1994

The effect of altered blood oxygen content on respiratory gas exchange dynamics

Mark Andrew Osborne
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The effect of altered blood oxygen content on respiratory gas exchange dynamics

A thesis submitted in partial fulfilment of the requirements for
the award of the degree

Master of Science (Honours)

from

The University of Wollongong.

Mark Andrew Osborne, B.App.Sc.

Department of Biomedical Science
1994
The effect of altered blood oxygen content on respiratory gas exchange dynamics.

ABSTRACT

Two theories describe the limiting factors existing during exercise, determined by factors effecting the entire respiratory gas exchange sequence from $O_2$ extraction to its use at the mitochondria. The first implicates $O_2$ transport (a central limitation), while the second focuses on oxygen utilisation at the periphery. This study aimed to identify which factors may limit gas exchange response dynamics during submaximal exercise. This was accomplished by altering blood oxygen content, either through manipulating haematocrit (Hct), or through changes in $F_{O_2}$. Six physically active male subjects (age $= 24.8 \pm 2.8$ (SD) yrs) undertook aerobic power ($V_O^{2peak}$) tests in each of three stages of altered haematocrit: (i) control (Hct $= 40.0 \pm 2.0\%$, $V_O^{2peak} = 5.58 \pm 0.6$ l.min$^{-1}$); (ii) anaemia (withdrawal of $-1350$ ml of whole blood: Hct $= 35.0 \pm 2.0\%$ (p<0.05), $V_O^{2peak} = 4.68 \pm 0.46$ l.min$^{-1}$ (p<0.05)); and (iii) polycythaemia (autologous reinfusion: Hct $= 44.0 \pm 2.0\%$ (p<0.05), $V_O^{2peak} = 5.06 \pm 0.74$ l.min$^{-1}$). Three submaximal tests, involving a single step-change in workrate from rest to $\approx 45\%$ of the condition-specific $V_O^{2peak}$, were completed in each state of altered Hct, with subjects breathing hyperoxic ($F_{O_2}=0.3$), normoxic ($F_{O_2}=0.2$) or hypoxic gases ($F_{O_2}=0.1$). Results show that following a step-change in workrate, there were no changes in oxygen consumption ($\dot{V}_O$), carbon dioxide elimination ($\dot{V}_{CO_2}$), minute ventilation ($\dot{V}_E$) or cardiac frequency ($f_C$) dynamics following blood withdrawal or subsequent reinfusion, suggesting a compensatory mechanism in $f_C$, may have nullified any change in oxygen delivery, or else oxygen utilization by the mitochondria may be rate limiting. Under hypoxic conditions, there were no differences in the times to reach 20%-80% of the $\dot{V}_O$ post-step plateau, although the time to reach 100% was longer in the anaemic state (p<0.05). Similar responses were observed for $f_C$ and $\dot{V}_E$ dynamics. The dynamics of $\dot{V}_{CO_2}$ for a step-increase in workrate, again showed that under hypoxic conditions, the increase tended to be faster than under normoxic or hyperoxic conditions. Following a step-decrease in workrate, hypoxia slowed all responses (p<0.05), while hyperoxia did not accelerate the responses relative to the normoxic responses (p>0.05). Cardiorespiratory response dynamics appear unaffected by minor changes in the Hct and blood $O_2$ content. This may imply that, the speed with which exercising tissues adjust to a step-function is influenced by peripheral factors, rather than by central factors, with $O_2$ delivery exceeding $O_2$ utilisation requirements. However, with a larger reduction in blood $O_2$ content, a slowing of the response dynamics may be ascribed to a predominantly central limitation. Thus, it appears that within certain $O_2$ delivery ranges, $\dot{V}_O$ dynamics are resilient to minor changes in $O_2$ delivery.
ACKNOWLEDGEMENTS:

Throughout the last three years, I have been guided and assisted by a number of people, and I wish to express my sincerest appreciation for their efforts and extend my gratitude to them. Firstly, a sincere thankyou to my supervisors, Dr. Nigel Taylor for his advice and encouragement throughout the last three years, and Dr. Ian Mackenzie also, for his advice and generosity in both time and equipment. I also wish to extend my thanks to Angus Taylor of Tuta Laboratories, and Des Comer of the Illawarra Regional Hospital, Wollongong Campus for their invaluable assistance, as well as the South Coast Blood Bank staff, for their friendliness, time and assistance. Thanks also to a number of friends and fellow students who have been helpful in both this and previous years, particularly Tara, Duncan, Patto, Jim, Jodie, Glenn, Jarrod, Bruce, Jenny and Graeme, for their assistance, some of whom were also willing to be subjected to such invasive research ..... and the fun. Good Luck in your own careers.

Finally, I wish to acknowledge a number of friends, apart from those previously mentioned, whose company I have had the pleasure to enjoy over the past few years and would not have missed for the world! Particularly Sloth, Shep, Lenny, Simone, Willy, and Sue.
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Item</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>i</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>ii</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>iii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xvi</td>
</tr>
</tbody>
</table>

CHAPTER ONE: INTRODUCTION..........................................................1

1.1 INTRODUCTION...........................................................................2

1.2 AIMS......................................................................................6

1.3 HYPOTHESES..........................................................................7

CHAPTER TWO: LITERATURE REVIEW..................................................8

2.1 INTRODUCTION......................................................................9

2.2 CARDORESPIRATORY EXERCISE ONSET RESPONSES..................11

2.3 CARDORESPIRATORY EXERCISE OFFSET RESPONSES...............13

2.4 DETERMINANTS OF EXERCISE ONSET PHASE TWO DYNAMICS:
    DELIVERY VERSUS UTILIZATION...........................................15

2.5 EXPERIMENTAL INTERVENTIONS PERMITTING EVALUATION OF THE
    OXYGEN DELIVERY HYPOTHESIS..........................................19
4.3.4 Overall cardiorespiratory responses

4.3.4 Respiratory responses to a step-increase in work rate

CHAPTER FIVE: DISCUSSION

5.1 EXPERIMENTAL MANIPULATIONS AND RESULTANT HAEMATOLOGICAL CHANGES

5.1.1 Blood volume following phlebotomy

5.1.2 Red blood cell turnover and blood volume following reinfusion

5.1.3 Blood viscosity

5.2 THE EFFECT OF ANAEMIA AND POLYCYTHAEMIA ON MAXIMAL EXERCISE

5.3 EFFECTS OF ALTERED OXYGEN TRANSPORT AND SUPPLY ON CARDIORESPIRATORY DYNAMICS DURING SUBMAXIMAL EXERCISE

5.3.1 Altering oxygen delivery by changing haematocrit

5.3.2 Altering oxygen supply by changing inspired oxygen concentrations

5.4 SUMMARY

CHAPTER SIX: CONCLUSIONS and RECOMMENDATIONS

6.1 CONCLUSIONS

6.2 RECOMMENDATIONS
REFERENCES............................................................................................................................138

APPENDICES........................................................................................................................................167

Appendix A. Determination of the number of trials required during submaximal testing...168

Appendix B. Determination of the optimal duration between consecutive submaximal
trials................................................................................................................................171

Appendix C. Test-retest reliability for the oxygen consumption dynamic procedure........174

Appendix D. Validation of the Ohmeda Biox 3700e pulse oximeter (with ear probe)
as a heart rate recording device during sub-maximal exercise........................................176

Appendix E. Quality control tests prior to reinfusing thawed blood.................................179

Appendix F. A case study of mild and severe anaemia on gas exchange dynamics........183

Appendix G. Results of blood tests determined by the Coulter Cell Counter......................189

Appendix H. Sum of integrated data averaged over 5 second intervals.............................191

Appendix I. Individual data for $\dot{V}_{O2peak}$ tests.................................................................195
### LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.1: Three phase response for $\dot{V}_{O_2}$ uptake following a step-increase in workrate</td>
<td>4</td>
</tr>
<tr>
<td>Figure 2.1: Possible sites in the oxygen transport and utilization processes which may limit the body's ability to instantaneously elevate its energy production</td>
<td>10</td>
</tr>
<tr>
<td>Figure 3.1: Experimental set-up of equipment for peak aerobic power tests: A: Quinton Q-Plex 1; B: Ohmeda Biox 3700e pulse oximeter; C: Lode electronically braked cycle ergometer; D: PE 3000 Sportstester</td>
<td>33</td>
</tr>
<tr>
<td>Figure 3.2: Validation of the Quinton Q-Plex 1 by comparisons of: (A) oxygen concentrations for Q-Plex I and Applied Electrochemistry analyser ($r=0.956$); (B) carbon dioxide concentrations for Q-Plex I and Beckman analyser ($r=0.972$); and (C) expired tidal volumes for Q-Plex I and water-filled, wedge spirometer ($r=0.999$). Data taken from Solomon (1991) with permission</td>
<td>36</td>
</tr>
<tr>
<td>Figure 3.3: Relationship between Hewlett-Packard oximetric and arterial blood $O_2$ saturation ($S_aO_2$) during hypoxic exercise. Data taken from Smyth et al., (1986)</td>
<td>39</td>
</tr>
<tr>
<td>Figure 3.4: Skin blood flow (SkBF) response to Metsal® application on the earlobe. Data are mean with standard error of the mean. Response was virtually complete within one minute of application. Note that treatment mean corresponds to saturation of Laser Doppler Flow (LDF) signal ($p&lt;0.05$), and volts are analogous to blood flow measured in ml.min$^{-1}$.gm$^{-1}$</td>
<td>40</td>
</tr>
<tr>
<td>Figure 3.5: Haemonetics® 30 Blood Processor</td>
<td>53</td>
</tr>
<tr>
<td>Figure 3.6: Schematic for pheresis kit for Haemonetics® 30 Blood Processor. Electromedics Inc</td>
<td>54</td>
</tr>
<tr>
<td>Figure 3.7: Reinfusion of deglycerolysed red blood cells (D-RBC's) through a 16 guage canular into the forearm</td>
<td>56</td>
</tr>
<tr>
<td>Figure 3.8: Summary of the five stages of data analysis for exercise onset data. Figure 3.8a is the raw data with the polynomial curve fit data, 95% confidence limits, and the data marked for exclusion. Figure 3.8b shows five breath running average to smooth data, and also shows plateaux. Figure 3.8c shows the final polynomial curve for these data, anchored at the pre-step plateau, and determined oxygen uptake at 20%, 40%, 60%, 80%, and 100% of the difference between pre-step and step-up plateaux</td>
<td>59</td>
</tr>
</tbody>
</table>
Figure 4.1: Arterial oxygen concentration ($C_{a}O_{2}$) averaged over 30 second intervals, during an exercise step-function from rest (5 min) to 55% aerobic power (6 min), and back to rest again (5 min). Graphs correspond with data collected during artificially-induced anaemia (A), control (B), and artificially-induced polycythaemia (C), and then across three levels of inspired oxygen: hypoxia (D), normoxia (E) and hyperoxia (F). Data are means with standard errors of the means.

Figure 4.2: Arterial oxygen saturation ($S_{p}O_{2}$) averaged over 30 second intervals, during an exercise step-function from rest (5 min) to ~45% aerobic power (6 min), and back to rest again (5 min). Graphs correspond with data collected during artificially-induced anaemia (A), control (B), and artificially-induced polycythaemia (C), and then across three levels of inspired oxygen: hypoxia (D), normoxia (E) and hyperoxia (F). Data are means with standard errors of the means.

Figure 4.3: The raw data (breath-by-breath) for oxygen uptake ($\dot{V}_{O_{2}}$; Graph A), carbon dioxide elimination ($\dot{V}_{CO_{2}}$; Graph B), minute ventilation ($\dot{V}_{E}$; Graph C), and cardiac frequency ($f_{C}$; Graph D), observed under hypoxic ($F_{IO_{2}}=0.1$), normoxic ($F_{IO_{2}}=0.2$) and hyperoxic ($F_{IO_{2}}=0.3$) conditions for Subject N3 during a submaximal step-exercise (cycling) forcing function from rest to ~45% $\dot{V}_{O_{2}peak}$, and returning to rest.

Figure 4.4: Oxygen uptake dynamics observed under hypoxic, normoxic and hyperoxic conditions. Data show oxygen uptake changes (%) between the pre-step baseline and the post-step plateau, expressed relative to the difference between these values, in the anaemic (A), normocythaemic or control (B), and polycythaemic states (C). Data are means with standard errors of the means. Significant differences ($p<0.05$) are indicated by '1', '2' and '3' for the comparisons between hypoxic and normoxic conditions, hypoxic and hyperoxic conditions, and normoxic and hyperoxic conditions respectively.

Figure 4.5: Carbon dioxide dynamics observed under hypoxic, normoxic and hyperoxic conditions. Data show carbon dioxide elimination changes (%) between the pre-step baseline and the post-step plateau, expressed relative to the difference between these values, in the anaemic (A), normocythaemic or control (B), and polycythaemic states (C). Data are means with standard errors of the means. Significant differences ($p<0.05$) are indicated by '1', '2' and '3' for the comparisons between hypoxic and normoxic conditions, hypoxic and hyperoxic conditions, and normoxic and hyperoxic conditions respectively.
Figure 4.6: Ventilation dynamics observed under hypoxic, normoxic and hyperoxic conditions. Data show minute ventilation changes (%) between the pre-step baseline and the post-step plateau, expressed relative to the difference between these values, in the anaemic (A), normocythaemic or control (B), and polycythaemic states (C). Data are means with standard errors of the means. Significant differences (p<0.05) are indicated by '1', '2' and '3' for the comparisons between hypoxic and normoxic conditions, hypoxic and hyperoxic conditions, and normoxic and hyperoxic conditions respectively..............77

Figure 4.7: Cardiac frequency dynamics observed under hypoxic, normoxic and hyperoxic conditions. Data show cardiac frequency changes (%) between the pre-step baseline and the post-step plateau, expressed relative to the difference between these values, in the anaemic (A), normocythaemic or control (B), and polycythaemic states (C). Data are means with standard errors of the means. Significant differences (p<0.05) are indicated by '1', '2' and '3' for the comparisons between hypoxic and normoxic conditions, hypoxic and hyperoxic conditions, and normoxic and hyperoxic conditions respectively.............78

Figure 4.8: Oxygen uptake dynamics observed under hypoxic, normoxic and hyperoxic conditions. Data show oxygen uptake changes (%) between the step-up plateau and the step-down plateau, expressed relative to the difference between these values, in the anaemic (A), normocythaemic or control (B), and polycythaemic states (C). Data are means with standard errors of the means. Significant differences (p<0.05) are indicated by '1', '2' and '3' for the comparison between hypoxic and normoxic conditions, hypoxic and hyperoxic conditions, and normoxic and hyperoxic conditions respectively..............79

Figure 4.9: Carbon dioxide dynamics observed under hypoxic, normoxic and hyperoxic conditions. Data show carbon dioxide elimination changes (%) between the step-up plateau and the step-down plateau, expressed relative to the difference between these values, in the anaemic (A), normocythaemic or control (B), and polycythaemic states (C). Data are means with standard errors of the means. Significant differences (p<0.05) are indicated by '1', '2' and '3' for the comparison between hypoxic and normoxic conditions, hypoxic and hyperoxic conditions, and normoxic and hyperoxic conditions respectively......80

Figure 4.10: Ventilation dynamics observed under hypoxic, normoxic and hyperoxic conditions. Data show minute ventilation changes (%) between the step-up plateau and the step-down plateau, expressed relative to the difference between these values, in the anaemic (A), normocythaemic or control (B), and polycythaemic states (C). Data are means with standard errors of the means. Significant differences (p<0.05) are indicated by '1', '2' and '3' for the comparison between hypoxic and normoxic conditions, hypoxic and hyperoxic conditions, and normoxic and hyperoxic conditions respectively..............81
Figure 4.11: Cardiac frequency dynamics observed under hypoxic, normoxic and hyperoxic conditions. Data show cardiac frequency changes (%) between the step-up plateau and the step-down plateau, expressed relative to the difference between these values, in the anaemic (A), normocythaemic or control (B), and polycythaemic states (C). Data are means with standard errors of the means. Significant differences (p<0.05) are indicated by '1', '2' and '3' for the comparison between hypoxic and normoxic conditions, hypoxic and hyperoxic conditions, and normoxic and hyperoxic conditions respectively.

Figure 4.12: Integrated cardiorespiratory responses obtained by summing raw data over 5 second intervals from the onset of cycle exercise (45% aerobic power), to the end of the physiological steady-state at minute 11. Subjects were studied while breathing hypoxic (10%), normoxic (20%) and hyperoxic gas mixtures (30%), while in three states of altered haematocrit. Data are means and standard error of the means. Significant differences (p<0.05) are indicated by '1', '2' and '3' for the comparison between hypoxic and normoxic conditions, hypoxic and hyperoxic conditions and normoxic and hyperoxic conditions respectively.

Figure 4.13: Integrated cardiorespiratory responses obtained by summing raw data over 5 second intervals from the onset of recovery following cycle exercise (45% aerobic power), at the end of the physiological steady-state at minute 11, to the end of a recovery period at minute 15. Subjects were studied while breathing hypoxic (10%), normoxic (20%) and hyperoxic gases (30%), during three states of altered haematocrit. Data are means and standard error of the means. Significant differences (p<0.05) are indicated by '1', '2' and '3' for the comparison between hypoxic and normoxic conditions, hypoxic and hyperoxic conditions, and normoxic and hyperoxic conditions respectively.

Figure 4.14: The time taken for cardiac frequency (f_C), oxygen consumption (\(\dot{V}_{O_2}\)), carbon dioxide elimination (\(\dot{V}_{CO_2}\)), and minute ventilation (\(\dot{V}_E\)) to reach 100% of the steady-state plateau following a step-exercise forcing function from rest to ~45% peak aerobic power. Subjects were studied while breathing hypoxic (F\(_{O_2}\)=0.1), normoxic (F\(_{O_2}\)=0.2) and hyperoxic gases (F\(_{O_2}\)=0.3), during three states of altered haematocrit, artificially-induced anaemia (A), normocythaemia (B) and artificially-induced polycythaemia (C). Data are means and standard error of the means. Significant differences (p<0.05) are indicated by '1' and '2' for the comparison between \(\dot{V}_{O_2}\) and \(\dot{V}_{CO_2}\), and \(\dot{V}_E\) and \(\dot{V}_{CO_2}\) respectively.
Figure 4.15: The time taken for cardiac frequency ($f_c$), oxygen consumption ($\dot{V}_{O_2}$), carbon dioxide elimination ($\dot{V}_{CO_2}$), and minute ventilation ($\dot{V}_E$) to reach 100% of the steady-state plateau under resting conditions following the completion of a step exercise forcing function at $\sim$45% peak aerobic power, to rest. Subjects were studied while breathing hypoxic ($F_iO_2=0.1$), normoxic ($F_iO_2=0.2$) and hyperoxic gases ($F_iO_2=0.3$), during three states of altered haematocrit, artificially-induced anaemia (A), normocythaemia (B) and artificially-induced polycythaemia (C). Data are means and standard error of the means. Significant differences ($p<0.05$) are indicated by '1' and '2' for the comparison between $f_c$ and $\dot{V}_E$, and $\dot{V}_{O_2}$ and $\dot{V}_E$ respectively.................................89

Figure 4.16: Oxygen uptake dynamics observed during anaemic, normocythaemic and polycythaemic states. Data show oxygen uptake changes (%) between the pre-step baseline and the post-step plateau, expressed relative to the difference between these values, when breathing hypoxic (A), normoxic (B) and hyperoxic gas mixtures (C). Data are means with standard errors of the means. Significant differences ($p<0.05$) are indicated by '1', '2' and '3' for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively.................92

Figure 4.17: Carbon dioxide elimination dynamics observed during anaemic, normocythaemic and polycythaemic states. Data show carbon dioxide elimination changes (%) between the pre-step baseline and the post-step plateau, expressed relative to the difference between these values, when breathing hypoxic (A), normoxic (B) and hyperoxic gas mixtures (C). Data are means with standard errors of the means. Significant differences ($p<0.05$) are indicated by '1', '2' and '3' for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively..........................................................93

Figure 4.18: Ventilation dynamics observed during anaemic, normocythaemic and polycythaemic states. Data show minute ventilation changes (%) between the pre-step baseline and the post-step plateau, expressed relative to the difference between these values, when breathing hypoxic (A), normoxic (B) and hyperoxic gas mixtures (C). Data are means with standard errors of the means. Significant differences ($p<0.05$) are indicated by '1', '2' and '3' for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively.............94
Figure 4.19: Cardiac frequency dynamics observed during anaemic, normocythaemic and polycythaemic states. Data show cardiac frequency changes (%) between the pre-step baseline and the post-step plateau, expressed relative to the difference between these values, when breathing hypoxic (A), normoxic (B) and hyperoxic gas mixtures (C). Data are means with standard errors of the means. Significant differences (p<0.05) are indicated by ‘1’, ‘2’ and ‘3’ for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively.............95

Figure 4.20: Oxygen uptake dynamics observed during anaemic, normocythaemic and polycythaemic states. Data show cardiac frequency changes (%) between the step-up plateau and the step-down plateau, expressed relative to the difference between these values, when breathing hypoxic (A), normoxic (B) and hyperoxic gas mixtures (C). Data are means with standard errors of the means. Significant differences (p<0.05) are indicated by ‘1’, ‘2’ and ‘3’ for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively.............96

Figure 4.21: Carbon dioxide dynamics observed during anaemic, normocythaemic and polycythaemic states. Data show carbon dioxide elimination changes (%) between the step-up plateau and the step-down plateau, expressed relative to the difference between these values, when breathing hypoxic (A), normoxic (B) and hyperoxic gas mixtures (C). Data are means with standard errors of the means. Significant differences (p<0.05) are indicated by ‘1’, ‘2’ and ‘3’ for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively.............97

Figure 4.22: Ventilation dynamics observed during anaemic, normocythaemic and polycythaemic states. Data show minute ventilation changes (%) between the step-up plateau and the step-down plateau, expressed relative to the difference between these values, when breathing hypoxic (A), normoxic (B) and hyperoxic gas mixtures (C). Data are means with standard errors of the means. Significant differences (p<0.05) are indicated by ‘1’, ‘2’ and ‘3’ for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively.............98

Figure 4.23: Cardiac frequency dynamics observed during anaemic, normocythaemic and polycythaemic states. Data show cardiac frequency changes (%) between the step-up plateau and the step-down plateau, expressed relative to the difference between these values, when breathing hypoxic (A), normoxic (B) and hyperoxic gas mixtures (C). Data are means with standard errors of the means. Significant differences (p<0.05) are indicated by ‘1’, ‘2’ and ‘3’ for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively.............99
Figure 4.24: Integrated cardiorespiratory responses obtained by summing raw data over 5 second intervals from the onset of cycle exercise (45% aerobic power), to the end of the physiological steady-state at minute 11. Subjects were studied when anaemic (A), normal (N) and polycythaemic (P), and were supplied with gas with three levels of fractional inspired oxygen concentration ($F_{1O_2}$). Data are means and standard error of the means. Significant differences ($p<0.05$) are indicated by '1', '2' and '3' for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively..............................100

Figure 4.25: Integrated cardiorespiratory responses obtained by summing raw data over 5 second intervals from the onset of recovery following cycle exercise (45% aerobic power), at the end of the physiological steady-state at minute 11, to the step-down plateau at minute 15. Subjects were studied when anaemic (A), normal (N) and polycythaemic (P), and were supplied with gas with three levels of fractional inspired oxygen concentration ($F_{1O_2}$). Data are means and standard error of the means. Significant differences ($p<0.05$) are indicated by '1', '2' and '3' for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively....................................................................................................................102

Figure 4.26: The relationship between minute ventilation ($\dot{V}_E$) and mean inspiratory flow rate ($V_{I/T}$) showing a linear response for hypoxia ($F_{1O_2}=0.1$), normoxia ($F_{1O_2}=0.2$) and hyperoxia ($F_{1O_2}=0.3$) until $\dot{V}_E$ reaches ~80 l.min$^{-1}$ BTPS, where the relationship becomes curvilinear under hypoxic conditions as $\dot{V}_E$ is elevated..........................................................................104

Figure 4.27: End tidal carbon dioxide pressure ($P_{ETCO_2}$) averaged over 30 second intervals, during an exercise step-function from rest (5 min) to 45% aerobic power (6 min), and back to rest again (5 min). Graphs correspond with data collected during artificially-induced anaemia (A), control (B), and artificially-induced polycythaemia (C). Data are means with standard errors of the means..................................................................................................................106

Figure A1: Each plot represents the mean of all previous trials............................................170

Figure B1: Three combined trials producing a single $\dot{V}_{O2}$ response curve with different durations between trials...............................................................173

Figure D1: A comparison of the cardiac frequency determined from the Ohmeda Biox 3700e pulse oximeter, PE3000 Sportstester, and that from a 5 lead ECG during a submaximal exercise step-forcing function..................................................178

Figure E1: Haemonetics® Color Comparator (18501 A).........................................................181
Figure F1: Venous haematocrit and haemoglobin concentration following each venesection...185

Figure F2: The effect of severe anaemia on step-up dynamics and step-down dynamics...187

Figure F3: The effects of mild and severe anaemia on pre-step baselines, step-up plateaux and step-down plateaux...188
LIST OF TABLES

Table 3.1: Physical characteristics of subjects........................................................................31
Table 3.2: Sequence of testing and associated time intervals....................................................42
Table 3.3: $\dot{V}_{O2\text{peak}}$ data for normocythaemic (control) conditions.................................46

Table 4.1: Haematological values for resting male subjects determined prior to (control), and following artificially-induced anaemia, and after artificially-induced polycythaemia (Coulter Cell Counter S PLUS IV). Values are means and standard deviations..................................................................................................64

Table 4.2: Cardiorespiratory responses to a maximal ramp exercise protocol (cycling; 36 W.min$^{-1}$) in control state, and then following artificially-induced anaemia, and artificially-induced polycythaemia. Data are means with standard errors of the means (anaemic and control conditions n=6; polycythaemia, n=5)..............................................66

Table 4.3: A comparison of cardiorespiratory function during a maximal ramp exercise protocol (36 Watts.min$^{-1}$), at different absolute workrates (50 Watt increments) under control (normocythaemic) conditions, isovolaemic anaemia and isovolaemic polycythaemia. Data are means and standard errors of the means..............................68

Table 4.4: Cardiorespiratory variables determined breath-by-breath during the submaximal exercise protocol. Pre-step baselines (PRE) are averaged between minutes 1 and 5, while the step-up plateaux (PLAT) are averaged between minutes 8 and 11, and step-down plateaux are averaged between minutes 14 and 16. Data are means with standard errors of the means. (anaemic and control conditions n=6; polycythaemia, n=5)................................................................................72

Table 4.5: Cardiorespiratory variables determined breath-by-breath for subjects during a submaximal step (cycling) forcing function. Pre-step baselines (PRE) are determined from data between minutes 1 and 5, while the step-up plateaux (PLAT) are determined between minutes 8 and 11, and step-down plateaux are determined between minutes 14 and 16. Data are means with standard errors of the means...........................................................90
Table 4.6: Cardiorespiratory variables following a step-increase in workrate, averaged over 30 second intervals, for the last 30 seconds of the step-up plateau. Subjects were studied when anaemic, normocythaemic, and polycythaemic, and were supplied with gas with three levels of fractional inspired oxygen concentration ($F_iO_2$). Data are means and standard error of the means (anaemic and control conditions n=6; polycythaemia, n=5)

Table A1: Differences between $\dot{V}_{O2}$ dynamics for single step data obtained from consecutive trials, averaged across three subjects. Data are times (decimal) to reach fixed points between resting and steady state $\dot{V}_{O2}$

Table B1: The time to reach 20%, 40%, 60%, 80% and 100% of the submaximal, steady-state plateau

Table C1: Pearson Product-Moment correlation coefficients for oxygen consumption dynamics, computed at five time points (time constants) in the response curves of subjects (N=5) during a single-step exercise protocol

Table E1: Quality control results carried out on deglycerolised red blood cells

Table F1: The effect of mild and severe haematocrit reductions by way of successive phlebotomies on one subject during maximal exercise

Table G1: Results of blood tests as determined by the Coulter Cell Counter

Table H1: The raw data, for each individual subject, averaged over 5 second intervals, integrated in the control state throughout a step-increase and step-decrease in workrate. Data are the total amount of oxygen consumed (l STPD), total amount of carbon dioxide eliminated (l STPD), total amount of ventilation (l BTPS) and total number of heart beats during the step-increase in workrate (73 data points from the onset of cycle exercise (45% aerobic power) at minute 5 to the end of the physiological steady-state at minute 11), and the step-decrease in workrate (49 data points from the onset of recovery following cycle exercise, at minute 11, to the end of a recovery period at minute 15). Subjects were also studied while breathing hypoxic (10%), normoxic (20%) and hyperoxic gases (30%)
Table H2: The raw data, for each individual subject, averaged over 5 second intervals, integrated in the anaemic state throughout a step-increase and step-decrease in workrate. Data are the total amount of oxygen consumed (1 STPD), total amount of carbon dioxide eliminated (1 STPD), total amount of ventilation (1 BTPS) and total number of heart beats during the step-increase in workrate (73 data points from the onset of cycle exercise (45% aerobic power) at minute 5 to the end of the physiological steady-state at minute 11), and the step-decrease in workrate (49 data points from the onset of recovery following cycle exercise, at minute 11, to the end of a recovery period at minute 15). Subjects were also studied while breathing hypoxic (10%), normoxic (20%) and hyperoxic gases (30%).

