge (Rongen et al, 1996). The latter action may explain why profound vasodilatation follows exercise in the absence of sympathetic vasoconstrictor withdrawal (Hara and Floras, 1992; 1994; 1996). Interestingly in the animal model, it has been reported that the BP-lowering effects of exercise training in older rats have been associated with an increased release of ATP and adenosine from vascular endothelial cells (Hashimoto et al, 1999). Its role in PEH may be limited however due to the fact that the re-uptake of adenosine is very rapid following exercise. Sparks (1980) suggested that during ischemic exercise, adenosine is responsible for an initial vasodilatation during the first few minutes of PEH in dogs. Notarius et al (2006) recently found that non-selective blockade of adenosine A1 and A2 receptors by caffeine ingestion attenuated early PEH in middle-aged normotensive humans. Indicating that endogenous adenosine contributed to the early PEH (up to 10-minutes post-exercise) in their cohort. However, the increase in vascular conductance underlying PEH lasts up to or longer that 2-hours (Halliwill, 2001), as data were only reported for 10-minutes post-exercise it is equivocal as to whether antagonism of adenosine A1 and A2 receptors would attenuate PEH for this entire extended time-period.

2.5.2 Prostaglandins

The enzyme cyclooxygenase catalyses the breakdown of arachidonic acid, producing prostaglandins (PGs), including several powerful vasodilators. A primary branch of this arachidonic acid cascade produces prostacyclin, a potent but unstable vasodilator (half-life ~ 3-minutes) that breaks down into the stable but less active 6-prostaglandin-$F_{1\alpha}$ (6-keto- $PGF_{1\alpha}$) (Nowak and Wennmalm, 1979; Salmon et al, 1979). This process occurs in the vascular endothelial cells in response to specific

stimuli including increases in blood flow and wall sheer stress (Koller et al, 1998; Koller and Kaley, 1990). Exercise produces such stimuli; in turn, PGs are liberated during exercise, causing vasodilatation of both arteries and veins (Ward, 1999) during and immediately after exercise in dogs (Young and Sparks, 1980) and humans (Ritter et al, 1983; Wennmalm and Fitzgerald, 1988; Duffy et al, 1999b; Boushel et al, 2002; Farouque and Meredith, 2003). PGEs, PGFs and to some extent PGAs are continually metabolised by the lungs, so for PGs to contribute to PEH to any degree a continued production would be necessary. Wilson and Kapoor (1993) showed that cyclooxygenase inhibition with indomethacin attenuated isometric-induced exercise hyperaemia of the forearm, suggesting that released PGs were directly responsible for vasodilatation and hyperaemia in the vascular bed. Utilising an ischaemic exercise model, Morganroth et al, (1977) concluded PGs mediate a decrease in peripheral resistance for 35-40-minutes post-exercise. Cowley et al, (1984; 1985) reported that calf blood flow immediately after exercise could be reduced by cyclooxygenase inhibition via aspirin and indomethacin. Moreover, Boushel et al, (2002) showed that exercise hyperaemia, induced by knee extensor exercise, was reduced by combined cyclooxygenase and NOS inhibition. Farouque and Meredith, (2003) repeated these findings, highlighting a reduced exercise hyperaemia, following wrist exercise with combined cyclooxygenase and NOS inhibition.

Whilst data have indicated that the plasma concentration of 6-keto- $PGF_{1\alpha}$ is increased up to 30-minutes post-exercise (Karamouzis et al, 2001) none of these previous studies directly assessed the contribution of PGs on PEH, i.e. whether the vasodilatory signal persists during the time frame associated with PEH. Lockwood et al, (2005a) directly investigated the effects of PGs on PEH in normotensive male subjects via cyclooxygenase inhibition with ibuprofen. They found no difference in vascular

conductance during PEH, following dynamic exercise, on control day or whilst supplemented with ibuprofen. These findings indicate that PG-dependent vasodilatation does not contribute to the extended increase in systemic vascular conductance underlying PEH.

2.5.3 Histamine

Histamine is stored and released by mast cells in most tissues and basophils in blood. It can also be synthesised (but not stored) in non-mast cell tissues such as the cells in the gastric mucosa and neurones of the CNS, among others, by the actions of $_{L}$-histidine decarboxylase (Brown and Roberts, 2001). Histamine levels have been reported as being increased during or following exercise (Harries et al, 1979; Campos et al 1999), but it is unclear if histamine plays a part in exercise hyperaemia or if it contributes to PEH via a vasodilatory process. Exercise may elicit histamine release through physical stimulators such as vibration and heat (Atkinson et al, 1992). There is also experimental evidence that sympathetic withdrawal augments histamine release (Rengo et al, 1978) and sympathetic withdrawal is a principle component of PEH (see section 2.4.4). When released, histamine binds to Histamine-1 receptors (H_1) located on vascular endothelial cells producing vasodilatation by the synthesis of local vasodilator metabolities such as NO· and prostacyclin (Hill, 1990; Brown and Roberts, 2001). H_2 receptors are found predominantly on smooth muscle cells producing vasodilatation by decreasing intracellular calcium levels (Hill, 1990; Brown and Roberts, 2001). H_3 receptor subtypes have been suggested to be located throughout tissues on pre-synaptic nerve terminals, causing vasodilatation by inhibiting nor-adrenaline release (Molderings et al, 1992) and/or by decreasing intracellular calcium release (Brown and Roberts, 2001). Thus, histamine causes vasodilatation through multiple pathways, implying that there are redundant

vasodilatation pathways; therefore, with blockade of one pathway, vasodilatation would ensue via recruitment of another pathway. This has recently been reported during exercise hyperaemia in humans (Schrage et al, 2004).

Morganroth et al (1977) utilising the animal model showed that a H_1 antagonist returned vascular resistance back to baseline quicker than control following exercise. Indicating that histamine acting at the H_1 receptor contributes to immediate post-exercise vasodilatation.

Lockwood et al (2005b) directly assessed the contribution of H_1 receptor-mediated vasodilatation to PEH. They found that selective blockade of the H_1 receptor with fexofenadine hydrochloride resulted in a significant reduction in post-exercise vasodilatation and markedly blunted the associated PEH, immediately (30-minutes) post-exercise. The blunting of PEH however, significantly reduced as the period of PEH continued, being minimal at 60- and 90-minutes post-exercise (Lockwood et al, 2005b). This may fit with data indicating a time course for differential vasodilatation produced from H_1 and H_2 receptor activation (Black et al, 1975; Chipman and Glover, 1976; Flynn and Owen, 1975; Pawlik et al, 1977). Early vasodilatation is resultant from H_1 receptor activation whereas later and sustained vasodilatation is due to H_2 receptor binding. Therefore, PEH could be mediated by both H_1 and H_2 receptor subtypes. McCord et al, (2006) directly investigated this possibility by blocking H_2 receptors with the H_2 selective antagonist ranitidine hydrochloride. They found that administration of ranitidine significantly reduced the vasodilatation after dynamic exercise in normotensive humans and markedly blunted the PEH especially during the later stages of the study i.e. 60- to 90-minutes post-exercise. Taken together the studies of Lockwood et (2005b) and McCord et al (2006) indicate that stimulation of H_1 receptors plays a more significant role in early (up to 30-minutes) PEH whereas H_2

receptors become involved in the later stages of PEH (60- to 120-minutes). McCord and Halliwill (2006) confirmed this by using a double blockade of H_1 and H_2 receptors in normotensive sedentary and endurance exercise-trained men and women. They found that the histamine-receptor antagonists abolished the entire 90-minute post-exercise hyperaemia and in addition the magnitude of the hypotension during recovery from exercise was blunted in all groups when administered with the H_1 and H_2 -receptor antagonists.

It must be noted however that in all of the above studies no increase in plasma or whole blood histamine concentration was noted following exercise. Several possibilities could explain this finding: Exercise could increase the sensitivity of the H_1 and H_2 receptors to histamine. Alternatively, histamine could be released locally and cleared before significant spillover into the circulation, which would explain the lack of vasodilatation in skin, splanchnic and renal vascular beds. Histamine and/or H_1 and H_2 receptor sensitivity thus appears to play a pivital role in the augmented post-exercise vascular conduction underlying PEH.

Table 2.1 provides a summary of the experimental evidence gathered towards potential local tissue vasodilators or metabolic parameters mediating the augmented vascular conductance inherent in PEH as highlighted in figure 3.7.

Table 2.1 Investigations into potential vasodilators mediating the augmented vascular conductance underlying PEH and/or skeletal muscle hyperaemia

Study	Significant Contribution To PEH	Investigated During Sustained PEH	Population
†Halliwill et al, 2001 *NOS Antagonism*	No	Yes	8 (4M/4F) Young Normotensive
‡Lockwood et al, 2005a *Prostaglandin inhibition*	No	Yes	11, Young Normotensive Males
§Notarius et al, 2006 *Adenosine blockade*	Yes ≤ 30-minutes post-exercise	Yes	14 (13M/1F) Middle-aged Normotensive
^Lockwood et al, 2005b *Histamine H_1 receptor Antagonism*	Yes ≤ 30-minutes post-exercise	Yes	14 (7M/7F) Young Normotensive

^McCord et al, 2006 *Histamine H_2 receptor Antagonism*	Yes 60-90-minutes post-exercise	Yes	10 (5M/5F) Young Normotensive
^McCord & Halliwill, 2006 *Histamine H_1 & H_2 receptor Antagonism*	Yes entire post-exercise period	Yes	28 (14M/14F) Young Normotensive
+Wray et al, 2007 *Endothelin-1*	Unknown but exerts significant vasoconstrictor tone on leg vasculature at rest and displays a profound exercise intensity-dependent "lysis" of peripheral vasoconstriction	No	8 Young Normotensive Males
*Schrage et al, 2006 *K_{ATP} Channel Antagonism*	Unknown but not obligatory in forearm exercise-induced hyperaemia	No	18 (M/F) Young Normotensive
♣♠♦Stowe et al, 1975 *Blood pH, O_2 &*	Unknown but acidosis, hypoxia &	No	Denervated canine gracilis

CO_2 fluctuation	hypercapnia contributes to the initiation and maintenance of exercise-induced hyperemia		muscle
♦Weisbrod et al, 2001 *Moderate Hypoxia*	Unknown but induces forearm hyperaemia at rest, which is augmented by local blockade of α–adrenergic receptors.	No	8 (5M/3F) Young Normotensive
♦Dinenno et al, 2003 *Mild to Moderate Hypoxia*	Unknown but induces forearm hyperaemia at rest whilst failing to blunt post-junctional α–adrenergic receptor vasoconstrictor responsiveness	No	18 (13M/5F) Young Normotensive

♦Wilkins et al, 2006 *Moderate Hypoxia*	Unknown but does not augment functional sympatholysis during induced hyperaemia throughout rest and forearm exercise	No	14 (10M/4F) Young Normotensive
~de Clerck et al, 2005 *Hyperosmolarity*	Unknown but induces long-lasting vasodilatation of skeletal muscle	No	Isolated rat skeletal muscle
#Richardson et al, 2007 *ROS Antagonism*	Unknown but important in exercise hyperaemia	No	25, Young Normotensive Males

2.6 Endocrine Responses

2.6.1 Noradrenaline and Adrenaline

An exercise bout causes increased sympathoadrenal drive, in turn, stimulating the release of noradrenaline and adrenaline in proportion to the intensity of the exercise bout. Hypertensive and older-aged humans are associated with marked elevations of sympathetic nervous system activity at rest (Seals and Dinenno, 2004; Neumann et al 2007). A decrease in circulating catecholamines after exercise could lead to PEH. However, measures of plasma noradrenaline levels as an indirect measure of 'spill over' from sympathetic activity are inconsistent during PEH. Levels have been documented as elevated (Landry et al 1992) or unchanged (Notarius et al, 2006) in normotensives, elevated (Paulev et al, 1984) and unchanged (MacDonald et al 2002) in borderline hypertensives and decreased in hypertensive individuals (Cleroux et al, 1992). Measures of circulating adrenaline indicate that levels are increased (Landry et al 1992) or unchanged (Notarius et al, 2006; MacDonald et al 2002; Wilcox et al, 1987) during the hypotensive period following exercise. This coupled with the findings that PEH persists during adrenaline infusion (Landry et al 1992) and β–receptor blockade (Wilcox et al, 1987) indicates that any role of adrenaline in PEH is minimal.

2.6.2 Renin-Angiotensin-Aldosterone System (RAAS)

As presented in section 2.2.4.1, the release of renin from the kidneys during periods of low perfusion pressure elicits reciprocal increases in arterial blood pressure in several ways. Increased sympathetic drive via exercise is a powerful stimulus for the RAAS (Maher, 1975). Thus it could be speculated that PEH is resultant from a blunting of the sustained elevation of RAAS following exercise. However, during PEH, unchanged (Wilcox et al, 1982) and increased (Paulev et al, 1984; Piepoli et al, 1993)

concentrations of circulating renin and increased angiotensin II (Paulev et al, 1984) concentrations have been found and therefore do not appear to play a significant role in PEH. Along these lines, a recent study by Wray et al (2008) indicated that a clear age-related hypersensitivity to angiotensin II in resting leg vasculature of humans occurs but that the elevation in angiotensin II type 1 receptor sensitivity does not contribute significantly to the blunted exercise hyperaemia experienced with senescence.

Data have also elucidated that in hypertensive men with RAAS polymorhisms a variant response in PEH was found between different RAAS genotypes (Blanchard et al, 2006), indicating that genetic variation of the RAAS affects the PEH response.

2.6.3 Anti-diuretic Hormone (ADH) [Arginine Vasopressin (AVP)]

ADH plays a primary role in controlling body water content but also contains a secondary function acting as a powerful vasoconstrictor of arterial smooth muscle (O'Leary & Potts, 2006). It is released from the neurohypophysis during periods of low pressure or increased osmolality providing a defence for restoring blood pressure and volume; together with its vasoconstricting properties this cardiovascular reflex is believed to be important in haemorrhagic situations (Voelckel et al, 2003). Prolonged moderate intensity exercise has been shown to increase circulating ADH concentration due to an exercise intensity-dependent plasma osmolality increase as a consequence of hypotonic fluid movement out of the vascular space (Takamata et al, 2000). Thus, PEH could be moderated via fluctuations in circulating ADH concentration. However, a hypotensive period has been reported following exercise in which insignificant changes in osmolality (Wilcox et al, 1982; Paulev et al, 1984) and increased (Wilcox et al, 1982) or unchanged (Paulev et al, 1984) levels of ADH are present. Furthermore, no significant correlations have been reported between levels of

ADH and the magnitude of PEH (Wilcox et al, 1982).

2.6.4 Atrial Natriuretic Peptide (ANP)

An atrial peptide with diuretic properties was first isolated from rat atrial muscle over 2 decades ago (Debold, 1985) and since then extensive natriuretic hormone peptides with broad physiological effects including diuresis, aldosterone inhibition and vasodilatation, have been documented (Mohapatra, 2007). The natriuretic peptide system (NP) contains a family of at least 8 structurally related peptides containing a 17-amino-acid core ring and a cysteine bridge and stored as 3 independent prohormones: pro-ANP (126 amino acids), b-type or brain NP (BNP, 108 amino acids) and C-type NP (CNP, 126 amino acids). Pro-ANP gives rise to 4 peptides: i) long acting natriuretic peptide; ii) vessel dilator; iii) kaluretic peptide; and iv) atrial natriuretic peptide (Vesely, 2001). Additonally, processing of Pro-ANP in renal tubular cells produces urodilatin that is released into the circulation (Forssmann et al, 2001). CNP comprises 2 peptides produced by many cell types (Levin et al, 1998). In concert with the diversity of the ANPs, there are 3 ANP receptor sub-types: natriuretic peptide receptor A (NPRA) and natriuretic peptide receptor B (NPRB) that are both coupled to guanylyl cyclase and the cyclic guanosine 3', 5'-monophosphate (cGMP)-independent receptor, natriuretic peptide receptor C (NPRC) (Misono, 2002). NPRA is the primary receptor for ANP and BNP and is constitutively expressed in the heart and other organs; binding of the ligand to NPRA increases cGMP and activates cGMP-dependent protein kinase (PKG). cGMP activation mediates natriuresis, inhibition of renin and aldosterone, vasorelaxation as well as anti-fibrotic, anti-hypertrophic and lusitropic effects (Lee and Burnett, 2007). PKG activation upregulates the ion transport mechanism activating specific transcription factors affecting a range of cellular activities including apoptosis and inflammation

(Mohapatra, 2007). NPRC functions mainly as a clearance receptor (Abbey and Potter, 2003).

NPs are synthesised in a variety of tissues of the mucosa, central nervous system and cardiovascular system and then released into the circulation. ANP and BNP are both found in cardiac tissue. ANP is synthesised in the atrial myocardium whilst BNP is synthesised in the ventricular myocardium. CNP is produced by the vascular endothelium with vasodilative properties. CNP is a novel endothelium-derived hyperpolarising factor that complements the actions of other endothelial vasorelaxant mediators such as NO and prostacyclin (Scotland et al, 2005), whilst also playing an important role in the pathophysiology of heart failure (Del Ry et al, 2006). Unlike ANP, which is rapidly metabolised, the other Pro-ANP peptides are slowly metabolised leaving their plasma concentration higher than that of ANP. This has been postulated as representative of these peptides' regulatory role in water electrolyte balance and vascular tone (Beltowski, 2000).

Investigations into ANP function utilising the genetic knockout mouse paradigm has demonstrated that disruption of the genes that encode for ANP or its receptors not only disrupts normal blood pressure and volume control but also induces hypertrophic effects in the heart. Furthermore, the data indicates that BNP and CNP and their receptors are unlikely to predominate in natriuretic renal effects but regulate autocrine and/or paracrine cGMP mediated vascular smooth muscle tone, water electrolyte homeostasis and cellular proliferation and differentiation in various tissues (Kuhn, 2005).

ANP possesses powerful natriuretic and vasodilatory properties, which assists in fluid regulation and BP control (DeBold, 1985). Distension of the cardiac atria causes granules to release ANP (Vollmar, 1990) and its secretion has been shown to increase

in the circulation following dynamic exercise (Perrault, 1991). The atrial distension resulting from increased venous return is accepted as the source of increased ANP release during upright exercise (Ray et al, 1990). Although ANP has a very short circulating half-life of 2-3-minutes it may still exert residual effects after it is cleared from the circulation (Davis, 1989), thus being capable of mediating PEH. However, ANP concentration was significantly reduced below baseline levels during a period of PEH (Hara and Floras, 1992). Moreover, ANP has previously been reported as having no effect on PEH (MacDonald et al, 1999b) although questions arise about the methodology of both the stimulus and measurement used in this study.

2.7 Summary of Literature Review

A review of the literature indicates that PEH is common after moderate-intensity dynamic exercise in both normotensive and hypertensive individuals and that the haemodynamic responses are greater in hypertensive subjects. The hypotension results from persistent reductions in vascular resistance, mediated by the autonomic nervous system and through vasodilator substances acting via presynaptic or post-synaptic modulation of the α–adrenergic pathway or by direct effects on the systemic vascular endothelium eliciting smooth muscle relaxation. With this in mind, study one utilised a dynamic cycle exercise protocol in humans to establish and confirm the existence of PEH in a cohort of prehypertensive men and compare post-exercise blood pressure changes to those determined during a control non-exercise day.

Study two utilised a dynamic cycle exercise protocol in the same group of prehypertensive men whilst augmenting or attenuating systemic oxygen flux via normobaric hypoxic or hyperoxic gas inspiration. To the authors' knowledge, this was

the first work to address the association between systemic oxygen flux and the resultant effect of changes elicited via the respiratory, cardiovascular and endocrine systems on PEH in any population.

As highlighted in the review of literature, there are little or no published reports on the interactive role of nitric oxide, reactive oxygen species, antioxidant status and clotting factors in post-exercise hypotension. Study 3 investigated these metabolic markers following acute dynamic exercise and was the first study to investigate these markers and the implications for the enhanced systemic vascular conductance that underlies post-exercise hypotension. Study 4 concluded the thesis by investigating loading or unloading systemic oxygen flux and the consequent result on the same metabolic markers as those investigated during study 3. The study followed the same paradigm as study 2 manipulating systemic oxygen flux during exercise to attenuate or potentiate O_2 tension. The novel function of this next study was to investigate these factors during loading or unloading of systemic oxygen flux, and to compare the responses to those highlighted in study 3 during normal exercising conditions. The implications for systemic vascular conductance and post-exercise hypotension was also considered.

2.8 Development of Null Hypothesis (H_O)

The following statements represent the experimental variables considered and analysed in this thesis. The hypothesis may be stated as follows:

H_0 – *null hypothesis*

H_1 – *alternative hypothesis*

Subsequently, depending on the results of the data collected and the statistical analysis performed, it was intended to reject or hold tenable the appropriate hypothesis at the level of significance established.

Hypothesis A – Study 1:

H_0 – An acute bout of dynamic cycle exercise does not induce sustained post-exercise hypotension in a cohort of prehypertensive males.

H_1 – An acute bout of dynamic cycle exercise induces sustained post-exercise hypotension in a cohort of prehypertensive males.

Hypothesis B – Study 2:

H_0 – Systemic oxygen tension during acute dynamic exercise does not mediate post exercise hypotension in a 'PEH-established' cohort of prehypertensive males.

H_1 – Systemic oxygen tension during acute dynamic exercise mediates post exercise hypotension in a 'PEH-established' cohort of prehypertensive males.

Hypothesis C – Study 3:

H_0 – Post-exercise hypotension is not associated with free radical-mediated oxidative stress.

H_1 – Post-exercise hypotension is associated with free radical-mediated oxidative stress.

Hypothesis D – Study 3:

H_0 – Post-exercise hypotension is not associated with redox regulation of circulating $NO^{\cdot}$ bioavailability.

H_1 – Post-exercise hypotension is associated with redox regulation of circulating $NO^{\cdot}$ bioavailability.

Hypothesis E – Study 4:

H_0 – Hyperoxia during acute dynamic exercise does not modify the redox regulation of circulating $NO^{\cdot}$ bioavailability coincident with blunted post-exercise hypotension.

H_1 – Hyperoxia during acute dynamic exercise modifies the redox regulation of circulating $NO^{\cdot}$ bioavailability coincident with blunted post-exercise hypotension.

Chapter 3

General Methodology

3.0 Research Methodology

This chapter includes a description of the procedures used in data collection. Full details of equipment used, testing procedures and statistical analyses are also included.

3.1 Subjects

Nine, sedentary, caucasian pre-hypertensive males, volunteered to participate in the studies. Their mean (SD) age, stature, mass, body mass index and maximum oxygen uptake ($\dot{V}O_{2\,max}$) were 50 (10) years, 175 (7) cm, 88 (17) kg, 29 (6) kg/m^2 and 25 (8) $ml \cdot kg^{-1} \cdot min^{-1}$. 10 subjects were initially recruited into the experimental cohort but one subject had to withdraw prior to commencement of the study. All subjects were free of pharmacological control of blood pressure (BP). Mean arterial pressure (MAP) was 106 (5) mmHg established according to stringent guidelines (Chobanian, et al. 2003) and procedures (section 3.5). Subjects were recruited from local community, general practitioner surgeries after confirmation of established prehypertension over 12-weeks prior to study commencement.

The local Ethical Committee, in accordance with the Declaration of Helsinki regarding the use of human subjects, approved all experimental protocols. The subjects were advised of the risks associated with the study and provided written informed consent.

3.2 Preliminary Testing: Functional Exercise Stress Test (FXT)

Prior to beginning the studies, subjects underwent a functional exercise stress test to preclude any adverse effects to maximal exercise. Prior to the test all subjects were

familiarised with the equipment and testing procedure. The subjects' peak oxygen uptake ($\dot{V}O_{2PEAK}$) was determined using an incremental cycle ergometry test to exhaustion (Monark 824ε, Ergomedic, Varberg, Sweden [See section 3.8]). Each subject started unloaded cycling exercise at a cadence of 70 rpm for the first 3-minutes (70 W). Power output increased by 27.5W at the completion of every 3-minute interval for the first 3 stages. Thereafter, power output increased by the same increment at the completion of every minute until volitional exhaustion. Each subject was instructed to signal clearly to the investigators when they considered they could continue at the specified power output for no longer than 60-seconds. Further criteria used to establish the achievement of a maximal test were a respiratory exchange ratio > 1.15 and establishment of the age predicted maximum heart rate within 10 b/min^{-1}. Systemic arterial BP and rating of perceived exertion (Borg, 1973) (as detailed in sections 3.5 and 3.7, respectively) were attained during the final minute of each stage. HR was determined via a 12-lead electrocardiograph (ECG) (see section 3.4) and expired respiratory gases were collected and analysed on-line (see section 3.6) continuously throughout the test.

There were no contraindications to the maximal exercise test as designated by the British Cardiac Sociey (2001) e.g. Severe angina/rest angina; angina < 1 month post-myocardial infarction; known left main stem stenosis; aortic stenosis; hypotension eg. SBP <90mm Hg; uncontrolled raised BP eg: SBP >180mm Hg, DBP >100mm Hg. The British Cardiac Society (2001) criteria were followed to determine immediate cessation of the test and subsequent exclusion from the study e.g. severe chest pain, dyspnoea or patient wanting to stop; SBP falling >20mm Hg; SBP rise to >230mm Hg; heart rate falling >20% of starting rate; severe dizziness or unsteadiness of gait;

>3mm ST depression; >2mm ST elevation; exercise induced arrhythmias. All subjects enrolled had negative findings for all criterions throughout the test.

3.2.1 Submaximal Workload

For each individual 70% of their $\dot{V}O_{2PEAK}$ was calculated as the target oxygen consumption during the submaximal dynamic exercise trials. The mass required to elicit each individuals' submaximal workload was calculated from linear regression (Excel, Microsoft, 2003) of the mass to $\dot{V}O_2$ relationship following the equation:

$Y = Mx + C$

Where: Y = mass

M = slope

x = $\dot{V}O_2$

C = intercept

A minimum of 5 data points obtained during the FXT were used to calculated submaximal workload. An average of 112 (35)W was required to elicit 70% $\dot{V}O_{2PEAK}$.

3.3 Anthropometric Measures

On all occasions upon arrival at the laboratory, subject body mass, stature, and BMI was determined. Each subject was instructed to undress to briefs and remove footwear prior to the measurement of body mass using a balanced weighing scales (Seca, Cardiokinetics, UK) and stature via a stadiometer (Seca, Cardiokinetics, UK). The weighing scales were calibrated, with a 5kg free mass, prior to all weighing procedures. The accuracy of the stadiometer was verified with a calibrated steel tape measure. Body mass index (BMI) was calculated by dividing subject mass in

kilograms by the square of the subject's stature in meters ($Kg.m^2$).

3.4 Electrocardiography (ECG) Preparation

During the FXT, HR was determined utilising a 12-lead (AT60 Schiller, Switzerland) ECG system and for all submaximal exercise trials HR was monitored via a three-lead, bi-polar, ECG (Lifepulse LP10, HME Limited, U.K.). Both methods were conducted in line with the position statement outlined by the British Cardiac Society (2001). The skin surface was prepared using a scouring pad, cotton wool and clinical swabs. To ensure accurate readings by minimising resistance across the electrodes, body hair was carefully removed using a razor (Wilkinson, UK) the skin was then prepared for electrode attachment by gently rubbing the skin with a scouring pad [to remove the superficial layer of skin] and then cleaning the site with a clinical sterilised swab saturated with 70% v/v isopropyl alcohol (Medi Swab, Smith and Nephew, UK). For the three-lead ECG, electrodes (Ag/AgCl, Skintact, Austria) were secured to the following anatomical sites: right chest (below right clavicle, midway between sternum and shoulder), left chest (below left clavicle, midway between sternum and shoulder), upper abdomen region (upper left side of abdomen). For the 12-lead ECG, electrodes were secured to the following standard anatomical sites:

Bipolar Electrodes (Limb Leads)

Right arm (below right clavicle, midway between the sternum and the shoulder). Left arm (below left clavicle, midway between the sternum and the shoulder). Right and left leg electrodes were placed in the upper abdomen region just under the ribcage (upper left side of abdomen).

Precordial Electrodes (Unipolar Leads)

V_1: 4th intercostal space (right sternal edge), V_2: 4th intercostal space (left sternal

edge), V_3: at the midpoint between V_2 and V_4, V_4: 5th intercostal space (in the left mid-clavicular line), V_5: on same horizontal as V_4 in anterior axillary line, V_6: on same horizontal as V_4 in mid-axillary line. The decision to place the electrodes on these sites was made on the basis of standard procedure (Meek and Morris, 2002) and of limiting the possibility of artefactual readings recorded during exercise. Transpore surgical tape (3M, USA) was used to secure the electrodes and trailing wires in place. The recording monitor was positioned out of subject sight. HR was recorded in beats per minute ($b.min^{-1}$) and the average HR during the last 60-seconds of either submaximal or maximal exercise was noted.

3.5 Systemic Arterial Blood Pressure

On all occasions, systemic arterial blood pressure (BP) (mmHg) was measured in the brachial artery via auscultation with a mercury sphygmomanometer (Accoson Freestyle, U.K.) and stethoscope (Litmann, 3M, USA) by the same experienced investigator according to stringent procedures outlined by Chobanian et al, (2003). For all BP readings a value for systolic blood pressure (SBP) was established 30 (4) s after inflation of the cuff [10(1) s] to a suprasystolic pressure and was noted at the first appearance of clear repetitive tapping sounds (Korotkoff phase 1). Diastolic blood pressure (DBP) was noted at the disappearance of repetitive sounds (Korotkoff phase 5). In some subjects in whom sounds continued until the zero point, diastolic pressure was determined when clear muffling of repetitive tapping sounds became apparent (Korotkoff phase 4) (Chobanian, et al. 2003). Therefore the entire procedure was completed in 40(3) s and was representative of a maximal value. The arm chosen for BP auscultation was the non-dominant arm and both arms were used in preliminary testing to check for variations between sites. The centre point of the cuff

was placed around the upper arm at a point midway between the antecubital fossa and the acromion. This point was marked in order for serial applications of the cuff. Cuff size (diameter) was determined by upper arm girth (anthropometric tape, Caltech, UK) as detailed in Chobanian et al, (2003) and Pickering et al, (2005) in order to control for artifactual readings due to the incorrect cuff size (Pickering et al. 2005). It has been shown that a cuff that is too small produces overestimations of arterial BP whereas a cuff that is too large produces underestimations of arterial BP (Pickering et al. 2005). Cuff size in heavily muscled or obese arms is additionally very important. For arm circumference of 35-44cm, the cuff should be "large adult" size: 16-36cm. For arm circumference of 45-52cm, the cuff should be "adult thigh" size: 16-42cm (Pickering et al. 2005). Arm circumferences in the cohort varied between "standard" cuff and "large adult" cuff size.

3.6 On-line Oxygen Uptake

During the FXT, on-line oxygen consumption ($\dot{V}O_2$) was determined via a metabolic cart system (Medgraphics, CPX/D, Cranleigh, UK), which generates real-time, breath by breath oxygen uptake. Subjects respired through a previously sterilised rubber mouthpiece attached to a 26g (0.9oz) 'prevent' pneumotach (valve dead space = 20ml) and transducer. The pneumotachograph was calibrated at 5 different flow rates using a 3-litre calibration syringe (Hans Rudolph, 5530 series, Kansas City, USA) to verify a linear response prior to exercise. Gas signals were directed to a waveform analyser, which subsequently transforms analog signals to correlate with flow. The volume was calculated by a computerised integration of flow relative to time. Dried expired gas samples were directed to fast responding infra red CO_2 and Zirconium O_2 analysers calibrated prior to and following each test using gases of known O_2

(12.02%, balanced N_2), CO_2 (5.10%, balanced N_2), reference gas, (21%, balanced N_2) (Medgraphics, USA) content. Respiratory parameters expressed at body temperature and pressure saturated (BTPS) were sampled every 30 seconds and printed on-line (Citizen, Swift 200, UK).

3.6.1 Off-line Semi-automated Oxygen Uptake

During the submaximal exercise trials, off-line respiratory gas analysis was determined using the Douglas Bag method. Subjects breathed through a 2-way non-returnable breathing valve (Hans Rudolph, 2400 series, Kansas City, USA). Expired air was directed into a Douglas Bag (150L), via a 1-meter length of Falconia tubing [3.18cm ID] (Cranleigh, UK) and a two-way stopcock valve (Hans Rudolph, Kansas City, USA). The collection of expired gas was hand timed using chronography (Timex, UK) to the nearest whole breath for a specific time period (*i.e.* 60 seconds). Samples of expired air were dried using 97% anhydrous calcium sulfate ($CaSO_4$) crystals (Drierite, USA). Gas fractions were determined using fast responding paramagnetic O_2 and infrared CO_2 analysers (Servomex 1400B4 series Analyser, Crowborough, UK). The precision of the O_2/CO_2 analysers has been documented as 0.1%. Prior to use the analysers were calibrated with precision- analysed gas mixtures containing pure Nitrogen and 17% O_2-5% CO_2 balanced N_2 (Spantek products Ltd, Surrey, UK). The volume of expired gas was measured using a dry gas meter (Harvard Ltd., Edenbridge, UK). Oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) corrected to standard temperature and pressure dry (STPD) was computed utilising the Haldane transformation (Wasserman et al, 1994).

3.7 Rating of Perceived Exertion

During the FXT and the submaximal exercise trials the rating of perceived exertion (RPE) was estimated using the Borg Scale (Borg, 1973). A numeric value, which

ranges from 6-20 may be selected to indicate a perceived rate of exertion from *"very very light"* to *"maximum"*.

3.8 Cycle Ergometer

The same cycle ergometer (Monark 824ε Ergomedic, Varberg, Sweden) was used for the FXT and all experiments. A complete calibration was performed prior to each experiment following the guidelines of Coleman (1996). The underlying principle involves the application of kinetic energy to the flywheel. The flywheel is braked by means of a brake belt located on the flywheel. Workload was adjusted by manually placing weights on the attached basket. A battery controlled electronic output monitor displayed pedalling velocity (rpm). The ergometer had an adjustable seat and handlebars, which were set to suit each individual subject. Seat height was adjusted so that partial knee flexion of between 170° to 175° (with 180° denoting a straight leg position) occurred during the down stroke. Toe-clips and straps attached to the pedals of the ergometer secured the subject's feet to the pedals. All subjects were instructed to remain seated during the test.

3.9 Arterial Pulse Wave Velocity (APWV) [Arterial Compliance]

APWV was measured simultaneously and non-invasively in the arm and leg of the supine subject by the relatively new computerised technique of oscillometry to detect wave timing (time resolution ± 2 ms; QVL SciMed [Bristol, UK]). The non-dominant arm and leg were used for all studies. This was ipsi-lateral for arterial BP measurement but contra-lateral to the venous cannulated limb. All measurements were performed by the same operator (author of thesis). Non-occlusive cuffs were placed over the brachial and radial arteries (in the antero-superior position of the right upper arm and the in the antero-inferior aspect of the right lower arm, at the wrist, respectively) and over the femoral and tibial arteries (at the antero-superior aspect of

the upper right mid thigh and ankle prior to the tibial artery becoming the dorsalis pedis artery, respectively). The cuffs were connected by "non-compliant" tubing to pressure transducers and inflated to 65–70 mmHg for measurement of pulse waves. Pulse pressure waveforms caused by the volume displacement of the traveling pulse wave were obtained from each of the four cuffs. A computer program was developed to characterize the waveform with respect to time at 30, 40, and 50% of peak pressure along its ascending limb and measure the transit time of normal pulse waves between the pairs of upper limb and of lower limb cuffs. The program was designed to discard ectopic beats and abnormal waveforms. Transit times between the proximal and distal sites of the upper and lower limb pairs of cuffs were measured as the average of the time delays between each of the three time points in each pulse and are given as the average of the transit times in 10 consecutive beats. Use of the cuffs over relatively large upper and lower limb arteries avoids complexities inherent in the incorporation of small arteries and assumptions inherent in measuring carotid to femoral pulse transit times (Naka et al, 2003).

PWV ($m.s^{-1}$) was derived as the distance between the proximal edges of each pair of cuffs (meters) divided by the transit time (s). Each recording of PWV took ~ 30 seconds. Measurements were made every minute throughout each study for 12 consecutive measurements (12-minutes). Measurements were taken pre and post exercise. Reproducibility of resting supine PWV was assessed by within-subject coefficients of variation over 30-minutes of consecutive measurements and of 4 separate measurements repeated 7 days apart over 4 weeks. All pre-exercise baseline PWV recordings were with subjects in the fasted state and at the same time on the morning of each study, having avoided strenuous exercise for the preceding 24-hours and abstained from caffeine containing beverages for 12-hours. Subjects were allowed

20-minutes of quiet, supine rest in a temperature controlled laboratory before determination of baseline PWV. Post-exercise measurements were recorded "immediately" after exercise (as soon as was practically possible, i.e., within 3-minutes after the end of exercise), and continuing for the same measurement period at 2-hours and 6-hours post-exercise. All Upper and lower limb PWV recordings were conducted at the same time of day, utilising the exact same methodology, by the same investigator [the author], between study days.

3.9.1 Flow Mediated Changes in APWV as a Marker of Endothelial Function

Upper and lower limb PWV responses (QVL SciMed; [Bristol, UK] time resolution ± 2 ms) to increased flow were studied during the APWV calibration studies (Section 3.9.2). Increased flow through the artery segment under study was provided by local hyperaemic responses to the abrupt release of a 5-minute distal cuff occlusion sited at the wrist and ankle. The cuffs were inflated to supra-systolic pressure (250mmHg) with their rapid release invoking reactive hyperaemia. Baseline PWV was recorded as the mean of a series of 10 consecutive measurements made at 1-minute intervals. For hyperaemic assessment, PWV was measured at 1-minute intervals for 10-minutes post-occlusion. Endothelial function (EF) was assessed via the difference in PWV (Pre-occlusion - Post-occlusion). The reduction in PWV relative to baseline ('the response') was recorded as: a) the maximum response ('peak') which occurs immediately (i.e. 1-minute) after deflation and b) as the overall 10-minute response measured as the mean of a series of 10 consecutive measurements made at 1-minute intervals. The latter approach also provides information about the duration of the response. This methodology and identical system has recently been reported as being a relatively robust and operator independent, non-invasive method for assessing endothelial function (Naka et al, 2006).

3.9.2 APWV Calibration & Standardisation Studies

The method and technique utilised in this thesis has been previously validated in that acute changes in vascular tone induce appropriate changes in PWV, independently of any associated change in systemic BP (Naka et al, 2000). For example, PWV in the upper limb is decreased or increased ~10% by local intra-arterial acetylcholine or N^G-mono-methyl-$_L$-arginine as echocardiographaically determined arterial diameter is increased or decreased, respectively, whereas lower limb PWV, HR and BP remain unchanged (Ramsey et al, 1995; Naka et al, 2000; Kinlay et al, 2001).

For calibration and standardisation in this thesis, APWV was measured in two distinct cohorts. Group one (n = 10, age = 50 ± 10 yr., mean ± SD) was classified as aged pre-hypertensive control and were not on prescribed anti-hypertensive or any other medications or antioxidants and were non-smokers. Group two (n = 8, age = 26.5 ± 7 yr.) who were classified as young normotensive control, non-smokers, free from medication, antioxidants, dyslipidaemia or diabetes. Systemic blood pressure, determined via mercury sphgmomanometry (Section 3.1.2), PWV and endothelial function (EF) were recorded at 09:00, 12:00, 15:00 and 17:00 hrs throughout a single day in group one and at 09:00 hrs the following day in group two. PWV and EF were determined using the SciMed QVL P84 system (on two separate occasions [averaged over 25 measurements at 1-minute intervals in each case]) with pressure cuffs placed proximally and distally to the right brachial and femoral artery as described above (Section 3.9). Subjects presented to the laboratory in a fasted state and group one remained in the laboratory for the day with a standardised meal provided at 13:00hrs. Group one subjects performed activities of daily living (free to move around, watch television, read, etc) whilst in the laboratory. Following confirmation or rejection of a

normal distribution, baseline characteristics between the groups were compared with an independent sample t-test or the non-parametric equivalent Mann-Whitney *U*-test. Experimental responses between subjects (prehypertensive *v.* normotensive) were assessed with an independent sample t-test or Mann-Whitney *U*-test whereas within subject responses (pre *v.* post occlusion) were evaluated with a paired-sample *t*-test or the Wilcoxon's matched-pairs signed rank test served as the nonparametric equivalent. The alpha level was established at $P<0.05$ for all two-tailed tests and values are reported as mean ± (1SD).

3.9.2.1 ***Regional Limb APWV Detection in Control & Prehypertensive Subjects***

Group one had significantly higher ($P<0.05$) resting SBP (133 ± 9.7 *v.* 121± 2.2 mmHg), mean arterial pressure (106 ± 5 *v.* 100 ± 0.9 mmHg) upper limb (UL) [14.2 ± 2.4 *v.* 8.4 ± 0.9 m s^{-1}, respectively] and lower limb (LL) [13.3 ± 2.4 *v.* 9.8 ± 1.3 m s^{-1}, respectively] PWV when compared to group two. As previously discussed (Section 2.2.7) increases in distending pressure increase PWV (Bramwell et al, 1922), therefore account should be taken of the level of systemic BP in studies that use PWV. In light of this, baseline PWV was normalised to systemic mean arterial BP (measured via sphygmomanometry in the brachial artery) in group one and two (PWV/MABP [m s^{-1} $torr^{-1}$]). This process failed to obviate the differences in PWV between the groups (Figure 3.1).

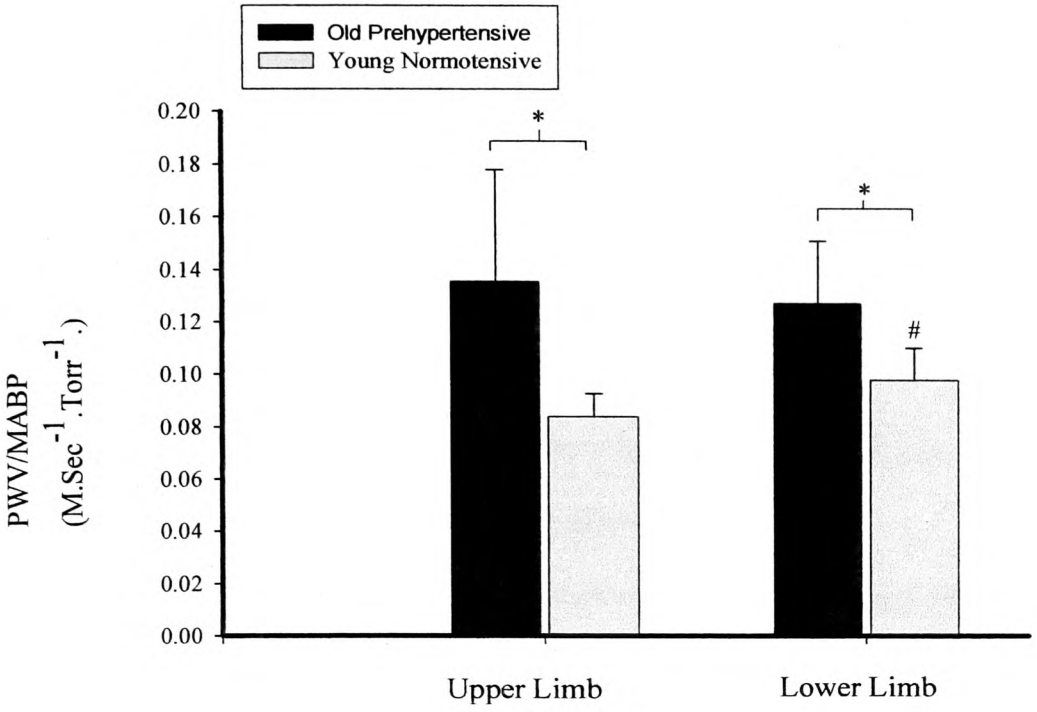


Figure 3.1. Normalised PWV for Differences in Systemic Mean Arterial Pressure. *n* = 10 for pre-hypertensive and *n* = 8 for normotensive subjects. PWV; pulse wave velocity; MABP = mean arterial blood pressure. **P*<0.05 normotensive *v.* pre-hypertensive; #*P*<0.05 *v.* upper limb.

Baseline supine UL normalised PWV was 0.14 ± 0.04 *v.* 0.08 ± 0.009 m s^{-1} $torr^{-1}$ (*P*<0.05) for group one and two, respectively. Similar differences were also noted for baseline supine LL normalised PWV (0.13 ± 0.02 *v.* 0.09 ± 0.01 m s^{-1} $torr^{-1}$) for group one and two, respectively (*P*<0.05). Within group, UL and LL normalised PWV was not different (*P*>0.05) for the hypertensive cohort whereas within group LL normalised PWV was augmented (*P*<0.05) in comparison to UL normalised PWV in the normotensive control group (0.09 ± 0.01 *v.* 0.08 ± 0.01 m s^{-1} $torr^{-1}$, respectively). The residual differences in UL and LL normalised PWV are likely due to the effects of both aging and hypertension on vascular function, *per se*. Normotensive controls

were younger ($P<0.05$) than the hypertensive cohort (50 ± 11 *v.* 26 ± 8 years). As previously discussed (section 2.2.8), senescence causes a decrease in arterial compliance (Vaitkevicius et al, 1993) as a result of progressive elastic fibre degeneration (Avolio et al, 1998) in the arterial wall. Moreover, hypertension is associated with a multitude of factors (see section 2.2.8) that lead to pathophysiological changes in the arterial wall and thus decreased arterial compliance.

3.9.2.2 *Regional Limb APWV Differences between Control & Hypertensive Subjects in Response to Reactive Hyperaemia*

Figure 3.2 details the UL and LL PWV response to flow-mediated dilatation. Groups displayed a regional limb difference in the PWV response. In the normotensive subjects post-occlusion LL PWV decreased ($P<0.05$) by 6 ± 1% compared to pre-occlusion LL PWV (Figure 3.2), whereas no differences ($P>0.05$) were observed for UL PWV measurements pre to post-occlusion. In the older pre-hypertensive subjects, post-occlusion UL PWV decreased by 11 ± 1% compared to pre-occlusion UL PWV (Figure 3.2), whereas no differences ($P>0.05$) were observed for LL PWV measurements pre to post-occlusion. These within and between group differences remained when PWV was normalised to changes in systemic arterial blood pressure. A site-specific difference was also noted when comparing velocities between limbs within normotensives either pre- or post occlusion. UL PWV in normotensive control subjects was attenuated in comparison to LL PWV both pre- and post-occlusion ($P<0.05$). In contrast, PWV in pre-hypertensive subjects was not different ($P>0.05$) between UL and LL pre or post-occlusion.

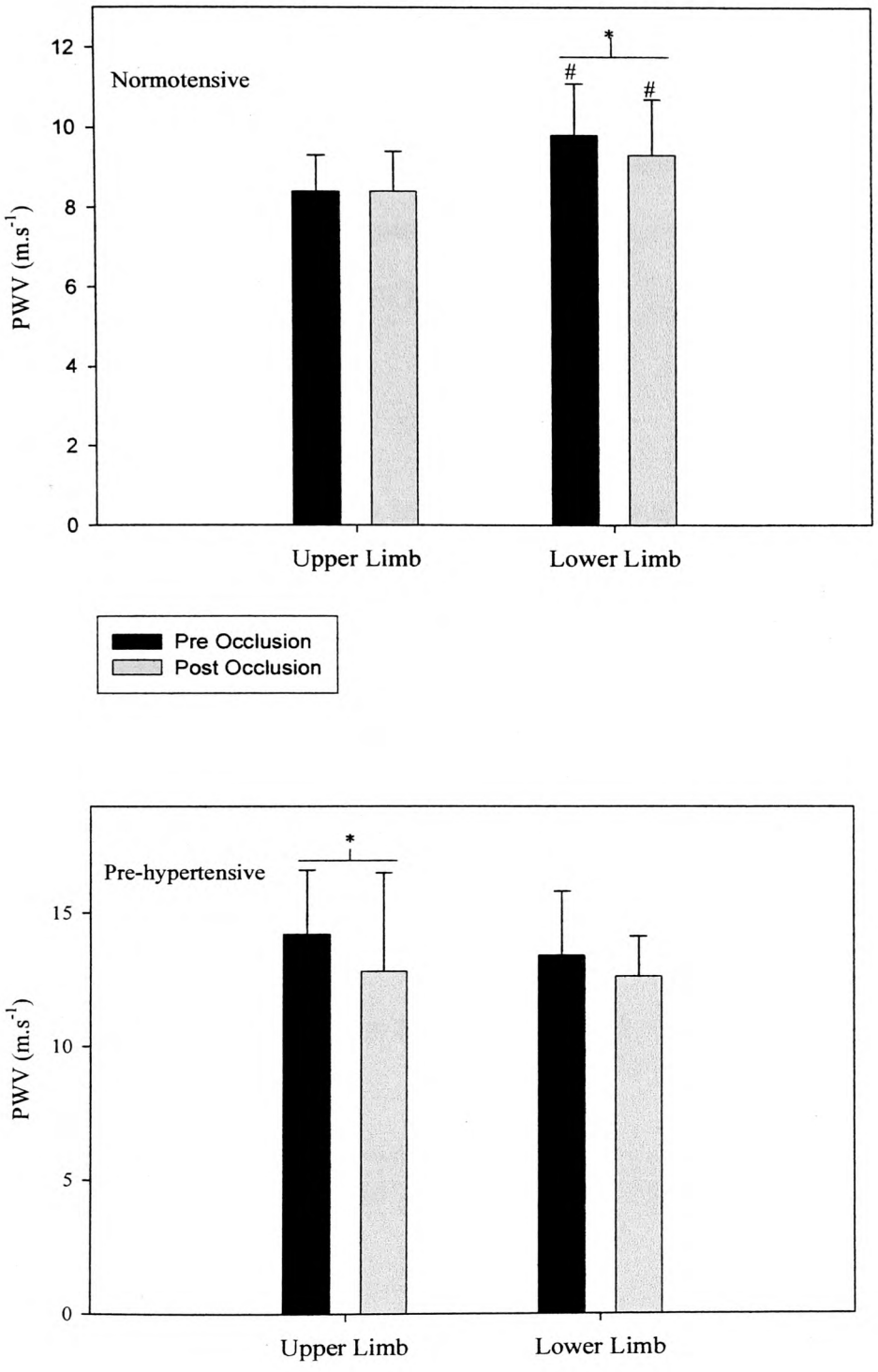


Figure 3.2. Regional PWV in response to reactive hyperaemia in normotensive (*top*) and pre-hypertensive (*bottom*) subjects. n = 10 for pre-hypertensive and n = 8 for normotensive subjects. PWV; pulse wave velocity. *P<0.05 pre *v.* post-occlusion; $^{\#}P$<0.05 *v.* upper limb.

3.9.2.3 *Reproducibility & Variability of PWV Measurement*

In group one, mean arterial pressure, UL, LL PWV and UL, LL EF showed little variability throughout the day (P>0.05) (Table 3.0). Values are an average of 12 readings pre- and 12 readings post 5-minute occlusion, thus total measurement time was 29-minutes at each time-point. Measurements were conducted on the hour at 4 time-points throughout the day (Table 3.0).

Table 3.0. Daytime Variation in Blood Pressure & Pulse Wave Velocity in Pre Hypertensive Subjects

Time (24hrs)	**09:00**	**12:00**	**15:00**	**17:00**
MABP (mmHg)	105 ± 6	104 ± 6	104 ± 6	104 ± 8
UL PWV ($M.S^{-1}$)	14.2 ± 4.4	12.8 ± 3.1	11.8 ± 3.5	12.9 ± 2.5
LL PWV ($M.S^{-1}$)	13.3 ± 2.5	12.9 ± 1.2	13.6 ± 2.2	13.1 ± 1.2

n = 10. MABP = Mean Arterial Blood Pressure; PWV = Pulse Wave Velocity; UL = upper limb; LL = lower limb.

The within-subject co-efficients of variation for PWV were *1)* 13% for the UL and 9% for the LL, measured for 10 consecutive measurements at 1-minute intervals (n = 17 subjects). *2)* 11% for the UL and 10% for the LL, measured for 10 consecutive measurements at 1-minute intervals once per hour, at 3 distinct hour points (09:00hrs, 12:00hrs and 15:00hrs) over 6-hours total duration (n = 9). *3)* 10% for the UL and

12% for the LL, for 10-minute averaged levels of measurements repeated four times at 7-day intervals (n = 9). This variation is higher (5-7%) than published values for coefficients of variation in "normal" subjects utilising the exact same PWV system and methodology (Naka et al, 2003). The one notable difference however with the study of Naka et al (2003) is that the current data is a mixture of "normal" and hypertensives thus increasing the variability in results due to labile pressure modulations in the latter cohort.

3.9.2.4 *Regional Limb PWV Detection when Fasted or Postpradial in Normotensive Controls*

A final validation study was conducted using group two to investigate any influence of the fasted v post-prandial state on PWV and EF determined via the SciMed QVL P84 system. PWV measurements were assessed on two separate occasions, separated by at least 5-days, as previously described (Section 3.9). Subjects presented to the laboratory following either an overnight 12-hour fast or following consumption of a standardised meal (that limited caffeine intake) in the preceding hour to the measurements. Subjects were instructed to refrain from vigorous physical activity for 24-hours prior to PWV measurements on both occasions. PWV and EF as determined by the SciMed QVL P84 system did not appear to be affected by consumption of a meal prior to assessment (Table 3.1). Values are an average of 12 readings pre- and 12 readings post 5-minute occlusion, thus total measurement time was 29-minutes at each time-point.

Fasted UL pre-occlusion PWV was not different from post-prandial UL pre-occlusion PWV [8.4 (0.9) *v.* 8.8 (1.2) m/s, respectively, $P>0.05$]. Likewise, Fasted LL pre-occlusion PWV was not different from post-prandial LL pre-occlusion PWV [9.81 (1.3) *v.* 9.91 (1.6) m/s, respectively, $P>0.05$]. Differences were noted however

between UL and LL PWV within conditions (e.g. UL pre-occlusion *v.* LL pre-occlusion within fasted condition and within post-prandial condition. (Table 3.1).

Table 3.1. Regional Limb PWV and Prior Meal Consumption in Normotensive Controls

Variable	Fasted	Postprandial
UL Pre-PWV ($M.S^{-1}$)	8.4 (0.9)	8.8 (1.2)
LL Pre-PWV ($M.S^{-1}$)	9.8 (1.3)*	9.9 (1.6)*
UL Post-PWV ($M.S^{-1}$)	8.4 (1.1)	8.4 (1.1)
LL Post-PWV ($M.S^{-1}$)	9.3 (1.1)*	9.4 (1.7)*

n = 8, UL, upper limb; LL, lower limb; pre-PWV, pre-occlusion pulse wave velocity; post-PWV, post-occlusion pulse wave velocity; *$P<0.05$ *v.* upper limb, within condition.

Thus it appears from the above experimental data that consumption of a standardised meal over the course of the experimental paradigm will have no bearing on subsequent detection of PWV using this system.

3.10 Central Haemodynamics

There are both invasive and non-invasive methods for determination of central haemodynamic function. Invasive measurements such as aortic and pulmonary artery catheterisation (direct Fick approach) are surgical procedures that carry elevated risk and ethical consideration. Whilst non-invasive methods such as acetylene re-breathing and echocardiography are associated with minimal risk to most populations. Both non-invasive measurements have been validated against the direct Fick approach (Johnson et al, 2000). Invasive and non-invasive methods provide information regarding central cardiac function and integrated physiology of the cardiorespiratory system, mathematically denoted by the Fick equation:

$\dot{V}O_2 = \dot{Q} \times (a - \bar{v})O_2 diff$ **Equation 3.**

$\dot{V}O_2$ is determined via direct assessment of oxygen uptake via metabolic cart or Douglas bag method.

$\dot{Q}$ is determined via direct or indirect method by measurement of stroke volume (SV) and its multiplication to HR.

$(a - \bar{v})O_2 diff$ (arterial venous oxygen difference) is determined via direct assessment of arterial and venous blood gasses or by mathematical reconfiguration of the equation when $\dot{V}O_2$ and $\dot{Q}$ are known.

3.10.1 Stroke Volume (SV)

SV was measured non-invasively in all subjects using echocardiography. All pre-exercise baseline SV recordings were with subjects in the fasted state and at the same time on the morning of each study, having avoided strenuous exercise for the preceding 24-hours and abstained from caffeine containing beverages for 12-hours. Subjects were allowed 20-minutes of quiet, supine rest in a temperature controlled

laboratory before determination of baseline SV. Post-exercise measurements were recorded "immediately" after exercise (as soon as was practically possible, i.e., within 10-seconds after the termination of exercise, [which was the similar time of day on each occasion]), and continuing for the same measurement period at 1-hour and 2-hours post-exercise. Subjects were placed on their left side with their left arm raised above the head to facilitate left lung displacement and opening of the acoustic windows. Ultrasound gel (Henleys Medical, Herts, UK) was placed on the 3-MHz transducer to maintain the interface between transducer and chest wall. The subject was imaged with an Accuson, Cypress (UK) portable echocardiography machine. 2-dimensional imaging in the parasternal long axis view was utilised to measure the diameter of the left ventricular outflow tract (LVOT). The apical 5-chamber view was obtained by placing the sample cursor in the LVOT. Pulsed-wave Doppler (PW) was measured in the LVOT to obtain the left ventricular velocity time integral (LVVTI). Specifically, the sample box was placed in the LVOT at a point 1cm sub-aortic valve. The negative Doppler signal was traced to form the velocity time integral of the aorta (VTI_{AO}).