Table H3: The raw data, for each individual subject, averaged over 5 second intervals, integrated in the polycythaemic state throughout a step-increase and step-decrease in workrate. Data are the total amount of oxygen consumed (1 STPD), total amount of carbon dioxide eliminated (1 STPD), total amount of ventilation (1 BTPS) and total number of heart beats during the step-increase in workrate (73 data points from the onset of cycle exercise (45% aerobic power) at minute 5 to the end of the physiological steady-state at minute 11), and the step-decrease in workrate (49 data points from the onset of recovery following cycle exercise, at minute 11, to the end of a recovery period at minute 15). Subjects were also studied while breathing hypoxic (10%), normoxic (20%) and hyperoxic gases (30%).

Table I1: Time to $\dot{V}_{O2peak}$ for each subject under anaemic, control and polycythaemic conditions

Table I2: Peak Watts for each subject under anaemic, control and polycythaemic conditions

Table I3: $\dot{V}_{O2peak}$ (l.min$^{-1}$) for each subject under anaemic, control and polycythaemic conditions

Table I4: Peak cardiac frequency (beats.min$^{-1}$) for each subject under anaemic, control and polycythaemic conditions

Table I5: Peak ventilation (l.min$^{-1}$) for each subject under anaemic, control and polycythaemic conditions
CHAPTER ONE: INTRODUCTION
1.1 INTRODUCTION

Exercise under non-steady state conditions provides the opportunity to investigate the physiological mechanisms that influence gas exchange dynamics. A step-increase in workrate\(^1\) results in immediate increases in energy demand, which cannot be supplied by aerobic metabolism alone (Henry, 1951), resulting in near exponential respiratory responses to the workrate increment (Whipp & Casaburi, 1982), and allows the evaluation of response dynamics of the physiological systems involved in exchanging, transporting and utilizing oxygen.

The rate that the physiological and cardiorespiratory mechanisms respond to increases in workrate, is governed by factors involved within the chain of events between the extraction of oxygen (O\(_2\)) from the atmosphere and its delivery to the mitochondria (Wagner, 1992), where it is serves as the final electron acceptor in the respiratory chain and combines with hydrogen to form water (McArdle et al., 1991).

In a perfect system, where supply could instantly satisfy demand, physiological systems would display a square-wave response pattern. However, the inability of aerobic

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\(^{1}\) A step-increase in workrate refers to the imposition of a single, constant load change in workrate.
mechanisms to instantly adjust to meet the energy requirements in response to a step-
increase in workrate results in one or more exponential\(^2\) response patterns in the variables
involved in energy production (Casaburi et al., 1989). The increase in the activity of each
variable is generally characterised by three temporal phases (Figure 1.1; Linnarsson, 1974;
Whipp & Ward, 1980; Bennett et al., 1981; Barstow & Molé, 1987; Hughson et al., 1988).

The first phase of oxygen uptake (\(\dot{V}_{\text{O}_2}\)) dynamics is the abrupt initial increase
evident at the onset of exercise, and sometimes at a change in workrate. It is maintained
for 10 to 20 seconds (Linarsson, 1974; Whipp and Ward, 1980; Whipp, 1987), and is
dominated by chronotropically-induced changes in pulmonary blood flow (Whipp et al.,
1982; Whipp & Ward, 1990). The second, slower phase extends from the end of phase one
through to the attainment of a new physiological steady-state, and represents the oxygen
transport and utilization aspects of oxygen uptake (Swanson, 1990). Phase three is
characterised as the steady-state or plateau, reached at the end of the exponential rise of
phase two, and is only seen when the magnitude of the step change in workrate permits the
responding physiological mechanisms to stabilize.

The speed with which the ventilatory, cardiovascular and metabolic systems adjust
to a change in workrate determine the relative contributions of the aerobic and anaerobic
metabolisms to the total work performed, and hence influence the efficiency with which
both energy substrates and oxygen are utilized to perform the necessary work. Since the

\(^2\) The response typifies an exponential shape.
Figure 1.1: Three phase response for $V_{\text{O}_2}$ uptake following a step-increase in workrate.
physiological responses follow an approximately exponential pattern, the rate of the phase two physiological adjustment to a change in workrate reflects the speed with which the system responds. In an exponential model this is quantified by its time constant ($\tau$)\(^3\) (Whipp & Casaburi, 1982; Hughson et al., 1988; Swanson & Hughson, 1988; Casaburi et al., 1989). The time constant for $\dot{V}_{O2}$ is believed to reflect changes in gas exchange at the active muscle (Raynaud et al., 1973; Diamond et al., 1977), and to be independent of pulmonary ventilation (Donald et al., 1955; Auchincloss et al., 1966).

There have been two hypotheses put forward to account for the imperfect nature of the gas exchange dynamics curve following a square-wave perturbation to the steady-state. The first implicates oxygen delivery, or transport mechanisms as rate limiting factors (Hughson et al., 1988; Murphy et al., 1989; Linnarsson, 1974). The second hypothesis is directed towards the limitation being centered on the utilization of oxygen by the mitochondria (Adams & Welch, 1980; Hickson et al., 1978; Pendergast et al., 1980; Sahlin et al., 1988; Whipp & Mahler, 1980).

Several groups have evaluated the roles of oxygen transport and utilization as determinants of cardiorespiratory dynamics. For example, Linnarsson (1974) and Dodd et al. (1988) have observed slower response dynamics following hypoxia, and $\beta$-adrenergic blockade; and faster response dynamics with hyperoxia (Linnarsson, 1974) and an increase in $\dot{V}_{O2max}$ (Powers et al., 1985), while Casaburi et al. (1980) and Cerretelli et al. (1979) have

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\(^3\) The time constant ($\tau$) is defined as the time taken for the variable to reach 63% of the difference between the pre-step baseline and the post-step plateau values, when an exponential curve is fitted to the data.
reported faster response dynamics following depleted oxygen and phospho-creatine stores, and training; and slower response dynamics following induced deconditioning (Convertino et al., 1984). However, research has not been located in which the role of the erythrocyte on phase two dynamics has been investigated.

1.2 AIMS

While it has been demonstrated that oxygen transport by the healthy pulmonary system is more than adequate to meet the increased metabolic demands imposed by submaximal steady-state exercise at sea level (Dempsey et al., 1984), the underlying physiology behind the dynamic responses of the cardiorespiratory systems to a step-change in workrate are less certain.

This study aimed to evaluate the effect of altered oxygen transport and oxygen availability on cardiorespiratory gas exchange dynamics. To evaluate these components, cardiorespiratory response dynamics were investigated during an exercise step-function in subjects with artificially-induced anaemia and polycythaemia, while breathing hypoxic, normoxic and hyperoxic gas mixtures.
1.3 HYPOTHESES

It was hypothesised that:

(1) the steady-state oxygen utilization, following a constant relative intensity step-change in exercise workrate, would be independent of inspired oxygen concentration and whole-body haematocrit, within the range of 35-45%.

(2) cardiorespiratory response dynamics to a constant intensity step-function exercise will be slowed when subjects are rendered mildly anaemic, and accelerated when made mildly polycythaemic; and

(3) the fractional concentration of inspired oxygen will act, independently of whole-body haematocrit, to delay (hypoxia) or accelerate (hyperoxia) cardiorespiratory response dynamics to a constant intensity step-change in exercise workrate.
CHAPTER TWO: REVIEW OF LITERATURE
CHAPTER TWO: REVIEW OF LITERATURE

2.1 INTRODUCTION

The transport of oxygen (O₂) to exercising muscle should generally be sufficient to ensure an adequate supply of energy during incremental exercise until near maximal workrates are attained. However, during step-changes in exercise intensity, there is generally a deficit period, during which the physiological responses cannot respond rapidly enough to meet the change in workrate (Åstrand & Saltin, 1961; Linnarsson, 1974). This deficit is due to the imperfect response of the oxygen transport and utilization systems. Energy supply in this period of oxygen deficit comes from the utilization of high-energy phosphate stores (adenosine-triphosphate (ATP) and phospho-creatine (CP)), glycolysis and the utilization of oxygen stores of venous blood (Murphy et al., 1989).

The rate of the cardiorespiratory response to an increase in workrate is governed by factors involved in the chain of events from alveolar ventilation and oxygen extraction, to its delivery and subsequent utilization in the mitochondria. Some of these factors are identified in Figure 2.1. In response to increasing demands in energy requirements, resulting from a step-increase in workrate, the physiological adjustments occur in an approximately exponential manner (Henry, 1951; Margaria et al., 1965; Linnarsson, 1974; Cerretelli et al., 1977). These response patterns are characterized by three temporal phases (Figure 1.1).
(1) LUNG: (a) Alveolar ventilation
- dead space ventilation
(b) Diffusion of Gases
- diffusion gradient
- thickness of alveolar and capillary membranes
- affinity of haemoglobin for oxygen
- surface area of the alveoli
- red blood cell numbers and haemoglobin concentration
- diffusion coefficient

(2) BLOOD TRANSPORT: (a) Central
- pulmonary capillary blood flow and transit time
- ventilation/perfusion ratio
- right-left shunting
- oxygen saturation
- carboxy-haemoglobin concentration
(b) Peripheral
- vasomotor tone
- blood pressure
- muscle capillary density and transit time

(3) PERIPHERAL GAS EXCHANGE:
- intracellular partial pressure of oxygen
- diffusion gradient
- diffusion distance
- muscle mass & fibre characteristics
- distribution of blood flow
- local tissue metabolism
- 2,3-diphosphoglycerate concentration

(4) \(O_2\) UTILIZATION
- number & density of mitochondria
- redox state of mitochondria
- myoglobin concentration
- substrate availability
- local tissue temperature
- local hydrogen ion concentration and acidity
- Adenosine-triphosphate generation
- Creatine phosphate hydrolysis

Figure 2.1: Possible sites in the oxygen transport and utilization processes which may limit the body's ability to instantaneously elevate its energy production.
2.2 CARDIORESPIRATORY EXERCISE ONSET RESPONSES

Early research indicated that the immediate or phase one oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide elimination ($\dot{V}_{CO_2}$) responses to a step-change in workrate were the result of a rapid increase in venous return, resulting in an increased pulmonary perfusion (Krogh and Lindard, 1913). This abrupt 'haemodynamic' or 'cardiogenic' change is proportional to the increase in $\dot{V}_{O_2}$ (Cummin et al., 1986; Miyamoto et al., 1982; Yoshida et al., 1993). It is influenced by both the intensity of the pre-step exercise and the intensity of the step itself; being reduced in both magnitude and duration when the step-increase in workrate is instigated from a baseline of loadless pedalling instead of rest (Whipp and Ward, 1980; Whipp et al., 1982), and is increased in magnitude, and delayed in duration, as the size of the step-workrate is increased (Sietsema et al., 1989).

The immediate response of minute ventilation ($\dot{V}_E$) is generally considered to result from neurogenic mechanisms originating in the limbs and the cerebral cortex, since it is too quick to be of humoural origin (Fujihara et al., 1973; Grucza et al., 1990; Whipp and Ward, 1980). Though others have suggested that exercise hyperpnoea is the result of a combination of cardiodynamic factors and changes in arterial carbon dioxide pressure ($P_{aCO_2}$) (Miyamoto et al., 1988; Miyamoto et al., 1989; Lamarra et al., 1989).

Phase two of the cardiorespiratory responses dominate the dynamic component of exercise-induced changes, reflecting the influence of tissue metabolism and factors which
facilitate gas exchange at the periphery (Hughson and Inman, 1985; Linnarsson, 1974; Whipp et al., 1982; Whipp and Ward, 1980; Whipp, 1987). This project focuses on phase two dynamics, looking specifically at factors which influence the transport of oxygen to the periphery (Figure 2.1), and how they affect the rapidity of the cardiorespiratory response to a step-change in exercise intensity.

The characteristics of the phase two response are determined by: (1) the intensity of the step increment itself; (2) the intensity of the pre-step exercise from which the change is initiated; and (3) the duration of this pre-step exercise. $\dot{V}_{O_2}$ is increasingly slowed as the relative intensity of the step-increment is elevated (Casaburi et al., 1989; Cerretelli et al., 1980). The kinetics of $\dot{V}_{O_2}$ are slower in the transition from light to moderate exercise than in the transition from rest to exercise (Hughson & Morrissey, 1982; Hughson & Morrissey, 1983), and $\dot{V}_{O_2}$ response dynamics are faster as a result of an extended period of exercise prior to the step-increase in workrate (Solomon & Taylor, 1994). The training status and fitness of the individual also effects the cardiorespiratory response dynamics (Powers et al., 1985; Hagberg et al., 1980).

Typically, these responses are described using an exponential model (Henry, 1951; Linnarsson, 1974), in which the time constant ($\tau$) is used to define systemic response speed. Though influenced by both the intensity of the step-change and the pre-step exercise level, several common response characteristics may be noted. Under normal conditions, the rate of increase of $\dot{V}_{O_2}$ is proportional to the metabolic demand and independent of oxygen...
supply (Henry, 1951). The $\dot{V}_{CO2}$ time constant ($\tau$~50-60 sec) is considerably longer than the $\dot{V}_{O2}$ time constant ($\tau$~30-40 sec), although not as long as $\dot{V}_E$ ($\tau$~60 sec; Casaburi et al., 1987; Linnarsson, 1974, Hughson and Inman, 1985; Karlsson et al., 1975; Miyamoto, 1992; Oren et al., 1982; Ward et al., 1987; Whipp and Mahler, 1980). The difference between the time courses of $\dot{V}_{O2}$ and $\dot{V}_{CO2}$ is believed to reflect the difference between the rate of change in carbon dioxide and oxygen stores, and the capacity of the tissues to store greater amounts of carbon dioxide (Farhi and Rahn, 1955; Hughson and Inman, 1985; Whipp and Mahler, 1980; Whipp and Ward, 1980).

Heart rate or cardiac frequency ($f_C$) dynamics follow a similar pattern to ventilatory mechanisms, with a very rapid initial increase, a slower rise, then a plateau. If the workload is maintained over an extended period of time, there may be a slow, almost linear drift lasting throughout the work period (Linnarsson, 1974; Shaffrath & Adams, 1984). The dynamics for cardiac output ($Q$) are faster than $\dot{V}_{O2}$, and the change in $f_C$ follows $\dot{Q}$ (Pendergast et al., 1980). Humoral factors have much greater role in phase two, as the chemosensitivity of the carotid bodies play an important contribution to the ventilatory control process (Boetger and Ward, 1986), as well as determining $f_C$ dynamics (Fujihara et al., 1973).

2.3 CARDIORESPIRATORY EXERCISE OFFSET RESPONSES

The inability of aerobic metabolism to instantly adjust to a change in workrate is
also evident in the cardiorespiratory response to a step-reduction in workrate. The dynamics of oxygen consumption are unable to decrease simultaneously with the reduction in workrate, and remain at a level in excess of that needed to meet energy transfer requirements of the active tissues following this reduction (Margaria et al., 1933). It has been assumed that the time constant for a step-decrease in workrate is the same as a step-increase (Miyamoto, 1992; Whipp & Ward, 1990; Solomon & Taylor, 1994), however, it appears that the step-down may be slightly slower (Hughson, 1990; Baum et al., 1992).

Following a step-reduction in workrate, the period of excess post-exercise oxygen consumption (EPOC⁴) reflects a disruption to cellular homeostasis, and the \( \dot{V}_\text{O}_2 \) dynamics are influenced by the rate at which homeostasis is regained. The direct control of mitochondrial respiration at the cellular level may be influenced by concentrations of adenosine-diphosphate, adenosine-triphosphate, inorganic phosphate and creatine phosphate, while the indirect control of cellular respiration may involve a variety of factors including catecholamines, thyroxine, glucocorticoids, fatty acids and calcium ions (Gaesser & Brooks, 1984). It has been calculated that the \( Q_{10} \) effect of temperature on metabolism could account for 60-70\% of the slow component of recovery \( \dot{V}_\text{O}_2 \) after exercise requiring 50-80\% \( \dot{V}_\text{O}_2\text{peak} \) (Hagberg et al., 1980). In addition, the time for the return of blood-borne norepinephrine to pre-exercise levels has been shown to be consistent with that of the slow

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⁴ "Excess post-exercise oxygen consumption" (EPOC) is used to replace the term "oxygen debt" because the latter implies that the removal of metabolic by-products and the replenishment of oxygen stores are the only mechanisms responsible for the increase in \( \dot{V}_\text{O}_2 \) above resting values following exercise (Gaesser & Brooks, 1984).
component of recovery $\dot{V}_{O_2}$ (Hagberg et al., 1979). In children and in adults, the cardiorespiratory dynamics of recovery are independent of workrate (Zanconato et al., 1991).

As exercise duration is lengthened, there is a corresponding increase in the magnitude and duration of the resulting EPOC (Chad and Wenger, 1988; Sedlock et al., 1989). The $\dot{V}_{O_2}$ dynamics in the transition from exercise to rest are faster than the transition from strenuous to mild work (di Prampero et al., 1970), while the time constants for $\dot{V}_{O_2}$ and $f_c$ in hypoxic and normoxic conditions are similar (Garner et al., 1986). Following exercise, $\dot{V}_E$ may be controlled by the regulation of both afferent and efferent neural mechanisms. As there is a slow decline of both $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$, based on the carbon dioxide flow hypothesis of $\dot{V}_E$ control, $\dot{V}_E$ also remains elevated following exercise while carbon dioxide remains above resting levels (Garner et al., 1986).

2.4 DETERMINANTS OF EXERCISE ONSET PHASE TWO DYNAMICS: DELIVERY VERSUS UTILIZATION.

Phase two dynamics during exercise onset reflects the combination of oxygen transport and the oxygen utilization aspects of oxygen uptake during submaximal exercise. The shape of the submaximal $\dot{V}_{O_2}$ response curve may be influenced by factors which affect oxygen supply or utilization. Support for the role of oxygen transport limiting $\dot{V}_{O_2}$ dynamics comes from studies showing faster dynamics when utilizing hyperoxic gas, and
slower dynamics under hypoxic conditions (Linnarsson, 1974). Oxygen delivery is thought to limit cardiorespiratory dynamics because artificially-induced polycythaemia has been used to show that the aerobic power of the exercising muscles is in excess of that measured under normal conditions (Spriet et al., 1986). This suggests that an increasing circulating red blood cells elevates the amount of useable oxygen transported to the muscles (Buick et al., 1980; Spriet et al., 1986). This however, has only been tested under maximal exercise conditions and not during submaximal exercise.

Since hypoxia results in the slowing of the $\dot{V}_{O_2}$ response (Linnarsson, 1974), it has been proposed that since there is a difference in the $\dot{V}_{O_2}$ response to a reduced inspired oxygen tension (hypoxia), or an increased workload, that the time course of $\dot{V}_{O_2}$ is determined by the rate of acceleration of the oxygen transport and not by any inertia of the peripheral processes of oxygen utilization (Linnarsson, 1974). Differences in the $\dot{V}_{O_2}$ response of step and ramp exercise protocols under hypoxic conditions have been interpreted to result from a reduced arterial oxygen content, which is not adequately compensated for by increased cardiac output ($\dot{Q}$) and blood flow distribution, to maintain oxygen transport during the exercise transition (Murphy et al., 1989).

On the other hand, it has been proposed that oxygen utilization, or the rate of cellular oxygen uptake, is the rate limiting factor in $\dot{V}_{O_2}$ dynamics. This hypothesis has support from several sources. For example, Pendergast et al. (1980), measuring muscle blood flow (MBF), where MBF was used as an indicator of peripheral circulation using
\({}^{133}\text{Xe}\) clearance techniques, compared trained and untrained skeletal muscle and found faster adjustment of MBF in the trained than in the untrained subjects, but concluded that \(\dot{Q}\) and MBF do not play a role in determining \(\dot{V}_{O_2}\) response dynamics. Training of specific muscles will accelerate the response dynamics (Cerretelli et al., 1979) by increasing the mitochondrial content and respiratory capacity of the muscle fibres, leading to a more efficient use of substrates (Holloszy and Coyle, 1984).

The rate of change of alveolar ventilation and \(\dot{Q}\) is faster than corresponding changes in \(\dot{V}_{O_2}\), resulting in elevated peripheral oxygen delivery (Cerretelli et al., 1980). Measurements of deep venous oxygen partial pressures (\(P_{O_2}\)) at rest and during intense exercise indicates that the \(P_{O_2}\) does not drop below 19 Torr (Jorfeldt and Wahren, 1971), a pressure which has been proposed as being above the critical pressure of diffusing limitation for oxygen (Cerretelli et al., 1980). Because this oxygen delivery at the onset of exercise rises more rapidly than oxygen extraction, and the rise in blood flow is faster than the rise in \(\dot{V}_{O_2}\), such data indicate that oxygen delivery is not a limiting factor in muscle \(\dot{V}_{O_2}\) dynamics (Barstow et al., 1990).

Exercise utilizing larger muscle masses may result in faster response dynamics (Cerretelli et al., 1980), although data from our laboratory do not support this. We have found no difference in \(\dot{V}_{O_2}\) dynamics when untrained subjects were tested across exercise modes (arm cranking, cycling and rowing) involving differing muscle masses (Osborne et al., 1991). Shephard et al. (1988) argue that because of the difficulty in perfusing small
muscles, arm work is limited by the intrinsic power of those muscles, single leg ergometry is equally limited by central circulatory and muscular factors, and that two leg ergometry is almost entirely dependent on the central circulatory transport of oxygen.

The adaption of oxygen consumption to a change in submaximal exercise stimulus appears to be limited by numerous factors. Reductions in oxygen transport do cause oxygen transport to appear as the rate limiting step, although there is limited data as to whether increased in oxygen delivery results in faster response dynamics. Since altering the concentration of inspired oxygen alters the partial pressure, altering the carrying capacity of the blood by increasing the red blood cell concentration, enhances the process of oxygen delivery. It is known that anaemia results in a reduction in \( \dot{V}_{O_2\text{peak}} \) and induced polycythaemia may result in greater \( \dot{V}_{O_2\text{peak}} \), primarily as a result of altered oxygen transport, yet the effects of these changes on oxygen delivery has not been demonstrated during submaximal exercise.

The current research aims at evaluating the role of oxygen delivery in determining cardiorespiratory response dynamics to a submaximal, step-increase in workrate. Oxygen delivery shall be manipulated by altering the partial pressure of inspired oxygen and the total circulating haemoglobin concentration [Hb].
2.5 EXPERIMENTAL INTERVENTIONS PERMITTING EVALUATION OF
THE OXYGEN DELIVERY HYPOTHESIS.

Numerous techniques have been employed to evaluate whether it is oxygen transport or oxygen utilization which affects the kinetics of cardiorespiratory adjustments to exercise transitions, and so determines the shape of the response curves. If oxygen delivery was the rate limiting factor, a change in systemic oxygen transport would result in altered response dynamics. Reducing systemic oxygen delivery using numerous methods has resulting in a slowing of cardiorespiratory response dynamics (Linnarsson, 1974). Likewise, if the rate of oxygen utilization was the limiting factor, modifying the ability of the body, or the mitochondria, to utilize oxygen would alter cardiorespiratory response dynamics. This study shall examine the effects of manipulating oxygen delivery, on cardiorespiratory response dynamics, while oxygen utilization shall not be modified across experimental conditions.

A change in the ability of the body to carry oxygen has been achieved where subjects undergo an artificially-reduced haemoglobin concentration (anaemia), or an altered ability of the haemoglobin to bind to oxygen, via carbon monoxide inhalation. Studies comparing anaemia, hypoxia and ischaemia, with matched oxygen delivery suggest that factors other than oxygen delivery are important determinants of $\dot{V}_{\text{O}_2}$ (Dodd et al., 1993), because while $\dot{V}_{\text{O}_2 \text{peak}}$ and the amount of tension developed was reduced under anaemic conditions, there may have been a decrease in diffusion capacity of the muscle.
A decrease in oxygen transport has been demonstrated by carbon monoxide inhalation, which is rapidly absorbed and readily combines with haemoglobin to produce carboxy-haemoglobin (COHb; Koike et al., 1990), also a by-product of cigarette smoking (Rotstein et al., 1991). This reduces the oxygen content of the blood without affecting the diffusion equilibrium between oxygen in the alveolar gas or the pulmonary capillary blood, although there is a small compensatory increase in $\dot{Q}$ for the reduced delivery. Carbon monoxide inhalation results in a slowing of the cardiorespiratory response dynamics (Rotstein et al., 1991; Koike et al., 1990).

A change in the amount of oxygen available for carriage, has been investigated in studies where the fractional concentration of inspired oxygen ($F_{1O_2}$) is altered. A slowing of cardiorespiratory response dynamics has been demonstrated by the use of hypoxia, which reduces arterial oxygen content, to show a transport limitation during exercise onset dynamics (Linnarsson, 1974; Ward and Whipp, 1989; Murphy et al., 1989; Springer et al., 1991). However, Garner et al. (1986) found no difference in step-down dynamics as a result of exposure to hypoxic conditions.

Systemic oxygen transport is elevated with the use of hyperoxia, which has been shown to result in faster cardiorespiratory response dynamics (Linnarsson et al., 1974; Ward and Whipp, 1989). Ward et al. (1987), measuring inspired ventilation ($\dot{V}_i$) only, and Nakazono & Miyamoto (1987), measuring $\dot{V}_E$ and $\dot{V}_{CO2}$ kinetics only, have demonstrated slower cardiorespiratory response dynamics under hyperoxic conditions. Though again,
Garner *et al.* (1986) and Robbins *et al.* (1992), found no difference in response dynamics in the step-down transition from exercise to rest.

Breathing hyperoxic gas may not necessarily directly increase oxygen delivery to active muscle. While the lungs are exposed to a higher $P_{O_2}$, leading to an increased alveolar $P_{O_2}$ (Morris, 1983; Graham *et al.*, 1987), studies by Wilson and Stainsby (1978) suggest there is a decrease in muscle blood flow in response to hyperoxic exposure, which may compensate for any increase in arterial oxygen concentration ($C_aO_2$), resulting in unaltered oxygen transport to muscle (Adams & Welch, 1980). Graham and Wilson (1983) and Adams and Welch (1980), suggest that acid-base shifts, and not $P_{O_2}$ mediate any effects related to hyperoxia. Evidence from this comes from studies showing similar responses in blood lactate suppression for hyperoxia and hypercapnia during exercise (Graham & Wilson, 1983), and at exhaustion, arterial hydrogen ion concentration ($[H^+]_a$) was not different under different inspired oxygen fractions (Adams and Welch, 1980; Byrnes *et al.*, 1984). Hyperbaric hyperoxia has been used to demonstrate an elevated $\dot{V}_{O_2\text{peak}}$ and $f_{C\text{peak}}$ during maximal exercise and reduced $\dot{V}_E$ and $f_C$ during submaximal exercise as a result of elevated arterial and tissue $O_2$ tension (Fagraeus *et al.*, 1972; Taunton *et al.*, 1970).

Welch *et al.* (1974), and Graham and Wilson (1983) have demonstrated an elevated $\dot{V}_{O_2}$ with hyperoxia which is most likely a result of factors other than an increased mitochondrial oxygen utilization. There may be an increase in the storage of oxygen within the body with hyperoxic inhalation, or some of the oxygen may be utilized in reactions
besides those in the respiratory chain, which may consume more oxygen when the $P_{O_2}$ is elevated (Welch et al., 1974). If the partial pressure of oxygen is increased under hyperoxic conditions, this should be evident in an elevated arterial oxygen saturation ($S_\text{a}O_2$), and if delivery is increased this would be noted in an elevated ($C_\text{a}O_2$), assuming $Q$ and muscle blood flow remained constant.

Changes in cardiovascular functioning that affect oxygen delivery has been investigated in studies using beta-blockade, and altered muscle perfusion. The ability of beta-adrenergic blockade to reduce systemic oxygen transport by reducing $f_C$, and therefore $\dot{Q}$, results in a slowing of the cardiorespiratory response dynamics because of a transport limitation (Hughson, 1990; Casaburi et al., 1989; Hughson, 1984; Dodd et al., 1988).

Muscle perfusion is an important process in the transport of oxygen to the working muscle. Eiken and Bjurstedt (1988) demonstrated severely impaired physical performance when positive pressure was applied to the legs. This intervention resulted in reduced blood perfusion to the working muscles. In contrast, they found improved performance with lower body negative pressure, which increased perfusion to the working muscles (Eiken, 1988). Hughson et al. (1993) have demonstrated faster cardiorespiratory response dynamics resulting of lower body negative pressure, believed to be the result of a more rapid increase in perfusion of the exercising muscles.

Four physiological interventions shall be used in the current project: (1) hyperoxia;
(2) hypoxia; (3) anaemia; and (4) polycythaemia. These manipulations are designed to help evaluate the role of oxygen delivery in determining cardiorespiratory dynamics to a step-exercise forcing function. These treatments will be investigated independently, and then in combination. While the effects of some of these manipulations have been studied previously, neither the reciprocal effects of haematocrit manipulation, nor the combined influence of these variables has been investigated.

2.5.1 Hypoxia

Breathing oxygen enriched gas mixtures (increased F\textsubscript{\text{1O}}\textsubscript{2}) has been found to improve endurance performance (Bannister and Cunningham, 1954; Plet et al., 1992; Welch, 1987). At workrates at or near maximal aerobic capacity, V\textsubscript{O2} may increase by adding oxygen to the inspired air (Welch et al., 1974; Yamaji and Shephard, 1985). In submaximal exercise the size of the oxygen deficit is increased with hypoxia and decreased with hyperoxia (Linnarsson et al., 1974). Therefore, under hyperoxic conditions, one may predict the reduced oxygen deficit to be a result of enhanced response dynamics. Faster V\textsubscript{O2} response dynamics under hyperoxic conditions (\(\tau=29\pm6s\)) have previously been demonstrated, compared to normoxic conditions (\(\tau=38\pm6s\); Linnarsson et al., 1974). The adjustment for f\textsubscript{c} and \(\dot{Q}\) also show a similar trend (Linnarsson et al., 1974). Though this is not a universal observation. Nakazono and Miyamoto (1987), however, found no difference in V\textsubscript{O2} or \(\dot{Q}\) dynamics, but a significant delay in V\textsubscript{E} and V\textsubscript{CO2} response dynamics.
A number of difficulties arise when determining oxygen consumption using elevated oxygen inspired tensions. These difficulties arise when the calculations for determining $\dot{V}_{O_2}$ are affected by variations in the volume of inspired and expired gases, and gas analysis error or contamination, and the uncertain assumption that there is no net nitrogen (N₂) exchange under hyperoxic conditions (Welch & Pedersen, 1981; Morris, 1983). Conventional use of the Douglas bag technique has resulted in an over-estimation of pulmonary $\dot{V}_{O_2}$ compared to those obtained using the mixing chamber method (Adams & Welch, 1980). When minimizing possible errors during submaximal steady-state exercise, Welch and Pedersen (1981) demonstrated no difference in $\dot{V}_{O_2}$ between hyperoxic and normoxic conditions, as did Linnarsson (1974), who also demonstrated improved cardiorespiratory dynamics under hyperoxic conditions (Nakazono & Miyamoto, 1987).

### 2.5.2 Hypoxia

Under hypoxic conditions, systemic oxygen transport may not be maintained because the decreased alveolar $P_{O_2}$ ($P_A O_2$) results in a reduced $C_sO_2$ (Welch, 1987), even though blood flow to working muscle is increased. Hypoxaemia leads to a reduction in the oxygen gradient between the capillaries and the muscle mitochondria, thus lowering tissue oxygen concentration.

An increased oxygen deficit, found under hypoxic conditions, implies that the additional energy was derived from sources other than aerobic metabolism (Linnarsson et
At very low workloads, steady-state exercise is able to be maintained under hypoxic conditions, with the cost being a greater oxygen deficit because of an elevated anaerobic metabolism, resulting in increased lactate production (Springer et al., 1991). This then results in an elevated $\dot{V}_E$ (Garner et al., 1986; Springer et al., 1989; Ward & Nguyen, 1991), allowing the greater removal of $H^+$ and an elevated $\dot{V}_{CO_2}$ (Adams and Welch, 1980).

Hypoxia has been shown to significantly slow cardiorespiratory response dynamics (Linnarsson, 1974; Ward & Whipp, 1989; Murphy et al., 1989; Springer et al., 1991), but no difference was shown in the response dynamics by Nakazono and Miyamoto (1987). The effect of hypoxia on the cardiorespiratory response dynamics has not yet been demonstrated when the oxygen transport capacity of the blood is reduced by normovolaemic anaemia.

### 2.5.3 Artificial normovolaemic anaemia

Under acute anaemic conditions, an increase in maximal cardiac output ($\dot{Q}_{\text{max}}$) during maximal exercise is thought to be a result of the lowered oxygen content of the blood and reduced viscosity (Nunn, 1993; Woodson et al., 1978). The loss of 800-1200ml of whole blood can reduce haemoglobin concentration by 13-18% after 24 hours, leading to a reduced $\dot{V}_{O_2\text{peak}}$ and performance; this decrease is proportional to the reduced haemoglobin concentration (Ekblom et al., 1972; Kanstrup and Ekblom, 1984) or oxygen carrying
capacity (Woodson et al., 1978).

In chronically anaemic subjects, $Q_{max}$ returns to nearly normal values during maximal exercise, as systemic vascular resistance decreases in response to tissue hypoxia, thus improving tissue oxygen flow (Nunn, 1993; Woodson et al., 1978). During submaximal exercise in the anaemic state, an increase in $Q$ is mainly the result of an elevated $f_c$. This increase in $Q$ does not totally compensate for the lower oxygen transporting capacity of the blood, therefore, the systemic oxygen transport remains lower (Celsing et al., 1986). Submaximal $\dot{V}_{O_2}$ has been shown to remain nearly constant with reduced oxygen delivery (Woodson et al., 1978; Duke & Abelman, 1969). This is the result of a reduction in mixed venous saturation due to an increase in oxygen extraction from arterial blood (Woodson et al., 1978; Nunn, 1993).

If the limiting factor for determining the rate of change in oxygen consumption is a transport mechanism, then an artificially reduced circulating haematocrit (and $C_{aO_2}$) should result in a slowing of the cardiorespiratory response dynamics during submaximal exercise. This hypothesis alone does not yet appear to have been tested. Combining a lowered haematocrit with altered $F_{O_2}$ during submaximal exercise, should either counteract or exaggerate the effects on the cardiorespiratory response dynamics. These manipulations have not been investigated.
2.5.4 Artificial normovolaemic polycythaemia

Blood volume and [Hb] are significant determinants of $\dot{V}O_{2\text{peak}}$ and endurance capacity (Kanstrup & Ekblom, 1984; Ekblom et al., 1972; Gledhill, 1982; Sawka et al., 1987; Williams et al., 1981; Woodson, 1984). Elevating systemic oxygen transport by increasing the number of circulating erythrocytes (polycythaemia) has been found to increase $\dot{V}O_{2\text{peak}}$ (Turner et al., 1993; Ekblom et al., 1972; Buick et al., 1980; Kanstrup & Ekblom, 1984; Thomson et al., 1982; Spriet et al., 1986; Robertson et al., 1979; Thomson et al., 1983), while others have found no effect (Celsing et al., 1986; Williams et al., 1978; Williams et al., 1973; Pate et al., 1979). Endurance time to exhaustion (Buick et al., 1980; Williams et al., 1981; Robertson et al., 1979), the time over a set distance (Berglund & Hemmingson, 1987; Brien & Simon, 1987; Williams et al., 1981), or the work capacity (Thomson et al., 1983; Robertson et al., 1984) may also increase with polycythaemia.

As for cardiorespiratory values during submaximal exercise, there has been few studies consistent in their results and conclusions. Early studies by Richardson & Guyton (1959) demonstrated a reduction in $\dot{Q}$ with a rise in haematocrit, accompanied by an increase in total peripheral resistance and a reduction in cell flow, indicating a reduction in $O_2$ delivery. Following the reinfusion of autologous red blood cell, Celsing et al. (1986) found no difference in submaximal $\dot{V}O_2$, $\dot{V}E$, $\dot{Q}$, $f_C$, stroke volume (SV) or $C_aO_2$, while Robertson et al. (1984) found no change in $\dot{V}O_2$ or SV, and Pate et al. (1979) demonstrated no change in $\dot{V}O_2$, $\dot{V}E$, blood lactate, $f_C$ or respiratory quotient. Williams et al. (1973) and
Videman and Rytömaa (1977), found a reduced $f_c$ as a result of reinfusion but suspected it was a training effect. Submaximal $f_c$ decreased following reinfusion under both normoxic and hypoxic conditions (Robertson et al., 1979). During intense submaximal exercise (91% $\dot{V}_{O_2\text{peak}}$; Spriet et al., 1986), $f_c$ was unchanged, $SV$ was elevated, resulting in an elevated $Q$.

Over a 90 minute protocol, the cardiorespiratory response was similar under conditions following reinfusion while $\dot{V}_E$ and pulmonary blood flow ($Q_c$) were slightly elevated (Thomson et al., 1983), while cardiorespiratory response dynamics have not been investigated when systemic oxygen transport has been elevated by increasing [Hb].

2.6 FOCUS OF CURRENT RESEARCH

The theme of this project is to test the hypothesis that cardiorespiratory response dynamics are a function of oxygen transport to the exercising muscle. Systemic oxygen transport has been altered by altering the $F_I O_2$, yet this method is insufficient to alter oxygen transport alone, since it involves altering the partial pressure of alveolar gases, which may be compensated for by changes in muscle blood flow. When maintaining the $F_I O_2$ constant, and altering the number of circulating red blood cells, systemic oxygen transport may be manipulated, as long as there is no subsequent increase in blood viscosity. If there is an oxygen transport limitation, then the latter changes should produce either an increase or decrease in cardiorespiratory response dynamics. When induced polycythaemia is combined with hyperoxia, one would predict faster response dynamics, while the combination of anaemia with hypoxia, should delay response dynamics.
CHAPTER THREE: METHODS
CHAPTER THREE: METHODS

3.1 SUBJECTS

Six male, non-smokers without a history or symptoms of cardiopulmonary or blood disorders participated in this study. The physical characteristics of the subjects are given in Table 3.1. Subjects were physically active cyclists, who trained on a regular basis (5-6 times per week). Subjects were screened for indications of gross pulmonary insufficiencies through tests of forced vital capacity (FVC), forced expiratory second volume (FEV$_{1.0}$), and maximal voluntary ventilation (MVV; Quinton Q-Plex I Spirometry package; Table 3.1).

Subjects were informed of the nature of the protocol, receiving a subject information package, and were required to complete a written informed consent for both the Department of Biomedical Science and the South Coast Blood Bank. All procedures were performed in accordance with requirements of the University of Wollongong Human Experimentation Ethics Commitee (approval number HE 92/48), and under the guidelines of the National Health and Medical Research Council (1987). Subjects were also required to satisfactorily complete the Physical Activity Readiness Questionnaire (Thomas et al., 1992).

Subjects took part in tests involving experimental manipulations of two independent variables: changes in the fractional concentration of oxygen in the breathing gas mixture; and changes in whole-body haematocrit using whole-blood withdrawal and autologous
Table 3.1: Physical characteristics of subjects.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (yrs)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>FVC (l) BTPS</th>
<th>FEV$_{1.0}$ (%)</th>
<th>MVV (l.min$^{-1}$) BTPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23</td>
<td>185.0</td>
<td>83.8</td>
<td>5.42</td>
<td>86.0</td>
<td>224.7</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td>178.0</td>
<td>74.0</td>
<td>4.42</td>
<td>79.0</td>
<td>204.2</td>
</tr>
<tr>
<td>3</td>
<td>27</td>
<td>169.0</td>
<td>72.2</td>
<td>4.13</td>
<td>87.0</td>
<td>212.3</td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>184.0</td>
<td>79.0</td>
<td>5.51</td>
<td>79.0</td>
<td>223.7</td>
</tr>
<tr>
<td>5</td>
<td>27</td>
<td>180.0</td>
<td>69.0</td>
<td>4.28</td>
<td>84.0</td>
<td>177.8</td>
</tr>
<tr>
<td>6</td>
<td>22</td>
<td>171.0</td>
<td>72.0</td>
<td>4.33</td>
<td>83.0</td>
<td>200.4</td>
</tr>
<tr>
<td>MEAN</td>
<td>24.8</td>
<td>177.8</td>
<td>75.0</td>
<td>4.68</td>
<td>83.0</td>
<td>207.2</td>
</tr>
<tr>
<td>SD</td>
<td>2.8</td>
<td>6.6</td>
<td>5.4</td>
<td>0.62</td>
<td>1.4</td>
<td>17.5</td>
</tr>
</tbody>
</table>
reinfusion. Subjects were tested at three levels of each manipulation, during a constant intensity step-forcing function exercise protocol. This protocol involved a sequence of pre-exercise rest (5 minutes seated on a cycle), cycling at a workrate corresponding to 45% of peak aerobic power (\(\dot{V}_{O2\text{peak}}\)) for 6 minutes, and a resting recovery (5 minutes seated on the cycle). The particular workrate of \(~45\%\) of peak aerobic power was selected so as not to shift the ventilatory response towards its alinear segment under normoxic conditions. The dynamics of several cardiorespiratory variables were studied during both the exercise and recovery periods.

3.2 APPARATUS

Data for oxygen consumption (\(\dot{V}_{O2}\)), carbon dioxide elimination (\(\dot{V}_{CO2}\)), and ventilation (\(\dot{V}_{E}\)) were collected on a pseudo breath-by-breath\(^5\) basis using a Quinton gas analysis system (Quinton Q-Plex I; Figure 3.1). Subjects breathed through a two-way, low resistance Hans-Rudolph non-rebreathing valve (2700 series, dead space 115 ml). Expired gas passed via low-resistance tubing (35mm internal diameter), through a Hans Rudolph pneumotachograph (Model no. 3813), to a gas mixing chamber. Gas samples were drawn from the mixing chamber, and passed through a zirconia oxide oxygen analyzer, and an infrared absorption carbon dioxide analyzer housed within the system, to determine

\(^5\) \(\dot{V}_{O2}\) and \(\dot{V}_{CO2}\) data generated by the Q-Plex 1 system are pseudo breath-by-breath because the gas concentrations are drawn from a mixing chamber over the expiratory duration of each breath. Using this technique, the breath volumes and durations are actual, while the concentrations are averaged over the breath duration, and may include gases from the previous breath.
Figure 3.1: Experimental set-up of equipment for peak aerobic power tests: A: Quinton Q-Plex 1; B: Ohmeda Biox 3700e pulse oximeter; C: Lode electronically braked cycle ergometer; D: PE 3000 Sportstester.
fractional concentrations.

Cardiac frequency ($f_c$) and arterial oxygen saturation ($S_pO_2$)\(^6\) was monitored continuously and recorded on a breath-by-breath basis using a pulse oximeter (Ohmeda Biox 3700e; Figure 3.1), attached to the pinna of the ear. Preparation of the site involved cleaning with isopropyl alcohol and gently massaging with a rubefacient cream (Metsal®) to make the ear hyperaemic. The pulse oximeter was linked with the Quinton Q-Plex I allowing direct downloading of the instantaneous data, in phase with each tidal volume excursion. The instruments cables were secured to eliminate movement artifact. During the maximal exercise tests, peak cardiac frequency ($f_{c_{max}}$) was determined using a Sportstester PE 3000 unit (Polar Electro KY).

Cycling was performed on a Lode electronically braked cycle ergometer (Lode Excalibur Sport; Figure 3.1). The seat and handlebars were adjusted on both the horizontal and vertical planes to suit the needs of each subject.

3.2.1 Validation of the gas analysis system

The Q-Plex I computerised gas analysis system had been previously been independently validated for measures of oxygen and carbon dioxide contents, $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and

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\(^6\) The arterial oxygen saturation ($S_pO_2$) read by pulse oximeters is referred to as $S_pO_2$ because the presence of dyshaemoglobins or other pigments cannot be determined by a two wavelength instrument, and may result in erroneous readings when present.
\( \dot{V}_E \) (Chypchar et al., 1990), while Solomon (1991) validated the unit in the current laboratory. This was completed by comparing expired gas volumes and concentrations to those determined independently.

Validation of the oxygen and carbon dioxide analyzers were performed using expired gas samples of varying concentrations. The samples were analyzed by the Q-Plex I analysers, and an Applied Electrochemistry oxygen analyser (Model no. S-3A) and a Beckman carbon dioxide analyser (Model no. LB-2). All analyzers were calibrated prior to use using two gas standards (\textit{alpha} Standard: Commonwealth Industrial Gases, Australia). Comparisons between the independent analyzers and the Q-Plex I resulted in correlation coefficients of 0.956 for oxygen and 0.972 for carbon dioxide (Figure 3.2; from Solomon, 1991).

Volume validation was performed by collecting expired gas samples in a water-filled, wedge spirometer (Phillip Harris Limited) connected in series with the Q-Plex I using low resistance tubing. The spirometer was calibrated using three syringes of known volume, having been previously calibrated using a multiple water-filling technique. Tidal volumes were then exhaled into the system as single breaths. Volumes from the spirometer were converted to body temperature and pressure saturated (BTPS) conditions. The comparison of these values with those corresponding to the tidal volumes from the Q-Plex I produced a correlation coefficient of 0.999 (Figure 3.2; from Solomon, 1991).
Figure 3.2: Validation of the Quinton Q-Plex 1 by comparisons of: (A) oxygen concentrations for Q-Plex I and Applied Electrochemistry analyser ($r=0.956$); (B) carbon dioxide concentrations for Q-Plex I and Beckman analyser ($r=0.972$); and (C) expired tidal volumes for Q-Plex I and water-filled, wedge spirometer ($r=0.999$). Data taken from Solomon (1991) with permission.
3.2.2 Validation of the Ohmeda Biox 3700e pulse oximeter and PE 3000 Sports tester

The cardiac frequency outputs of the pulse oximeter and sportstester were validated with two subjects performing a submaximal step-protocol over a period of 36 minutes. Throughout the tests, subjects were connected to the pulse oximeter via an ear probe, and also connected to a 5 lead Electrocardiogram (Quinton 5000) where cardiac frequency was determined from leads II, V and VIII, using the R-R interval. Cardiac frequency from both systems was regressed against that obtained from ECG analysis. Analysis indicated that, during submaximal exercise, the pulse oximeter and the sportstester provided reliable determinations of cardiac frequency ($r=0.999$ and $r=0.996$ respectively; Appendix D).

Validation of arterial oxygen saturation was not able to be undertaken. However, such validation has been performed by other laboratories for apparatus using both this technique and this model of instrument. Continuous measurement by the Ohmeda 3700 pulse oximeter gives consistently higher values (4.7% in black subjects; 1.6% in white subjects) than those obtained using the Hewlett-Packard ear oximeter\(^7\) (Cahan et al., 1990), and as $S_aO_2$ decreases, there is an increasing difference between pulse oximeters and the HP oximeter. The accuracy of the pulse oximeters equipped with transmittance sensors is

\(^7\) Hewlett Packard oximeters have previously been validated against arterial blood gas measurements. "The difference between the HP and arterial blood gas levels were $0.9 \pm 4.3\%$, indicating good correlation and 95% confidence limits of agreement of about 4%" (Cahan et al., 1990).
however, within the limits of clinical acceptance for monitoring and trending of $S_aO_2$ during exercise in healthy subjects (Figure 3.3; Decker et al., 1990; Powers et al., 1989; Severinghaus & Kelleher, 1992). During maximal exercise, $S_pO_2$ tend to be lower than $S_aO_2$ measured from arterial blood, although the difference is small and not significant (Powers et al., 1989). Pulse oximeters tend to fail during systolic hypotension due to inadequate perfusion except when vasoconstriction is present (Severinghaus & Spellman, 1990), although reliable $S_pO_2$ and $f_C$ readings were still obtained when cardiac output and peripheral temperature were low (Pälve & Vuori, 1990). Smyth et al. (1986) suggest that during exercise under hypoxic conditions, the Ohmeda Biox 3700e should be used as a qualitative evaluation of oxygen desaturation rather than as a measure of the degree of hypoxia induced by exercise. On the basis of this work, it was believed that further validation of the Ohmeda Biox 3700e was not warranted.

The effect of the rubefacient cream (Metsal®) to arterialize earlobe capillary blood was determined on 6 subjects for 20 minutes post-application of the cream, using a Blood Perfusion Monitor (Laserflo BPM², Vasamedics, USA) to determine skin blood flow (SkBF; Figure 3.4). Saturation of the Laser Doppler Flow (LDF) signal indicated the response was virtually complete within one minute of application. The application of the rubefacient cream was therefore assumed to be sufficient to fully arterialize the capillaries of the earlobe prior to connection of the ear probe.
Figure 3.3: Relationship between Hewlett-Packard oximetric and arterial blood O$_2$ saturation (S$_a$O$_2$) during hypoxic exercise. Data taken from Smyth et al., (1986).
Figure 3.4: Skin blood flow (SkBF) response to Metsal® application on the earlobe. Data are mean with standard error of the mean. Response was virtually complete within one minute of application. Note that treatment mean corresponds to saturation of Laser Doppler Flow (LDF) signal (p<0.05), and volts are analogous to blood flow measured in ml.min⁻¹.gm⁻¹.
3.2.3 Calibration

Volume calibration of the gas analysis system was performed using a known volume standard (3012ml), which had been previously determined through a water-filling technique (Solomon, 1991). This standard was passed through the system six times at various flow rates. Calibration was repeated if the error was greater than 3%. Reference gases\(^8\) were used to calibrate the gas analysis system before each test. The reference gases had previously been calibrated against alpha standard reference gases (gravimetrically determined: Commonwealth Industrial Gases).

3.3 PROCEDURES

Subjects were involved in six days of testing: three maximal tests to determine peak aerobic power (\(\dot{V}_{O_2}\text{peak}\)) in each state of altered haematocrit; and three days of submaximal tests, where subjects were exposed to hypoxic, normoxic and hyperoxic gas mixtures, in each state of altered haematocrit. Haematocrit changes were carried out in the same order, while gases were random. The sequence is summarised in Table 3.2.

\(^8\) The low calibration gas was 10.27% oxygen with the balance nitrogen, while the high calibration gas was comprised of 5.03% carbon dioxide, 25.09% oxygen and the balance nitrogen.
### Table 3.2: Sequence of testing and associated time intervals.

<table>
<thead>
<tr>
<th>STEP</th>
<th>TASK</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Initial $\dot{V}_{O2peak}$ test</td>
</tr>
<tr>
<td>2.</td>
<td>One day interval</td>
</tr>
<tr>
<td>3.</td>
<td>First series of submaximal tests at normal haematocrit.</td>
</tr>
<tr>
<td>4.</td>
<td>First unit of blood withdrawn (~450 cc)</td>
</tr>
<tr>
<td>5.</td>
<td>Twenty four hour interval</td>
</tr>
<tr>
<td>6.</td>
<td>Second unit of blood withdrawn (~450 cc)</td>
</tr>
<tr>
<td>7.</td>
<td>Twenty four hour interval</td>
</tr>
<tr>
<td>8.</td>
<td>Third unit of blood withdrawn (~450 cc)</td>
</tr>
<tr>
<td>9.</td>
<td>Forty-eight hour interval</td>
</tr>
<tr>
<td>10.</td>
<td>Second $\dot{V}_{O2peak}$ test in anaemic state</td>
</tr>
<tr>
<td>11.</td>
<td>Twenty four hour interval</td>
</tr>
<tr>
<td>12.</td>
<td>Second series of submaximal tests in anaemic state.</td>
</tr>
<tr>
<td>13.</td>
<td>Twelve week interval</td>
</tr>
<tr>
<td>14.</td>
<td>Reinfusion of packed red blood cells</td>
</tr>
<tr>
<td>15.</td>
<td>Twenty four hour interval</td>
</tr>
<tr>
<td>16.</td>
<td>Third $\dot{V}_{O2peak}$ test</td>
</tr>
<tr>
<td>17.</td>
<td>Twenty four hour interval</td>
</tr>
<tr>
<td>18.</td>
<td>Third series of submaximal tests in polycythaemic state.</td>
</tr>
</tbody>
</table>
3.3.1 Pre-experimental standardisation

The temperature and humidity of the Applied Physiology Research Laboratory at the University of Wollongong were controlled to ~20°C and ~55% humidity. To minimize effects of circadian rhythms on the subjects' responses, each subject reported to the laboratory at approximately the same time of day for each of the experimental sessions. Subjects were asked not to take caffiene in the 4 hours prior to each testing session; not to take any food in the 4 hours prior to testing; and not to exercise strenuously in the 24 hours prior to each session. To standardise the hydration status of each subject throughout testing, subjects consumed 400 ml of water prior to exercise. For all experimental sessions, subjects wore cycle shorts, t-shirt, socks and sports shoes only.

On arrival at the laboratory, subjects were asked to void and were measured for height and weight. Each subject then performed standardised pulmonary function tests. Three trials were performed for forced vital capacity and the results averaged, and two maximum voluntary ventilation tests were performed, and the results averaged. Following instruction on the various test protocols, subjects performed the cycle exercise tests.

3.3.2 Maximal aerobic power test

Standard open-circuit spirometry techniques were used to determine $\hat{V}_{O2\text{peak}}$. Subjects cycled on an electrically braked ergometer, performing a ramp protocol, where the workrate
increased at the rate of 36 W.min\(^{-1}\). The seat height of the ergometer was adjusted so that the legs were in a position of slight flexion (170°) at the nadir of the downstroke, while the handlebars were set according to the needs of the subject. Once positioned on the ergometer, the head gear which supported the two-way valve, was fitted. Expired gas analysis started simultaneously with the commencement of cycling.