Stroke volume (SV) was calculated by measuring aortic flow via the equation:

$$SV\ (ml) = \frac{\pi D^2}{4} \times VTI_{AO} \qquad \textbf{Equation 4.}$$

Where: D = diameter of LVOT (cm or mm)

VTI_{AO} = velocity time integral of the aorta

As with all Doppler measurements care was taken to ensure the angle of incidence was as close to 0° compared to the line of action of the musculature. All

measurements were taken as the mean of 3 consecutive cardiac cycles, and together with the calculations were performed by the same experienced investigator, over the course of 3-weeks, utilising the same equipment on each occasion.

3.10.2 Cardiac Output ($\dot{Q}$)

$\dot{Q}$ was calculated from the measured SV values by the equation:

$\dot{Q}$ (L/min) = SV × HR / 1000. **Equation 5.**

HR was determined simultaneously utilising a three lead, bi-polar, ECG (Section 3.4).

3.10.3 Rate-Pressure Product (RPP)

RPP provides an indirect marker of myocardial work. Myocardial oxygen uptake is determined by interactions between several mechanical factors, of which the most important are the development of tension within the myocardium, its subsequent contraction and HR. RPP was derived from the product of HR and SBP [measured at the brachial artery], which were calculated as previously described (Section 3.4 and 3.5, respectively). Values were divided by 1000 by convention. The RPP index is highly related to directly-measured myocardial oxygen uptake and coronary blood flow in healthy subjects across a wide range of exercise intensities (Nelson et al, 1974).

3.10.4 Stroke Volume Index

Stroke volume index is computed by dividing stroke volume into body surface area (BSA) [ml/m^2] and a further derivative cardiac index is computed by dividing $\dot{Q}$ into BSA (l/m^2). BSA provides an approximation of blood volume and body size, thus providing an index that can be used to compare subjects. BSA was calculated by the formula of DuBois and DuBois (1916) which itself has been further validated by Wang et al (1992):

$BSA = (W^{0.425} \times H^{0.725}) \times 0.007184$ **Equation 6.**

Where: W = weight (kg)

H = height (cm)

3.10.5 O_2 Pulse

O_2 pulse provides a quantitative measure regarding the efficiency of the cardiovascular system. O_2 pulse is essentially the amount of oxygen taken up by metabolically active tissue with each ejection of the left ventricle.

Manipulation of the Fick equation as presented in Section 3.10 above yields determination of $(a - \bar{v})O_2$diff by:

$$(a - \bar{v})O_2\text{diff} = \frac{\dot{V}O_2}{\dot{Q}} \qquad \textbf{Equation 7.}$$

O_2 pulse was then calculated as a product of SV and $(a - \bar{v})O_2$diff this equates to the alternative equation for O_2 pulse $\dot{V}O_2 \div HR$.

3.10.6 Systemic Vascular Resistance (SVR) & Conductance (SVC)

As discussed in section 2.2, mean arterial pressure is the product of $\dot{Q}$ [heart rate (HR) × stroke volume (SV)] and systemic vascular resistance (SVR). Therefore a mathematical derivative of the above equation was used for calculation of SVR and SVC:

$SVR = MAP / \dot{Q}$ **Equation 8.**

$SVC = \dot{Q} / MAP$

Thus calculated values for $\dot{Q}$ and MAP were inputted to derive SVR and SVC values pre- and post-exercise. Values are presented as resistance units (RU) and (ml/min/mmHg), respectively.

3.11 Gas Delivery System

During studies 2 and 4, subjects were exposed to normobaric hypoxia or hyperoxia to manipulate systemic oxygen tension. Normobaric hypoxic or hyperoxic medical grade gas (British Oxygen Company, U.K.) was delivered from 5000 L cylinders, at the prevailing barometric pressure, to a 3000 L Douglas bag reservoir connected to the inspiratory port of a 2-way, non-rebreathing T- valve (Hans Rudolph, 2400 series) via a 2 m length of Falconia tubing [3.18cm ID] (Cranleigh, UK). The Douglas bag was controlled by means of a four-way valve that allowed reserve Douglas bags to be connected in series. Normoxic inspirate was delivered by leaving the four-way valve open to the ambient oxygen fraction (20.93%). The gas was presented to the subject in a double-blind manner. Barometric pressure was determined (Section 3.14) during every testing session to calculate the inspired partial pressure of oxygen. Gas fractions were obtained from a port in the four-way valve and measured using a fast responding paramagnetic O_2 and infrared CO_2 analyser as previously described (see section 3.6.1), blind to the exercising subjects, to monitor inspired partial pressure of O_2. Expired respiratory gases were collected by connecting to the expiratory port of the 2-way, non-rebreathing T- valve, as previously detailed (see section 3.6.1). All equipment (tubing, Douglas bags, valves) was identical across trials. Figure 3.3 displays a schematic overview of the gas delivery system.

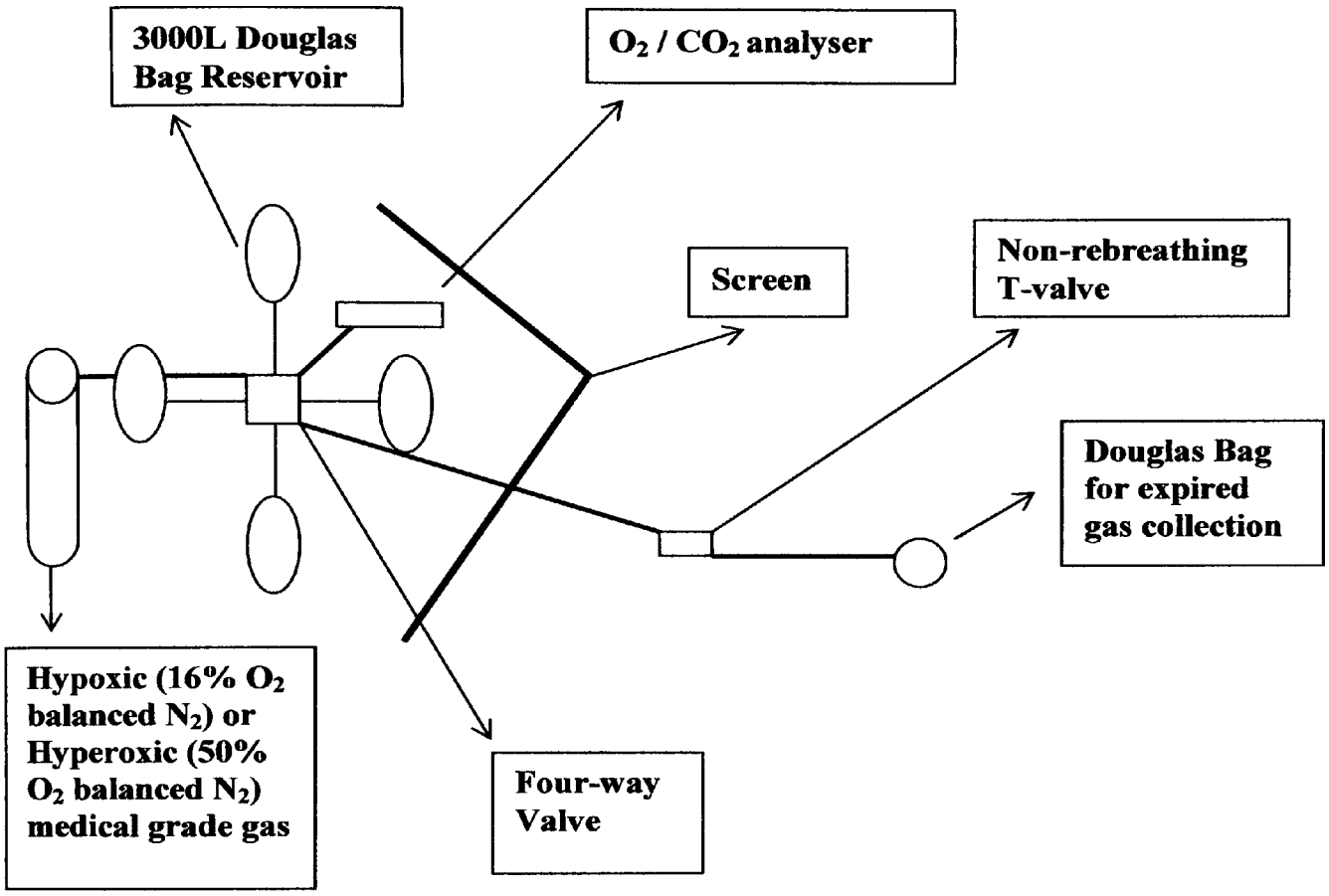


Figure 3.3 Schematic overview of gas delivery system

3.12 Arterial Haemoglobin Oxygen Saturation ($S_{a,O2}$)

Arterial haemoglobin oxygen saturation ($S_{a,O2}$) was measured using finger pulse oximetry (Nonin Model 8800, cardiorespiratory Minnesota, USA). Care was taken to position the sensor on the top and bottom of the end of an index finger. The light emitter portion was placed on the fingernail side and the detector on the side opposite to the nail. The pulse oximeter shines red and infrared light through the tissue and detects the fluctuating signals caused by arterial blood pulses. The ratio of fluctuation

of the two colour signals received determines the $S_{a,O2}$:

$$S_{a,O2} = f \quad \frac{\text{In(min/max)Red}}{\text{In(min/max)IR}}$$

f = function

Red/IR = Red light measured at 660 nm/infrared light at 910nm

The accuracy of this measurement has been estimated at ± 2% (at an $S_{a,O2}$ of between 70-100% [Nonin Model 8800, Laboratory Manual, Minnesota, USA]). Correlation between arterial O_2 saturation calculated from end tidal gas measurement and similar pulse oximeter systems to this one is very good ($r^2 = 0.9$) (Haseler et al, 1998; 1999).

3.12.1 Arterial Blood Gases & O_2 Content

Arterial P_{O2} ($P_{a,O2}$) was calculated from $S_{a,O2}$ using the Hill equation (Equation 9) assuming a normal O_2 half-saturation pressure of Hb (P50) of 26.8 Torr (Wagner, 1996).

$$P_{a,O2} = \left[\frac{S_{a,O2} \times 9971.9}{100 - S_{a,O2}} \right]^{L}$$

L = 0.357 (Hill Constant)

Equation 9.

Arterial O2 content was calculated as:

(1.39 × [Haemoglobin] × measured O_2 saturation) – (0.003 × calculated $P_{a,O2}$)

Equation 10.

3.13 Dietary Analysis

Dietary analysis was conducted before all studies. For 4 days (3 weekdays, 1 weekend day) and for the 48 hours preceding the experimental phase, each volunteer recorded

the quantity and type of food/beverage consumed. The data was analysed using a commercial software package (Balanced Diet version 1.12). Subjects were instructed to maintain their usual dietary pattern. From these, their average daily energy intake was calculated and dietary supplements were designed in order to control for caffeine and sodium intake. During the test day the subjects consumed the prepared meal containing 28% carbohydrate, 3% fat and 60% protein and 0 grams of sodium (Vydex Nutrition, Cardiff, U.K.).

3.14 Laboratory Temperature, Humidity and Barometric Pressure

For all studies laboratory temperature and humidity was determined using a wall-mounted temperature and humidity gauge (Thermo-Hydro) that measured temperature and humidity to the nearest 0.1°C and 0.1%, respectively. Barometric pressure was obtained from a wall-mounted mercury Fortin barometer (Cranleigh, U.K.).

3.15 Haematological Measurements & Biochemistry

3.15.1 Blood Sampling

All blood sampling was carried out by the author, with previous experience in the methods employed, at the same time of day throughout studies. This practice helped standardise blood handling and minimise inter-subject analytical variation (Reilly et al, 1984). It has been shown that diet may adversely affect several blood borne metabolites, in particular plasma lipids and lipoproteins (Pronk et al, 1993), thus all blood sampling was completed after a 12-hour overnight fast. Blood was obtained from a sterilised earlobe (arterialised capillary blood) and from a forearm ante-cubital vein (venous blood). Pre-exercise capillary and venous blood samples were taken after the subject was allowed 20-minutes of seated rest. This procedure in conjunction

with the equations of Dill and Costill (1974) (See Section 3.9.6) was used in an attempt to correct and control for plasma volume shifts.

3.15.2 Collection of Venous Blood

For all exercise trials a 1.2 × 45 mm, 18-gauge cannula (Becton Dickenson, Oxford, U.K.) was inserted into an ante-cubital vein for the collection of blood samples. Each subject assumed a supine position and a tourniquet was fixed above the distal region of the subjects' *m. bicep brachii* (Bachorik, 1982). The arm chosen for cannulation was the contra-lateral limb to that being used for BP measurement. Venous blood was drawn, after cleaning a prominent ante-cubital forearm vein with a sterilised swab saturated with 70% v/v isopropyl alcohol (Medi Swab, Smith and Nephew, UK), into glass collection vials using the vacutainer^{TM} method (Becton Dickinson, Oxford, UK). The cannula was connected to a 3-way sterile stopcock (Connecta plus 3, Ohmeda, Sweeden) allowing connection of a vacutainer via a vacutainer holder with luer adapter (Becton Dickinson, vacutainer systems, NJ, USA). Following blood withdrawal, the cannula was kept patent by flushing the line isovolumically (3-5ml) with physiological saline (0.9% NaCl, Maco Pharma, London, U.K.). The first vial (2ml) of subsequent blood withdrawals was discarded.

Immediately following blood collection, lithium heparin (for ANP, AVP, adrenaline and noradrenaline) and ethylene-diamine-tetra-acetate (EDTA) vacutainers (for NO, renin, angiotensin II, ascorbic acid, and lipid soluble antioxidants) were placed on ice, whilst serum separation tube (SST) vacutainers (for aldosterone, lipids, lipidhydroperoxides, Na^+ and K^+) were allowed to clot for 10-minutes at room temperature. All samples were then centrifuged at 3000 revolutions per minute (RPM) at 4°C for 10-minutes (Centra CL3R, IEC, USA) and the plasma or serum was extracted using a 1ml pipette (Gilson Medical Electronics, France), and transferred to

1.5 ml plastic vials (Eppednorf, Germany). Aliquots were stored at -70°C until assayed to minimise peroxidation during storage (Young and Trimble, 1991). Sodium citrate vacutainers (Fibrinogen and clotting time) were allowed to stand at room temperature. The time from blood withdrawal to storage was no longer than 15-minutes. Assays were performed within six weeks of the study completion and all samples from the same subject were analysed within the same batch. Sodium citrate vacutainers for the determination of fibrinogen concentrations and clotting factors were stored at room temperature until analysis within 2-hours.

3.15.3 Collection of Arterialised Capillary Blood

The volunteer's right earlobe was sterilised with 70% v/v isopropyl alcohol (Medi Swab, Smith and Nephew, UK). Before exercise began, the sample site was punctured using a sterile stainless steel lancet (Lance, Sheffield, UK), and subsequent blood was wiped clean with medical grade cotton wool. In order to obtain a blood sample, gentle pressure was applied to the site using the thumb and index finger, to make the lobe hyperaemic. To overcome any peripheral vasoconstriction following exercise, it was necessary to increase the pressure applied to the sample site to increase blood flow. Extra care was taken to remove excess sweat from the earlobe before blood sampling in order to offset diluting the sample.

3.15.4 Packed Cell Volume (PCV)

A 75mm (59µl) heparinised capillary tube (Hawksley and Sons Ltd, Sussex, UK) was used to collect arterialised capillary blood from the subjects' earlobe. An air bubble free sample was sealed at the distal end with cristaseal (Hawksley and Sons Ltd, Sussex, UK) and carefully inserted into a micro haematocrit centrifuge (Hawksley and Sons Ltd, Sussex, UK) with the sealed end facing outwards. The capillary sample was immediately centrifuged at 11,800 RPM for four minutes and the subsequent packed

erythrocytes were measured using the standard microcapillary reader technique (Hawksley and Sons Ltd, Sussex, UK). The value expressed in L/L of whole blood was subsequently corrected by 1.5% for plasma trapped between erythrocytes (Dacie and Lewis, 1968). Duplicate samples were analysed and the mean of the two was taken as the definitive value.

3.15.5 Haemoglobin (Hb)

The concentration of Hb in whole blood was measured photometrically following the method outlined by Vanzetti (1966). The principle procedure involves the release of haemoglobin from haemolysed erythrocytes by sodium deoxycholate. Hb is converted to methaemoglobin by sodium nitrite, which together with sodiumazide forms azidemethaemoglobin. The absorbance is subsequently measured at two wavelengths (570 and 880 nm). This method has been validated against the established haemiglobincyanide method. Following calibration with an optical interference filter (Hb = 13.7 g/dl^{-1} [8.7 mmol/l] ± 0.3 g/dl^{-1} [0.2 mmol/l]), 10µl of arterialised capillary blood (earlobe) was collected in a microcuvette (Hemocue Ltd, Angleholm, Sweden). The microcuvette was inserted into the β–haemoglobin photometer (Hemocue Ltd, Angleholm, Sweden) and a digital result was presented in approximately 25 seconds. Duplicate samples were measured and the mean of the two was taken as the definitive value.

3.15.6 Percent Change in Plasma Volume

Percent change in plasma volume (ΔPV) and blood volume (ΔBV) were calculated from changes in PCV and Hb (Dill and Costill, 1974). PCV and Hb samples were obtained at the exact same time of each venous blood withdrawal. Blood metabolites were corrected for ΔPV following exercise.

3.15.7 Plasma Atrial Natriuretic Peptide (ANP) and Arginine Vasopressin (AVP)

Venous plasma ANP and ADH were determined using the method of Penney et al (1992). Whereby, a rapid vacuum-driven procedure, using pre-treated Sep-Pak C18 cartridges, elicits the simultaneous extraction of AVP and ANP from plasma. Non-specific interference was removed by fractional elution with an aqueous methanol/trifluoroacetic acid (TFA) mixture. AVP and ANP were coeluted under positive pressure with a methanol/TFA mixture and the eluates air-dried before measurement using separate radioimmunoassay (Penney et al, 1992). The extraction and assay procedures have been validated by observing the changes in plasma AVP and ANP concentrations in normal subjects at different stages of hydration and in elderly patients during treatment for congestive cardiac failure.

3.15.8 Plasma Catecholamines

Venous plasma was assayed for adrenaline and nor-adrenaline via reverse-phase high-performance liquid chromatograpy using electrochemical detection (Bouloux, et al. 1985).

3.15.9 Plasma Renin

Venous plasma was assayed for renin via an Active Renin kit (Nichols Institute Diagnostics, Ca. USA) utilising an immunoradiometric assay using antibodies directed against the active renin molecule, rather than measuring the enzymatic activity of renin.

3.15.10 Plasma Angiotensin II

Angiotensin II concentrations were determined in venous plasma via Euria-Angiotensin II (Euro-Diagnostica AB, Malmo, Sweeden). Following an extraction procedure, angiotensin II was measured by a double-antibody radioimmunoassay.

3.15.11 Serum Aldosterone

Venous serum aldosterone concentrations were determined using a solid-phase radioimmunoassay (Diagnostic Products Corporation, Ca. USA).

3.15.12 Plasma Nitric Oxide Assessment

Over 23 years ago it was demonstrated that both rodents and humans excrete much larger amounts of nitrate (NO^-_3) than could be accounted for by ingestion of food. Subsequent studies converged to identify $NO^\cdot$ as the precursor of NO^-_3 (Tarpey et al, 2004). $NO^\cdot$ is relatively unstable in the presence of molecular oxygen, rapidly and spontaneously autoxidizing to yield a variety of nitrogen oxides, such as nitrogen dioxide (NO_2), dinitrogen trioxide (N_2O_3) and nitrite (NO^-_2). The only stable product formed by the spontaneous autoxidation of NO in oxygenated solutions is NO^-_2. However, analysis of urine or plasma predominantly detects NO^-_3. The mechanisms by which $NO^\cdot$ is converted to NO^-_3 in vivo are not fully elucidated, although two possibilities exist (Tarpey et al, 2004). Ignarro et al (1993) indicated that the NO^-_2 derived from NO autoxidation is rapidly converted to NO^-_3 via oxidation by oxyhaemoproteins ($P\text{-}Fe^{2+}O_2$) such as oxyhaemoglobin or oxymyoglobin. There are however, some limitations to this work, most notably the supraphysiological dose of $NO^\cdot$ (300μm) utilised and the very long reaction time in the order of 2-3-hours (Ignarro et al 1993). A more reasonable explanation for the preponderance of NO^-_3 *in vivo* is related to the low levels of $NO^\cdot$ produced from NOS eliciting a longer half-life of $NO^\cdot$. Under these conditions $NO^\cdot$ would react directly and extremely rapidly with $P\text{-}Fe^{2+}O_2$ to yield NO^-_3 before it can autoxidise to NO^-_2 (Tarpey et al, 2004). Determination of urinary or plasma levels of NO^-_2 or NO^-_3 provides a useful method to quantify systemic $NO^\cdot$ production *in vivo*. Importantly urinary or plasma NO^-_3

levels reflect both endogenous NO· production and total ingestion from the diet including the minor contribution made by bacteria in the gut. Therefore, subjects should be fasted before analysis. Care must also be taken when collecting blood samples for NO· determination. Samples must be free from any source of heparin, as heparinised plasma may form a precipitate on addition of the highly acidic Griess reagent, rendering the samples unusable (Grisham et al, 1996). Two separate methods were utilised in this thesis for the indirect measurement of NO·.

3.15.12.1 Determination of Plasma Nitrate/Nitrite

NO^-_3 and NO^-_2 concentrations were determined using the diaminonaphtalene fluorometric assay and fluorescence spectroscopy. Although the Griess reaction is a simple, rapid and inexpensive assay for NO^-_2 and NO^-_3 in physiological fluids, it has a practical sensitivity limit of only 2-3 μM (Tarpey et al, 2004). More sensitive fluorometric assays have been developed that exploit the ability of NO· to produce N-nitrosating agents. The current assay employed the use of the aromatic diamino compound 2.3-Diaminonapalene (DAN) as an indicator of NO· formation (Miles et al, 1996). The relatively nonflourescent DAN reacts rapidly with the NO-derived N-nitrosating agent dinitrogen trioxide (N_2O_3) generated from the interaction of NO· with O_2 or from the acid-catalysed formation of nitrous acid from nitrite to yield the highly fluorescent product 2,3-naphthotriazole (NAT) (Figure 3.4). This assay offers additional advantages of specificity, sensitivity and versatility with detection limits as little as 10-30 nM (10-30 pmol/ml) NAT and can be used to quantify NO· generated under physiologically relevant conditions (Tarpey et al, 2004).

The detailed methodology employed was as follows: Blood samples were collected in EDTA vacutainers and all chemicals were purchased from Sigma-Aldrich (Dorset, UK) unless otherwise stated. All solutions were prepared fresh on the same day as

analysis. The method was as follows: 500µl of plasma was filtered through membranes (Sartorius, Vivaspin 500, Epsom, UK) by centrifuging at 10,000g for 30-minutes (Sorvall Micro Max, Jencons PLS).

The membranes were washed three times by centrifuging at 10,000g for 10-minutes each time with distilled water (Sigma-Aldrich, Dorset, UK). Filtrate was removed after each wash. This procedure ensures removal of 99% of sodium azide from the membranes to enable nitrate reduction to take place. 50µl of plasma filtrate was added via a pipette (Gilson Medical Electronics, France) to 96 well-plates (Dynatech, Nunc Laboratories, UK) in triplicate wells for both nitrite and total nitrite + nitrate measurements (i.e. 6-wells per sample).

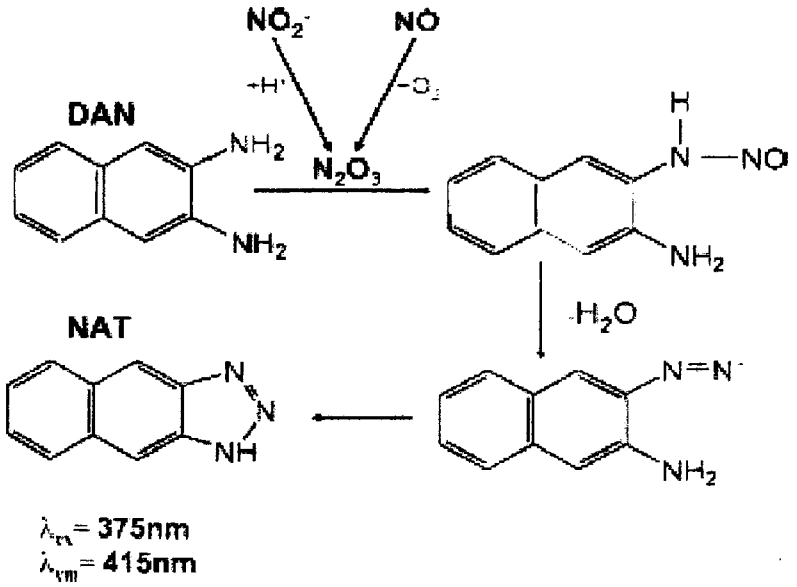


Figure 3.4. Fluorometric detection of NO or nitrite using 2.3-Diaminonapalene (DAN). Taken from Tarpey et al, (2004). The nitrosating agent dinitrogen trioxide (N_2O_3) generated from the autoxidation of NO· or from the acid-catalysed formation of nitrous acid from nitrite to yield the highly fluorescent product 2,3-naphthotriazole (NAT).

Nitrite (10mM [0.069g/100ml double distilled H_20] and nitrate (10mM [0.085g/100ml double distilled H_20] standard solutions (Sigma-Aldrich, Dorset, UK) were made

fresh at the start of each analysis. 200μM solutions of nitrate and nitrite were prepared by diluting the standards (100μl into 5ml double distilled H_20) and were kept on ice. Standard solutions were added in triplicate 50μl volumes into the 96 well-plate (including negative controls). 10μl of 10μM NADPH was added to the 96 well-plate. 40μl of master mix (sodium phosphate buffer [pH 7.4], 5mM glucose-6-phosphate, 1.6μl/ml glucose-6-phosphate-dehydrogenase, 0.8U/ml nitrate reductase) was added to the 96 well-plate. Plates were covered in cling film (3M, UK) then incubated (Laboratory Thermal Equipment Ltd, UK) for 1 – 1.5 hours at 37°C. During this time DAN (Sigma-Aldrich, Dorset, UK) reagent (5ng/ml DAN, 0.62M HCl [Fisher, UK]) was prepared and mixed for 30-minutes, protected from the light by covering with aluminium foil (3M, UK). Following incubation, 10μl of DAN reagent was added to the 96 well-plate and incubated for a further 10-minutes at room temperature in the dark (by covering with foil). Once 10-minutes had elapsed the reaction was stopped by the addition of 5μl of sodium hydroxide (NaOH) solution (46-48% NaOH ¼ diluted in distilled water) to the well plate. Fluorescence was then measured spectrophotometrically (Perkin Elmer, UK) at 450nm against the standard curve (FL WINLAB), 10-minutes following addition of NaOH to allow maximum fluorescence to develop.

3.15.12.2 Detection of Plasma S-Nitrosothiols (RSNO)

The formation and biological properties of NO· -derived RSNO's play an important part of the physiological significance of NO· (Stamler et al, 1992; Arnelle and Stamler, 1995; Stamler, 1995). The autoxidation of NO· in presence of thiols (RSH) generates RSNOs via the following mechanism:

$$2NO + O_2 \longrightarrow 2NO_2$$

$$2NO + 2NO_2 \longrightarrow 2N_2O_3$$

$$2N_2O_3 + 2RSH \longrightarrow 2RSNO + 2NO_2^-$$

RSNOs stimulate guanylate cyclase thereby triggering vasorelaxation. S-nitrosohaemoglobin adducts have also been suggested to be involved in homeostasis of vascular tone and oxygen delivery (Jia et al, 1996; Gow and Stamler, 1998; James et al, 2004).

There are two main methods of measuring RSNO-derived nitrosating species at neutral pH, in extracellular fluids (i.e. plasma), the fluorometric and colourimetric (Wink et al, 1999) techniques. The colourimetric method utilises the components of the Griess reaction whilst the fluorometric method uses the conversion of DAN to its fluorescent triazole derivative (Wink et al, 1999). By using either of these methods at neutral rather than acidic pH, eliminates the interference of contaminating nitrite and allows the detection of nitrosation mediated by the presence of NO·. The colourimetric assay has a detection range of 0.5 - 100μM. The colourimetric methodology was employed in the present study to quantify plasma concentrations of RSNOs.

The original method of Saville was developed to assay thiols, and further adapted to RSNOs. The basis of the method is quantification of the nitrosating specie nitrosonium (NO^+) displaced from the thiol by mercuric ion. The displaced NO^+ reacts, under acidic conditions, with sulfanilamide (Ar-NH_2) (Equation 11,12; Figure 3.5). The resulting diazonium salt (which is formed in amounts equivalent to the thionitrite) is then coupled with the aromatic amine, *N*-(1-napthyl)-ethylenediamine (Ar') to form a coloured azo complex which can be measured at 540nm (Equation 13; Figure 3.5). In principle, the second part of the Saville procedure is analogous to the

classical Griess reaction for the detection of nitrite.

$2RSNO + Hg^{2+} \longrightarrow Hg(RS)_2 + 2NO^+$ **(Equation 11)**

$NO^+ + Ar\text{-}NH_2 \longrightarrow Ar\text{-}N_2^+ + H_20$ **(Equation 12)**

$Ar' + Ar\text{-}N2^+ \longrightarrow Ar\text{-}N{=}N\text{-}Ar'$ **(Equation 13)**

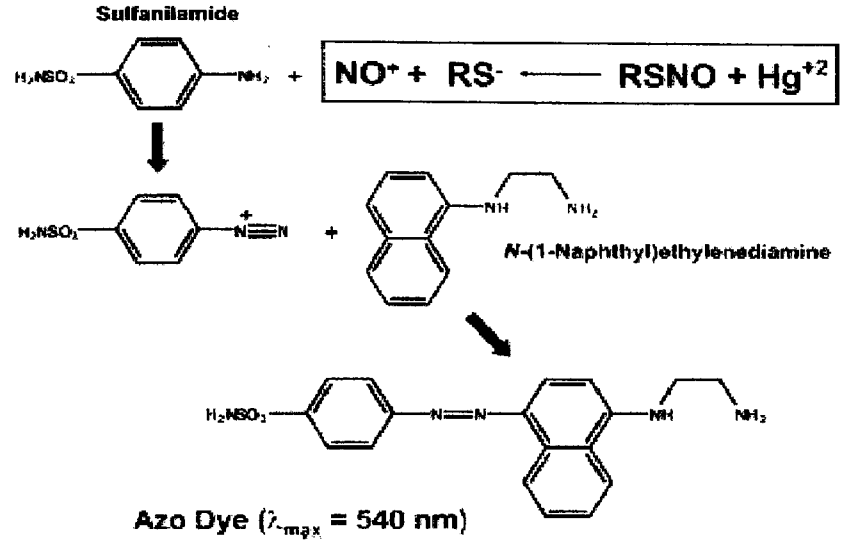


Figure 3.5. Detection of S-Nitrosothiols by the Saville reaction. Taken from Tarpey et al, (2004). Liberation of the nitrosating specie nitrosonium (NO^+) by interaction of RSNO with mercury salts in the presence of the Griess reagents, results in the formation of the diazo product that absorbs strongly at 540nm.

The specific method was as follows: All chemicals were purchased from Sigma-Aldrich (Dorset, UK) unless otherwise stated. All water used was HPLC grade water and care was taken not to contaminate solutions with nitrite as this causes interference at high concentrations. Solutions A, B and C were all prepared fresh on the day of analysis and solutions B and C were protected from ambient light by covering with aluminium foil (3M, UK). Standard solutions were made to calculate the standard curve from 1.34mg G-SNO + 20ml H_20 (200μM) diluted to produce a standard curve

between 0-50μM. Plasma was diluted 1 in 4 with H_20. The assay procedure was as follows: 0.5ml of solution A (1g sulfanilamide dissolved in 100ml 0.5M HCl [Fisher, UK) was added to 0.5ml plasma (in duplicate). 0.5ml of solution B (1g sulfanilamide + 0.2g $HgCl_2$ dissolved in 100ml 0.5M HCl) was added to 0.5ml plasma sample (in duplicate). The solution was then incubated in darkness (by covering with foil) at room temperature for 5-minutes. Following incubation, 0.5ml of solution C (0.02g Naphthylethylenediamine hydrochloride dissolved in 100ml 0.5M HCl) was added to all samples and then incubated for a further 5-minutes. After 5-minutes had elapsed the absorbance of the sample was read spectrophotometrically at 540nm (Perkin Elmer, UK) against the standard curve in the range 0-50μM. The RSNO is quantified as the difference in absorbance between solution B ($+HgCl_2$) and A ($-HgCl_2$) (i.e. B – A) corrected for the dilution factor of the plasma.

Variations of this and the fluorometric RSNO assay have been used successfully to quantify high and low molecular weight RSNOs in human and rat plasma as well as the S-nitrosated derivatives of human and rat haemoglobin (Jourd'heuil et al, 2000a; 2000b).

3.15.13 Oxidative Stress: Serum Lipid Peroxidation Assessment

Lipid peroxidation is used as a secondary marker to indicate damage to cellular membranes following attack from highly volatile reactive oxygen species (Ashton et al, 1999; Davison et al, 2002; 2006; 2008; Bailey et al, 2006). These 'metabolic footprints' signal the end result of a repetitive process whereby polyunsaturated fatty acid (PUFA) molecules are degraded to a variety of end products. The by-products of lipid peroxidation are formed at various stages of the lipid oxidative process and include in order of production, conjugated dienes, lipid hydroperoxides,

malondialdehyde and 4-hydroxynonenal (Davison et al, 2002). The following technique was utilised, in this investigation, to determine peroxidation of lipids in human venous blood.

3.15.13.1 Serum Lipid Hydroperoxides (LOOH)

Hydroperoxides in biological systems can be measured by HPLC coupled with electrochemical determination, chemiluminescence, activation of cyclooxygenase and various methods that incorporate thiobarbituric acid (TBA). Of these, the TBA methods are most widely used, but are also the most criticised on grounds of their underestimation and ambiguity of extent of lipid hydroperoxides (Nourooz-Zadeh et al, 1994). There are currently two simple and reliable spectrophotometric methods in which lipid hydroperoxides may be determined *in vivo*. Both methods are collectively known as 'FOX' (Ferrous Oxidation of Xylenol Orange) assays and may be differentiated by the nomenclature FOX 1 and 2 (Wolff, 1994).

The FOX 1 assay is best suited for the determination of small levels of H_2O_2 in aqueous samples (Wolff, 1994). It is relatively free from interference by other compounds, such as proteins and sodium. The FOX2 assay is used to study H_2O_2 in concentrations of non-peroxidised background lipids. In this instance, alkoxyl radicals generated during the ferrous oxidation step react with native lipid, generating further hydroperoxides in a chain reaction. However, inclusion of a suitable chain breaking antioxidant such as butylated hydroxyl toluene (BHT) overcomes this problem by repairing alkyl radicals produced by the reaction of alkoxyl radicals with unsaturated lipids (Wolff, 1994).

Following the success of the original assay, (Nourooz-Zad eh et al, 1994) have modified the FOX 2 assay by using triphenylphosphine to discriminate between background signal generated by ferric ions present in plasma and that generated by

hydroperoxides in plasma.

Aqueous phase lipid hydroperoxides were determined using a modification of the methods of Wolff (1994) and Nourooz-Zadeh et al, (1994) FOX 1 assay. Hydroperoxides oxidise ferrous ions to ferric ions in dilute acids and the resultant ferric ions can be determined using ferric-sensitive dyes as an indirect measure of hydroperoxide concentration. A blue-purple coloured complex is produced with the selective binding of xylenol orange to the ferric ions produced. The absorption can be measured at 560nm.

$$Fe^{3+} + XO \longrightarrow \text{blue-purple complex (560nm)}$$

(Equation 14)

Plasma samples are spiked with the enzyme catalase (Sigma, Dorset, UK) to discriminate between authentic hydroperoxides reacting with ferrous ions, and H_2O_2 which may be present in the sample. All chemicals were purchased from Sigma-Aldrich (Dorset, UK) unless otherwise stated. The method was as follows: 90µl serum was incubated with 10µl catalase for 30-minutes at room temperature. To this solution 900µl FOX 1 reagent (250 µmol/l ammonium ferrous sulphate, 100 µmol/l xylenol orange, 100 µmol/l sorbitol, 25 µmol/l sulphuric acid [H_2SO_4]), was added via pipette (Gilson Medical Electronics, France), mixed and incubated for a further 30-minutes at room temperature in the dark. Standard solutions were prepared from H_2O_2 in the range 0–5.0 µmol/l and also incubated for 30-minutes with the FOX 1 reagent, after which samples were centrifuged in a Beckman microfuge for 5-minutes to remove any flocculated material. The absorbance of the supernatant was read spectrophotometrically (U-2001, Hitachi, England) at 560nm against the standard curve that was linear in the range 0-5µmol/l. The inter-assay and intra-assay CV was

less than 4% ($n = 9$) and 2% ($n = 9$), respectively.

3.15.14 Determination of Selected Antioxidant Concentration

Antioxidant status of all subjects was obtained by the measurement of selected antioxidants. Following is a description of the procedures and techniques employed in determining selected antioxidant activity in venous blood.

3.15.14.1 Plasma Ascorbic Acid

Ascorbic acid concentrations were determined as described by Vuilleumier & Keck (1989). The procedure is based on the enzymatic oxidation of ascorbic acid and subsequent quinoxaline formation to generate a fluorescent derivative measured on the Cobas Fara centrifugal analyser. The whole blood sample, stabilised with dithiothreitol (Fisher Scientific), was centrifuged and separated upon arrival at the laboratory. The plasma was precipitated by adding 100 µl of plasma to 900 µl of 5% metaphosphoric acid (Fisher Scientific) in a conical microcentrifuge tube and vortex mixed. Once precipitated the sample was stable for 1 year at -70°C. Our samples were assayed within 6 months of collection. Working standards (0, 1, 5, 10, 15, 20 µmol/l) were prepared for each run from a freshly made stock standard of 20 µmol/l ascorbic acid in 5% metaphosphoric acid. Approximately 200 µl of standards and samples were placed in Cobas cups, and oxidising reagent (9.9 ml 2 mol/l acetate buffer and stock ascorbate oxidase) and coupling reagent (20 mg of 1,2-phenylenediamine dissolved in 20 ml deionised water) added to appropriate reagent wells. The assay was then started. The intra-assay CV was calculated for several different serum samples. At a mean concentration of 4.2 µmol/l, the inter-assay CV was 6.80% (n=9), at 50.6 µmol/l the inter-assay CV was 0.72%, while at 151.0 µmol/l

the inter-assay CV was 1.50%.

3.15.14.2 Lipid Soluble Antioxidants

Lipid soluble antioxidant levels were measured using HPLC as described by Craft (1992). Briefly, the method was as follows: Samples or standards (300 µL) were pipetted into glass tubes (4 mL). 250 uL ethanol containing internal standard, α-tocopherol acetate, and 0.25 g/L butylated hydroxy toluene (BHT) was added, followed by 500 ul heptane, the tubes were vortexed vigorously for 1 minute to extract the lipid soluble vitamins into the heptane. The sample tubes were then centrifuged for 5 minutes at 1200 x g and 350 µL of the resulting heptane layer, located at the top, was transferred into a clean set of glass tubes. The original tube was re-extracted and again 350ul removed and added to first extraction giving a final volume of 700 ul. Theses tubes were evaporated to dryness in a rotary evaporator under vacuum for 1.2 – 2 hours. Samples were stored frozen –70^{O} C until analysed. For HPLC analysis, both samples and standards were reconstituted in 150 µL methanol, vortexed and assayed by HPLC using a diode array detector (Thermo Separation Products). Samples and standards were injected onto a Hypersil ODS 5 µm column. The mobile phase contained 97 % methanol: 3 % tetrahydrofurin (all solvents were HPLC grade). Prior to use the mobile phase was filtered and degassed. A quality control serum sample was assayed at the start of every batch of samples. The inter-assay CV for α-tocopherol was 5.4% (n = 7) and 10% (n = 7) for β-carotene. The addition of internal standard allowed for the calculation of intra-assay CV of 4.9% (n = 10).

3.15.15 Serum Lipoproteins and Lipids

Serum concentrations of total cholesterol and triacylglycerol (TAG) were determined via routine enzymatic techniques using a dry film analyzer (Ortho Clinical Diagnostics Ltd, Amersham, UK). The intra-assay and inter-assay CV values were 2% and 4%, respectively. The cholesterol content of serum high-density lipoprotein (HDL-C) was assayed enzymatically after chemical precipitation of other lipoproteins from serum with dextran sulfate and magnesium by using an ILab 600 analyzer (Instrumentation Laboratory, Warrington; Cheshire, UK). The intra-assay and inter-assay CV values were both 3%. Serum low-density lipoprotein cholesterol (LDL-C in mmol/l) was calculated as:

LDL-C = total cholesterol - triglycerides/2.2 - HDL-C (Friedewald et al, 1972)

3.15.16 Haemostatic Factors

Venous citrated plasma was assayed for coagulation and fibrinolysis parameters utilising turbidimetric (coagulometric) analysis (ACL Futura, Instrumentation Laboratory, Warrington; Cheshire, UK). The principle of turbidimetric (coagulometric) clot detection is used in the system to measure and record the amount of time required for a plasma specimen to clot. This technique assesses coagulation endpoint by measuring change in optical density. Light passing through a medium in which fibrinogen is converted to fibrin will be absorbed by the fibrin strands. Light (880nm) transmitted through the plasma sample is monitored by a sensitive photodetector positioned 180° to the incident source. Light absorption increases as fibrin clot formation progresses; consequently light transmittance through the sample continuously decreases. Specific coagulation markers determined were: fibrinogen, international normalized ratio (INR), activated partial thromboplastin time (aPTT),

activated partial thromboplastin time ratio (aPTTr), and prothrombin time (PT). Between assay CV values for fibrinogen was 5.35% at 272, INR was 3.83% at 1, aPTT was 2.29% at 27.5 seconds, aPTTr was 5.86% at 0.96 and PT was 2.84% at 13 seconds.

3.15.17 Homocysteine, Folate, Vitamin B12 & Glucose

Pre-exercise venous plasma concentrations of Homocysteine, Folate and Vitamin B12 were determined via imunochemiluminometric analysis on an Advia Centaur analyser (Bayer Diagnostics, Newbury, U.K.). Between assay CV values were 6.3% at 9.9, 3.6% at 20.4 and 4.7% at 35.2 μmol/l. Pre-exercise fasted glucose was determined in capillary blood via a Reflotron analyser (M06.02). Prior to analysis, the instrument was calibrated with a calibration standard of known concentration (643mmol/l) and repeated 3 times. This method of glucose analysis has been validated against a standard spectrophotometric method.

3.15.18 Whole Blood Lactate ($[La^-]_B$) Analysis

Approximately 50 μl of arterialised capillary blood was collected in a capillary tube (Analox Instruments Ltd) lined with heparin, fluoride and nitrate (glycolytic inhibitors) and mixed for 4 minutes. The appropriate volume of blood required for analysis was achieved with a micropipette (Gilson Microman, France), which was calibrated to 7 μl. The concentration of $[La^-]_B$ was measured using an automated electrochemical analyser (Analox PGM7 Champion, London, UK). Fresh buffer was added to L-lactate:oxygen reductase (LOD) at an ambient temperature of 21°C and placed inside the analyser. Injection of the sample into the cuvette activates an oxidation-reduction reaction catalysed by LOD at a pH of 6.5. The maximum rate of oxygen consumption during the reaction is directly related to the concentration of lactate in the sample. The analyser was calibrated prior to blood sampling using 7μl of

a known calibration standard (8 mmol/l of bovine $[La^-]_B$). 7 µl of a quality control sample (Analox lactate/pyruvate quality control serum) was used to ensure reagent activity and to correct calibration. Duplicate samples were analysed and the mean of the two was taken as the definitive value.

This method of $[La^-]_B$ analysis has been validated against a standard spectrophotometric method (Sigma-Aldrich, Dorset, UK). The interpolation of lactate concentration using this standard has been identified as linear up to a concentration of 10 mmol/l.

3.15.19 Sodium (Na^+) and Potassium (K^+) Analysis

Na^+ and K^+ concentrations were measured using thin film ion specific electrodes on a Vitros 950 analyser (Ortho Clinical Diagnostics Ltd, Amersham, UK). Between assay CV values for Na^+ are 0.8% at 144 mmol/l and 0.99% at 127 mmol/l. For K^+ between assay CV values are 1.33% at 3.8 mmol/l and 1.19% at 6.2 mmol/l.

3.15.20 Plasma Osmolality

Plasma samples were analysed for osmolality utilising the method of freezing point depression on an Advanced Micro Osmometer 3300 (Advanced Instruments Inc. Lab Products, Mass. USA) The principle of operation is based on a supercooling procedure and the subsequent measurement of the freezing point of a sample. Prior to analysis, the instrument was calibrated with 2 or more calibration standards of known concentrations (NaCl ± 2 mOsm-kg H_2O) at each of 2 calibration levels (50 and 850 mOsm-kg H_2O) and 1 reference level (290 mOsm-kg H_2O). A sampler (Advanced Instruments Inc. Mass. USA) was used to inject 20µl of plasma into the micro-osmometer. Sampling repeatability is reported to be ± 2 mOsm-kg H_2O (0-400 mOsm-kg H_2O) and ± 0.5% at 400-2000 mOsm-kg H_2O (Advanced Micro

Osmometer 3300, laboratory manual [Advanced Instruments Inc. Mass. USA]).

3.16 Statistical Analysis

Statistical analysis was performed using the SPSS social statistics package (Version 15, Surrey, U.K.).

3.16.1 Power of the Test

A preliminary attempt was made to assess the sample size required to detect an intervention effect at $P<0.05$, however it must be emphasised that power calculations from complex MANOVA analysis are inherently difficult. The method of Altman (1982) was employed incorporating the critical difference for the main biochemical and cardiovascular parameters (Fraser and Fogerty, 1989). Where appropriate, retrospective power calculations are included.

3.16.1.2 Critical Difference (CD) Determination

CD was assessed using the equation of Fraser and Fogerty (1989):

$CD = K\sqrt{CVa^2 + CVw^2}$ **(Equation 15)**

Where: K = factor dependent on the probability level selected (2.77 at $P<0.05$).

CVa^2 = coefficient of analytical variation

CVw^2 = within subject biological coefficient of variation

3.16.1.3 Coefficient of Variation (CV)

CV was calculated using the following equation:

$$CV = [SD \div Mean] \times 100 \quad \textbf{(Equation 16)}$$

The coefficient of analytical variation (Cva) for biochemistry was determined during analysis. For analysis of other variables CVa was determined by ten consecutive measurements of that specific variable on one subject.

Assay data collected from each subject, at three distinct time points, was used in the calculation of within subject biological coefficient of variation (CVw) utilising the following equation:

CVw (%) = Total variation (%) – CVa (%) **(Equation 17)**

All data was tested for the presence of outliers among mean values according to Reeds criterion (Reed et al, 1971). This criterion considers the difference between the extreme value and the next highest or lowest value, rejecting the extreme if this difference exceeds one-third the range of all values.

3.16.2 Descriptive Statistics

Data were analysed using parametric or non-parametric statistics following mathematical confirmation or rejection of a normal distribution by repeated Shapiro-Wilk W tests and P-P plots. The latter method plots the cumulative proportion for a single numeric variable against the cumulative proportion expected if the sample were from a normal distribution. If the sample is from a normal distribution, points will cluster around a straight line. The alpha level was established at $P< 0.05$ for all two-tailed tests and values were reported as mean ± (1SD).

3.16.3 Comparative Statistics

Data were analysed using parametric or non-parametric statistics following mathematical confirmation or rejection of a normal distribution by repeated Shapiro-Wilk W tests and P-P plots. *Study 1 and 2:* Pre and post-exercise data (or the corresponding time-points for the non-exercise trial) were assessed with a two-way [A × (B)] repeated measures analysis of variance (ANOVA) with one between (*study 1,*

state: exercise *v.* non-exercise) (*study 2 & 4,* state: normoxia, hypoxia, hyperoxia) and one within (time of measurement: pre *v.* post-exercise time points) subjects factor as the repeated measures.

Following a significant interaction, grouped means for time and state were analysed using a one-factor repeated measures ANOVA with *a posteriori* Bonferonni corrected paired samples *T*-tests or Wilcoxon Signed Ranks tests. Pre and post exercise blood metabolites (*study 1 & 3)* were assessed with a one-factor repeated measures ANOVA with *a posteriori* Bonferonni corrected paired samples *T*-tests or Wilcoxon Signed Ranks tests. $S_{a,O2}$ during exercise were analysed with the Friedman test. Following a significant interaction (*time × state*) means were analysed using the Wilcoxon Signed Ranks Test. The alpha level was established at $P< 0.05$ for all two-tailed tests.

The linear relationship between two dependent variables was established using Pearsons Product Moment Correlation Coefficients or the Spearman Rank Order correlation. The absolute values and relative change (Δ; pre-exercise – post-exercise) was used particularly when performing correlations between two dependent metabolites.

Chapter 4

Research Studies

STUDY 1: THE EFFECTS OF ACUTE MODERATE INTENSITY EXERCISE ON POST EXERCISE HAEMODYNAMICS IN THE PRE-HYPERTENSIVE HUMAN

4.0 Introduction

An acute exercise bout produces profound 'after-effects' on systemic arterial pressure that culminates in a sustained depression of arterial pressure for at least 2-hours but may persist for up to 6-hours post-exercise in normotensive sedentary and endurance trained men and women (Kenney and Seals, 1993; Halliwill, 2001; MacDonald, 2002) and hypertensive subjects (Pescatello, 1999). Findings also suggest that hypertensive subjects display a greater post-exercise haemodynamic response (MacDonald, 2002). Several mechanisms have been put forth to explain the sustained peripheral vasodilatation occurring post-exercise such as: baroreflex resetting (Halliwill et al, 1996a; 1996b), attenuated vascular responsiveness to a given level of sympathetic nerve activity (Halliwill et al, 2003a) and a persistent histamine-receptor dependent augmentation of vascular conductance (Lockwood et al, 2005; McCord et al, 2006; McCord and Halliwill, 2006). Thus, factors that modulate smooth muscle tone or vascular responses may modify the post-exercise response.

Along these lines, many metabolites with either direct or indirect effects on the endothelium and/or smooth muscle of the peripheral vasculature have been postulated as potentially mediating PEH. MacDonald (2002) provided an overview of experimental evidence regarding many of these candidate metabolites; however some of the evidence is both dated and lacks a comprehensive analysis of the full role of the metabolites during an extended period of PEH. Furthermore, all of these prior studies have used a single study day to compare post-exercise arterial pressure responses to baseline arterial pressure without an additional non-exercise control day. An

additional control condition allowS haemodynamic changes occurring post-exercise to be ascribed to the exercise bout *per se* above the typical changes that may occur hourly with such a labile parameter as systemic arterial pressure.

Therefore the purpose of the present investigation was to evaluate the response of systemic arterial blood pressure following a single bout of acute dynamic exercise against the response of systemic arterial blood pressure following a control protocol in pre-hypertensive subjects. A further aim was to quantify the effects of an acute bout of dynamic exercise on the peripheral venous plasma concentration of adrenaline, noradrenaline, renin, angiotensin II, aldosterone, ANP and AVP implicated in the vasodilatory response underlying PEH, analysing the relationship between these key blood-borne metabolites and the magnitude of PEH.

The final aim of the present study was to measure and quantify both upper limb and lower limb arterial pulse wave velocity (APWV) following a period of acute dynamic exercise and investigate the relationship between APWV and the magnitude of PEH to determine whether execise-induced modulations in arterial compliance occur over a similar time-course and to a similar degree as to changes in SVR/SVC.

4.1 Methodology

Experimental Protocol

The subjects attended the laboratory over two visits each separated by approximately one week. The randomised visits were: 1) 30-minutes of cycle exercise (Monark 824ε) at 70% of pre-determined $\dot{V}O_{2PEAK}$ [See general methodology section 3.2.1]. 2) A non-exercise, control day following the exact time-course, location and subject positioning as the exercise day. The subjects were then followed post-exercise or post-control for 6-hours. Subjects were randomly assigned to either condition

followed by cross-over. On both occasions after an 8-hour overnight fast, the subjects presented to the laboratory at 8:30 am and underwent baseline assessment of physiological parameters. During the day the subjects consumed the provided standardised meal (see section 3.13) and the laboratory was regulated for temperature (21± 2°C) and humidity (65± 3%) as described in methodology section 3.14.

All subjects attended the laboratory for a familiarisation session 1-week prior to the commencement of the experimental protocol. Subjects were instructed to refrain from exercise and alcohol for 48-hours prior to both days.

Fasting plasma electrolyte concentrations and osmolality across the trial were assessed as detailed in methodology section 3.15.19 and 3.15.20 , respectively. Values were within normal ranges (Table 4.0). Likewise, fasting homocysteine, folate and glucose concentrations, assayed as detailed in methodology section 3.15.17 were within normal ranges (Table 4.1), excluding the possibility of existing co-morbidities.

Table 4.0 Plasma Na^+/K^+ Concentration & Osmolality Pre-Exercise

Variable	Control Study	Exercise Study
$Na^+(mmol.l^{-1})$	142.7 (1.2)	136.2 (5.6)
$K^+(mmol.l^{-1})$	4.5 (0.2)	4.3 (0.4)
Osmolality (mOsm-kg H_2O)	280 (9)	284 (18)

($n = 9$) All data are expressed as mean (SD).

Table 4.1 Fasted Plasma Concentrations of Selected Metabolic Cardiovascular Risk Factors

Metabolic Marker	Concentration	Reference Value[#]
Vitamin B12 ($mmol.l^{-1}$)	288 (61)	180 – 600
Folate ($mmol.l^{-1}$)	8.5 (2.4)	2.7 – 14.0
Homocysteine ($mmol.l^{-1}$)	13.3 (2.2)	<16
Glucose ($mmol.l^{-1}$)	4.5 (1.4)	4.5 – 5.6
TC ($mmol.l^{-1}$)	5.1 (0.6)	3.5 – 6.5
LDL-C ($mmol.l^{-1}$)	3.2 (0.6)	1.55 – 4.4
HDL-C ($mmol.l^{-1}$)	1.3 (0.2)	0.95 – 2.15
Tg ($mmol.l^{-1}$)	1.3 (0.6)	0.7-2.1

All data are expressed as mean (SD). n = 9. TC, total cholesterol; Tg, triacylglycerol; HDL-C/LDL-C, high/low-density lipoprotein cholesterol. [#]Values are typical laboratory reference levels (Kumar & Clark, 1999).

Anthropometric Measures

On arrival at the laboratory, subject body mass, stature, and BMI was determined as outlined in the methodology section 3.3.

Systemic Arterial Pressure & Heart Rate

BP was measured by the same experienced investigator according to stringent procedures outlined in methodology section 3.5. Auscultatory BP readings averaged 131(8)/87(5) mmHg for systolic blood pressure (SBP) and diastolic blood pressure

(DBP), respectively at baseline. Heart rate (HR) was determined with a three lead, bi-polar, ECG as detailed in methodology section 3.4 Baseline recordings were taken after the subjects were allowed 20-minutes of seated rest.

Exercise Protocol

Subjects began a warm-up of unloaded cycle exercise at a cadence of 70 rpm for one minute (70 W) at which point the appropriate weight was applied to elicit 70% of pre-determined normoxic $\dot{V}O_{2PEAK}$. SBP, HR and RPP (see section 3.10.3) were measured every 10-minutes during the 30-minute trial. $\dot{V}O_2$ and $[La^-]_B$ were determined midway through the exercise bout (~15-minutes). At the cessation of exercise the subject was transferred to a couch where they remained quietly seated, measurements of BP, HR, and RPP were taken, following which venous blood was withdrawn. For the first three hours following exercise subjects remained seated with BP, HR, and RPP recorded every 15-minutes. Venous blood was sampled on the hour for the first 2 hours following exercise. After 3 hours the subjects were given their standardised meal and were then allowed to walk and relax (e.g. read, watch television) around the laboratory and adjacent lounge. BP, HR, and RPP were recorded every hour for a further 3-hours and blood was drawn at the 6-hour post-exercise time point. PWV was recorded pre, post, 1-hour, 2-hours and 6-hours post-exercise. Central haemodynamic recordings were taken pre, post, 1-hour post and 2-hours post-exercise. Figure 4.1 displays an overview of the experimental design.