The criterion for \(\dot{V}_{O_2}\)peak test termination was volitional fatigue. Verbal encouragement was provided throughout all tests. \(\dot{V}_{O_2}\)peak was calculated by averaging the breath-by-breath \(\dot{V}_{O_2}\) data over 15 second periods, with the highest value taken as the peak.

3.3.3 Submaximal Exercise Forcing Function

All subjects performed the submaximal series of tests within a two hour period to minimize the effect of circadian rhythms of the dynamics of oxygen uptake. In the control state the mean and standard deviation for temperature and humidity was 21.0° ± 1.5°C and 54.8 ± 7.8%. Subsequently, temperature and humidity averaged 19.7° ± 1.5°C and 62.2 ± 7.7% in the anaemic state, and 23.2° ± 1.3°C and 54.0 ± 4.1% in the polycythaemic state.

Subjects performed three sets of submaximal exercise on an electronically braked cycle ergometer, using a square-wave step forcing function. Positional set-up was the same as for the \(\dot{V}_{O_2}\)peak test. Following five minutes of seated rest on the ergometer, subjects started pedalling at a cadence of 70 rev.min\(^{-1}\) (after Coast et al., 1986). The cadence was
visible to the subject on a tachometer mounted on the cycle ergometer. Submaximal exercise lasted six minutes, and was undertaken at a workrate corresponding to 45.38% of the subjects' $\dot{V}_{O2peak}$ (Table 3.3). This was followed by a step-decrease to rest for a further five minutes, where the subjects remained seated quietly on the ergometer. Expired gases and $f_c$ were monitored for the entire period of this protocol.

This protocol was repeated twice for each breathing gas mixture, to ensure adequate data collection across the full test period and to permit higher resolution in the curve fitting procedures (see: Appendix A). Pilot testing determined that tests could be carried out consecutively with at least 45 minutes complete rest between each test, without affecting the gas exchange dynamics (Appendix B).

Approximately 15 minutes prior to each series of submaximal tests and after 20 minutes sitting, blood samples were withdrawn from a venapuncture (with tourniquet) to determine haemoglobin concentration and venous haematocrit immediately prior to each test battery. Full blood counts were calculated on a 5 ml sample using a Coulter Counter.

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9 The workrate and exercise duration selected were chosen to ensure that the workrate was on the linear phase of the $\dot{V}_E/W$ response curve, and to minimize core temperature elevation, plasma catecholamines, and metabolic acidosis which accompany high work rates (Garner et al., 1986). It was determined that this workrate elicited 36.02% of $\dot{V}_{O2peak}$ during the ramp protocol. Under steady-state conditions, these workrates resulted in 45.38% of $\dot{V}_{O2peak}$ (Table 3.3).
Table 3.3: $\dot{V}_\text{O}_2\text{peak}$ data for normocythaemic (control) conditions.

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>$\dot{V}_\text{O}_2\text{peak}$ (l.min$^{-1}$)</th>
<th>Maximum Workrate (Watts)</th>
<th>40% Maximum Workrate (Watts)</th>
<th>$\dot{V}_\text{O}_2$ AT 40% Max Workrate During Ramp Test (l.min$^{-1}$)</th>
<th>$\dot{V}_\text{O}_2$ Plateau During Submaximal Step-test (l.min$^{-1}$)</th>
<th>% of $\dot{V}_\text{O}_2\text{peak}$ During Submaximal Plateau</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.06</td>
<td>436.0</td>
<td>135.0</td>
<td>1.85</td>
<td>2.41</td>
<td>39.77</td>
</tr>
<tr>
<td>2</td>
<td>4.85</td>
<td>360.0</td>
<td>150.0</td>
<td>1.42</td>
<td>2.64</td>
<td>54.43</td>
</tr>
<tr>
<td>3</td>
<td>6.13</td>
<td>490.0</td>
<td>166.0</td>
<td>2.24</td>
<td>2.37</td>
<td>38.66</td>
</tr>
<tr>
<td>4</td>
<td>6.06</td>
<td>430.0</td>
<td>172.0</td>
<td>2.40</td>
<td>2.62</td>
<td>43.23</td>
</tr>
<tr>
<td>5</td>
<td>5.48</td>
<td>420.0</td>
<td>168.0</td>
<td>2.22</td>
<td>2.53</td>
<td>46.17</td>
</tr>
<tr>
<td>6</td>
<td>4.88</td>
<td>375.0</td>
<td>150.0</td>
<td>1.92</td>
<td>2.44</td>
<td>50.00</td>
</tr>
<tr>
<td>MEAN</td>
<td>5.58</td>
<td>418.5</td>
<td>156.8</td>
<td>2.01</td>
<td>2.50</td>
<td>45.38</td>
</tr>
<tr>
<td>SD</td>
<td>0.60</td>
<td>46.6</td>
<td>14.2</td>
<td>0.36</td>
<td>0.11</td>
<td>5.30</td>
</tr>
</tbody>
</table>
Venous haematocrits were corrected for venous-to-whole body ratio (Gibson et al., 1946). Whole-body haematocrit \( [Hb_{wb}] \) was determined using the equation: \( Hb= \) Venous haematocrit \( \times 0.91 \) (Walker, 1990; Mollison, 1979). Arterial oxygen concentration \( (C_aO_2) \) was estimated using the equation:

\[
C_aO_2=([Hb] \times S_pO_2 \times 1.31) + 0.3 \quad (Nunn, 1993).
\]

### 3.3.4 Modifying breathing gas composition

For each variation in haematocrit, subjects underwent submaximal tests under each of the following conditions: normoxic gas mixture (20% \( O_2 \)), hypoxic gas mixture (10% \( O_2 \)), and hyperoxic gas mixture (30% \( O_2 \)), with the balance being nitrogen in each case. These gas mixtures were selected as the extreme gas concentrations the software for the Q-Plex I was capable of calculating. Prior to starting the exercise test, each subject breathed the gas mixture for approximately five minutes to obtain equilibration of body gas stores (Garner et al., 1986). Exposure to the gas mixtures was performed in random order to minimize sequence effects.

The breathing gas supplies (beta standard: Commonwealth Industrial Gases) were calibrated against alpha standard reference gases, and were administered to each subject in a single blind fashion to minimize psychological effects of breathing gas mixtures containing different oxygen concentrations (Buick et al., 1980). The different oxygen

---

\(^{10}\) A full blood count included: white blood cells (WBC), red blood cells (RBC), haemoglobin concentration ([Hb]), venous haematocrit (Hct), mean cell volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), and platelet count (PLT).
concentrations were supplied from storage cylinders into a 170 litre Douglas bag (Hans Rudolph) which served as a continually refilled reservoir of gas from which the subjects were able to breathe.

3.3.5 Modifying red blood cell count

The first series of submaximal tests were carried out at each subject's normal (control) venous haematocrit (Hct: 40.0 ± 0.02%) and haemoglobin concentration ([Hb]: 142.0 ± 8.8g.dL⁻¹). After completing the submaximal tests, subjects had three units (~1350ml) of whole blood withdrawn. The blood was withdrawn over a period of five days (Table 3.2), with a minimum of 48 hours between venesections. Plasma expansion was not employed between venesections because the blood letting was spread over the period of a week, and blood volume is normally restored within 24-48 hrs (Gregersen and Dawson, 1959; Walker, 1990). As the blood volume withdrawn was only small, and was completed over the period of five days, problems of hypovolaemia following venesection were minimised (Warren and Cureton, 1989).

One day later, when alterations in blood volume had been negated by the regulatory mechanisms of the kidneys, and possible ill effects of the blood withdrawal overcome, another $\dot{V}_{O_2\text{peak}}$ test was performed to determine the workrate for the second series of submaximal tests. Peak tests were undertaken in a state of relative anaemia (Hct: 35.0 ± 0.02%; [Hb]: 122.2 ± 6.6g.dL⁻¹). The 24-hour delay ensured the re-equilibration of the intravascular and extravascular fluid volumes.
Iron deficiency as a result of blood donation was eliminated by the consumption of ferrous iron (e.g. 100 mg Fe\(^{2+}\)) taken daily for the weeks following the final phlebotomy.

Following these tests, subjects resumed normal training for a period of twelve weeks, which let the body naturally replace the lost volume of red blood cells. This period permitted the red blood cell volume to return to normal (determined from control Hct and [Hb]), and the subjects to train at their normal haematological values for a number of weeks. The autologous red blood cell mass (~600ml) was then reinfused over a period of three to four hours (Buick et al., 1980). The subjects then repeated the \(\dot{V}_\text{O}_2\text{peak}\) test and the submaximal protocols 24 and 48 hours later, when blood volume would have returned to normal. The final series of submaximal tests were undertaken with the venous haematocrit at 43.0 ± 0.02% and haemoglobin concentration at 149.0 ± 10.2g.dL\(^{-1}\).

3.3.5.1 Phlebotomy

Blood was drawn from the antecubital vein following scrubbing with a soap solution, cleaning with a 10% acetone in 70% isopropyl alcohol solution, and a local anaesthetic (5mls: Lignocaine). Blood was withdrawn from a single venipuncture by nursing staff of the South Coast Blood Bank (Illawarra Regional Hospital, Wollongong Campus) using a sterile, closed system. During collection, blood was mixed with an
anticoagulant (CPDA-1)\textsuperscript{11}, and the volume of blood collected was measured. When the appropriate volume had been collected, pilot tubes and segments were collected, and subject identification was confirmed.

3.3.5.2 Red blood cell freezing

To minimize the destruction of erythrocytes during blood storage, red blood cells were stored using the high glycerol freezing technique (after: Åkerblom and Högman, 1974; Mollison, 1979; NSW Blood Transfusion Service, Immunohaematology Protocols, 1991). With freeze preserved red blood cells, it is possible to expect nearly 100\% \textit{in vivo} survival at 24 hours and 7 days post-reinfusion (Buick \textit{et al.}, 1980)\textsuperscript{12}.

After collection into a double dry blood pack (Tuta\textsuperscript{®}), the whole blood was packed by centrifuging at 2500 rev.min\textsuperscript{-1} for 20 minutes. A plasma separator was used to remove the plasma component and supernatant. The red cell volume was estimated by suspending the pack from a spring balance and subtracting the mass of the empty pack. Using a 45cm link set (Reference No. 72-118), the donor unit was connected to

\textsuperscript{11} Citrate Phosphate Dextrose Adenine-1 anticoagulant (CPDA-1) contains additional adenine which provides a substrate from which the red blood cells (RBC's) can synthesize adenosine triphosphate during storage until frozen, resulting in improved viability.

\textsuperscript{12} The percentage of red cell recovery can be estimated by the formula: \% red cell recovery=weight (g) of deglycerolized RBC/weight (g) of RBC to be frozen. To determine the mass of the red blood cells to be frozen, the mass of the red cell container is subtracted from the gross mass of the bag plus the red cell component before glycerolization. This net mass is then multiplied by the measured haematocrit of the red cell component. To determine the mass of the deglycerolized-red blood cells, the mass of the red cell container is subtracted from the gross mass of the bag plus the cells. This net mass is then multiplied by the measured haematocrit of the cells in the bag (Walker, 1990).
a bag of cryopreservative solution (38% glycerol, and 2.9% mannitol in 0.63% saline). To the estimated volume of packed red cells, an equivalent volume of freezing solution was added during constant mixing. The pack was agitated gently for 10-15 minutes to ensure thorough mixing and to avoid damage caused by osmotic changes (Åkerblom and Högman, 1974; Walker, 1990).13

A Gambro® Hemofreeze bag was labeled with the subjects' name, date of freezing, venesection number and any special characteristics. The link set was disconnected from the freezing solution and connected into the entry port of the Gambro® Hemofreeze bag. After the bag had been checked for leakages, the remainder of the glycerolised blood was allowed to run into the bag. The air in the Gambro® Hemofreeze bag was squeezed out through the entry port before the link set was closed. The Gambro WD-2® welder was used to heat seal the teflon bag below the level of the entry port, so the unit was fully contained. The seal integrity was tested by holding the bag upside down. The link set was disconnected from the Gambro® bag, and the blood in the link set was used to fill two labelled Nunc tubes for pilot specimens. The two cell samples were placed into the sample pocket and sealed using the welder.

For rapid and even freezing of the unit, the Hemofreeze® bag was placed into a wire grid which was locked flat over the bag. The metal cage and bag were immersed in liquid nitrogen (-196.5°C) to a level just below the outlet ports of the bag. After

13 Glycerol is used in concentrations hypertonic to blood in order to penetrate the cell and alter the tonicity of the cells' interior to prevent severe freezing injury by altering its freezing rate, however its rapid introduction can cause damage to red cells due to incomplete equilibration, which manifests itself as haemolysis after thawing (Walker, 1990).
approximately 15 minutes, the cage was removed from the nitrogen, and the Gambro bag removed from the cage. The bag was stored standing in a liquid nitrogen freezer with labels and ports above the level of the liquid nitrogen (after: Åkerblom and Högman, 1974; NSW Blood Transfusion Service, Immunohaematology Protocols, 1991).

3.3.5.3 Red cell thawing

Before autologous reinfusion, the subject-matched frozen red cells were thawed and reconstituted with a 3.6% and then 0.9% saline solution. The Hemofreeze® bag was placed in a polyethylene bag and thawed in a water bath (37° to 40°C) under gentle agitation. Thawing time was at least 10 minutes, or until room temperature was reached. Hypertonic sodium chloride solution (500ml at 3.6%) was added directly to the thawed cells and carefully agitated. The whole volume of red blood cells in solution was then siphoned into the bowl kit of the Haemonetics®30 Blood Processor (Figure 3.5) at a slow rate, while the bowl was being centrifuged at approximately 1500 rev.min⁻¹. After removal of the haemolysed cells and supernatant, the blood was washed with 3000ml of sterile solution of 0.9% Saline while being centrifuged (Figure 3.6). Finally, the red cells remaining in the Haemonetics® bowl kit were resuspended in a volume of saline to a packed cell volume of approximately 60%. The tubing from the collection pack was heat sealed using the Fenwal Hematron® making 3 numbered segments (2 for cross-matching and an 11cm segment for quality control purposes (simulated transfusion)). The remaining red cells (~5ml) in the line were placed into a dry 10ml specimen container for further quality control tests. Standard quality control tests were carried out prior to that particular pack of deglycerolised red blood cells being cleared.
Figure 3.5: Haemonetics® 30 Blood Processor.
Figure 3.6: Schematic for pheresis kit for Haemonetics® 30 Blood Processor. Electromedics Inc.
for reinfusion\textsuperscript{14}.

\subsection*{3.3.5.4 Reinfusion}

The red blood cells were transfused into the original donor within 8-12 hours from the time of thawing (after: Ákerblom and Högman, 1974; NSW Blood Transfusion Service, Immunohaematology Protocols, 1991), by nursing staff (Illawarra Regional Hospital, Wollongong Campus). The packed deglycerolised red blood cells (D-RBC's) were reinfused through a Blood/Solution Infusion Set (Baxter, Code FMC 5833) and a 2 inch, 16 guage canular (Deseret Medical Inc.), inserted under medical supervision into one of the prominent forearm veins. Each unit of red blood cells took approximately 60 minutes for reinfusion (Figure 3.7).

\section*{3.4 SAFETY DURING RED BLOOD CELL MANIPULATION}

Three units (~1350 ml) of whole blood were donated in three separate phlebotomies over a period of one week, eliminating the risk of hypovolaemia. The phlebotomies were carried out under supervision of the nursing staff at the South Coast Blood Bank at the Illawarra Regional Hospital. The donor was subsequently given instructions about post-phlebotomy care regarding fluid replacement and possible symptoms of hypovolaemia (Ákerblom and Högman, 1974).

\textsuperscript{14} Quality control tests included tests for excessive haemolysis, a simulated transfusion in 0.7\% saline at 37°C, osmolality, pH, supernatant [Hb] in the effluent line of the washing kit, and electrolytes such as [K\textsuperscript{+}] and [Na\textsuperscript{+}], and cross-matching for subject verification (see Appendix E).
Figure 3.7: Reinfusion of deglycerolised red blood cells (D-RBC's) through a 16 guage canular into the forearm.
Blood handling was at a minimum for both the nursing staff carrying out the phlebotomy and those involved in processing the blood during freezing. Nursing staff did not come into direct contact with any blood but were required to wear gloves to minimize the risk of contamination. Prior to phlebotomy, storage bags were thoroughly checked for sterility, leaks, cracks and the condition of the anticoagulant, which should have been clear. Throughout the process of withdrawal, the blood remained in a closed series of interconnecting bags. These seals were only broken when the glycerol was being added prior to freezing in liquid nitrogen, and after storage to allow the washing of the red cells. However maximum possible sterility was achieved as the seals were broken in a sterile laminar flow cabinet.

Most of the haemolysed red blood cells were removed prior to reinfusion during the washing process. To ensure that the level of circulating free haemoglobin in the red cell free supernatant would be limited, a number of quality control tests were carried out (Appendix E).

To eliminate the remote risk of incorrect blood reinfusion, a number of safety checks were carried out prior to reinfusion. All bags were be labelled with both the subjects' name and identification number. Prior to reinfusion, a blood test was carried out to ensure accurate cross-matching of autologous blood. This was overseen by the Director of the Department of Haematology. Following the insertion of a canular under medical supervision, the red cells were reinfused over a period of 3-4 hours with regular observations being carried out by nursing staff.
3.5 SAFETY DURING THE EXERCISE TEST

Subjects were instructed to stop pedalling immediately, should unusual symptoms (other than normal shortness of breath or leg discomfort) develop. These criteria included overt signs of syncope, nausea, dizziness, cyanosis of peripheral tissues, abnormal tachycardia, progressive angina or a desire to stop.

Sterility of laboratory equipment such as mouth pieces, valves and hoses, was carried out by the rinsing the equipment in water, followed by soaking the equipment in an aqueous instrument disenfectant (Cidex®) for a minimum of 20 minutes, prior to further rinsing in water and storage.

3.6 DETERMINING CARDIORESPIRATORY DYNAMICS

Raw data files were created for the breath-by-breath data for time, $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, $\dot{V}_E$, and $f_C$. These were analysed to derive mathematical equations which most closely described the dynamics of the exercise on-set and off-set responses. Several stages of analysis were required before these equations were computed. First, data from each of the three stages of the submaximal protocol were separated and curve fitted using least squares, best-fit polynomials (Tablecurve 3.01; Jandel Scientific)$^{15}$. Data points, for all variables, falling outside of the 95% confidence limits were deleted (Figure 3.8a). Such data are typically artifactual, resulting from swallowing, coughing, or prematurely ending

$^{15}$ The use of polynomial regression equations to describe curve shape allows more precise fitting of a curve to the data, therefore allowing more accurate determination of various increments throughout the response.
Figure 3.8: Summary of the five stages of data analysis for exercise onset data. Figure 3.8a is the raw data with the polynomial curve fit data, 95% confidence limits, and the data marked for exclusion. Figure 3.8b shows five breath running average to smooth data, and also shows plateaux. Figure 3.8c shows the final polynomial curve for these data, anchored at the pre-step plateau, and determined oxygen uptake at 20%, 40%, 60%, 80%, and 100% of the difference between pre-step and step-up plateaux.
the breath for some other reason (Lamarra et al., 1987). Second, the remaining data were smoothed using a five-breath running average. Third, mean plateau values for each variable were obtained for the periods between minute one and five, eight and eleven, and fourteen and sixteen (Figure 3.8b). Fourth, these values were used to anchor both ends of onset and recovery curves for each variable\textsuperscript{16}. Fifth, these anchored curves, representing cardiorespiratory transients, were then modelled using least squares, best-fit polynomials, and the resulting polynomials were evaluated to determine the time to reach 20\%, 40\%, 60\%, 80\% and 100\% of the difference between baseline and post-step plateau for the variable of interest (Figure 3.8c).

Response dynamics were also analyzed by integrating the raw data, averaged over five second periods, from the period of exercise onset at minute 5, to the completion of the particular cardiorespiratory plateaux at minute 11 (73 data points), and for the step-down data, between minutes 11 and 15 (49 data points).

3.7 DESIGN AND ANALYSIS

The experimental design was based on a fully crossed 3 x 3 factorial design. There were 3 levels of factor A (fractional concentration of inspired oxygen: 10\%, 20\% and 30\% O\textsubscript{2}), as well as three levels of factor B (haematocrit: anaemia, normocythaemia and polycythaemia). Testing sequence was determined using a Latin Square model to minimize the order effects of gas administration. All dynamics data were analysed

\textsuperscript{16} Anchoring was necessary since analysis of curve segments without data before and after the segment of interest, invariably altered the shape of the curve from that which was obtained during the experiment.
using MANOVA. Post hoc analyses were performed using Tukey's HSD to identify the sources of significant difference. ANOVA was used to analyse differences between pre-step baselines and between post-step plateaux. Alpha was set at the 0.05 level for all analyses.
CHAPTER FOUR: RESULTS
4.1 HAEMATOLOGICAL RESULTS

The withdrawal of three units of blood over the six day period resulted in a number of haematological changes in the anaemic state. Table 4.1 summarises the resting haematological measurements for pre-withdrawal (control), post-withdrawal (anaemic) and post-reinfusion (polycythaemic) states; complete blood test results can be found in Appendix G. Following blood withdrawal, Hct, [Hb] and red blood cell concentration each experienced a significant reduction from the control state (p<0.05; Table 4.1). Blood reinfusion resulted in elevations in Hct, [Hb] and RBC concentration, such that the new values were significantly greater than observed in both the control and anaemic states (p<0.05; Table 4.1).

4.2 MAXIMAL EXERCISE

Haematocrit manipulations resulted in significant changes to physiological function during maximal exercise. In the anaemic state, the time to volitional fatigue did not change, although \( \dot{V}_{\text{O}_2\text{peak}} \) and peak power were significantly reduced below corresponding control variables (p<0.05). However, \( f_{\text{Cpeak}} \) increased by about 4% in

\[ 17 \text{ Overall } MANOVA F = 5.66474 (4,28), p=0.002 \\
18 \text{ Overall } MANOVA F = 7.55161 (4,28), p<0.001 \\
19 \text{ Overall } MANOVA F = 6.54218 (4,28), p=0.001 \\
20 \text{ see Footnotes } 18, 19 \text{ and } 20. \\
21 \text{ Overall } MANOVA F = 7.55161 (4,28), p<0.001 \\
22 \text{ Overall } MANOVA F = 6.54218 (4,28), p=0.001 \]
Table 4.1: Haematological values for resting male subjects determined prior to (control), following artificially-induced anaemia, and after artificially-induced polycythaemia (Coulter Cell Counter S PLUS IV). Values are means and standard deviations.

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>ANAEMIA (n=6)</th>
<th>CONTROL (n=6)</th>
<th>POLYCYTHAEMIA (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HAEMATOCRIT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(venous)</td>
<td>0.34 ± 0.02</td>
<td>0.40 ± 0.02</td>
<td>0.43 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>**</td>
<td>#</td>
</tr>
<tr>
<td><strong>HAEMATOCRIT</strong></td>
<td>0.31 ± 0.02</td>
<td>0.36 ± 0.02</td>
<td>0.39 ± 0.02</td>
</tr>
<tr>
<td>(whole-body)</td>
<td>*</td>
<td>**</td>
<td>#</td>
</tr>
<tr>
<td><strong>HAEMOGLOBIN</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONCENTRATION (g.l⁻¹)</td>
<td>122.16 ± 6.62</td>
<td>142.00 ± 8.88</td>
<td>151.60 ± 9.79</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>**</td>
<td>#</td>
</tr>
<tr>
<td><strong>RBC</strong></td>
<td>4.06 ± 0.22</td>
<td>4.70 ± 0.21</td>
<td>5.16 ± 0.28</td>
</tr>
<tr>
<td>(x10¹².l)</td>
<td>*</td>
<td>**</td>
<td>#</td>
</tr>
<tr>
<td><strong>MCV</strong></td>
<td>85.85 ± 1.97</td>
<td>85.03 ± 1.70</td>
<td>82.76 ± 2.30</td>
</tr>
<tr>
<td>(fL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MCH</strong></td>
<td>30.05 ± 0.52</td>
<td>30.15 ± 0.71</td>
<td>29.16 ± 1.04</td>
</tr>
<tr>
<td>(pg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MCHC</strong></td>
<td>350.00 ± 5.62</td>
<td>354.83 ± 6.52</td>
<td>352.20 ± 4.38</td>
</tr>
<tr>
<td><strong>CₐO₂</strong></td>
<td>15.47 ± 1.66</td>
<td>17.94 ± 1.09</td>
<td>19.38 ± 2.49</td>
</tr>
<tr>
<td>(ml.dl⁻¹)</td>
<td></td>
<td></td>
<td>#</td>
</tr>
</tbody>
</table>

Abbreviations:
RBC = red blood cell
MCV = mean cell volume
MCH = mean corpuscular haemoglobin
MCHC = mean corpuscular haemoglobin concentration
CₐO₂ = arterial concentration of oxygen

Significant differences (p<0.05) are indicated by `*`, `#` and `**` for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively.
the anaemic state (p>0.05; Table 4.2). Following blood reinfusion, polycythaemic subjects performed equally well during maximal exercise, with \( \dot{V}_{O2\text{peak}} \), time to fatigue, peak power and \( f_{C\text{peak}} \) all being equivalent to values obtained during control conditions (p>0.05; Table 4.2). There were no significant changes in peak ventilation across conditions (p>0.05; Table 4.2). \( f_c \), \( \dot{V}_E \) and \( \dot{V}_{O2} \) at various absolute workrates during the ramp exercise protocol are shown in Table 4.3. There were no significant differences in \( \dot{V}_E \) or \( \dot{V}_{O2} \) between the anaemic, control or polycythaemic conditions, while \( f_c \) was elevated by about 15 beats under anaemic conditions compared to both control and polycythaemic conditions from 100 to 300W (p<0.05).

### 4.3 Submaximal Exercise

\( S_pO_2 \) and \( C_aO_2 \) (averaged over 30 seconds) were determined throughout the submaximal tests to evaluate the effects of haematocrit reduction on oxygen transport. Arterial oxygen concentration (\( C_aO_2 \)) was significantly reduced as a result of hypoxic gas (p<0.05) during each stage of altered haematocrit, while there was no difference between the normoxic and hyperoxic conditions (p>0.05; Figure 4.1). Under normoxic conditions, \( C_aO_2 \) was reduced as a result of anaemia (p<0.05), but there was no difference between control and polycythaemic values (p>0.05). \( S_pO_2 \) was significantly reduced (p<0.05) when inspiring hypoxic gas, while there was no desaturation during submaximal exercise when breathing normoxic or hyperoxic gas (p>0.05; Figure 4.2).

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23 Overall MANOVA \( F = 2.45757 \) (4,28), p=0.069  
24 Overall MANOVA \( F = 3.44828 \) (10,16), p=0.014  
25 Overall MANOVA \( F = 1.81433 \) (24,10), p=0.164  
26 Overall MANOVA \( F = 1.85768 \) (24,8), p=0.184  
27 Overall MANOVA \( F = 1.57965 \) (24,10), p=0.229
Table 4.2: Cardiorespiratory responses to a maximal ramp exercise protocol (cycling; 36 W.min\(^{-1}\)) in the control state, and then following artificially-induced anaemia, and artificially-induced polycythaemia. Data are means with standard errors of the means.

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>ANAEMIA (n=6)</th>
<th>CONTROL (n=6)</th>
<th>POLYCYTHAEMIA (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to (\dot{V}<em>O</em>{2\text{peak}}) (minutes)</td>
<td>10.54 ± 0.47</td>
<td>11.54 ± 1.24</td>
<td>11.11 ± 0.58</td>
</tr>
<tr>
<td>Peak power (Watts)</td>
<td>380.33 ± 17.05</td>
<td>418.50 ± 19.03</td>
<td>394.00 ± 20.7</td>
</tr>
<tr>
<td>(\dot{V}<em>O</em>{2\text{peak}}) (l.min(^{-1}))</td>
<td>4.63 ± 0.19</td>
<td>5.58 ± 0.25</td>
<td>5.00 ± 0.29</td>
</tr>
<tr>
<td>(\dot{V}<em>O</em>{2\text{peak}}) (ml.kg(^{-1}).min(^{-1}))</td>
<td>62.25 ± 2.72</td>
<td>74.48 ± 3.02</td>
<td>67.54 ± 4.09</td>
</tr>
<tr>
<td>f_{C\text{peak}} (beats.min(^{-1}))</td>
<td>189.33 ± 5.07</td>
<td>182.167 ± 3.28</td>
<td>180.60 ± 4.64</td>
</tr>
<tr>
<td>(\dot{V}<em>E</em>{\text{peak}}) (l.min(^{-1}))</td>
<td>193.92 ± 9.99</td>
<td>196.12 ± 9.21</td>
<td>185.90 ± 10.81</td>
</tr>
</tbody>
</table>

Abbreviations:
- \(\dot{V}_O_{2\text{peak}}\) = peak oxygen consumption
- f_{C\text{peak}} = peak cardiac frequency
- \(\dot{V}_E_{\text{peak}}\) = peak ventilation

Significant differences (p<0.05) are indicated by `*`, `#` and `**` for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively.

Under each condition of altered \(F_1O_2\), there were no differences in \(S_pO_2\) between anaemia, control and polycythaemic conditions (p>0.05).
4.3.1 Typical subject data during submaximal exercise

The raw datum for one subject (S3; \( \dot{V}_{O2\text{peak}} = 6.13 \text{ l.min}^{-1} \)) are shown in Figure 4.3 illustrating the effect of mild hyperoxia and extreme hypoxia on \( \dot{V}_{O2} \), \( \dot{V}_{CO2} \), \( \dot{V}_E \) and \( f_c \) during a step-exercise forcing function. There did not appear to be a difference in the pre-step baselines from minute 0 to minute 5, between the different inspired gas concentrations for \( \dot{V}_{O2} \), \( \dot{V}_{CO2} \) or \( \dot{V}_E \), although \( f_c \) was elevated under hypoxic conditions. \( \dot{V}_{CO2} \), \( \dot{V}_E \) and \( f_c \) were also elevated under hypoxic conditions from the onset of exercise at minute 5, through to the beginning of the recovery period, and only returning to normal values in the last two minutes of the step-down protocol, with the exception of \( f_c \) which remained elevated throughout data collection. Hyperoxia tended to result in an elevated \( \dot{V}_{O2} \), and depress \( \dot{V}_{CO2} \) and \( \dot{V}_E \), although there does not appear to be a difference between normoxia and hyperoxia during the step-forcing function.

4.3.2 Effects of altered inspired oxygen concentrations

4.3.2.1 Baselines and plateaux

In the anaemic state, hypoxia resulted in a significantly reduced \( \dot{V}_{O2} \) pre-step baseline and step-up plateau (p<0.05; Table 4.4)\(^{28} \), while hypoxia resulted in elevated

\(^{28}\) Overall MANOVA \( F = 3.51941 \) (24,126), p<0.001
Table 4.3: A comparison of cardiorespiratory function during a maximal ramp exercise protocol (36 Watts.min\(^{-1}\)), at different absolute workrates (50 Watt increments) under control (normocythaemic) conditions, isovolaemic anaemia and isovolaemic polycythaemia. Data are means and standard errors of the means.

<table>
<thead>
<tr>
<th>Work rate (Watts)</th>
<th>Anaemia (n=6)</th>
<th>Control (n=6)</th>
<th>Polycythaemia (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( f_c )</td>
<td>( \dot{V}_E )</td>
<td>( \dot{V}_{O_2} )</td>
</tr>
<tr>
<td>50</td>
<td>107.50 ± 4.10</td>
<td>28.98 ± 3.58</td>
<td>1.36 ± 0.13</td>
</tr>
<tr>
<td>100</td>
<td>111.17 ± 4.71 * #</td>
<td>36.15 ± 2.67</td>
<td>1.57 ± 0.09</td>
</tr>
<tr>
<td>150</td>
<td>127.50 ± 4.62 * #</td>
<td>47.67 ± 2.84</td>
<td>2.07 ± 0.09</td>
</tr>
<tr>
<td>200</td>
<td>147.83 ± 4.62 * #</td>
<td>60.33 ± 3.39</td>
<td>2.54 ± 0.11</td>
</tr>
<tr>
<td>250</td>
<td>161.33 ± 6.62 * #</td>
<td>82.23 ± 3.21</td>
<td>3.09 ± 0.07</td>
</tr>
<tr>
<td>300</td>
<td>170.67 ± 6.21 * #</td>
<td>103.08 ± 5.87</td>
<td>3.66 ± 0.10</td>
</tr>
</tbody>
</table>

Abbreviations:
\( f_c \) = cardiac frequency
\( \dot{V}_E \) = minute ventilation
\( \dot{V}_{O_2} \) = oxygen consumption

Significant differences (p<0.05) are indicated by `*`, `#` and `**` for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively.
Figure 4.1: Arterial oxygen concentration ($CaO_2$) averaged over 30 second intervals, during an exercise step-function from rest (5 min) to ~45% aerobic power (6 min), and back to rest again (5 min). Graphs correspond with data collected during artificially-induced anaemia (A), control (B), and artificially-induced polycythaemia (C), and then across three levels of inspired oxygen: hypoxia (D), normoxia (E) and hyperoxia (F). Data are means with standard errors of the means.
Figure 4.2: Arterial oxygen saturation ($S_pO_2$) averaged over 30 second intervals, during an exercise step-function from rest (5 min) to -45% aerobic power (6 min), and back to rest again (5 min). Graphs correspond with data collected during artificially-induced anaemia (A), control (B), and artificially-induced polycythaemia (C), and then across three levels of inspired oxygen: hypoxia (D), normoxia (E) and hyperoxia (F). Data are means with standard errors of the means.
Figure 4.3: The raw data (breath-by-breath) for oxygen uptake ($\dot{V}_O_2$; Graph A), carbon dioxide elimination ($\dot{V}_C02$; Graph B), minute ventilation ($\dot{V}_E$; Graph C), and cardiac frequency ($f_c$; Graph D), observed under hypoxic ($F,O_2=0.1$), normoxic ($F,O_2=0.2$) and hyperoxic conditions ($F,O_2=0.3$) for Subject N3 during a submaximal step-exercise (cycling) forcing function from rest to ~45% $V_{O2peak}$ and returning to rest.
Table 4.4: Cardiorespiratory variables determined breath-by-breath during the submaximal exercise protocol. Pre-step baselines (PRE) are averaged between minutes 1 and 5, while the step-up plateaux (PLAT) are averaged between minutes 8 and 11, and step-down plateaux are averaged between minutes 14 and 16. Data are means with standard errors of the means. (anaemic and control conditions n=6; polycythaemia, n=5).

<table>
<thead>
<tr>
<th>Condition</th>
<th>10% O_2</th>
<th>20% O_2</th>
<th>30% O_2</th>
<th>10% O_2</th>
<th>20% O_2</th>
<th>30% O_2</th>
<th>10% O_2</th>
<th>20% O_2</th>
<th>30% O_2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaemic</td>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td></td>
<td></td>
<td>Polycythaemic</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.38 ± 0.02</td>
<td>0.53 ± 0.02</td>
<td>0.54 ± 0.03</td>
<td>0.37 ± 0.04</td>
<td>0.42 ± 0.01</td>
<td>0.47 ± 0.03</td>
<td>0.34 ± 0.02</td>
<td>0.45 ± 0.02</td>
<td>0.51 ± 0.01</td>
</tr>
<tr>
<td>PLAT</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.40 ± 0.02</td>
<td>0.31 ± 0.01</td>
<td>0.30 ± 0.01</td>
<td>0.34 ± 0.03</td>
<td>0.27 ± 0.01</td>
<td>0.31 ± 0.03</td>
<td>0.37 ± 0.02</td>
<td>0.29 ± 0.01</td>
<td>0.31 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>15.94 ± 1.23</td>
<td>11.64 ± 0.91</td>
<td>12.16 ± 0.68</td>
<td>14.67 ± 0.68</td>
<td>11.37 ± 0.28</td>
<td>11.93 ± 1.12</td>
<td>14.35 ± 0.97</td>
<td>11.32 ± 0.51</td>
<td>11.32 ± 0.20</td>
</tr>
<tr>
<td>POST</td>
<td>109.76 ± 8.59</td>
<td>57.24 ± 1.26</td>
<td>55.73 ± 1.31</td>
<td>103.48 ± 7.82</td>
<td>57.57 ± 2.38</td>
<td>56.29 ± 2.38</td>
<td>107.89 ± 6.42</td>
<td>61.35 ± 1.58</td>
<td>59.09 ± 2.39</td>
</tr>
<tr>
<td></td>
<td>164.65 ± 5.03</td>
<td>140.51 ± 5.18</td>
<td>136.65 ± 3.14</td>
<td>157.44 ± 4.70</td>
<td>127.46 ± 3.16</td>
<td>124.72 ± 2.54</td>
<td>153.99 ± 3.62</td>
<td>125.98 ± 5.61</td>
<td>121.44 ± 5.13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Condition</th>
<th>10% O_2</th>
<th>20% O_2</th>
<th>30% O_2</th>
<th>10% O_2</th>
<th>20% O_2</th>
<th>30% O_2</th>
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<th>20% O_2</th>
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<tr>
<td>Anaemic</td>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td></td>
<td></td>
<td>Polycythaemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.49 ± 0.04</td>
<td>0.38 ± 0.02</td>
<td>0.34 ± 0.02</td>
<td>0.43 ± 0.02</td>
<td>0.32 ± 0.01</td>
<td>0.35 ± 0.03</td>
<td>0.43 ± 0.03</td>
<td>0.34 ± 0.01</td>
<td>0.42 ± 0.03</td>
</tr>
<tr>
<td>PLAT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>26.36 ± 3.11</td>
<td>14.39 ± 1.03</td>
<td>14.36 ± 0.86</td>
<td>22.34 ± 1.79</td>
<td>12.77 ± 0.78</td>
<td>13.62 ± 0.95</td>
<td>21.22 ± 0.94</td>
<td>13.67 ± 0.25</td>
<td>15.72 ± 0.76</td>
</tr>
<tr>
<td>POST</td>
<td>109.31 ± 5.89</td>
<td>86.60 ± 6.70</td>
<td>83.05 ± 5.51</td>
<td>95.40 ± 3.76</td>
<td>72.36 ± 4.01</td>
<td>71.68 ± 3.51</td>
<td>91.90 ± 4.73</td>
<td>66.98 ± 6.73</td>
<td>67.59 ± 5.72</td>
</tr>
</tbody>
</table>

Abbreviations: \( \dot{V}_O_2 \) = oxygen consumption  \( \dot{V}_{CO_2} \) = carbon dioxide elimination  \( \dot{V}_e \) = minute ventilation  \( f_c \) = cardiac frequency
PRE = pre-step baseline  PLAT = step-up plateau  POST = step-down plateau

Significant differences (p<0.05) are indicated by '1' and '2' for the comparison between 10% O_2 and 20% O_2 conditions, 10% O_2 and 30% O_2 conditions, and 20% O_2 and 30% O_2 conditions respectively.
\( \dot{V}_{O_2} \) step-down plateau (p<0.05)\(^{29}\). In the control state, there were no differences in the pre-step baselines, step-up plateaux or the step-down plateaux between the different inspired gases, and in the polycythaemic state. \( \dot{V}_{O_2} \) was significantly lower under hypoxic conditions than under hyperoxic conditions during the pre-step and post-step-up periods (p<0.05)\(^{30}\). For \( \dot{V}_{CO_2} \)\(^{31}\) and \( \dot{V}_E \)\(^{32}\) in the anaemic state, hypoxia resulted in significantly elevated baselines and plateaux (p<0.05; Table 4.4). In the control state, \( \dot{V}_{CO_2} \) and \( \dot{V}_E \) was elevated under hypoxic conditions following both the step-up and step-down functions\(^{33,34}\). In the polycythaemic state, hypoxia resulted in significantly elevated \( \dot{V}_E \) for the step-up plateau, while for the step-down plateau, \( \dot{V}_E \) was only elevated more during hypoxic than normoxic gas (p<0.05; Table 4.4)\(^{35}\). In each state of altered haematocrit, there were no significant differences in \( f_c \) pre-step baselines between any of the different inspired oxygen concentrations (p>0.05; Table 4.4), while hypoxic stimulation resulted in a significant elevation in \( f_c \) during the step-up plateau across the three levels of altered haematocrit (p<0.05)\(^{36}\), and remained elevated during the step-down plateau under hypoxic conditions in the anaemic and control states (p<0.05)\(^{37}\).

\(^{29}\) Overall MANOVA \( F = 3.51941 \) (24,126), p<0.001
\(^{30}\) Overall MANOVA \( F = 3.51941 \) (24,126), p<0.001
\(^{31}\) Overall MANOVA \( F = 2.25512 \) (24,126), p=0.002
\(^{32}\) Overall MANOVA \( F = 2.76419 \) (24,126), p<0.001
\(^{33}\) Overall MANOVA \( F = 2.25512 \) (24,126), p=0.002
\(^{34}\) Overall MANOVA \( F = 2.76419 \) (24,126), p<0.001
\(^{35}\) Overall MANOVA \( F = 2.76419 \) (24,126), p<0.001
\(^{36}\) Overall MANOVA \( F = 2.58801 \) (24,126), p<0.001
\(^{37}\) Overall MANOVA \( F = 2.58801 \) (24,126), p<0.001
4.3.2.2 Step-up dynamics

In the anaemic state, breathing hypoxic gas resulted in slower $\dot{V}_o_2$ dynamics as the subjects approached steady-state, evident by the longer time to reach 100% of the difference between the pre-step baseline and the step-up plateau (p<0.05)\(^{38}\). This was not significant in the normocythaemic or polycythaemic states, nor were there any of the differences in $\dot{V}_{co_2}$, $\dot{V}_e$ and $f_c$ dynamics between hypoxic, normoxic and hyperoxic gas inhalation significant across the haematocrit modifications (see Figures 4.4 to 4.7; p>0.05).

4.3.2.3 Step-down dynamics

For $\dot{V}_o_2$, $\dot{V}_{co_2}$ and $f_c$ step-down dynamics, hypoxia resulted in a significantly slower response in the early stages of the recovery curve, as evident by the slower times to reach 20%\(^{39}\), 40%\(^{40}\), 60%\(^{41}\) and 80%\(^{42}\) of the difference between the step-up (baseline) plateau and the step-down plateau during each of the three states of altered haematocrit (p<0.05), while the times to reach 100% of the difference were not significantly different for $\dot{V}_o_2$ or $\dot{V}_{co_2}$ (p>0.05; Figures 4.8 to 4.11). In the control state, $f_c$ dynamics were slower under hypoxic than under normoxic conditions (p<0.05)\(^{43}\), while in the polycythaemic state, hypoxia was significantly slower (p<0.05)\(^{44}\) than hyperoxia in the

\(^{38}\) Overall MANOVA $F = 1.54686$ (32,168), p=0.041
\(^{39}\) Overall MANOVA $F = 2.69400$ (32,168), p<0.001
\(^{40}\) Overall MANOVA $F = 2.57365$ (32,168), p<0.001
\(^{41}\) Overall MANOVA $F = 2.46252$ (32,168), p<0.001
\(^{42}\) Overall MANOVA $F = 2.42231$ (32,168), p<0.001
\(^{43}\) Overall MANOVA $F = 1.58532$ (32,168), p=0.033
\(^{44}\) Overall MANOVA $F = 1.58532$ (32,168), p=0.033
Figure 4.4: Oxygen uptake dynamics observed under hypoxic, normoxic and hyperoxic conditions. Data show oxygen uptake changes (%) between the pre-step baseline and the post-step plateau, expressed relative to the difference between these values, in the anaemic (A), normocythaemic or control (B), and polycythaemic states (C). Data are means with standard errors of the means. Significant differences (p<0.05) are indicated by '1', '2' and '3' for the comparisons between hypoxic and normoxic conditions, hypoxic and hyperoxic conditions, and normoxic and hyperoxic conditions respectively.
Figure 4.5: Carbon dioxide dynamics observed under hypoxic, normoxic and hyperoxic conditions. Data show carbon dioxide elimination changes (%) between the pre-step baseline and the post-step plateau, expressed relative to the difference between these values, in the anaemic (A), normocytic or control (B), and polycytic states (C). Data are means with standard errors of the means. Significant differences (p<0.05) are indicated by '1', '2' and '3' for the comparisons between hypoxic and normoxic conditions, hypoxic and hyperoxic conditions, and normoxic and hyperoxic conditions respectively.
Figure 4.6: Ventilation dynamics observed under hypoxic, normoxic and hyperoxic conditions. Data show minute ventilation changes (%) between the pre-step baseline and the post-step plateau, expressed relative to the difference between these values, in the anaemic (A), normocythaemic or control (B), and polycythaemic states (C). Data are means with standard errors of the means. Significant differences (p<0.05) are indicated by ‘1’, ‘2’ and ‘3’ for the comparisons between hypoxic and normoxic conditions, hypoxic and hyperoxic conditions, and normoxic and hyperoxic conditions respectively.
Figure 4.7: Cardiac frequency dynamics observed under hypoxic, normoxic and hyperoxic conditions. Data show cardiac frequency changes (%) between the pre-step baseline and the post-step plateau, expressed relative to the difference between these values, in the anaemic (A), normocythaemic or control (B), and polycythaemic states (C). Data are means with standard errors of the means. Significant differences (p<0.05) are indicated by '1', '2' and '3' for the comparisons between hypoxic and normoxic conditions, hypoxic and hyperoxic conditions, and normoxic and hyperoxic conditions respectively.
Figure 4.8: Oxygen uptake dynamics observed under hypoxic, normoxic and hyperoxic conditions. Data show oxygen uptake changes (%) between the step-up plateau and the step-down plateau, expressed relative to the difference between these values, in the anaemic (A), normocyaemic or control (B), and polycyaemic states (C). Data are means with standard errors of the means. Significant differences (p<0.05) are indicated by '1', '2' and '3' for the comparison between hypoxic and normoxic conditions, hypoxic and hyperoxic conditions, and normoxic and hyperoxic conditions respectively.
Figure 4.9: Carbon dioxide dynamics observed under hypoxic, normoxic and hyperoxic conditions. Data show carbon dioxide elimination changes (%) between the step-up plateau and the step-down plateau, expressed relative to the difference between these values, in the anaemic (A), normocythaemic or control (B), and polycythaemic states (C). Data are means with standard errors of the means. Significant differences (p<0.05) are indicated by '1', '2' and '3' for the comparison between hypoxic and normoxic conditions, hypoxic and hyperoxic conditions, and normoxic and hyperoxic conditions respectively.
Figure 4.10: Ventilation dynamics observed under hypoxic, normoxic and hyperoxic conditions. Data show minute ventilation changes (%) between the step-up plateau and the step-down plateau, expressed relative to the difference between these values, in the anaemic (A), normocytic or control (B), and polycytic states (C). Data are means with standard errors of the means. Significant differences (p<0.05) are indicated by '1', '2' and '3' for the comparison between hypoxic and normoxic conditions, hypoxic and hyperoxic conditions, and normoxic and hyperoxic conditions respectively.
Figure 4.11: Cardiac frequency dynamics observed under hypoxic, normoxic and hyperoxic conditions. Data show cardiac frequency changes (%) between the step-up plateau and the step-down plateau, expressed relative to the difference between these values, in the anaemic (A), normocythaemic or control (B), and polycythaemic states (C). Data are means with standard errors of the means. Significant differences (p<0.05) are indicated by '1', '2' and '3' for the comparison between hypoxic and normoxic conditions, hypoxic and hyperoxic conditions, and normoxic and hyperoxic conditions respectively.
time to reach 100% of the difference (Figure 4.11). Under anaemic and polycythaemic conditions, there were significant differences (p<0.05) between hypoxic and normoxic gases in the time for $V_e$ to reach 20%-80%\(^{45,46,47,48}\) of the step-down plateau (p<0.05; Figure 4.10), although under control conditions, there was only a significant difference between hypoxic and normoxic gases in the time to reach 80% of the step-down plateau (p<0.05)\(^{49}\).

4.3.2.4 Overall cardiorespiratory responses

The overall cardiorespiratory responses were evaluated by interpreting the data for each variable over the duration of each of the test phases. Integrated data for step-up dynamics, obtained by summing the data averaged over five second intervals between the start of exercise at minute 5 and the completion of exercise at the post-step plateau at minute 11, for a total of 73 data points per subject, are shown in Figure 4.12. For the total amount of oxygen consumed, hypoxia resulted in a significantly lower $V_{O_2}$ than normoxic and hyperoxic gas in the anaemic state (p<0.05)\(^{50}\), although there were no significant differences between the control and polycythaemic states. Hypoxia also resulted in an elevated total pulmonary ventilation and total number of cardiac contractions under all conditions of altered haematocrit (p<0.05; Figure 4.12)\(^{51}\).

\(^{45}\) Overall MANOVA $F = 2.69400 (32,168)$, p<0.001
\(^{46}\) Overall MANOVA $F = 2.57365 (32,168)$, p<0.001
\(^{47}\) Overall MANOVA $F = 2.46252 (32,168)$, p<0.001
\(^{48}\) Overall MANOVA $F = 2.42231 (32,168)$, p<0.001
\(^{49}\) Overall MANOVA $F = 2.42231 (32,168)$, p<0.001
\(^{50}\) Overall MANOVA $F = 2.79893 (32,168)$, p<0.001
\(^{51}\) Overall MANOVA $F = 2.79893 (32,168)$, p<0.001
Figure 4.12: Integrated cardiorespiratory responses obtained by summing raw data over 5 second intervals from the onset of cycle exercise (45% aerobic power), to the end of the physiological steady-state at minute 11. Subjects were studied while breathing hypoxic (10%), normoxic (20%) and hyperoxic gas mixtures (30%), while in three states of altered haematocrit. Data are means and standard error of the means. Significant differences (p<0.05) are indicated by '1', '2' and '3' for the comparison between hypoxic and normoxic conditions, hypoxic and hyperoxic conditions and normoxic and hyperoxic conditions respectively.
Integrated data for step-down dynamics, obtained by summing the data averaged over five second intervals between the end of exercise at minute 11 and the completion of the rest period at minute 15, for a total of 49 data points per subject, are shown in Figure 4.13. Hypoxia resulted in significantly more oxygen being consumed during the recovery period under anaemic and control conditions (p<0.05)$^{52}$ as well as more carbon dioxide being eliminated, air ventilated and total number of cardiac contractions, across the three levels of altered haematocrit (p<0.05)$^{53}$.

4.3.2.5 Comparison between cardiorespiratory variables in the time to plateau

A comparison of the time taken for each of the four main cardiorespiratory variables to reach 100% of the difference between the pre-step baseline and the step-up plateau are shown in Figure 4.14. There were no significant differences between $f_c$, $\dot{V}_{O2}$, $\dot{V}_{CO2}$ or $\dot{V}_E$ in the time to reach the steady-state plateau under normoxic or hyperoxic conditions in the normocytthaemic or polycytthaemic states (p>0.05), although under normoxic conditions, $f_c$ and $\dot{V}_{O2}$ did tend to reach a plateau faster than $\dot{V}_{CO2}$ and $\dot{V}_E$ (p>0.05). Breathing hypoxic gas in the anaemic state, $\dot{V}_{CO2}$ did reach the steady-state plateau significantly faster than $\dot{V}_{O2}$ and $\dot{V}_E$ (p<0.05)$^{54}$. The time taken for these same variables to reach 100% of the difference between the step-up plateau and the step-down plateau is shown in Figure 4.15. There were no significant differences under hypoxic or hyperoxic conditions in any state of altered haematocrit (p>0.05). While breathing normoxic gas under normocytthaemic or control conditions, $f_c$ and $\dot{V}_{O2}$ were both

$^{52}$ Overall MANOVA $F = 2.51081 \ (32,168)$, p<0.001

$^{53}$ Overall MANOVA $F = 2.51081 \ (32,168)$, p<0.001

$^{54}$ Overall MANOVA $F = 3.91767 \ (3,16)$, p=0.002

Page 85
Figure 4.13: Integrated cardiorespiratory responses obtained by summing raw data over 5 second intervals from the onset of recovery following cycle exercise (45% aerobic power), at the end of the physiological steady-state at minute 11, to the end of a recovery period at minute 15. Subjects were studied while breathing hypoxic (10%), normoxic (20%) and hyperoxic gases (30%), during three states of altered haematocrit. Data are means and standard error of the means. Significant differences (p<0.05) are indicated by `1`, `2` and `3` for the comparison between hypoxic and normoxic conditions, hypoxic and hyperoxic conditions, and normoxic and hyperoxic conditions respectively.
Figure 4.14: The time taken for cardiac frequency ($f_c$), oxygen consumption ($\dot{V}_{O_2}$), carbon dioxide elimination ($\dot{V}_{CO_2}$), and minute ventilation ($\dot{V}_E$) to reach 100% of the steady-state plateau following a step-exercise forcing function from rest to ~45% peak aerobic power. Subjects were studied while breathing hypoxic ($F_{O_2}=0.1$), normoxic ($F_{O_2}=0.2$) and hyperoxic gases ($F_{O_2}=0.3$), during three states of altered haematocrit, artificially-induced anaemia (A), normocythaemia (B) and artificially-induced polycythaemia (C). Data are means and standard error of the means. Significant differences (p<0.05) are indicated by `1' and `2' for the comparison between $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$; and $\dot{V}_E$ and $\dot{V}_{CO_2}$ respectively.
4.3.3 Effects of altered haematocrit

4.3.3.1 Baselines and Plateaux

For $\dot{V}_{O2}$ under normoxic conditions, there was no significant difference between the pre-step baseline in the anaemic state and the polycythaemic state (Table 4.5; $p>0.05$), although in the anaemic state ($\dot{V}_{O2} = 0.53 \text{ l.min}^{-1}$), it was significantly higher than in the control state ($\dot{V}_{O2} = 0.42 \text{ l.min}^{-1}; p<0.05$). There were no differences across the haematocrit range in the pre-step baselines for $\dot{V}_{CO2}$, $\dot{V}_{E}$ or $f_c$ ($p>0.05$). Similarly, there were no differences in the step-up or step-down plateaux across the haematocrit range for $\dot{V}_{O2}$, $\dot{V}_{CO2}$, $\dot{V}_{E}$ and $f_c$ ($p>0.05$; Table 4.5) while breathing any of the three gases.