Control Trial

For the non-exercise day subjects followed the exact same time course but without the stimulus of exercise or blood sampling. At the same time of day the subjects

performed 3-hours of seated rest followed by a standardised meal and then 3-hours of the same activities encountered on the exercise trials

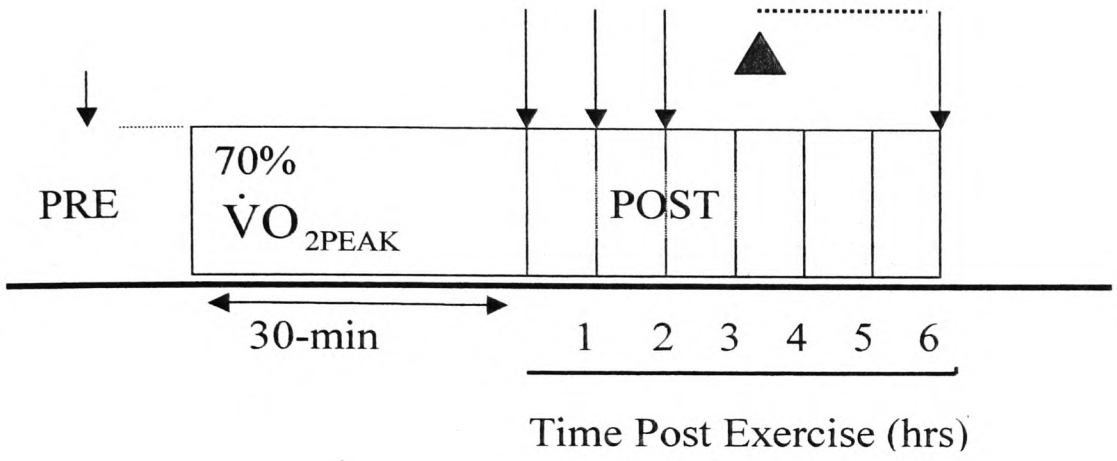


Figure 4.1. Overview of the Experimental Design. Subjects (n = 9) were randomised to either condition and counter-balanced. Workload is a percentage of pre-determined peak oxygen uptake. Arrows indicate timing of venous blood collection [see text for details]. Systemic blood pressure, heart rate and rate pressure product were measured at baseline, during exercise, every 15-minutes for the first three hours and every hour thereafter post-exercise [see text for details]. APWV was recorded pre, immediately post, 2-hours and 6-hours post-exercise. Central heamodynamic recordings were taken pre, immediately post, 1-hour post and 2-hours post-exercise. Triangle indicates the time point at which the standardised meal was given. Dotted line highlights the time period that the subjects were free to move around the laboratory. The non-exercise day followed the same procedure minus the exercise stimulus and blood sampling. $\dot{V}O_{2PEAK}$, Peak oxygen uptake.

Venous Blood Collection & Analysis

Pre- and post-exercise venous blood samples were collected using the cannula method outlined in methodology section 3.15.1. ΔPV and ΔBV (i.e. percent change in PV and BV) following exercise were calculated as described in methodology section 3.15.6.

At every blood collection time-point all blood metabolites were corrected for haemodilution or haemoconcentration.

Adrenaline & Noradrenaline Analysis

Systemic venous blood plasma was assayed for adrenaline and noradrenaline via the method outlined in methodology section 3.15.8.

Renin & Angiotensin II Analysis

Systemic venous blood plasma was assayed for renin and angiotensin II via the method outlined in methodology section 3.15.9 and 3.15.10, respectively.

Aldosterone Analysis

Aldosterone quantification was performed on systemic venous serum utilising the methodology described in section 3.15.11.

ANP & AVP Analysis

ANP & AVP concentrations were determined in systemic venous plasma by the methodology outlined in section 3.15.7.

$[La^-]_B$

Arterialised capillary blood was collected and analysed for whole blood lactate as outlined in methodology section 3.15.18.

Plasma Osmolality

Plasma osmolality was assessed by the methodology outlined in section 3.15.20.

Central Haemodynamics

Central haemodynamic data was collected on subjects following the procedures laid out in methodology section 3.10.

APWV

Pre and post-exercise or sham APWV was determined in subjects as detailed in

methodology section 3.9.

Statistical Analyses

Statistical analysis was performed using the methodology outlined in section 3.16.3.

4.2 Results

4.2.1 Cardiorespiratory Response To Exercise

Table 4.2 displays the cardiorespiratory responses to sub-maximal exercise. Expected increases were noted in the major cardiorespiratory variables at the termination of exercise. The goal was to have subjects exercise for 30-minutes at 70% $\dot{V}O_{2PEAK}$. Four subjects failed to complete the full 30-minute exercise bout, mean (SD) exercise duration was 25 (6) minutes. Exercise intensity equated to 75 (15)% of previously determined values with an average workload of 112 (35)W. Heart rate was 140 (17) bpm during exercise. This represented, on average, 86 (11) % of maximal heart rate determined during $\dot{V}O_{2PEAK}$ testing and is consistent with the target workload.

4.2.1.2 Blood Pressure Response

Mean arterial blood pressure (MABP) was unmodified throughout the day following a period of 30-minutes quiet seated rest ($P>0.05$) (Figure 4.2). Thirty minutes of dynamic cycle exercise, performed at the same time of day as the seated rest but on a different day, produced a 5% reduction of MABP versus pre-exercise. MABP was reduced by ~5mmHg at the 1-hour post-exercise time-point ($P<0.05$) (Figure 4.2). The PEH persisted for the entire 6-hour post-exercise period, i.e. until cessation of recordings, with a nadir at 60-minutes post-exercise. This fall in MABP resulted in a significantly lower MABP at the 1 and 2-hour recovery time-points during the exercise trial as compared to the same time-points in the non-exercise sham trial ($P<0.05$) (Figure 4.2). The reduction in MABP resulted from a post-exercise fall in

systolic blood pressure (SBP) whilst diastolic blood pressures remained invariant following exercise (Table 4.3). SBP was attenuated by ~10% 1-hour post-exercise and remained ~5% below pre-exercise levels until cessation of recordings 6-hours following exercise ($P<0.05$). SBP values were lower ($P<0.05$) at 1 and 2-hours post-exercise when evaluated against the control trial (Table 4.3).

Table 4.2. Cardiorespiratory responses to 30-minutes sub-maximal cycle exercise in Pre-hypertensives

Variable	Pre	Termination^
VO_2 (l min^{-1})	0.4 (0.5)	1.6 (0.4)†
V_E (l min^{-1})	-	42.5 (7.6)
Heart Rate (bpm)	69 (7)	146 (18)†
Systolic BP (mmHg)	128 (8)	180 (18)†
Diastolic BP (mmHg)	85 (4)	84 (4)
RPP (mmHg/min)	9 (1)	26 (4)†
RPE (unitless)	-	14 (4)
$[La^-]_B$ (mmol/l)	1.2 (0.3)	2.7 (1.1)†

All data are expressed as mean (SD). n = 9 except for V_E where n = 5. Termination indicates the termination of exercise ^(apart from V_E and lactate which was determined at the mid-point of the exercise bout); VO_2, oxygen uptake; V_E, Ventilation; BP, blood pressure; RPP, rate pressure product; RPE, rating of perceived exertion; $[La^-]_B$, whole blood lactate. †$P< 0.05$ *v.* pre-exercise.

Table 4.3. Cardiovascular responses to either 30-minutes sub-maximal exercise or control condition in Pre-hypertensive subjects

Variable	Pre		Post		60-min		120-min		360-min	
	Ex	Control	Ex	Control	Ex	Control	Ex	Control	Ex	Control
HR	69 (7)	70 (7)	102 (14)*	-	70 (8)	70 (7)	65 (5)	70 (7)	69 (10)	69 (7)
SBP	128 (8)	132 (10)	128 (10)	-	116 (7)*	133 (11)†	119 (9)*	133 (9)†	122 (8)*	132 (11)†
DBP	85 (4)	90 (5)	84 (4)	-	82 (3)	89 (4)	83 (4)	89 (5)	84 (4)	89 (6)
RPP	8.8 (1.2)	9.2 (1.3)†	13.1 (2.4)*	-	8.1 (1.1)	9.4 (1.4)†	7.8 (1)*	9.3 (1.3)†	8.6 (1.6)	9.2 (1.4)

All data are expressed as mean (SD). n = 9. HR, heart rate (bpm); SBP, systolic blood pressure (mmHg); DBP, diastolic blood pressure (mmHg); RPP, rate pressure product (mmHg/min). Ex, exercise; control, non-exercise. Post, immediate (i.e. within 1-minute) post-exercise. No immediate (i.e. within 1-minute) post-exercise data was available in the control trial. 60-min, 60-minutes post-exercise; 120-min, 120-minutes post-exercise; 360-min, 360-minutes post-exercise. * $P<0.05$ *v.* pre-exercise within condition; † $P<0.05$ *v.* corresponding time-point between conditions.

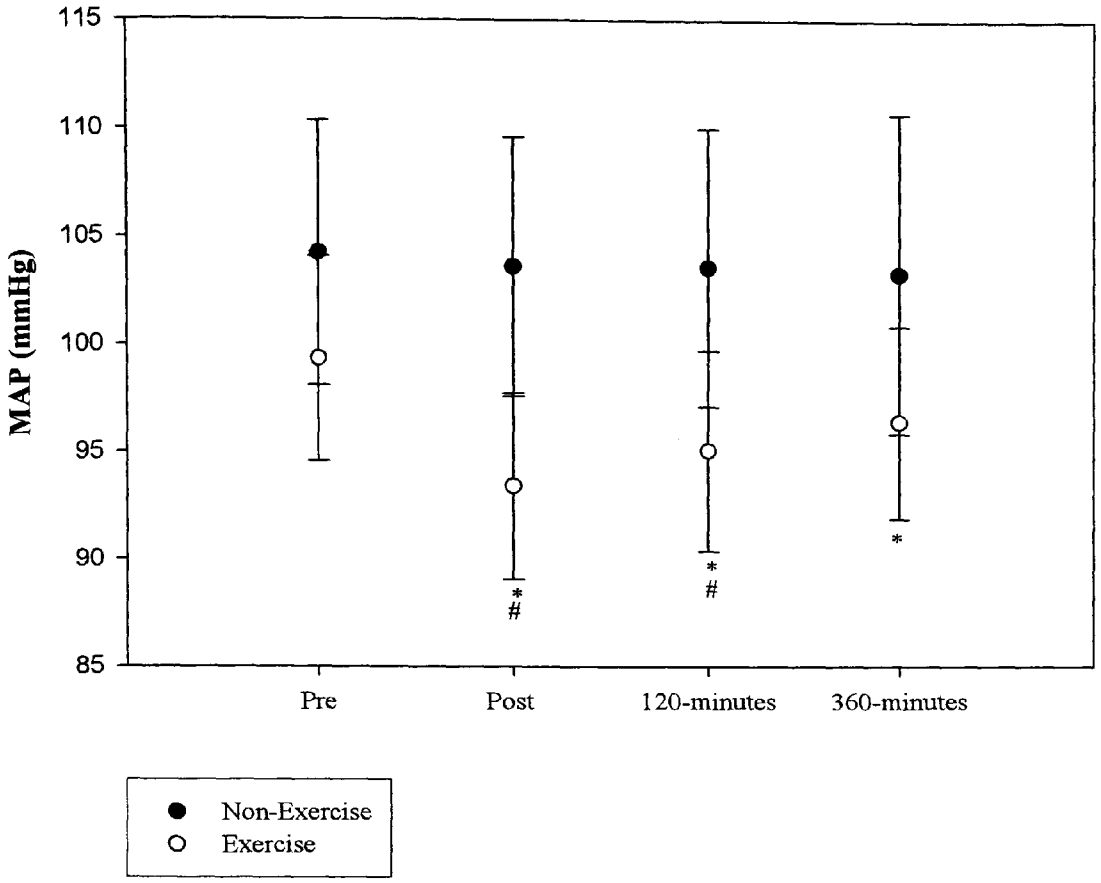


Figure 4.2 Mean arterial blood pressure response to either 30-minutes dynamic cycle exercise (open symbols) or 30-minutes quiet seated rest (shaded symbols). Values are Mean ± (SD). MABP, mean arterial blood pressure. Post, within 3-minutes post-exercise; 120-min, 120-min post-exercise; 360-min, 360-min post-exercise. * $P<0.01$ *v.* pre within condition. $^{\#}P<0.05$ *v.* same time-point between conditions.

4.2.1.3 Heart Rate (HR) & Rate Pressure Product (RPP)

Immediately following exercise (i.e. within 1-minute) HR remained elevated ($P<0.05$) but returned to baseline values by the 1-hour post-exercise recovery time point (Table 4.3). HR values were not different between the exercise and sham conditions at the 1, 2 and 6-hour post-exercise time points ($P>0.05$). In line with SBP data, RPP values differed between conditions (Table 4.3) being augmented by 14 and 16% at the post-1 and –2 hour time-points, respectively, in the sham trial. Of note, baseline RPP values differed between conditions.

4.2.2 Postexercise Haemodynamics

Table 4.4 displays post-exercise *versus* pre-exercise haemodynamics. Immediately post-exercise both HR and CO were elevated above baseline ($P<0.05$) but returned to pre-exercise levels by 1-hour post-exercise. SVR was reduced during recovery from exercise throughout the entire post-exercise recording period. SVR was attenuated by ~30% immediately post-exercise and remained blunted, in comparison to baseline, by ~13 and 8% at 60- and 120-minutes post-exercise, respectively ($P<0.05$). The return in SVR back towards baseline was significant at both the 60- and 120-minutes post-exercise time-points when compared to immediately post-exercise (both $P<0.05$). Likewise systemic vascular conductance was augmented by ~28 ml/min/mmHg remaining elevated above baseline until the cessation of recording at 120-minutes post-exercise ($P<0.05$; Figure 4.4). At 120-minutes post-exercise SVC was significantly blunted when compared to the immediate post-exercise values ($P<0.05$). Both arterial-venous oxygen difference and O_2 pulse increased (both $P<0.05$) during the initial post-exercise period by 43% and 49%, respectively but returned to baseline levels by 1-hour post-exercise.

Table 4.4. Exercise Trial Haemodynamics

Variable	Pre	Post	60-min	120-min
HR (bpm)	69 (7)	102 (14)*	70 (8)	65 (5)
Stroke Volume (ml $beat^{-1}$)	92.6 (30.7)	83.3 (42.2)	95.2 (31.2)	96.5 (32.4)
SV Index (ml/m^2)	48.2 (16.1)	43.1 (21.4)	49.6 (16.5)	50.2 (16.9)
Cardiac Output (l min^{-1})	6.5 (2.5)	11.3 (5.3)*	6.7 (2.5)	6.6 (2.6)
SVR (RU)	17.1 (4.5)	12.1 (3.9)*	15.1 (3.9)*	15.8 (3.8)*
$(a-\bar{v})O_2$diff (ml/100ml)	7.4 (9.5)	17.1 (8.5)*	7.9 (8.8)	6.7 (9)
O_2 Pulse (ml O_2 $beat^{-1}$)	5.8 (7.2)	11.8 (3.5)*	6.5 (6.9)	5.5 (7.3)

All data are expressed as mean (SD). n = 9. HR, heart rate (bpm); SVR, systemic vascular resistance; RU, resistance units; $(a-\bar{v})O_2$diff ; arterial-venous oxygen difference; SV Index, stroke volume index. For explanation of time points (e.g. post, 60-min) see Table 4.3.
*$P<0.05$ *v.* pre-exercise.

Figure 4.3 shows the changes in MAP and systemic vascular resistance from baseline to immediately post-, 60-minutes post- and120-minutes post-exercise. Sampling restraints prevented SVR/SVC from being obtained at the final 360-minute point. The MAP response is also displayed including the final time point at 360-minutes post-exercise. MAP was reduced ~6 mmHg following exercise from 60-minutes post-exercise onwards.

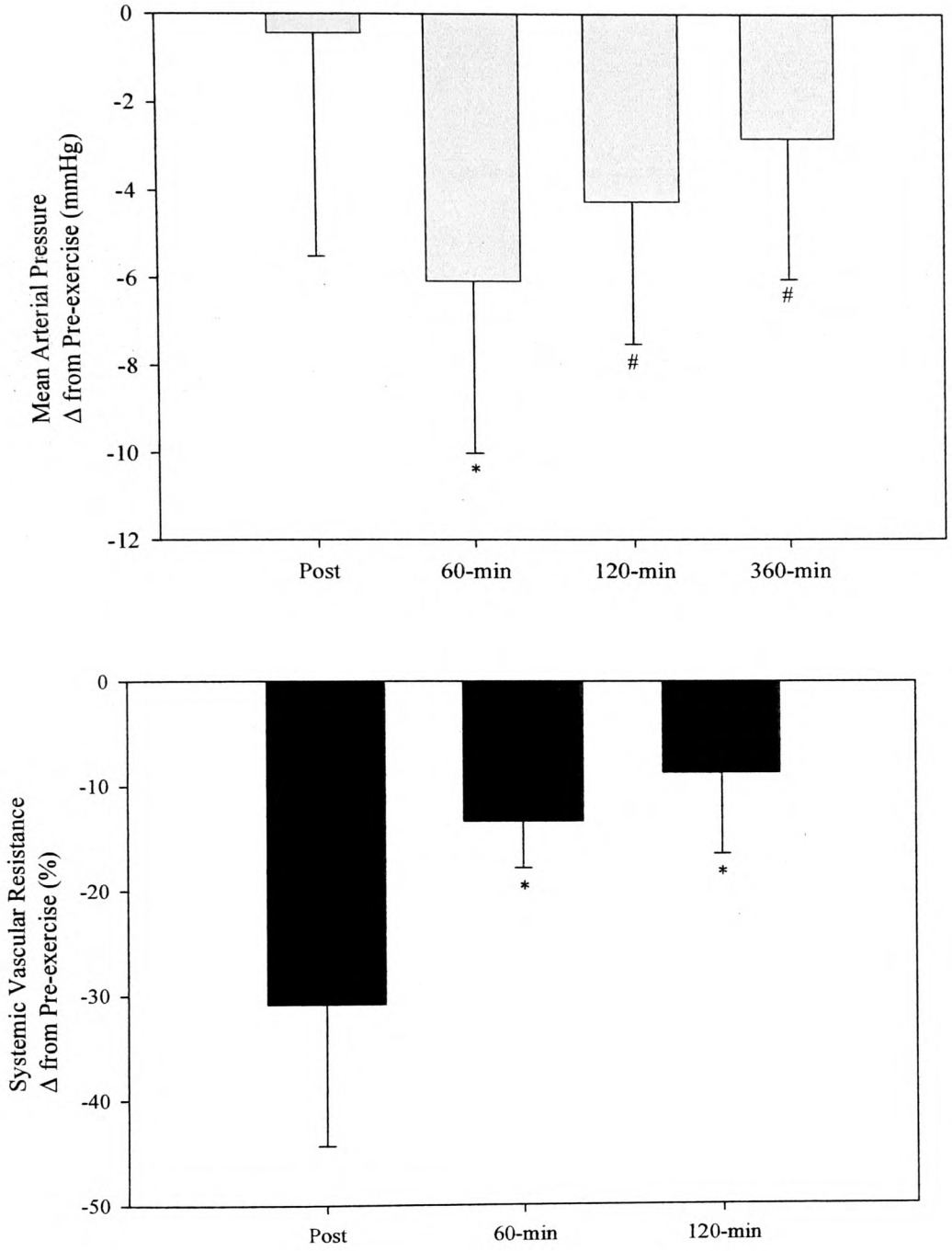


Figure 4.3. Absolute change (Δ) in mean arterial pressure from pre-exercise to immediately post-, 1-hour post-, 2-hours post- and 6-hours post-exercise and relative change (Δ%) in systemic vascular resistance from pre-exercise to immediately post-, 1-hour post-, and 2-hours post-exercise. *Top*: absolute decrease in mean arterial pressure. *Bottom*: % reduction in systemic vascular resistance. Values are Mean ± (SD); *P<0.05 *v.* Post; $^{\#}$ P<0.05 *v.*60-min.

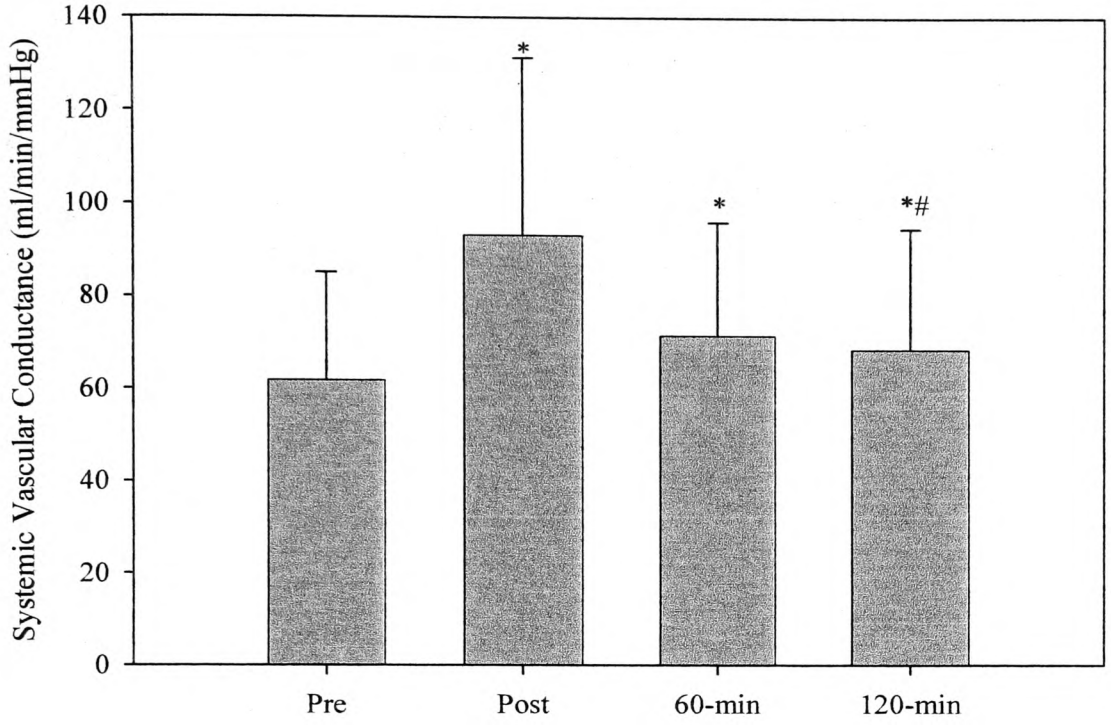
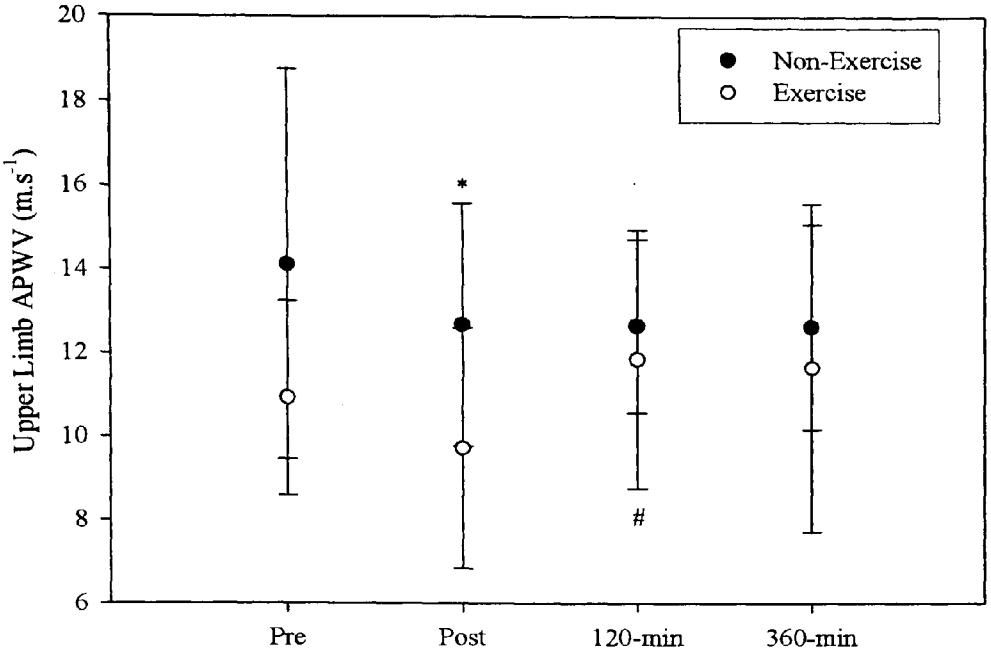


Figure 4.4. Systemic vascular conductance response to 30-minutes sub-maximal dynamic exercise. Values are Mean ± (SD); post, immediately post-; 60-min, 60-min post-; 120-min, 120-min post-exercise. *$P<0.05$ *v.* Pre; $^{\#}$ $P<0.05$ *v.* Post.

4.2.3 Arterial Pulse Wave Velocity (APWV)

Upper limb (UL) APWV analysis revealed a significant main effect of exercise over non-exercise ($P<0.05$). *Post hoc* analysis revealed that at the immediate (within 3-minutes) post-exercise time-point UL APWV was 23% lower in the exercise trial compared to the non-exercise trial (Figure 4.5). Following exercise a significant increase in UL APWV was recorded from immediately post-exercise to 120-minutes post-exercise. Lower limb (LL) APWV responses were unremarkable ($P>0.05$) between conditions and across the protocol indicating no modulation of LL APWV following exercise (Figure 4.5). Further analysis of normalised APWV (APWV/MABP) to control for differences in prevailing arterial pressure between conditions, revealed that the 23% reduction in UL APWV immediately post-exercise was obviated [0.10 (0.3) *v.* 0.12 (0.3) m/s. $torr^{-1}$ in exercise and control, respectively; $P>0.05$]. There was no main effect for exercise or time across conditions nor was there an interaction effect (state x time) for normalised UL & LL APWV (all $P>0.05$). Thus the absolute reduction in UL APWV following exercise as opposed to control is due to the lowering of MAP and SBP not inherent APWV *per se*.



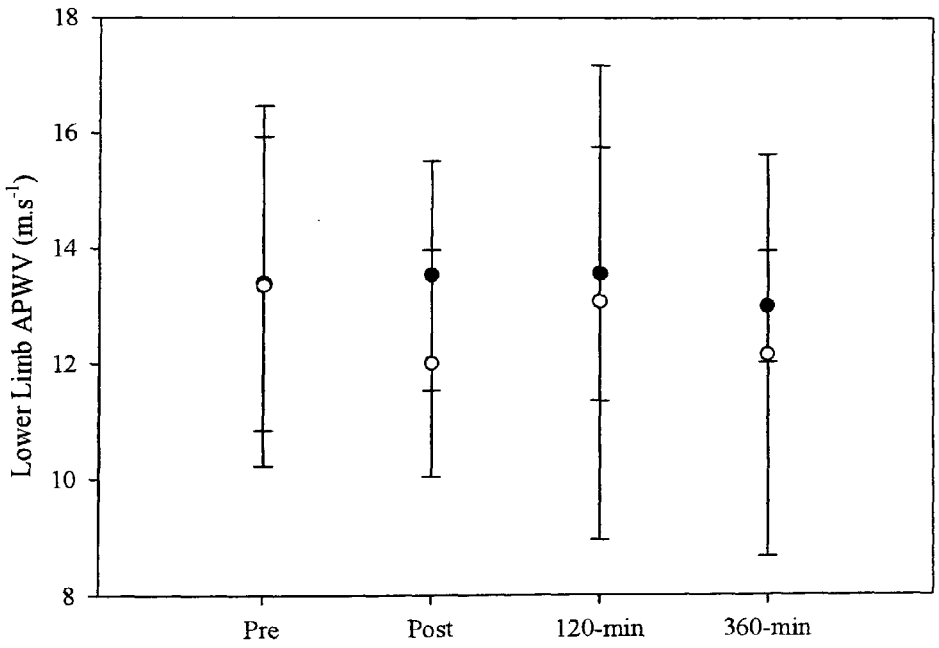


Figure 4.5. *Top:* Upper limb and *Bottom:* Lower limb arterial pulse wave velocity (APWV), averaged over 10-minutes at 1-minute intervals in each case, following either 30-minutes dynamic cycle exercise (open symbols) or 30-minutes quiet seated rest (shaded symbols). Values are Mean ± (SD). Post, within 3-minutes post-exercise; 120-min, 120-min post exercise; 360-min, 360-min post-exercise. $^{*}P<0.05$ *v.* same time-point between conditions. $^{\#}P<0.05$ *v.* prior time-point within condition.

4.2.4 Plasma, Blood Volume & Plasma Osmolality

Immediately following exercise PV was Δ-1.6 (11.4)%, where Δ is change, in comparison to baseline and blood volume (BV) was Δ0.7 (11.6)% (both, $P>0.05$). At 1-hour post-exercise PV was Δ20 (27.5)% ($P>0.05$) and BV was Δ13 (19)%. For the remainder of the protocol at both 2- and 3-hours post-exercise PV remained at pre-exercise values [Δ-0.3 (14.3)% and Δ-5.3 (13.6)%, respectively, both $P>0.05$]. Likewise, BV was unchanged from baseline at 2 and 3-hours post-exercise [Δ0.7 (9.3)% and Δ-5.6 (11.5)%, respectively, both $P>0.05$]. Analysis of the relative PV shifts revealed that the haemodilution between baseline and 1-hour following exercise [Δ20 (27.5)%] was greater than at all other time points ($P<0.05$).

Immediately following exercise plasma osmolality was 310.6 (3.2) mosm/kg H_20 in comparison to 309.3 (2.9) mosm/ kg H_20 at baseline ($P>0.05$). For the remainder of the protocol plasma osmolality remained at baseline levels [308.5 (3.3), 309.3 (4.6) and 311 (5.3) mosm/ kg H_20 at Post1, Post2 and Post3, respectively, all $P>0.05$].

4.2.5 Plasma Catecholamines

Venous plasma noradrenaline concentration was un-modified ($P>0.05$) by exercise (Figure 4.6). Likewise, venous plasma adrenaline concentration was also unremarkable following exercise ($P>0.05$) (Figure 4.7).

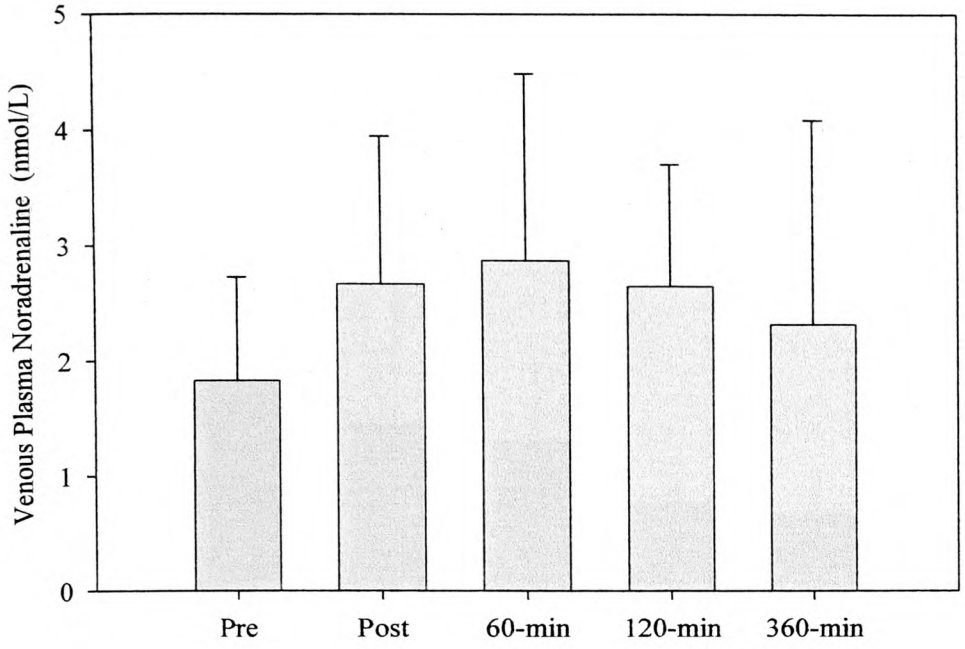


Figure 4.6. Venous plasma noradrenaline response to 30-minutes dynamic cycle exercise. Values are Mean ± (SD). Post, within 1-minute post-exercise; 60-min, 60-min post-exercise; 120-min, 120-min post-exercise; 360-min, 360-min post-exercise.

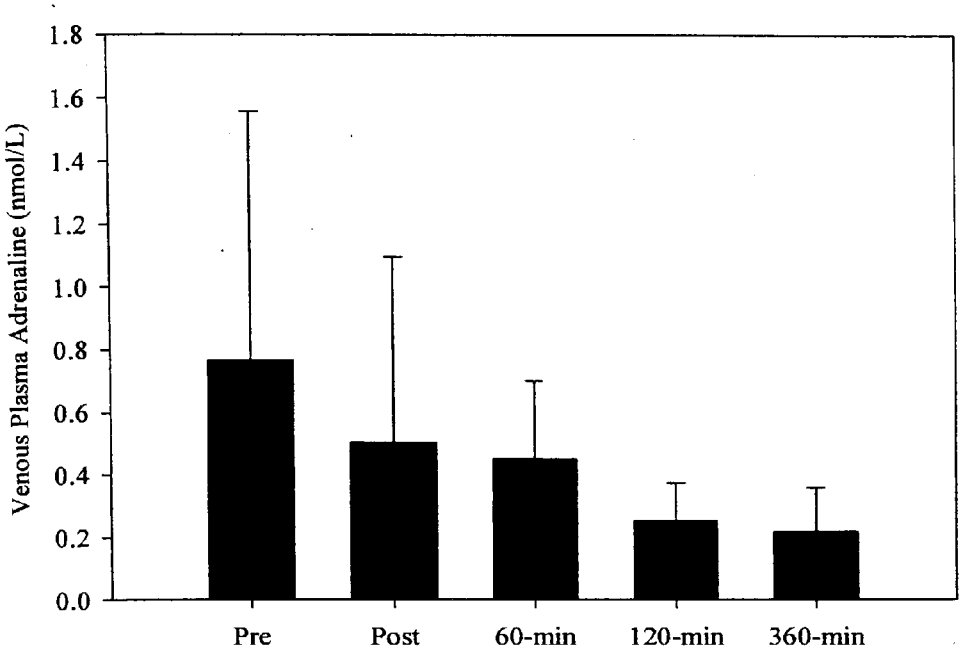


Figure 4.7. Venous plasma adrenaline response to 30-minutes dynamic cycle exercise. Values are Mean ± (SD). Post, within 1-minute post-exercise; 60-min, 60-min post-exercise; 120-min, 120-min post-exercise; 360-min, 360-min post-exercise.

4.2.6 Renin & Angiotensin II Response

Exercise augmented venous plasma renin concentration by ~ 31% ($P<0.05$) but values returned to baseline by 60-minutes post-exercise (Figure 4.8). However, Angiotensin II concentrations increased ($P<0.05$) following exercise and continued to be elevated by ~25% at the 1-hour post-exercise time-point ($P<0.05$). Values returned to baseline by 2-hours post exercise, remaining un-modified until cessation of recordings (Figure 4.8).

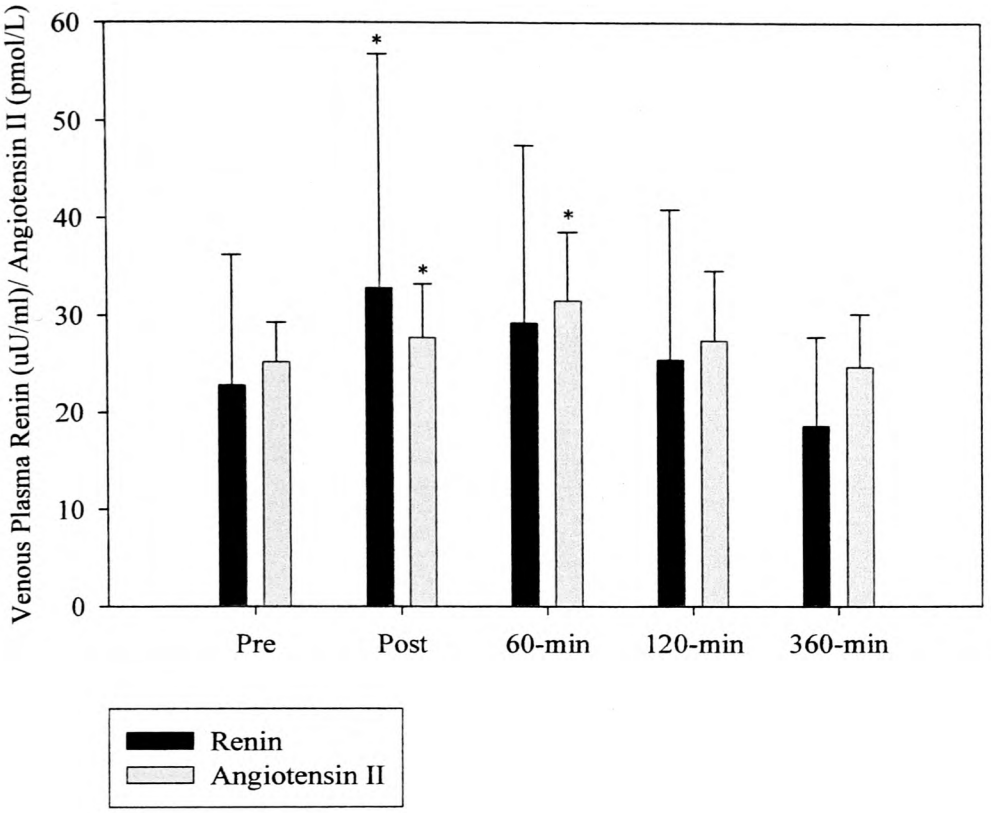


Figure 4.8. Venous plasma renin and angiotensin II response to 30-minutes dynamic cycle exercise. Values are Mean ± (SD). Post, within 1-minute post-exercise; 60-min, 60-min post-exercise; 120-min, 120-min post-exercise; 360-min, 360-min post-exercise.

4.2.7 Aldosterone Response

As shown in Figure 4.9 venous aldosterone levels increased 38% ($P<0.05$) following exercise, remaining elevated at the 1-hour post-exercise point but returned to baseline by 2-hours post-exercise.

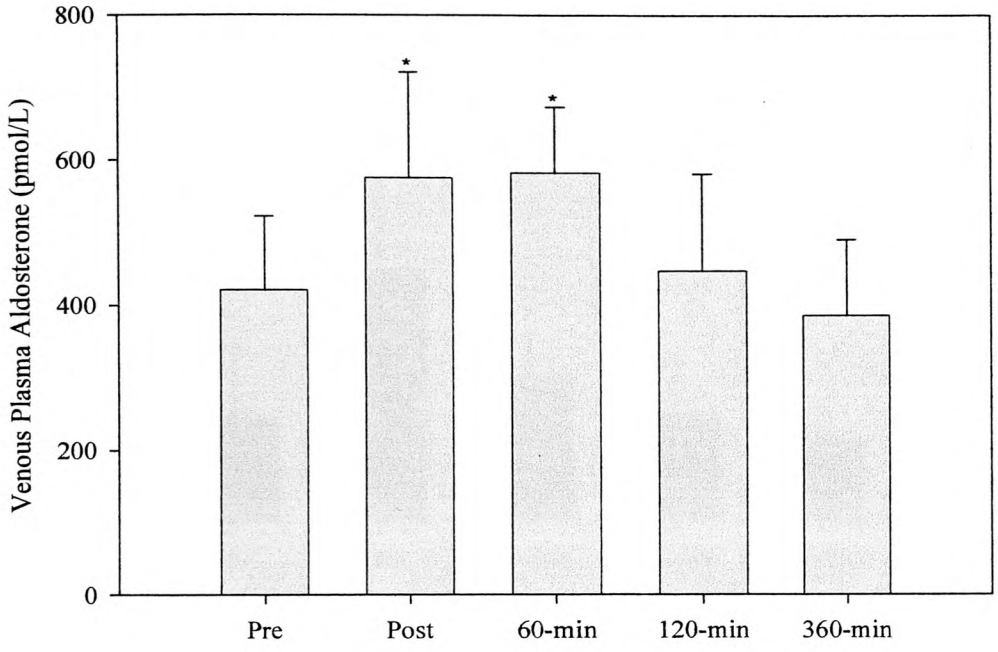


Figure 4.9 Venous serum aldosterone response to 30-minutes dynamic cycle exercise. Values are Mean ± (SD). Post, within 1-minute post-exercise; 60-min, 60-min post-exercise; 120-min, 120-min post-exercise; 360-min, 360-min post-exercise. *$P<0.05$ *v.* pre.

4.2.8 Arginine Vasopressin & Atrial Natriuretic Peptide Response

Figure 4.10 illustrates the changes in venous plasma AVP concentration. Analysis revealed that exercise increased circulating venous AVP levels by ~100% ($P<0.05$). Values then returned to baseline by 60-minutes post-exercise ($P>0.05$).

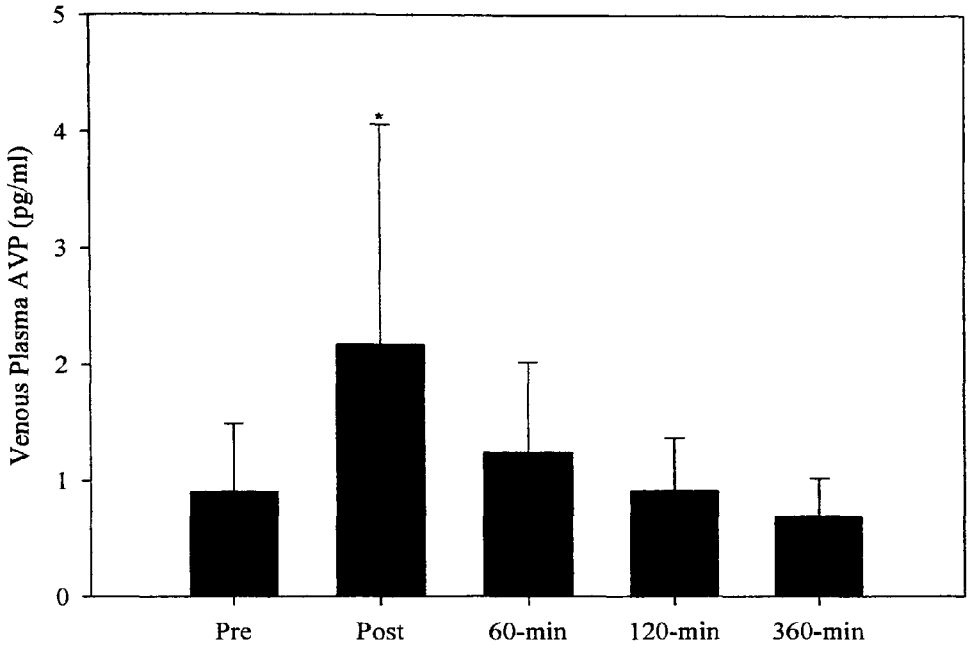


Figure 4.10 Venous plasma AVP response to 30-minutes dynamic cycle exercise. Values are Mean ± (SD). Post, within 1-minute post-exercise; 60-min, 60-min post-exercise; 120-min, 120-min post-exercise; 360-min, 360-min post-exercise. *$P<0.05$ *v.* pre-exercise.

Analysis of the venous plasma ANP response produced a significant effect for exercise, with values increasing by ~37% following exercise ($P<0.05$). ANP concentration significantly fell ($P<0.05$) from peak post-exercise values decreasing ($P<0.05$) below baseline at the 120-min and 360-min time-points by 33% and 28%, respectively (Figure 4.11).

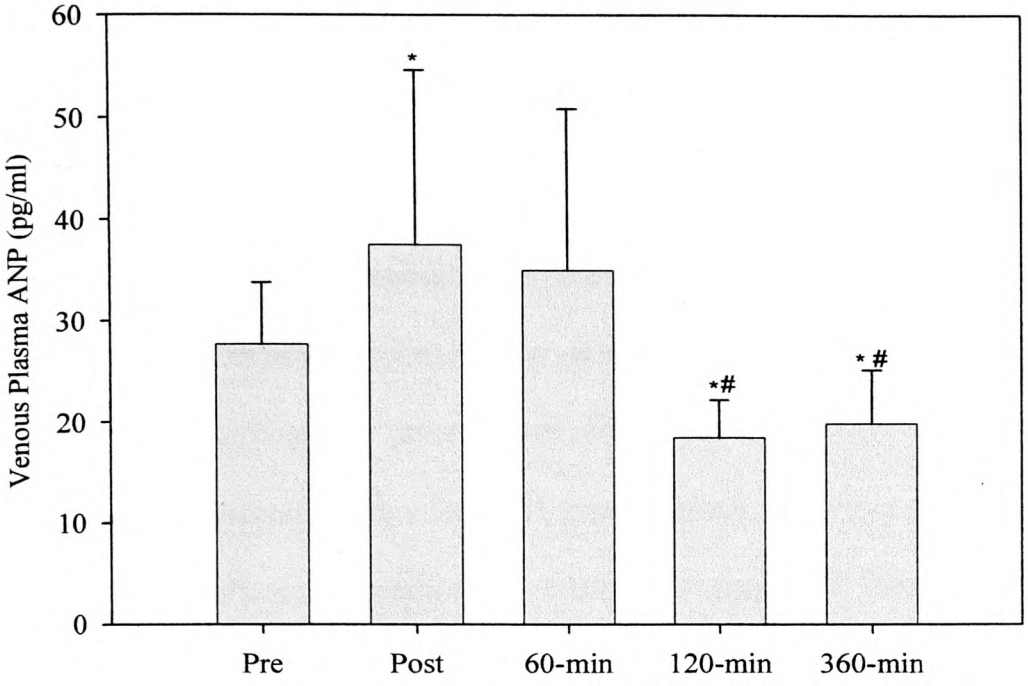


Figure 4.11 Venous plasma ANP response to 30-minutes dynamic cycle exercise. Values are Mean ± (SD). Post, within 1-minute post-exercise; 60-min, 60-min post-exercise; 120-min, 120-min post-exercise; 360-min, 360-min post-exercise. $^{*}P<0.05$ *v.* pre-exercise; $^{\#}P<0.05$ *v.* post.

4.2.9 Experimental Correlations

Systemic Arterial Pressure, Haemodynamics & Biochemistry

In the present investigation there was no correlation evident between systemic MAP, catecholamines, renin, angiotensin II, aldosterone, AVP or ANP (all $P>0.05$) indicating that the systemic venous concentration of these metabolites were not associated to the prevailing systemic mean arterial pressure. Likewise, correlational analysis between Δ (change = pre-exercise minus post-exercise) MAP, Δcatecholamines, Δrenin, Δangiotensin II, Δaldosterone, Δ AVP or Δ ANP failed to elucidate any significant correlations ($P>0.05$). SBP and ΔSBP failed to demonstrate any correlation to the metabolites studied (both, $P>0.05$). Of note, systemic DBP was weakly and positively correlated to only one of the metabolites studied, renin (r = 0.30, $P<0.05$) but the ΔDBP response failed to demonstrate this association to the Δrenin response ($P>0.05$). This indicates that although DBP was weakly associated to the systemic venous concentration of renin over the course of the protocol, the changes in DBP following exercise were unrelated to the changes in renin concentration post-exercise.

No association was found between the change in SVR and the change in any of the metabolites investigated following exercise ($P>0.05$). However, ΔSVR was positively associated with ΔCO ($r = 0.78$, $P<0.01$).

Biochemical Response

Inter-assay correlations revealed several observations. A positive correlation was noted between the systemic venous concentration of AVP to both aldosterone (r = 0.49, $P<0.01$) and ANP ($r = 0.57$, $P<0.01$) concentrations. Similar results were displayed for Δ AVP to both Δaldosterone ($r = 0.47$, $P<0.01$) and Δ ANP ($r = 0.59$,

$P<0.01$) concentrations. Δ ANP concentration was associated with Δrenin ($r = 0.39$, $P<0.05$), Δangiotensin II ($r = 0.41$, $P<0.05$) and Δaldosterone ($r = 0.70$, $P<0.01$) concentration.

Expected responses were noted between the individual metabolites of the renin-angiotensin-aldosterone axis. A positive correlation was observed between the systemic venous concentrations of renin to both angiotensin II ($r = 0.65$, $P<0.01$) [Δrenin to Δangiotensin II ($r = 0.60$, $P<0.01$)] and aldosterone ($r = 0.42$, $P<0.01$) [Δrenin to Δaldosterone ($r = 0.67$, $P<0.01$)]. Similarly the systemic venous concentration of aldosterone was positively correlated to angiotensin II ($r = 0.53$, $P<0.01$) [Δaldosterone to Δangiotensin II ($r = 0.63$, $P<0.01$)] concentration.

Systemic Arterial Pressure, Arterial Pulse Wave Velocity & Biochemistry

Upper limb and lower limb APWV failed to demonstrate any relationship to MAP, SBP or DBP (all, $P>0.05$). Likewise Δupper limb APWV and Δlower limb APWV failed to demonstrate any correlation to ΔMAP, ΔSBP or ΔDBP (all, $P>0.05$). Upper limb APWV was correlated to systemic venous angiotensin II concentration ($r = 0.49$, $P<0.01$), whereas lower limb APWV marginally failed to display the same relationship ($r = 0.30$, $P=0.07$). Δupper limb APWV was correlated to the Δangiotensin II response ($r = 0.49$, $P<0.01$) whilst there was no correlation between Δlower limb APWV and Δangiotensin II ($P>0.05$).

4.3 Discussion

The present study aimed to investigate the haemodynamic response to an acute bout of exercise in older pre-hypertensive humans. The data indicate that when the present pre-hypertensive subjects performed 30-minutes of fixed relative intensity (70%

$\dot{V}O_{2PEAK}$) cycle ergometer exercise, MAP decreased significantly below baseline levels for up to 6-hours post-exercise. This time-frame and magnitude of reduction is similar to previous studies, which have shown a fall in systemic arterial pressure following dynamic exercise using various modalities (Halliwill 2001; MacDonald 2002). Questions have been raised as to whether decrements in blood pressure last for an extended period or through post-exercise activities (Somers et al, 1991) the present study controlled the activities and environment for 6-hours following both exercise and control conditions, elucidating reductions in MAP following exercise only. Whilst the activities encountered may not fully reflect those of daily living it is apparent that in the present subjects a moderate PEH ensues for a considerable time and is resistant to some typical activities encountered.

The present investigation included an additional non-exercise control day to isolate the carryover effects of prior exercise *per se* on haemodynamic parameters. The findings demonstrate that MAP is lowered following an acute exercise bout beyond the normal variation that occurs throughout a non-exercising day.

Mechanisms Of PEH

PEH following a single bout of aerobic exercise is caused by an unexplained peripheral vasodilatation. The fundamental characteristic of PEH is a persistent rise in systemic vascular conductance (or reduction in SVR) that is not completely offset by rises in $\dot{Q}$ (Halliwill, 2001) and that the underlying attenuation in systemic vascular resistance is not solely attributable to the active skeletal muscles (Halliwill et al, 2000). One possible explanation for the attenuation in SVR following exercise is that the baroreflex was reset to a lower pressure following exercise so that sympathetic vasoconstrictor outflow was reduced (Halliwill et al, 1996a). The present findings are in agreement with this previous finding. At both 60- and 120-minutes after exercise

SVR was blunted by 14 and 11%, respectively (Figure 4.3), when compared to pre-exercise, whilst $\dot{Q}$ had returned to baseline over the same time-period. Although it must be noted that the reduction in SVR cannot be ascribed to a reduction in sympathetic outflow in the current protocol as no recordings of this parameter were undertaken.

Biochemical Response & PEH

The individual response of each metabolite is discussed below.

Catecholamines

PEH ensued regardless of an unchanged concentration in circulating venous plasma adrenaline concentration. This would agree with previous studies reporting unchanged concentrations of adrenaline during the hypotensive episode in hypertensive subjects (Wilcox et al, 1987; MacDonald 2002; Notarius et al 2006). Moreover, Landry (1992) reported elevated levels of circulating adrenaline in normotensive individuals during PEH. This, coupled with the fact that PEH persists during adrenaline infusion (Landry 1992) and β–adrenergic receptor blockade (Wilcox et al, 1987) indicates that any role of adrenaline in PEH is minimal. The lack of change in noradrenaline throughout the PEH period is in agreement with the results of MacDonald (2002) in borderline hypertensives and Notarius et al (2006) in normotensive subjects. Prior findings have demonstrated that during the post-exercise period vascular responsiveness to sympathetic vasoconstrictor outflow is impaired so that vascular resistance is reduced for a given level of sympathetic nerve activity independent of changes in α-adrenergic receptor responsiveness (Halliwill et al, 2003a). This is suggestive of pre-synaptic inhibition of noradrenaline release from sympathetic vasoconstrictor nerves following exercise. The lack of change in post-exercise noradrenaline in the present study could possibly be indicative of such pre-synaptic inhibition of noradrenaline release.

Together the findings from the catecholamine data adds to the already considerable body of literature, some in disagreement, indicating that changes in sympathetic catecholamine activity is not a key mediator of PEH.

RAAS

Initiation of an acute exercise bout stimulated the RAAS in the present study. Renin, angiotensin II and aldosterone were elevated following exercise consistent with earlier data on the response of the RAAS to an acute exercise bout (Maher, 1975). The return to baseline of renin by 1-hour, angiotensin II by 2-hours and aldosterone by 2-hours post-exercise indicates that the concentration of these metabolites in the systemic circulation plays no part in sustained PEH in the present subjects. Of note, inter-assay correlations highlighted predicted responses between the individual components of the RAAS following exercise. The positive associations noted between renin, angiotensin II and aldosterone and angiotensin II and aldosterone highlights an intact RAAS in the experimental cohort.

The present results confirm the findings of Wilcox et al (1982) who found unchanged concentrations of renin in hypertensives following brief exercise and the study of Paulev et al (1984) who elucidated increased angiotensin II concentrations during sustained PEH, suggesting that these components of the RAAS do not play a significant role in PEH. To this authors knowledge this is the first study to display the response of the complete RAAS axis during an extended period of PEH. The additional aldosterone data in the present study indicates that there is a complete dissociation of the RAAS axis to PEH.

As with earlier studies, PEH was sustained in spite of increases in venous angiotensin II concentration over the initial 60-minutes post-exercise. The octapeptide angiotensin II, the main effector of the RAAS, exerts its vasoconstrictor and trophic effects on

smooth muscle cells via the AT1 receptor (Dihn et al, 2001). However, angiotensin II acts on endothelial cells as they also express AT1 and the second subtype (AT2) receptors (Pueyo and Michel, 1997). Furthermore, AT1 receptors may mediate the release of vasodilator substances, such as $NO^{\cdot}$, by the endothelium (Pueyo et al, 1998) and they may modulate the effects of angiotensin II on smooth muscle cells. AT2 receptors have been shown to possibly induce vasodilatation (Horiuchi, 1996; Csikós et al, 1998). Targeted deletion of the AT2 receptor gene in mice produces raised blood pressure and enhanced sensitivity to the pressor effects of angiotensin II (Hein et al, 1995; Ichiki et al, 1995). This indicates that the AT2 receptor mediates a vasodepressor effect and functionally opposes the AT1 receptor possibly via bradykinin and $NO^{\cdot}$ (Siragy et al, 1999).

Whether there is a decrease in the sensitivity of AT1, an up-regulation of AT2 receptors following exercise or that there is a pronounced vasodilator signal mediating the increase in vascular conductance following exercise is not completely known. However, findings clearly support that of the latter possibility of an overriding vasodilator signal mediating PEH when α-adrenergic receptors are blocked (Halliwill et al, 2003a). Furthermore, it has been clearly documented that with advancing age there is a notable decline in plasma renin activity (Tsunoda et al, 1986; Duggan et al, 1993) accompanied by small decrements in circulating angiotensin II (Duggan et al, 1992) and an increase in AT1 receptor density (Siebers et al, 1990). Thus, as the current cohort were of advancing age this may have been a prominent factor. Along these lines Wray et al (2008) displayed a marked age-related increase in AT1 receptor sensitivity to exogenous infusion of angiotensin II in the vasculature of the resting leg in humans but an augmented reduction in AT1 receptor sensitivity during exercise in older subjects. It is possible that such blunting of receptor sensitivity may continue for

some time following the exercise stimulus. This provides some evidence that at least one of the first two scenarios of receptor sensitivity may have also existed during the first hour post-exercise whilst angiotensin II was elevated.

The elevation of aldosterone concentration following exercise was concomitant to angiotensin II levels. This can be explained by the stimulation of AT1 receptors by angiotensin II eliciting release of aldosterone over the same time frame.

AVP & ANP Responses

In the present study PEH was recorded up to 6-hours post-exercise whilst systemic venous AVP concentration returned to baseline by 60-minutes and osmolality remained invariant following exercise. This would corroborate the findings of Wilcox et al. (1982) who found no significant correlations between levels of antidiuretic hormone (ADH; an analogue of AVP) and the magnitude of PEH. Paulev et al. (1984) also showed a lack of change in plasma osmolality or AVP during PEH. The failure of plasma osmolality to increase following the acute exercise bout in the present study is likely explained by the fact that an increase in plasma osmolality following exercise is dependent on reaching a critical exercise intensity causing hypotonic fluid movement out of the vascular space. Thus, the author postulates that subjects in the present study failed to reach an exercise intensity capable of inducing increases in plasma osmolality.