4.3.3.2 Step-up cardiorespiratory dynamics

When breathing the normoxic gas mixture, there were no significant differences across the haematocrit range for $\dot{V}_{O2}$, $\dot{V}_{CO2}$, $\dot{V}_{E}$ or $f_c$, in the time to reach 20%, 40%, 60% 80% and 100% of the difference between the pre-step baseline and the step-up plateau (see Figures 4.16 to 4.19; $p>0.05$). Similarly, there were no changes for any of the four cardiorespiratory variables across haematocrit modifications when the subjects were supplied with hyperoxic gas. A difference was found when breathing

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$^{55}$ Overall MANOVA $F = 0.62422 (3,16), p=0.028$

$^{56}$ Overall MANOVA $F = 3.51941 (24,126), p<0.001$

$^{57}$ Overall MANOVA $F = 1.54686 (32,168), p=0.041$
Figure 4.15: The time taken for cardiac frequency ($f_C$), oxygen consumption ($\dot{V}_{O2}$), carbon dioxide elimination ($\dot{V}_{CO2}$), and minute ventilation ($\dot{V}_E$) to reach 100% of the steady-state plateau under resting conditions following the completion of a step-exercise forcing function at ~45% peak aerobic power, to rest. Subjects were studied while breathing hypoxic ($F_{iO2}=0.1$), normoxic ($F_{iO2}=0.2$) and hyperoxic gases ($F_{iO2}=0.3$), during three states of altered haematocrit, artificially-induced anaemia (A), normocythaemia (B) and artificially-induced polycythaemia (C). Data are means and standard error of the means. Significant differences ($p<0.05$) are indicated by ‘1’ and ‘2’ for the comparison between $f_C$ and $\dot{V}_E$, and $\dot{V}_{O2}$ and $\dot{V}_E$ respectively.
Table 4.5: Cardiorespiratory variables determined breath-by-breath for subjects during a submaximal step (cycling) forcing function. Pre-step baselines (PRE) are determined from data between minutes 1 and 5, while the step-up plateaux (PLAT) are determined between minutes 8 and 11, and step-down plateaux are determined between minutes 14 and 16. Data are means with standard errors of the means.

<table>
<thead>
<tr>
<th></th>
<th>( F_1O_2=0.2 )</th>
<th>( F_1O_2=0.2 )</th>
<th>( F_1O_2=0.2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ANAEMIC (n=6)</td>
<td>CONTROL (n=6)</td>
<td>POLYCYTHAEMIC (n=5)</td>
</tr>
<tr>
<td>( \dot{V}_O2 )-PRE</td>
<td>0.53 ± 0.02 *</td>
<td>0.42 ± 0.01 *</td>
<td>0.45 ± 0.02</td>
</tr>
<tr>
<td>( \dot{V}_CO2 )-PRE</td>
<td>0.31 ± 0.01</td>
<td>0.27 ± 0.01</td>
<td>0.29 ± 0.01</td>
</tr>
<tr>
<td>( \dot{V}_E )-PRE</td>
<td>11.64 ± 0.91</td>
<td>11.37 ± 0.28</td>
<td>11.32 ± 0.51</td>
</tr>
<tr>
<td>( f_c )-PRE</td>
<td>73.37 ± 7.11</td>
<td>66.86 ± 5.26</td>
<td>62.84 ± 4.00</td>
</tr>
<tr>
<td>( \dot{V}_O2 )-PLAT</td>
<td>3.02 ± 0.09</td>
<td>2.83 ± 0.17</td>
<td>3.02 ± 0.13</td>
</tr>
<tr>
<td>( \dot{V}_CO2 )-PLAT</td>
<td>2.10 ± 0.06</td>
<td>2.18 ± 0.11</td>
<td>2.30 ± 0.06</td>
</tr>
<tr>
<td>( \dot{V}_E )-PLAT</td>
<td>57.24 ± 1.26</td>
<td>57.57 ± 2.74</td>
<td>61.35 ± 1.58</td>
</tr>
<tr>
<td>( f_c )-PLAT</td>
<td>140.51 ± 5.18</td>
<td>127.46 ± 3.16</td>
<td>125.98 ± 5.61</td>
</tr>
<tr>
<td>( \dot{V}_O2 )-POST</td>
<td>0.60 ± 0.03</td>
<td>0.47 ± 0.03</td>
<td>0.50 ± 0.02</td>
</tr>
<tr>
<td>( \dot{V}_CO2 )-POST</td>
<td>0.38 ± 0.02</td>
<td>0.32 ± 0.01</td>
<td>0.34 ± 0.01</td>
</tr>
<tr>
<td>( \dot{V}_E )-POST</td>
<td>14.39 ± 1.03</td>
<td>12.77 ± 0.78</td>
<td>13.67 ± 0.25</td>
</tr>
<tr>
<td>( f_c )-POST</td>
<td>86.60 ± 6.70</td>
<td>72.36 ± 4.01</td>
<td>66.98 ± 6.73</td>
</tr>
</tbody>
</table>

Abbreviations:
\( \dot{V}_O2 \) = oxygen consumption
\( \dot{V}_CO2 \) = carbon dioxide elimination
\( \dot{V}_E \) = minute ventilation
\( f_c \) = cardiac frequency
PRE = Pre-step baseline
PLAT = Step-up plateau
POST = Step-down plateau

Significant differences (p<0.05) are indicated by `*`, `#` and `**` for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively.
hypoxic gas. $\dot{V}_o2$ dynamics were slowed as subjects approached steady-state, and the time to reach 100% of the difference between the pre-step baseline and the step-up plateau was slower in the anaemic state, relative to the control state ($p<0.05$)

### 4.3.3.3 Step-down cardiorespiratory dynamics

When breathing any of the three different gas mixtures, there were no significant differences between the anaemic, control and polycythaemic states for $\dot{V}_o2$, $\dot{V}_{CO2}$, $\dot{V}_E$ or $f_C$, in the time to reach 20%, 40%, 60% 80% and 100% of the difference between the pre-step baseline and the step-up plateau (see Figures 4.20 to 4.23; $p>0.05$)

### 4.3.3.4 Overall cardiorespiratory responses

Integrated data for step-up dynamics, obtained by summing the data averaged over five second intervals between the start of exercise at minute 5 and the completion of exercise at the post-step plateau at minute 11, for a total of 73 data points per subject, for each variable of interest, are shown in Figure 4.24. There were no significant differences ($p>0.05$) between anaemic, control and polycythaemic conditions for any cardiorespiratory variables under hypoxic, normoxic or hyperoxic conditions.

Integrated data for step-down dynamics, obtained by summing the data points, averaged over five second intervals between the end of exercise at minute 11 and the

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58 Overall $MANOVA F = 1.54686 (32,168)$, $p=0.041$

59 Overall $MANOVA F = 1.54686 (32,168)$, $p=0.041$
Figure 4.16: Oxygen uptake dynamics observed during anaemic, normocytthaemic and polycythaemic states. Data show oxygen uptake changes (%) between the pre-step baseline and the post-step plateau, expressed relative to the difference between these values, when breathing hypoxic (A), normoxic (B) and hyperoxic gas mixtures (C). Data are means with standard errors of the means. Significant differences (p<0.05) are indicated by '1', '2' and '3' for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively.
Figure 4.17: Carbon dioxide elimination dynamics observed during anaemic, normocytthaemic and polycytthaemic states. Data show carbon dioxide elimination changes (%) between the pre-step baseline and the post-step plateau, expressed relative to the difference between these values, when breathing hypoxic (A), normoxic (B) and hyperoxic gas mixtures (C). Data are means with standard errors of the means. Significant differences (p<0.05) are indicated by '1', '2' and '3' for the comparisons between anaemic and control conditions, anaemic and polycytthaemonic, and control and polycytthaemonic conditions respectively.
Figure 4.18: Ventilation dynamics observed during anaemic, normocythaemic and polycythaemic states. Data show minute ventilation changes (%) between the pre-step baseline and the post-step plateau, expressed relative to the difference between these values, when breathing hypoxic (A), normoxic (B) and hyperoxic gas mixtures (C). Data are means with standard errors of the means. Significant differences (p<0.05) are indicated by '1', '2' and '3' for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively.
Figure 4.19: Cardiac frequency dynamics observed during anaemic, normocytthaemic and polycythaemic states. Data show cardiac frequency changes (%) between the pre-step baseline and the post-step plateau, expressed relative to the difference between these values, when breathing hypoxic (A), normoxic (B) and hyperoxic gas mixtures (C). Data are means with standard errors of the means. Significant differences (p<0.05) are indicated by '1', '2' and '3' for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively.
Figure 4.20: Oxygen uptake dynamics observed during anaemic, normocythaemic and polycythaemic states. Data show cardiac frequency changes (%) between the step-up plateau and the step-down plateau, expressed relative to the difference between these values, when breathing hypoxic (A), normoxic (B) and hyperoxic gas mixtures (C). Data are means with standard errors of the means. Significant differences (p<0.05) are indicated by ‘1’, ‘2’ and ‘3’ for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively.
Figure 4.21: Carbon dioxide dynamics observed during anaemic, normocytthaemic and polycythaemic states. Data show carbon dioxide elimination changes (%) between the step-up plateau and the step-down plateau, expressed relative to the difference between these values, when breathing hypoxic (A), normoxic (B) and hyperoxic gas mixtures (C). Data are means with standard errors of the means. Significant differences (p<0.05) are indicated by '1', '2' and '3' for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively.
Figure 4.22: Ventilation dynamics observed during anaemic, normocythaemic and polycythaemic states. Data show minute ventilation changes (%) between the step-up plateau and the step-down plateau, expressed relative to the difference between these values, when breathing hypoxic (A), normoxic (B) and hyperoxic gas mixtures (C). Data are means with standard errors of the means. Significant differences (p<0.05) are indicated by '1', '2' and '3' for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively.
Figure 4.23: Cardiac frequency dynamics observed during anaemic, normocythaemic and polycythaemic states. Data show cardiac frequency changes (%) between the step-up plateau and the step-down plateau, expressed relative to the difference between these values, when breathing hypoxic (A), normoxic (B) and hyperoxic gas mixtures (C). Data are means with standard errors of the means. Significant differences (p<0.05) are indicated by ‘1’, ‘2’ and ‘3’ for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively.
Figure 4.24: Integrated cardiorespiratory responses obtained by summing raw data over 5 second intervals from the onset of cycle exercise (45% aerobic power), to the end of the physiological steady-state at minute 11. Subjects were studied when anaemic (A), normal (N) and polycythaemic (P), and were supplied with gas with three levels of fractional inspired oxygen concentration (F\textsubscript{I\textsubscript{O}2}). Data are means and standard error of the means. Significant differences (p<0.05) are indicated by '1', '2' and '3' for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively.
completion of the rest period at the step-down plateau at minute 15, for a total of 49 data points, are shown in Figure 4.25. There were no significant differences (p>0.05) between anaemic, control and polycythaemic conditions for any cardiorespiratory variables under hypoxic, normoxic or hyperoxic conditions.

4.3.4 Respiratory responses to a step-increase in work rate

Respiratory variables, averaged over 30 second intervals, for the last 30 seconds of the step-up plateau are shown in Table 4.6. The respiratory exchange ratio was significantly elevated under hypoxic conditions compared to normoxic and hyperoxic conditions, in all states of altered haematocrit (p<0.05). Breathing frequency (f_b) was also significantly increased under hypoxic conditions, as was the mean inspiratory flow rate (V_t/T; p<0.05). The relationship between f_b and V_t under hypoxic conditions during the step-increase in workrate is shown in Figure 4.26A. Inspiratory time, expiratory time, the total time of the respiratory cycle, and the duty cycle (T_r/T_tot) were all significantly reduced under hypoxic conditions in each state of altered haematocrit (p<0.05). The relationship between V_E and the mean inspiratory flow rate is shown in Figure 4.26B. The response is linear under normoxic, hypoxic and hyperoxic conditions at lower levels of ventilation (up to ~80 litres), however, at greater levels which were not achieved under normoxic or hyperoxic conditions, the hypoxic response appears curvilinear. End tidal carbon dioxide pressure (P_{ET}CO_2) was determined throughout the submaximal tests to evaluate the effects of different F_iO_2 on respiratory control. P_{ET}CO_2 was significantly reduced during exercise and recovery under hypoxic conditions in each state of altered haematocrit (p<0.05; Figure 4.27), while hyperoxia had no effect on
Figure 4.25: Integrated cardiorespiratory responses obtained by summing raw data over 5 second intervals from the onset of recovery following cycle exercise (45% aerobic power), at the end of the physiological steady-state at minute 11, to the step-down plateau at minute 15. Subjects were studied when anaemic (A), normal (N) and polycythaemic (P), and were supplied with gas with three levels of fractional inspired oxygen concentration ($F_iO_2$). Data are means and standard error of the means. Significant differences ($p<0.05$) are indicated by '1', '2' and '3' for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively.
Table 4.6: Cardiorespiratory variables following a step-increase in work rate, averaged over 30 second intervals, for the last 30 seconds of the step-up plateau. Subjects were studied when anaemic, normocythaemic, and polycythaemic, and were supplied with gas with three levels of fractional inspired oxygen concentration (FiO₂). Data are means and standard error of the means (anaemic and control conditions n=6; polycythaemia, n=5).

| Variable | ANAEMIC | | | CONTROL | | | POLYCYTHAEMIC | | |
|----------|---------|---------|---------|---------|---------|---------|
|          | F₁O₂=0.1 | F₁O₂=0.2 | F₁O₂=0.3 | F₁O₂=0.1 | F₁O₂=0.2 | F₁O₂=0.3 | F₁O₂=0.1 | F₁O₂=0.2 | F₁O₂=0.3 |
| RER      | 0.92 ± 0.03 | 0.70 ± 0.01 | 0.72 ± 0.02 | 0.93 ± 0.05 | 0.78 ± 0.02 | 0.75 ± 0.02 | 0.96 ± 0.02 | 0.75 ± 0.02 | 0.74 ± 0.01 |
|          | I, 2 (p<0.001) | I | 2 | I, 2 (p<0.001) | I | 2 | I, 2 (p<0.001) | I | 2 |
| Vₜ       | 2.73 ± 0.17 | 2.33 ± 0.17 | 2.34 ± 0.18 | 2.70 ± 0.20 | 2.40 ± 0.21 | 2.46 ± 0.13 | 2.50 ± 0.18 | 2.14 ± 0.13 | 2.15 ± 0.12 |
| fₜ       | 42.83 ± 4.52 | 26.00 ± 2.10 | 25.67 ± 1.31 | 41.83 ± 5.35 | 25.50 ± 2.95 | 25.5 ± 1.93 | 47.60 ± 7.20 | 29.80 ± 1.93 | 27.40 ± 1.40 |
|          | I, 2 (p<0.001) | I | 2 | I, 2 (p<0.007) | I | 2 | I, 2 (p<0.001) | I | 2 |
| Tₜ       | 0.55 ± 0.08 | 1.07 ± 0.10 | 0.98 ± 0.08 | 0.58 ± 0.08 | 1.08 ± 0.16 | 1.05 ± 0.09 | 0.50 ± 0.07 | 0.84 ± 0.05 | 0.90 ± 0.06 |
|          | I, 2 (p<0.001) | I | 2 | I, 2 (p<0.013) | I | 2 | I, 2 (p<0.001) | I | 2 |
| Tₑ       | 0.94 ± 0.07 | 1.46 ± 0.13 | 1.41 ± 0.07 | 0.97 ± 0.09 | 1.48 ± 0.16 | 1.49 ± 0.10 | 0.88 ± 0.11 | 1.31 ± 0.06 | 1.27 ± 0.07 |
|          | I, 2 (p<0.002) | I | 2 | I, 2 (p<0.011) | I | 2 | I, 2 (p<0.006) | I | 2 |
| Tₜ/ₜₜₒᵣₜ | 1.49 ± 0.14 | 2.52 ± 0.23 | 2.39 ± 0.12 | 1.55 ± 0.17 | 2.57 ± 0.32 | 2.54 ± 0.18 | 1.38 ± 0.18 | 2.11 ± 0.11 | 2.21 ± 0.10 |
|          | I, 2 (p<0.001) | I | 2 | I, 2 (p<0.012) | I | 2 | I, 2 (p<0.002) | I | 2 |
| Tₗ/ₜₜₒᵣₜ | 0.36 ± 0.02 | 0.43 ± 0.01 | 0.42 ± 0.02 | 0.36 ± 0.02 | 0.42 ± 0.01 | 0.42 ± 0.01 | 0.36 ± 0.01 | 0.40 ± 0.01 | 0.40 ± 0.01 |
|          | I, 2 (p<0.010) | I | 2 | I, 2 (p<0.002) | I | 2 | I, 2 (p<0.014) | I | 2 |
| Vₜ/ₗₜ | 5.49 ± 0.77 | 2.20 ± 0.11 | 2.38 ± 0.10 | 5.13 ± 0.76 | 2.30 ± 0.18 | 2.33 ± 0.12 | 5.41 ± 0.59 | 2.52 ± 0.09 | 2.43 ± 0.14 |
|          | I, 2 (p<0.001) | I | 2 | I, 2 (p<0.001) | I | 2 | I, 2 (p<0.001) | I | 2 |

Abbreviations:
RER = respiratory exchange ratio  Vₜ = tidal volume  fₜ = frequency of breathing  
Tₗ = inspiratory time  Tₑ = expiratory time  Tₜ/ₜₜₒᵣₜ = total respiratory cycle duration  
Tₗ/ₜₜₒᵣₜ = duty cycle  Vₜ/ₗₜ = mean inspiratory flow rate

Significant differences (p<0.05) are indicated by '1', '2' and '3' for the comparisons between 10% O₂ and 20% O₂ conditions, 10% O₂, 20% O₂ and 30% O₂ conditions respectively.
Figure 4.26: (A) The relationship between tidal volume ($V_T$) and breathing frequency ($f_B$), with the percentage of vital capacity (%VC), with the data averaged over 30 second intervals during a step-increase in workrate from rest to $-45\% \dot{V}O_{peak}$ while breathing hypoxic gas ($F_iO_2=0.1$). Data are means and standard error of the means. (B) The relationship between minute ventilation ($V_E$) and mean inspiratory flow rate ($V_i/T_i$) showing a linear response for hypoxia ($F_iO_2=0.1$), normoxia ($F_iO_2=0.2$) and hyperoxia ($F_iO_2=0.3$) until $V_E$ reaches $-80 \text{ l.min}^{-1} \text{ BTPS}$, where the relationship becomes curvilinear under hypoxic conditions as $V_E$ is elevated.
$P_{ET}CO_2$ in any state of altered haematocrit ($p>0.05$; Figure 4.27).
Figure 4.27: End tidal carbon dioxide pressure ($P_{ET\,CO_2}$) averaged over 30 second intervals, during an exercise step-function from rest (5 min) to 45% aerobic power (6 min), and back to rest again (5 min). Graphs correspond with data collected during artificially-induced anaemia (A), control (B), and artificially-induced polycythaemia (C). Data are means with standard errors of the means.
CHAPTER FIVE: DISCUSSION
CHAPTER FIVE: DISCUSSION

5.1 EXPERIMENTAL MANIPULATIONS AND RESULTANT HAEMATOLOGICAL CHANGES

The removal, storage and subsequent reinfusion of red blood cells has significant acute and chronic effects on haematological status. Before discussing how these experimental manipulations influenced cardiorespiratory responses to maximal and submaximal exercise, it is important to identify their effects on blood volume, red blood cell (RBC) turnover and blood viscosity of the subjects at rest.

5.1.1 Blood volume following phlebotomy

Though variable between subjects, blood withdrawal acutely lowers blood volume and oxygen delivery. Homeostatic processes ensure the rapid regulation of blood volume, so that blood loss is quickly countered. Following afferent input from the atrial stretch receptors, the carotid sinus and aortic baroreceptors, and the hypothalamic osmoreceptors, there ensues a series of hormonal responses (involving antidiuretic hormone, aldosterone and renin) which result in the restoration of the blood volume (Gauer et al., 1970). For these reasons, it was assumed that, for the purposes of this study, the change in blood volume accompanying blood withdrawal would be negated during the 24-36 hours between the third phlebotomy and the maximal exercise test, and subsequent submaximal tests. This volume regulation was also aided by fluid replacement procedures employed by all subjects following each venesection. Therefore,
at the time of testing, it was assumed that the principle haematological change would be the reduction in the total number of circulating RBC's, which would occur under isovolaemic conditions.

5.1.2 Red blood cell turnover and blood volume following reinfusion

Following the withdrawal of three units of whole blood, a significant reduction in the number of circulating RBC's was achieved (Table 4.1). The resultant isovolaemic anaemia would have stimulated erythropoietin production. This hormone would be released within minutes, peaking after about 24 hours, and circulate through the blood to the red bone marrow (Gregersen & Rawson, 1959). It stimulates haemocytoblasts to develop into RBC's, resulting in erythrocyte numbers rising gradually, with an increase in circulating erythrocytes approximately 5 days later, and haemoglobin returning to normal 5-10 weeks after withdrawal (Buick et al. 1980; Gledhill, 1982).

After thirteen weeks of storage, the deglycerolised red blood cells and saline (Hct~70%) were reinfused over a period of 3-4 hours. Gregerson & Dawson (1959) showed that blood volume could be stabilised within hours following acute increases or decreases, through altered renal filtration (Gauer et al., 1970). The body experienced an acute normocythaemic hypervolaemia, which would have been countered by the rapid shift of excess plasma fluid into the intercellular compartments, following reinfusion to return blood volume to normal. Sawka et al. (1987) found that about 15-20 g of protein also left the blood during this period. This would enable the maintenance of haemoconcentration during polycythaemia and result in a progression towards
isovolaemic polycythaemia, distinguished by the increase in the number of circulating RBC's (Table 4.1). Polycythaemia, through negative feedback control, precipitates a reduction in erythropoietin production, which occurs rapidly, with production first peaking ~5 hours after reinfusion (Berglund et al., 1989), then falling to less than 50% of the pre-infusion baseline within 24 hours (Berglund et al., 1987). Erythropoietin production falls to below control levels, slowly returning to normal over a period of ~28 days (Berglund et al., 1989). However, given the 120 day life span of the RBC, there is a delay between reduced erythropoietin production and the return to normocythaemia, ranging from 60-120 days (Sawka & Young, 1989).

With a life span of ~120 days, ~0.83% of erythrocytes die daily, whether they are in the body or not. Freezing suspends the rate of erythrocyte destruction. Handling destroys about ~15% of erythrocytes, permitting ~85% erythrocyte viability after extended storage using low or high glycerol freezing (Valeri, 1976).

Blood storage alters erythrocyte 2,3-diphosphoglycerate (2,3-DPG) levels. Refrigerated blood at 4°C loses 60% of its 2,3-DPG after 1 week and 90% after 2 week (Mollison, 1979; Williams et al., 1983). However, when blood is stored using the low glycerol freezing method, there is little change in 2,3-DPG levels during storage (Gledhill et al., 1978). Although 2,3-DPG was not measured in this study, previous studies have shown there is no loss of 2,3-DPG following the freezing of RBC's (Mollison, 1979; Åkerblom & Högman, 1974; Williams et al., 1981). A significant change in the total number circulating RBC's, and therefore potential oxygen transport capacity, was achieved by both sequential blood withdrawal and subsequent reinfusion
after the subjects had replenished their natural stores of RBC's.

5.1.3 Blood Viscosity

Blood is a suspension of cells within a fluid which does not behave as an ideal fluid\(^60\), and its viscosity, \(\sim 2-4\) times greater than water, is not constant. Arterial blood viscosity is determined by temperature, the relative volume occupied by suspended cells and platelets, protein concentration, and osmolarity. However, within smaller blood vessels, viscosity is affected by tube dimensions, RBC aggregation and RBC deformability (Somer & Meiselman, 1993). Since flow in tubes is inversely proportional to the viscosity of the liquid (Poiseuille's law), it has been suggested that polycythaemic-induced elevations in haematocrit will affect blood viscosity, to the extent that it may compromise muscle perfusion (Richardson & Guyton, 1959), thereby negating any benefits of the elevated oxygen transport capacity at the tissue level. The intra-muscular capillaries may actually become hypoxic (Tenney, 1974). In chronically polycythaemic people where muscle perfusion may have been a problem, blood withdrawal and artificial haemodilution has been used successfully to improve exercise capacity (Chetty \textit{et al.}, 1990).

Richardson & Guyton (1959) suggested that oxygen delivery during rest was optimal at an haematocrit of \(~40\%\) (dogs), describing an inverted 'U' relationship between the variables. A lower haematocrit reduced oxygen carriage, while an elevated

\(^60\) A fluid is described as being 'ideal' or 'Newtonian' if its viscosity remains constant, and independent of velocity.
haematocrit depressed muscle blood flow, as a result of elevated blood viscosity. Buick et al. (1980) and Robertson et al. (1984) demonstrated that previous calculations for an optimal haematocrit were derived from data obtained during rest. Since exercise is accompanied by changes to blood vessel diameter, elevated muscle blood flow, muscle and body core temperatures, and a redistribution of blood towards active tissues, extrapolating resting data to exercise is inappropriate (Buick et al., 1980). Robertson et al. (1984) found that an elevation in haematocrit from $38.1\% \pm 2.8$ to $44.9\% \pm 2.7$, did not reduce $\dot{Q}_{\text{max}}$, therefore haemoglobin flow to skeletal muscle during maximal exercise was increased following reinfusion, and aerobic power was increased. Both Robertson et al. (1984) and Buick et al. (1980) concluded that the optimal haematocrit was in excess of that obtained during exercise under normocytthaemic conditions.

While it has been demonstrated that haemoconcentration and polycythaemia are accompanied by rises in blood viscosity, increases in viscosity remain relatively small when haematocrit is below 50%, rising exponentially beyond this level (Stone et al., 1968). Since most reinfusions in previous research have involved less than four units of reconstituted erythrocytes, haematocrits will typically be less than 50% after reinfusion, and viscosity changes will remain relatively small.

Furthermore, since the in vivo haematocrit at the microvascular level is unknown, the influence of viscosity on muscle capillary perfusion cannot be determined. It is generally accepted that reinfusions of 3 units, or less, result in viscosity changes which do not significantly impair blood flow in active tissues (Stone et al., 1968). For this reason, viscosity was not thought to be a limiting factor in maximal exercise in this
study, particularly when the haematocrits were only elevated to the higher end of the normal haematocrit range, and were below the 50% threshold where blood viscosity is thought to increase exponentially. Furthermore, the subject group came from a well trained sub-group who tend to have lower plasma viscosity and higher red cell flexibility under normocythaemic conditions, than non-athletic subjects, which would further reduce compromising influences of blood viscosity changes (Ernst et al., 1985).

In summary, following the withdrawal of the three units of blood over a period of five days, blood volume was rapidly restored, and oxygen carrying capacity was significantly reduced in the new state of normovolaemic anaemia. Over the following weeks, erythropoetin concentration would increase resulting in the number of circulating RBC's returning to normal within weeks. After the thirteen weeks of storage using the low glycerol technique, the deglycerolised RBC's were reinfused over a period of 3-4 hours, resulting in a state of hypervolaemia. Over the following 24 hours, blood volume would be restored to normal resulting in a new state of normovolaemic polycythaemia. Following reinfusion, there would have been no change in 2,3 DPG concentration, or any change in blood viscosity.

5.2 THE EFFECT OF ANAEMIA AND POLYCYthaEMIA ON MAXIMAL EXERCISE

The withdrawal of three units of whole blood resulted in significant reductions in $C_sO_2$ (Figure 4.1), resulting in the earlier attainment of volitional fatigue, reduced peak power, and consequently a reduced aerobic power ($V_{O_2peak}$). Such changes are
almost universal observations (Celsing et al., 1986; Ekblom et al., 1972; Kanstrup & Ekblom, 1984; Woodson, 1984), suggesting a central transport limitation to exercise. During early pilot testing in one subject, the withdrawal of three units similarly reduced $\dot{V}_{O_2peak}$, while further reductions\(^{61}\) in the circulating number of red blood cells led to more dramatic reductions in $\dot{V}_{O_2peak}$ (Appendix F). A decrease in $\dot{V}_{O_2peak}$ is the result of a significantly reduced $O_2$ transport. Compensatory increases in $\dot{Q}$ were probably inadequate to make up for the reduced $C_aO_2$, resulting in less oxygen available for aerobic metabolism. This decrease in $C_aO_2$ reduces the ability of the anaemic person to draw on their reserve of mixed venous oxygen content by increasing the amount of oxygen extracted (Celsing et al., 1986; Nunn, 1993).