The relationship between exercise stimulus and AVP secretion is well documented (Bouissou et al, 1987; Convertino et al, 1981; Freund et al, 1991; Wade, 1984). It is accepted that the key stimulus to AVP secretion during exercise is an intensity-dependent plasma osmolality increase (Takamata et al, 2000). Takamata et al (2000) failed to elicit any changes in AVP secretion following exercise until relative exercise intensity reached values above ~70% $\dot{V}O_{2PEAK}$. An exercise intensity of 75 (15)%

$\dot{V}O_{2PEAK}$ in the current study stimulated AVP secretion without changes in plasma osmolality. This discrepancy may be explained by the fact that the subjects in the present study were prehypertensive. Debate has existed as to the exact role of circulating AVP levels in essential hypertension (Ando et al, 1983; Morton and Padfield, 1986; Puri, 1987). Higher circulating levels of AVP found in hypertensive individuals could reset the operating point of the functional antagonism between plasma osmolality and AVP secretion, or elicit an uncoupling of the plasma osmolality – AVP secretion relationship. In support of this, De Lima et al (1981) found that volume-dependent ADH secretion is inhibited in subjects with essential hypertension.

Of interest, changes in AVP concentration were related to changes in aldosterone release ($r = 0.47$, $P<0.01$). Previous reports have indicated similar findings between the RAAS and vasopressin response to exercise (Perrault et al, 1991; Stebbins et al, 1993; 1994).

AVP is a complex hormone that has multiple effects including water reabsorption at the distal and collecting renal tubules as well as vascular smooth muscle constriction (Collins et al, 2001). Systemic AVP also contributes to an increase in arterial pressure and redistribution of $\dot{Q}$ during dynamic exercise via its potent vasoconstrictor actions (Stebbins and Symons, 1993; Stebbins et al, 1994; Symons and Stebbins, 1995). AVP can cause marked elevations in vascular resistance and decreases in blood flow to vascular beds such as skeletal muscle and skin (Schmid et al, 1974; Hoffman, 1980). These cardiovascular effects are mediated by vasopressin V_1 receptors located in the vascular smooth muscle (Cowely and Laird, 1988) whilst the anti-diuretic effects of ADH (AVP) are exerted by V_2 receptors. Stimulation of V_2 receptors has also been shown to have opposing effects to V_1 receptor stimulation producing a vasodilatation

(Schwartz et al, 1985). Under 'normal' basal conditions the pressor effect of AVP predominates over the weaker vasodilator effect.

Interestingly, AVP also interacts with cardiopulmonary and arterial baroreceptors to mediate cardiovascular regulation at rest and in exercise. AVP acts on the central nervous system, specifically the area postrema, via V_1 receptors to enhance arterial-baroreflex induced sympathoinhibition during static exercise (Bonigut et al, 1997). This sympathoinhibition may be due to resetting of the arterial baroreflex operating point to a lower pressure. Furthermore, this mechanism of facilitation of baroreceptor afferent input has been postulated to mediate PEH (Collins et al, 2001).

Collins et al (2001) reported an attenuation of PEH by central blockade of vasopressin V_1 receptors with the V_1 antagonist, d(CH3)5 Tyr(Me)-AVP, in the spontaneously hypertensive rat. The authors postulated that the central effects of ADH on V_1 receptors induced facilitation of baroreceptor afferent input, which mediated sympathoinhibition and PEH. Although, it is important to note that differences do exist between the central and systemic effects of AVP (Stebbins and Symons, 1993) stimulation of V_1 receptors and possible species differences in the effect of central V_1 receptor blockade.

AVP has a systemic half-life of ~3-4 minutes so the effects of AVP on the modest PEH immediately (3-5 minutes) post-exercise could not be discounted in the present study. However, as the PEH ensued well beyond the 1-hour post-exercise time point it can be concluded with some certainty that the enhanced systemic AVP secretion immediately post-exercise could not be responsible for the reductions noted in MAP beyond this time frame via interaction with central V_1 or systemic V_2 receptors.

ANP is a cardiac hormone that has direct renal and cardiovascular actions mediating natriuresis, diueresis and vasodilatation. Thus, this metabolite may be responsible for modulating, at least in part, post-exercise haemodynamics during PEH. In the present study exercise elicited an increase in circulating ANP concentration ($P<0.05$). The finding that ANP was raised immediately post-exercise supports the work of Somers et al. (1986) who found that a progressive exercise test to $\dot{V}O_{2MAX}$ resulted in a pronounced increase in plasma ANP and Schmidt et al. (1990) who highlighted an exercise-dependent increase in plasma ANP. An important finding in the study by Schmidt et al. (1990) was that exercise intensity is the key stimulus for ANP release rather than exercise duration. Work by Lawrence and Schenker (1991) and Milledge et al. (1989) lend further agreement to an increase in plasma ANP following exercise. The increased atrial distension produced by augmented venous return, heart rate, atrial pulsatility and atrial pressure during dynamic exercise is the most obvious explanation for the increased levels of circulating ANP immediately post-exercise (Perrault et al, 1989). Furthermore, Nishikimi et al. (1986) highlighted similar increases in circulating plasma ANP in patients with essential hypertension and postulated that the elevations may be due to elevated left atrial pressure or left ventricular systolic function.

The concentration of ANP was associated ($r = 0.57$, $P<0.01$) with AVP concentration. Likewise, the change in ANP concentration was correlated to changes in the individual components of the RAAS (see results section for individual correlations). This is similar to the findings reported by Perrault et al (1989; 1991) where the pattern of changes in ANP and vasopressin were similar throughout exercise and recovery and the changes in ANP were related to changes in the RAAS.

Plasma ANP returned to baseline levels following 1-hour post-exercise in the present study, similar to that occurring in the study by Somers et al (1986). Interestingly, the venous plasma ANP levels continued to fall being blunted below baseline levels at the post-2 hour and post-6 hour time points, in the present study. This is in agreement with the findings of Hara and Floras (1992), who found significant reductions below baseline levels of ANP during an episode of PEH. Whereas, Macdonald et al. (1999b) showed a lack of change in PCV or ANP during PEH measured for 1-hour post-exercise.

The falls in plasma ANP, in the present study, were not paralleled by concomitant increases in AVP, whilst ΔPV and ΔBV exhibited non-significant haemoconcentrations at the same time points. A large haemoconcentration would be indicative of a decreased cardiac preload and in turn inhibit ANP release. Thus, it does not appear that this was the case in the present trial. One explanation could account for the postural adjustments after the 3-hour post-exercise period (i.e. semi-recumbent to standing, walking, sitting when the subjects were free to move around the laboratory). Again, the lack of change in ADH and/or ΔPV and ΔBV over the same post-exercise time course together with the fact that a significant decline in ANP concentration occurred between 60 and 120-minutes post-exercise when the subject was held in constant position (semi-recumbent), and thus the right atrium was subjected to the same distension, would negate this influence. It may be reasonable to speculate that the significant decline in ANP concentration below baseline was related to the fall in pressure observed within the present study. A drop in pressure would lower the stretch on the atria which in-turn would inhibit ANP secretion. A study investigating cardiac dimensions and pulmonary wedge pressures would lend further support to this supposition.

A final possibility explaining the decline in ANP values below baseline is that of increased extraction of the metabolite. Plasma concentrations reflect not only hormonal release but also removal from the blood stream. Results obtained from subjects undergoing routine cardiac catheterisation indicate that plasma ANP is rapidly extracted with a metabolic clearance rate of ~2.4 l/minute by vascular beds of the lower limbs, kidney and the liver but not changed within the pulmonary circulation (Espiner et al, 1986). Increases in systemic vascular conductance were displayed in the current investigation and have been reported in numerous investigations during PEH for up to 90-minutes post-exercise (Lockwood et al, 2005a; 2005b; McCord et al, 2006; McCord and Halliwill, 2006). However, both the active (femoral) and inactive (brachial) muscle vascular beds are the sites of pronounced increases in vascular conductance (Lockwood et al, 2005a; 2005b; McCord et al, 2006), contributing ~56% to the rise in systemic vascular conductance (Pricher et al, 2004), whereas splanchnic (Pricher et al, 2004) and cutaneous (Wilkins et al, 2004) vascular conductance returns to baseline before PEH resolves. Thus, the increase in muscle vascular conductance post-exercise could have accounted for increased clearance of ANP.

It is reported that ANP has a half-life of 2-3 minutes in the systemic circulation and may still exert residual effects after it is completely cleared from the circulation (Davis, 1989; Freund et al, 1991). However, the PEH persisted up to the 6-hour post-exercise time point and ANP values had significantly decreased below baseline after 2-hours post-exercise. Thus it appears that the significant decline in ANP values in the post exercise period occurs over a similar time-frame to the blunting of systemic vasodilatation. It can be concluded that the enhanced ANP secretion or its residual

effects may be required to iniate and maintain a degree of hyperaemia but can not be solely responsible for the reductions noted in MAP over this time period.

Plasma & Blood Volume Changes

The indwelling cannula provided a saline flush, however the volume infused over the testing session was minimal (<6ml). This would therefore argue against a hypotensive effect due to decreased total blood volume. Moreover, the fact that PCV was unaltered during the non-exercise control trial (data not shown), nor ΔPV or ΔBV during the recovery portion of the exercise trials after 1-hour post-exercise, argues against vascular pooling as a causal mechanism. It could be purported that maintaining a relatively static body position during the initial recovery phase would cause a shift in plasma volume and thus a decreased central blood volume resulting in a concomitant drop in blood pressure. This potential mechanism can be dismissed.

However, this scenario is distinctly different from the suggestion that PEH plays a role in the allowance of PV recovery following exercise (Hayes et al, 2000). Although the present study failed to report any significant changes in PV from baseline throughout the protocol, analysis revealed that the PV expansion between baseline and 60-minutes post-exercise was different to the shifts (mild PV losses) reported at all other time-points (see results) and that the nadir in MAP reduction occurred concomitantly. Water movement between the interstitial and intravascular compartments is due to a balance between hydrostatic and oncotic forces. PV increases could be due to increased salt retention, plasma protein content or decreased capillary hydrostatic pressure (i.e. due to peripheral vasodilatation and hypotension). Therefore the difference in PV shift between pre and 60-minutes post-exercise could have resulted from the peak decline in MAP at this time point. Of note, aldosterone concentrations were elevated following exercise during this same epoch. Increased

aldosterone concentration produces salt retention that may have also elicited PV expansion at this time.

APWV Responses

To this authors knowledge this is the first study to document simultaneous measurements of APWV during PEH and to also compare the responses to a control condition in any subject population. The results indicate that immediately following exercise APWV is significantly attenuated in the upper limb region but not the lower limb region when compared against a non-exercise control. APWV provides a quantitative assessment of arterial distensibility, in-turn distensibility is influenced by dynamic changes in vascular tone. Thus, APWV may be used to investigate acute flow-related modulations in vascular tone without pharmacological provocation (Naka et al, 2006). Previous findings utilising the exact methodology as that used in the present study but in normotensive subjects highlighted a 6-10% and 23-10% decline in upper and lower limb APWV, respectively, from 10 to 60-minutes post-exercise, with a concomitant decline in SBP only (Naka et al 2003).

The authors attributed the observed changes in APWV to responses in vascular tone in recovery from net systemic vasoconstrictor and local vasodilator signals elicited via exercise, culminating in persistent post-exercise vasodilatation.

The lack of change in both UL and LL APWV following exercise in the present study may result from the sampling time post-exercise. In the study of Naka et al (2003) declines in UL and LL APWV post-exercise were not apparent until ~10-minutes post-exercise where it reached a nadir between ~10-12-minutes, remaining significantly attenuated by ~10% by 1-hour post-exercise. Thus, it is possible that declines in APWV occurred in the present study between the immediate post-exercise (3-minutes) recordings and the next serial measurement at 2-hours post-exercise.

Naka et al (2003) only recorded APWV post-exercise for 60-minutes so the point of return to baseline values is unknown in normotensive subjects, however the current study indicates that if any changes in APWV occurred in the present protocol they were resolved by 120-minutes post-stimulus.

Notwithstanding, difficulty arises in a direct comparison of the current study with that of Naka et al (2003) due to the distinct cohorts enrolled in the two studies. Young (31 ± 6 years), normotensive (MAP <100 mmHg) subjects of average fitness were studied by Naka et al (2003), whereas older (50 ± 10 years), pre-hypertensive (MAP 106 ± 5 mmHg) sedentary subjects were enrolled for the present study.

Arterial distensibility and arterial compliance decrease with age and pathological conditions such as atherosclerosis and hypertension (Laogun and Gosling, 1982). Data indicates that both APWV and arterial compliance are modified early in the course of essential hypertension (Brinton et al, 1996) and that the decrease in arterial compliance with age (Vaitkevicius et al, 1993) is the result of progressive elastic fibre degeneration (Avolio et al, 1998) in the arterial wall. APWV in the brachial artery has also been reported as increasing with age, indicative of worsening arterial compliance (Avolio et al, 1983) and pathological changes to arterial smooth muscle. Thus, endothelial 'dysfunction' is noted to play a seminal role in the attenuation of circulatory 'efficiency' (Henderson, 1997) with the key characteristic being an impairment of flow-mediated arterial dilatation.

Validation studies carried out with the same pre-hypertensive cohort and the APWV system (presented in section 3.9) demonstrate that similar decreases (~6%) in UL APWV with no change in LL APWV were noted in response to reactive hyperaemia in the pre-hypertensives, indicative of preserved endothelial function in the UL (Naka et al, 2006) and consistent limb heterogenity.

A further notable difference between the study of Naka et al (2003) and the current data is that of exercise intensity. The current protocol used a 30-minute fixed intensity (70% $\dot{V}O_{2PEAK}$) cycling stimulus whereas Naka et al (2003) utilised a progressive intensity to $\dot{V}O_{2PEAK}$ (14.5 ± 2.6 min) on the treadmill. It may be that the post-exercise reductions in APWV noted by Naka et al (2003) were due to a larger vasodilatation elicited via greater flow-mediated responses to maximal exercise. Despite the lack of change in UL or LL APWV following exercise the present study does highlight a 23% lowering of UL APWV immediately following exercise when compared to the same time-point determined following 30-minutes of non-exercise. However this difference can be solely attributed to the changes in distending MAP measured in the brachial artery, as normalising the APWV to the prevailing systemic arterial pressure completely obviated the absolute differences in APWV. It is interesting to note that in the study of Naka et al (2003) only absolute APWV values were recorded while there was a significant attenuation in systolic arterial pressure over the same period in the protocol. It is tempting to speculate that normalising the APWV to SBP may abolish the post-exercise declines in APWV highlighted by Naka et al (2003). Notwithstanding, it is apparent that not only does exercise significantly lower arterial blood pressure but also an additional benefit is a lowering of UL APWV. A lower APWV coupled with PEH would enable reflected pressure waves to return to the central aorta in diastole, in turn augmenting DBP and coronary blood flow, ultimately improving myocardial oxygen consumption (Bogren et al, 1989; Ohtsuka et al, 1994).

Why the modulation of vascular tone in the upper limb, non-active vascular bed was greater than in the lower limb, active vascular bed can not be resolved but as stated previously similar responses were seen in the same cohort to a reactive hyperaemic

stimulus. Moreover, recent data does support the contention that the UL vasculature remains responsive to flow-mediated stimuli whilst LL function deteriorates with age (Wray et al, 2005; 2006; Donato et al, 2006; Nishiyama et al, 2007).

A novel finding of the present study is that APWV was disassociated with PEH. This is surprising as recent studies of haemodynamics during PEH have reported increases in femoral (Pritcher et al, 2004; Lockwood et al, 2005a; 2005b; Lynn et al, 2007) and femoral and brachial (McCord et al, 2006; McCord and Halliwill, 2006) vascular conductance determined via ultrasonography of arterial diameter changes. Whilst vasodilatation during PEH occurs in both active and non-active vascular beds, up to ~56% of the rise in systemic vascular conductance is attributable to muscle hyperaemia, ~8% to splanchnic and renal hyperaemia, with ~36% of the augmented vascular conductance unaccounted for (Pritcher et al, 2004). Increased vascular conductance in turn is a consequence of vasodilatation, to which modulations in APWV have been shown to be related (Naka et al, 2000; 2006). Thus, changes in APWV would be expected during PEH, especially at the sites where clearly established increases in vascular conductance have been documented. One possible explanation could be an impaired LL endothelial response in the present cohort. An ineffective endothelial response would be counterintuitive and difficult to comprehend in the face of the current data highlighting a sustained vasodilatation. Although, it cannot be overlooked that endothelial-independent dilatation can also take place. Moreover, APWV determined via exactly the same methodology as that utilised in the present study has been shown to respond accordingly to both endothelial-dependent (Ach intra-arterial infusion) and endothelial-independent (*N*-monomethyl-L-arginine intra-arterial infusion; sublingual glyceryl trinitrate) stimulation (Naka et al, 2006). The significant positive correlation between UL APWV and the systemic venous

concentration of angiotensin II indicates that modulations in vascular tone elicited via angiotensin II transcend into appropriate responses in UL APWV.

4.2 Conclusion

In summary, the present study demonstrated that 30-minutes of moderate intensity dynamic exercise produces PEH in borderline hypertensive subjects that lasts up to 6-hours post-exercise. The data also highlights that the blood pressure-lowering effect of dynamic exercise is beyond the variation that occurs over the same time course in the same subjects following a period of non-exercise.

Similar to previous findings (Halliwill et al, 2000; Halliwill, 2001) the current data support the conclusion that PEH results from a sustained systemic vasodilatation that is not offset by increases in $\dot{Q}$, significantly reducing SVR. The present findings indicate that changes in the systemic concentration of catecholamines, the RAAS, AVP or ANP cannot be responsible for the sustained reduction in SVR noted and are dissociated with PEH. The lack of association between these metabolites and PEH would fit with the latest data indicating that a persistent histamine-receptor dependent augmentation of vascular conductance (Lockwood et al, 2005b; McCord et al, 2006a; McCord and Halliwill, 2006) is operating during PEH at least up to 2-hours post-exercise. Only ANP has been shown to have the potential to interact with histamine receptors.

It is also apparent that changes in APWV do not relate to PEH over the course of the current protocol. Although an important secondary finding is that additional to the decline in systemic arterial blood pressure following an acute bout of exercise, a decrease in the absolute APWV immediately post-exercise is found when compared to

the control condition. This lowering of absolute APWV following exercise has important ramifications for circulatory efficiency.

It could be speculated that additional recordings of sympathetic outflow e.g. muscle sympathetic nerve activity (MSNA) via microneurography would have substantiated the findings of blunted SVR. Human data does indicate that MSNA is reduced during PEH in normotensive (Halliwill et al, 1996a) and borderline hypertensive (Floras et al, 1989) subjects where the decrements in MAP are similar to those reported presently. However, other workers have recorded no changes in MSNA during the post-exercise period (Hara and Floras, 1992; Floras and Senn, 1991) indicating that reductions in MSNA may not be obligatory for PEH and the primary determinant to be ascertained is systemic arterial pressure.

STUDY 2: THE EFFECTS OF SYSTEMIC OXYGEN TENSION DURING EXERCISE ON POST EXERCISE HAEMODYNAMICS IN HYPERTENSION

5.0 Introduction

Variations in oxygen tension beyond the physiological range exert complex effects on the human circulation. Hypoxia is a powerful sympathetic stimulant in healthy (Calbet 2003) and diseased (Fletcher 2000; Heindl, et al. 2001) populations capable of mediating heart rate, $\dot{Q}$ and vascular resistance. In support of this, Somers et al, (1988) found enhanced sympathetic activity when borderline hypertensives were exposed acutely at rest to hypoxic gas mixtures (14% and 10% O_2). However, potentially beneficial modulations in vascular function can occur in response to systemic hypoxia, such as increased vascular conductance in many diverse vascular beds to offset the decrease in arterial oxygen content thus maintaining convective O_2 delivery (Rowell and Blackmon, 1987; Weisbrod et al, 2001; Dinenno et al, 2003). Hyperoxia on the other hand has been shown to produce both favourable effects on cardiovascular/arterial-cardiac baroreflex function (Shibata, et al. 2005), via decreased sympathetic and increased parasympathetic tone, and adverse effects on the circulatory system. For example, Ciarka and co-workers (2005) reduced mean arterial pressure in hypertensive subjects when breathing hyperoxic gas (100% O_2) at rest. Whilst, Izdebska and collegues (1998) elicited a potentiation of PEH in hypertensive subjects when inspiring a hyperoxic gas mixture in the post-exercise period, indicating that attenuation of the sympathoexcitatory arterial chemoreceptor reflex may play a role in PEH. There is no further data investigating the effect of systemic oxygen flux on PEH. Conversely, several studies have highlighted the undesirable outcome of hyperoxic exposure on cardiovascular variables, notably MAP, SVR and central haemodynamics in healthy subjects (Saadjian et al, 1999; Mak et al, 2001;

Warring et al, 2003; Thomson et al, 2006) and in heart failure patients (Haque et al, 1996) independent of changes in sympathetic activity or ventilation at rest.

Systemic oxygen flux mediates a cascade of metabolic functions and intiates a profound integration of many physiological systems especially if further perturbations such as exercise are coupled to the stimulus. A variety of mechanisms contribute to the observed changes in cardiovascular variables following modulations in oxygen tension. Vascular smooth muscle cell tone is directly affected by oxygen tension through a variety of smooth muscle cell ion channels (Welsh et al, 1998; Daut et al, 1990; Coppock et al, 2001). The synthesis and/or release of many circulating metabolites are also potentially mediated by O_2. For example systemic oxygen flux is known to disassociate the renin-angiotensin-aldosterone system (RAAS) axis (Bouissou et al, 1987; 1988), and exert independent effects on the circulating concentration of ANP (Lawrence and Shenker, 1991), catecholamines (Calbet et al, 2003; Hansen & Sander, 2003) and endothelin-1. At the level of the vascular endothelium a number of vasoactive metabolites are synthesised in an oxygen-sensitive manner, such as prostaglandins, adenosine, $NO^{\cdot}$ and ROS (Rubanyi and Vanhoutte, 1986; Wink et al, 1996; Bailey et al, 2003b). ROS in turn may subsequently alter cellular function (Drummond et al, 2000) and mediate cellular signalling (Quintero et al 2006; Pacher et al, 2007).

To date it remains unknown if hypertensive subjects will display PEH following exercise when exposed to acute variations in systemic oxygen tension during exercise. To test the hypothesis that augmented or attenuated systemic O_2 flux modulates PEH in pre-hypertensives, the current investigation exposed 9 such subjects to dynamic exercise in acute hypoxia, hyperoxia and normoxia and tracked haemodnamic variables for 2-hours during normoxic recovery.

5.1 Methodology

Experimental Protocol

The subjects attended the laboratory over three visits each separated by approximately two to three weeks. The randomised visits were: 1) 30-minutes of cycle exercise (Monark 824ε Ergomedic, Varberg, Sweden) at 70% of pre-determined normoxic $\dot{V}O_{2PEAK}$ [See general methodology section 3.2.1] [normoxia]; 2) whilst inspiring 16% O_2 [hypoxia] and 3) whilst inspiring 50% O_2, [hyperoxia]. The subjects were then followed post-exercise for 2-hours. Subjects were randomly assigned to either condition followed by cross-over. On all occasions after an 8-hour overnight fast, the subjects presented to the laboratory at 8:30 am and underwent baseline assessment of physiological parameters. During the day the subjects consumed the provided standardised meal (see section 3.13) and the laboratory was regulated for temperature (21± 2°C) and humidity (65± 3%) as described in methodology section 3.14. All subjects were familiarised with the protocol and methodology employed prior to the commencement of the experimental protocol. Subjects were instructed to refrain from exercise and alcohol for 48-hours prior to study days.

Fasting plasma electrolyte concentrations and osmolality across the trial were assessed as detailed in methodology section 3.15.19 and 3.15.20, respectively. Values were within normal ranges but did vary slightly throughout the protocol (Table 5.0). The only deviation was the significant elevation of sodium concentration at baseline on the hyperoxic study day when compared to the normoxia trial. Fasting Hcy, folate and glucose concentrations, assayed as detailed in methodology section 3.15.17 were

within normal ranges (as recorded previously in Table 4.1), excluding the possibility of existing co-morbidities.

Table 5.0 Plasma Na^+/K^+ Concentration & Osmolality Pre Exercise

	0.21	**0.16**	**0.50**
$Na^+(mmol.l^{-1})$	136.2 (5.6)	140.6 (2.2)	142.6 (2.9)*
$K^+(mmol.l^{-1})$	4.3 (0.4)	4.5 (0.3)	4.4 (0.2)
Osmolality (mOsm-kg H_2O)	284.1 (18)	263.4 (20)	279.3 (7.7)

(n = 9) All data are expressed as mean (SD). 0.21, Normoxic trial; 0.16, Hypoxic trial; 0.50, Hyperoxic trial. *P<0.05 *v.* Normoxia.

Systemic Arterial Pressure & Heart Rate

BP was measured by the same experienced investigator according to stringent procedures outlined in methodology section 3.5. Auscultatory BP readings averaged 128(7)/85(3) mmHg for systolic blood pressure (SBP) and diastolic blood pressure (DBP), respectively at baseline. Heart rate (HR) was determined with a three lead, bi-polar, ECG as detailed in methodology section 3.4 and arterial haemoglobin oxygen saturation ($S_{a,O2}$) was determined as outlined in methodology section 3.12. The recordings were taken after the subjects were allowed 20-minutes of seated rest.

Exercise Protocol

Subjects underwent the same exercise protocol as that outlined in Section 4.1 with the additional factor of gas delivery. Normobaric normoxic, hypoxic and hyperoxic gas was delivered to subjects after 1-minute of initial warm-up on the cycle ergometer, as outlined in methodology section 3.11. Gas delivery occurred concomitantly with the application of the mass to the cycle ergometer basket. The delivery of gas to the subject was double-blind, thus the investigator determining the arterial pressure

responses (author of the thesis) and the subject were blinded from gas delivery. Physiological parameters measured and the timing of measurements during and post-exercise were as those described previously in section 4.1 and Figure 4.1 with the additional determination of $S_{a,O2}$ recorded continuously throughout the trial. Arterial P_{O2} ($P_{a,O2}$) was calculated from $S_{a,O2}$ and arterial O_2 content was calculated from $P_{a,O2}$ and arterial saturations using the method laid out in section 3.12.1. Gas delivery was terminated at the end of the exercise period and subjects remained in a post-exercise normoxic environment following every trial. This methodological approach was utilised to completely isolate the effects of systemic oxygen flux during exercise on post-exercise responses.

Venous Blood Collection & Analysis

Pre- and post-exercise venous blood samples were collected using the cannula method outlined in methodology section 3.15.1. ΔPV and ΔBV (i.e. percent change in PV and BV) following exercise were calculated as described in methodology section 3.15.6. At every blood collection time-point all blood metabolites were corrected for haemodilution or haemoconcentration.

Adrenaline & Noradrenaline Analysis

Systemic venous blood plasma was assayed for adrenaline and noradrenaline via the method outlined in methodology section 3.15.8.

Renin & Angiotensin II Analysis

Systemic venous blood plasma was assayed for renin and angiotensin II via the method outlined in methodology section 3.15.9 and 3.15.10, respectively.

Aldosterone Analysis

Aldosterone quantification was performed on systemic venous serum utilising the methodology described in section 3.15.11.

ANP & AVP Analysis

ANP & AVP concentrations were determined in systemic venous plasma by the methodology outlined in section 3.15.7.

$[La^-]_B$

Arterialised capillary blood was collected and analysed for whole blood lactate as outlined in methodology section 3.15.18.

Plasma Osmolality

Plasma osmolality was assessed by the methodology outlined in section 3.15.20.

Central Haemodynamics

Central haemodynamic data was collected on subjects following the procedures laid out in methodology section 3.10.

APWV

Pre and post-exercise APWV was determined in subjects as detailed in methodology section 3.9.

Statistical Analyses

Statistical analysis was performed using the methodology outlined in section 3.16.3.

5.2 Results

5.2.1 Cardiorespiratory & Blood Gas Response to Exercise Conditions

Cardiorespiratory responses to sub-maximal exercise under the varying conditions are shown in Table 5.1. Expected increases were noted in the major cardiorespiratory variables at the termination of exercise. The goal was to have subjects exercise for 30-minutes at 70% of normoxic $\dot{V}O_{2PEAK}$ i.e relative exercise intensity was held constant between conditions. Four subjects failed to complete the full 30-minute exercise bout, mean (SD) exercise duration was 25 (6) minutes in normoxia, and 23 (9) minutes in

hypoxia. Whereas only 3 subjects failed to complete the full 30-minute exercise bout in hyperoxia, mean exercise duration was 26 (7) minutes ($P>0.05$). Exercise intensity equated to 79 (15)% of previously determined normoxic $\dot{V}O_{2PEAK}$ values in normoxia, 74 (9)% in hypoxia and 74 (14)% in hyperoxia ($P>0.05$), with an average workload of 112 (35)W. Heart rate was 140 (17)bpm during normoxic exercise, 147 (22)bpm during hypoxic exercise and 135 (14)bpm during hyperoxic exercise. This represented, on average, 86 (11)%, 90 (11)% and 83 (10)%, respectively ($P>0.05$), of maximal heart rate determined during normoxic $\dot{V}O_{2PEAK}$ testing and is consistent with the target workload.

Heart rate was not different at the termination of exercise between trials ($P>0.05$). Likewise, SBP and DBP did not differ at the termination of exercise ($P>0.05$) between exercise conditions despite the expected decrease and increase in $S_{a,O2}$ and $P_{a,O2}$ during hypoxic and hyperoxic exercise, respectively ($P<0.05$) (Table 5.1). RPP values increased following exercise ($P<0.05$) but coincident with the lack of variation in heart rate or systolic pressure between trials were not modified by the changes in the fraction of inspired oxygen (F_{IO2}). Whole blood lactate increased during exercise (significant main effect for time $P<0.05$) with grouped mean values being significantly lower in the hyperoxic condition when compared to the normoxic trial (significant main effect for state $P<0.05$). However, there was no significant interaction effect (state x time $P>0.05$) displayed.

Table 5.1. Cardiorespiratory and blood gas responses to sub-maximal cycle exercise in normobaric normoxia, hypoxia and hyperoxia.

Variable	0.21		0.16		0.50	
	Pre	Term^	Pre	Term	Pre	Term
$\dot{V}O_2$ (l min^{-1})	-	1.7 (0.5)	-	1.4 (0.7)	-	1.5 (0.4)
V_E (l min^{-1})	-	42.5 (7.6)	-	47.4 (7.0)*	-	43.3 (5.4)
Heart Rate (bpm)	69 (7)	146 (18)†	68 (7)	138 (20)†	70 (8)	140 (13)†
Systolic BP (mmHg)	128 (8)	180 (18)†	129 (8)	180 (15)†	128(5)	183 (9)†
Diastolic BP (mmHg)	85 (4)	84 (4)	87 (2)	85 (6)	84 (4)	85 (4)
MAP (mmHg)	99 (5)	99 (5)	101 (3)	100 (8)	99 (4)	102 (3)
RPP (AU)	9 (1)	26 (4)†	9 (1)	25 (4)†	9 (1)	26 (3)†
RPE (AU)	-	14 (4)	-	14 (2)	-	13 (2)
$[La^-]_B$ (mmol/l)$^{\#}$	1.2 (0.3)	2.7 (1.1)†	1 (0.6)	4.4 (3.2)†	0.2 (0.2)	1.9 (1.3)†
$S_{a,O2}$ (%)	96 (1)	96 (1)	96 (2)	92 (2)‡	97 (0.9)	97 (1)§
$P_{a,O2}$ (Torr)	88 (10)	88 (9)	89 (14)	66 (6)‡	90 (8)	109 (12)§
$C_{a,O2}$ (ml/dl)	19 (2)	19 (0)	19 (3)	16 (0)‡	19 (2)	19 (0)

All data are expressed as mean (SD). $n = 9$ except for V_E, where $n = 5$. 0.21, Normoxic trial; 0.16, Hypoxic trial; 0.50, Hyperoxic trial. ^Term, termination of exercise (apart from V_E and $[La^-]_B$ which was determined at the mid-point of the exercise bout); $\dot{V}O_2$, pulmonary oxygen uptake; V_E, Ventilation; BP, blood pressure; MAP, mean arterial pressure; RPP, rate pressure product; AU, arbitary units; $[La^-]_B$, whole blood lactate; $S_{a,O2}$, arterial O_2 saturation; $P_{a,O2}$, arterial partial pressure of O_2; $C_{a,O2}$ arterial O_2 content. *$P<0.05$ *v.* normoxia at the same time point. $^{\#}$ Significant main effect for state, $P<0.05$ normoxia *v.* hyperoxia. $^{\dagger}P<0.05$ *v.* pre within-condition. $^{\ddagger}P<0.05$ v. normoxia and hyperoxia at the same time point; $^{\S}P<0.05$ v. normoxia at the same time point.

5.2.1 Blood Pressure Response

Analysis of MAP produced a significant main effect for time ($P = 0.00$), indicating a reduction from baseline of 5 mmHg at the 30-minutes post-exercise time point onwards until cessation of recordings (Figure 5.1). Moreover, analysis of the absolute change in MAP from baseline between trials revealed a significant main effect for oxygenation state ($P<0.05$) whereas no interaction effect was observed (state x time; $P>0.05$). Similar to study 1, exercise *per se* produced a 5% reduction of MAP versus pre-exercise. MAP was reduced by ~5mmHg at its nadir at the 1-hour post-exercise time-point ($P<0.05$).

Figure 5.2 displays the absolute reduction (Δ) in MAP from baseline. A negative Δ value indicates an increase in MAP post-exercise. The MAP response across the trial was blunted following hyperoxic exercise in comparison to normoxic and hyperoxic exercise (both $P=0.00$) whereas no differences were observed between normoxic and hypoxic exercise (grouped means for state; 4.5 (4) following normoxia, 5.7 (6) following hypoxia and 1.4 (4) mmHg following hyperoxia, change from baseline). The reduction in MAP resulted from a post-exercise fall in systolic blood pressure (SBP) and diastolic blood pressure (DBP) (Table 5.2). SBP was attenuated by ~10mmHg 1-hour post-exercise and remained ~8mmHg below pre-exercise levels until cessation of recordings 2-hours following exercise ($P<0.01$; pooled for time). DBP was attenuated by ~4% 60-minutes following exercise remaining blunted by ~3% at 120-minutes post-exercise ($P<0.01$; pooled for time). Unlike MAP, the absolute reduction in SBP from baseline was not different between conditions ($P=0.07$) but did show a main effect for time ($P<0.05$) displaying an elevated SBP [~2 (8)mmHg] immediately (within 1-minute) post-exercise then a decline in SBP from 15-minutes post-exercise to the nadir of ~10 (4)mmHg at 1-hour post-exercise and

continuing to be significantly depressed ($P<0.05$) below the immediate post-exercise response until completion of the trial. In contrast, the absolute reduction in DBP from baseline failed to display any differences between conditions or across the trial ($P>0.05$).

5.2.2.1 Heart Rate (HR), Rate Pressure Product (RPP) & Blood Gases

Immediately following exercise (i.e. within 1-minute) HR remained elevated ($P<0.05$; pooled values) but returned to baseline by the 1-hour post-exercise recovery time point (Table 5.2). HR values were not different between the exercise conditions at the 1 and 2-hour post-exercise time points ($P>0.05$). In line with HR data, RPP values increased following exercise ($P<0.05$; pooled values) but returned to pre-exercise values by 60-minutes post-exercise and failed to differ between conditions (Table 5.2; $P>0.05$ for state and state x time).

As expected, termination of gas delivery at the completion of exercise reverted both $S_{a,O2}$ and $P_{a,O2}$ back to normoxic baseline values with no carryover effect. Thus, from the immediate post-exercise (within 1-minute) time-point until completion of the protocol both indicies of blood gas status remained at pre-exercise levels ($P>0.05$ for time, state and state x time). This is in contrast to the expected variations in blood gases during the exercise conditions as displayed in Table 5.1.

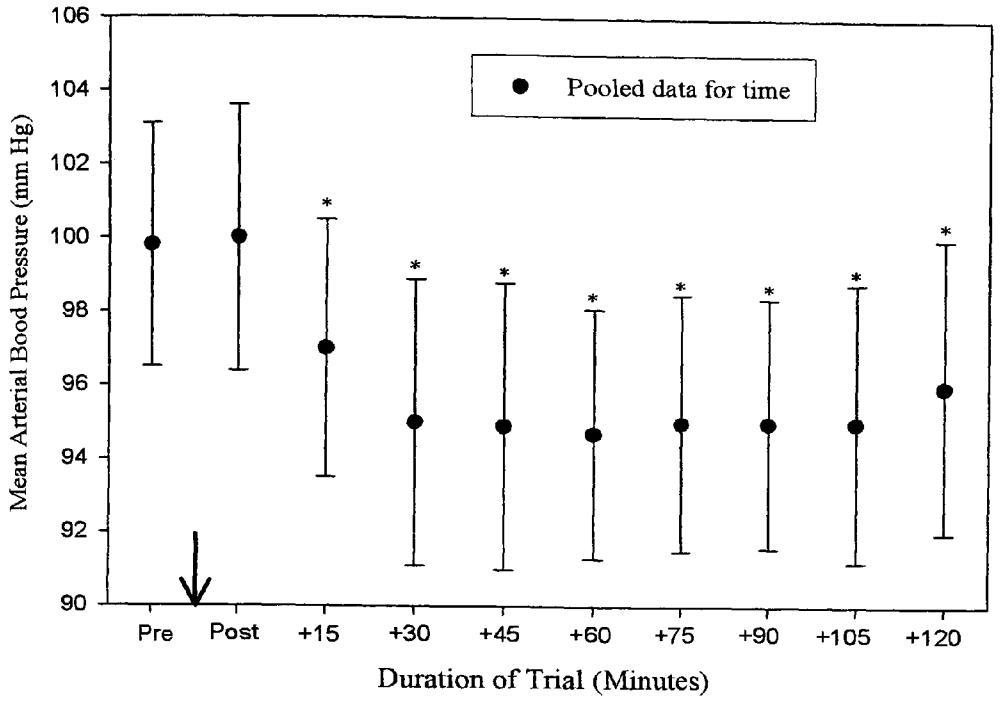


Figure 5.1. Mean arterial pressure response, pooled for time, following cycle ergometry. Data are expressed as mean (SD); $n = 9$. Arrow indicates exercise. Post (within 1-minute post-exercise). +, Cumulative time following exercise. *$P<0.01$ *v.* pre-exercise.

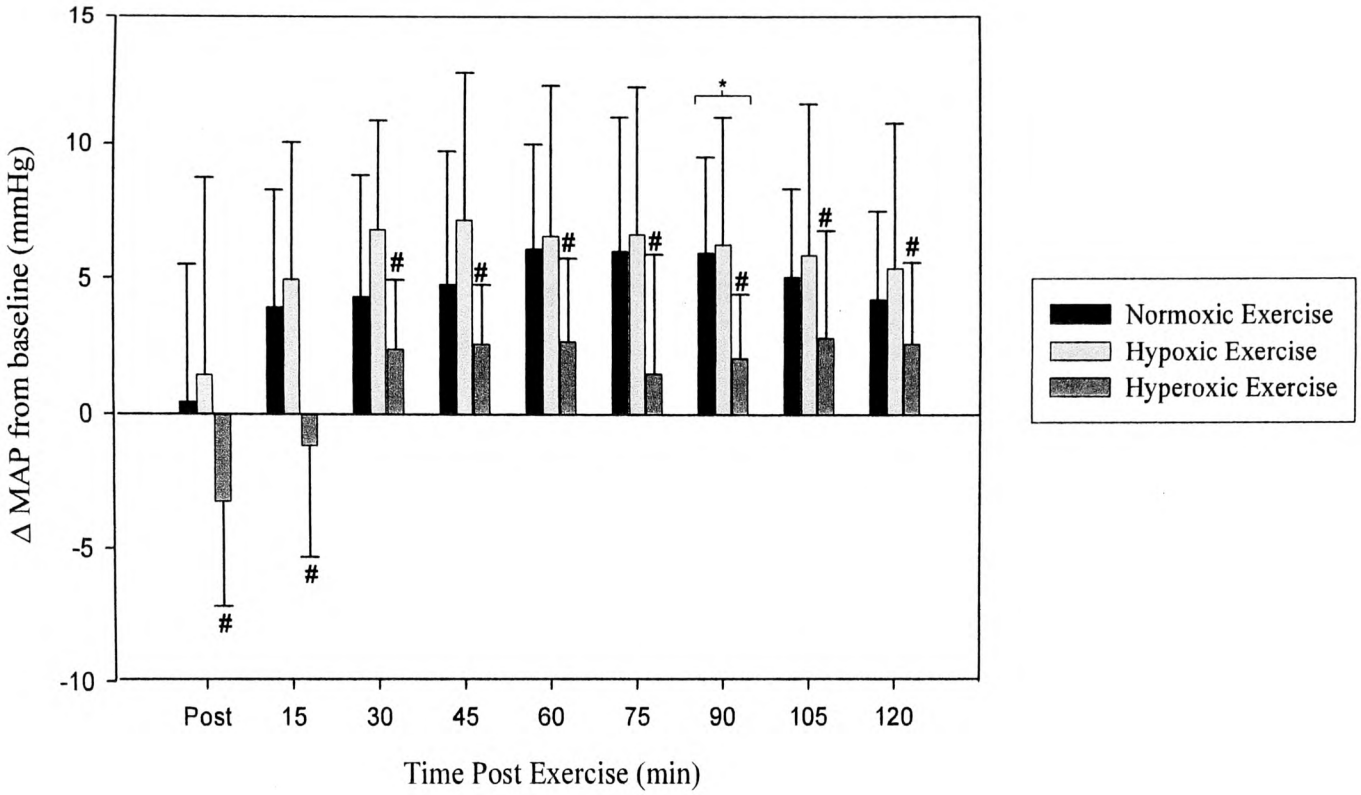


Figure 5.2. Absolute change (Δ) in mean arterial pressure from pre-exercise baseline. Post, immediately (within 1-minute) post-exercise. *$P<0.05$ *v.* post, pooled for time, $^{\#}P<0.05$ hyperoxia *v.* normoxic and hypoxic exercise, pooled for oxygenation state. Values are Mean ± (SD); $n = 9$ for each group.

Table 5.2. Cardiovascular and blood gas responses to 30-minutes normobaric normoxic, hypoxic or hyperoxic sub-maximal exercise

	Pre			Post			60 min			120 min		
	0.21	**0.16**	**0.50**	**0.21**	**0.16**	**0.50**	**0.21**	**0.16**	**0.50**	**0.21**	**0.16**	**0.50**
HR* (bpm)	69 (7)	68 (7)	70 (8)	102 (14)	101 (11)	104 (10)	70 (8)	72 (8)	70 (6)	65 (5)	67 (5)	69 (8)
SBP† (mmHg)	128 (8)	129 (8)	128 (5)	128 (10)	128 (18)	136 (9)	116 (7)	118 (10)	121 (8)	119 (9)	123 (11)	122 (7)
DBP† (mmHg)	85 (4)	87 (2)	84 (4)	84 (4)	85 (7)	85 (4)	82 (3)	83 (4)	83 (4)	83 (4)	84 (4)	
RPP* (AU)	9 (1)	9 (1)	9 (1)	13 (2)	13 (2)	14 (2)	8 (1)	9 (2)	8 (1)	8 (1)	8 (1)	8 (1)
$S_{a,O2}$ (%)	96 (1)	96 (2)	97 (1)	96 (1)	96 (1)	97 (0.5)	96 (1)	96 (1)	96 (1)	97 (1)	97 (1)	96 (1)
$P_{a,O2}$ (Torr)	88 (10)	89 (14)	90 (8)	84 (9)	85 (6)	90 (5)	82 (9)	86 (9)	89 (11)	90 (11)	96 (17)	85 (9)
$C_{a,O2}$ (ml/dl)	19 (2)	19 (3)	19 (2)	19 (3)	19 (3)	19 (2)	17 (3)	19 (3)	18 (4)	19 (2)	19 (2)	18 (3)

All data are expressed as mean (SD). 0.21, Normoxic trial; 0.16, Hypoxic trial; 0.50, Hyperoxic trial. Pre, pre-exercise; Post, immediately (within 1-minute post-exercise); 60 min, 60-minutes post-exercise; 120 min, 120-minutes post-exercise. HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; RPP, rate pressure product; AU, arbitary units; $S_{a,O2}$, arterial O_2 saturation; $P_{a,O2}$, arterial partial pressure of O_2; $C_{a,O2}$, arterial O_2 content..

*$P<0.05$, Post *v.* pre pooled for time. †$P<0.05$, 60 min & 120 min *v.* pre pooled for time. $n = 9$ for all conditions.

5.2.1 Postexercise Haemodynamics

Table 5.3 displays post-exercise *versus* pre-exercise haemodynamics. Immediately post-exercise both HR and $\dot{Q}$ were elevated above baseline ($P<0.05$, pooled for time) by 148% and 68%, respectively, but returned to pre-exercise levels by 1-hour post-exercise. SV, SVI and $\dot{Q}$ values were reduced during the hypoxic trial when compared to the normoxic trial. SVR was decreased from a baseline value of 19.3 (4.6) resitance units (RU) ($P<0.05$, pooled for time) during the entire post-exercise phase being 12.7 (3.4), 16.2 (3.4) and 16.6 (3.4) RU immediately post-, 60-minutes post- and 120-minutes post-exercise, respectively, but failed to demonstrate any variation between exercise conditions. Arterial-venous oxygen difference (pooled for time) showed a significant decrease over time being ~13% reduced below baseline at the 120-minute sampling point. O_2 pulse increased ($P<0.05$, pooled for time) during the immediate post-exercise period by 50% but returned to baseline levels by 1-hour post-exercise.

Figure 5.3 shows the relative changes in systemic vascular resistance and conductance from baseline to immediately post-, 60-minutes post- and120-minutes post-exercise. Systemic vascular resistance was attenuated by ~32% (across conditions) immediately post-exercise and remained blunted, in comparison to baseline, by ~15 and 13% at 60- and 120-minutes post-exercise, respectively. The rise in SVR back towards baseline level caused the vasodilatation at 60- and 120-minutes post-exercise to be attenuated when compared to the immediate post-exercise point ($P<0.05$). In line with the systemic mean arterial pressure response the reduction in SVR from baseline was significantly less following hyperoxic exercise as compared to normoxic or hypoxic exercise ($P<0.05$, pooled for state). SVC values (Figure 5.3b) highlighted a ~53% augmentation (across conditions) over baseline immediately post-exercise but was

significantly blunted at 60- and 120-minutes post-exercise (18 and 14%, respectively) compared to the immediate post-exercise response. SVC however failed to display a sensitivity to systemic oxygenation across the trial ($P>0.05$).

Table 5.3. Haemodynamics

	Pre			Post			60 min			120 min		
	0.21	**0.16**	**0.50**	**0.21**	**0.16**	**0.50**	**0.21**	**0.16**	**0.50**	**0.21**	**0.16**	**0.50**
HR* (bpm)	69 (7)	68 (7)	70 (8)	102 (14)	101 (11)	104 (10)	70 (8)	72 (8)	70 (6)	65 (5)	67 (5)	69 (8)
SV‡ (ml $beat^{-1}$)	93 (31)	71 (17)	90 (34)	83 (42)	75 (31)	68 (20)	95 (31)	80 (15)	91 (27)	95 (30)	80 (15)	92 (24)
SV Index‡ (ml/m^2)	48 (16)	37 (9)	47 (18)	43 (21)	39 (16)	35 (10)	50 (16)	42 (9)	48 (14)	50 (17)	42 (8)	48 (13)
$\dot{Q}$ *‡ (l min^{-1})	7 (3)	5 (1)	7 (3)	11 (5)	10 (4)	9 (3)	7 (3)	6 (1)	6 (2)	7 (3)	6 (2)	6 (2)
SVR†# (RU)	17 (5)	21 (5)	18 (7)	12 (4)	13 (4)	14 (3)	16 (4)	17 (3)	16 (4)	16 (4)	18 (3)	16 (5)
SVC†# (ml/min/mmHg)	62 (23)	50 (13)	61 (25)	93 (38)	86 (31)	77 (21)	71 (25)	61 (14)	67 (23)	68 (26)	59 (14)	68 (23)
$(a-\bar{v})O_2$diff * (ml/100ml)	9 (9)	12 (10)	9 (7)	17 (9)	20 (13)	19 (9)	9 (8)	11 (9)	9 (6)	7 (8)	10 (10)	9 (6)
O_2 Pulse* (ml O_2 $beat^{-1}$)	8 (6)	8 (6)	8 (6)	12 (3)	12 (4)	11 (3)	6 (7)	8 (7)	8 (6)	5 (7)	8 (7)	8 (6)

All data are expressed as mean (SD). HR, heart rate; SV, stroke volume; SV Index, stroke volume index; $\dot{Q}$, cardiac output; SVR, systemic vascular resistance; RU, resistance units; $(a-\bar{v})O_2$diff ; arterial-venous oxygen difference. 0.21, Normoxic trial; 0.16, Hypoxic trial; 0.60, Hyperoxic trial. Pre, pre-exercise; Post, immediately (within 1-minute post-exercise); 60 min, 60-minutes post-exercise; 120 min, 120-minutes post-exercise. *$P<0.05$, Post *v.* pre pooled for time. ‡$P<0.05$, normoxia *v.* hypoxia pooled for state. †$P<0.05$, Post, 60 min & 120 min *v.* Pre, pooled for time. #$P<0.05$, 60 min & 120 min *v.* post pooled for time. ♠$P<0.05$, 120 min *v.* pre pooled for time. $n = 9$ for all conditions.

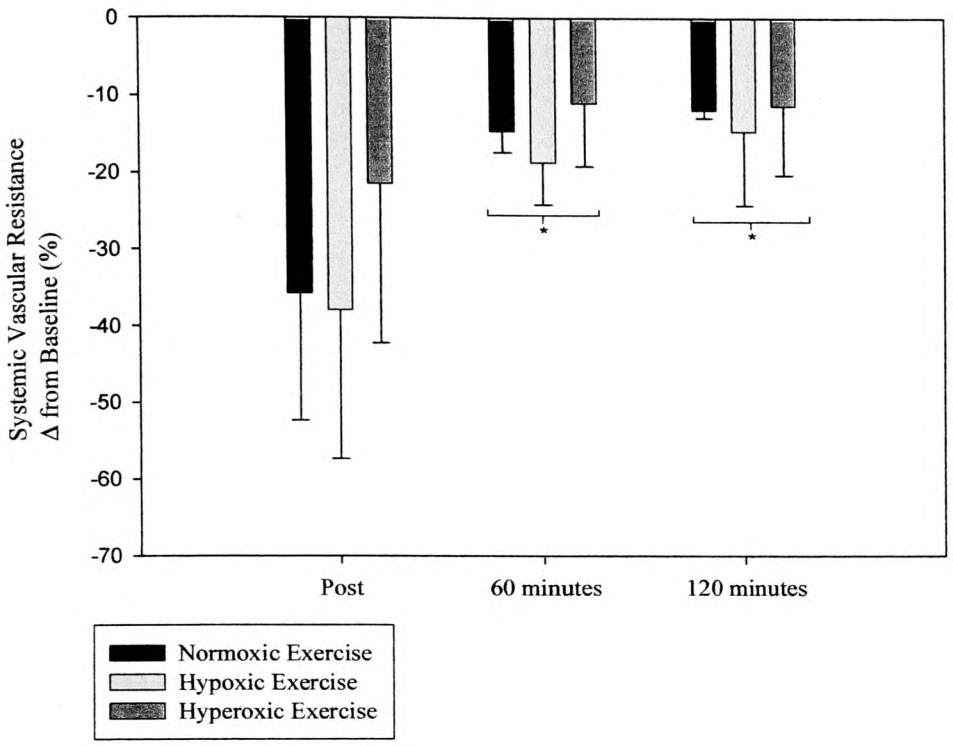


Figure 5.3A. Relative change (Δ%) in systemic vascular resistance from pre-exercise baseline. Post, immediately (within 1-minute) post-exercise; 60 minutes post-exercise; 120 minutes post-exercise. *P<0.05 *v.* post, pooled for time; P<0.05 main effect for state, hyperoxic *v.* normoxic and hypoxic exercise. Values are Mean ± (SD); n = 9 for each group.

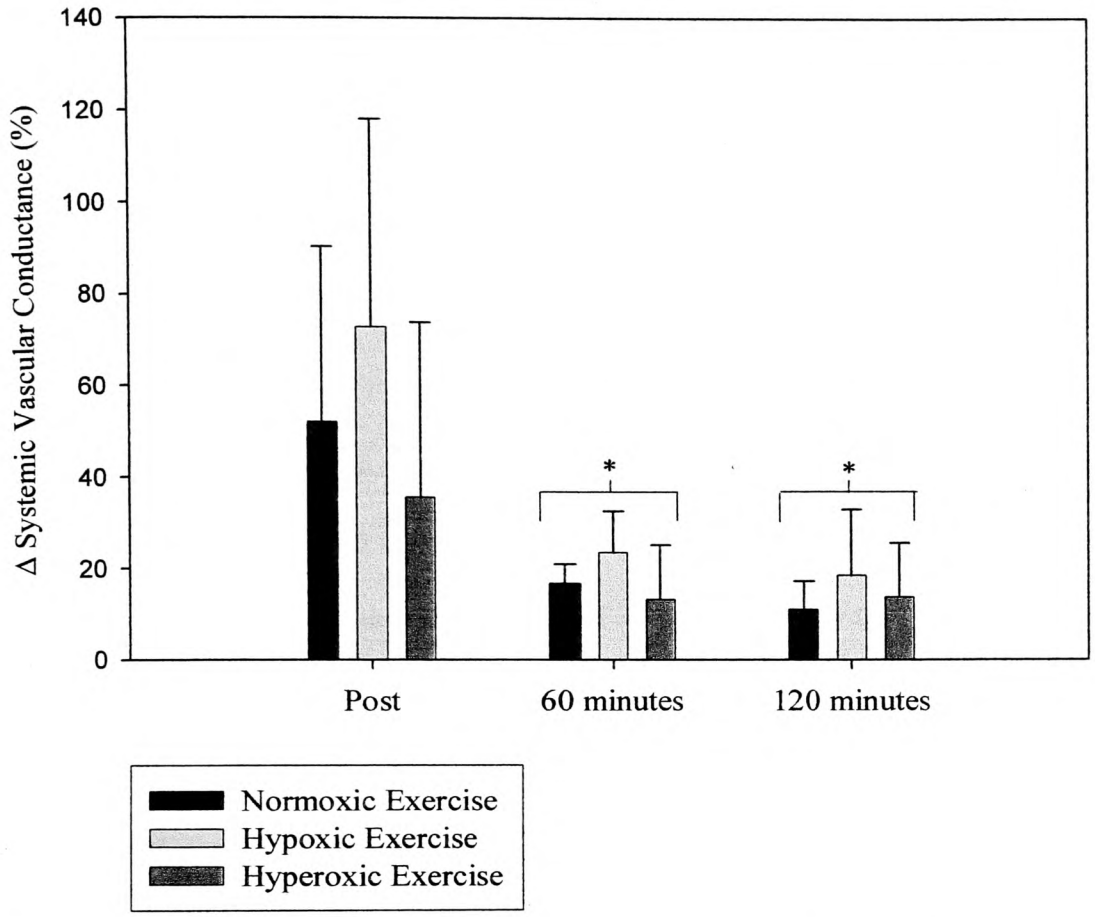


Figure 5.3B. Relative change (Δ%) in systemic vascular conductance from pre-exercise baseline. Post, immediately (within 1-minute) post-exercise; 60 minutes post-exercise; 120 minutes post-exercise. *$P<0.05$ *v.* post, pooled for time. Values are Mean ± (SD); $n = 9$ for each group.

5.2.4 Arterial Pulse Wave Velocity (APWV)

Upper limb (UL) APWV analysis revealed a significant main effect of exercise ($P<0.05$; main effect for time) although there was no effect across the conditions ($P>0.05$; main effect for state) or interaction effect (state x time, $P>0.05$). *Post hoc* analysis revealed that at the immediate (within 3-minutes) post-exercise time-point UL APWV was 18% lower compared to baseline ($P<0.05$) (Figure 5.4). Interestingly, by 120-minutes post-exercise, UL APWV had recovered and was 9% elevated above baseline UL APWV levels ($P<0.05$). Lower limb (LL) APWV between conditions and across the protocol were unremarkable, indicating no modulation of LL APWV following exercise (Figure 5.4).