With the reduction in oxygen delivery to the exercising muscles, one would predict significant elevations in $f_C$ during exercise, while this was generally observed, such changes were not significant at maximal workrates (Table 4.2), but they were at lesser absolute workrates (Table 4.3). The inability to elevate $f_{Cpeak}$ may have resulted from the subjects ceasing exercise prior to reaching the $f_{Cmax}$. This result is consistent with others who demonstrated no change in $\dot{Q}$, $f_C$ and stroke volume (SV) during maximal exercise under anaemic conditions (Ekblom et al., 1976), and inconsistent with observations of a reduced $f_{Cpeak}$ during maximal exercise in anaemic conditions (Celsing et al., 1986, Woodson, 1984; Woodson et al., 1978). At lesser absolute workrates (Table 4.3) the data are consistent with those who have demonstrated an elevated $f_C$ under anaemic conditions during similar absolute workrates (Woodson et al., 1978; Ekblom

\(^{61}\) Additional units were withdrawn to evaluate the amount of blood removal necessary to reduce haematocrit in a well-trained subject.
et al., 1972; Ekblom et al., 1976), which is a compensatory mechanism for the reduced \( C_aO_2 \).

Following reinfusion, there was no statistical change in \( \dot{V}_{O2\text{peak}} \). This was despite the elevation in \( C_aO_2 \) resulting in a significant difference between the anaemic and polycythaemic states (Table 4.1), which indicates that red blood cell numbers were elevated in this state and did affect blood oxygen content. It was generally reported by the subjects that they felt greater local muscular fatigue or "heaviness" in the legs relative to the previous trials, and this effect resulted in greater discomfort and an earlier voluntary termination of the maximal tests. Such observations have not been reported previously, and while it is possible that such localised "heaviness" may be related to the polycythaemic manipulation, it is not possible to explain these subjective impressions. This result is inconsistent with previous observations of an increase in \( \dot{V}_{O2\text{peak}} \) when employing the low glycerol freezing technique for increasing the total number of circulating red blood cells (Jones & Tunstall Pedoe, 1989), as it suggests aerobic power may be limited by the ability of the mitochondria to utilize oxygen, or some methodological weakness. The results obtained may be due to the relatively small change in haematocrit (4 points), since others have found up to a 6-7 point increase using 3 units of whole blood and similar freezing processes. This may be due to the loss of red blood cells in the storage and washing process, which may have been greater than the standard 15%, also one subject (S4) only received two units of deglycerolised red blood cells due to excessive haemolysis in the washing process in one unit, and another subject (S6), had his \( \dot{V}_{O2\text{peak}} \) test delayed seven days due to complications accompanying reinfusion.
In summary, in the anaemic state, it appears that the ability to transport oxygen to the exercising muscle limited maximal aerobic power, as evident by the reduced \( \dot{V}_{O_2 \text{peak}} \). In the polycythaemic state, while the oxygen transport capacity was elevated, distinguished by the elevated \( C_aO_2 \), local fatigue may have limited maximal aerobic power prior to the development of any central limitation.

5.3 EFFECTS OF ALTERED OXYGEN TRANSPORT AND SUPPLY ON CARDIORESPIRATORY DYNAMICS DURING SUBMAXIMAL EXERCISE

5.3.1 Altering oxygen delivery by changing haematocrit

The reinfusion of the deglycerolised red blood cells, resulted in a significant difference in \( C_aO_2 \) between the anaemic and polycythaemic states (Figure 4.1). While there were no significant changes in pre-step baselines for \( \dot{V}_{CO_2}, \dot{V}_E \) or \( f_C \), prior to the step-increase in workrate, \( \dot{V}_{O_2} \) was significantly elevated in the anaemic state, compared to the control state (Table 4.4). This indicates more oxygen was consumed maintaining resting metabolism which may be associated to the elevated resting \( f_C \) and \( \dot{V}_E \) in the anaemic state (\( p>0.05 \)). Murray and Rapaport (1972) attribute this rise in resting \( \dot{V}_{O_2} \) under anaemic conditions to an increase in myocardial oxygen consumption. In the anaemic state, \( f_C \) was elevated, and in the polycythaemic state, \( f_C \) was reduced. Although these changes were not significant, they were consistent across the different inspired oxygen concentrations (Table 4.4). There were no significant differences in the post-step plateaux following the workrate increase and decrease, across the haematocrit range for \( \dot{V}_{O_2}, \dot{V}_{CO_2} \) or \( \dot{V}_E \) either (\( p>0.05 \)), indicating that the relative workrates of the
submaximal tasks were similar across the three levels of altered haematocrit. Therefore partially supporting the acceptance of the first hypothesis, which states the steady-state oxygen utilization would be independent of whole-body haematocrit or $F_1O_2$ following a constant relative intensity step-change in workrate.

There were no significant changes in the times to reach various percentages of the step-up or step-down plateaux for $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, $\dot{V}_E$ or $f_C$ during this experimental manipulation (haematocrit), indicating that $O_2$ transport must have been able to meet the demands imposed by the step-exercise forcing function, at least when haematocrit was within the range of 35-45%. The second hypothesis, that cardiorespiratory response dynamics would be slowed when rendered anaemic and accelerated when polycythaemic, is therefore unable to be accepted. It would appear that $O_2$ transport may not be rate limiting within this haematocrit range, at least not during submaximal step-function exercise (~45% $\dot{V}_{O_2peak}$), demonstrating the possibility that intracellular factors affecting $O_2$ utilization (Figure 2.1) may be the prime rate limiting factors.

In each state of altered haematocrit, under normoxic conditions, $f_C$ and $\dot{V}_{O_2}$ were the fastest to plateaux, while $\dot{V}_{CO_2}$ tended to be fractionally faster than $\dot{V}_E$ dynamics, following a step-increase in exercise workrate. Under hyperoxic conditions in each state of altered haematocrit, there were no clear differences between the four cardiorespiratory variables in the time to 100% of the step-up plateaux. Under normoxic conditions, $\dot{V}_{CO_2}$ rises slower than $\dot{V}_{O_2}$ during phase 2 because the high $CO_2$ solubility allows for a relatively larger $CO_2$ storage capacity in the exercising muscle and blood, thereby allowing a proportion of the metabolically produced $CO_2$ to be transiently stored during
the exercise transition (Whipp, 1987; Wasserman et al., 1987). The dynamics of \( \dot{V}_E \) are
normally some 10% slower than those of \( \dot{V}_{CO2} \), with these two variables having
relatively close temporal response characteristics (Miyamoto et al., 1982; Whipp et al., 1982; Miyamoto et al., 1983). The small disparity between the dynamics of these two
variables during phase two is likely to elicit a small transient rise in \( P_{\dot{a}CO2} \) and a transient
fall in \( P_{\dot{a}O2} \) (Whipp and Ward, 1980). Wasserman and Whipp (1983), and Diamond et
al. (1977) suggest that a signal related to \( CO2 \) delivery to the lungs, and in proportion
to the increase in \( \dot{V}_{CO2} \), causes the increase in \( \dot{V}_E \), and is responsible for a high
correlation between these two variables. During moderate exercise, most of the increase
in ventilation is a result of an increase in tidal volume (\( V_T \)), until it reaches about 50%
of vital capacity, then follows an increase in respiration rate (Figure 4.26A; Lind, 1984;
Pardy et al., 1984). Under hypoxic conditions, \( \dot{V}_{CO2} \) dynamics were much faster than \( f_C \),
\( \dot{V}_{O2} \) and \( \dot{V}_E \), being significantly different than \( \dot{V}_{O2} \) and \( \dot{V}_E \) in the anaemic state (Figure
4.14). The faster \( \dot{V}_{CO2} \) response in the hypoxic condition has not been demonstrated or
reported previously.

Altering haematocrit resulted in no difference in the time to reach 100% of the
steady-state plateau between the step-decrease in exercise workrate across \( f_C, \dot{V}_{O2}, \dot{V}_{CO2} \)
and \( \dot{V}_E \) under hypoxic conditions (Figure 4.15). Under normoxic and hyperoxic
conditions, the time to reach 100% of the step-down plateaux were fastest for \( f_C \) and \( \dot{V}_{O2} \)
followed by \( \dot{V}_{CO2} \) and \( \dot{V}_E \). The slight delay in \( \dot{V}_{O2} \) dynamics (p>0.05) during the step-
decrease, compared to the step-increase in workrate, indicates there is a slow component
of the post-exercise \( \dot{V}_{O2} \), which may last for hours and may be attributable to the \( Q_{10} \)
effect of elevated muscle temperature (Hagberg et al., 1980), elevated concentrations of
catecholamines (Gaesser & Brooks, 1984), or substrate cycling (Gaesser & Brooks, 1984; Newsholme, 1978).

With the change in oxygen transport capacity, there was no change between haematocrits in the total amount of oxygen consumed, carbon dioxide eliminated, ventilation or cardiac beats, when integrated across time, during either the step-up or step-down phases (Figure 4.24; Figure 4.25). A mild reduction or mild increase in haematocrit had little effect on these cardiorespiratory variables even though significant changes in $C_aO_2$ and oxygen transport capacity were achieved, confirming that $O_2$ transport in these circumstances was not necessarily a rate limiting factor. This observation also indicates that integrating time averaged data, which is unique to this study, may reflect a change in response dynamics at least as well as curve analysis techniques for evaluating respiratory dynamics. Under each condition of altered $F_iO_2$, there were a greater number of cardiac contractions in the anaemic state, and slightly less in the polycythaemic state, relative to the control or normocythaemic state (Table 4.5), indicative of the altered plateau $f_c$ levels in each state of altered haematocrit. The reduced $C_aO_2$ in anaemic subjects is probably compensated for by an elevated cardiac output ($\dot{Q}$), with a higher than normal $f_c$ for a given workrate, and a normal or even increased stroke volume (Wasserman et al., 1987). While an elevated $C_aO_2$ may result in the reverse situation to maintain normal tissue oxygenation.

5.3.2 Altering oxygen supply by changing inspired oxygen concentrations

There were no differences in the response dynamics between the step-up and
step-decrease in workrate, as observed by Karlsson et al. (1975) and Miyamoto (1992). The exercise onset and offset appear symmetrical during submaximal exercise, although some cardiorespiratory variables tended to be slower for the step-down (p > 0.05; Hughson, 1990; Linnarsson, 1974).

There were no differences between pre-step baseline, step-up plateaux and step-down plateaux between normoxic and hyperoxic gases, when analyzed on a breath-by-breath basis (Table 4.4). While hyperoxia has been shown to induce various changes, such as a reduced $\dot{V}_E$, and delayed $\dot{V}_E$ and $\dot{V}_{CO_2}$ dynamics (Byrnes & Mullin, 1981, Byrnes et al., 1984, Nakazono & Miyamoto, 1987), in this study, the hyperoxic gas did not elicit such changes (Figure 4.4 to Figure 4.11). It is possible that the $F_{I}O_2$ was not great enough to alter dynamics or plateau levels. It has been reported that a $F_{I}O_2$ of 0.66 provides an optimal level of alveolar tension for improving respiratory mechanisms, and that a $F_{I}O_2$ of 0.3-0.35 (the range employed here) will result in smaller changes in cardiorespiratory variables (Bannister & Cunningham, 1954). With the relatively small subject numbers in this study, such changes did not attain significance.

Chapler et al. (1984) found that following induced anaemia, $\dot{Q}$ increased in an effort to maintain oxygen delivery, as indicated in this study by a consistently elevated $f_C$ (p > 0.05), and following subsequent hyperoxic exposure, $\dot{Q}$ was reduced. This would most likely have been the result of a local peripheral vasoconstrictor response. In response to an elevated $P_{aO_2}$, capillary blood flow would have been redistributed away from exchange vessels, which would reduce oxygen uptake and maintain tissue $P_{O_2}$ (Chapler et al., 1984). A lower $\dot{Q}$ could result from a redistribution of capillary blood...
flow away from exchange vessels that would minimize any change in tissue oxygen uptake in response to the elevated $P_{aO_2}$, and from a reduction in $f_c$. The decrease in $f_c$ normally associated with hyperoxia (Plet et al., 1992; Byrnes & Mullin, 1981; Adams & Welch, 1980), was not evident in the current study, and may have resulted from baroreceptor feedback accompanying an increase in blood pressure. A local vasoconstrictor effect has been shown to accompany an increased $P_{aO_2}$, which produces a small decrease in blood flow to the active muscle mass (Byrnes & Mullin, 1981; Yamaji & Shephard, 1985). During exercise, where the exercising muscles may become hypoxic, hyperoxia alleviates this state of hypoxia in the muscle tissue (Welch & Pedersen, 1981).

$V_e$ and blood lactate concentration ([HL$_a$]) plateaux are normally reduced during steady-state exercise under hyperoxic conditions (Byrnes & Mullin, 1981; Plet et al., 1992) while there may be a slight decrease in $V_{CO_2}$ (Welch et al., 1974). No such changes were evident in this study (Table 4.4), even though [HL$_a$] was not measured. The lack of any effect under hyperoxic conditions may possibly be due to the compensatory effects of a reduced $f_c$, and possibly $Q$, which probably resulted in no change in oxygen delivery with the increase in $F_iO_2$. A lower [HL$_a$], seen in previous studies under hyperoxic conditions, may reflect an enhanced glucose-alanine cycle, where the conversion of pyruvate to alanine may be faster or more efficient (Byrnes & Mullin, 1981). An elevated arterial hydrogen ion concentration ([H$^+$]$_a$), $P_{aCO_2}$ and bicarbonate concentration ([HCO$_3^-$]$_a$) accompanying hyperoxic exposure are most likely the cause of some of the effects previously seen under hyperoxic conditions, such as a reduced $V_e$ and [HL$_a$] (Nakazono & Miyamoto, 1987; Ward & Nguyen, 1991; Plet et
Under hyperoxic conditions, an increase in $\dot{V}_{O_2}$ during submaximal exercise may be the result of something other than increased mitochondrial oxygen utilization. Welch et al. (1974) found an increase in metabolic rate and $\dot{V}_{O_2}$, but not $\dot{V}_{CO_2}$ during hyperoxia. These observations were not supported by data from the current study (Table 4.4). The possible reasons for an elevated $\dot{V}_{O_2}$ during submaximal exercise in previous studies may have been due to the use of the Douglas bag method to determine $\dot{V}_{O_2}$, rather than the mixing chamber method (which gives consistently lower $\dot{V}_{O_2}$ values) (Adams & Welch, 1980). As hypothesized by Welch et al. (1974), an elevated $\dot{V}_{O_2}$ during steady-state submaximal exercise, may have resulted from $O_2$ storage during inhalation, or some $O_2$ might be utilized in reactions other than those in the respiratory chain which normally do not produce $CO_2$. These reactions may be altered under conditions of elevated $P_{O_2}$ (Welch et al., 1974). In the current study, hyperoxia did not result in an elevated $\dot{V}_{O_2}$ during the submaximal step-up plateau (Table 4.4). Byrnes and Mullin (1981) demonstrated similar observations, and suggested that the metabolic responses to submaximal exercise is the same under hyperoxic and normoxic conditions. In submaximal exercise, oxygen enriched breathing may cause a decrease in carbohydrate utilization, with an increase in fat utilization. This change may account for any of the observed changes in $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ in the study by Byrnes and Mullin (1981), because $\dot{V}_{CO_2}$ was slightly lower under hyperoxic conditions compared to $\dot{V}_{O_2}$. Such differences could result from an increase in the body's store of $CO_2$ under hyperoxic conditions (Byrnes et al., 1984). These stores tend to hide abrupt changes in peripheral carbon dioxide production (Sherrill et al., 1982). However, such differences did not appear in
the current study, as $\dot{V}_{CO2}$ was equivalent under normoxic and hyperoxic conditions, suggesting substrate utilization was the same for both conditions.

Hyperoxia resulted in no change in steady-state respiratory exchange ratio (RER; Table 4.6). Previous research, using greater oxygen concentrations, has demonstrated an increase in fat utilization with the use of hyperoxic gas mixtures (Byrnes & Mullin, 1981). Hypoxia results in a much higher RER (Table 4.6), under all conditions of altered haematocrit, probably due to elevated carbohydrate utilization. This possible substrate shift, or a greater aerobic utilization of carbohydrate, could account for the observed changes in $\dot{V}_{O2}$ and $\dot{V}_{CO2}$ (Byrnes & Mullin, 1981). If $\dot{V}_{CO2}$ does not increase in proportion to $\dot{V}_{O2}$, there must be an increase in the body's store of CO$_2$ under hyperoxic conditions (Byrnes et al., 1984). However, whole-body $\dot{V}_{O2}$, when derived from pulmonary measures, tends to overestimate $\dot{V}_{O2}$ when the P$_{aO2}$ is elevated by hyperoxic exposure (Graham & Wilson, 1983), which may account for suggested substrate shifts, or changes in the body's store of CO$_2$. Current data, however, reflects a greater amount of total oxygen consumed breathing hyperoxic gas in the anaemic state (Figure 4.12; p>0.05), as well as in control and polycythaemic conditions.

Under hyperoxic gas conditions, the step-up dynamics for all four variables were similar, although $f_C$ dynamics tended to be marginally faster than $\dot{V}_{O2}$ in the time to reach its plateau under hypoxic and normoxic conditions (Figure 4.14; p>0.05), while under hypoxic conditions, $\dot{V}_{CO2}$ dynamics tended to plateau fastest, being significant in the anaemic state (p<0.05). Therefore, the third hypothesis, that hyperoxia will accelerate cardiorespiratory response dynamics independently of whole-body
haematocrit, is not totally accepted.

Breathing hyperoxic gas mixtures after exercise does not alter the recovery dynamics of $\dot{V}_E$ or $f_c$ (Figure 4.10 and Figure 4.11; Robbins et al., 1992), or $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ (Figure 4.8 and Figure 4.9; Garner et al., 1986). Under the mild hyperoxic conditions used in this study, there were no improvements in cardiorespiratory response dynamics (Figure 4.20 to Figure 4.23) following a step-decrease in workrate, under any stage of altered haematocrit, again, not supporting the third hypothesis. This may be due to mechanisms which compensate for the increase in $C_aO_2$ and ensure a constant delivery of oxygen to the exercising muscle (Wilson and Stainsby, 1978). Thus, hyperoxic gas may not necessarily increase $O_2$ delivery to the exercising muscle. Such compensatory mechanisms may include a reduction in blood flow to the exercising muscle (Wilson & Stainsby, 1978) and a reduction in $\dot{Q}$, possibly indicated by the reduced $f_c$ in this study. If the combined compensatory effects of a reduced $\dot{Q}$, and reduced muscle blood flow do not reduce $C_aO_2$ to normal levels, the muscle would still be exposed to a slightly higher $P_{O_2}$ (Morris, 1983; Graham et al., 1987; Welch, 1982).

Under normocythaemic conditions, and while breathing normoxic gas mixtures, $\dot{V}_{O_2}$ and $f_c$ dynamics were the fastest to reach a steady-state plateau following a step-decrease in workrate, with both $\dot{V}_{O_2}$ and $f_c$ being significantly faster than the ventilatory response (Figure 4.15). This indicates that the $CO_2$ stored during exercise is removed, or "blown off" during recovery. Under normoxic conditions, Wasserman and Whipp (1983), suggest that a signal related to the $CO_2$ delivery to the lungs, and in proportion to the elevated $\dot{V}_{CO_2}$ causes $\dot{V}_E$ to remain elevated, and is consequently responsible for
a high correlation between these two variables (Diamond et al., 1976). If \( \dot{V}_E \) did not remain elevated in proportion to the rate of CO\(_2\) production, respiratory acidosis would occur, resulting in cellular disturbances (Wasserman et al., 1986). In the polycythaemic and anaemic states, similar patterns of \( f_c \) and \( \dot{V}_{o2} \), being faster than \( \dot{V}_{co2} \) and \( \dot{V}_E \), were seen, but the differences were not statistically significant. Under hyperoxic conditions, the timing of the cardiorespiratory variables followed a similar sequence to that seen under normoxic conditions. The similarity between \( \dot{V}_{o2} \) and \( f_c \) has also been demonstrated by Garner et al. (1986). Under hypoxic conditions, there were no differences between \( f_c, \dot{V}_{o2}, \dot{V}_{co2} \) or \( \dot{V}_E \), in the time to reach 100% of the steady-state plateau, suggesting either a large compensatory effect in the elevated \( f_c \) for the reduced \( C_aO_2 \) in all states of altered haematocrits under hypoxic conditions, or, the factors that affect peripheral gas exchange or oxygen utilization (Figure 2.1), which were not measured in this study, play a more important role under hypoxic conditions.

While breathing normoxic gas in each state of altered haematocrit, \( \dot{V}_{o2} \) and \( f_c \) had the fastest time to various percentages of the cardiorespiratory response curve for the step-increase in workrate (Figure 4.14). \( \dot{V}_{co2} \) dynamics were much longer, while \( \dot{V}_E \) was delayed further, as has previously been demonstrated (Casaburi et al., 1987; Linnarsson, 1974; Hughson & Inman, 1985; Karlsson et al., 1975; Miyamoto, 1992; Oren et al., 1982; Ward et al., 1987; Whipp & Mahler, 1980). While breathing hypoxic gas, \( \dot{V}_{co2} \) dynamics were very rapid in each state of altered haematocrit, being significantly faster than \( \dot{V}_{o2} \) and \( \dot{V}_E \) in the anaemic state. This may result from the acute hyperventilation which accompanies hypoxia, evident by the reduced P\(_{ET}CO_2\) (Figure 4.27), eliminating CO\(_2\) from the body's CO\(_2\) stores at a rate in excess of \( \dot{V}_{o2} \). CO\(_2\) can
be unloaded in proportion to the increased ventilation and level of $P_{CO_2}$. Whereas, in contrast, at sea level under normoxic conditions, the ability of haemoglobin to take up oxygen is limited because the haemoglobin molecules have only a limited number of binding sites (four) on the iron atoms that make up the haeme pigment, and these binding sites are almost completely saturated with $O_2$ in most people at the end of the pulmonary capillaries (~97%), even during maximal exercise. Similarly, additional $CO_2$ may be produced from bicarbonate buffering of hydrogen ions produced in association with the elevated $[HL_a]$ shown to occur under hypoxic conditions (Adams & Welch, 1980).

Hypoxia resulted in significantly reduced $C_aO_2$ compared to normoxic and hyperoxic conditions under anaemic, control and polycythaemic conditions. Previous research has demonstrated that this results in an elevated $[HL_a]$ in venous blood from exercising muscle during submaximal exercise (Yoshida et al., 1989a), along with decreased hydrogen ion concentration, $P_{aCO_2}$ and bicarbonate concentration (the opposite trends are associated with hyperoxia, Adams & Welch, 1980; Yoshida et al., 1986b). Adams and Welch (1980), using the same relative workrate to determine steady-state, submaximal effects of various inspired oxygen fractions, suggest that hydrogen ion concentration is a possible limiting factor during exercise and the effect of oxygen on submaximal exercise, with regards to intensity and duration, may be related to the control of hydrogen ion concentration. The increased $\dot{V}_E$ (Table 4.4), which lowers the $P_{aCO_2}$, reflected in the reduced $P_{ET}CO_2$ (Figure 4.27), probably lowers arterial hydrogen ion concentration, while hypoxic exposure increases $[HL_a]$ produced by glycolysis, resulting in an increased hydrogen ion concentration (Adams & Welch, 1980).
reduction in $C_aO_2$ (Figure 4.1) is the cause of the reduced $O_2$ availability to the exercising muscle, which probably further resulted in elevated anaerobic metabolism and lactate production. The reduction in $C_aO_2$ was probably inadequately compensated for by an increase in $\dot{Q}$ as there was only a small increase in $f_C$, assuming stroke volume remained constant (Table 4.4). This has also been demonstrated by Murphy et al. (1989). Assuming that $\dot{Q}$, muscle perfusion or blood flow, and blood pressure could remain constant, exposure to hyperoxic gas would increase oxygen delivery to the exercising muscle, exposing it to a higher $P_aO_2$. This results in a greater concentration gradient across the membrane, and thus more oxygen available for mitochondrial utilization. As no changes in cardiorespiratory response dynamics were demonstrated with hyperoxia, this suggests that the rate limiting factors may be in the ability to utilize the available oxygen (Figure 2.1), or that muscle blood flow decreased.

Hypoxia resulted in a greater oxygen deficit, evident by the delayed cardiorespiratory response dynamics following a step-increase in workrate (Figure 4.4), and while the steady-state may have been achieved (Table 4.4), there would have been a greater fall in creatine phosphate stores (Springer et al., 1991). Hypoxia resulted in a greater excess post-exercise oxygen consumption (Gaesser & Brooks, 1984), and an increase in $\dot{V}_E$ (Garner et al., 1986; Springer et al., 1989). The elevated $\dot{V}_E$ allowed the greater removal of $CO_2$ and most likely a reduction in hydrogen ion concentration [$H^+$] as well (Adams & Welch, 1980). The increase in minute ventilation, during the hypoxic stimulus (Table 4.4), is primarily the result of an increased respiration rate (Table 4.6), while tidal volume increases minimally. This is evident by reductions in the total time of respiration ($T_{TOT}$), brought about by the reduction in both inspiratory ($T_I$) and
expiratory time ($T_E$; Table 4.6). The relationship between $\dot{V}_E$ and the mean inspiratory flow rate ($V_t/T_i$) showing a curvilinear response for hypoxia, beyond $\sim 80 \text{ l.min}^{-1}$, where a linear relationship for hyperoxia, normoxia and hypoxia was evident (Figure 4.26B).

This indicates that for hypoxic stimulation above a $\dot{V}_E$ of $\sim 80 \text{ l.min}^{-1}$, the increase in $\dot{V}_E$ was achieved by increasing mean inspiratory flow at a greater rate than $T_i/T_{TOT}$ is being reduced. This represents increased central inspiratory activity, or an increased rate of rise of inspiratory alpha-motoneuron activity (Milic-Emili, 1981). The cause of this increased respiratory drive would have been the reduced $P_{aO_2}$ associated with hypoxic exposure. Lahiri (1991) suggests the chemoreceptor response is determined not only by blood gas concentration, but also temperature, osmolarity, and humoral substances. The reduced $P_{aO_2}$ would have been detected by the peripheral chemoreceptors, causing the increase in $\dot{V}_E$ (Table 4.6), "blowing off" CO$_2$ from the blood and therefore reducing $P_{aCO_2}$, verified by the reduced $P_{ET}CO_2$ under hypoxia (Figure 4.27; Lahiri, 1991). This would also have reduced arterial hydrogen ion concentration (Lahiri, 1991). The CO$_2$ being "blown off" was also verified by the elevated $\dot{V}_{CO_2}$ during the step-up plateaux (Table 4.4). At the times that mean inspiratory flow was at its highest, $T_i/T_{TOT}$ was beginning to decrease. This tended to reduce minute ventilation and cause the curvilinear response. As a result of enhanced expiratory activity, increased expiratory braking or a decreased mid-expiratory lung volume, $T_E$ will decrease at a slower rate than $T_i$, or mean expiratory flow ($V_t/T_E$) will decrease more rapidly than $V_t/T_i$, causing a decrease in $T_i/T_{TOT}$, minimizing the oxygen cost of breathing (Lind, 1984).