Further analysis of normalised APWV (APWV/MABP) to control for differences in prevailing arterial pressure pre- to post-exercise, revealed that the 18% reduction in UL APWV immediately post-exercise remained [0.11 (0.02) *v.* 0.09 (0.01) m/s. $torr^{-1}$ pre and post-exercise, respectively; $P<0.05$]. Likewise the 9% elevation above baseline by 120-minutes post-exercise also remained intact when normalised for changes in arterial pressure [0.11 (0.02) *v.* 0.13 (0.02) m/s. $torr^{-1}$ pre and 120-minutes post-exercise, respectively; $P<0.05$]. There was no main effect for state nor was there an interaction effect (state x time) for normalised UL APWV (all $P>0.05$). Thus, the absolute reduction in UL APWV following exercise is due to inherent mechanisms of arterial pulse wave propagation other than the lowering of MAP and SBP. In similarity to the absolute LL APWV analysis there was no main effect for state nor was there an interaction effect (all $P>0.05$) for normalised LL APWV. Interestingly, a significant main effect for time was elucidated for normalised LL APWV indicating a significant elevation between the immediate post-exercise and 120-minutes post-exercise values ($P<0.05$)

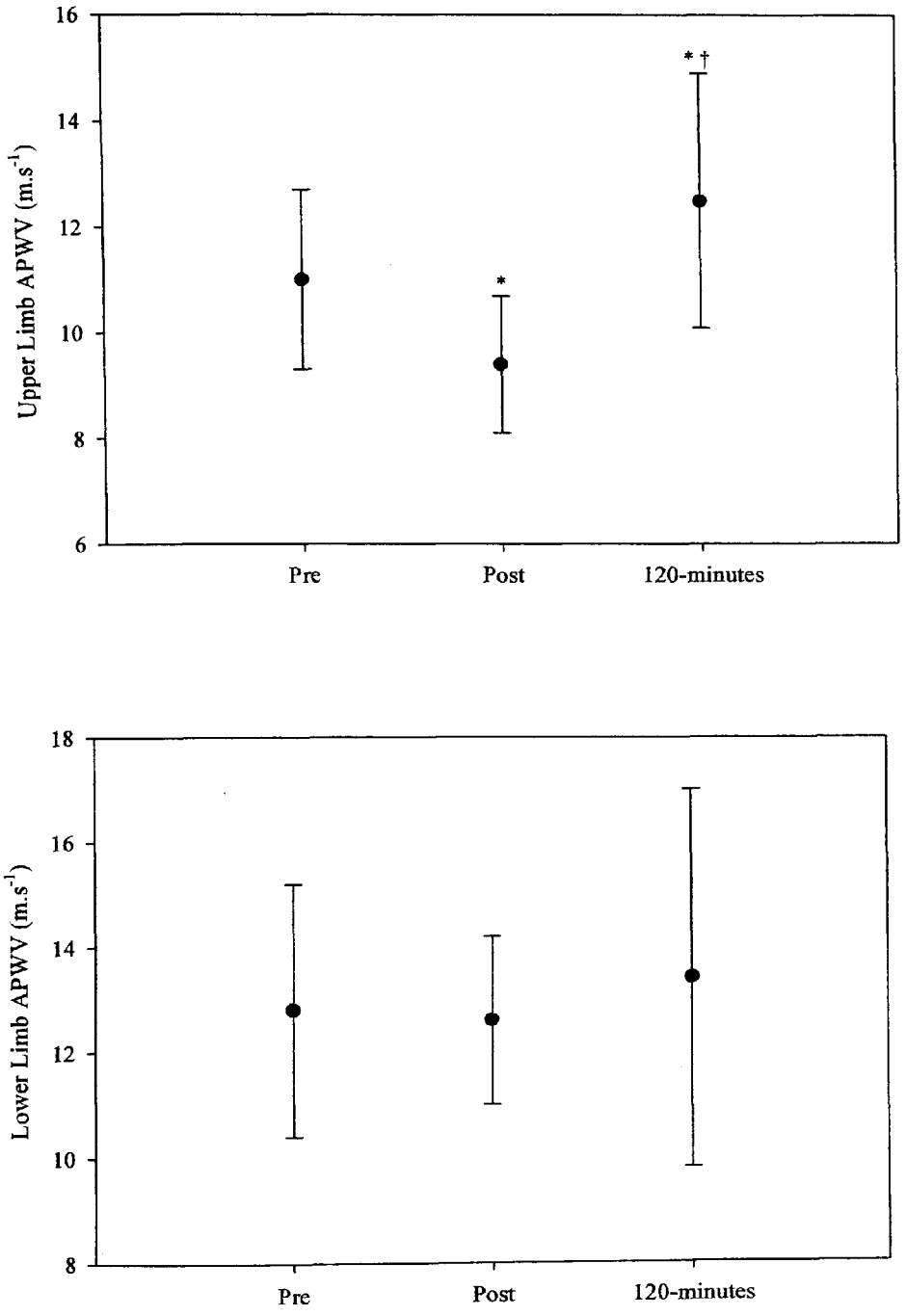


Figure 5.4. *Top:* Upper limb and *Bottom:* Lower limb arterial pulse wave velocity (APWV) averaged over 10-minutes at 1-minute intervals in each case and pooled for time, following 30-minutes dynamic cycle exercise. Values are Mean ± (SD); $n = 9$. Post, within 3-minutes post-exercise; 120-minutes post-exercise. $^{*}P < 0.05$ *v.* pre-exercise; $^{\dagger}P < 0.05$ *v.* post.

5.2.5 Plasma, Blood Volume & Plasma Osmolality

There was no main effect for time, state or interaction effect (time x state; all $P>0.05$) across the protocol for ΔPV or Δblood volume (BV), where Δ is change.

Systemic oxygen flux failed to elicit any changes in plasma osmolality ($P>0.05$). However, exercise *per se* evoked plasma osmolality increases. Immediately following exercise plasma osmolality was 310.6 (2.9) mosm/kg H_20 in comparison to 308 (1.6) mosm/ kg H_20 at baseline ($P<0.05$; Pooled for time). For the remainder of the protocol plasma osmolality remained elevated [310 (1.8) and 310 (1.7) mosm/ kg H_20 at Post1 and Post2, respectively, both $P<0.05$; Pooled for time].

5.2.6 Plasma Catecholamines

Venous plasma noradrenaline concentration was un-modified by systemic oxygen flux ($P>0.05$; Figure 5.5). Likewise, venous plasma adrenaline concentration was also unremarkable across the protocol ($P>0.05$; Figure 5.6).

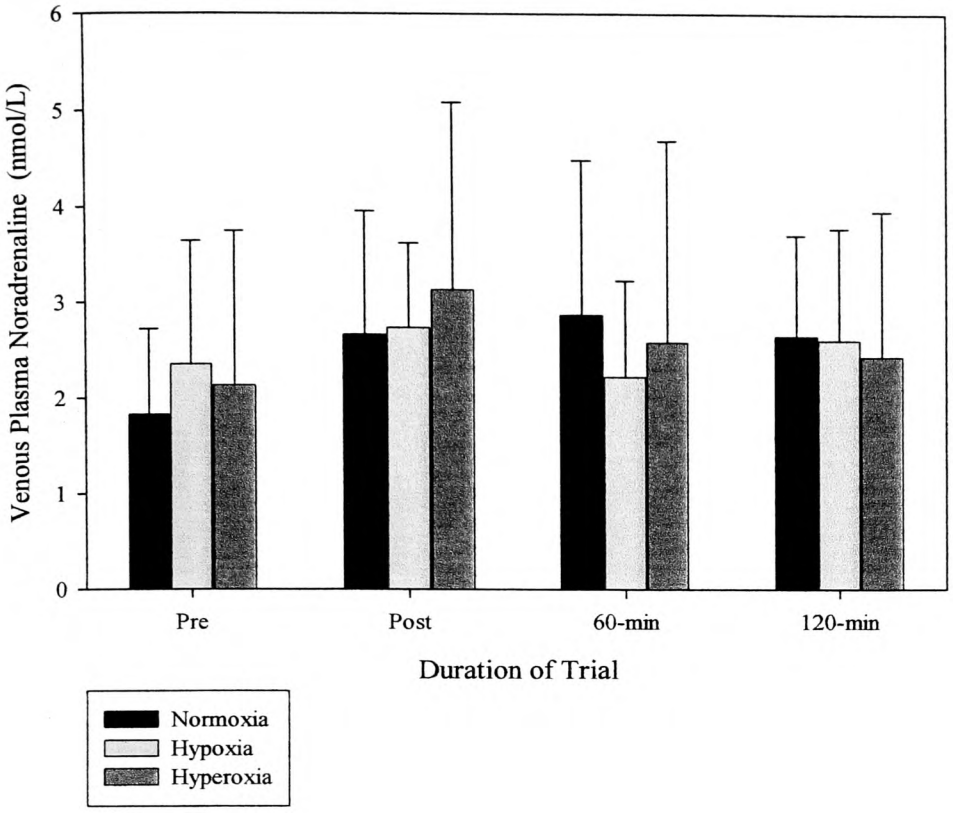


Figure 5.5 Venous plasma noradrenaline response to 30-minutes dynamic cycle exercise under varying degrees of systemic oxygen tension. Values are Mean ± (SD); $n = 9$. Post, within 1-minute post-exercise; 60-min, 1-hour post-exercise; 120-min, 2-hours post-exercise.

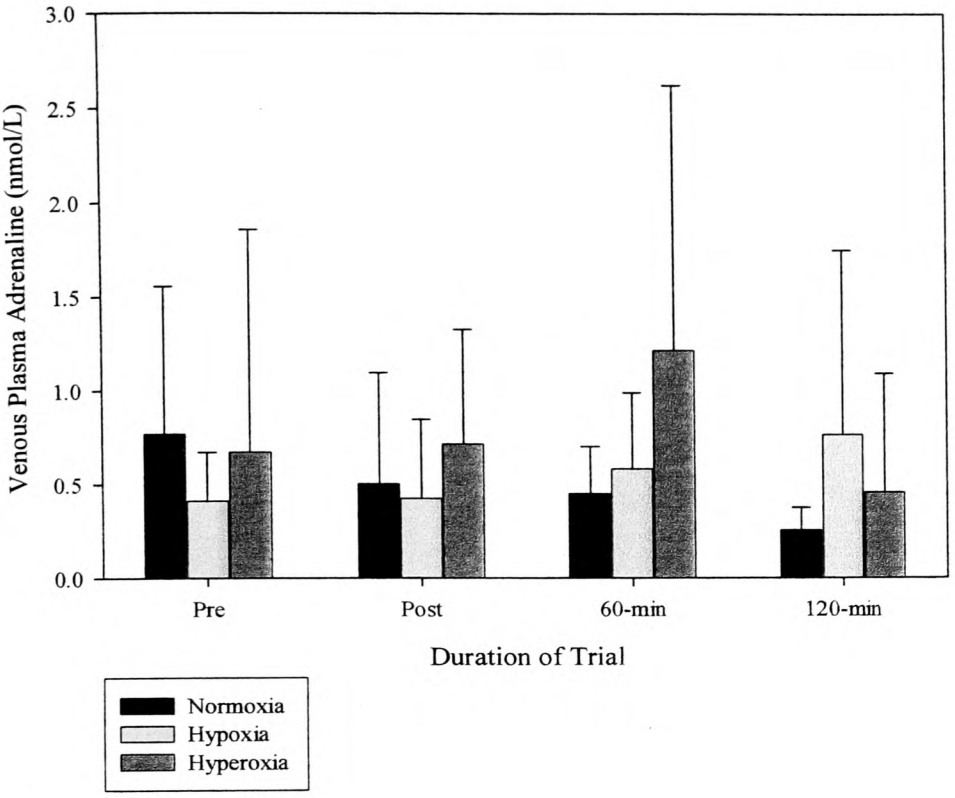


Figure 5.6 Venous plasma adrenaline response to 30-minutes dynamic cycle exercise under varying degrees of systemic oxygen tension. Values are Mean ± (SD); $n = 9$. Post, within 1-minute post-exercise; 60-min, 1-hour post-exercise; 120-min, 2-hours post-exercise.

5.2.7 Renin-Angiotensin-Aldosterone System (RAAS)

Systemic oxygen flux during exercise failed to elicit any changes in the RAAS ($P>0.05$ for state and interaction effect) whereas the acute exercise bout induced changes across the individual metabolites of the RAAS ($P<0.05$ for time). Exercise *per se* augmented venous plasma renin concentration by ~ 52% ($P<0.05$; Pooled for time), values then decreased towards baseline remaining significantly elevated by ~39% at the termination of the protocol 120-minutes post-exercise (Figure 5.7).

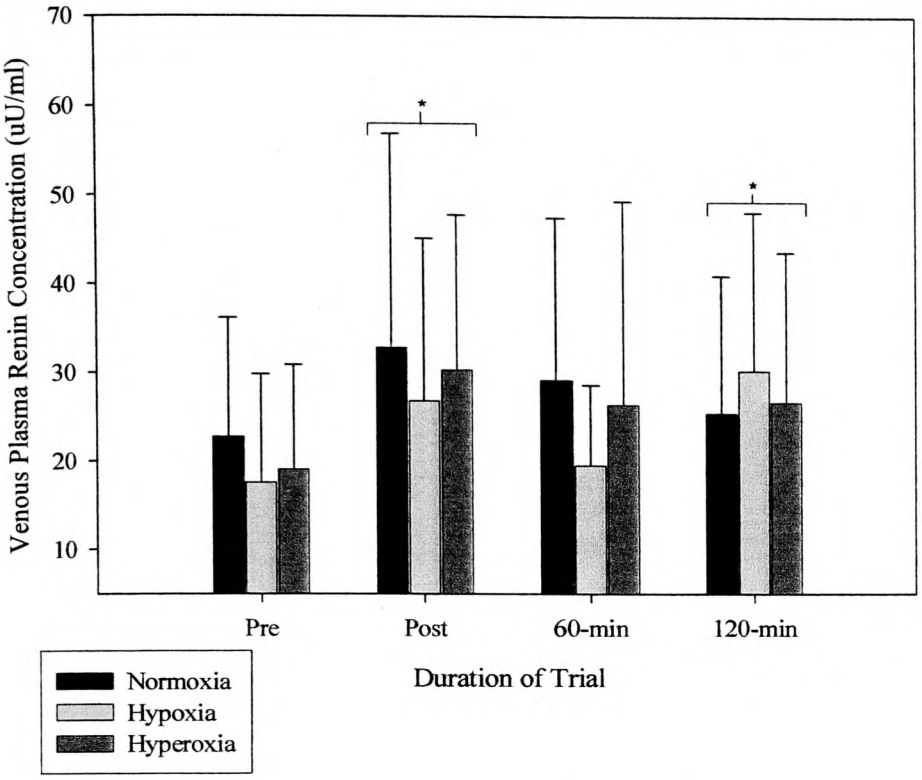


Figure 5.7 Renin response to 30-minutes dynamic cycle exercise under varying degrees of systemic oxygen tension. Values are Mean ± (SD); $n = 9$. Post, within 1-minute post-exercise; 60-min, 1-hour post-exercise; 120-min, 2-hours post-exercise. *$P<0.05$ *v.* pre-exercise, pooled for time.

Angiotensin II concentrations increased ($P<0.05$; Pooled for time) ~11% following exercise and continued to be elevated by ~18% at the 1-hour post-exercise time-point ($P<0.05$). Values returned to baseline by 2-hours post exercise (Figure 5.8).

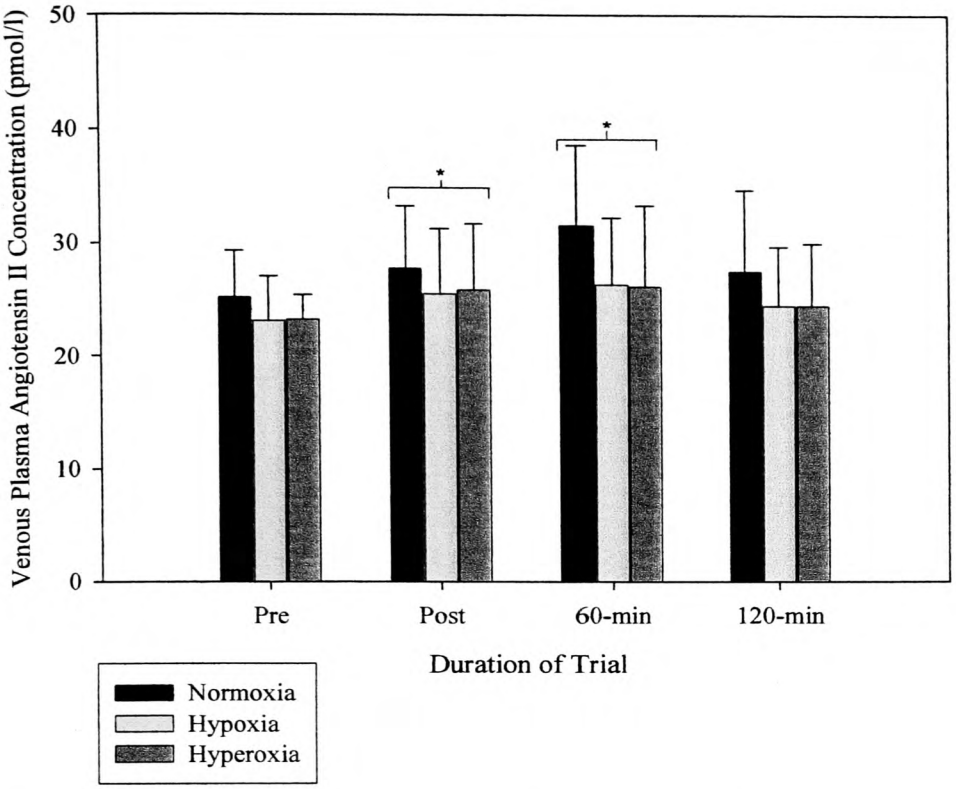


Figure 5.8 Venous plasma angiotensin II response to 30-minutes dynamic cycle exercise under varying degrees of systemic oxygen tension. Values are Mean ± (SD); $n = 9$. Post, within 1-minute post-exercise; 60-min, 1-hour post-exercise; 120-min, 2-hours post-exercise. * $P<0.05$ *v.* pre-exercise, pooled for time.

Similarily, aldosterone displayed exercise-induced increases up until the 1-hour post-exercise time point ($P<0.05$; Pooled for time) and then declined to baseline values by conclusion of the protocol (Figure 5.9).

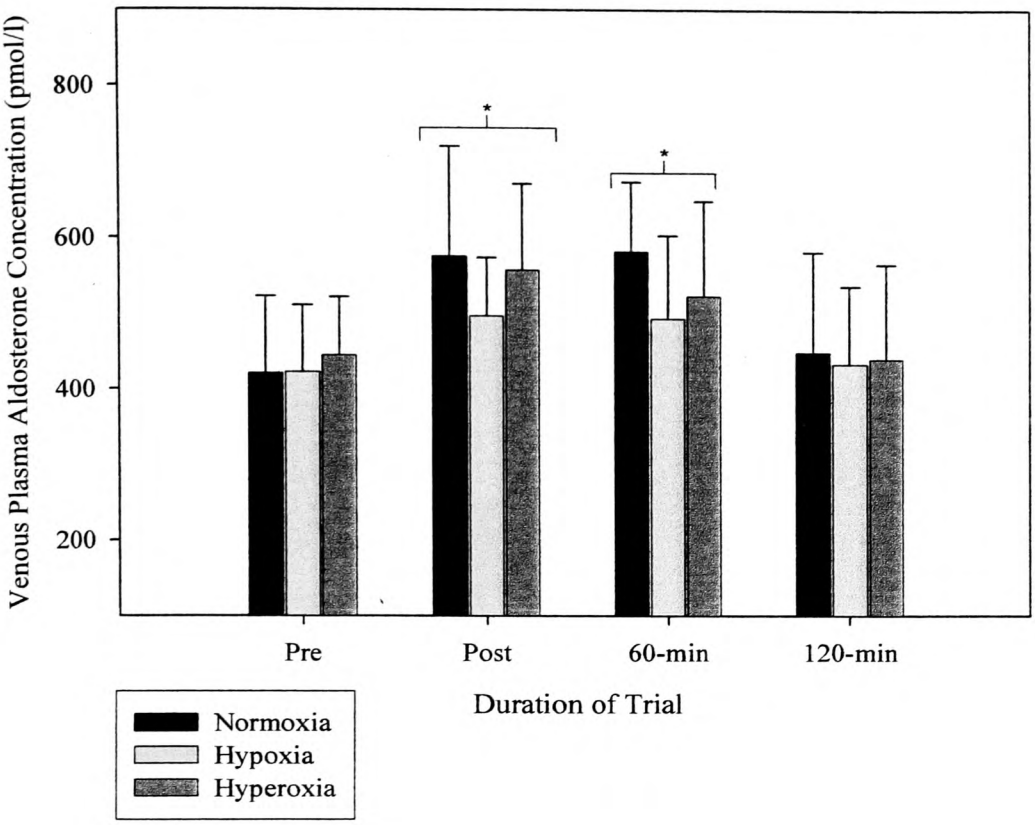


Figure 5.9 Aldosterone response to 30-minutes dynamic cycle exercise under varying degrees of systemic oxygen tension. Values are Mean ± (SD); $n = 9$. Post, within 1-minute post-exercise; 60-min, 1-hour post-exercise; 120-min, 2-hours post-exercise. *$P<0.05$ *v.* pre-exercise, pooled for time.

5.2.8 Arginine Vasopressin & Atrial Natriuretic Peptide Response

Figure 5.10 illustrates the changes in venous plasma AVP concentration throughout the protocol. Analysis revealed that varying systemic oxygen flux during exercise failed to modify the venous concentration of AVP ($P>0.05$ for state and interaction effect). However, there was a significant effect for time, indicating an exercise-induced response throughout the trial. Exercise *per se* increased circulating venous

AVP levels almost twofold ($P<0.05$; Pooled for time). Values then returned to baseline by 60-minutes post-exercise ($P>0.05$; Pooled for time).

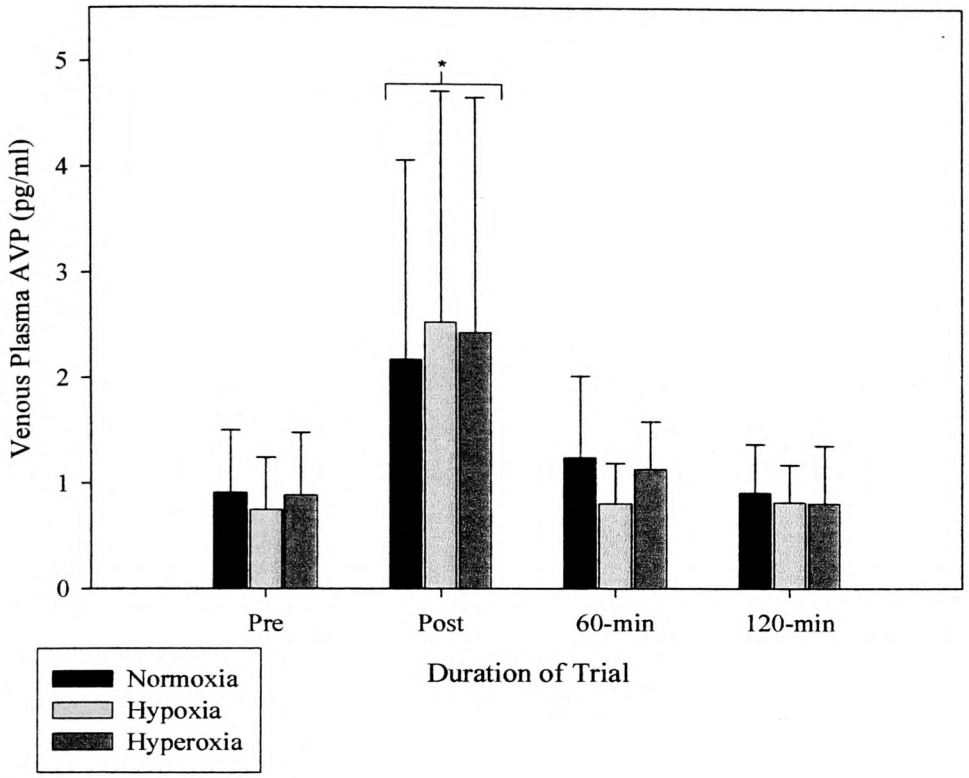


Figure 5.10 Arginine Vasopressin (AVP) response to 30-minutes dynamic cycle exercise under varying degrees of systemic oxygen tension. Values are Mean ± (SD); $n = 9$. Post, within 1-minute post-exercise; 60-min, 1-hour post-exercise; 120-min, 2-hours post-exercise. *$P<0.05$ *v.* pre-exercise, pooled for time.

Manipulation of systemic oxygen flux during exercise elicited a variant response in ANP concentration ($P<0.05$ for state) but failed to induce an interaction effect ($P>0.05$). ANP values were significantly attenuated in the hyperoxic condition when compared to the hypoxc and normoxic trials [24.3 (13.4) *v.* 31.5 (16.3) and 29.6 (13.9)

pg/ml, respectively; Pooled for state]. Analysis of the venous plasma ANP response produced a significant main effect for exercise, with values increasing by ~44% following exercise ($P<0.05$; Pooled for time). ANP concentration returned towards baseline by 60-minutes post-exercise, significantly falling from peak values immediately post exercise ($P<0.05$) to be reduced below pre-exercise values ($P<0.05$) at the 120-minute time-point by ~35% (Figure 5.11).

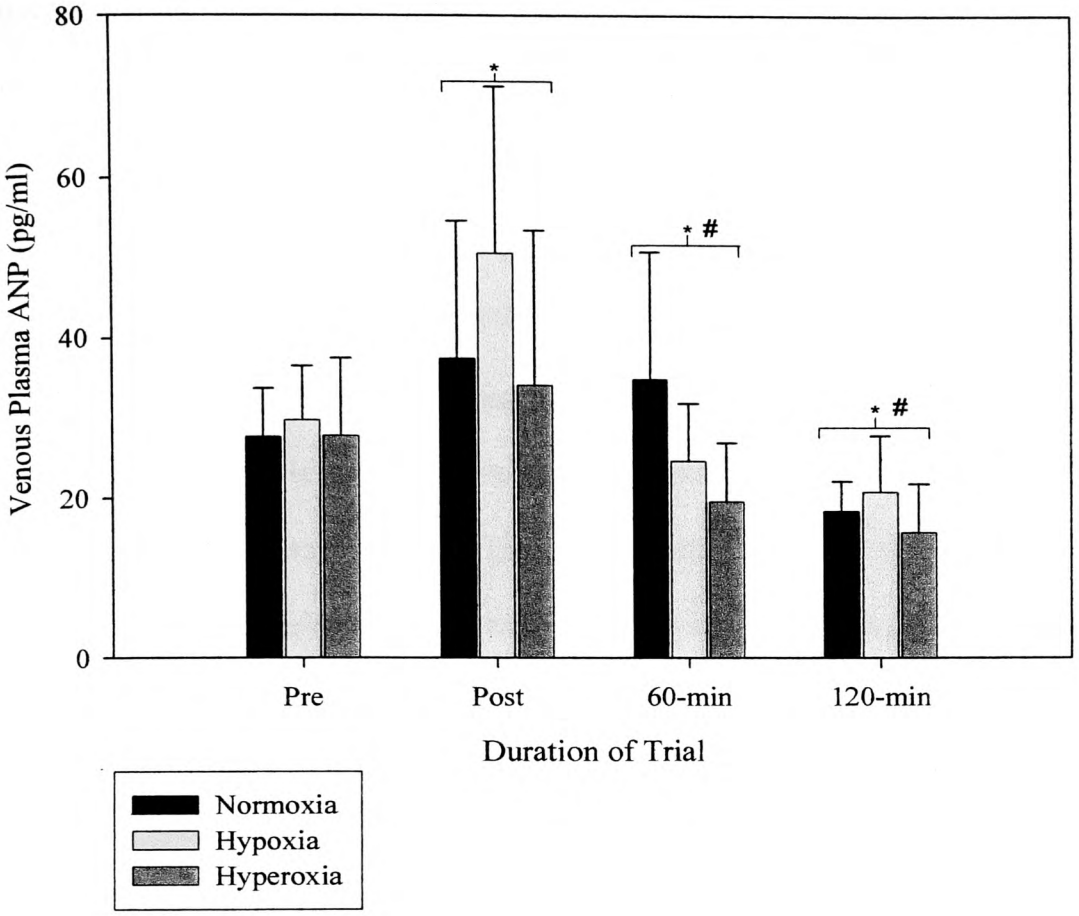


Figure 5.11 Atrial Natriuretic Peptide (ANP) response to 30-minutes dynamic cycle exercise under varying degrees of systemic oxygen tension. Values are Mean ± (SD); $n = 9$. Post, within 1-minute post-exercise; 60-min, 1-hour post-exercise; 120-min, 2-hours post-exercise. $^{*}P<0.05$ *v.* pre-exercise, pooled for time. $^{\#}P<0.05$ *v.* post, pooled for time. $P<0.05$ main effect for state, hyperoxic *v.* normoxic and hypoxic exercise.

5.2.9 Experimental Correlations

Systemic Oxygen Flux, Haemodynamics & Biochemistry

Following hypoxic and normoxic exercise there were no correlations ($P>0.05$) between Δ MAP, Δrenin, Δangiotensin II, Δaldosterone, Δ AVP or Δ ANP (change = pre-exercise minus post-exercise). Both ΔSBP and ΔDBP also failed to demonstrate

any correlation to the above metabolites (both, $P>0.05$). Systemic oxygen flux did produce a differential response in the hyperoxic condition. A positive correlation was observed between ΔMAP and Δ ANP ($r = 0.50$, $P<0.01$). The ΔMAP response, results from a similar association between ΔSBP and Δ ANP ($r = 0.51$, $P<0.01$) whilst ΔDBP was unrelated to any metabolite investigated ($P>0.05$). In the hypoxic trial a positive relationship was recorded between ΔSVR and Δangiotensin II ($r = 0.50$, $P<0.05$).

Systemic Oxygen Flux & the Biochemical Response

Inter-assay correlations revealed several observations. Normoxic exercise produced similar responses to those reported in Chapter 4, section 4.2.9. In similarity to normoxic exercise, a positive correlation was noted in the hypoxic trial between Δ ANP and Δaldosterone ($r = 0.41$, $P<0.05$, normoxia; $r = 0.44$, $P<0.05$, hypoxia) and Δ AVP ($r = 0.67$, $P<0.01$, normoxia; $r = 0.57$, $P<0.01$, hypoxia). There was no such association following hyperoxic exercise (all $P>0.05$).

Unlike the expected responses noted between the individual metabolites of the renin-angiotensin-aldosterone axis following normoxic exercise (Chapter 4, section 4.2.9.), there was no association between Δrenin and Δangiotensin II or Δaldosterone following hypoxic exercise. However a positive correlation was noted between Δaldosterone and Δangiotensin II ($r = 0.77$, $P<0.01$). Following hyperoxic exercise, Δrenin was positively correlated to Δangiotensin II ($r = 0.42$, $P<0.05$) and Δangiotensin II to Δaldosterone ($r = 0.67$, $P<0.01$).

Systemic Arterial Pressure, Arterial Pulse Wave Velocity & Biochemistry

Systemic oxygen flux produced a differential response in absolute APWV. ΔUpper limb and Δlower limb APWV failed to demonstrate any relationship to ΔMAP, ΔSBP or ΔDBP following both hypoxic and normoxic exercise (all, $P>0.05$). Hyperoxic

exercise produced a negative relationship between Δupper limb APWV and ΔMAP and ΔSBP (r = -0.81, P<0.01 and r = -0.77, P<0.01, respectively) whereas Δlower limb APWV was unrelated to changes in systemic arterial pressure.

5.3 Discussion

The present data indicate that when borderline hypertensive subjects perform 30-minutes of fixed relative intensity (70% normoxic $\dot{V}O_{2PEAK}$) cycle ergometer exercise MAP decreased significantly below baseline levels for up to 2-hours post-exercise. This time-frame is similar to previous studies, which have all shown a fall in blood pressure following dynamic exercise using various modalities (Pescatello et al, 1991; Halliwill et al, 1996a; 1996b; 2001; MacDonald, 2002).

The novel finding of this investigation is that systemic oxygen flux during exercise appears to modulate subsequent PEH. The present study investigated the contributory role of systemic oxygen flux by both reducing and increasing $P_{a,O2}$ during hypoxic and hyperoxic exercise, respectively. Hyperoxia elicited a blunting of the PEH response and attenuated the decline in systemic vascular resistance following exercise. Taken together these results would suggest that hyperoxic exercise elicits a deleterious carryover effect that reflexly attenuates PEH in these subjects.

Peripheral Chemoreceptor Drive

The current findings are in disagreement with Izdebska et al, (1998) who lowered systemic blood pressure and total peripheral resistance with a period of hyperoxic breathing in hypertensive subjects. Two differences are of note between the present investigation and the study of Izdebska et al (1998). In the present investigation subjects received a greater exposure (30-minutes compared to 3-minutes) to hyperoxia. Moreover, the gas was delivered while subjects exercised in the present investigation rather than passive exposures at rest and post-exercise in the study of

Izdebska et al. Thus, in essence the present investigation is a unique paradigm focusing on the carryover effects of systemic O_2 flux *during exercise* whilst prior studies have focused on manipulation of *in vivo* O_2 levels *during* the post-exercise period (i.e. the period in which PEH occurs) or in the *resting situation* (Somers et al, 1988; Izdebska et al, 1998; 2006; Saadjian et al, 1999; Mak et al, 2001; Warring et al, 2003; Ciarka et al, 2005; Thomson et al, 2006).

Exercise clearly induces a vast array of perturbations when compared with the resting state. One example could be the exercise pressor reflex (Mitchell et al, 1983), with the potential to attenuate any further reductions in blood pressure due to hyperoxia. Interestingly, the effects of the blood pressure reduction via hyperoxia were less during the post-exercise period compared to pre-exercise in the investigation of Izdebska et al (1998), indicating that the carryover effects of prior exercise blunt the augmentation of PEH during hyperoxic inspiration. Thus, it appears possible that in the present study the exercise pressor reflex could be one mechanism that obviates the potentiation of PEH occurring with hyperoxic exercise.

The present investigation is also at odds with the findings of Ciarka et al (2005) who found a decrease in sympathetic nerve activity (SNA) in essential hypertensives following acute hyperoxia (100% O_2) and that of Somers et al (1988) who highlighted increases in SNA in borderline hypertensives following acute hypoxia (10% O_2) at rest. Again the critical factor between these former studies and the present study is that of the exercise versus resting condition. Furthermore, Izdebska et al (1998) and other studies (Izdebska et al, 2006; Ciarka et al, 2005; Somers et al, 1988) have linked the modulations in systemic arterial pressure and SNA following manipulation of systemic oxygen flux to peripheral chemoreceptor function. Again, this situation is in contrast to the exercising protocol adopted in the present study. To this authors

knowledge there is no experimental data to support the rationale for a carry-forward of chemoreflex activation during exercise to the post-exercise period or that arterial chemoreceptors are engaged during normal cycling exercise. Along these lines, hypoxic exercise failed to demonstrate an augmented sympathoadrenergic drive, as highlighted by the lack of difference in the cardiovascular variables, displayed in table 5.1, which would be indicative of increased chemoreflex activity. Moreover, the fact that $P_{a,O2}$ did not decrease during normoxic exercise (Table 5.1) would argue against activation of arterial chemoreceptors during the experimental condition. An additional confounding factor in relation to arterial chemoreceptor drive is that of controlling inspired CO_2. In the present study CO_2 was not titrated between conditions to maintain isocapnic hypoxia or hyperoxia. Poikilocapnia induces effects on arterial chemoreceptors such that chemoreflex inhibition may have occurred by hypocapnia during hypoxic exercise, whereas at rest cardiovascular activation is similar between isocapnia and poikilocapnia (Thomson et al, 2006).

Thus, the attenuation or augmentation via hypoxia or hyperoxia, respectively, of SNA, MAP or PEH in the resting condition is a vastly different scenario to the experimental rationale adopted in the present study and is resultant from differing mechanistic pathways to those under investigation in this experimental paradigm.

Indeed, Seals and co-workers (1991) some 17 years ago, highlighted the differing effects of hyperoxia (100% O_2) at rest or during exercise on sympathetic function in healthy volunteers. The authors failed to find any modulatory effect of hyperoxia on the nonactive muscle sympathetic nerve adjustments to rhythmic exercise, whereas a lowering of efferent SNA to skeletal muscle was noted under resting conditions. The authors attributed the lack of effect on sympathetic drive during hyperoxic exercise to

several factors, notably a similar chemoreflex drive and mechanoreceptor stimulus under normoxic and hyperoxic trials.

Moreover, a recent study by Houssière et al (2006) revealed that in healthy subjects hyperoxic (100% O_2) isometric forearm exercise *actually* enhanced the sympathetic and blood pressure reactivity to metaboreflex activation. The augmented responses were resultant from an increase in metaboreflex sensitivity by hyperoxia that overrules the sympathoinhibitory and blood pressure lowering effects of chemoreflex inhibition.

Potential mechanisms of the carry-over effect of hyperoxic exercise eliciting an attenuation of PEH

Skeletal Muscle Metabolism

A substantial component of PEH could be resultant from the metabolites accumulated in skeletal muscle during exercise (Halliwill, 2001). Hypoxic and/or Hyperoxic exercise, in turn, affect muscle metabolism (Richardson et al, 1998; Stellingwerff et al, 2005) and hence has the potential capacity to modify PEH. Hypoxia generally vasodilates the muscle vascular bed (Rowell and Blackmon, 1987; Weisbrod et al, 2001; Dinenno et al, 2003) in the face of increased sympathetic activity/noradrenaline spillover whilst hyperoxia has a propensity to vasoconstrict arterial smooth muscle at rest (Duling, 1974; Jackson and Duling, 1983), possibly decreasing limb blood-flow (Reich et al, 1970). Several lines of evidence ascribe the metabolic implications of varying systemic oxygen flux on the vascular smooth muscle (Bachofen et al, 1971; Duling, 1974; Jackson and Duling, 1983; Bredle et al 1988). Vascular smooth muscle cell tone is directly affected by altered conduction through L-type Ca^{2+} channels (Welsh et al, 1998) and ATP-sensitive (Daut et al, 1990) and voltage-dependent K^+ channels (Coppock et al, 2001).

Muscle metabolism is ultimately the end result of O_2 delivery to the active muscle mass and it has been previously reported that O_2 delivery to the exercising limb is not different between systemic hyperoxia versus normoxia (Welch et al, 1977), although this data was speculative at best. At maximal effort in whole body cycle (Knight et al, 1993) and isolated quadriceps exercise (Richardson et al, 1999) there appears to be increased O_2 delivery to the exercising limb with unchanged leg blood flow during hyperoxia: i.e. the increased O_2 delivery, leg/muscle $\dot{V}O_{2MAX}$ and work rate is due solely to an increase in O_2 content of arterial blood.

During whole body upright exercise with moderate actue hypoxia (12% O_2) maximal leg blood flow (LBF) and O_2 delivery is reported to be similar to normoxia (Bender et al, 1988; Knight et al, 1993), yet with a slightly more aggressive hypoxia (11% O_2) reductions in maximal LBF and O_2 delivery have been reported (Koskolou et al, 1997). Utilising the knee extension exercise model, Richardson et al (1999) found reduced muscle blood flow and O_2 delivery at maximal exercise in acute hypoxia (12% O_2). The latest data regarding submaximal exercise eminates from the well-executed and thorough study of Calbet et al, (2003) who reported increases in both systemic and leg vascular conductances and hence LBF in response to severe acute hypoxia (10% O_2). However, the increased conductance was not enough to offset the reduction in arterial oxygen content and consequently O_2 delivery both systemically and to the exercising limb was attenuated with a concomitant marked lactic acidosis (Calbet et al, 2003). It must be noted that the present study used a much lower level of hypoxia (16% O_2) and hyperoxia (50% O_2) thus the haemodynamic effects were expected to be of smaller magnitude.

As can be seen in Table 5.1, systemic oxygen flux was moderated between conditions (decreases and increases in arterial oxygen saturation and PO_2 in hypoxia and

hyperoxia, respectively). While an overall main effect of a reduced $\dot{Q}$, SV and SVI was recorded in the hypoxic trial, no specific interaction effect was noted indicating that the grouped values across time points were likely blunted in hypoxia due to the ~24 and ~29% declines in SV and $\dot{Q}$, respectively, in the resting state before initiation of the hypoxic exercise in comparison to the hyperoxic and normoxic trials. Indeed, similar increases were noted for the above variables immediately following exercise in all 3 conditions with much less variability between trials (~9%, $P>0.05$). Notwithstanding, it is possible and even likely that differences in O_2 delivery to the exercising muscle mass occurred between the hypoxic, normoxic and hyperoxic conditions and therefore modest differences in muscle metaboreflex drive may have occured between conditions in the present study.

Muscle metaboreceptors regulate sympathetic activation during exercise and are activated by metabolites released from exercising skeletal muscle (Mark et al, 1985; Houssière et al 2005). Several substances, such as lactate, phosphate, K^+, H^+, adenosine, prostaglandins, and bradykinin have now been shown to be stimulators of this pressor response (Rotto et al, 1988; Sinoway et al 1994; Scott et al, 2002). These metabolites simulate group III and IV chemosensitive afferents in the working muscles (Ray and Mark, 1995). Activation in both exercising and non-exercising limbs provokes a reflex response increasing $\dot{Q}$ and vasoconstriction of the non-ischaemic vascular beds producing elevations in blood pressure and perfusion pressure during exercise (Rotto et al, 1988; Victor et al, 1988; Sinoway et al 1994; Ray and Mark, 1995).

Failure of enhanced sympathetic drive during hyperoxia and/or hypoxia when compared to the normoxic trial (Table 5.1) indicates that even though modest differences in muscle metaboreflex drive may have occurred between conditions it

was not evidenced with reciprocal changes in cardiovascular variables. There are several possibilities to explain this outcome.

Whereas, Houssière et al (2006) reported an independent effect of hyperoxia in sensitising metaboreceptors, the lack of effect in the current protocol is similar to that of Seals et al (1991) and is potentially related to the mode of exercise. The study of Houssière et al (2006) adopted an isometric forearm exercise protocol whereas Seals et al (1991) and the present study utilised rhythmic, dynamic exercise. The importance of these differing modes of exercise is highlighted by the increased accumulation of muscle metabolites during isometric exercise (Astrand et al, 1960; Sabapathy et al, 2004) and hence the potential for metaboreflex drive. A further study by Houssière et al (2005) demonstrated that with isometric exercise in hypoxia, the response to the combined stimulation was not significantly larger than the response elicited by handgrip alone. Thus, while hypoxia does increase MSNA at rest and is enhanced with isometric exercise the results suggest against an enhanced metaboreflex sympathetic response in the presence of hypoxic exercise.

The clearest indication against a carry-over effect from a modified metaboreceptor drive is in the response of whole blood lactate $[La^-]_B$ across the trial. Whilst $[La^-]_B$ was significantly less for grouped mean values in hyperoxia when compared to normoxia this is likely due in part to the lower values before the hyperoxic challenge such that there was no difference in values at the mid-point of the exercise bout between conditions (state x time, $P>0.05$). Although, increased sensitivity of the metaboreceptor has been noted in the face of decreased lactate production in hyperoxic isometric exercise (Houssière et al, 2006).

Furthermore, other molecular candidates implicated in the metaboreceptor reflex, namely adenosine and prostaglandins, have been shown to be dissociated with the

increased vascular conductance underlying PEH. Blockade of adenosine receptors (Notarius et al, 2006) and cyclooxygenase, a precursor of prostaglandin formation, (Lockwood et al, 2005a) has shown to have a small effect during the early period or no effect at all, respectively, on PEH in healthy subjects.

Therefore, taking all of the above findings into consideration a carryover effect of hyperoxic exercise on PEH appears unlikely to originate from the metaboreflex pathway.

Intensity of Exercise

Directly linked to differences in muscle metabolism is the aspect of variations in the intensity of exercise between trials within the current protocol. In the present study relative exercise intensity was clamped at 70% of normoxic $\dot{V}O_{2PEAK}$ between trials, thus absolute exercise intensity was likely to have varied between trials and therefore it could be argued that the blunted haemodynamics post-hyperoxic-exercise were resultant from the decreased exercise intensity/workload in hyperoxia. It must be noted however that the calculated $\dot{V}O_2$ failed to reach statistical significance between trials (Table 5.1). Using data from 3 prior studies of endurance trained or habitual athletes there would be expected differences in pulmonary $\dot{V}O_2$ (Peltonen et al, 2001) and leg $\dot{V}O_2$ (Knight et al, 1993; Richardson et al, 1999) with hypoxic and hyperoxic exercise. At a similar workload to that utilised in the present study (100W), pulmonary $\dot{V}O_2$ has been recorded as being elevated by 35.2% in hyperoxia (35% O_2) when compared to normoxia and by 40.4% when compared to hypoxia (16% O_2) (Peltonen et al, 2001). At a higher exercise intensity than that experienced in the present study, maximal leg $\dot{V}O_2$ has been reported as being increased by 17% in hyperoxia (100% O_2) when compared to normoxia and by 39.4% when compared to

hypoxia (12% O_2) (Richardson et al, 1999). Knight and collegues (1993) recorded an elevation in leg $\dot{V}O_2$ of 8.1% with the same hyperoxic stimulus when compared to normoxia.

However, the weight of evidence suggests that PEH persists independently of exercise intensity. One study (Piepoli et al, 1994) has reported that PEH may be exercise intensity dependent. The study of Piepoli et al (1994) used normotensive subjects and only found PEH following maximal exercise. A possible explanation is that in sedentary, normotensive individuals maximal exercise could have a greater effect on the haemodynamic response to exercise than in other studies utilising normotensive trained or hypertensive subjects. The latest investigation into exercise intensity and the magnitude of PEH was conducted by Jones and co-workers (2007). The authors found that the acute post-exercise reduction in BP was clinically similar following high intensity short duration exercise and moderate intensity longer duration exercise that was matched for total work done. Taken together, the previous literature indicates that the observed blunting in the peripheral haemodynamic response following hyperoxic exercise cannot solely be attributed to an effect of a difference in absolute exercise intensity encountered. Moreover, as detailed in the results (Section 5.2.1) exercise duration also failed to differ across conditions so could not be associated with the observations noted.

Resetting of the Arterial Baroreflex

It has previously been speculated that both the sensitivity and resetting of the baroreflex may play a role in PEH (Halliwill, 2001; Section 2.4.4). Whereby, following an acute bout of normoxic exercise the baroreceptor is reset to a lower pressure, consequently efferent vasoconstrictor outflow is reduced for a set pressure (Halliwill et al 1996a). Exercise under varying systemic oxygen flux has the potential

to exert an additional perturbation to the baroreflex. Therefore could this additional factor have elicited the carryover effects of hyperoxic exercise on PEH?

It has been documented that an acute exposure (18-minutes) to hypoxia (12% O_2) at rest resets baroreflex control of both heart rate (HR) and sympathetic activity to higher pressures without changes in sensitivity in normotensives, such that HR and MSNA activity increase (Halliwill and Minson, 2002; Halliwill et al, 2003). Acute exposure to hyperoxia (70-100% O_2) at rest on the other hand, has been shown to decrease HR through increased parasympathetic tone and arterial-cardiac baroreflex function (Shibata et al, 2005). Warring et al (2003) recorded a reduction in HR and an increase in MAP, SVR, large artery stiffness and baroreflex sensitivity in response to hyperoxia (85% end tidal O_2 concentration). Thomson et al (2006) controlled for potential poikilocapnic effects of hyperoxia on cardiovascular function. Isocapnic hyperoxia (80% end tidal O_2 concentration) depressed HR, and stroke index and elevated systemic vascular resistance index (SVRI) in healthy men at rest. Of interest the effects of hyperoxia on cardiac index and SVRI persisted for up to 1-hour after return to normoxic inspiration.

In heart failure patients at rest, Haque et al (1996) elucidated that hyperoxia (100% O_2) decreased SV and $\dot{Q}$ whilst increasing SVR and pulmonary capillary wedge pressure independent of changes in sympathetic activity or ventilation. Likewise, Mak et al (2001) demonstrated that 100% O_2 increased systemic vasoconstriction and decreased $\dot{Q}$ in CHF and normal subjects at rest. Interestingly, the authors noted a carryover effect of hyperoxia on central diastolic cardiac performance when subjects reverted to normoxic breathing. Finally, Saadjian et al (1999) noted a reduction in HR and $\dot{Q}$ and an increase in SVR following a 30-minute exposure to 100% O_2 at rest.

Data from animal and human models of PEH also indicates that MSNA is reduced in the post-exercise period following normoxic exercise (Halliwill et al, 1996a; Kulics et al, 1999; Halliwill, 2001).

Although the present study did not investigate baroreflex sensitivity *per se* or record MSNA throughout the study it appears that the increased SVR following hyperoxic exercise could have emanated from changes in baroreflex sensitivity and as previously documented that these changes persist for an extended period post-exposure.

The lack of change in haemodynamic parameters (other than SVR) post-exposure (Table 5.3) is in agreement with previous results (Thomson et al, 2006) whilst the failure of modification of cardiovascular parameters during hyperoxic exposure (Table 5.1) is, as previously discussed, likely resultant from the exercise as opposed to resting protocol. The lack of variance in haemodynamics or PEH following hypoxic exposure could be explained on several fronts; one, the modest degree of hypoxia (16% O_2) utilised in the present study may not have caused a pronounced resetting of the baroreflex and two, unlike hyperoxia there is no experimental evidence to explain a carry-over effect of hypoxic exposure i.e. baroreceptor sensitivity is unchanged in hypoxia (Halliwill and Minson, 2002; Halliwill et al, 2003b).

It would be neglectful not to mention that previous studies have demonstrated that the baroreceptor is not the sole determinant of PEH in hypertensive and normotensive individuals (Bennet et al, 1984; Somers et al, 1985). The current findings are in line with this in that whilst some of the modulation in PEH could have been resultant from modified baroreceptor sensitivity additional mechanisms may have also been operant.

Circulating Biochemistry: Catecholamines

Venous adrenaline and noradrenaline (NA) were unmodified across the protocol. This observation must be taken with caution. As an indicator of sympathetic activity plasma NA concentration has some drawbacks; one, no discernable information can be gleaned concerning regional sympathetic activation and two, NA concentration is determined not only by its rate of neuronal release but also by its reuptake, spillover, and degradation (Esler, 2000). Whilst it is accepted that better alternatives such as determination of NA spillover rate via isotope dilution or MSNA would have provided a much more robust probe of sympathetic tone, in some studies a correlation between resting NA levels and MSNA has been demonstrated (Schobel et al, 1998). The additional concern with systemic oxygen flux is that hypoxia induces altered NA kinetics, i.e. reduced re-uptake, enhanced spillover or increased clearance (Leuenberger et al, 1991; Calbet et al, 2003; Rostrup, 1998; respectively). Hence, post-exercise/exposure increases may have occurred throughout the hypoxic/hyperoxic trials but were unable to be detected.

Alternatively it may have been the case that the modest hypoxia was insufficient to produce an exaggerated increase in circulating catecholamines. In support of this, the majority of data showing hypoxia-induced catecholamine/sympathetic activation have used a hypoxic stimulus less than 12% O_2 (Leuenberger et al, 1991; Mazzeo et al, 1991, 1995; Antezana et al, 1994; Kanstrup et al, 1999; Calbet et al, 2003; Hansen & Sander, 2003).

The lack of increase in NA following hyperoxic exercise is in line with previous studies showing an absence of a potentiated response following hyperoxia (60-100% O_2) in comparison to normoxia (Howley et al, 1983; Seals et al, 1991). In other

studies utilising 70-100% O_2 differences in NA following exercise have indicated tendencies for an attenuated rather than an exaggerated response (Hesse et al, 1981; Byrnes et al, 1984). In one study that determined arterial catecholamine responses to exercise, NA was increased following the dual bolus of hyperoxia (90-99% O_2) and carbon monoxide (19%) (Gonzalez-Alonso et al, 2001). Therefore it is likely that with more aggressive hyperoxia reductions in NA would be seen.

The normoxic trial produced analogous results to those observed in study 1 (see section 4.2.5 & 4.3). As discussed previously the weight of evidence (adrenaline infusion; β–adrenergic receptor blockade) limits there being any meaningful contribution of adrenaline to PEH. Moreover, the repeated finding of unchanged NA during PEH (MacDonald, 2002; Notarius et al, 2006) and the inference of pre-synaptic inhibition of NA release from sympathetic vasoconstrictor nerves following exercise (Haliwill et al, 2003a) lends itself to the current findings. This suggests that catecholamines play little or no role in PEH and are not responsible for the blunting of PEH following hyperoxia as seen in the present study.

Renin-Angiotensin-Aldosterone System (RAAS)

Analagous to study 1, initiation of the acute exercise bout stimulated the RAAS in the present study. Renin, angiotensin II and aldosterone displayed exercise-induced elevations when pooled across trials. The variation in response of renin post-exercise and the return to baseline of angiotensin II by 1-hour and aldosterone by 1-hour post-exercise indicates that the RAAS is completely dissociated from the sustained PEH in the present subjects. Critically, varying the systemic oxygen flux during exercise failed to elicit any variation in the response of the RAAS or its individual components

across the protocol. Ergo, the differences in SVR and PEH highlighted in the present findings cannot be ascribed to a divergent response in the RAAS.

The lack of change in the RAAS or its individual components during acute hypoxaemia is in agreement with the majority of the literature. Data utilising an acute (1-hour) hypobaric exposure (equivalent to 16% O_2) has shown that levels of plasma renin activity (PRA) and aldosterone following dynamic exercise are dependent on absolute workload without any specific effect of hypoxia (Bocqueraz et al, 2004). Kjaer et al (1999) displayed a potentiated response in PRA to a 20-minute bout of exercise in an acute and severe hypoxia (11.5% O_2). At rest, Cargill and co-workers (1996) reported that 30-minutes of hypoxia had no effect on PRA or angiotensin II whereas aldosterone levels were blunted. Likewise in the resting state, Millar and co-workers (1995) observed no change in the levels of PRA or angiotensin II in response to 30-minutes of hypoxia (12% O_2). Swenson et al (1995) utilised an extended (6-hours) acute exposure to hypoxia (12% O_2) at rest and found that neither PRA or aldosterone were modified by the exposure but that the natriuretic response (% change in sodium excretion) was mediated by the peripheral arterial chemoreceptors.

Earlier data indicates that an acute exposure to hypoxia (<2-hours; ~11.9 O_2) failed to have any effect on PRA whilst aldosterone was significantly attenuated following exercise (Rock et al, 1993). Similarly, Lawrence and Shenker (1991); Lawerence et al (1990) displayed a reduced aldosterone response to acute (30-minutes; 1-hour) hypoxaemic exercise (16%; 12% O_2) whilst PRA was unchanged. Moreover, an extended (6-hour) exercise bout during moderate hypoxia (12.5% O_2) failed to induce a potentiated response for PRA or aldosterone above exercise alone (Meehan, 1986). Interestingly, the majority of the early data are suggestive of the apparent dissociation of aldosterone from the RAAS following exercise in acute and/or chronic hypoxia

(Sutton et al, 1977; Milledge and Catley, 1982; Milledge et al, 1983a; 1983b; Colice and Ramirez, 1985; Shigeoka et al, 1985: Bouissou et al, 1987; 1988). Animal data indicates that the reduction in plasma renin and angiotensin II observed with an acute (1-hour) aggressive hypoxia (10% O_2) is adenosine mediated whereas reductions in aldosterone are independent of circulating adenosine concentrations (Hohne et al, 2001).

Data on the RAAS response to exercise or at rest in hyperoxia is extremely sparse. Limited animal findings have indicated that an extended (8-hour) exposure to hyperoxia (100% O_2) at rest sensitises cardiovascular responsiveness to the RAAS in the dog (Sventek & Zambraski, 1985). Again using the conscious dog model, Walker et al (1981) highlighted a reduction in renin secretory rate during hyperbaric oxygenation.

Therefore taken together the above data fit with previous results of a lack of effect of hypoxia on PRA and angiotensin II and provide additional data on the response of the RAAS to hyperoxaemia being suggestive of an unmodified response to hyperoxic exercise. Of note, the early data regarding the effects of acute hypoxaemia on the RAAS elucidating a detachment of aldosterone from the axis were reciprocated in the present findings by the lack of correlation between the response of angiotensin II and aldosterone to renin in the hypoxic trial. The data also indicate, in line with prior findings (Wilcox et al, 1982; Paulev et al 1984), that the RAAS is dissociated from the haemodynamic events underlying PEH.

As discussed in Section 4.3, the role of a decrease in the sensitivity of angiotensin II type 1 (AT1) or an up-regulation of the AT2 receptor cannot be delineated or discounted from the present results. There also appears to be an intimate relationship between systemic hypoxia, AT1 and AT2 upregulation and inflammation (Phillips et

al, 2004; Smilth & Missailidis, 2004). However, as the hypotensive effects persisted for a further hour following normalisation of angiotensin II levels and that angiotensin II was invariant between trials it is likely that a more prominent vasodilator signal was operant during the post-exercise period and differed between conditions.

AVP

AVP responses were unmodified by changes in systemic oxygen flux during exercise and as such changes in the SVR and MAP response following hyperoxic exercise cannot be afforded to changes in circulating AVP in the hyperoxic trial. When grouped together for state, PEH was observed concomitantly with increases in AVP concentration post-exercise but was then discordant with the normalisation of AVP by 60-minutes post-exercise. The findings of an increase in AVP secretion following exercise and a prevailing PEH during augmented AVP concentrations are both in agreement with the literature (Bouissou et al, 1987; Convertino et al, 1981; Freund et al, 1991; Wade, 1984) and (Wilcox et al, 1982), respectively. AVP can induce a sympathoinhibitory (Collins et al, 2001) and vasodilatory (Schwartz et al, 1985) effect via stimulation of central V_1 or systemic V_2 receptors, respectively. The continuation of PEH beyond the normalisation of AVP and the lack of change in AVP concentration across the protocol leads to the conclusion that AVP is not responsible for the modulation in haemodynamics following exercise or between trials in the present investigation.

The failure of acute systemic oxygen flux to modify AVP concentration is in agreement with prior work. Bocqueraz et al, (2004) recorded unchanged AVP levels following dynamic hypoxaemic exercise (equivalent to 16% O_2). Takamata et al, (2000) also failed to elicit any changes in AVP secretion between hypoxia (13% O_2) and normoxia until relative exercise intensity reached values above ~70% $\dot{V}O_{2PEAK}$

concluding that hypoxia itself has no direct effect on AVP release nor does it modify the AVP-plasma osmolality relationship during exercise.

The present study built on the work of previous investigations to include a hyperoxic stimulus. The hyperoxic stimulus also produced an invariant response in AVP secretion. Prior work documenting the response of AVP to hyperoxia is extremely limited with the report of just one animal study. Walker et al (1980) failed to produce any difference in AVP secretion in dogs exposed to hyperbaric oxygenation. Thus, the present study shows that a brief exposure to wide ranging variations in systemic oxygen flux during dynamic exercise fails to modulate the AVP response in mildly hypertensive men.

ANP

In the present experimental paradigm ANP displayed both a temporal effect and responded to systemic oxygen tension during exercise. Similar to study 1, exercise led to increases in ANP concentration in the systemic circulation in line with numerous prior investigations (Somers et al, 1986; Milledge et al, 1989; Schmidt et al, 1990; Lawrence and Schenker, 1991). Grouped for time across trials the ANP response mirrored study 1 with values returned to baseline by 1-hour post-exercise and reduced below baseline levels at the post 2-hour time-point. Again, as found with study 1 this is in agreement with previous findings displaying significant reductions or no change in ANP during an episode of PEH (Hara and Floras, 1992; Macdonald et al, 1999). Furthermore, as previously outlined in the preceeding study, the fall in plasma ANP post-exercise is not likely to originate from a decreased cardiac preload or postural adjustments (subjects remained in the same semi-recumbent position throughout the post-exercise period) but could be associated with the drop in systemic arterial pressure and/or increased clearance of ANP mediated via the increase in systemic and

muscle (Pricher et al, 2004; Lockwood et al, 2005a; 2005b; McCord et al, 2006) vascular conductance post-exercise.

ANP was the only measured metabolite to respond to the variation in systemic oxygen flux during exercise. Whilst failing to display individual interaction effects (time x state), grouped for state the systemic venous concentration of ANP was significantly attenuated in the hyperoxic trial in similarity to the blunting of ΔSVR and ΔMAP in the same trial. A positive correlation was observed between ΔMAP and ΔANP only in the hyperoxic condition. This is an intriguing finding.

The present results showed no difference in the venous concentration of ANP following hypoxic exercise. The literature yields inconsistent results regarding the effect of acute normobaric hypoxia on plasma ANP concentrations. A plethora of *in vivo* and *in vitro* data from both humans and animals has indicated that ANP synthesis and secretion is increased during chronic hypoxaemia. The data shows that ANP plays an important role in the normal adaptation to hypoxia and in the pathogenesis of cardiopulmonary diseases, including chronic hypoxia-induced pulmonary hypertension, vascular remodeling, right ventricular hypertrophy and right heart failure (Chen, 2005). Although it is important to note that chronic hypoxaemia secondary to pathological insult is a distinctly differing condition to that of acute physiological hypoxaemia.

Previous work has highlighted a pronounced increase in ANP release on exposure to hypoxia at rest (Lawrence et al, 1990). Discrepancies with the present study may result from the strength of the hypoxic stimulus, 12% O_2 in the former study and 16% O_2 in the present, the time of the exposure, 1 hour in the former and 30-minutes in the present study and the fact that the former study differed on other levels such as dietary restrictions [low salt diet], premedication with dexamethasone, and resting state

throughout the study. It does appear that there is a graduated response of ANP to the level of hypoxic exposure with increases in release occurring at a threshold point lying between 15% and 10% O_2 (Kawashima et al, 1989). Studies reporting increases in ANP concentrations with hypoxia have used a stimulus between 10-12% O_2. Further studies investigating resting exposure to hypoxia (~10% O_2) have reported no significant effect on ANP levels (Ramirez et al, 1992) or an elevated ANP concentration following acute hypoxaemia (Ramirez et al, 1988). A very acute (30-minute) study with ~11% O_2 highlighted an increase in ANP but not b-type natriuretic peptide [BNP] (Cargill et al, 1996) whilst a 20-minute exposure to hypoxaemia failed to generate any differences in ANP concentrations (Morice et al, 1990). A longer (3-hour) exposure to hypoxaemia (10.5% O_2) at rest has been reported as producing significant elevations in ANP (De Angelis et al, 1996), whereas a more extended (4-day) chronic trial at rest in hypoxia (16.75% O_2) with concomitant hypercapnia lowered ANP levels (Castellani et al, 2005).

The present study combined the double stimulus of exercise and hypoxia and failed to elicit any differential response for hypoxaemia ($P = 0.09$). Utilising the exact same exercise and hypoxic stimulus as the present study, Lawrence and Shenker (1991) produced a 45% increase in ANP release during hypoxic exercise over normoxic exercise. The key finding may be that the subjects employed by the authors were significantly younger (18-24 years) and healthy in comparison to the older pre-hypertensive subjects utilised presently. Thus, it makes direct comparisons between studies difficult but it is apparent that the younger, healthy subjects in the study of Lawrence and Shenker (1991) were capable of tolerating larger workloads during the hypoxic challenge and as such the augmented ANP release during hypoxic exercise could be driven by an absolute workload stimulus. Along these lines, Lawrence and

Shenker (1991) adjusted workload to equate heart rate between trials whereas the present study fixed relative intensity at 70% of normoxic peak between conditions so that absolute workload varied, although no differences in exercising heart rates between trials were delineated in the present study. The blunted release of ANP after maximal hypobaric hypoxic (14% O_2) exercise (Vuolteenaho et al, 1992) supports a workload threshold conclusion. However, Bocqueraz et al, (2004) displayed a slight increase in ANP concentrations following normobaric hypoxaemic (~16% O_2) exercise for 1-hour in comparison to normoxic exercise of the same relative intensity. Other reports of ANP release during hypoxaemic exercise are also inconclusive. Lordick et al (1995) reported no changes in venous plasma ANP at rest following 30-minutes of 11% O_2 but did see elevations following a 5-minute 25W exercise bout. To the contrary, Schmidt et al (1990) failed to induce any modulation in ANP release following 12% O_2 inspiration for 90-minutes at rest but significantly attenuated the ANP response to exercise (both maximal and 60-minutes sub-maximal [60% maximal performance] exercise).

Therefore, taken together the above data suggest that the failure to evoke a change in ANP following the mild hypoxaemic exercise in the present study could be explained by several factors relating to the strength of the hypoxic stimulus, time of exposure and absolute workloads encountered during the present study.

Only one prior study has investigated ANP release in humans under hyperoxic exposure. Skwarski et al. (1998) found a lowering of ANP back to baseline levels when subjects inspired 40% O_2 following a prior hypoxic (12% O_2) exposure at rest. The present study found a differential response in attenuated ANP concentrations in the hyperoxic exercise trial that were related to the changes in MAP across the trial.

Whilst study 1 and prior reports (Hara and Floras, 1992; Macdonald et al, 1999b) indicate that ANP, via its powerful vasodilatory effects, is unlikely to be responsible for the entire, extended haemodynamic changes post-exercise due to its relatively short half-life of 2-3 minutes in the systemic circulation (Freund et al, 1988; Davis, 1989) and the fact that levels decline below baseline during PEH conmitant with blunted hyperaemia; it may also be the case that in circumstances in which ANP release is compromised (such as hyperoxia) part of the beneficial vasodilatory network mediating vascular tone is also compromised. The reciprocal hypothesis of this relates to the well-established detrimental effects of hyperoxia on haemodynamics, in particular increased SVR and vascular stiffness, which has been shown to have an extended carryover on return to normoxia (Haque et al, 1996; Saadjian et al, 1999; Mak et al, 2001; Waring et al, 2003; Thomson et al, 2006). It is plausible that the negative changes in SVR following hyperoxia could be resultant from a decrease in the concentration of circulating ANP.

Differences in grouped values for state incorporate pre-exercise values as well as all time-points post-exercise. The fact that there was no significant interaction effect at this time point and *post hoc* analysis revealed that ANP values were no different in the hyperoxic protocol pre-exercise compared to the hypoxic and normoxic trials (P = 0.51 and P = 0.96, respectively) highlights that subjects entered the hyperoxic condition without a pre-set reduction in ANP concentration. Furthermore, analysis of pre-exercise plasma osmolality and electrolytes revealed that the only difference found was in the slightly elevated Na^+ concentration in the hyperoxic trial compared to the normoxic trial. An elevated sodium level would serve to augment ANP rather than the blunted responses noted. Hence a variation in ANP values before exercise

cannot account for the overall reduction of ANP in the hyperoxic condition and the slight elevation of Na^+ before exercise did not lead to increased ANP concentration. The present results indicate that the attenuated reductions in MAP following hyperoxic exposure, which is also paralleled by a blunting in the reduction of SVR post-exercise, are due, in part, to a decrease in the concentration of circulating ANP. Thus, whilst ANP is not the exclusive, prominent mediating factor of PEH over an extended period, failure to substantially increase circulating levels of ANP during exercise has a deleterious effect on post-exercise haemodynamics.

Hyperoxic Governance of ANP-Mediated Vasodilatation: Potential Mechanisms

It has clearly been established that the NPs represent a compensatory mechanism in vivo to attenuate cardiac overload by reducing vascular resistance and effective blood volume (Kuhn, 2005). In essence the NP system acts as a negative feedback loop to limit cardiac myocyte expression and hypertrophy secondary to cardiac overload and to maintain endothelial function (Booz, 2005). In the present study the main point of interest is the reliance of ANP on the same second messenger system as $NO^{\cdot}$ – cGMP. On this note ANP has been shown to potentially ameliorate endothelial dysfunction by upregulating eNOS and downregulating cytokine-mediated iNOS expression (Calderone, 2003).

$NO^{\cdot}$ can be chemically degraded by oxygen (Rubanyi and Vanhoutte, 1986; Wink et al, 1996). Therefore in hyperoxia an oxygen-induced reduction of $NO^{\cdot}$ could account for the oxygen-induced vasoconstriction in the vasculature. Hyperoxia also appears to negatively influence [downregulate] cGMP, jointly utilised by ANP and $NO^{\cdot}$ (Sopi et al, 2007). Thus, it appears that there is an intimate relationship between ANP and $NO^{\cdot}$ together with a shared reliance on an intracellular messaging pathway. In turn, exposure to hyperoxia modulates the bioavailability of both vasodilators together with

a potential for mediating cGMP sensitivity, although early data indicates that the vascular and haemodynamic effects of ANP may still persist in the face of ANP - cGMP signal transduction ihibition (vonGeldern et al, 1990). The present study only quantified the ANP response, similar changes may have also occurred in vessel dilator, BNP and the endothelial para/autocrine effector CNP which would further explain the negative changes in MAP and SVR following hyperoxic exercise.

Natriuretic Peptide Signaling & Histamine

Of further interest is the fact that PEH was noted in the face of unchanged plasma and whole blood histamine concentrations (Lockwood et al, 2005b; McCord et al, 2006 and McCord and Halliwill, 2006). It could be argued that a direct influence on the cGMP pathway sensitivity via hyperoxia and reduced ANP and/or $NO^{\cdot}$ affects activation of H_1 receptors located on vascular endothelial cells and H_2 receptors, on smooth muscle cells (Hill, 1990; Brown and Roberts, 2001). H_1 receptors produce vasodilatation by the synthesis of local metabolities such as $NO^{\cdot}$ and prostacyclin (Hill, 1990; Brown and Roberts, 2001). H_2 receptors may be effected by hyperoxic modulation of their intracellular calcium dilator mechanism, in similarity to hyperoxic modulation of smooth muscle cell L-type Ca^{2+} channels (Welsh et al, 1998). Interestingly, hyperoxia also appeared to dissasociate responses between ANP and other circulating metabolites when compared to normoxic and hypoxic exercise such that following hyperoxic exercise there was no association between ΔANP and both Δaldosterone and ΔAVP whereas the RAAS axis remained intact.

APWV Responses & Systemic Oxygen Flux

The present results demonstrate that systemic oxygen flux failed to moderate APWV between conditions. To this authors knowledge this is the first study reporting compliance of peripheral arteries, via oscillometry, in response to systemic oxygen

flux. There appears to be little data available on systemic APWV in response to hypoxia and a paucity of data available on measures of arterial stiffness in response to hyperoxia. Thomson et al (2006) reported central aortic APWV-derived augmentation index (AI) values in response to isocapnic hypoxia (80% arterial saturation) and hyperoxia (85% end-tidal O_2 concentration). AI fell markedly during hypoxia but was unresponsive to hyperoxia. In opposition to these results, an earlier study from the same laboratory elucidated a marked increase in AI following poikilocapnic hyperoxia (Waring et al, 2003). The authors ascribe the differences in results to the hypocapnia elicited by hyperventilation during the poikilocapnic study and hence the direct effect of carbon dioxide tension on arteriolar resistance. In a study investigating the effects of severe (12% O_2) and moderate (15% O_2) intermitant hypoxia, Wang et al (2007) reported decreased arterial compliance and endothelium-dependent vasodilatation following severe exposure only. Rossi and co-workers (2007) highlighted a hyperoxia-induced decrease in arterial compliance and distensibility co-efficients in the upper limbs, calculated with pulsed-doppler and ultrasonography of the brachial artery, when septic patients inhaled 100% O_2 for 20-minutes. Earlier work by the same group (Rossi and Boussuges, 2005) found a similar result of decreased brachial artery cross-section compliance co-efficient when healthy subjects were exposed to the same hyperoxic stimulus. Thus, it appears that although the decline in SVR was blunted following hyperoxia in the present study, these were not paralleled by comparable changes in APWV. This finding is similar to the adverse changes in vascular resistance and cardiac index following hyperoxia being uncoupled from AI in the study of Thomson et al (2007). Also, it may be the case that the oscillometry system utilised in the present study was incapable of detecting any subtle changes in

APWV that could have occurred in response to hyperoxia whereas ultrasonography of arterial wall responses may have differed in hypeoxia.

When the APWV responses were grouped for time a significant effect of exercise was noted. Exercise *per se* caused an 18% attenuation in UL APWV immediately post-exercise with recovery and elevation (9%) above baseline by 120-minutes post-exercise, whereas LL APWV failed to respond to exercise. Furthermore, normalising UL APWV to control for differences in prevailing arterial pressure pre- to post-exercise had no effect on the differences indicating that there was an exercise-induced modulation of the inherent mechanisms of arterial pulse wave propagation independent of systemic arterial pressure.

The 18% reduction in UL APWV is substantially larger than previously reported values of 6-10% utilising the exact methodology as that used in the present study but in normotensive subjects (Naka et al 2003). As discussed in chapter 4, Naka and co-workers (2003) also displayed a 23-10% decline in LL APWV, from 10 to 60-minutes post-exercise, with a concomitant decline in SBP but not DBP or MAP. The 18% reduction in UL APWV is also markedly elevated in comparison to the ~6% blunting in UL APWV in response to flow-induced changes during reactive hyperaemia carried out in validation studies with the same pre-hypertensive cohort and APWV system (presented in section 3.9).

The present results, i.e. APWV responses to 3 acute dynamic exercise bouts separated by at least one week, differ from those presented in Chapter 4 (Section 4.3) i.e. APWV responses to 1 acute bout of dynamic exercise. The failure of UL APWV to change in study 1 could be explained by two main factors: one, the power of the first study (54% at $P<0.05$) was insufficient to elicit changes in UL APWV compared to the power of pooled responses for time in study 2 (100% at $P<0.05$), and/or that the

repeated exercise bouts produced an accumulative effect on UL APWV i.e. that a 'training effect' was observed. Both factors are likely to have played a part in the observed differences between studies. Whilst prescriptive exercise was strictly abstained from throughout the current protocol (other than the 3 acute experimental bouts) it is possible that these acute trials had an accumulative effect. As discussed in the review of literature, evidence indicates that aerobic exercise training increases arterial compliance. Cross-sectional studies indicate that aerobically trained athletes have higher arterial compliance than sedentary individuals (Mohiaddin et al, 1989; Kingwell et al, 1995; Vaitkevicius et al, 1993; Bertovic et al, 1999). Distensibility has also been shown to increase following as little as 4-weeks of exercise training (Cameron and Dart, 1994).

A novel finding of the present study is that the blunting of UL APWV was reversed and significantly elevated above baseline by 120-minutes post-exercise. In the study of Naka et al (2003) declines in UL and LL APWV post-exercise remained significantly attenuated by ~10% until 1-hour post-exercise. Thus, it is unknown if the normotensive population employed by Naka et al (2003) would have displayed similar results, although the present finding suggests several intruiging possibilities. It could be that, as suggested by Naka et al (2003), the observed changes in APWV immediately post-exercise are attributable to responses in vascular tone in recovery from net systemic vasoconstrictor and local vasodilator signals elicited via exercise. The current data suggest that contrary to the original suggestion from Naka et al (2003) of a persistant increase in peripheral arterial compliance and vasodilatation that SVR, SVC and APWV significantly recover towards and above baseline, respectively. Thus the local vasodilatory signals immediately post-exercise are short-lived and overcome by systemic vasoconstrictor mechanisms. The responses of angiotensin II,

AVP, nor-adrenaline and adrenaline cannot explain the blunting of arterial compliance, SVC and SVR as both angiotensin II and AVP decreased over the post-exercise period from peak concentrations immediately post-exercise and the catecholamine response was indifferent across the protocol. Therefore, further vasocontrictor mechanisms and pathways must be operating during the post-exercise period. Such possibilities could be the declining concentration of ANP in the systemic circulation over the post-exercise period being markedly depressed from its peak concentration recorded immediately post-exercise and below baseline by 120-minutes post-exercise. The vasodilatory influences of ANP, BNP, CNP or vessel dilator may provide a substantial controlling influence over APWV.

Likewise, further metabolic vasodilator pathways could be implicated such as the individual contribution and combined interaction of endothelial protagonists such as ROS and $NO^{\cdot}$. The pathophysiological effects of increased oxidative stress on the vasculature are well documented (Jablonski et al, 2007; Förstermann and Münzel, 2006). For example, the deleterious effects of oxidative stress on SVR and arterial compliance in the present study could be attributed to the well-established quenching of circulating $NO^{\cdot}$ and production of peroxynitrite that is capable of mediating vascular tone (Faraci 2006). Moreover, it may be that the pre-hypertensive state and age of the subjects recruited in the present study dictated that there was an underlying pathophysiological response in the mechanisms governing APWV (Vaitkevicius et al, 1993; Brinton et al, 1996). This can be noted in the normalised response of APWV, in the present subjects controlling for differences in distending systemic arterial pressure, when compared to a young, normotensive control group (Section 3.9.2).

Whilst there was no modulation of APWV with systemic oxygen flux, hyperoxia did modify the relationship between ΔUL APWV, ΔMAP and ΔSBP eliciting a significant negative correlation.

Therefore, the current APWV findings indicate that the decrease in UL APWV immediately following exercise is short-lived and becomes elevated over baseline by the end of a 120-minute recovery period. The deleterious vasoconstrictor pathway mediating such changes could be related to the withdrawal of circulating ANP or be more specifically targeted at the level of the endothelium through such modulators as $NO^{\cdot}$ and ROS.

Clinical Significance

The opportunities to be exposed to a hypoxic stimulus are much greater than those of exposure to hyperoxia. The two most common areas associated with hyperoxia are in those populations involved in recreational sport diving or occupational sub-aquatic work [deep-sea diving], (e.g. military, police service) and clinical based oxygen therapy, first used over 200 years ago. The limited data available that withstands scientific rigour of being randomised, double-blind, and controlled, indicates that patients with uncomplicated myocardial infarction randomised to receive supplemental oxygen tended to have higher mortality and more ventricular tachycardia than those randomised to normoxia (Rawles and Kenmure, 1976). Prior work has also indicated that oxygen therapy must be treated with caution in patients with congestive heart failure due to the potentially undesirable haemodynamic effects (Haque et al, 1996; Sadijan et al, 1999; Mak et al, 2001).

Supplemental oxygen is also used in neonatal and obstetric units; in particular hyperoxia is used routinely at caesarean section performed under regional anaesthesia. This practice has recently been questioned after increased levels of lipid peroxidation were noted in the foetoplacental unit with little gain in umbilical oxygenation (Khaw et al, 2002). The present results suggest that antagonism of circulating ANP levels may play a role in the adverse effects of hyperoxia on the circulation. Therefore, pharmacological intervention with ANP-mimetics, or known protagonists of ANP release, e.g. the current experimental medications aimed at prophylaxis of congestive heart failure and states of blood volume expansion, could prove beneficial in ameliorating the pathophysiological effects of hyperoxic exposure.

This study chose to investigate the effects of changes in oxygenation on cardiovascular function solely in the male population. This allowed better comparison with previous literature on haemodynamic function and oxygenation and also excluded the possibility of hormonal fluctuations superimposing onto the study results. Investigating a single gender makes it difficult to fully extrapolate the present findings.

Whilst the clinical utility of the very brief exposure to hypoxia during exercise and return to normoxia in recovery is difficult to determine, the present results also indicated that acute, modest, hypoxic exercise fails to have any detrimental effect on post-exercise haemodynamics even in a distinct population with partially-compromised physiological cardiovascular function at rest.

5.4 Conclusion

The present study confirmed that hyperoxia, even in the face of powerful integrated vasodilator and vasocontrictor stimuli occuring during exercise, has significant long-

lasting effects on the human circulation. The present study also suggests that circulating ANP concentration may play a central role in the deleterious effects of hyperoxia on cardiovascular function. Finally, the current results show that the beneficial effects of acute exercise on peripheral UL APWV are short-lived and in fact recover adversely over an extended recovery period, again potentially mediated by circulating ANP concentration.

STUDY 3: FREE RADICAL-MEDIATED OXIDATIVE STRESS & REDOX REGULATION OF NO· BIOAVAILABILITY: IMPLICATION FOR POST EXERCISE HAEMODYNAMICS IN HYPERTENSION

6.0 Introduction

PEH following a single bout of aerobic exercise is caused by an unexplained peripheral vasodilatation modulated by metabolic factors acting at the vascular endothelium eliciting a sustained reduction in SVR (Halliwill, 2001). The putative metabolic vasodilator mechanism underlying the augmented vascular conductance post-exercise remains an intense area of investigation. Recent work has elucidated a persistent histamine-receptor dependent augmentation of vascular conductance (McCord & Halliwill, 2006) as being a primary mechanism.

Oxidative stress is a (patho) physiological state in which the bioavailability of ROS is increased relative to antioxidant defences. To date the oxidative stress response has not been documented during a sustained period of PEH. This is surprising as ROS have clearly been implicated in the regulation of vascular tone in health (Richardson et al, 2007), disease (Gokce et al, 1999; Förstermann & Münzel, 2006; Félétou & Vanhoutte, 2006) and senescence (Eskurza et al, 2004; 2004b, 2005; Moreau et al, 2007; 2005; Jablonski et al, 2007; Donato et al, 2007). Moreover, there is strong experimental evidence for a redox regulation pathway of NO·-bioavailability (Faraci, 2006; Félétou & Vanhoutte, 2006; Pacher et al, 2007), mast cell-mediated histamine release (Son et al, 2006; Suzuki et al, 2005; Di Bello et al, 1998; Masini et al, 1990), α-adrenergic vasoconstriction (Girouard & de Champlain, 2005) and endothelial derived (Camacho et al, 1998; Ungvari et al, 2006) and circulating (Tabet et al, 2008; Montezano et al, 2008) vasoactive factors. Thus, the balance between proxidants and antioxidants following exercise may potentially have a profound effect on recovery

haemodynamics and PEH. To this authors' knowledge no prior study has evaluated the endogenous redox state (i.e. balance of ROS to antioxidant defences), potential redox-mediated regulation of NO·-bioavailability and consequent association with post-exercise haemodynamics.

To this end, using an acute bout of dynamic exercise the present study sought to determine redox-mediated regulation of circulating NO· -bioavailability and the consequent effects on post-exercise haemodynamics and arterial compliance in pre-hypertensive men.

6.1 Methodology

Experimental Protocol

The subjects attended the laboratory and performed a single bout of 30-minute cycle exercise (Monark 824ε) at 70% of pre-determined $\dot{V}O_{2PEAK}$ [See general methodology section 3.2.1]. The subjects were then followed post-exercise for 6-hours.

Anthropometric Measures

On arrival at the laboratory, subject body mass, stature, and BMI was determined as outlined in the methodology section 3.3.

Systemic Arterial Pressure & Heart Rate

BP was measured by the same experienced investigator according to stringent procedures outlined in methodology section 3.5. Heart rate (HR) was determined with a three lead, bi-polar, ECG as detailed in methodology section 3.4. The recordings were taken after the subjects were allowed 20-minutes of seated rest.

Exercise Protocol

Subjects began a warm-up of unloaded cycle exercise at a cadence of 70 rpm for one minute (70 W) at which point the appropriate weight was applied to elicit 70% of pre-determined normoxic $\dot{V}O_{2PEAK}$. SBP, HR and RPP (see section 3.10.3) were measured every 10-minutes during the 30-minute trial. $\dot{V}O_2$ and $[La^-]_B$ were determined midway through the exercise bout (~15-minutes). At the cessation of exercise the subject was transferred to a couch where they remained semi-recumbent, measurements of BP, HR, and RPP were taken, following which venous blood was withdrawn. For the first three hours following exercise subjects remained seated with BP, HR, and RPP recorded every 15-minutes. Venous blood was sampled on the hour point for the first 2-hours following exercise. After 3-hours the subjects were given their standardised meal and were then allowed to walk and relax (e.g. read, watch television) around the laboratory and adjacent lounge. BP, HR, and RPP were recorded every hour for a further 4-hours and blood was drawn at the 6-hour post-exercise time point. PWV was recorded pre, post, 1-hour, 2-hours and 6-hours post-exercise. Central haemodynamic recordings were taken pre, post, 1-hour post and 2-hours post-exercise.

Venous Blood Collection & Analysis

Pre- and post-exercise venous blood samples were collected using the cannula method outlined in methodology section 3.15.1. ΔPV and ΔBV (i.e. percent change in PV and BV) following exercise were calculated as described in methodology section 3.15.6. At every blood collection time-point all blood metabolites were corrected for haemodilution or haemoconcentration.

Nitrate and Nitrite (NO^-_3 and NO^-_2)

Systemic venous blood was assayed for NO^-_3 and NO^-_2 utilising the methodology described in section 3.15.12.1.

S-Nitrosothiols (RSNO)

RSNO's were determined in systemic venous blood via the procedure detailed in section 3.15.12.2.

Markers of Oxidative Stress: Lipid Hydroperoxides (LOOH)

LOOH concentrations were determined in systemic venous serum by the methodology outlined in section 3.15.13.

Antioxidant Status

Ascorbic acid concentrations were determined on stabilised venous plasma following the methodology laid out in section 3.15.14.1. Lipid soluble antioxidants were assayed following the procedure detailed in section 3.15.14.2.

Lipids

Venous serum concentrations of total cholesterol (TC) and triacylglycerol (TAG) were determined via the procedure detailed in section 3.15.15.

Fibrinogen and Clotting Factors

Venous citrated plasma was assayed for coagulation and fibrinolysis as detailed in section 3.15.16.

$[La^-]_B$

Arterialised capillary blood was collected and analysed for whole blood lactate as outlined in methodology section 3.15.18.

Central Haemodynamics

Central haemodynamic data was collected on subjects following the procedures laid out in methodology section 3.10.

APWV

Pre and post-exercise APWV was determined in subjects as detailed in methodology section 3.9.

Statistical Analyses

Statistical analysis was performed using the methodology outlined in section 3.16.3.

6.2 Results

6.2.1 Cardiorespiratory Response To Exercise

The cardiorespiratory responses to sub-maximal exercise were as those depicted in Table 4.2. Expected increases from baseline were noted in oxygen uptake, ventilation, HR, SBP, and RPP at the termination of exercise.

6.2.2 Blood Pressure Response

Mean arterial blood pressure (MAP) was blunted by ~5% when compared to pre-exercise. MAP was reduced by ~5mmHg at the 1-hour post-exercise time-point ($P<0.05$) (Figure 6.1). The PEH persisted for the entire 6-hour post-exercise period, i.e. until cessation of recordings, with a nadir at 60-minutes post-exercise. The reduction in MAP resulted from a post-exercise fall in systolic blood pressure (SBP) whilst diastolic blood pressures remained invariant following exercise (Figure 6.1). MAP returned towards baseline over the course of the post-exercise period; with the recovery being significant between 60-minutes and 360-minutes post-exercise (Figure 6.1). SBP was attenuated by ~10% 1-hour post-exercise and remained ~5% below pre-exercise levels until cessation of recordings 6-hours following exercise ($P<0.05$).

6.2.3 Heart Rate (HR) & Rate Pressure Product (RPP)

HR remained elevated ($P<0.05$) within 1-minute post-exercise (Post) but returned to baseline values by the 1-hour post-exercise recovery time point remaining at baseline

levels for the remainder of the recovery period. Exercise induced an expected rise in RPP which then recovered and was blunted below baseline by 120-minutes post-exercise (Table 6.0).

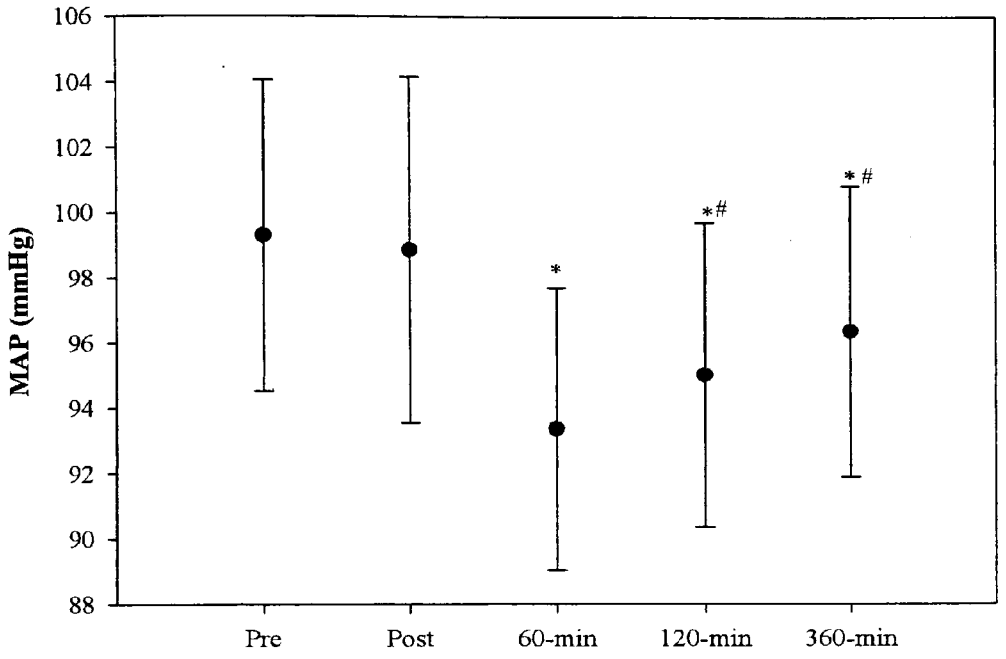


Figure 6.1 Absolute mean arterial pressure response following cycle ergometry. Data are expressed as mean (SD); $n = 9$. Post (within 1-minute post-exercise); 60-min, 1-hour post-exercise; 120-min, 2-hours post-exercise; 360-min, 6-hours post-exercise. *$P<0.05$ *v.* pre-exercise; $^{\#}P<0.05$ *v.* 60-minutes post-exercise.

Table 6.0. Cardiovascular responses to 30-minutes sub-maximal exercise in Pre-hypertensive subjects

Variable	Pre	Post	60-min	120-min	360-min
HR	69 (7)	102 (14)*	70 (8)	65 (5)	69 (10)
SBP	128 (8)	128 (10)	116 (7)*	119 (9)*	122 (8)*
DBP	85 (4)	84 (4)	82 (3)	83 (4)	84 (4)
RPP	8.8 (1.2)	13.1 (2.4)*	8.1 (1.1)	7.8 (1)*	8.6 (1.6)

All data are expressed as mean (SD). n = 9. HR, heart rate (bpm); SBP, systolic blood pressure (mmHg); DBP, diastolic blood pressure (mmHg); RPP, rate pressure product (AU). Post, immediate (i.e. within 1-minute) post-exercise. 60-min, 1-hour post-exercise; 120-min, 2-hours post-exercise; 360-min, 3-hours post-exercise. *$P<0.05$ *v.* pre-exercise within variable.

6.2.4 Postexercise Haemodynamics

Post-exercise *versus* pre-exercise haemodynamics were as those displayed in Table 4.4 and Figures 4.3 and 4.4. SV remained at baseline level from immediately (within 1-minute) post-exercise to the 360-minute time-point. Q was elevated immediately post-exercise but returned to baseline by 1-hour post exercise. SVR recovered from a nadir of ~ 31% reduction immediately post-exercise to ~13 and 8% at 60- and 120-minutes post-exercise, respectively ($P<0.05$). The return in SVR back towards baseline was significant at both the 60- and 120-minutes post-exercise time-points

when compared to immediately post-exercise (both $P<0.05$). SVC was augmented following exercise remaining elevated throughout recording although values were blunted compared to post-exercise by 2-hours post exercise ($P<0.05$; Figure 4.4).

6.2.5 Arterial Pulse Wave Velocity (APWV)

Upper limb (UL) APWV analysis revealed a significant effect of exercise on subsequent UL arterial compliance ($P<0.05$). A 14.5% fall was recorded immediately following exercise whilst over the time-course of the recovery period UL APWV increased by 33% and 24% from immediately post-exercise to 120-minutes and 360-minutes post-exercise, respectively. The increase in UL APWV over the recovery period led to values being elevated by 9% above baseline at the 120-minute stage. Lower limb (LL) APWV responses were unremarkable ($P>0.05$) following exercise (Figure 6.2).

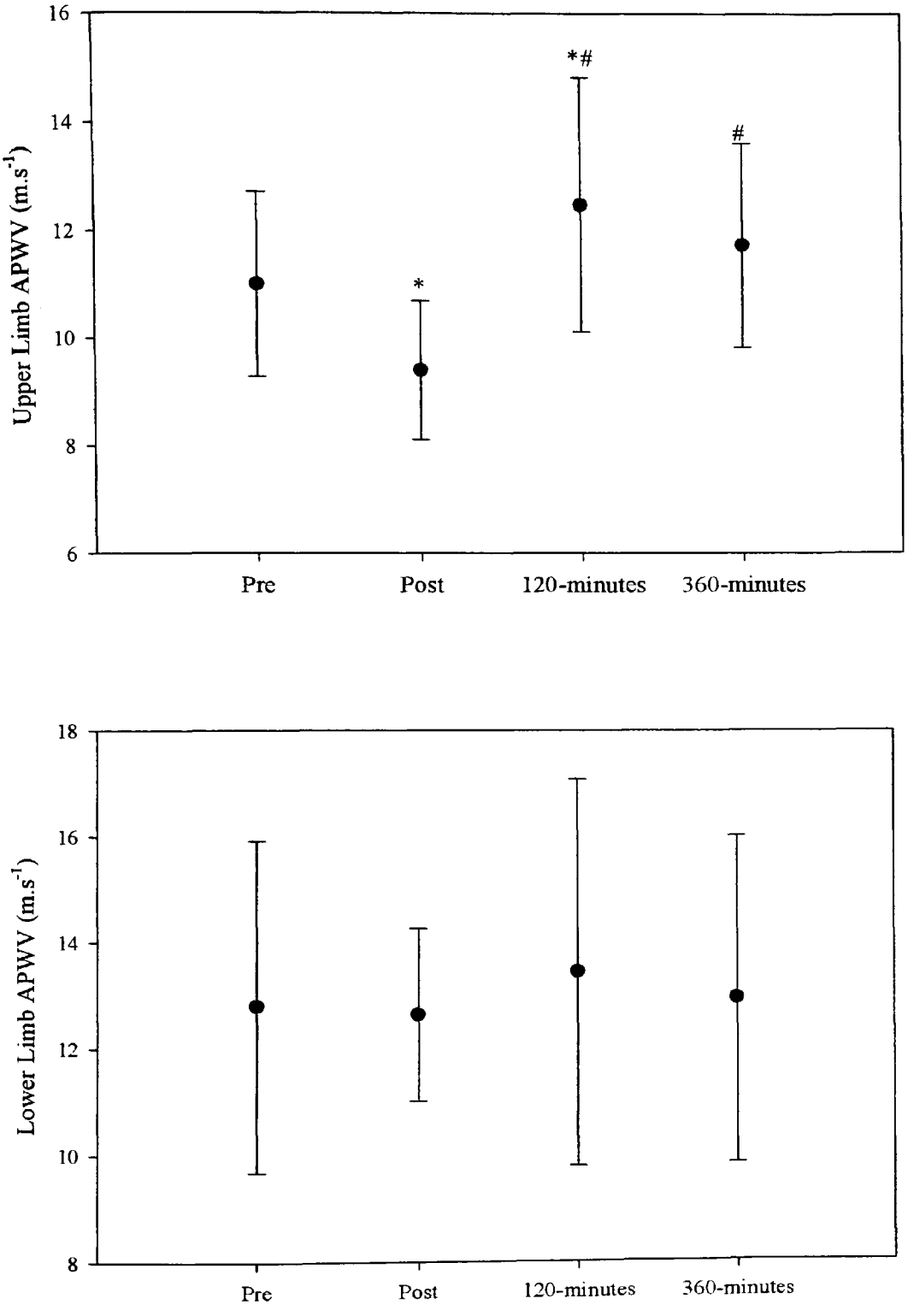


Figure 6.2. *Top:* Upper limb and *Bottom:* Lower limb arterial pulse wave velocity (APWV), averaged over 10-minutes at 1-minute intervals in each case, following 30-minutes dynamic cycle exercise. Values are Mean ± (SD); $n = 9$. Post, within 1-minute post-exercise; 60-min, 1-hour post-exercise; 120-min, 2-hours post-exercise; 360-min, 6-hours post-exercise. $^{*}P<0.05$ *v.* baseline; $^{\#}P<0.05$ *v.* post.

Further analysis of normalised APWV (APWV/MABP) to control for differences in prevailing arterial pressure revealed that the increase in UL APWV from immediately post-exercise to 120-minutes post-exercise was magnified further to an 8-fold increase [0.12 (0.3) *v.* 0.98 (0.3) m/s. $torr^{-1}$, respectively; $P<0.05$]. Similar increases were noted for normalised APWV at the final recording point when compared to the immediate post-exercise fall. Thus, the absolute increase in UL APWV during recovery following exercise is due to modulations of inherent factors determining APWV and cannot be apportioned to changes in systemic arterial pressure over the same time course.

6.2.6 NO^-_3 and NO^-_2

The acute exercise bout failed to modify the venous plasma concentration of NO^-_3 over the first 1-hour post-exercise period but values were significantly attenuated by ~24% and ~31% at the 2- and 6-hour post-exercise time-points, respectively ($P<0.05$). Systemic venous NO^-_2 concentration however was unaltered across the trial ($P>0.05$) (Figure 6.3).

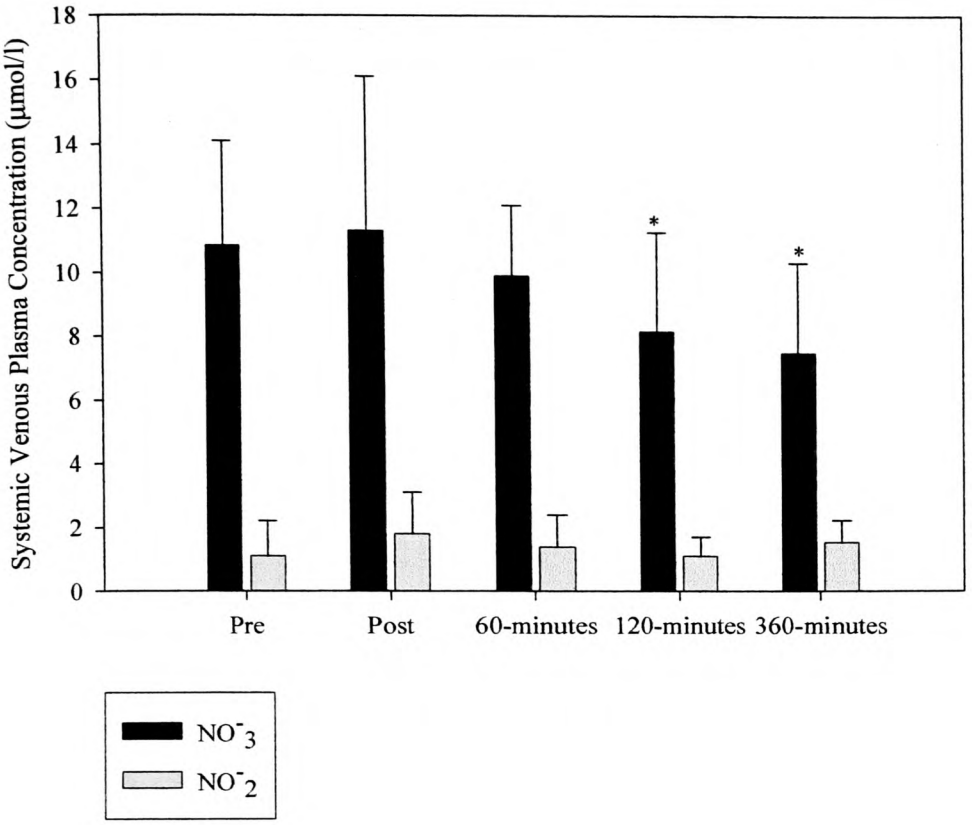


Figure 6.3. Venous plasma NO^-_3 and NO^-_2 response to 30-minutes dynamic cycle exercise. Values are Mean ± (SD); $n = 9$. Post, within 1-minute post-exercise; 60-min, 1-hour post-exercise; 120-min, 2-hours post-exercise; 360-min, 6-hours post-exercise. *$P<0.05$ *v.* pre-exercise.

6.2.6.1 RSNO

Exercise failed to modulate systemic venous RSNO concentration with both absolute concentration and the relative change from baseline remaining unmodified across the protocol ($P>0.05$) (Figure 6.4).

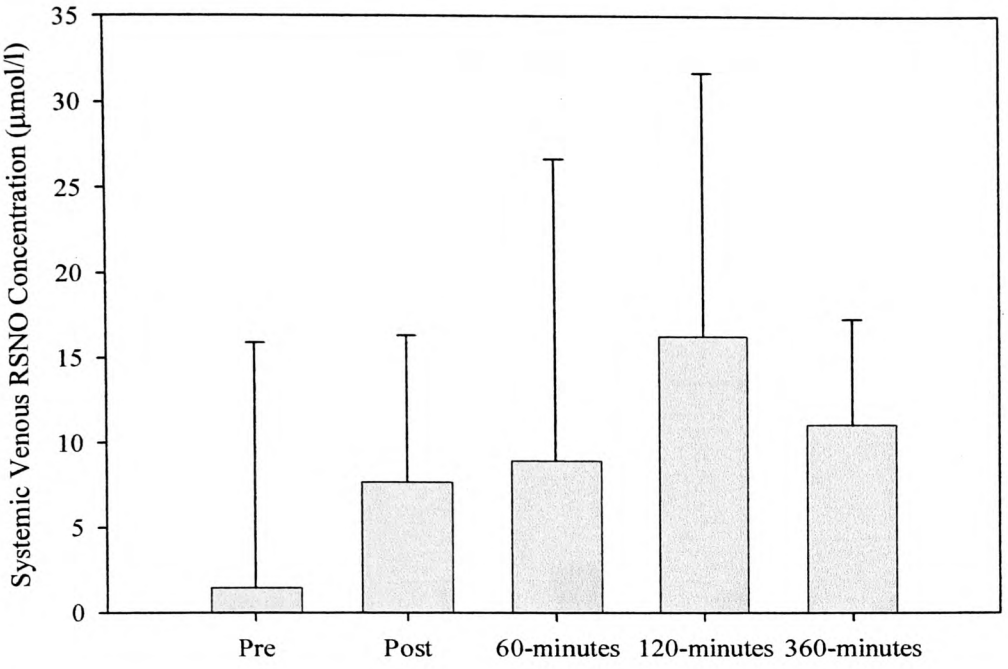


Figure 6.4. Effect of 30-minutes dynamic cycle exercise on systemic venous plasma S-Nitrosothiols (RSNO). Values are Mean ± (SD); $n = 9$. Post, within 1-minute post-exercise; 60-min, 1-hour post-exercise; 120-min, 2-hours post-exercise; 360-min, 6-hours post-exercise.

6.2.6.2 Total Plasma NO·

Total plasma NO· (the sum of NO^-_3, NO^-_2 and RSNO) also failed to respond to the exercise challenge ($P>0.05$).

6.2.7 Lipid Hydroperoxides (LOOH)

Absolute LOOH concentration across the trial is displayed in Figure 6.5 A. Exercise elicited an increase in circulating biomarkers of oxidative stress with elevated LOOH by 60-minutes post-exercise ($P<0.05$) until termination of the protocol.

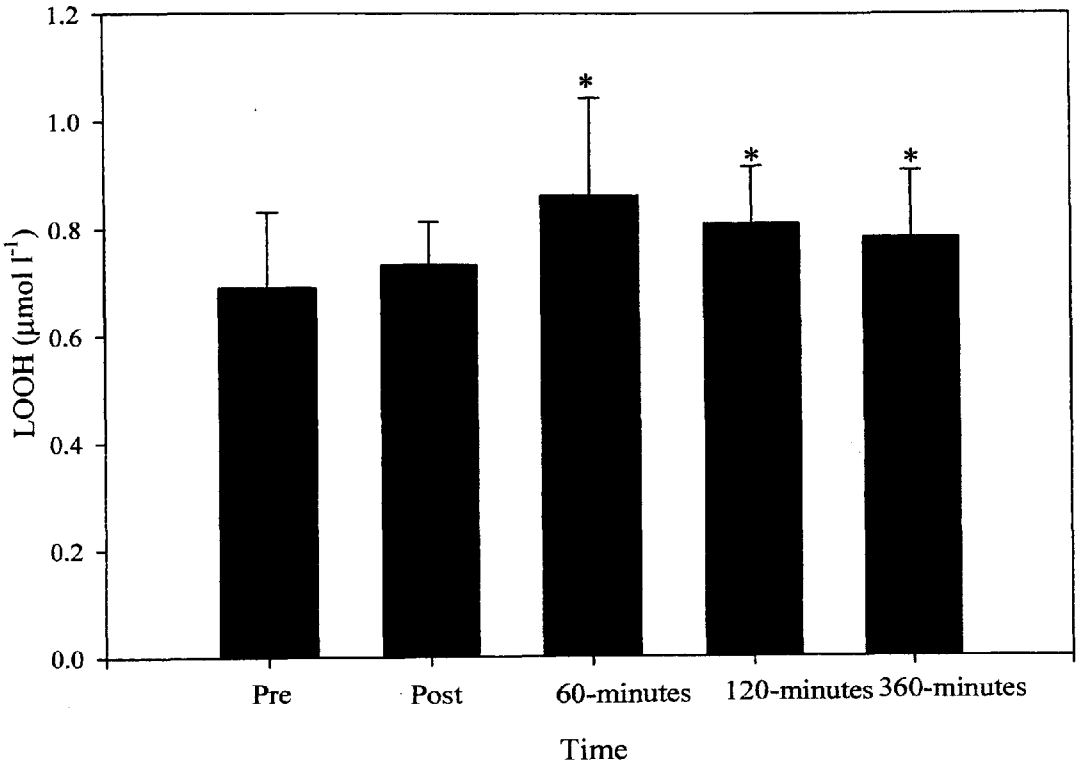


Figure 6.5. Effect of 30-minutes dynamic cycle exercise on systemic venous plasma lipid hydroperoxide (LOOH) concentration. Values are Mean ± (SD); $n = 9$. Post, within 1-minute post-exercise; 60-min, 1-hour post-exercise; 120-min, 2-hours post-exercise; 360-min, 6-hours post-exercise. *$P<0.05$ *v.* pre-exercise.

6.2.8 Venous Lipid-lipoproteins

Table 6.1 displays the lipid-lipoprotein concentration across the trial. With the exception of a 22% increase in TC and a 13% decline in derived LDL-C at the 60 & 120-minute post-exercise sampling point, respectively ($P<0.05$), acute dynamic exercise did not influence the remaining lipids or lipoproteins.

Table 6.1. Blood Lipid-lipoproteins

	Pre	Post	60-min	120-min	360-min
TC, mmol/l	5.1 (0.6)	5.7 (0.8)	6.2 (0.9)*	5.5 (1.1)	5.4 (1.3)
Tg, mmol/l	1.3 (0.6)	1.5 (0.6)	1.6 (1)	1.6 (0.3)	1.5 (0.6)
HDL-C, mmol/l	1.3 (0.2)	1.3 (0.2)	1.3 (0.2)	1.1 (0.2)	1.3 (0.3)
LDL-C, mmol/l	3.2 (0.6)	3.5 (0.8)	3.5 (0.7)	2.8 (0.5)*	3.6 (0.7)

All data are expressed as mean (SD). n = 9. TC, total cholesterol; Tg, triacylglycerol; HDL-C/LDL-C, high/low-density lipoprotein cholesterol. Post, immediate (i.e. within 1-minute) post-exercise. 60-min, 1-hour post-exercise; 120-min, 2-hours post-exercise; 360-min, 3-hours post-exercise. *$P<0.05$ *v.* pre.

6.2.9 Antioxidants

There were no apparent changes in the venous concentrations of ascorbic acid and the majority of the lipid-soluble antioxidants (Table 6.2). The two exceptions were zeaxanthin and β-Cryptoxanthin, which decreased between the 60-minute and 120-minute post-exercise sampling points ($P<0.05$).

Table 6.2. Antioxidants

	Pre	Post	60-min	120-min	360-min
Ascorbic Acid (μmol/l)	42.9 (18.9)	44.8 (27.4)	55.6 (24)	47.3 (29.8)	36.3 (18.1)
α-Tocopherol (μmol/l)	31 (7)	33 (7.6)	36.6 (7.5)	33 (10.1)	31.1 (8.4)
γ-Tocopherol (μmol/l)	3.5 (0.8)	3.5 (0.7)	3.8 (0.7)	3.3 (0.9)	3.5 (1)
α-Carotene (μmol/l)	0.12 (0.8)	0.11 (0.6)	0.12 (0.7)	0.09 (0.7)	0.14 (0.7)
β-Carotene (μmol/l)	0.28 (0.18)	0.26 (0.09)	0.30 (0.13)	0.26 (0.9)	0.34 (0.11)
Retinol (μmol/l)	2.17 (0.37)	2.32 (0.53)	2.42 (0.47)	2.2 (0.62)	2.11 (0.51)
Lycopene (μmol/l)	0.31 (0.21)	0.44 (0.29)	0.29 (0.17)	0.30 (0.11)	0.69 (0.49)
Lutein (μmol/l)	0.2 (0.07)	0.22 (0.08)	0.23 (0.07)	0.19 (0.06)	0.20 (0.07)
Zeaxanthin (μmol/l)	0.039 (0.02)	0.041 (0.02)	0.044 (0.02)	0.036 (0.02)*	0.04 (0.02)
β-Cryptoxanthin (μmol/l)	0.07 (0.04)	0.08 (0.04)	0.09 (0.04)	0.07 (0.04)*	0.08 (0.04)

All data are expressed as mean (SD). n = 9. Post, immediate (i.e. within 1-minute) post-exercise. Post1, 1-hour post-exercise; Post2, 2-hours post-exercise; Post3, 3-hours post-exercise. *$P<0.05$ *v.* preceeding time-point within antioxidant.

6.2.10 Haemostatic Factors

Exercise marginally failed to modify ($P = 0.06$) the venous concentration of fibrinogen as displayed in Table 6.3. International normalized ratio (INR), activated partial thromboplastin time (aPTT), activated partial thromboplastin time ratio (aPTTr), and prothrombin time (PT) were unremarkable across the protocol.

Table 6.3. Haemostasis Profile

	Pre	Post	60-min	120-min
Fibrinogen	267 (39)	296.5 (34.3)	295.4 (26.9)	274.2 (31.5)
INR	0.95 (0.08)	0.97 (0.06)	0.98 (0.03)	0.95 (0.07)
aPTT (s)	31.1 (4.2)	29.4 (3.1)	30.3 (2)	29.2 (1.9)
aPTTr	0.97 (0.11)	0.92 (0.11)	0.96 (0.06)	0.92 (0.07)
PT (s)	12.7 (0.87)	12.7 (0.59)	13.1 (0.38)	12.7 (0.73)

All data are expressed as mean (SD) [Fibrinogen displayed in mmol/l] n = 9. Post, immediate (i.e. within 1-minute) post-exercise. Post1, 1-hour post-exercise; Post2, 2-hours post-exercise; Post3, 3-hours post-exercise. INR, international normalized ratio; aPTT, activated partial thromboplastin time; aPTTr, activated partial thromboplastin time ratio; PT, prothrombin time. s, seconds.

6.2.11 Experimental Correlations

Systemic Arterial Pressure, Haemodynamics & Biochemistry

In the present investigation there was no correlation evident between systemic MAP, NO^-_3, NO^-_2, RSNO, LOOH or any antioxidant (all $P>0.05$) indicating that the systemic venous concentration of these metabolites were not associated to the

prevailing systemic mean arterial pressure. Likewise, correlational analysis between Δ (change = pre-exercise minus post-exercise) MAP, ΔNO^-_3, ΔNO^-_2, ΔRSNO, ΔLOOH, failed to elucidate any significant correlations ($P>0.05$). ΔZeaxanthin and ΔMAP displayed a positive relationship ($r = 0.44$, $P<0.01$), indicating that exercise-induced changes in this metabolite are moderately related to exercise induced modulations in systemic arterial pressure. Table 6.4 displays the correlation coefficients between DBP, as measured at the brachial artery, and circulating venous biochemistry or the exercise-induced change in both variables (Δ).

Table 6.4. Pearson product moment correlation coefficients between diastolic blood pressure (DBP) and circulating venous biochemistry

Variables	Correlation Coefficient
DBP *v.* NO^-_2	*(r = 0.32)**
ΔDBP *v.* ΔLycopene	(*r* = 0.39)*
ΔDBP *v.* ΔZeaxanthin	(*r* = 0.46)**
ΔDBP *v.* Δβ-Cryptoxanthin	(*r* = 0.34)*

*($P<0.05$); **($P<0.01$); (Δ) exercise-induced change in variables.

Although DBP was weakly associated to the systemic venous concentration of NO^-_2 over the course of the protocol, the changes in DBP following exercise were unrelated to the changes in NO^-_2 concentration post-exercise. The table also indicates that the changes in the systemic concentration of the particular antioxidants post-exercise are related to changes in systemic arterial DBP following exercise.

Biochemical Response: NO-Bioactivity ROS & Redox Balance

Table 6.5 displays the correlation coefficients between circulating venous NO bioactivity, lipid peroxidation and antioxidants or the exercise-induced change in variables (Δ).

Table 6.5. Pearson product moment correlation coefficients between circulating venous NO-bioactivity lipid peroxidation and antioxidants

Variables	Correlation Coefficient
NO_3^- *v.* ascorbic acid	(*r* = 0.30)*
NO_2^- *v.* leutin	(*r* = 0.38)*
ΔNO_2^- *v.* Δα-carotene	(*r* = 0.33)*
ΔLOOH *v.* Δα-tocopherol	(*r* = -0.34)*
ΔLOOH *v.* Δβ-cryptoxanthin	(*r* = -0.40)*

*(*P*<0.05); (Δ) exercise-induced change in variables.

Correlational analysis revealed that the venous concentration of nitrate and nitrite are associated with certain antioxidants and that the exercise-induced modulations in nitrite are moderately associated with the post-exercise changes in α-carotene. LOOH, a circulating biomarker of oxidative stress, was related to reciprocal changes in the bioavailability of two of the antioxidants investigated.

Lipid-Lipoprotein Metabolism

Table 6.6 highlights the association between circulating venous triglyceride concentration (Tg) and antioxidants or the exercise-induced change in variables (Δ).

Table 6.6. Pearson product moment correlation coefficients between circulating venous triglyceride concentration and antioxidants

Variables	Correlation Coefficient
Tg *v.* ascorbic acid	(r = 0.71)**
Tg *v.* retinol	(r = 0.64)**
Tg *v.*lycopene	(r = 0.42)**
Tg *v.* leutin	(r = 0.41)**
Tg *v.* zeaxanthin	(r = 0.44)**
Tg *v.* γ–tocopherol	(r = 0.39)**
Tg *v.* α-carotene	(r = 0.38)*
ΔTg *v.* Δleutin	(r = 0.40)*
ΔTg *v.* Δretinol	(r = 0.45)**
ΔTg *v.* Δα–tocopherol	(r = 0.45)**
ΔTg *v.* Δγ–tocopherol	(r = 0.43)**

*(P<0.05), **(P<0.01); (Δ) exercise-induced change in variables

The correlations highlight that the circulating venous concentration of Tg is directly related to the circulating concentration of a number of lipid-soluble antioxidants. The associations between the exercise-induced changes signify that the exercise-induced modulation in Tg metabolism is directly related to the exercise-induced changes in a number of lipid-soluble antioxidants.