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62 This curvilinear pattern is not peculiar to hypoxia, but could also have been observed had $\dot{V}_E$ been elevated beyond $\sim 80 \text{ l.min}^{-1}$ in the other two conditions.
Hypoxia resulted in an elevated $\dot{V}_E$ and $\dot{V}_{CO2}$ step-down plateau (Garner et al., 1986; Springer et al., 1989), which resulted in significantly slower cardiorespiratory response dynamics following a step-decrease in workrate (Figure 4.8 to Figure 4.11). Therefore, it is possible to partially accept the third hypothesis that: cardiorespiratory response dynamics following a step-decrease in exercise under hypoxic conditions would be delayed. Thus, there was a greater amount of oxygen consumed post-exercise (Figure 4.8). The $\dot{V}_{O2}$ dynamics would have been delayed because of the greatly elevated recovery metabolism following exercise (Gaesser & Brooks, 1984). The increased $\dot{V}_E$ during recovery allowed the greater removal of CO$_2$, which would have accumulated throughout the exercise protocol. Hence there was an elevated $\dot{V}_{CO2}$, and probably a reduction in hydrogen ion concentration [H+] as well (Adams & Welch, 1980). Whipp & Ward (1980) found the ventilatory dynamics to be faster in the hypoxic state and slowed in the hyperoxic state following a step-increase in workrate, although, in this study, no difference was found for a step-increase, and hypoxia was slower than normoxia during the recovery period following exercise. The delay in fc dynamics would have been a consequence of the reduced C$_a$O$_2$ and the body's effort to maintain an elevated $\dot{Q}$ to maintain oxygen transport throughout the recovery period to compensate for the low P$_{aO2}$.

While breathing the hypoxic gas mixture, polycythaemia was not able to negate any of the hypoxic effects despite raising C$_a$O$_2$. Accompanying polycythaemia, there is a compensatory reduction in blood flow to the exercising muscle and a subsequent decrease in the oxygen extraction reserve or the store of O$_2$ available for use by the muscle mitochondria (Stork et al., 1989). When there is an increase in oxygen demand,
the extraction reserve is soon depleted, followed by a reduction in vascular resistance which only allows an increase in muscle blood flow at the expense of other areas of the body (Stork et al., 1989). Under hypoxic conditions, reflex vasoconstrictor tone is insufficient to redistribute the limited oxygen supply away from the exercising muscles to more vital organs such as the brain and heart. Thus, polycythaemia appears to offer no advantage to oxygen delivery during hypoxia (Stork et al., 1989).

5.4 SUMMARY

The withdrawal of three units of whole blood, and subsequent reinfusion of an equivalent volume of packed red blood cells, was able to significantly alter red blood cell and haemoglobin concentration, and thus oxygen carrying capacity. Assuming that blood volume, viscosity, and the ability of the red blood cells to transport and dissociate from oxygen, remains constant during all three stages of altered haematocrit, then one may expect that the exercising tissues were exposed to altered oxygen concentration prior to any circulatory compensatory effects.

Reducing the oxygen transport capacity resulted in significant reductions in maximal aerobic power. During anaemia, this suggests that, during maximal exercise, there may be a central transport limitation to exercise. A subsequent increase in the transport of oxygen did not result in improvements in aerobic power. This may reflect that, within the range of haematocrit elevation investigated, maximal exercise was associated with an oxygen utilization limitation, or that the change in oxygen transport was inadequate to show a transportation effect.
The submaximal relative workrates in each state of altered haematocrit were not different, as indicated by the step-up plateau for $\dot{V}_{O_2}$ being the same in each condition (Table 4.4). This suggests the steady-state oxygen utilization requirements were independent of haematocrit. This trend allows the partial acceptance of the first hypothesis that: following a constant relative intensity step-change in exercise workrate, the steady-state oxygen utilization requirements would be independent of whole body haematocrit and $F_{I}O_2$. However, the influence of hypoxia on gas exchange dynamics during recovery does not permit complete acceptance of this hypothesis.

Altering haematocrit did not alter cardiorespiratory response dynamics during a submaximal step-forcing function during either a step-increase or a step-decrease in workrate. This observation does not support the second hypothesis that: cardiorespiratory dynamics would be slowed when rendered mildly anaemic, and faster when rendered mildly polycythaemic. From these observations, it is apparent that a small change in oxygen transport capacity, did not result in delayed cardiorespiratory dynamics, indicating the utilization of oxygen by the mitochondria may be the site of factors which determine response dynamics, at least within the haematocrit range of 35-45%.

Changing the $C_aO_2$, by altering the supply of oxygen during submaximal exercise step-forcing functions, had a different effect to altering $C_aO_2$ by changing haematocrit. Hypoxic gas resulted in a significant delay in $\dot{V}_{O_2}$ response dynamics following a step-increase in workrate and significantly delayed the response dynamics of $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, $\dot{V}_E$ and $f_C$ following a step-decrease in workrate. The third hypothesis stating that: $F_{I}O_2$ acts
independently of whole-body haematocrit to alter cardiorespiratory response dynamics to a constant relative intensity step-change in workrate, is therefore partially accepted. Hypoxic exposure also resulted in elevated $\dot{V}_E$ and $f_C$ plateaux. There were no differences between hyperoxic and normoxic gas during the submaximal exercise, possibly because the hyperoxic stimulus was not great enough to elicit a significant reduction in $\dot{V}_E$ or $f_C$. From these observations, it would appear that severe hypoxia results in a transport limitation during exercise transitions, despite a compensatory elevation of $f_C$. However the failure of mild hyperoxia to affect either exercise onset or offset dynamics shows that oxygen transport may not influence $\dot{V}_{O_2}$ dynamics when $C_aO_2$ is only slightly elevated by this method.
CHAPTER SIX: CONCLUSIONS and RECOMMENDATIONS
6.1 CONCLUSIONS

In each state of altered haematocrit, the oxygen utilization requirements of the exercise workrate was independent of whole-body haematocrit. However, while the relative workrate was the same in each state of altered haematocrit, the reduced $F_iO_2$ resulted in a significant reduction in $\dot{V}_{o2}$, while an elevated $F_iO_2$ resulted in no change in $\dot{V}_{o2}$. Therefore, the first hypothesis which states that following a constant relative intensity step-change in workrate, the steady-state oxygen utilization requirements would be independent of whole-body haematocrit and $F_iO_2$, is therefore only able to be partially accepted.

Cardiorespiratory dynamics, in response to a constant intensity step-forcing function, were unaltered when subjects were rendered mildly anaemic or mildly polycythaemic. Thus, the second hypothesis stating that the cardiorespiratory response dynamics following a constant intensity step-change in workrate would be slowed when rendered mildly anaemic and accelerated when rendered mildly polycythaemic, is not accepted. One may suggest that the oxygen transport capacity, within the narrow range of 35-45%, was able to meet the demands placed on the cardiovascular system by a step-increase and step-decrease in exercise workrate. This indicates oxygen utilization may be a rate limiting factor in cardiorespiratory response mechanisms during submaximal exercise transitions. Dodd et al. (1993), with their work using ischaemia, hypoxaemia and anaemia, supports the notion that factors other than the rate of oxygen
delivery to muscle are determinants of maximal $\dot{V}_{O_2}$. Reinfusion of red blood cells only provides a means for arguing the central (oxygen conductance) case, if neither the redistribution of blood flow nor $\dot{Q}$ are impeded with normovolaemic polycythaemia (Gledhill, 1982). The hypothesis that oxygen transport is a cardiorespiratory limiting factor in submaximal exercise has generally, though not unequivocally, been supported (Gledhill, 1982; Sawka & Young, 1989), but is not supported by data from this study. This is most likely due to the compensatory mechanisms that occur under anaemic and polycythaemic conditions, such as a possible change in muscle blood flow, which was not determined in the current study, and an elevation in $f_C$ ($p > 0.05$) in an effort to elevate $\dot{Q}$ in the anaemic state, and a reduction in $\dot{Q}$ in the polycythaemic state.

Altering the fractional concentration of inspired oxygen did act independently of whole-body haematocrit to affect cardiorespiratory response dynamics. Severe hypoxia ($F_iO_2 = 0.1$) did delay the response dynamics of $\dot{V}_{O_2}$ following a step-increase in workrate, allowing only partial acceptance of the third hypothesis. That is, hypoxia delayed cardiorespiratory response dynamics independent of whole-body haematocrit following a constant intensity step-decrease in exercise workrate, although significant only under anaemic conditions. This demonstrates that submaximal exercise may be limited by the reduced oxygen transport capability, suggesting a transport limitation. As the difference was only significant in the time to 100% of the plateau, this difference may have been due to cardiorespiratory drift and the inability of $\dot{V}_{O_2}$ to plateau under hypoxic conditions. The part of the third hypothesis regarding hyperoxic exposure accelerating cardiorespiratory response dynamics, independently of whole-body haematocrit, was able to be partially accepted. This suggests that either the stimulus was not great enough to
elicit any significant changes, or that the ability to utilize the extra available oxygen was a rate limiting factor during submaximal exercise beyond a normal $F_1O_2$.

Following a step-decrease in workrate, hypoxia did significantly delay the early stages of the response dynamics for $\dot{V}_O_2$, $\dot{V}_{CO_2}$, $\dot{V}_E$ and $f_c$ under all conditions of altered haematocrit. This suggests that although $f_c$ and probably $\dot{Q}$, remain elevated, a transport delay may exist during the early stages of the recovery period following moderate submaximal exercise, which would permit a faster return of respiratory variables to resting values. Since hyperoxia had no effect on the step-up or step-down dynamics, and hypoxia had virtually no effect on the step-up and slowed most (but not all) of the variables for the step-down, the overall evidence supports rejecting the third hypothesis.

6.2 RECOMMENDATIONS FOR FUTURE RESEARCH

A larger decrease in the number of circulating red blood cells would be achieved by increasing number of phlebotomies. The data obtained in a case study performed as pilot work, where acute anaemia was established (Appendix F) could be substantiated by further work. The data indicates that while further reductions in haematocrit may not alter $\dot{V}_O_2$ dynamics, $\dot{V}_{CO_2}$ and $\dot{V}_E$ response dynamics may be slowed.

The effects of altering inspired oxygen concentration on step-up and step-down dynamics could be further investigated. More extreme concentrations of hyperoxic gas may have significant effects on the polycythaemic and anaemic states which were not evident here. Under hypoxic conditions, the cause of increased $\dot{V}_{CO_2}$ dynamics, and the
patterns of desaturation in maximal and submaximal exercise could also be further investigated. The faster $\dot{V}_{\text{CO}_2}$ dynamics may be due to the increased anaerobic metabolism in the exercising muscle as a result of the reduced oxygen delivery under hypoxic conditions. A probable increase in CO$_2$ production as a result of the increased buffering of bicarbonate and hydrogen ion concentration would occur under similar exercise conditions.

Since haemoglobin and blood volume are prime determinants of aerobic power, the effects of alterations in blood volume should also be investigated for its effect on respiratory mechanisms, in conjunction with further investigations of the effects of haematocrit. The total amount of circulating haemoglobin may have a greater influence compared to haemoglobin concentration (Kanstrup & Ekblom, 1984), so an increase in blood volume, by isotonic plasma volume expansion, may have significant, and different effects on maximal and submaximal aerobic performance (Kanstrup & Ekblom, 1984).

In the current project many trends were evident, but few were significant, probably due to the small population sub-group with a relatively high variance. Further work in this area may involve repeating the study with an increase in subject numbers from a more homogenous population group. This would help reduce the variance that resulted from having small subject numbers with a high variance in a number of physiological variables, such as $\dot{V}_{\text{O}_2}$peak, $f_{\text{C}}$peak or cardiorespiratory response dynamics.
REFERENCES


APPENDICES
APPENDIX A

Determination of the number of trials required during submaximal testing.
Determination of the number of trials required during submaximal testing.

The purpose of this pilot study was to determine the number of single step forcing function trials required during a single data collection session, in order to minimize variability in $\dot{V}_{O_2}$ dynamics data.

Three subjects performed four submaximal trials, with a minimum inter-trial duration of 45 minutes, using a step exercise protocol. Each trial consisted of five minutes of seated rest, followed by six minutes of cycling at a workrate corresponding to $60\% \dot{V}_{O_2\text{peak}}$, followed by a further five minutes of seated rest. $\dot{V}_{O_2}$ dynamics data were analyzed both as a single trial and as averages of two, three and four trials (Figure A1). Data from each trial (averaged across subjects) was used to determine the time to reach 20%, 40%, 60%, 80% and 100% of the difference between the pre-step baseline and the submaximal plateau $\dot{V}_{O_2}$ (Table A1). Since the subject number was small (N=3) no statistical analysis was performed.

Table A1: Differences between $\dot{V}_{O_2}$ dynamics for single step-data obtained from consecutive trials, averaged across three subjects. Data are times (decimal) to reach fixed points between resting and steady state $\dot{V}_{O_2}$.

<table>
<thead>
<tr>
<th>PERCENTAGE</th>
<th>TRIAL 1</th>
<th>TRIAL 2</th>
<th>TRIAL 3</th>
<th>TRIAL 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>20%</td>
<td>0.19 min</td>
<td>0.16 min</td>
<td>0.13 min</td>
<td>0.11 min</td>
</tr>
<tr>
<td>40%</td>
<td>0.38 min</td>
<td>0.36 min</td>
<td>0.29 min</td>
<td>0.25 min</td>
</tr>
<tr>
<td>60%</td>
<td>0.62 min</td>
<td>0.55 min</td>
<td>0.49 min</td>
<td>0.43 min</td>
</tr>
<tr>
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<td>0.85 min</td>
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<td>1.50 min</td>
<td>1.48 min</td>
<td>1.43 min</td>
<td>1.31 min</td>
</tr>
</tbody>
</table>

While the $\dot{V}_{O_2}$ raw data appeared to be superimposed (Figure A1), there appeared to be a change in the $\dot{V}_{O_2}$ dynamics of each consecutive test (Table A1). The faster dynamics was attributed to a warm-up effect. It was concluded that more than one test would be required during data collection to provide higher resolution in curve fitting procedures, whilst minimizing possible inter-subject differences in the warm up effect.
Figure A1: Each plot represents the mean of all previous trials.
APPENDIX B

Determination of the optimal duration between consecutive submaximal trials.
Determination of the optimal duration between consecutive submaximal trials.

With multiple trials required to provide average $\dot{V}_{O2}$ response curves within a given experimental session (Appendix A), it was necessary to determine the optimal duration between consecutive repetitions in a single test session, to minimize any warm-up effect on $\dot{V}_{O2}$ uptake dynamics.

One subject performed three repeats of a step function, submaximal exercise test. Each repetition consisted of three single step exercise loads, separated by either 30, 45 or 60 minutes of seated rest. Each step forcing function involved 5 minutes of rest with the subject seated on the cycle, 6 minutes cycling at a workload corresponding to 60% $\dot{V}_{O2}$, and a further 5 minutes of rest, over three time periods. The first three trials were performed with 60 minutes duration between each test. On the following day, the three tests were performed with 30 minutes between each test, and on the third day, tests were performed 45 minutes between each test. Each set of three trials was then combined to produce a single $\dot{V}_{O2}$ response curve (Figure B1), and the time to reach 20%, 40%, 60%, 80% and 100% of the submaximal, steady state plateau was then determined (Table B1).

<table>
<thead>
<tr>
<th>PERCENTAGE CHANGE: $\dot{V}_{O2}$</th>
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<th>TIME (45 min)</th>
<th>TIME (60 min)</th>
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<td>80%</td>
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<td>0.91 min</td>
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<tr>
<td>100%</td>
<td>1.35 min</td>
<td>1.41 min</td>
<td>1.57 min</td>
</tr>
</tbody>
</table>

A significant difference was found in the first five minute period in the 30 minute and 60 minute trial. This is most likely due to the reduced time allowed for recovery in the 30 minute test which is too short to allow a full recovery. $\dot{V}_{O2}$ data showed a tendency towards a faster response with the 30 minute rest periods, and was attributed to warm-up factors determining $\dot{V}_{O2}$ dynamics. This trend was not evident for the 45 minute session, consequently it was decided that a 45 minute duration between tests would be used.
Figure B1: Three combined trials producing a single $\dot{V}_{O_2}$ response curve with different durations between trials.
APPENDIX C

Test-retest reliability for the oxygen consumption dynamics procedure.
Test-retest reliability for the oxygen consumption dynamics procedure.

The test-retest reliability for the $\dot{V}_{O2}$ dynamics procedure was determined using five subjects. Each subject underwent two identical submaximal, single-step forcing function exercise protocols, from rest, on two separate days. The test protocol consisted of five minutes of seated rest on a cycle ergometer, followed by a step-increase in workrate to a load corresponding to 60% of the subjects $\dot{V}_{O2peak}$. Following six minutes of cycling, subjects ceased exercising and remained seated for a further five minutes. The tests were then repeated after a minimum of 48 hours, at a similar time of day.

The time to reach various percentages of the steady-state $\dot{V}_{O2}$ plateau, were not statistically significant different between tests one and test two ($p>0.05$), and the correlation coefficients between tests exceeded 0.998 for times to reach 20%, 40%, 60%, 80% and 100% of the difference between the pre-step baseline and the plateau (Table C1).

**Table C1**: Pearson Product-Moment correlation coefficients for oxygen consumption dynamics, computed at five time points (time constants) in the response curves of subjects (N=5) during a single-step exercise protocol.

<table>
<thead>
<tr>
<th>Time Constant</th>
<th>Correlation Coefficient</th>
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<tbody>
<tr>
<td>20%</td>
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</tr>
<tr>
<td>40%</td>
<td>1.000</td>
</tr>
<tr>
<td>60%</td>
<td>0.999</td>
</tr>
<tr>
<td>80%</td>
<td>1.000</td>
</tr>
<tr>
<td>100%</td>
<td>1.000</td>
</tr>
</tbody>
</table>
APPENDIX D

Validation of the Ohmeda Biox 3700e pulse oximeter (with ear probe) as a heart rate recording device during sub-maximal exercise.
Validation of the Ohmeda Biox 3700e pulse oximeter (with ear probe) as a heart rate recording device during sub-maximal exercise.

A simple method for recording heart rate \( (f_c) \) on a breath-by-breath basis was required to obtain the fast response times of heart rate dynamics during submaximal exercise. An analogue interface with the Q-Plex I enabled the Ohmeda Biox 3700e pulse oximeter to directly download both \( f_c \) and arterial oxygen saturation \( (S_pO_2) \) data in phase with each tidal volume excursion, however, prior to use, cardiac frequency needed to be validated.

Two subjects were required to remain seated on a bicycle for a period of 10 minutes, followed by a step-increase in workrate to 150W for a further 17 minutes. This was then followed by a further period of rest where the subject remained seated. Throughout the test, subjects were connected to the Ohmeda Biox 3700e pulse oximeter by way of an ear probe attached to the pinna of the ear. Prior to attaching the probe to the ear the site was massaged with isopropyl alcohol and then with a rubefacient cream (Metsal®). Subjects were also connected to a 5 lead ECG (Quinton Q5000), where \( f_c \) was determined from leads II, V and VDI, using the R-R interval. A linear regression analysis was performed between the two sets of \( f_c \) data, and it was found that for submaximal exercise, the pulse oximeter was a reliable indicator of cardiac frequency \( (r=0.999; \text{Figure D1}) \).
Figure D1: A comparison of the cardiac frequency ($f_c$) determined from the Ohmeda Biox 3700e pulse oximeter, PE3000 Sportstester, and that from a 5 lead ECG during a submaximal exercise step-forcing function.
APPENDIX E

Quality control tests prior to reinfusing thawed blood.
Quality control tests prior to reinfusing thawed blood.

SIMULATED TRANSFUSION METHOD

- Obtain an 11cm (3 in) segment of the tubing from the collection bag containing washed, deglycerolysed blood. The volume needed is 0.6ml of blood in the wash solution.

- Dilute the contents of the segment into 7mls of 0.7% saline.

- Mix well.

- Centrifuge immediately at high speed for 5 minutes.

- Compare the hue of the supernate with those on the Haemonetics colour comparator, using tubing of equal diameter to the comparator.

- A hue of less than 6 on the colour comparator is acceptable. If a hue is greater than 6, the osmolality of the supernate must be performed, which must be less than 500 mOsm/kg. If greater, further washing is required.

OSMOLALITY, pH, [K⁺], [Na⁺]

- Quality control tests for these electrolytes are performed on the 5mls collected in a dry 10 ml tube, and are performed on the supernate of the washed blood immediately after washing.

- Acceptable ranges are:
  Osmolality less than 500mOsm/kg
  [Na⁺] 210 ± 10 meq/l
  [K⁺] less than 1.5 meq/l
  pH 6.0 to 7.5

SUPERNATANT HAEMOGLOBIN

- After washing, the effluent line is compared to a colour comparator chart to determine supernatant haemoglobin.

- Plasma Hb less than 2000mg/l is deemed acceptable.
<table>
<thead>
<tr>
<th>COLOR COMPARATOR</th>
<th>MILLIGRAM % FREE HAEMOGLOBIN</th>
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</tr>
<tr>
<td>8</td>
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Figure E1: Haemonetics® color comparator (18501A)
Table E1: Quality control results carried out on deglycerolised RBC's. ** indicates did not reinfuse this unit

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<th>SUBJECT</th>
<th>DONATION UNIT No.</th>
<th>INITIAL VOLUME</th>
<th>SIMULATED TRANSFUSION</th>
<th>pH</th>
<th>[Na⁺] mmol.L⁻¹</th>
<th>[K⁺] mmol.L⁻¹</th>
<th>OSMOLALITY mOsm.kg⁻¹</th>
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<td>236</td>
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APPENDIX F

A case study of mild and severe anaemia on gas exchange dynamics.
A case study of mild and severe anaemia on gas exchange dynamics.

A pilot project was undertaken on one subject to evaluate the effects of anaemia on respiratory gas exchange dynamics, and to determine the number of blood units to be withdrawn to elicit a haemoglobin concentration of less than 10 g.dL\(^{-1}\). This subject was required to undergo maximal and submaximal exercise step-tests to determine the effects of severe anaemia on respiratory gas exchange dynamics.

Exercise tests were performed prior to withdrawal, following the withdrawal of three units and following the withdrawal of a total of nine units of whole blood. Blood withdrawal occurred over a 23 day period with a minimum of 48 hours between each venesection. The maximal exercise tests; performed 24 hours following blood withdrawal, were a standard incremental ramp protocol, with the workload increasing at 36 Watts.min\(^{-1}\). Submaximal tests were performed 24 hours later with the subject seated on a cycle ergometer for a five minute period, followed by cycling at a workload corresponding to 45% \(\dot{V}_{O_2}\text{peak}\) for six minutes, then a further five minute period remaining seated on the cycle ergometer.

The reduction in venous haematocrit and haemoglobin concentration as a result of compounding venesections are shown in Figure F1. The effects of a mild and severe reduction in haematocrit on maximal exercise are shown in Table F1.

During submaximal exercise oxygen carrying capacity \(\left[C_aO_2\right]\) was dramatically reduced. This had no effect on step-up dynamics for \(\dot{V}_{O_2}\) or \(f_c\), although for \(\dot{V}_{CO_2}\) and \(\dot{V}_E\), it appears that the time to plateau is inversely proportional to haematocrit (Figure F2). For step-down dynamics there appears no influence on \(\dot{V}_{O_2}\), \(\dot{V}_{CO_2}\) or \(\dot{V}_E\) dynamics, while for \(f_c\) dynamics the pre-withdrawal dynamics were faster than following any blood withdrawal (Figure F2). There appear to be no major changes in the pre-step baselines, step-up plateaux or the step-down plateaux across the reductions in haematocrit for any of the variables except for \(f_c\) which appeared to be increasing as haematocrit was reduced (Figure F3). Therefore cardiorespiratory dynamics appear insensitive to both mild and severe changes in \(C_aO_2\) by way of altering haematocrit, and any effect this reduced oxygen transport capability does present, is countered by minor alterations to \(f_c\) to maintain \(C_aO_2\) at its normal levels.
Figure F1: Venous haematocrit and haemoglobin concentration following each venesection.
Table F1: The effect of mild and severe haematocrit reductions by way of successive phlebotomies on one subject during maximal exercise.

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<td>( f_c ) (beats.min(^{-1}))</td>
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Figure F2: The effect of severe anaemia on step-up dynamics and step-down dynamics.
Figure F3: The effects of mild and severe anaemia on pre-step baselines, step-up plateaux and step-down plateaux.
APPENDIX G

Results of blood tests as determined by the Coulter Cell Counter (S PLUS IV)
Table G1: Results of blood tests as determined by the Coulter Cell Counter. ** INDICATES DID NOT REINFUSE THIS SUBJECT.

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APPENDIX H

Sum of integrated data averaged over 5 second intervals.
Table H1: The raw data, for each individual subject, averaged over 5 second intervals, integrated in the control state throughout a step-increase and step-decrease in workrate. Data are the total amount of oxygen consumed (1 STPD), total amount of carbon dioxide eliminated (1 STPD), total amount of ventilation (1 BTPS) and total number of heart beats during the step-increase in workrate (73 data points from the onset of cycle exercise (45% aerobic power) at minute 5 to the end of the physiological steady-state at minute 11), and the step-decrease in workrate (49 data points from the onset of recovery following cycle exercise, at minute 11, to the end of a recovery period at minute 15). Subjects were also studied while breathing hypoxic (10%), normoxic (20%) and hyperoxic gases (30%).

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Table H2: The raw data, for each individual subject, averaged over 5 second intervals, integrated in the anaemic state throughout a step-increase and step-decrease in workrate. Data are the total amount of oxygen consumed (1 STPD), total amount of carbon dioxide eliminated (1 STPD), total amount of ventilation (1 BTPS) and total number of heart beats during the step-increase in workrate (73 data points from the onset of cycle exercise (45% aerobic power) at minute 5 to the end of the physiological steady-state at minute 11), and the step-decrease in workrate (49 data points from the onset of recovery following cycle exercise, at minute 11, to the end of a recovery period at minute 15). Subjects were also studied while breathing hypoxic (10%), normoxic (20%) and hyperoxic gases (30%).

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Table H3: The raw data, for each individual subject, averaged over 5 second intervals, integrated in the polycythaemic state throughout a step-increase and step-decrease in workrate. Data are the total amount of oxygen consumed (l STPD), total amount of carbon dioxide eliminated (l STPD), total amount of ventilation (l BTPS) and total number of heart beats during the step-increase in workrate (73 data points from the onset of cycle exercise (45% aerobic power) at minute 5 to the end of the physiological steady-state at minute 11), and the step-decrease in workrate (49 data points from the onset of recovery following cycle exercise, at minute 11, to the end of a recovery period at minute 15). Subjects were also studied while breathing hypoxic (10%), normoxic (20%) and hyperoxic gases (30%).

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APPENDIX I

Individual data for $\dot{V}_{O2peak}$ tests
Table I.1: Time to \( \dot{V}_{O2\text{peak}} \) for each subject under anaemic, control and polycythaemic conditions.

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Significant differences (p<0.05) are indicated by `*`, `#` and `**` for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively.
Table 1.2: Peak Watts for each subject under anaemic, control and polycythaemic conditions.

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Significant differences (p<0.05) are indicated by `*`, `#` and `**` for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively.
Table 1.3: $\dot{V}_{O_2\text{peak}}$ (L.min$^{-1}$) for each subject under anaemic, control and polycythaemic conditions.

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Significant differences (p<0.05) are indicated by `*`, `#` and "**" for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively.
Table L4: Peak cardiac frequency (beats.min\(^{-1}\)) for each subject under anaemic, control and polycythaemic conditions.

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Significant differences (p<0.05) are indicated by `*`, `#` and `**` for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively.
Table 1.5: Peak ventilation (l.min$^{-1}$) for each subject under anaemic, control and polycythaemic conditions.

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Significant differences (p<0.05) are indicated by `*`, `#` and `**` for the comparisons between anaemic and control conditions, anaemic and polycythaemic, and control and polycythaemic conditions respectively.