Clotting Factors

The relationship between lipid peroxidation, circulating antioxidants and haemostasis is shown in table 6.7.

Table 6.7. Pearson product moment correlation coefficients between venous haemostasis lipid peroxidaton and antioxidants

Variables	Correlation Coefficient
Δfibrinogen *v.* Δleutin	(r = 0.74)*
Δfibrinogen *v.* Δretinol	(r = 0.45)*
Δfibrinogen *v.* Δγ–tocopherol	(r = 0.53)**
Δfibrinogen *v.* Δα–tocopherol	(r = 0.43)*
Δfibrinogen *v.* Δascorbic acid	(r = 0.45)*
Δfibrinogen *v.* ΔLOOH	(r = 0.45)*
INR *v.* α-carotene	(r = 0.35)*
INR *v.* lycopene	(r = 0.49)**
ΔINR *v.* Δ LOOH	(r = 0.59)**
APTT *v.* LOOH	(r = -0.40)*
APTT *v.* α–tocopherol	(r = -0.35)*
APTT *v.* γ–tocopherol	(r = -0.33)*
Δ APTT *v.* ΔLOOH	(r = 0.57)**
ΔAPTTr *v.* Δretinol	(r = 0.52)**
ΔAPTTr *v.* Δγ–tocopherol	(r = 0.51)**
ΔAPTTr *v.* Δα–tocopherol	(r = 0.47)*
ΔPT *v.* ΔLOOH	(r = 0.55)**
ΔPT *v.* ΔRSNO	(r = 0.39)*

*(P<0.05), **(P<0.01); (Δ) exercise-induced change in variables. For description of variables refer to Figure 6.3.

The analysis highlights that the exercise-induced modulation of fibrinogen concentration was directly related to the exercise-induced variations in venous redox balance. The LOOH data indicate that the systemic venous concentration of LOOH is inversely related to APTT and that the exercise-induced modulation of LOOH is moderately related to the post-exercise change in APTT. The exercise-induced change in venous PT was moderately related to the modulation of both venous LOOH and RSNO following acute exercise.

Arterial Pulse Wave Velocity, Systemic Arterial Pressure & Biochemistry

The relationship between arterial pulse wave velocity (APWV) and circulating biochemistry is shown in table 6.8.

Table 6.8. Pearson product moment correlation coefficients between arterial pulse wave velocity and circulating venous biochemistry

Variables	Correlation Coefficient
UL APWV *v.* TC	(r = 0.34)*
UL APWV *v.* Tg	(r = 0.58)**
UL APWV *v.* ascorbic acid	(r = 0.39)*
UL APWV *v.* α-tocopherol	(r = 0.69)**
UL APWV *v.* γ–tocopherol	(r = 0.53)**
UL APWV *v.* retinol	(r = 0.62)**
UL APWV *v.* NO^-_2	(r = -0.48)**
LL APWV *v.* LOOH	(r = 0.43)**
Δ UL APWV *v.* Δascorbic acid	(r = -0.53)**
Δ UL APWV *v.* Δβ-cryptoxanthin	(r = -0.40)*

*(P<0.05), **(P<0.01); (Δ) exercise-induced change in variables

The table highlights that UL APWV is associated with redox state and NO· bio-availability, whilst LL APWV is related to systemic oxidative stress.

6.3 Discussion

The present study aimed to investigate redox-mediated regulation of post-exercise haemodynamics, NO· bioavailability and arterial compliance in pre-hypertensive men. As previously established in studies 1 and 2, and repeated in the present study, the subjects elicited a favourable haemodynamic response to an acute bout of exercise [MAP and SVR decreases/ SVC increases significantly below/above baseline levels for up to 6-hours and 2-hours, respectively, post-exercise]. This study demonstrates for the first time that the attenuated SVR and augmented SVC following exercise occur from a pathway independent to increased ROS and the return of values towards baseline in the post-exercise period appears to coincide with increased oxidative stress. Moreover, the present study is the first to elucidate a potential redox mediated regulation of circulating NO·-bioavailability during a sustained period of PEH. This suggests that elevated oxidative stress reduces the bioavailability of NO· but a persistant hypotension remains resultant from a metabolic pathway autonomous of NO·.

Redox Regulation of PEH: Role of ROS in Post-Exercise Vascular Function

Vascular tone is critically determined by the balance between the bioavailability of pro-oxidants and antioxidants. Such that, at the level of the vascular endothelium, oxidative stress plays a regulatory role in normal vascular function (Eskurza et al, 2004; 2006; Förstermann & Münzel, 2006; Jablonski et al, 2007). The overall effect on haemodynamics and arterial pressure will be determined by how these various pathways and mechanisms integrate at the level of the vascular smooth muscle and

endothelium; thus factors that modulate smooth muscle tone or vascular responses may modify the post-exercise response (Lynn et al, 2007). There is both salutary and contrary evidence on the role of ROS on vascular function.

Beneficial Role of ROS in the Vascular Bed

There is an abundance (Section 2.4.5.3) of data suggesting that ROS, particularly H_2O_2, play an essential role in eliciting vasodilatation in several vascular beds (Zhang & Gutterman, 2007) from humans (Matoba et al, 2002; Liu et al, 2003; Miura et al, 2003; Sato et al, 2003) and animals (Wei et al, 1996; 1998; Matoba et al, 2000; Yan et al, 2005). The present findings suggest that in the distinct cohort investigated in this study that PEH and attenuated SVR/augmented SVC occur through a pathway unrelated to augmented ROS production. LOOH concentrations failed to increase in the epoch immediately following exercise when the nadir in MAP and SVR and peak augmentation of SVC occured. In fact, the current results indicate that increased lipid peroxidation (i.e. LOOH) above baseline levels occurring at the 60-minute post-exercise time-point coincided with a return of SVR, SVC and MAP back towards baseline values. This is suggestive of ROS imparting a negative rather than a positive effect on post-exercise haemodynamics.

A critical factor may be that the exact response of the vasculature to redox activity is dependent on a number of variables such as the age of the vasculature, pre-existing pathophysiological state of the experimental cohort, the vascular bed sampled, the redox species and integrity of endogenous antioxidant pathways. To this end it is interesting to note that Richardson et al, (2007) utilised a cohort of young healthy subjects performing a sub-maximal forearm handgrip exercise investigating the brachial artery. Liu et al (2003) [coronary resistance arteries], Miura et al (2003) [coronary arterioles] and Sato et al (2003) [coronary arterioles] all studied 60+-year-

old subjects with extant heart disease and Matoba et al (2002) investigated human mesenteric arteries.

The varying effects of ROS on the vasculature also relates to the multitude of pathways and effectors that exist to modulate vascular smooth muscle tone. The beneficial effects of H_2O_2 on the vessels studied has been purported to relate to the specific effects of this radical species on the endothelium-independent pathway of potassium channel opening in vascular smooth muscle cells, in-turn eliciting a hyperpolarisation (Gutterman et al, 2005). Gutterman and co-workers (2005) argue that this mechanism is of crucial importance at the level of the microvasculature where hyperpolarisation pathways predominate and often compensate for loss of $NO^{\cdot}$-dependent dilatation. It is also highlighted that these hyperpolarisation pathways, originating from sarcolemmal K^{+}-channels, are open to the same oxidative milieau of the general vasculature lumen. Thus, certain dilator mechanisms may be in tact while others may be impaired depending on the nature of the redox species present and the integrity of endogenous antioxidant mechanisms (Gutterman et al, 2005).

Richardson and co-workers (2007) suggest that there is an exercise-induced reliance upon pro-oxidant-stimulated vasodilatation revealing an important and beneficial role of free radicals at the vascular endothelium.

Deleterious Role of ROS in the Vascular Bed

With regard to the modulating influence of ROS on vascular function a vast body of literature pertains to the equally plausible but contrasting proposal that increased production of oxidative stress and/or impaired quenching of radical species unbalances vasculature redox state, which attenuates vasodilatation and blood flow by reducing $NO^{\cdot}$-bioavailability (Eskurza et al, 2004a; 2004b, 2005; Moreau et al, 2005; 2007; Faraci, 2006; Förstermann & Münzel, 2006; Jablonski et al, 2007; Donato et al,

2007). Thus, in this situation redox imbalance abolishes an essential endothelium-dependent pathway.

A key factor that must also be highlighted is the age and gender of the current experimental cohort. Both the study of Jablonski et al (2007) and Moreau et al (2007) indicate that the tonic vasoconstrictor effects of oxidative stress appear to be greatest in middle-aged and older men than their female counterparts. Thus, the current study employed such a population with vasculature at the greatest susceptibility to ROS-mediated tonic reactance. Furthermore, the current experimental cohort had pre-existing compromised cardiovascular function with demonstration of pre-hypertension. There is sound experimental support for compromised antioxidant defences in the progression and maintenance of hypertension (Duffy et al, 1999a; Tian et al, 2005; Davis Manning Jr et al, 2005). On this note, the current experimental cohort demonstrated lower baseline levels of ascorbic acid when compared to similarly aged but healthier cohorts published in the literature (Eskurza et al, 2004a; Eskurza et al, 2004b; Monahan et al 2004; Jablonski et al, 2007) but comparable levels to younger but type I diabetic individuals assessed in the authors' laboratory (Davison et al, 2008b). Thus, the lower baseline levels of ascorbic acid in the present study could be indicative of compromised defences towards hypertensive disease progression.

What is clear from the present study is that endogenous ascorbic acid and antioxidant levels were insufficient to fully quench ROS and lipid peroxidation occurred. Moreover, the increase in LOOH appeared to coincide with a significant attenuation of exercise-induced hyperaemia and amelioration of blunted SVR and MAP. Thus, the results indicate that in this experimental cohort increased ROS generation imparts a deleterious effect on systemic haemodynamics. This is in line with a host of recent

studies indicating the beneficial effects of ROS antagonism on vascular haemodynamics and baroreflex sensitivity in senescence (Eskurza et al, 2004; 2004b; Monahan et al 2004; Moreau et al, 2005; 2007; Jablonski et al, 2007).

Previous findings have elucidated a significant inverse correlation between baseline oxidised LDL (a marker of systemic oxidative stress) and vascular flow/conductance. In the present study both the absolute and Δ (pre to post exercise changes) LOOH values failed to show any significant relationship to either absolute baseline SVC/SVR values or ΔSVC/SVR across the trial (both $P>0.05$). This may be a result of the experimental paradigm of a single homogenous small cohort whereas prior studies (Donato et al, 2007; Jablonski et al, 2007; Moreau et al, 2007) utilised a larger but, critically, heterogenous experimental sample (old *v.* young) where large differences in limb blood flow and oxidative stress were noted between groups. Or lack of an association may indicate that oxidised LDL is a more sensitive marker in relation to vascular dysfunction than that of LOOH or that "tighter" associations exist between peripheral vascular flow (i.e. brachial/femoral conductance) and ROS than central large elastic arterial (aortic) flow as utilised presently. Evidence towards the latter point can be found in the study of Eskurza et al (2004b) who failed to find any influence of intravenous ascorbic acid infusion on carotid artery stiffness or aortic pulse wave velocity.

Exercise & ROS generation

A full evaluation of exercise and ROS generation is beyond the scope of this discussion and thesis but there is clear experimental evidence for the increase in circulating ROS following whole body aerobic exercise (Bailey et al, 2001; Davison

et al, 2002; 2006; 2008a; 2008b; Vasilaki et al, 2006) and release of free-radicals from isolated exercising muscle beds (Bailey et al, 2003a; 2003b; 2004; 2007; McArdle et al, 2005; Close et al, 2006). In the current study there appeared to be a delay in the elevation of LOOH (circulating biomarker of oxidative stress) following whole body dynamic aerobic exercise such that there was an increase in LOOH only from the 60-minute post-exercise time-point until termination of the protocol (Figure 6.5). It is reasonable to suggest that the lack of elevation immediately post-exercise is related to exercise intensity. Indeed, Bailey et al (2004) failed to display any change in the absolute venous concentration of LOOH from exercising human quadricep muscle between 25 and 100% work rate max (WRmax) but that the outflow (arterial-venous exchange) was significantly increased at 70% WRmax compared to 25% with no differences at higher exercise intensities. In an earlier study, Bailey and co-workers, (2003a) displayed an association between leg $\dot{V}O_2$ and free-radical outflow (net PBN spin-adduct [*ex-vivo* "spin trap"] outflow) such that higher convective O_2 flux to the muscle increased free-radical outflow into the femoral venous circulation. Unfortunately the authors did not report LOOH data. It is important to note that in the majority of previous studies from our laboratory the PBN spin adduct concentration positively responds to exercise stimuli across exercise intensities from 25 to 100% WRmax (Bailey et al, 2003a; 2003b; 2004; 2007) whereas absolute venous LOOH concentrations can appear unchanged (Bailey et al, 2004).

Bailey et al, (2001) and Davison et al, (2002) found a significant main effect of whole body dynamic exercise for increasing venous concentrations of LOOH following maximal aerobic exercise, whilst Davison and colleagues (2006) reported elevated circulating LOOH following 2-hours of submaximal (55% $\dot{V}O_{2PEAK}$) cycle exercise. Thus, the short sub-maximal exercise intensity (30-minutes at 75 (15)% of $\dot{V}O_{2PEAK}$,

[86 (11) % of maximal heart rate]) and paradigm (time-line [post to 60-minutes post-exercise]; sample site [peripheral venous circulation distal to locus of generation]) utilised in the present study may have precluded the observation of increased circulating LOOH at the immediate post-exercise time-point. Possible increases may have been observed following maximal exercise or longer sub-maximal exercise; with sampling from leg vasculature as these were the principle muscle beds engaged in energetic work and with a serial sampling point closer to the termination of exercise than the 60-minutes recorded presently. However, even though no elevation was recorded immediately post-exercise, elevations were seen from 60-minutes post-exercise onwards.

Source of increased circulating biomarkers of oxidative Stress

LOOH is a biomarker of oxidative stress resultant from lipid peroxidation, a self-perpetuating chain reaction, with oxidation byproducts and free radical intermediaries, initiated by primary oxygen-centred radical attack of cellular membranes and subsequent polyunsaturated fatty acid (PUFA) decomposition (Duthie, 1993; Bailey et al, 2004; Davison et al, 2008a). Thus, there are many sites *in vivo* (cells; organs) that possess such a lipid bi-layer with double bond structures susceptible to ROS assault. The two most prominent potential sources of ROS generation following whole body aerobic exercise are the skeletal myocyte mitochondria and the systemic circulation (Bailey et al, 2004; Davison et al, 2008a).

Skeletal Muscle Mitochondria

The primary ROS generated by skeletal muscle during contractions are $O_2^{\cdot-}$ and $NO^{\cdot}$ with rapid dismutation of $O_2^{\cdot-}$ to H_2O_2 (McArdle and Jackson, 2000). Mitochondria have frequently been cited as the source of generation during contractile activity (Davies et al, 1982; Bailey et al, 2003b; 2004) but other data also indicate that skeletal

muscle has additional sources of generation including NAD(P)H oxidases and other muscle plasma membrane oxidoreductases (Vasilaki et al, 2006). There are two primary muscle mitochondrial mechanisms, eluded to previously, that are potentially responsible for exercise-induced ROS production. These are $O_2^{\cdot-}$ from mitochondrial electron transport chain leakage, subsequent to increased muscle blood flow and O_2 consumption within muscle fibres. Along this line, in the present study clear increases in $\dot{Q}$ and SVC were noted following exercise. The second purported muscle mitochondrial mechanism is that of decreased muscle intracellular PO_2 (Bailey et al, 2004). Although different modalities of exercise were utilised between the present study and that of Bailey et al (2004) (whole body two leg cycling *v.* single-leg knee extensor, respectively) it would be anticipated that reductions in mitochondrial intracellular PO_2 would also have occurred in the present paradigm potentially eliciting ROS generation via this mechanism. Thus, it is implied that the observed increase in circulating LOOH concentrations following exercise could be the result of primary radical species attacking intra- or extracellular PUFA (Davison et al, 2008a).

Systemic Circulation & Vascular Endothelium

An equally acceptable contention is that the peripheral systemic circulation and vascular endothelium was the locus of ROS generation. Established evidence exists that the peripheral circulation plays an important role in ROS generation through such mechanisms as ischaemia/reperfusion (Bailey et al, 2006), catecholamine auto-oxidation reactions (Bors et al, 1978), respiratory burst involving phagocytic cells and continuous production of $O_2^{\cdot-}$ by the vascular endothelium to neutralise $NO^{\cdot}$ (Young and Woodside, 2001; Davison et al, 2008a).

Auto-oxidation of adrenaline to adrenachrome can yield adrenaline semi-quinone and increase $O_2^{\cdot-}$ (Bors et al, 1978). However, it is unlikely that this was the mechanism

responsible for increased LOOH in the present study as adrenaline failed to be significantly elevated over baseline in the post-exercise period. Humoral influences can also have a profound effect on ROS stimulation with a key circulating trigger being angiotensin II (Taniyama and Griendling, 2003). *In vitro* evidence points to the stimulation of NAD(P)H oxidase by angiotensin II to the release of increased $O_2^{\cdot-}$ (Landmesser et al, 2002; Haque and Majid, 2004). Thus, in the present study exercise stimulated an increase in angiotensin II until 60-minutes post-exercise so this could have been a mechanism for the increases in LOOH noted in the present study. Erythrocyte membranes are rich sources of PUFA and highly susceptible to oxidative damage (Clemens and Waller, 1987) making them a principal candidate for the increase in venous LOOH in the present study. Furthermore, blood-borne free iron is a catalyst for the production of the extremely pernicious $OH^{\cdot}$, by the transition metal catalysed (e.g. copper and iron Fenton chemistry) decomposition of $O_2^{\cdot-}$ and H_2O_2 (Young, 1994; Bailey et al, 2004; 2006).

Additionally, lipid radicals are known to travel within the blood rendering them liable to propagate and attack PUFA other than at their site of formation (Davison et al, 2008a) thus giving them a "double-edged" potency causing mass destruction systemically and concomitantly eliciting augmented propagation and detection.

ROS & Histamine Release: Potential Redox-Mediated Histamine Release & Modulation of Vascular Tone

The current findings of increased oxidative stress in the post-exercise period provide some additional insight to the recent data highlighting a histamine receptor-dependent mechanism mediating PEH (McCord & Halliwill, 2006; McCord et al, 2006; Lockwood et al, 2005b). It is known that ROS are involved in mast cell activation and

facilitate histamine release (Son et al, 2006; Suzuki et al, 2005; Drábiková et al, 2002; Di Bello et al, 1998; Masini et al, 1990). The finding that blockade of histamine receptors ameliorates PEH may be indicative of 'downstream' inflammatory events and that ROS are the principle governor of events. However, the lack of increase in LOOH immediately post-exercise when the greatest attenuation/augmentation of SVR/SVC, respectively, occurred argues against a ROS-induced histamine release and that increased ROS in the recovery period mediates deleterious effects on systemic haemodynamics that outweigh any potential benefits of histamine release. Thus, the current findings would tentatively suggest that rather than being obligatory, 'metabolic triggers' for histamine release and post-exercise hypereamia, ROS appear concomitantly with blunted hyperaemic responses.

This appears counter-intuitive to the parallel hypothesis that H_1 and H_2-receptor antagonists serve as antioxidants (Kesiova et al, 2006). Hence, the finding that blockade of H_1 and H_2 receptors ameliorates PEH and augments vascular conductance could also be indicative of their antioxidant effects *per se* and not just their histamine antagonism qualities.

A fuller evaluation of ROS – histamine interrelationships is warranted and further work should involve quantification of "direct" (electron paramagnetic spectroscopic) and "indirect" (metabolic biomarkers) circulating ROS/oxidative stress with concomitant quantitative histamine evaluation. Of note, in all of the studies showing a clear involvement of histamine receptors in post-exercise hyperaemia, no increase in circulating histamine has been found (Lockwood et al 2005b; McCord et al 2006; McCord & Halliwill, 2006). Thus, the most complete assessment of this potential interaction is likely to result from a pharmacological"double-blockade" of both ROS and histamine receptors and to note the response of post-exercise haemodynamics

compared to "single-blockade" of either metabolite. Interestingly ascorbate appears to increase H_1 receptor sensitivity, intimating towards a close relationship between histaminergic receptors and ascorbate (Dillon et al, 2006).

Redox Regulation of Circulating NO·-Bioavailability: Implication for PEH

NO· is a vasodilator that plays a role in blood flow and oxygen delivery (Ray & Marshall, 2005). However, elevated NO· concentrations can interact with the superoxide anion *in vivo* leading to the formation of the powerful oxidant species peroxynitrite (Moncada & Balanos, 2006; Pacher et al, 2007). Also, NO· bioavailability is reduced with increased oxidative stress (Félétou & Vanhoutte, 2006; Yung et al, 2006). The present NO· data lend support to Halliwill et al (2000) who found that systemic NO· synthase inhibition did not reverse PEH in normotensives following brief exercise; suggesting that NO· production and sympathoinhibition do not play a significant role in PEH. The present study endorses these findings indicating that augmented circulating bioavailability of NO· is not obligatory for the observed hypotension in our pre-hypertensive subjects.

The negative (i.e. blunting of exercise-induced benefits) effects of oxidative stress on MAP, SVR and SVC in the current study could be ascribed to the well-established quenching of circulating NO· by superoxide and production of peroxynitrite either of which is capable of mediating vascular tone (Engelke et al, 1996; Brock et al, 1998; Rådegran & Saltin, 1999; Faraci, 2006; Pacher et al, 2007). It must be noted, however, that in previous studies investigating the direct effects of pharmacological antagonism of NO· on exercise haemodynamics (Engelke et al, 1996; Brock et al, 1998; Rådegran & Saltin, 1999; Halliwill et al, 2000) no baseline or experimental determination of circulating NO· bioavailability was made.

In the present study, venous nitrate demonstrated a significant dimunition by the end of the trial concomitant with a signficiant blunting of the favourable haemodynamic responses. Thus, to this authors' knowledge this is the first study to document circulating markers of circulating NO· bioavailability during a period of PEH and detail the associated effects on systemic haemodynamics.

Venous plasma nitrate is considered a marker of total endogenous NO· production (Tarpey & Fridovich, 2001; Tarpey et al, 2004). Dietary intake can dictate plasma NO· concentrations, however subjects presented to the trial fasted and were provided with a control meal during the study that minimised nitrate/nitrite consumption. Therefore, the author is confident that nitrate values in the current protocol reflect, in part, endogenous NO· bioactivity. It has been argued that the anion nitrite is a stable storage form of intravascular NO· (Gladwin & Schechter, 2004) and as such similar modulations in this marker as well as RSNO concentration and total plasma NO· (the sum of nitrate, nitrite and RSNO) would have provided more compelling evidence. Notwithstanding, this study is the first to provide a 'metabolic snapshot' of NO· bioactivity and its redox regulation during a period of PEH. An intriguing antagonism exists whereby elevated oxidative stress in the post-exercise period reduces the bioavailability of NO·, blunting systemic vasodilatation, but a sustained hypotension remains.

Redox Regulation of arterial compliance – Relation to PEH

Exercise and the subsequent systemic vasodilatation imparted modulations in UL APWV compared to baseline, whilst LL APWV remained invariant. A full appraisal of the APWV response and the relevant literature was undertaken in the discussion of chapter 4 and 5. Hence, this discussion will focus on arterial compliance in the

context of any potential susceptibility to redox regulation. As previously stated, APWV provides a quantitative assessment of arterial distensibility used to investigate acute flow-related modulations in vascular tone without pharmacological provocation (Naka et al, 2006). The current findings indicate that whilst LL APWV displayed no change across the trial that UL APWV was significantly blunted and then elevated over the post-exercise period between exercise termination and 2-hours post-exercise. Over this time-period a simultaneous increase in UL venous LOOH and fall in NO^-_3 was also noted. Furthermore, correlational analysis revealed several intruiging findings with UL APWV being inversely associated to systemic venous NO^-_2 concentration (r = -0.48, P<0.01), LL APWV was related to systemic venous LOOH (r = 0.43, P<0.01) and Δupper limb APWV was inversely correlated to Δascorbic acid (r = -0.53, P<0.01) and Δβ-cryptoxanthin (r = -0.40, P<0.05).

Previous findings utilising an assessment of peripheral UL APWV, slightly dissimilar to the current protocol, found that increases in UL APWV occurred with concomitant elevations in LOOH following consumption of a high-fat meal (Clegg et al, 2007). Work from the same laboratory highlighted a significant inverse association between UL APWV and blood SOD concentration (McClean et al, 2007). Utilising other peripheral APWV measures, data has consistently demonstrated deterioration in vascular compliance with reciprocal increases in oxidative stress (Wilkinson et al, 1999; Mullan et al, 2002; Plantinga et al, 2007). Thus, the present results appear to complement recent data suggesting a deleterious effect of oxidative stress on UL vascular distensibility. UL APWV remained augmented during the full time-course of increased LOOH and critically decreased NO^-_3 concentration. The same APWV methodology has been shown to respond accordingly to both endothelial-dependent and -independent stimulation (Naka et al, 2006), although the authors did not report

endogenous levels of systemic NO· -bioavailability during the trial. The specificity of the UL response also fits with prior data indicating that this vascular bed, under this methodology, is more responsive than the simulutaneously assessed LL vascular bed in response to both exercise and pharmacological provocation (Naka et al, 2003; 2006). Interestingly, it may be the case that APWV is sensitive to redox mediation dependent on the specific vascular bed as findings indicate that unlike peripheral (limb) APWV, central aortic APWV is unaffected by chronic or acute ascorbate exposure (Eskurza et al, 2004b).

The unique finding of this study is that following acute exercise UL APWV is elevated along with increased oxidative stress and reduced NO·-bioavailability over a similar time-course to the recovery of reduced SVR, MAP and augmented SVC. Moreover, this is the first data to report simultaneous quantification of UL/LL APWV, systemic LOOH and NO·-bioavailability at baseline or during recovery from exercise.

Fibrinogen, Coagulation & PEH

The current findings show that blood haemostatic markers were unchanged across the study. Changes in fibrinogen and tissue plasminogen activator activity have been shown to increase more following moderate (60% $\dot{V}O_{2PEAK}$) compared to light (40% $\dot{V}O_{2PEAK}$) exercise, explaining a portion of the hypotensive response after light but not moderate exercise (Blanchard et al, 2007). Whilst blood viscosity was not directly assessed in the current study it can have a profound influence on vasodilatation and APWV. Increasing blood viscosity increases endothelial sheer stress, NO· release and consequent vasodilation. It may also directly decrease APWV (Naka et al, 2003). A significant component of increased viscosity is due to increased concentration of blood-bound proteins with fibrinogen and red corpuscle mass being the largest

intravascular circulating proteins (el-Sayed & Davies, 1995). However, fibrinogen and haematocrit (data not shown) remained stable throughout the recovery period whilst APWV, SVR and MAP fell and SVC increased. Thus variations in haemostatic factors do not appear to play a part in the post-exercise hamodynamic profile noted presently. Futhermore, ROS and NO·-bioavailability have been purported to modify haemostatic factors (Herkert et al, 2004; Görlach et al, 2005). Several significant relationships were highlighted between the absolute haemostatic markers and indicators of redox state across the trial.

Clinical & Physiological Significance

These findings have potential physiological significance for both health and disease as the ability to generate an appropriate hyperaemic response is compromised in healthy aging (Dinenno et al, 2001; Wray et al, 2008) and numerous cardiovascular maladies (Ramsey et al, 1995; Wilson et al, 1999; Naka et al, 2006).

Whilst the quenching of circulating NO·-bioavailability is an attractive hypothesis for the mechanism of ROS-mediated tonic control, presently, other possibilities can not be excluded. It is quite possible that the increase in circulating ROS imparted an effect on α-adrenergic receptor responsiveness rather than directly influencing α-adrenergic signalling (Girouard & de Champlain, 2005) as noradrenaline levels, an indirect measure of sympathetic nerve activity, were unchanged following exercise. This concept is in support of prior findings (Bell et al, 2003). Furthermore, the possibility of ROS-mediated influences on non-adrenergic receptor responsiveness is also tenable as exercise stimulated increases in angiotensin II, aldosterone and AVP, thus ROS may potentiate the effects of these metabolites on the systemic circulation.

Either via direct or indirect factors the deleterious effects of increased ROS in the recovery period following exercise have some potential clinical implications. Firstly, supplementation with prophylactic antioxidants could play a role in extending the beneficial reductions in SVR and MABP and augmented SVC following an acute bout of exercise in all populations but may be of critical importance in older hypertensive individuals. Secondly, whilst susceptible individuals prone to orthostatic intolerance (Holtzhausen et al, 1995) and vasovagal syncope (Krediet et al, 2004) following physical exertion have been recommended to take anti-histamine products, due to histaminergic receptor involvement in hyperaemia, they may also benefit from abstaining from supplemental antioxidants to counter any potentiating effects these substances may impart. Finally, there is a potential dual function of ROS in the vasculature linked to the integrity of SOD defences. In those studies showing beneficial vasodilatory effects of increased ROS (Richardson et al, 2007; Gutterman et al, 2005) the effects may be attributable to SOD conversion to H_2O_2 and its hyperpolarising capabilities. Whereas in older, diseased systems with depressed SOD increased superoxide quenches $NO^{\cdot}$-bioactivity, eliciting impaired circulatory responses (Donato et al, 2007; Jablonski et al, 2007). Hence, exercise training (increased SOD; Eskurza et al, 2004b; Donato et al, 2007) and antioxidant supplementation may give the greatest benefits to aged and diseased individuals with regards to circulatory function.

6.4 Conclusion

To this author's knowledge, this is the first study to report ROS (LOOH) data during a period of PEH. The present findings extend previous results reporting the pathophysiological effects of increased oxidative stress on the vasculature. Increased oxidative stress in the recovery period appears to coincide with a return of SVR, SVC

and MAP towards baseline. This suggests that PEH can be regulated from a metabolic pathway associated with ROS and with potential involvement from $NO^{\cdot}$ bioactivity. These findings have implications for the mechanisms by which ROS contribute to normal and pathophysiological vascular function.

STUDY 4: EFFECT OF HYPEROXIC EXERCISE ON REDOX REGULATION OF CIRCULATING NO· BIOAVAILABILITY: IMPLICATIONS FOR POST-EXERCISE HAEMODYNAMICS IN HYPERTENSIVE MAN

7.0 Introduction

The exact mechanisms underlying the vascular tonic responses to hyperoxia and hypoxia have yet to be clearly defined. Whilst study 2 suggests that ANP may play a role in blunted vasodilatation following hyperoxia, O_2 tension may influence one or more of the endothelium-derived factors that contribute to the maintainence of vascular tone. Such that, in line with the findings from study 3, the increased production of reactive oxygen species, oxidative stress and reduced NO· bioavailability could also be responsible (Rubanyi & Vanhoutte, 1986; Pries et al, 1995; Mouren et al, 1997; Mak et al, 2002). Both hypoxic (Simon-Schnass & Pabst, 1988; Simon-Schnass, 1994; Duranteau et al, 1998; Bailey, 2001; Bailey et al, 2000; 2001; 2003a; 2003b; 2004; 2006; Chandel & Schumacker, 2000; Liu et al, 2003) and hyperoxic (Jamieson et al, 1986; Loiseaux-Meunier et al, 2001; Khaw et al, 2002; Bandali et al, 2004; Carpagnano et al, 2004; Solberg et al, 2007) conditions have been documented to increase formation of ROS that may subsequently alter cell function by reacting with various cellular components including the plasma membrane, enzymes and ion channels. ROS created in this way have also been speculated to engage in intracellular signalling (Duranteau et al, 1998; Chandel & Schumacker, 2000). The clearest evidence that hyperoxic vasoconstriction is mediated via upregulated ROS production and reduced NO· bioavailability is in the study of Mak et al (2002) where intra-arterial ascorbic acid infusion in the forearm negated hyperoxic vasoconstriction and reversed the decline in endothelial-derived vasodilatation in

healthy volunteers but not in older subjects with compromised cardiovascular function. The study also failed to document systemic markers of oxidative stress, antioxidant status or $NO^{\cdot}$ bioavailability. Later data has emerged showing no increase in free-radical mediated lipid peroxidation during hyperoxic inspiration with concomitant increases in SVR (Thomson et al, 2006) but the authors failed to report $NO^{\cdot}$ metabolism in the experiment.

Therefore, the purpose of this study was to determine the association between a redox-mediated regulation of circulating $NO^{\cdot}$ bioavailability and the augmented vasoconstriction following hyperoxic exercise.

7.1 Methodology

Experimental Protocol

The experimental and exercise protocols were as those detailed in section 5.1. Identical physiological measures were obtained along the same experimental time-line as those previously displayed in section 5.1.

Nitrate and Nitrite (NO^{-}_3 and NO^{-}_2)

Systemic venous blood was assayed for NO^{-}_3 and NO^{-}_2 utilising the methodology described in section 3.15.12.1.

S-Nitrosothiols (RSNO)

RSNO's were determined in systemic venous blood via the procedure detailed in section 3.15.12.2.

Markers of Oxidative Stress: Lipid Hydroperoxides (LOOH)

LOOH concentrations were determined in systemic venous serum by the methodology outlined in section 3.15.13.

Antioxidant Status

Ascorbic acid concentrations were determined on stabilised venous plasma following the methodology laid out in section 3.15.14.1. Lipid soluble antioxidants were assayed following the procedure detailed in section 3.15.14.2.

Lipids

Venous serum concentrations of total cholesterol (TC) and triacylglycerol (TAG) were determined via the procedure detailed in section 3.15.15.

Fibrinogen and Clotting Factors

Venous citrated plasma was assayed for coagulation and fibrinolysis as detailed in section 3.15.16.

Statistical Analyses

Statistical analysis was performed using the methodology outlined in section 3.16.3.

7.2 Results

7.2.1 Physiological Data

The physiological data collected in response to the experimental trials is that presented in section 5.2.1 through to 5.2.5. Analysis of MAP produced a significant main effect for time ($P = 0.00$), indicating a reduction from baseline of 5 mmHg at the 30-minutes post-exercise time point onwards until cessation of recordings. Moreover, analysis of the absolute change in MAP from baseline between trials revealed a significant main effect for oxygenation state ($P<0.05$) whereas no interaction effect was observed (state x time; $P>0.05$). Exercise *per se* produced a 5% reduction of MAP versus pre-exercise. MAP was reduced by ~5mmHg at its nadir at the 1-hour post-exercise time-point ($P<0.05$).

The MAP response across the trial was blunted following hyperoxic exercise in comparison to normoxic and hyperoxic exercise (both P=0.00) whereas no differences were observed between normoxic and hypoxic exercise (grouped means for state; 4.5 (4) following normoxia, 5.7 (6) following hypoxia and 1.4 (4) mmHg following hyperoxia, change from baseline). The reduction in MAP resulted from a post-exercise fall in systolic blood pressure (SBP) and diastolic blood pressure (DBP). SBP was attenuated by ~10mmHg 1-hour post-exercise and remained ~8mmHg below pre-exercise levels until cessation of recordings 2-hours following exercise (P<0.01; pooled for time). DBP was attenuated by ~4% 60-minutes following exercise remaining blunted by ~3% at 120-minutes post-exercise (P<0.01; pooled for time). Unlike MAP, the absolute reduction in SBP from baseline was not different between conditions (P=0.07) but did show a main effect for time (P<0.05) displaying an elevated SBP [~2 (8)mmHg] immediately (within 1-minute) post-exercise then a decline in SBP from 15-minutes post-exercise to the nadir of ~10 (4)mmHg at 1-hour post-exercise and continuing to be significantly depressed (P<0.05) below the immediate post-exercise response until completion of the trial. In contrast, the absolute reduction in DBP from baseline failed to display any differences between conditions or across the trial (P>0.05).

Immediately post-exercise both HR and $\dot{Q}$ were elevated above baseline (P<0.05, pooled for time) by 148% and 68%, respectively, but returned to pre-exercise levels by 1-hour post-exercise. SV, SVI and $\dot{Q}$ values were reduced during the hypoxic trial when compared to the normoxic trial [76.4 (19.7) *v.* 91.6 (32) ml beat^{-1}, 39.8 (10.4) *v.* 47.8 (16.9) ml/m^2, 6.7 (3.1) *v.* 7.8 (3.8) l/min, respectively; P<0.05, pooled for state]. SVR was decreased from a baseline value of 19.3 (4.6) resitance units (RU) (P<0.05, pooled for time) during the entire post-exercise phase being 12.7 (3.4), 16.2 (3.4) and

16.6 (3.4) RU immediately post-, 60-minutes post- and 120-minutes post-exercise, respectively, but failed to demonstrate any variation between exercise conditions. Arterial-venous oxygen difference showed a tendency to be elevated immediately following exercise ($P = 0.06$, pooled for time) but interestingly showed a significant decrease over time being ~13% reduced below baseline at the 120-minute sampling point. O_2 pulse increased ($P<0.05$, pooled for time) during the immediate post-exercise period by 50% but returned to baseline levels by 1-hour post-exercise. Systemic vascular resistance was attenuated by ~32% (across conditions) immediately post-exercise and remained blunted, in comparison to baseline, by ~15 and 13% at 60- and 120-minutes post-exercise, respectively. The rise in SVR back towards baseline level caused the vasodilatation at 60- and 120-minutes post-exercise to be attenuated when compared to the immediate post-exercise point ($P<0.05$). In line with the systemic mean arterial pressure response the reduction in SVR from baseline was significantly less following hyperoxic exercise as compared to normoxic or hypoxic exercise ($P<0.05$, pooled for state). SVC values highlighted a ~53% augmentation (across conditions) over baseline immediately post-exercise but was significantly blunted at 60- and 120-minutes post-exercise (18 and 14%, respectively) compared to the immediate post-exercise response. SVC however failed to display a sensitivity to systemic oxygenation across the trial ($P>0.05$).

7.2.2 NO^-_3 and NO^-_2

Both hypoxic and hyperoxic exercise failed to modify the venous plasma concentration of NO^-_3 over the trial but a significant main effect was noted for pooled values over time with a dimunition between immediately post-exercise and termination of the trial ($P<0.05$). Systemic venous NO^-_2 concentration however was unaltered across the trial ($P>0.05$) (Figure 7.1, 7.2).

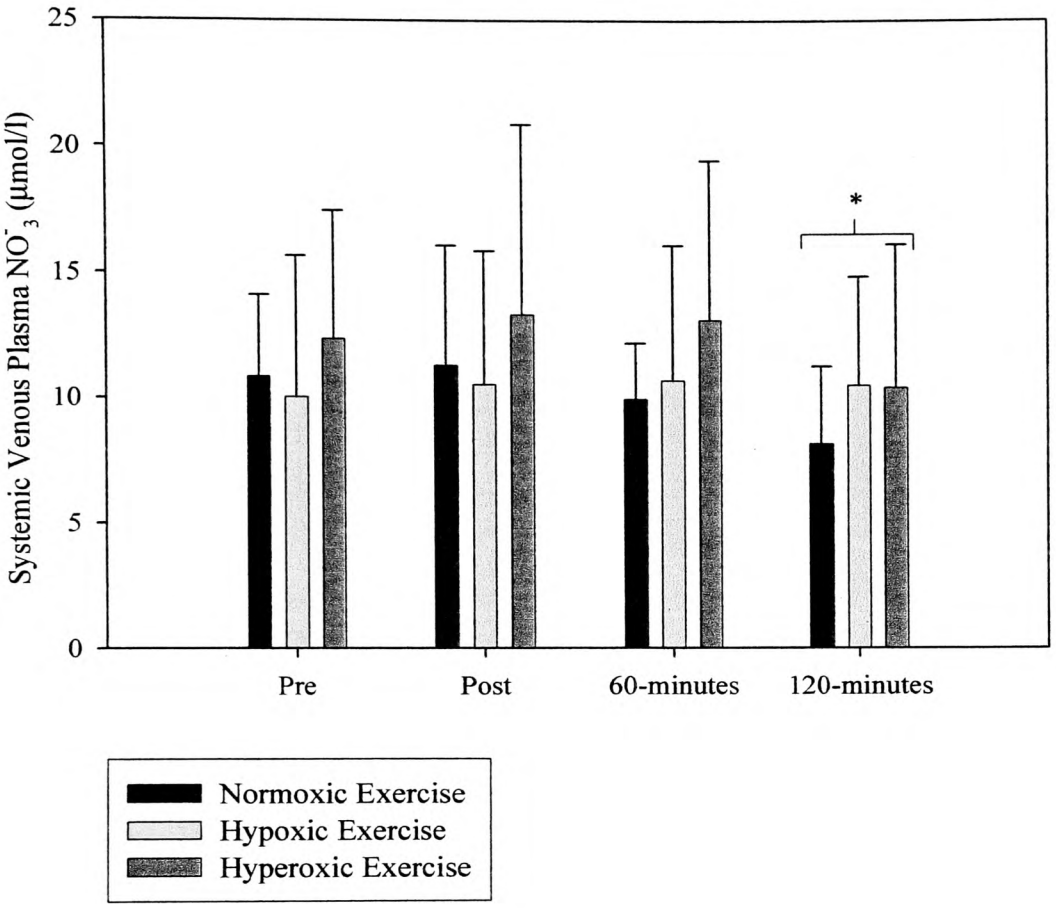


Figure 7.1. Systemic Venous Plasma NO^-_3 in response to exercise under varying F_IO_2. Values are Mean ± (SD); $n = 9$. Post, within 1-minute post-exercise; 60-min, 1-hour post-exercise; 120-min, 2-hours post-exercise. *$P<0.05$ *v.*post, pooled for time.

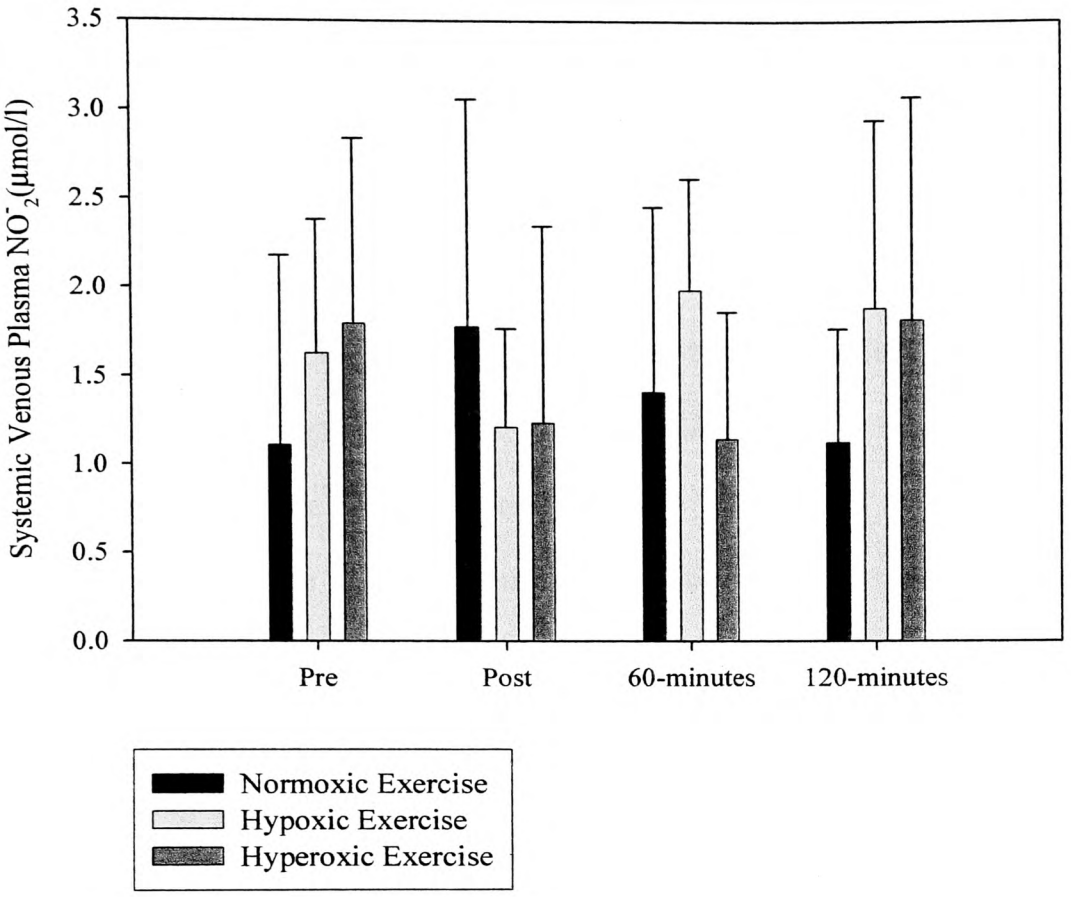


Figure 7.2. Systemic Venous Plasma NO^-_2 in response to exercise under varying F_IO_2. Values are Mean ± (SD); n = 9. Post, within 1-minute post-exercise; 60-min, 1-hour post-exercise; 120-min, 2-hours post-exercise.

7.2.2.1 RSNO

Systemic venous RSNO was unaltered by the exercise stimulus *per se* with time (pre to post exercise) failing to demonstrate a significant effect ($P>0.05$). Across the trial an elevation in RSNO concentration was noted in the hypoxic condition in comparison to the normoxic and hyperoxic trials [18.6 (9) *v.* 12.8 (9.6) and 12 (9.3) μmol/l, respectively; $P<0.05$, pooled for state]. Removing baseline levels from the analysis revealed that, in the post-exercise period, values were elevated following the hypoxic condition relative to the hyperoxic condition only [18.9 (9) *v.* 11.1 (9) μmol/l, respectively; $P<0.05$, pooled for state]. A significant time by state effect was apparent with the systemic venous concentration of RSNO following hypoxic exercise being significantly elevated immediately post-exercise when compared to normoxic and hyperoxic exercise (Figure 7.3).

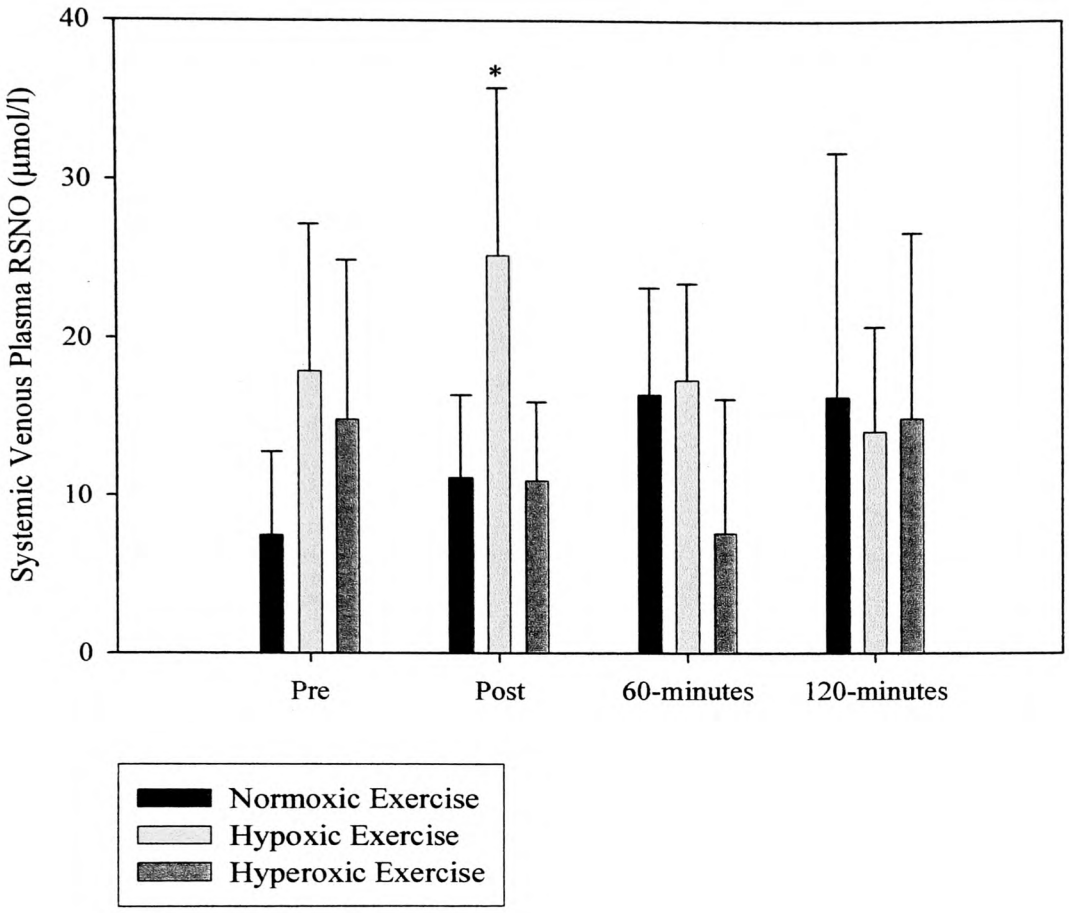


Figure 7.3. Systemic Venous Plasma RSNO in response to exercise under varying F_IO_2. Values are Mean ± (SD); $n = 9$. Post, within 1-minute post-exercise; 60-min, 1-hour post-exercise; 120-min, 2-hours post-exercise. *$P<0.05$ *v.* normoxic and hyperoxic conditions, within time-point.

7.2.2.2 Total Plasma $NO^{\cdot}$

Total plasma $NO^{\cdot}$ (the sum of NO^{-}_3, NO^{-}_2 and RSNO) also failed to respond to the exercise challenge *per se* ($P>0.05$) but displayed an elevation in the hypoxic trial immediately post-exercise when compared to normoxic and hyperoxic exercise [36.97 (7.8) *v.* 24.21 (6.38) & 25.47 (10.44) µmol/l, respectively; $P<0.05$].

7.2.3 Lipid Hydroperoxides (LOOH)

Venous plasma LOOH concentration across the protocol is displayed in Figure 7.4. LOOH concentration was unmodified by varying inspired oxygen fraction but exercise *per se* elicited an increase in lipid peroxidation with elevated LOOH by 60-minutes post-exercise ($P<0.05$, pooled for time) until termination of the protocol.

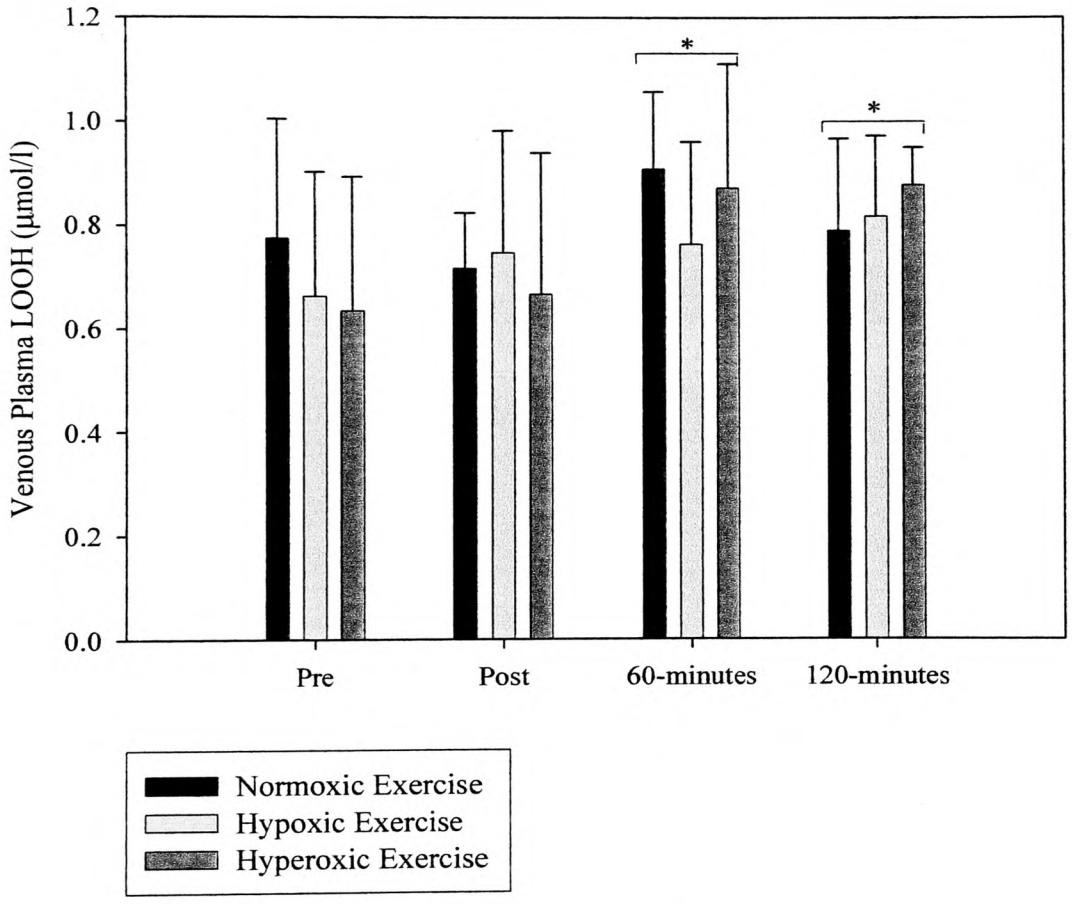


Figure 7.4. Systemic Venous Plasma LOOH in response to exercise under varying F_IO_2. Values are Mean ± (SD); $n = 9$. Post, within 1-minute post-exercise; 60-min, 1-hour post-exercise; 120-min, 2-hours post-exercise. *$P<0.05$ *v.*pre, pooled for time.

7.2.4 Venous Lipid-lipoproteins

Table 7.0 displays the lipid-lipoprotein concentration across the trial. Exercise failed to modify HDL-C *per se*, but values were elevated in the normoxic trial in comparison to the hypoxic and hyperoxic conditions. LDL-C demonstrated a 9 and a 13% decline in concentration at post and 120-minute post-exercise, respectively, pooled for time ($P<0.05$). Values were also elevated by 6% in the hypoxic condition compared to normoxia, with a specific augmentation in venous LDL-C at baseline in the hyperoxic trial respective to the normoxic baseline. The other lipid-lipoproteins were unremarkable following the experimental paradigm.

7.2.5 Antioxidants

The venous plasma concentration of selected antioxidants is shown in Table 7.1.

Table 7.0. Blood Lipid-lipoproteins

	Pre			Post			60-min			120-min		
	0.16	**0.21**	**0.50**	**0.16**	**0.21**	**0.50**	**0.16**	**0.21**	**0.50**	**0.16**	**0.21**	**0.50**
TC	4.9 (0.9)	5.1 (0.6)	5.3 (0.7)	5.5 (1.1)	5.7 (0.8)	5.2 (1.1)	4.8 (1.3)	6.2 (0.9)	5.5 (1)	4.8 (1.4)	5.5 (1.1)	5.5 (1)
Tg	1.5 (0.9)	1.3 (0.6)	1.7 (1.0)	1.6 (1.0)	1.5 (0.6)	1.6 (0.7)	1.4 (0.7)	1.8 (0.4)	1.5 (0.6)	1.4 (0.7)	1.5 (0.6)	2.0 (1.6)
HDL-C‡	1.2 (0.1)	1.3 (0.2)	1.2 (0.1)	1.3 (0.2)	1.4 (0.2)	1.3 (0.3)	1.3 (0.3)	1.5 (0.3)	1.3 (0.3)	1.2 (0.2)	1.3 (0.2)	1.3 (0.2)
LDL-C$^{\dagger *\#}$	3.6 (0.6)	3.2 (0.6)	3.9 (0.7)	3.2 (0.8)	3.5 (0.8)	3.1 (0.7)	3.5 (0.9)	3.5 (0.7)	3.2 (0.7)	3.3 (0.6)	2.8 (0.5)	3.3 (0.9)

All data are expressed as mean (SD) [mmol/l]. n = 9. TC, total cholesterol; Tg, triacylglycerol; HDL-C/LDL-C, high/low-density lipoprotein cholesterol. Post, immediate (i.e. within 1-minute) post-exercise. 60-min, 1-hour post-exercise; 120-min, 2-hours post-exercise; 0.21, Normoxic trial; 0.16, Hypoxic trial; 0.60, Hyperoxic trial. *P<0.05, Pre *v.* post & 120-min, pooled for time. $^{\ddagger}P$<0.05, normoxia *v.* hypoxia & hyperoxia, pooled for state. $^{\dagger}P$<0.05, normoxia *v.* hypoxia, pooled for state. $^{\#}P$<0.05, normoxia *v.* hyperoxia, within pre-exercise time-point.

Table 7.1 Anitoxidant Profile

	Pre			Post			60-min			120-min		
	0.16	**0.21**	**0.50**	**0.16**	**0.21**	**0.50**	**0.16**	**0.21**	**0.50**	**0.16**	**0.21**	**0.50**
Asc A[‡#]	52.2 (18.6)	42.9 (18.9)	47.1 (11.8)	64.9 (23.7)	44.8 (27.4)	55.6 (16)	51.7 (15)	50.7 (15.9)	52.2 (20)	46.7 (14.2)	39.5 (18.3)	75.1 (31)
α-carot	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)
β-carot	0.2 (0.1)	0.3 (0.2)	0.3 (0.2)	0.3 (0.1)	0.3 (0.1)	0.3 (0.2)	0.3 (0.1)	0.3 (0.1)	0.3 (0.2)	0.3 (0.1)	0.3 (0.1)	0.3 (0.2)
α-toco	30.7 (7)	31 (7)	30.6 (8)	33 (10)	33 (8)	31.6 (9)	32.4 (9.5)	36.6 (7.5)	32.4 (8.1)	30.1 (7.2)	33 (10)	32.7 (7.5)
γ-toco	3.9 (1.1)	3.5 (0.8)	3.7 (1)	4 (1)	3.5 (0.7)	3.3 (0.9)	3.5 (0.7)	3.8 (0.7)	3.3 (0.9)	3.6 (0.7)	3.3 (0.9)	3.5 (1)
Retinol	2.1 (0.3)	2.2 (0.4)	2.1 (0.5)	2.3 (0.3)	2.3 (0.5)	2.4 (0.6)	2.1 (0.4)	2.4 (0.5)	2.4 (0.6)	2.1 (0.3)	2.2 (0.6)	2.3 (0.7)
Lycop	0.2 (0.1)	0.3 (0.2)	0.5 (0.6)	0.3 (0.2)	0.4 (0.3)	0.5 (0.4)	0.3 (0.1)	0.3 (0.2)	0.4 (0.3)	0.3 (0.2)	0.3 (0.1)	0.3 (0.1)
Lutein*	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)

Zeaxa	0.4 (0.2)	0.4 (0.2)	0.5 (0.3)	0.5 (0.3)	0.4 (0.2)	0.4 (0.2)	0.5 (0.2)	0.4 (0.2)	0.4 (0.2)	0.4 (0.2)	0.4 (0.2)	0.5 (0.2)
β-crypt	0.1 (0.04)	0.1 (0.04)	0.1 (0.09)	0.1 (0.04)	0.1 (0.04)	0.1 (0.08)	0.1 (0.04)	0.1 (0.04)	0.1 (0.08)	0.I (0.04)	0.1 (0.04)	0.1 (0.09)

All data are expressed as mean (SD) [μmol/l]. n = 9. Post, immediate (i.e. within 1-minute) post-exercise. 60-min, 1-hour post-exercise; 120-min, 2-hours post-exercise. Asc A, ascorbic acid; α-carot; α-carotene; β-carot, β-carotene; α-toco; α-tocopherol; γ-toco, γ-tocopherol; Lycop, lycopene; Zeaxa, zeaxanthin; β-crypt, β-Cryptoxanthin. $^{\ddagger}P<0.05$, normoxia *v.* hypoxia & hyperoxia, pooled for state. $^{\#}P<0.05$, hyperoxia *v.* normoxia & hypoxia, within 120-min, time-point. $^{*}P<0.05$, Pre *v.* post, pooled for time.

Ascorbic acid was elevated across both the hypoxic and hyperoxic exercise trials in comparison to the normoxic condition by 20 and 29%, respectively ($P<0.05$, pooled for state). Furthermore, at the 120-minute post-exercise sample point there was a selective increase in ascorbic acid concentration in the hyperoxic trial. Leutin values displayed an 8% increase following exercise ($P<0.05$, pooled for time) that resolved by 60-minutes post-exercise. All other antioxidants sampled were unremarkable across the conditions.

7.2.6 Haemostasis

International normalized ratio (INR) and prothrombin time (PT) responded to exercise *per se*. INR displayed a ~3% elevation over the post-exercise period between post and 120-minutes post and PT was elevated by ~3% between post and 60-minutes post ($P<0.05$, pooled for time). Activated partial thromboplastin time (aPTT) and activated partial thromboplastin time ratio (aPTTr) decreased over the protocol with an ~6% fall from pre to post and pre to 60-minutes post-exercise, respectively ($P<0.05$, pooled for time). Fibrinogen concentration was unremarkable across the protocol (Table 7.2).

Table 7.2 Haemostasis Profile

	Pre			Post			60-min			120-min		
	0.16	**0.21**	**0.50**	**0.16**	**0.21**	**0.50**	**0.16**	**0.21**	**0.50**	**0.16**	**0.21**	**0.50**
Fib	305 (79)	267 (39)	328 (76)	327 (83)	297 (34)	325 (86)	308 (50)	295 (27)	337 (78)	296 (52)	274 (32)	325 (99)
INR*	0.97 (0.04)	0.95 (0.08)	0.98 (0.09)	0.97 (0.04)	0.97 (0.06)	0.97 (0.06)	1 (0.06)	0.98 (0.03)	1 (0.08)	1 (0.04)	0.95 (0.07)	1 (0.09)
aPTT[#]	32.1 (3.5)	31 (3.2)	30.1 (3.1)	29.1 (1.8)	29.4 (3.1)	28.7 (1.4)	29.9 (2.8)	30.3 (2)	29 (1.5)	30.1 (2.1)	29.2 (1.9)	29.6 (1.1)
aPTTr[‡]	0.99 (0.1)	0.99 (0.1)	0.92 (0.1)	0.91 (0.1)	0.92 (0.1)	0.89 (0.1)	0.91 (0.1)	0.96 (0.6)	0.89 (0.1)	0.93 (0.1)	0.92 (0.1)	0.93 (0)
PT[†]	13 (0.7)	12.7 (0.9)	12.7 (0.9)	13 (0.4)	12.7 (0.6)	12.9 (0.8)	13.5 (0.6)	13.1 (0.4)	13.2 (0.9)	13.5 (0.6)	12.7 (0.7)	13.2 (0.8)

All data are expressed as mean (SD) n = 9. Post, immediate (i.e. within 1-minute) post-exercise. 60-min, 1-hour post-exercise; 120-min, 2-hours post-exercise. Fib, fibrinogen (mmol/l); INR, international normalized ratio; aPTT, activated partial thromboplastin time (s); aPTTr, activated partial thromboplastin time ratio; PT, prothrombin time (s). *$P<0.05$, Post *v.* 60-min & 120-min, pooled for time; [#]$P<0.05$, Pre *v.* post, pooled for time; [‡]$P<0.05$, pre *v.* post & 60-min, pooled for time; [†]$P<0.05$, post *v.* 60-min, pooled for time.

7.2.7 Experimental Correlations

Systemic Arterial Pressure, Haemodynamics & Biochemistry

There was no correlation evident between systemic MAP, NO^-_3, NO^-_2, RSNO, LOOH or any antioxidant (all P>0.05) across the conditions indicating that the systemic venous concentration of these metabolites were not associated to the prevailing systemic mean arterial pressure. No association was found between SVR values or SVC and the absolute concentrations in any of the metabolites investigated (P>0.05) throughout the protocol.

Biochemical Response to F_IO_2

No relationship was found between NO^-_3, NO^-_2 or RSNO and arterial oxygen saturation, calculated arterial O_2 content or arterial PO_2. LOOH displayed a significant relationship towards arterial O_2 content (r = -0.20, P<0.05) across the trial. With regards to the antioxidant profile α-carotene, β-carotene and lutein showed an inverse association with arterial O_2 content (r = -0.27, r = -0.26 and r = 0.25, P<0.01) respectively. Lipid-lipoprotein analysis revealed that the venous concentration of HDL-C was negatively related to the systemic arterial O_2 content (r = -0.36, P<0.01). No associations were found between any marker of haemostasis and those of *in vivo* arterial oxygenation.

Interassay Correlations

Inter-assay correlations revealed several observations that in the majority are analogous to those reported previously in section 6.2.11 between markers of $NO^{\cdot}$ metabolism, LOOH, antioxidants, lipid-lipoproteins and haemostatic factors. Additional novel relationships elucidated in this study were that systemic venous fibrinogen concentration was correlated to venous plasma NO^-_3 (r = 0.45, P<0.01), TC (r = 0.41, P<0.01), LDL (r = 0.19, P<0.05) and TAG (r = 0.37, P<0.01) whilst

failing to display any relationship to the other haemostatic markers. APTT was correlated to venous plasma $NO^{\cdot}_2$ ($r = 0.20$, $P<0.05$) and PT was inversely related to to venous plasma $NO^{\cdot}_3$ ($r = -0.21$, $P<0.05$).

7.3 Discussion

Previous studies in this thesis have established the existence of PEH, blunted SVR and augmented SVC following dynamic cycle exercise in this specific cohort of pre-hypertensives. Study 2 clearly demonstrated that following exercise in hyperoxia there is a mildly hypertensive effect which translates into an attenuation of the reduction in SVR following hyperoxic exercise. The potential roles of muscle metabolism, baroreflex resetting, peripheral chemoreceptor activity and exercise intensity were thoroughly evaluated in the discussion to chapter 5 (Section 5.3). Likewise, analysis of several purported circulating vasoactive factors revealed that the response of ANP may explain part of the blunted haemodynamic response.

The aim of this final study was to evaluate the role of a redox-mediated regulation of circulating $NO^{\cdot}$ bioavailability as a modulator of the augmented vasoconstriction following hyperoxic exercise. This aim built on the findings of the previous study (study 3) that elucidated the deleterious effects of ROS on circulating $NO^{\cdot}$ metabolism and haemodynamics in the post-exercise period. The novel finding of the current study is that the haemodynamic responses following hyperoxic exercise appear to be independent of any change in systemic oxidative stress and $NO^{\cdot}$ metabolism.

Oxygen Tension & ROS Production

This study analysed LOOH as a stable, reliable marker of lipid peroxidation that has been shown to represent free-radical-catalysed peroxidation of PUFA (Davison et al,

2008a). In the present study no differential response was found in venous LOOH concentration following hyperoxic exercise. This is in agreement with the most recent study investigating O_2 tension and heamodynamics. Thompson et al (2006) found no change in 8-Iso-Prostaglandin F2α (F2α), formed by free-radical-induced modification of arachidonic acid within cell membranes, following isocapnic hyperoxia at rest with concomitant increased SVR in 8 healthy men. Taken together with the current results it appears that acute hyperoxia, which imposes significant haemodynamic effects, does not emanate from concomitant oxidative stress. Although the mechanisms by which hyperoxia generates ROS at or near the vascular wall are unclear several possibilities exist (Freeman & Crapo, 1981; Wolin et al, 1999). The oxidation of catecholamines (Jamieson et al, 1986) has been postulated as a prominent mediator. As both adrenaline and nor-adrenaline displayed no changes following hyperoxic exercise (study 2: chapter 5) this latter possibility appears unlikely.

Un-changed oxidative stress following acute hyperoxia, whilst supportive of previous findings, is somewhat surprising but can be ascribed to several possibilities. Both animal (Bandali et al, 2004; Solberg et al, 2007) and human studies (Jamieson et al, 1986; Loiseaux-Meunier et al, 2001; Khaw et al, 2002; Carpagnano et al, 2004) have previously documented increased peroxidation following hyperoxia but it appears that there is a dose-response relationship (Solberg et al, 2007), such that the modest hyperoxia utilised presently (50% O_2) would elicit a diminished oxidative perturbation as that reported in previous studies with 100% O_2 (Solberg et al, 2007; Bandali et al, 2004). However, it should also be noted that such changes could have been too subtle to detect systemically or may occur in regional circulations, the microcirculation or the lung. Indeed, reduced levels of hyperoxia have been reported

to induce oxidative stress in such local circulations. In the study of Carpagnano et al (2004) ~50% increases were recorded in F2α and interleukin-6 concentrations from breath condensate following 1-hour exposure to 28% O_2 in normals and chronic obstructive pulmonary disease patients. Utilising the same hyperoxic stimulus, Phillips et al (2003) reported increased breath markers (breath methylated alkane contour) of oxidative stress in 31 healthy volunteers following 30-minutes passive exposure. In a further human study, Khaw et al (2002) utilised 60% O_2 during caesarean section and determined F2α, organic hydroperoxides and malondialdehyde in maternal arterial blood and foetal umbilical arterial and venous blood. Results indicated that in comparison to a normoxic control, hyperoxia induced increases in maternal arterial oxidation and foetal umbical arterial and venous oxidation.

The current results also demonstrate a failure of hypoxia (hypoxic exercise) *per se* to increase lipid peroxidation. This is in agreement with prior work which reported that hypoxic exposure at rest (Thompson et al, 2006) and during exercise (Davison et al, 2006) failed to augment F2α or LOOH, MDA and directly assessed (via electron spin resonance spectroscopy) free radical concentration, respectively, in healthy males compared to normoxia. Previous work from the authors' laboratory (Bailey 2001; Bailey et al, 2000; 2001; 2003a; 2003b; 2004; 2006) and others (Simon-Schnass & Pabst, 1988; Simon-Schnass, 1994; Duranteau et al, 1998; Chandel & Schumacker, 2000; Liu et al, 2003) have implicated hypoxia as an independent stimulator of free radical generation and release from exercising muscle. Bailey et al (2004) postulated a mechanistic relationship between intracellular (mitochondrial PO_2) and mitochondrial ROS generation related to a decrease in diffusive oxygen delivery to myocytes causing a decrease in the V_{max} of cytochrome oxidase and an increase in univalent reduction.

Of particular interest, the study of Davison et al (2006) employed the same hypoxic stimulus (16% O_2) and exercise mode to that of the present study but exercised subjects at 55% $\dot{V}O_{2PEAK}$ for 2-hours. The authors attribute the lack of response to absolute and relative hypoxic exercise to an insufficient decrease in intracellular PO_2 as a consequence of using 16% O_2 at 55% $\dot{V}O_{2PEAK}$ that ultimately elicited similar $\dot{V}O_2$ and workrate across trials. Indeed in the studies of Bailey et al, alluded to previously, a much more aggressive hypoxia (12% O_2 or higher), or in a study that used 16% O_2, maximal exercise was utilised (Bailey et al, 2001). Whereas the current protocol employed a higher target relative $\dot{V}O_{2PEAK}$ than that of Davison et al (2006) it was undertaken for a short time (30-minutes) and similar $\dot{V}O_2$ was also noted between trials (Table 5.1), thus it is likely that the lack of effect was due to the same factors as those reported by Davison et al (2006).

Notwithstanding the lack of interaction effect in LOOH concentration between trials, a correlation was observed between LOOH and $C_{a,O2}$ ($r = -0.20$, $P<0.05$) across the trial together with a selective modulation in $S_{a,O2}$, $P_{a,O2}$ (hypoxia & hyperoxia) and $C_{a,O2}$ (in hyperoxia) lending support to the hypothesis promulgated by Bailey et al (2004) for a regulation of augmented systemic ROS concentration by decreasing arterial PO_2 and perhaps this mechanism partially contributed to the increase in LOOH observed following exercise *per se*.

A further moderating factor potentially underlying the lack of effect of oxygen tension on LOOH presently is that of the integrity of the antioxidant defences throughout the protocol. In the prior literature cited pertaining to hyperoxic exposure antioxidant status was either unchanged or unreported. This factor will be discussed in the following section.

Oxygen Tension & Antioxidants

In the study of Thompson et al (2006) no change was found in the venous concentration of ascorbic acid, urate and total antioxidant capacity. The authors apportion the lack of change in both F2α and antioxidant concentrations to a sufficient antioxidant reserve capable of buffering small changes in oxidative stress. The present results demonstrate a specific increase in ascorbic acid concentration in both the hypoxic and hyperoxic trials with an interaction effect resulting in a selective increase in ascorbic acid concentration at 2 hours post-hyperoxic exercise. Thus the selective increase in circulating ascorbate concentration would likely have served to sufficiently quench a significant increase in systemic ROS and as such limit lipid peroxidation in the hypoxic and hyperoxic trials.

The potent aqueous phase antioxidant ascorbic acid has been shown to scavenge and reduce the bioavailability of $O_2^{\cdot-}$ (Jackson et al, 1998) and attenuate free radical and LOOH concentration in type I diabetic humans (Davison et al, 2008b), thus increased circulating concentrations of this species would provide a powerful defence against an increased oxidant stress induced by hypoxic and hyperoxic exercise.

Along these lines, the only prior study investigating hyperoxia-mediated vasoconstriction, ROS and ascorbate revealed a clear role for ascorbic acid in attenuating the vascular response to hyperoxic exposure (Mak et al, 2002). The study revealed that infusion of a supraphysiological dose of ascorbate into the brachial artery of healthy, relatively young (35 ± 3 years) and older (55± 3 years) CHF subjects abolished hyperoxia-induced (100% O_2) vasoconstriction at rest in both cohorts and reversed the acute impairment in endothelium-dependent vasodilatation during hyperoxia in healthy subjects only. Therefore, it appears that ascorbate is a

powerful defence against increased ROS generation during hyperoxia that has consequent effects on limb vascular tone.

It is important to acknowledge however, some differences between the study of Mak et al (2002) and the present protocol. No systemic markers of antioxidant status, free radicals or lipid peroxidation were assessed in the prior study, hence the exact response of ROS, the induced peroxidation and circulating ascorbic acid concentrations elicited by hyperoxia were undocumented. The response of the biochemical variables of interest is important for several reasons not least to account for the efficacy of the intervention (ascorbic acid) to blunt the primary metabolic variable of interest (ROS).

Also infusion of supraphysiological doses would not only have ablated augmented ROS production but could potentially mediate a multitude of systemic effects. With regards to vascular tone this is important due to the fact that ascorbate appears to increase H_1 receptor sensitivity (Dillon et al, 2006) and the H_1 receptor is a primary mediator of endothelial vasodilatation (Brown & Roberts, 2001) and increased vascular conductance (McCord & Halliwill, 2006). Thus blunted vasoconstriction during ascorbate infusion may also be due to increased histamine- and/or –receptor-sensitivity – induced vasodilatation. Intriguingly, Mak et al (2002) reported the reversal of impaired endothelium-dependent vasodilatation with ascorbate infusion suggestive of involvement of ascorbate at the level of the H_1 receptor.

Mak et al (2002) also used a greater degree of hyperoxia which has been shown to elicit greater ROS generation (Solberg et al, 2007) and as such in this scenario ROS may play a central role in the physiological derangement in response to hyperoxia. Furthermore, the present study investigated the response of ROS and antioxidants in the post-exposure period whereas Mak and co-workers infused ascorbic acid during

the exposure where peak ROS concentrations would be expected and therefore most susceptible to mediating deleterious vascular responses.

What is clear from the present findings is that there is an apparent mobilisation of ascorbic acid in the hyperoxic and hypoxic trials and that this likely serves to prevent an increase in lipid peroxidation, suggesting that in the period following hyperoxic exposure the compromised haemodynamic responses are independent of increased ROS concentration.

There is limited data available from studies that have also reported antioxidant indices in response to normobaric systemic O_2 flux during exercise. Bailey et al (2001) documented an increase in LOOH and MDA in the face of increased mobilisation of the lipid-soluble antioxidant α-tocopherol following hypoxic exercise. Davison et al (2006) failed to detect any differences in antioxidant indices in their experimental hypoxic protocol. Animal data indicates that moderate hyperoxia (40% O_2) stimulates increases in tissue accumulation of α–tocopherol and glutathione but not ascorbic acid (Lee et al, 2005). Other than the increase in leucine following exercise *per se* no other notable change was found in venous plasma antioxidant concentrations in the present study. Therefore ascorbic acid appears to be the primary modulator of ROS between conditions in this particular paradigm.

Oxygen Tension & NO· metabolism

The present study also aimed to investigate the effects of systemic O_2 tension on circulating NO· metabolism, finding an increase in RSNO and total plasma NO· concentration only, immediately following hypoxic exercise. Hypoxia has been shown to augment circulating NO· (Pohl & Busse, 1989; Park et al, 1992; Blitzer et al, 1996; Ray & Marshall, 2005; Morgan, 2007; Panesar, 2008; Bertuglia, 2008; Maher et al, 2008). Furthermore, recent data implicates red blood corpuscle (RBC) bound NO· (*S*

nitrosothiol-haemoglobin) as a circulating signal for RBC-induced hypoxic vasodilatation (Rogers et al, 2005; Singel & Stamler, 2005; Angelo et al, 2006; 2008; González-Alonso et al, 2006; Diesen et al, 2008) and that the haeme globin family subserve a critical function as an intrinsic nitrite reductase, regulating responses to hypoxia (Hengden-Cotta et al, 2008). A further recent postulate is that under hypoxic conditions $NO^{\cdot}$ becomes reappointed between metabolite species that correlates with haemoglobin oxygen saturation (Rogers et al, 2007). Thus, whilst an increase in circulating NO_2^- would have added further support to the data, $NO^{\cdot}$ metabolism did appear to respond to the mild hypoxic exercise condition.

The novel and intriguing finding of the present study is that hyperoxic exercise failed to selectively reduce $NO^{\cdot}$ metabolism either in the immediate post-exercise or the extended post-exercise epoch where concomitant haemodynamics revealed a blunted response. Experimental *in vitro* data have previously postulated that hyperoxia degradates $NO^{\cdot}$, mediated via an increase in superoxide (Rubanyi & Vanhoutte, 1986). It does however appear to be vascular bed dependent as some but not all later animal studies implicate hyperoxia in quenching $NO^{\cdot}$ bioavailability (Pries et al, 1995; Mouren et al, 1997; Sauls & Boegehold, 2000; Mak et al, 2002; Zhilyaev et al, 2003; Pasgaard et al, 2007; Taraldsøy et al, 2007). It may be that in similarity to ROS and lipid peroxidation in response to hyperoxia a more aggressive increase in O_2 tension would have caused a significant fall in $NO^{\cdot}$ metabolism and a proportionally augmented SVR. Moreover, the selective increase in ascorbic acid in the hyperoxic exercise condition may have served to preserve the venous concentrations of $NO^{\cdot}$ metabolism via quenching of superoxide but the physiological characteristics of hyperoxia remained.

Therefore, the current results suggest that in support of the previous study in this thesis (study 3) that the vasoconstriction following hyperoxic exercise is mediated via a pathway independent of $NO^{\cdot}$. A critical difference between this study and that of Mak et al (2002) who showed a blunting of endothelial-dependent vasodilatation in hyperoxia was their use of a young healthy cohort. Indeed hyperoxia failed to effect endothelial-dependent dilatation when they tested older subjects with clinical disease (CHF) in the same study. Thus, the use of older pre-hypertensives, in the current paradigm, who demonstrated increased normalised APWV (study 1,2 & methodology) and who likely would have further decreased vascular function in comparison to younger subjects (Jablonski et al, 2007; Moreau et al, 2007; 2005) , indicates that in this cohort control of vascular tone is more complex, involving additional and/or redundant pathways.

As discussed previously post-exercise haemodynamics have been shown to be independent of $NO^{\cdot}$ metabolism in young healthy humans (Halliwill et al, 2000), this study extends these findings and previous hyperoxic vasconstriction studies to report an alternative pathway to $NO^{\cdot}$ metabolism as being responsible for hyperoxia-mediated increases in vascular tone and blood pressure in an older pre-hypertensive population.

O_2 Tension & Haemostasis

A further finding of the present study is that O_2 tension during exercise failed to modulate any effect on haemostatic parameters. Thus while intravascular proteins and increasing blood viscosity may potentially induce effects on endothelial sheer stress, $NO^{\cdot}$ release and consequent vasodilation, this mechanism can be discounted in the present study. Thus, variations in haemostatic factors do not appear to play a part in the post-exercise haemodynamic profile noted presently.

Physiological & Clinical Implications

This study has highlighted the deleterious effects that a relatively short exposure to hyperoxic exercise has on vascular tone and systemic haemodynamics that persists upon return to normoxia. Whilst the role of ROS and redox-mediated reductions in $NO^{\cdot}$ bioavailability cannot be completely excluded as metabolic mediators (due to an increase in circulating ascorbic acid in hyperoxic trial) the findings suggest that further endothelial vasoactive factors are responsible for a significant portion of the vasoconstriction. Thus, whilst prophylactic supplementation (principally with ascorbic acid) may combat against some of the hyperoxic vasoconstriction, especially in the young population, other factors need to be explored to protect against the physiological malfunction noted in the older and/or diseased cohort which appears to be independent of $NO^{\cdot}$ degradation. Likewise, $NO^{\cdot}$ donors (GTN) may be efficacious in modulating some of the vasoconstriction in certain populations but would be anticipated, based on the current findings, to be unsuccessful in specific groups. Clearly there is a need to perform larger pharmacological blockade and infusion studies to fully investigate the role of these metabolites. Previous findings from this thesis suggest that angiotensin II does not play a prominent part in the vasoconstriction but the response of ANP may be crucial. The role of the powerful endothelial vasoconstrictor endothelin 1 has not been documented in this thesis but may play a vital role in the underlying vasoconstriction. Thus, future work should investigate the response of this vasoconstrictor to the current paradigm. Whilst clinical consensous is moving towards the use of lower hyperoxic inspirates the utility of supplemental O_2 to correct hypoxia needs to be cautiously considered due to the physiological consequences of exposure particularly in those people with clinical cardiovascular maladies.

7.4 Conclusion

In conclusion, the present results do not support a role for a redox-mediated regulation of circulating $NO^{\cdot}$ bioavailability as being a principle governor of the attenuated vasodilatation following hyperoxic exercise. This suggests that the vasoconstriction is resultant from a metabolic pathway principally independent of $NO^{\cdot}$.

Chapter 5

8.0 Testing of Null Hypothesis (H_O)

The following section will consider the five hypotheses tested in this thesis.

Hypothesis A – Study 1:

H_0 – An acute bout of dynamic cycle exercise does not induce sustained post-exercise hypotension in a cohort of prehypertensive males.

HYPOTHESIS REJECTED

An acute bout (30-minutes) of dynamic cycle exercise produced a 5% reduction of MABP versus pre-exercise. MABP was reduced by ~5mmHg at the 1-hour post-exercise time-point ($P<0.05$). The PEH persisted for the entire 6-hour post-exercise period, i.e. until cessation of recordings, with a nadir at 60-minutes post-exercise. This fall in MAP resulted in a significantly lower MAP at the 1 and 2-hour recovery time-points during the exercise trial as compared to the same time-points in the non-exercise sham trial ($P<0.05$). SVR was attenuated by ~30% immediately post-exercise and remained blunted, in comparison to baseline, by ~13 and 8% at 60- and 120-minutes post-exercise, respectively ($P<0.05$). This data provides the basis to reject the alternative hypothesis previously stated as *an acute bout of dynamic cycle exercise does not induce sustained post-exercise hypotension in a cohort of prehypertensive males.*

Hypothesis B – Study 2:

H_0 – Systemic oxygen tension during acute dynamic exercise does not mediate post exercise hypotension in a 'PEH-established' cohort of prehypertensive males.

HYPOTHESIS REJECTED

Analysis of the absolute change in MAP from baseline between trials revealed a significant main effect for oxygenation state ($P<0.05$) whereas no interaction effect was observed (state x time; $P>0.05$). The MAP response across the trial was blunted following hyperoxic exercise in comparison to normoxic and hyperoxic exercise (both $P=0.00$) whereas no differences were observed between normoxic and hypoxic exercise (grouped means for state; 4.5 (4) following normoxia, 5.7 (6) following hypoxia and 1.4 (4) mmHg following hyperoxia, change from baseline). In line with the systemic MAP response the reduction in SVR from baseline was significantly less following hyperoxic exercise as compared to normoxic or hypoxic exercise ($P<0.05$, pooled for state). This data provides the basis to reject the alternative hypothesis previously stated as *systemic oxygen tension during acute dynamic exercise does not mediate post exercise hypotension in a 'PEH-established' cohort of prehypertensive males.*

Hypothesis C – Study 3:

H_0 – Post-exercise hypotension is not regulated via free radical-mediated oxidative stress.

HYPOTHESIS REJECTED

Exercise elicited an increase in circulating biomarkers of oxidative stress with elevated LOOH by 60-minutes post-exercise ($P<0.05$) until termination of the protocol. SVR recovered from a nadir of ~ 31% reduction immediately (within 1-minute) post-exercise to ~13 and 8% at 60- and 120-minutes post-exercise, respectively ($P<0.05$). The return in SVR back towards baseline was significant at both the 60- and 120-minutes post-exercise time-points when compared to immediately post-exercise (both $P<0.05$). SVC was augmented following exercise remaining elevated throughout recording although values were blunted compared to post-exercise by 2-hours post exercise ($P<0.05$). MAP fell to its nadir of ~6 mmHg following exercise between immediately post- and 60-minutes post-exercise ($P<0.05$). MAP returned towards baseline over the course of the post-exercise period; with the recovery being significant between 60-minutes and 360-minutes post-exercise. This data provides the basis to reject the alternative hypothesis previously stated as *post-exercise hypotension is not regulated via free radical-mediated oxidative stress.*

Hypothesis D – Study 3:

H_0 – Post-exercise hypotension is not mediated via redox regulation of circulating $NO^{\cdot}$ bioavailability.

HYPOTHESIS REJECTED

The acute exercise bout failed to modify the venous plasma concentration of NO^{-}_3 over the first 1-hour post-exercise period but values were significantly attenuated by 24% and ~31% at the 2- and 6-hour post-exercise time-points, respectively ($P<0.05$). Systemic venous NO^{-}_2 concentration however was unaltered across the trial ($P>0.05$). Exercise also failed to modulate systemic venous RSNO and total plasma $NO^{\cdot}$ concentration with both absolute concentration and the relative change from baseline remaining unmodified across the protocol ($P>0.05$). The data tentatively provides the basis to reject the alternative hypothesis previously stated as *post-exercise hypotension is not mediated via redox regulation of circulating $NO^{\cdot}$ bioavailability.*

Hypothesis E – Study 4:

H_0 – Hyperoxia during acute dynamic exercise does not modify redox regulation of circulating $NO^{\cdot}$ bioavailability coincident with blunted post-exercise hypotension.

HYPOTHESIS ACCEPTED

Hyperoxic exercise failed to modify the venous plasma concentration of NO^{-}_3 over the trial but a significant main effect was noted over time ($P<0.05$; pooled values for time) with a dimunition between immediately post-exercise and termination of the

trial. Systemic venous NO^{-}_{2} concentration however was unaltered across the protocol (P>0.05). Across the trial an elevation in systemic venous RSNO concentration was noted in the hypoxic condition in comparison to the normoxic and hyperoxic trials [18.6 (9) v. 12.8 (9.6) and 12 (9.3) μmol/l, respectively; P<0.05, pooled for state]. A significant time by state effect was apparent with the systemic venous concentration of RSNO following hypoxic exercise being significantly elevated immediately post-exercise when compared to normoxic and hyperoxic exercise. Total plasma $NO^{\cdot}$ displayed an elevation in the hypoxic trial immediately post-exercise when compared to normoxic and hyperoxic exercise (P<0.05). Venous plasma LOOH concentration across the trial was unmodified by varying inspired oxygen fraction but exercise *per se* elicited an increase in lipid peroxidation with elevated LOOH by 60-minutes post-exercise (P<0.05, pooled for time) until termination of the protocol. This data provides the basis to reject the alternative hypothesis stated previously as *hyperoxia during acute dynamic exercise modifies redox regulation of circulating $NO^{\cdot}$ bioavailability coincident with blunted post-exercise hypotension.*

8.1 General Discussion & Realisation of Aims

The main objectives of this section are to: (a) integrate and summarise the research findings of all studies and consider the biochemical and physiological implications of exercise and O_2 tension on post-exercise haemodynamics; and (b) provide direction for future research relating to the investigation of oxidative stress, NO· bioavailability and post-exercise haemodynamics.

8.1.1 Integration & Summary of Research Findings

The overriding characteristic of PEH is a fall in SVR (augmented SVC) in the region of ~30% in both active and non-active skeletal muscle vascular beds (see chapter 2). Whilst neurological contributions to the post-exercise response have been established such as baroreceptor resetting, decreased SNA outflow and modulation of α – adrenergic receptor responsiveness more recent focus has been adjusted towards the potential 'metabolic governors' of the hyperaemic response (see chapter 2).
Strong experimental data now exists that the histamine receptors H_1 and H_2 are implicated in the augmented vascular conductance following exercise (McCord & Halliwill, 2006). Systemic O_2 tension is an independent modulator of vascular tone exerting its effects via multiple pathways. The current research was conducted in order to investigate: (1) the post-exercise haemodynamic response of older pre-hypertensive humans over an extended period in comparison to a non-exercise control condition, (2) the effect of systemic O_2 tension during exercise on post-exercise haemodynamics and circulating vasoactive factors and (3) the role of free-radical mediated oxidative stress and redox regulation of NO· metabolism on the post-exercise response.

Exercise & Arterial Pressure Control: Establishment of PEH in Prehypertension

The findings from study 1 suggest that an acute bout of moderate intensity exercise exerts a modest fall (~6 mmHg) in arterial pressure that is sustained for an extended period of time (6-hours). The fall in MAP equates to a significantly lower afterload when compared to both pre-exercise baseline and non-exercise control data taken at the same time of day. The clinical utility of a modest fall in MAP requires further investigation but the pressure responses transcend into a sustained reduction (~20%) in systemic vascular resistance and reciprocal increase in vascular conductance that is maintained up to 2-hours post-exercise but significantly recovers towards baseline.

For the first time simultaneous determination of systemic arterial pulse wave velocity during PEH revealed that compared to the non-exercise control condition upper limb velocity is reduced (23%) immediately following exercise but significantly recovers within 2-hours. Normalised (for arterial pressure changes) values indicate that the differences are solely due to changes in distending arterial pressure and not inherent mechanisms of arterial wall wave propagation. Lower limb velocity appears resistant to exercise (study 1). The upper limb velocity (and normalised values) appears to decrease significantly (18%) following exercise and significantly recover above baseline (9%) over the post-exercise period (pooled values for state: study 2).

Circulating Vasoactive Factors Mediating Post Exercise Hyperaemia

Findings from this research (study 1 & 2) reveal that several 'conventional' circulating vasoactive factors cannot be implicated as essential candidates required for mediating the haemodynamic response. In support of prior work the catecholamines adrenaline & noradrenaline, the RAAS axis and arginine vasopressin are dissociated with the hyperaemic response. Atrial natriuretic peptide may exert an effect on the

noted responses. A significant increase in venous concentration following exercise was noted followed by a decline in post-exercise concentrations with a concomitant blunting of the vasodilatory response (study 1 & 2). Thus, it appears that ANP secretion or its residual effects may be required to initiate and maintain a degree of hyperaemia but cannot be completely responsible for the reductions noted in MAP over this time period.

The finding that ANP played a role in modulating vascular tone and haemodynamics fits well with data highlighting naturiuretic peptides as anti-inflammatory regulators of macrophage function, directly influencing histamine release from mast cells. Therefore a decreased bioavailability of ANP would potentially mediate histamine release from mast cells and basophils limiting the potential for binding of histamine to its H_1 and H_2 receptors. It may be that increases in ANP immediately post-exercise stimulate histamine release and that ANP mimetics may potentiate PEH.

O_2 Tension & Vascular Tone: Implications for Post-Exercise Haemodynamics

The results of study 2 demonstrate the vasoconstrictive effects of acute moderate hyperoxia. In line with the systemic mean arterial pressure response the reduction in SVR from baseline was significantly less following hyperoxic exercise as compared to normoxic or hypoxic exercise ($P<0.05$, pooled for state). In agreement with previous findings increased vascular tone persisted during recovery in normoxia. Thus, hyperoxia exerts a reflex vasoconstrictor effect that persists for up to 2-hours post-exposure/exercise and must be used with caution especially in the hypertensive population. The finding that ANP was selectively decreased in hyperoxia and that changes in MAP were related to changes in ANP concentration only following

hyperoxic exercise ($r = 0.50$, $P<0.01$), reveals that this metabolite may be a dominant vasoactive factor responsible for the deleterious effects of hyperoxic exposure.

Circulating 'Novel' Vasoactive Factors Mediating Post Exercise Hyperaemia

Results from study 3 indicate that free radical-mediated oxidative stress exerts a decline in post-exercise haemodynamics. Free-radical mediated lipid peroxidation increased in from 60-minute post-exercise until cessation of the trial. The increase in lipid peroxidation appeared concomitantly with a blunting of vasodilatation in the post-exercise period. Whilst certain radical species such as H_2O_2 may exert vasodilatation in specific vascular beds (coronary; forearm skeletal muscle) it appears that in the present paradigm augmented oxidative stress exerted a deleterious effect on post-exercise hyperaemia. In parallel to this finding the data also suggest that endogenous antioxidant reserves in older pre-hypertensive individuals are inadequate to fully buffer and ameliorate free-radical induced vasoconstriction. ROS have previously been shown to facilitate histamine release from mast cells, such that the augmented ROS production may potentiate vasodilatation by both primary mechanisms and secondary effects on histamine activation of H_1 and H_2 receptors. The discovery that increased lipid peroxidation occurred with negative rather than salutary effects on post-exercise haemodynamics (study 3) implies that the vasoconstrictive effect of increased ROS predominates any potential secondary dilative effect of histamine receptor sitmulation.

Study 3 also highlighted a significant attenuation of circulating nitrate, a principle component of $NO^{\cdot}$ metabolism, during the post-exercise recovery period with a blunting of peak hyperaemic responses. This finding implicates a potential redox

regulation pathway of $NO^{\cdot}$ as being a mechanism by which free radical-induced oxidative stress blunts the degree of PEH over the recovery period.

Free Radical Mediated Oxidative Stress & Redox Regulation of $NO^{\cdot}$ metabolism: Role in Hyperoxia-induced vasoconstriction

Study 4 demonstrated that free radical mediated oxidative stress is not an essential 'metabolic governor' of the increased vascular tone following hyperoxia. Furthermore, the data show that an increase in O_2 tension induced a mobilisation of ascorbic acid in hyperoxia that served to lessen any further damaging effects of increased oxidative stress on the vasculature. In accordance with a mobilisation of ascorbic acid, study 4 shows that markers of $NO^{\cdot}$ metabolism were not attenuated following hyperoxia. This argues against a redox regulation pathway of $NO^{\cdot}$ metabolism as a primary mediator of blunted vasodilatation in this scenario and is in agreement with previous results of a more complex regulation of arterial tone in older subjects with predisposing malformation in cardiovascular function (study 4).

It is important to note that the studies presented in this thesis utilised a very distinct cohort (male, pre-hypertensive, non-smokers, not on antihypertensive medication or any form of supplements and free from any other overt cardiovascular disease) that precluded the use of a large subject group. Prospective power of the test calculations were made in all studies and repeated measures tests have inherently higher degrees of power (Altman, 1991). Retrospective calculations revealed generally acceptable power in all studies. Thus conclusions drawn in this thesis may be treated with a

minor degree of caution, whilst it is hoped that future work may add to the findings included in this thesis.

As evident in the preceeding and following sections numerous questions still remain in the area of PEH, vascular tone, O_2 tension and metabolic mediators of the response. It is therefore hoped that the work contained in this thesis provides a strong foundation and helps to generate future research studies.

8.1.2 Directions for Further Research

I. This work was conducted on a relatively small homogenous sample, recent data has suggested that there is no gender difference in the post-exercise haemodynamic response (Lynn et al, 2007) while other data suggests that older males exhibit the greatest sensitivity to free-radical modulation of arterial tone (Moreau et al, 2007). Therefore there is a general need to compare these findings to those when investigating a young 'healthy' sample population.

II. There is a need to extend the protocol utilised in the present study to control gas inspiration in baseline and recovery periods to evaluate the potentiated effect of O_2 tension at these time-points. Furthermore, this adapted paradigm would allow a full dissociation of O_2 tension from relative work-load to be made.

III. There is a general need to provide further quantification of the free radical response during the post exercise period. This is recommended to be achieved through implementation of concomitant *ex vivo* spin trapping and electron spin resonance spectroscopic (ESR) detection of free radical species together with lipid peroxidation, protein and DNA oxidation markers. ESR is regarded as the

most sensitive, specific and direct method of measuring free radical species (Davison et al, 2008a). This would provide additional information on the free radical response post-exercise.

IV. It is suggested that the natural progression from this body of work is to conduct an ascorbic acid infusion trial during whole body normoxic exercise and document the consequent effects on post-exercise haemodynamics, ideally in both cohorts noted in point I. This would provide powerful data on the modulatory influence of ROS on post-exercise haemodynamics in health, hypertension and senesence. Furthermore, biochemical determination of histamine should occur simultaneously and blockade of H_1 & H_2 receptors in a second arm of investigation to study the potential interactions between these metabolites alluded to previously in this thesis.

V. With regards to normoxic exercise and PEH, future work should investigate the role of metabolite receptor sensitivity in modulating post-exercise haemodynamics. Little data currently exists documenting the endogenous response of the metabolites reported in this thesis and the role of receptor sensisitivity. Therefore, trials should investigate the role of angiotensin II, AVP and ANP receptor sensitivity in aging and hypertension. Moreover, endothelin-I was not measured in this thesis. It is recommended that the response of this powerful vasoconstrictor be documented when adopting the present protocol and in the future trials suggested in this section.

VI. This thesis only used venous markers of biochemistry that are distal to the sites of the active vasculature/musculature. Therefore future studies should attempt to replicate the findings but with arterial and venous samples taken

from the active sites together with a distal venous source. This would provide a complete picture of the circulatory response.

VII. The role of ANP infusion and receptor sensitivity need to be investigated in hyperoxic vasoconstriction together with an evaluation of free-radical antagonism and $NO^{\cdot}$ donor infusion and blockade. This would provide a complete analysis of the function of these factors in this physiological phenomenon.

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Appendices

Appendix I - Medical History Questionnaire

STRICTLY PRIVATE AND CONFIDENTIAL

MEDICAL QUESTIONNAIRE

Please complete the following as fully as possible:-

Last Name _____ Initials _____ Mr./Mrs./Miss

Forenames _____ Previous Names _____

Address _____

_____ Postcode _____

Telephone _____ Birthdate _____

Sex _____ Occupation _____

Nationality _____ Religion _____

Marital Status _____ No. of Children _____

Date of Last Screen _____

Name of General Practitioner _____

Full Address _____

_____ Postcode _____

Telephone _____

N.B. Part of our service is to report to your G.P., therefore it is very important for you to provide the information when you attend.

2. Please list any major illnesses, operations, hospitalisations, major injuries or other important medical problems that you have had throughout your life.

DATE	OPERATION OR MEDICAL PROBLEM

3. Are any of these conditions still troublesome at the present time? No ☐ Yes ☐

If so, which ones? _____

4. Are there any drugs, insect stings or other substances to which you have had an adverse or allergic reaction? If so, what? No ☐ Yes ☐

Drug or other substance	Reaction (e.g. skin rash, breathing difficulty)

5. Please list all medication which you take regularly (more than once a month) including all non-prescription medications such as laxatives, painkillers, sleeping pills, aspirins, vitamins, etc.

Medicine	Frequency of use (No. of times per day or week)

6. Are you on any special diets? If yes, give brief details. No ☐ Yes ☐

_____

7. Have any close relatives had high blood pressure, a stroke, heart attack or angina occurring before the age of 55? If so, list relationship, disease and age of occurrence. No ☐ Yes ☐

_____

8. Are there any of the following diseases in your parents, brothers or sisters? No ☐ Yes ☐

Epilepsy, fits or fainting No ☐ Yes ☐

Alcoholism No ☐ Yes ☐

Thyroid problems No ☐ Yes ☐

Gout No ☐ Yes ☐

Glaucoma No ☐ Yes ☐

Mental Illness No ☐ Yes ☐

Cancer No ☐ Yes ☐

Other No ☐ Yes ☐

9. Do you smoke? No ☐ Yes ☐

If so, what? _____

What quantity? _____

How many years have you smoked? _____

If you have stopped smoking, how long since you smoked? _____

10. Do you have any regular form of exercise? If so, describe. No ☐ Yes ☐

_____

_____

_____

_____

_____

11. How much of the following alcoholic beverages do you drink on average per week?

Bottles of wine _____

Cans or bottles of beer _____

Spirits _____

If you drink alcohol:

a) Have you ever felt that you should cut down on your drinking? No ☐ Yes ☐

b) Have people annoyed you by criticising your drinking? No ☐ Yes ☐

c) Have you ever felt guilty about drinking? No ☐ Yes ☐

d) Have you ever had a drink first thing in the morning to steady your nerves? No ☐ Yes ☐

e) Does your drinking tend to conflict with family, friends or at work? No ☐ Yes ☐

f) Because of drink have you ever lost your driving licence or job? No ☐ Yes ☐

12. List your approximate weight _____ No ☐ Yes ☐

Over the last year has your weight increased/decreased/remained stationary?

_____

13. Are you having any problems with your eyesight? If so, describe. No ☐ Yes ☐

_____

_____

14. Are you having difficulty with your hearing? If so, describe. No ☐ Yes ☐

_____

_____ No ☐ Yes ☐

15. Are you having any problems with your nose, throat, mouth or ears (other than hearing)? No ☐ Yes ☐

16. Do you now or have you ever suffered from asthma or become unusually short of breath? No ☐ Yes ☐

17. Do you have a persistent cough producing phlegm or sputum at times other than when you have a chest cold? No ☐ Yes ☐

18. Have you ever had tuberculosis? No ☐ Yes ☐

19. Have you ever been told that you have a heart murmur? No ☐ Yes ☐

20. Do you regularly get chest pain or discomfort from activity or at other times? No ☐ Yes ☐

21. Have you ever had rheumatic fever? No ☐ Yes ☐

22. Have you ever had a low blood count, anaemia or any other form of blood disorder?

23. Do you have unusual and persistent stomach pain, indigestion or trouble with bowel movements, such as constipation or diarrhoea? If so, describe.

_____

_____

_____

24. Has there ever been blood in your motions or have your motions ever been black? No ☐ Yes ☐

25. Have you ever had hepatitis, liver disease, jaundice or gall bladder disease? No ☐ Yes ☐

26. Have you ever had a venereal disease (V.D.)? If so, describe disease, year and treatment given. No ☐ Yes ☐

_____

_____

_____

_____

_____

10. Do you have any persistent trouble with urination or kidney, bladder or prostate problems? No ☐ Yes ☐

11. Do you suffer from any skin diseases? If yes, give details. No ☐ Yes ☐

_____

_____

12. Are there any health hazards that you are exposed to at work such as toxic chemicals, dusts, fumes, etc.? No ☐ Yes ☐

13. Have you ever had any x-rays? If so, give details and any dates. No ☐ Yes ☐

_____

_____

_____

_____

14. Do you suffer from any disorder of your bones, joints, ligaments or muscles? No ☐ Yes ☐

15. Do you suffer from backache, lumbago or sciatica? No ☐ Yes ☐

16. Have you begun to have regular headaches or are you having a different kind of headache or more severe headaches than you have had previously? If so, describe. No ☐ Yes ☐

_____

_____

_____

_____

17. Stress and Personality Evaluation.

Please tick whichever of the following applies to you:

Are you?

a) Rarely tense or anxious ☐

b) Calmer than average, very occasionally feel tense. ☐

c) Tense or anxious two or three times per week. ☐

d) Quite tense, usually rushed, feel tense two or three times a day. ☐

e) Extremely tense, often feel that life is hard to cope with. ☐

Have you ever been hospitalised or treated for anxiety depression or other mental disorder? If so, describe.

_____

_____

_____

_____

_____

Do you feel that you are more nervous or depressed than other people? No ☐ Yes ☐

Do you have regular difficulty with sleeping? No ☐ Yes ☐

Do you find yourself waking early? No ☐ Yes ☐

Do you find yourself waking up feeling unrested? No ☐ Yes ☐

Have you ever felt constantly under strain? No ☐ Yes ☐

Have you recently felt unable to enjoy your normal activities? No ☐ Yes ☐

Have you recently been losing confidence in yourself? No ☐ Yes ☐

Do you worry about your health? No ☐ Yes ☐

Has your libido (sexual desire) decreased recently? No ☐ Yes ☐

Are there any sexual problems you would like to discuss? No ☐ Yes ☐

What bodily and mental signs do you get to help you to know when you are under stress?

_____

_____

Have any major life events or crises happened to you in the last year or so? If so, describe. No ☐ Yes ☐

_____

_____

What are your usual methods of coping with stress, unwinding or relaxing?

_____

_____

35. Employment

If you are in full-time or part-time employment, please answer the following:

Present occupation _____

Company _____

Salaried No ☐ Yes ☐

Self-employed No ☐ Yes ☐

Length of time with present company _____

Do you enjoy your work? No ☐ Yes ☐

Are you given enough responsibility? No ☐ Yes ☐

Do you take work home with you at nights or at weekends? No ☐ Yes ☐

Are you worried about the security of your job? No ☐ Yes ☐

Are you too tired by your work to do anything active afterwards? No ☐ Yes ☐

Do you have time to eat properly at work? No ☐ Yes ☐

Is your present workload light/moderate/heavy? _____

How many hours do you spend each day at work? _____

How much time do you take off for holidays each year? _____

36. Domestic and Social History

Marital status _____

Are there any problems with the health of your spouse/partner? No ☐ Yes ☐
If so, please give details.

_____

_____

Please state ages and sex of any children.

_____

_____

Are there any problems with the health of your child/children? No ☐ Yes ☐

_____

_____

What leisure activities and hobbies do you enjoy?

_____

_____

37. Lastly, what are your reasons for choosing to come for a health screening and what do you hope to gain from it?

_____

_____

_____

_____

FOR WOMEN ONLY

1. At what age did your menstrual periods start? _____

2. Have your periods stopped? No ☐ Yes ☐
If so, when. _____

3. Are you having any problems with the menopause (change of life)? No ☐ Yes ☐
If so, describe _____

_____

4. Are you having any trouble with your periods, such as excessive bleeding, pain, etc? If so, describe. No ☐ Yes ☐

_____

5. Are your periods regular? No ☐ Yes ☐

6. Is there any bleeding between periods? No ☐ Yes ☐

7. Is there any bleeding after intercourse? No ☐ Yes ☐

8. Have you any persistent or unusual vaginal discharge? No ☐ Yes ☐

9. Do you have any other gynaecological problems that you would like to discuss with your doctor? If so, describe No ☐ Yes ☐

_____

_____

_____

10. Have you had any gynaecological operations? If so, please list below.

DATE	OPERATION

11. Family planning method (if applicable)

a) Birth Control Pill ☐

b) Intrauterine Device (IUCD) ☐

c) Diaphragm (Cap) ☐

d) Condom ☐

e) Vaginal Spermicide ☐

f) Rhythm Method ☐

g) Sterilisation ☐

h) Partner Vasectomy ☐

12. If you have been on "the Pill",
How long for? _____
When did you stop? _____

13. Are you taking any form of hormone therapy? No ☐ Yes ☐

14. How many times have you been pregnant? Number: _____

15. Any miscarriages? No ☐ Yes ☐

16. Any abortions? No ☐ Yes ☐
Number _____

17. When was your last cervical smear? _____ No ☐ Yes ☐
Was it normal?

18. Are you having any problems with your breasts? If yes, describe _____ No ☐ Yes ☐

_____

_____

19. Have you had any previous breast surgery? If yes, please give details _____ No ☐ Yes ☐

_____

_____

20. Is there any history of breast disease in your family? If yes, please describe _____ No ☐ Yes ☐

_____

_____

Appendix II – Subject Information Sheet & Informed Consent

Informed Consent

Explanation of the Tests

Protocols Used: Incremental exercise stress test. Exercise to maximal volitional exhaustion; constant load sub-maximal exercise on a cycle ergometer at 70% of maximum for 30-minutes.

Number of Tests: 1 maximal test; 3 sub-maximal tests.

Other Measurements being taken: Metabolic and respiratory exchange capacities [online analysis], 12-lead ECG, manual sphymomanometry, psychological perceived exertion, echocardiography, pulse wave analysis, indwelling venous cannula for multiple blood withdrawals, ear-lobe capillary sampling, finger prick sampling.

Risks and Discomforts

Abstention from foods etc. Nil for maximal test but each of the sub-maximal tests requires an overnight fast.

Major risks, discomforts and potential harmful occurrences: Local muscular fatigue in the lower extremities, high blood pressure, heart conduction anomalies, 'light headedness' and adverse cardiovascular regulation in response to exercise or changes in oxygen inspired, pain from venepuncture.

Safety measures being taken: ECG recording continuously [resting and exercise], blood pressure monitoring, semi-automatic defibrillator, medical supervision.

Confidentiality
The researching party, on co-signing this form with you, the subject, shall use the information collected for purposes of their research alone and in the strictest confidence, divulging no personal or physiological data to other parties at any time.

Inquiries
Questions about the procedures carried out are encouraged. If you have questions about the tests or need additional information please ask for further explanation.

Freedom of Consent
Your permission to perform these tests is strictly voluntary. You are free to stop the tests at any point if you so desire.

I have read this form carefully and I fully understand the test procedures that I will perform and the risks and discomforts that I may experience. Knowing these and having had the opportunity to ask questions that have been answered to my satisfaction, I consent to participate in these tests.

Signature of subject Signature of investigator/witness Date

Subject Information Sheet

'Exercise Performance for High Blood Pressure: Varying Inspired Oxygen as a Potential Mediator'

Thank you for expressing an interest in this study. The following information explains why the investigators are doing the study, the benefits and possible discomforts of your participation and what would be required from you during this study. If you are willing to take part you will be asked to sign a consent form.

WHY IS THIS STUDY BEING DONE?

Exercise performance has been implemented as a potent mediator for borderline [pre] hypertension. The modulation in blood pressure has been noted for several hours after a single exercise bout. A reduction in the inspired fraction of oxygen in the air is termed hypoxia whereas an increase is hyperoxia. Both hypoxia and hyperoxia can have an effect on several hormones and related factors in the body that may alter the blood pressure response to exercise in these conditions. Little information exists on the exact response of post exercise blood pressure and cardiovascular function following hypoxic and hyperoxic exercise.

We would like to investigate the effect of exercise (fixed cycling at 70% of your maximum capability for 30-minutes) breathing varying percentages of oxygen (1: normal 21% atmospheric oxygen, 2: hypoxic 16%, 3: hyperoxic 60%) on subsequent blood pressure, cardiovascular and metabolic responses.

We hope the results will contribute towards gaining more information about the control of blood pressure following exercise and elucidate potential mechanisms in the response to the additional variation of oxygen levels.

WHAT ARE WE ASKING YOU TO DO?

Subjects should arrive at the lab fully hydrated after an overnight fast.

Week One: Subjects will undergo a series of measurements including height and weight. Subjects will also undergo an incremental test to exhaustion to determine their individual maximum oxygen uptake. Subjects will have a 12-lead ECG to monitor cardiovascular responses to the test and blood pressure monitored throughout. This will be randomised with week two.

Week Two: Subjects will be required to remain in the laboratory for 8-hours to track the blood pressure responses throughout the non-exercise day. The first three hours will involve constant adoption of a semi-recumbent position. The additional time will require remaining in the laboratory with minimum activity and returning to the semi-recumbent position 30-minutes before the hour mark.

Week Three: Subjects will be randomly assigned to either week two, three or four. Cycling exercise will be performed at 70% of your determined maximum for 30-minutes in 16% oxygen [hypoxia]. Cannulation of an antecubital vein will be

performed by a trained technician prior to the test to sample blood. Blood samples will also be taken at six time points post-exercise. At every time point of venous blood sampling you will also have an ear lobe puncture to obtain information regarding various compartments of the blood.
You will have blood pressure recorded throughout the trial by manual sphygmomanometry. You will have a measurement of arterial reactivity ; in essence a marker of arterial 'health' throughout the trial as well as echocardiographic Doppler imaging of the heart to obtain additional information regarding the status of the cardiovascular system.
You will be required to remain fairly stationary during the first three hours following exercise and will then be allowed some movement around the laboratory. On some occasions the total time you will be in the laboratory will be 9am to 5pm but a standardised lunch will be provided on all occasions.

Week 4: The test is the same as week three but the exercise will be performed in 60% oxygen [hyperoxia]. All measurements and times are exactly the same.

Week 5: Same as weeks three and four but perform the remaining test in 'normal' sea-level conditions 21% oxygen [normoxia].

DO I NEED TO TAKE PART?

No. If you decide to volunteer but then subsequently change your mind you can withdraw at any stage. If you do decide to then you do not have to give a reason.

WHAT MIGHT HAPPEN TO ME?

As the initial exercise test involves exercising to maximum then subjects will probably feel very fatigued at the end of exercise. Possible implications may be high blood pressures during the initial and subsequent exercise tests. The hypoxia trial represents a modest altitude of ~2500m. If you have experienced any medical complications with airline travel or on holiday in altitude regions you **MUST** inform the investigator prior to undertaking this study. Constant supervision from our experienced investigators will ensure the subject's well-being via continual monitoring of the subject and specific physiological function throughout the test.

WHO WILL SEE ALL THE INFORMATION ABOUT ME?

In accordance with the Data Protection Act (1973) data will be confidential and subjects will only in general be identified by a subject number. Personal details, which may indentify an individual, will be held separately from the rest of the research data and will only be held to enable any questions, which arise during the research, to be answered. This data will only be seen by the main researchers and used for statistical analyses.

WHAT INFORMATION WILL I RECEIVE?

Upon completion of the study each subject will receive a complete breakdown of the results and a consultation with the investigator regarding the findings.

CONTACTS

Further information on the study can be obtained from any one of the investigators present or alternatively contact:

Professor Bruce Davies
Health and Exercise Sciences
University of Glamorgan
CF37 1DL

APPENDIX 3

DIETARY QUESTIONNAIRE

Name: **Date:**

Try to recall exactly what you have consumed over the last 48-hours, including the approximate time. Identify all food and drinks including alcoholic beverages. Indentify food portions as: small (S), medium (M) or large (L).

Day:

Breakfast:

Lunch:

Evening Meal:

Snacks: