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Effects of Human Recombinant Erythropoietin on Endurance Performance: Real and Imagined

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Submitted in fulfilment of the requirements for the
Degree of Doctor of Philosophy

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Abstract

The overall aim of this thesis was to determine the effects of administration of (recombinant human erythropoietin) r-HuEpo on endurance performance and to explore the psychosocial effects of taking this drug. In addition, the effects of injecting a Placebo, believed to be an erythropoietin-like substance was assessed, quantitatively and qualitatively, to determine what role the placebo effect may play in mediating the effects of r-HuEpo on endurance performance. All tests were also carried out in the field to ensure that studies had high ecological validity.

The first study, presented in this thesis (Chapter 3), aimed to assess the validity of the Cosmed K4b2 portable metabolic analyser (K4b2) in measuring $\dot{V}O_2$ during submaximal and maximal running velocities in an outdoor environment. Nineteen trained male volunteers (age: 22.9 ± 1.0 years; weight: 74.1 ± 1.8 kg; height: 179.2 ± 1.4 cm; mean \pm SD; $\dot{V}O_{2\max}$ 59.37 ± 0.30 ; mean \pm SEM) completed maximal continuous incremental running tests which involved 3 minute exercise stages at running speeds between 8 to 16 $\text{km}\cdot\text{h}^{-1}$ in 2 $\text{km}\cdot\text{h}^{-1}$ increments. Measured gas exchange variables included fractional content of expired oxygen ($F_{E}O_2$) and fractional content of expired carbon dioxide ($F_{E}CO_2$), oxygen uptake ($\dot{V}O_2$), carbon dioxide output ($\dot{V}CO_2$), ventilation (\dot{V}_E) and respiratory exchange ratio (RER). The Douglas bag method was used for comparison purposes. The typical error in $\dot{V}O_2$ measurement for the K4b2 outdoors compared to the Douglas bag method was $6.5 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. This degree of error for the measurement of was considered unacceptable and it was therefore decided that the K4b2 was not accurate enough to justify its use in subsequent studies.

The aim of Chapter 4 was to assess the effect of r-HuEpo administration on haematological, psychological and performance measures. Seven well-trained males (age: 25.7 ± 2.2 y; BMI $22.5 \pm 0.7 \text{ kg}\cdot\text{m}^{-2}$; $\dot{V}O_{2\max}$: $57.7 \pm 2.9 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) participated in a 10 week protocol divided into three phases; baseline (2 weeks), r-HuEpo administration (4 weeks) and post administration (4 weeks). Fifty $\text{IU}\cdot\text{kg}^{-1}$ subcutaneous injections were administered every two days during the r-HuEpo administration phase. Blood measures included the determination of hemoglobin concentration ([Hb]), haematocrit (Hct), reticulocytes (ret) and total haemoglobin mass (tHb). Cognitive State Anxiety Inventory (CSAI-2) and Profile of Mood States (POMS) were utilised for psychological measures.

Maximal oxygen uptake was measured and 3 km time trial performance was assessed at baseline, immediately post (i.e. at 4 weeks) and 4 weeks post r-HuEpo administration. Cognitive State Anxiety questionnaires were obtained immediately prior $\dot{V}O_{2max}$ and 3 km time trials. Profile of Moods States questionnaires were obtained at baseline, immediately and 4 weeks post. At immediately the post r-HuEpo administration time-point, [Hb], Hct, ret and tHb significantly increased compared to baseline ($P < 0.05$) by 21.2%, 21.3%, 75.0% and 11.7%, respectively. At 4 weeks post r-HuEpo administration, [Hb], Hct and tHb decreased compared to the immediately post time-point, but remained significantly elevated compared to baseline by 7.9%, 9.3% and 6.9%, respectively ($P < 0.05$). Ret decreased to below baseline levels (by 41.7%) 4 weeks post r-HuEpo administration ($P < 0.05$). $\dot{V}O_{2max}$ did not significantly change ($P = 0.07$) over the course of the protocol, but was numerically 5.2% higher immediately post r-HuEpo administration when compared to baseline. Running speed at which the LT occurred was significantly higher by 6.4% ($P < 0.05$) immediately post r-HuEpo administration when compared to baseline. Running speed at OBLA was significantly higher immediately post r-HuEpo administration when compared to baseline by 5.9% ($P < 0.05$) and this remained elevated 4 weeks post, by 5.3%, when compared to baseline ($P < 0.05$). The $\% \dot{V}O_{2max}$ at LT was significantly higher immediately post r-HuEpo administration when compared to baseline by 6.6% ($P < 0.05$). Lactate turn-point values were also significantly higher ($P < 0.05$) immediately post r-HuEpo administration when compared to baseline and 4 weeks post by 9.6 and 7.6%, respectively. The CSAI-2 cognitive subscale decreased significantly (18.3%; $P < 0.05$) immediately post. The POMS tension subscale decreased significantly immediately and 4 weeks post baseline (61% and 50% respectively; $P < 0.05$). Three km time trial performance significantly improved immediately post r-HuEpo administration by 4.9% ($P < 0.05$) when compared to baseline. Faster times were sustained 4 weeks post r-HuEpo administration, when compared to baseline, by 3.9% ($P < 0.05$). Changes in [Hb] significantly correlated with changes in $\dot{V}O_{2max}$ ($r = 0.985$; $P = 0.016$) with a tendency for changes in Hct to correlate with changes in $\dot{V}O_{2max}$ ($r = 0.807$; $P = 0.053$). A significant *negative* relationship was found between changes in $\% \dot{V}O_{2max}$ at LT with changes in [Hb] ($r = -0.882$; $P = 0.048$) and Hct ($r = -0.926$; $P = 0.024$) immediately post r-HuEpo administration. This study confirmed r-HuEpo increases [Hb] and Hct, and these were associated with improvements in $\dot{V}O_{2max}$. Three km time trial performance improved immediately post r-HuEpo administration by 4.9%. However, no clear association was found between [Hb], Hct or $\dot{V}O_{2max}$ and 3 km performance, suggesting that factors other than changes in [Hb], Hct and $\dot{V}O_{2max}$ are likely to have influenced running performance.

Positive changes were also observed in psychological components which warrant further investigation. Performance benefits persisted 4 weeks post r-HuEpo administration.

Chapter 5 aimed to assess the effect of r-HuEpo use on psychosocial factors associated with the practice. The use of substance abuse in sport, with the aim of improving performance, has been prevalent in sport for decades. Recent studies, have attempted to measure athlete viewpoints on doping in sport. However, few studies have been able report viewpoints related to illegal substance use, whilst participants are actually being administered with a banned substance. Six well-trained males (age: 26 ± 2 y; BMI: 22 ± 1 $\text{kg}\cdot\text{m}^{-2}$; $\dot{V}\text{O}_{2\text{max}}$: 58 ± 3 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) individually participated in semi-structured interviews after completing the trial involving the administration of r-HuEpo by subcutaneous injection for 4 weeks. Participants individually participated in semi-structured interviews which were carried out within 2 weeks of completing the study. Varied responses were given in relation to psychosocial impacts and experiences. Five participants reported feeling a substantial performance enhancing effect. One individual expected a greater performance effect. Four individuals stood by their initial stance of 'drugs should be banned in sport'. One individual entertained the idea that drugs should be allowed, but in a 'controlled' manner. One participant suggested that his views had changed, since being involved in the trial, sighting being geographically disadvantaged in the UK in comparison competitors with access to higher altitude. However, the emotions of guilt, shame and/or embarrassment were prominent themes which may be considered as a significant deterrent to doping.

Chapter 6 aimed to determine whether an injectable placebo, claiming to be a legal substance with similar effects to r-HuEpo, would improve endurance running performance in simulated race conditions. Fifteen endurance-trained male volunteers (age: 27.5 ± 6.8 years, height: 1.79 ± 0.05 m, body mass: 73.4 ± 7.6 kg: BMI 22.9 ± 2.0 $\text{kg}\cdot\text{m}^{-2}$) successfully completed the randomised cross-over study design consisting of 3 km competition races before and after a 7-day 'control' phase and 'placebo' phase. During the placebo phase, participants self-administered daily subcutaneous saline injections in the belief that they were receiving a performance enhancing drug called OxyRBX. At the start and end of each 7 day phase haematological variables, CSAI-2, POMS and competitive 3 km race performance were assessed. Participants were interviewed throughout the study and upon study completion. Participants completed the 3 km race distance significantly

faster by 1.2% post the placebo condition (652.4 ± 13.1 s) when compared to the control condition (664.0 ± 13.6 s; $p < 0.05$). An explanation as to these findings remains unknown but may be related to the notion of a 'dissociative task' which, in turn, may have limited the participant's ability to pick up distressing cues during running. This may have diminished or attenuated the perception of fatigue during the races immediately post placebo administration.

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Figure 6.4: This diagram shows the major variables which determine $\dot{V}O_{2\max}$ and the maximum sustained running velocity. The known interactions are shown as black lines. The known interactions with the Central Governor Model (CGM) are shown as orange lines. The known influences of r-HuEpo injections are shown by red arrows. Potential influences of placebo injections are shown by blue arrows with question mark. Red ticks mark variables found to be of significance immediately post placebo administration and red crosses mark variables which were not found to be of significance immediately post placebo administration. HR_{\max} = maximal heart rate; SV_{\max} = maximal stroke volume; [Hb] = haemoglobin concentration; %SaO₂ = percent oxygen saturation; CO_{\max} = maximal cardiac output; $(Ca-CvO_2)_{\max}$ = maximal arterial-venous difference; $\dot{V}O_{2\max}$ = maximal oxygen uptake; % $\dot{V}O_{2\max}$ at LT = percentage of maximal oxygen uptake at lactate threshold. This figure is adapted from (Bassett and Howley, 2000).

Figure 7.1: Summary of determined and potential effects of r-HuEpo and injected r-HuEpo placebo immediately post administration on endurance performance capability. Red coloured ticks, crosses and question marks refer to r-HuEpo administration. Blue coloured ticks, crosses and question marks refer to injected r-HuEpo placebo administration.

Figure 7.2: Simplified summary of selected outcomes associated with r-HuEpo administration and believing r-HuEpo was being administered

Figure 7.3: Finishing line photo shot at a) Beijing Olympics 2008 and b) London Olympics 2012

Other

Box 1.1: Substances and Methods Prohibited In- and Out-Of-Competition

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Author's declaration

I hereby declare that this thesis has been composed by myself, and that the work of which it is a record has been done by myself, except where specifically acknowledged. I also confirm that it has not been submitted in any previous application for a higher degree and that all sources of information have been specifically acknowledged by means of references.

Ramzy Ross _____ **Date** _____

Some of the results contained in this thesis have been presented at conferences as follows:

1. **Ross, R.**, Fudge, B., Gibson, A., Ojiambo, R., Pitsiladis, Y. K4b2 Validity and Reliability during Fast Outdoor Running. Presented as a thematic poster at the ACSM Annual Conference, Denver, USA. May 31st - June 4th 2011. Published in *Medicine & Science in Sports & Exercise*: 2011 - Volume 43 - Issue 5 - p 80.

Dedications

The most exciting phrase to hear in science, the one that heralds new discoveries, is not, “Eureka!” (“I found it!”), but rather, “Hmm...that’s funny...”

- Isaac Asimov

المعرفة القليلة التي تعمل بلا حدود يستحق أكثر من ذلك بكثير
المعرفة التي خامل.

A little knowledge that acts is worth infinitely more than much
knowledge that is idle.

- Khalil Gibran

Definitions/Abbreviations

% SaO ₂	Percent Arterial Oxygen Saturation
%ret	Percent reticulocytes
[Ca ²⁺]	Calcium Concentration
[Hb]	Haemoglobin Concentration
ΔHbCO	Delta Carboxy-haemoglobin
[La]	Blood Lactate Concentration
% $\dot{V}O_{2max}$	Percentage of Maximal Oxygen Uptake
A-aO ₂	Alveolar to Arterial Oxygen Exchange
ATP	Adenosine Tri-Phosphate
ADP	Adenosine diphosphate
A-vO ₂	Arterio-venous Oxygen Difference
BMI	Body Mass Index
CaO ₂	Arterial Oxygen Content
CaO ₂ – CvO ₂	Arterio-venous Oxygen Difference
CGM	Central Governor Model
CNS	Central Nervous System
CO	Carbon Monoxide
Cr	Creatine
CSAI-2	Competitive State Anxiety Inventory
DB	Douglas Bag
EMG	Electromyogram
EPO	Erythropoietin
F _E CO ₂	Fractional Carbon Dioxide
F _E O ₂	Fractional Expired Oxygen
F _i O ₂	Fraction Inspired Oxygen
Hb	Haemoglobin
HbCO	Carboxy-haemoglobin
Hct	Haematocrit
HR	Heart Rate
LT	Lactate Threshold
LTP	Lactate Turnpoint
MCV	Mean Cell Volume
MCH	Mean Corpuscular Volume

MLSS	Maximal Lactate Steady State
O ₂	Oxygen
OBLA	Onset of Blood Lactate
PCr	Phospho-creatine
Pi	Inorganic Phosphate
POMS	Profile of Mood Scales
PO ₂	Oxygen Partial Pressure
RBC	Red-blood cell count
RE	Running economy
RER	Respiratory exchange ratio
Ret	Reticulocytes
r-HuEpo	Recombinant human erythropoietin
RPE	Rate of Perceived Exertion
SV	Stroke Volume
tHb	Total Haemoglobin Mass
$\dot{V}CO_2$	Carbon Dioxide Production
V _D	Dead space
\dot{V}_E	Minute Ventilation
$\dot{V}O_2$	Oxygen Uptake
$\dot{V}O_{2max}$	Maximal Oxygen Uptake
$\dot{V}O_{2peak}$	Peak Oxygen Uptake
WADA	World Anti-Doping Agency

1. General Introduction

1.1 Overview

Research into factors which limit human endurance performance dates back centuries. For example, questions were raised about human physiological limitations, as far back as 490BC, when it was reported that Pheidippides ran 40 km from Marathon to Athens. However, it was the Nobel Laureate in Physiology, Professor Archibald Vivian Hill (A.V. Hill), inspired and persuaded by the works of British physiologists Sir F.G. Hopkins and W.M. Fletcher, who in 1923, truly sparked the debate on ‘what limits human performance’. Hill proposed exercise performance was limited by anaerobiosis in exercising muscle due to inadequate delivery of oxygen (O₂) by the cardiovascular system (Hartree and Hill, 1923). This model has been challenged and refined over time, but it remains clear that O₂ supply and utilisation by skeletal muscle plays a key role in determining endurance performance.

This thesis is about the potential effects of the drug erythropoietin – which stimulates bone marrow to increase red blood cell production and therefore increases O₂ delivery to muscle – on endurance performance. The purpose of this introduction is to provide the background and rationale for the research studies that will be described in subsequent Chapters. The Chapter has been divided into several sections. After a brief overview of the energy systems used to sustain muscular contractions, the limitations to endurance performance will be considered, with an initial emphasis on the importance of maximal oxygen uptake ($\dot{V}O_{2max}$), and the key factors influencing this parameter. Then, factors other than $\dot{V}O_{2max}$ that influence endurance performance capability will be discussed. The effects of human recombinant erythropoietin (r-HuEpo) administration on the endurance performance and the potential physiological and psychological mechanisms responsible will then be addressed whilst also discussing psychosocial considerations. The placebo effect will then be discussed. The final section will address the role of field-based approaches, for improved ecological validity, when assessing exercise performance.

1.2 Energy for Muscular Contractions

Energy, in the form of adenosine triphosphate (ATP), is essential for muscular contractions to enable external work to be done. Adenosine triphosphate is composed of an adenine nucleotide, a ribose sugar (i.e. forming adenosine) and 3 phosphate groups (i.e. forming the

triphosphate segment). Adenosine triphosphate is hydrolysed, with the breakage of the high energy yielding phosphate bonds, in order to release the required stored energy and this also results in the formation of adenosine diphosphate (ADP). As ATP is required to provide energy, ATP is resynthesised from ADP by the addition of a third phosphate group, thus, allowing continuation of the ATP cycle. There is only sufficient ATP in the cell to sustain 1-2 seconds of contraction. Thus, the resynthesis of ATP is vital in order to enable a sustained energy supply to exercising muscle to enable them to continually contract which then allows for endurance activities to be performed.

Humans are reliant on three interactive metabolic systems to resynthesise ATP and their relative contributions to energy provision, during exercise over time, is illustrated in Figure 1.1. The first metabolic system involves the hydrolysis of phosphocreatine which provides an immediate energy source of energy. The phosphocreatine (PCr) metabolic system resynthesises ATP by hydrolysing creatine phosphate, located in the cytoplasm of the myocyte, which provides energy to add the third phosphate group to ADP in order to form ATP. This is carried out in the absence of O₂ and allows ATP to be synthesised at a fast rate with no metabolic by-products formed. However, the phosphocreatine system is only capable of providing energy for ~10-15 seconds (due to the depletion of muscle phosphocreatine) and so its importance in endurance performance, where prolonged energy supply is essential, is limited.

The second metabolic system for regenerating ATP, anaerobic glycolysis, is slower acting, with its contribution to energy provision peaking after muscle phosphocreatine is depleted. Anaerobic glycolysis/glycogenolysis involves the hydrolysis of glucose/glycogen, to pyruvic acid which provides energy to resynthesise two ATP molecules per glucose molecule and three per glycogen molecule. Again, this metabolic pathway occurs in the absence of O₂, in order to provide energy to muscles relatively quickly (although at a slower rate in comparison to phosphocreatine). This is the main energy system when ATP requirements are high; however, debilitating metabolic by-products are produced, during the hydrolysis of glucose to pyruvic acid, which interfere with the muscle contraction cycle and ultimately causes muscle fatigue within 1-3 minutes. Thus, this pathway is also limited in its ability to regenerate ATP for prolonged endurance activities.

Aerobic metabolism, involving the oxidation of carbohydrates and fats, and providing 38/39 and 129 ATP molecules per glucose/glycogen and fat molecule, respectively, is slower than the two anaerobic energy systems, but has a capacity for ATP regeneration that

is many hundred-fold greater. Further, there is no production of the debilitating metabolites that interfere with the muscle contraction cycle. Thus, the aerobic metabolic system is predominantly relied upon to provide energy during prolonged endurance activities. As the reaction of metabolic fuels with O_2 is fundamental to this pathway, factors associated with O_2 delivery and utilisation are key in determining endurance exercise performance capability. For example, oxygen uptake ($\dot{V}O_2$) increases linearly with work rate or running speed (Brooks, 1985, Maloney et al., 2007), thus, the ability to utilise O_2 at high rates is associated, in part, with high performance in endurance events.

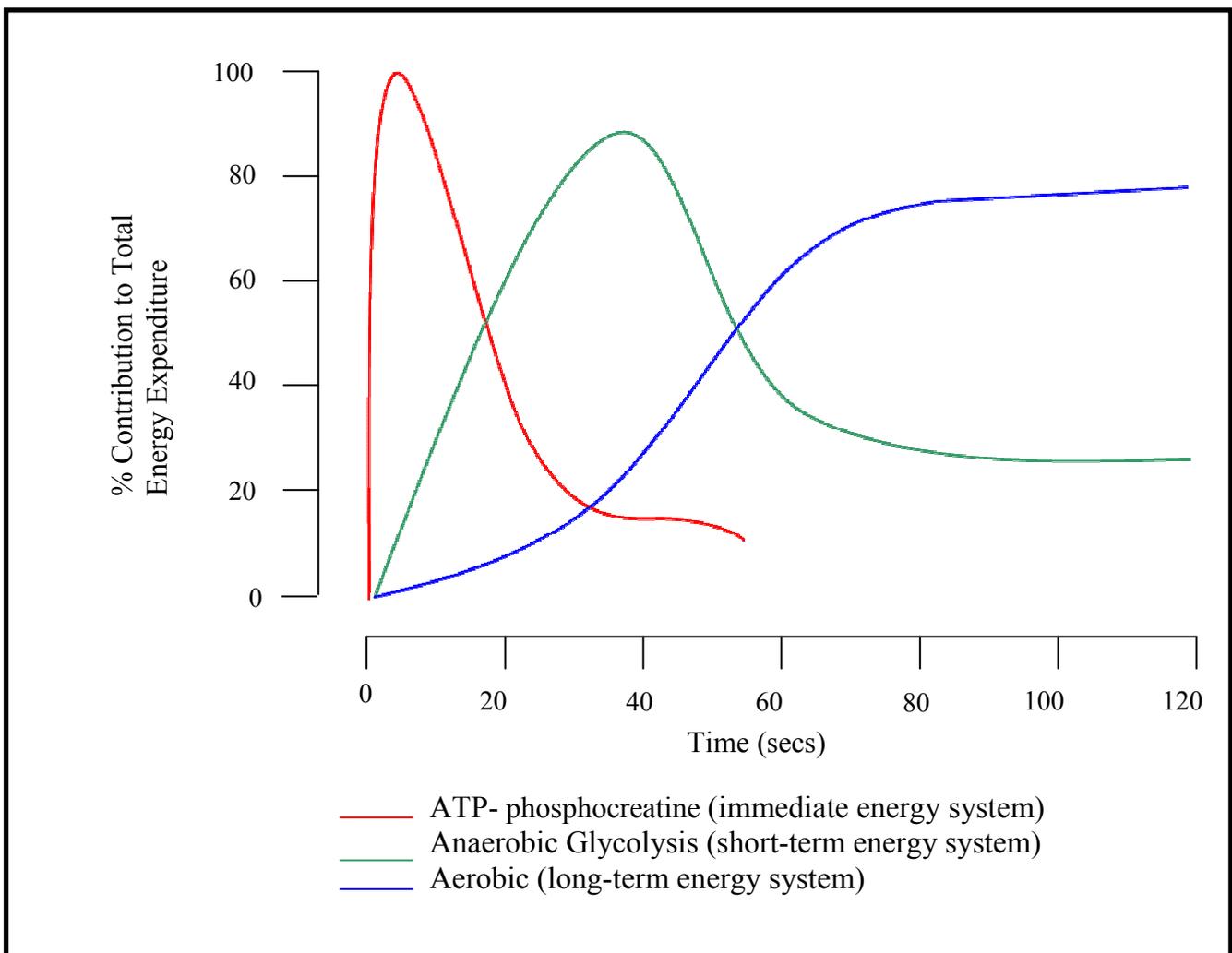


Figure 1.1: Metabolic energy systems and their respective percent contribution to total energy expenditure over time during exercise. Modified from Davis et al. (2000).

1.3 Maximal Oxygen Uptake

Maximal oxygen uptake is defined as the highest rate at which O_2 can be taken up and used by the body during severe exercise (Bassett and Howley, 2000). To accurately measure $\dot{V}O_{2max}$, a graded exercise protocol is carried out where exercise intensity is progressively increased with time, to progressively tax the aerobic system to a point where the exercise intensity can no longer be voluntarily maintained. Substantial evidence exists indicating a strong relationship between $\dot{V}O_{2max}$ and endurance performance. Researchers in the first half of the 20th century first observed that a high $\dot{V}O_{2max}$ was a key physiological characteristic of elite endurance athletes (Hill and Lupton, 1923, Robinson et al., 1937). Over the next half century a number of studies confirmed this with high correlations observed between $\dot{V}O_{2max}$ and endurance performance over a range of running distances (Table 1.1). Thus, given the strong relationship between $\dot{V}O_{2max}$ and endurance performance, understanding limitations to endurance performance requires an understanding of the factors which determine and limit $\dot{V}O_{2max}$.

Table 1.1. Summary of study correlations between $\dot{V}O_{2max}$ and performance at different running distances

Study	Subject Type	Age range (years)	N & Sex	$\dot{V}O_{2max}$ Range (ml·kg ⁻¹ ·min ⁻¹)	Performance Distance (km)	Performance Time range (min)	Race Pace range (m·min ⁻¹)	Correlation between $\dot{V}O_{2max}$ and Performance Time (r)
Costill et al., 1973	Highly trained runners	25-48	16 males	54.8-81.2	16.1	48.9-67.8	237-329	-0.91*
Farrell et al., 1979	Well-trained endurance athletes	18-54	18 males	46.3-73.7	42.2	138-229	184-306	-0.91*
					19.3	60-98	196-322	-0.91*
					15	46-74	203-326	-0.89*
					9.7	29-44	218-340	-0.86*
Ramsbottom et al., 1987	PE students	N/A	69 males 55 females	35.0-78.0	5	15.0-34.0	147-333	-0.89*
					3.2	12.4-20.3	158-258	-0.91*
					3.2	9-14	230-374	-0.83*
Mello et al., 1987	Various fitness levels	22-51	44 males 17 females	36.0-58.1	3.2	12.4-20.3	158-258	-0.91*
Ramsbottom et al., 1988	Well-motivated volunteers	19-36	36 males 38 females	45.9-77.8	5	14.8-21.9	228-338	-0.94*

*Significant at p <0.05; PE: Physical Education; mean ± SD

1.4 What Limits $\dot{V}O_{2\max}$?

The transport of O_2 from the atmosphere to the mitochondrion in muscles involves several stages: these are illustrated in Figure 1.2. Air enters the body via the nose and/or mouth, passes through the larynx, trachea, bronchi and bronchioles before arriving at the alveoli in the lungs. The alveoli are the site of diffusion of O_2 into the pulmonary circulatory system where the heart is then responsible for pumping oxygenated blood around the systemic circulatory system. Diffusion of O_2 then takes place between systemic blood vessels and muscle tissue.

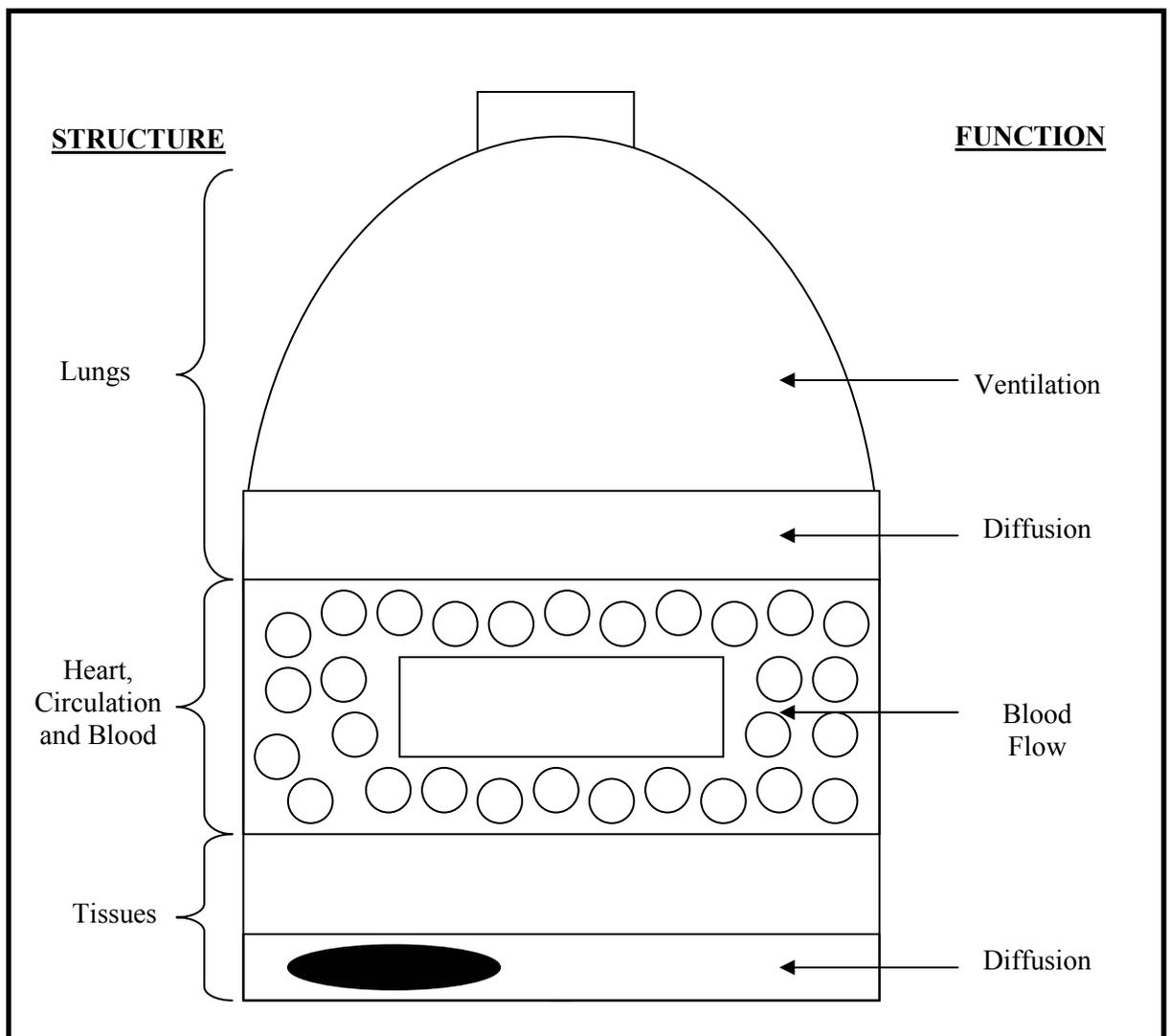


Figure 1.2: Simplified model of the O_2 transport pathway. Modified from Wagner (2008).

The ability of the cardiorespiratory system (i.e. lungs, heart and consequent oxygenated blood supply) to transport O_2 is described as the ‘central component’ of O_2 transport (Robergs and Roberts, 1997, Bassett and Howley, 2000). Thus the potential ‘central’ factors that could limit $\dot{V}O_{2\max}$ include: 1) pulmonary diffusion capacity, 2) cardiac output and 3) O_2 carrying capacity of the blood (Bassett and Howley, 2000). The ability of exercising muscles (i.e. skeletal muscle) to then extract and utilise O_2 supplied by the cardiorespiratory system is described as the ‘peripheral’ component (Robergs and Roberts, 1997, Bassett and Howley, 2000) of $\dot{V}O_{2\max}$. Figure 1.3 illustrates this classic cardiovascular model, often described as a ‘central dogma’, which is widely taught and accepted in exercise physiology.

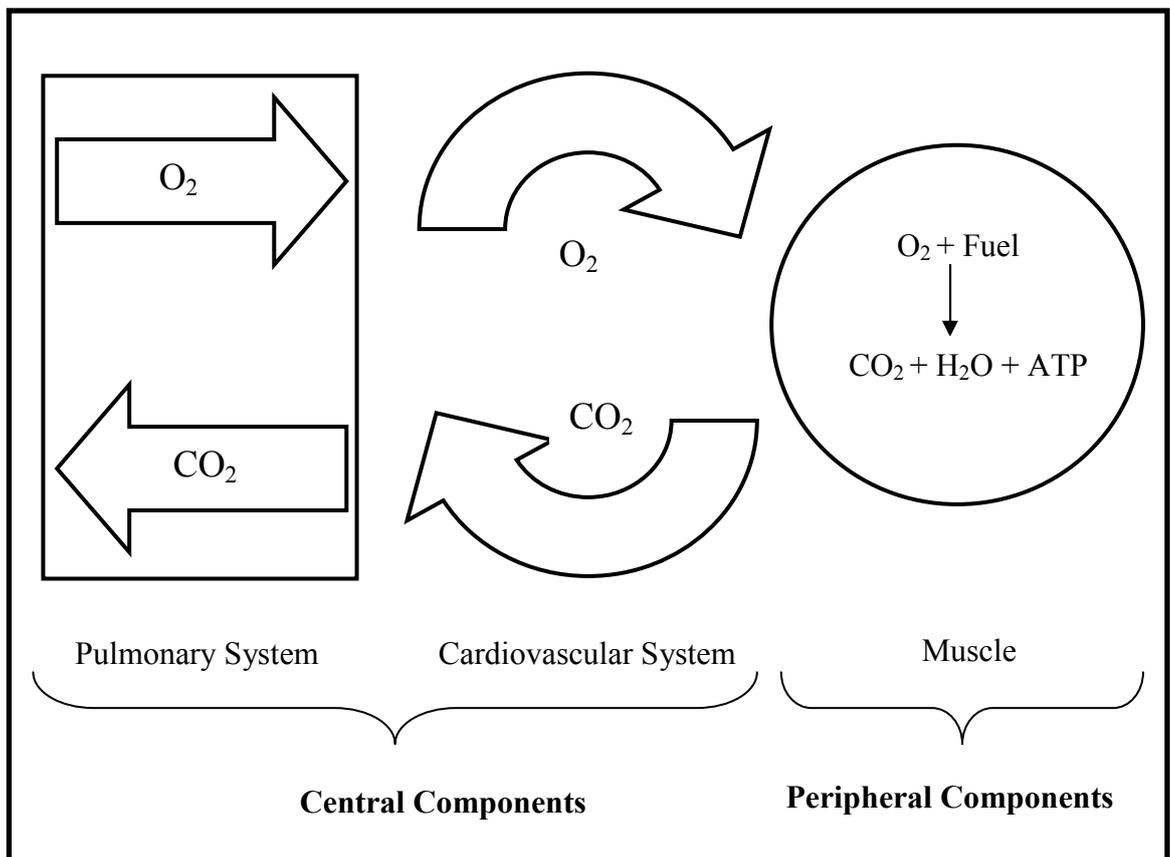


Figure 1.3: ‘Central Dogma’ approach to explaining fatigue.

According to the *Fick Principle* $\dot{V}O_{2\max}$ is the product of the volume of blood pumped by the heart in one minute and the difference in arterial and venous blood O₂ content (Rose, 1956):

$$\dot{V}O_{2\max} = \text{Cardiac Output} \times \text{Arterio-venous Oxygen Difference (CaO}_2 - \text{CvO}_2)$$

Cardiac output refers the amount of blood pumped by the heart per minute and CaO₂ – CvO₂ refers to the difference in O₂ content between arteries and veins. Thus, factors which influence the right side of the Fick equation (i.e. cardiac output x (CaO₂ – CvO₂)) are the key factors which limit $\dot{V}O_{2\max}$.

1.4.1 Pulmonary System Limitations

The pulmonary system is responsible for delivering atmospheric O₂ into the blood. Oxygen diffuses from the alveoli in the lungs into red blood cells where it binds with haemoglobin to form the oxy-haemoglobin complex. The proportion of haemoglobin molecules in the oxygenated form is termed O₂ saturation (%SaO₂). The %SaO₂ is one of the two key determinants of the ‘CaO₂’ (the total number of O₂ molecules in arterial blood – both bound and unbound to haemoglobin) component of the Fick equation (the other being O₂ carrying capacity of the blood). Thus, any potential pulmonary system limitation to $\dot{V}O_{2\max}$ would manifest as a reduction in %SaO₂ (Dempsey et al., 1990).

In average, healthy individuals at sea level the weight of evidence supports the lungs’ ability to effectively saturate arterial blood with O₂ during submaximal and maximal exercise, as %SaO₂ is maintained at ~95% (Powers et al., 1989, Rowell et al., 1964, Koskolou and McKenzie, 1994, Prefaut et al., 2000, Dempsey et al., 1984, Dempsey et al., 1980, Wasserman et al., 1981). In highly trained individuals at sea level, however, there is evidence of arterial hypoxemia at high exercise intensities (Amann et al., 2006, Romer and Dempsey, 2006). This is a consequence of the higher maximal cardiac output in highly trained individuals leading to a lower transit time of red blood cells in the pulmonary circulation. This resulting in insufficient time to effectively saturate haemoglobin (Hb) in red blood cells in the pulmonary circulation (Hammond et al., 1986).

This specific pulmonary limitation in highly trained athletes with high maximal cardiac output was further illustrated by Powers and colleagues (Powers et al., 1989) who demonstrated that breathing a hyperoxic gas mixture (26% O₂) – which increases the alveolar-arterial diffusion gradient – increased %SaO₂ from 90.6% to 95.9% and $\dot{V}O_{2\max}$

from 70.1 to 74.7 ml·kg⁻¹·min⁻¹ in highly trained athletes, but did not alter these factors in subjects with more modest $\dot{V}O_{2\max}$ values (~56.5 ml·kg⁻¹·min⁻¹).

Pulmonary limitations are also evident in conditions of moderately high altitude, where atmospheric O₂ partial pressure (PO₂) is reduced, thereby reducing the alveolar-arterial diffusion gradient (West et al., 1962, Daniels and Oldridge, 1970). For example, West and colleagues (West et al., 1962) measured six participants, from the Himalayan Scientific and Mountaineering Expedition (1960-1961), at an elevation of 5800 m (equivalent to a barometric pressure of 380 mmHg) at rest and during a range of exercise intensities up to maximal efforts. These authors reported average %SaO₂ values of 67% at rest and ≤50% during maximal exercise, indicating a limitation of pulmonary diffusing capacity at altitude. This is further illustrated by data, from various studies, showing a linear decrease in $\dot{V}O_{2\max}$ in endurance athletes with increasing altitudes (Wehrlin and Hallen, 2006) (Figure 1.4).

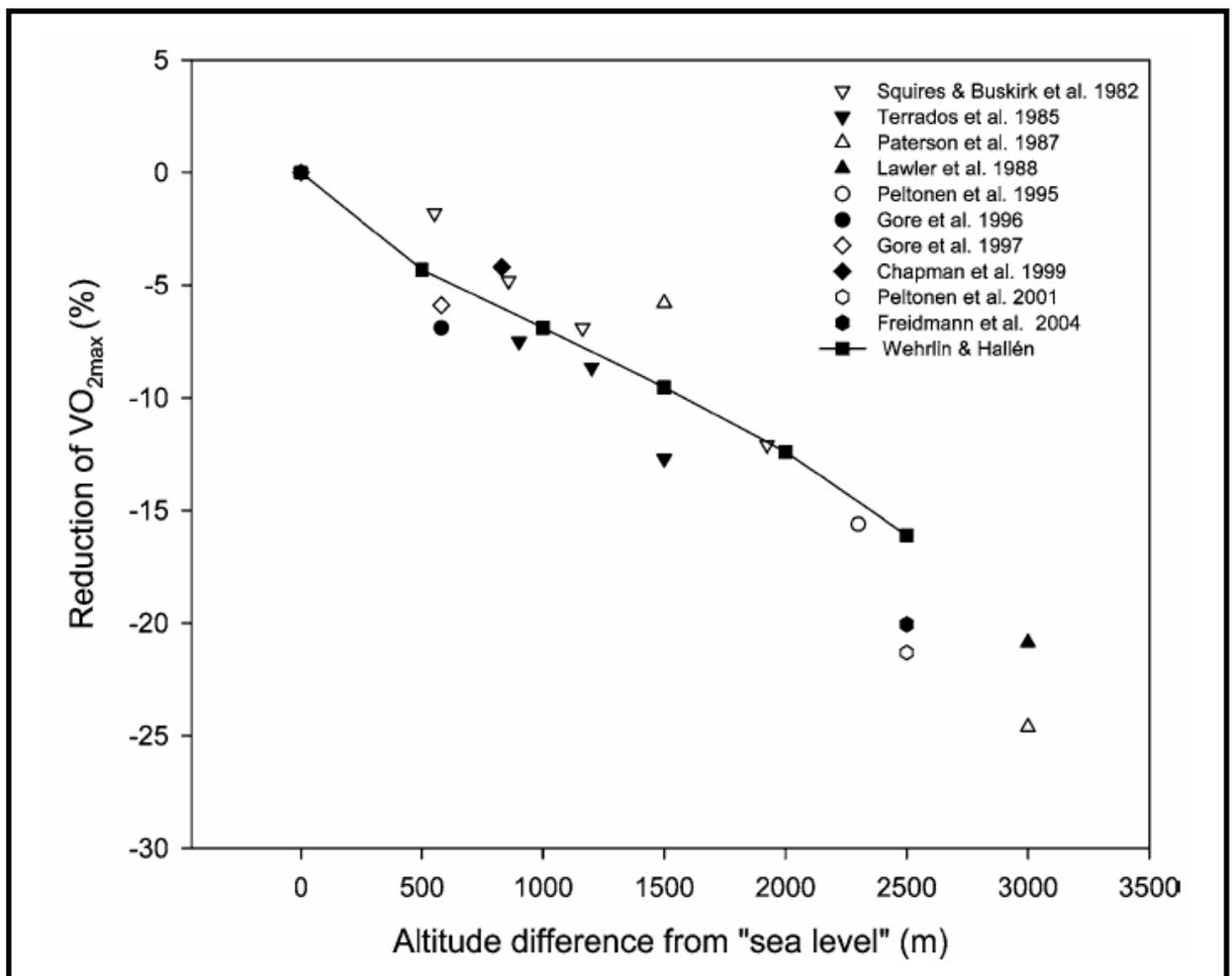


Figure 1.4: Percent decrease in $\dot{V}O_{2\max}$ from sea level (Wehrlin and Hallen, 2006).

As part of the assessment of pulmonary limitations, the trainability of lung parenchyma has also been investigated. Overall, the trainability of the lung parenchyma by respiratory muscle training remains controversial. The general consensus suggests that respiratory muscle training influences physical performance measures to only a limited extent, if at all (Sheel, 2002, McKenzie, 2012). For example, no differences in structural properties were found in healthy individuals after an exercise training programme according to Dempsey & Wagner (Dempsey and Wagner, 1999). Research by Sonetti and colleagues (Sonetti et al., 2001) concluded a very limited effect of respiratory muscle training on exercise performance in highly fit and competitive subjects.

Thus, the weight of evidence suggests that pulmonary diffusion capacity does not limit $\dot{V}O_{2\max}$ in individuals with 'normal' $\dot{V}O_{2\max}$ levels when exercising at sea level. However, in highly trained endurance athletes, where cardiac output is high and pulmonary transit time for red blood cells is low at maximal intensities, arterial hypoxemia may occur, limiting $\dot{V}O_{2\max}$ to some degree (<10%). In addition, at altitude, where there is a reduced alveolar-arterial O_2 gradient, a clear pulmonary limitation to $\dot{V}O_{2\max}$ is evident.

1.4.2 Cardiovascular System Limitations

1.4.2.1 Cardiac Output

The ability of the cardiovascular system to deliver O_2 to the exercising muscles, after pulmonary diffusion has occurred, is highly dependent on cardiac output. As the Fick equation illustrates, $\dot{V}O_2$ is directly proportional to cardiac output (for a given arterio-venous O_2 difference). Indeed, a linear relationship between $\dot{V}O_2$ and cardiac output over a wide range of submaximal work rates has been reported (Astrand et al., 1964). Furthermore, the performance enhancing effects of endurance training on $\dot{V}O_{2\max}$ has been highly correlated to increasing cardiac output (Saltin and Strange, 1992).

Cardiac output is described as the product of heart rate (HR) multiplied by stroke volume (SV) (i.e. cardiac output = HR x SV) and it has been suggested to account up to 85% of the limitation of $\dot{V}O_{2\max}$ (di Prampero and Ferretti, 1990, Bassett and Howley, 2000). This has been demonstrated during maximal exercise where the majority of O_2 present in arterial blood, $\sim 200 \text{ ml } O_2 \cdot \text{l}^{-1}$, is extracted and perfused into exercising muscles with only $\sim 25 \text{ ml } O_2 \cdot \text{l}^{-1}$ remaining in venous blood (Shephard, 1970). The main training adaptation that facilitates this is the expanded capacity of both ventricles in the heart which, in turn, elicits a greater elastic recoil for oxygenated blood delivery to the periphery – an improved

Starling effect (Starling and Visscher, 1927, La Gerche and Gewillig, 2010). Greater elastic recoil allows for a greater amount of oxygenated blood to be delivered, per heartbeat, to exercising muscles as well as increasing venous return. An increase in venous return also contributes to increasing ventricular filling and therefore preload, which in turn increases SV and, consequently, cardiac output (Starling and Visscher, 1927, La Gerche and Gewillig, 2010).

A number of studies, utilising various testing protocols, have indicated cardiac output to be the key limiter of $\dot{V}O_{2\max}$. Saltin and colleagues demonstrated that the difference in $\dot{V}O_{2\max}$ between deconditioned and conditioned individuals was explained predominantly by a difference in cardiac output (Saltin et al., 1968). Interestingly, in the 40-year follow-up of this study it was reported that the age-related decline in $\dot{V}O_{2\max}$ was primarily due to declines in maximal cardiac output (McGavock et al., 2009), primarily as a consequence of reduced maximal HR (Fleg et al., 2005). Ekblom and colleagues (Ekblom et al., 1968) also assessed changes in $\dot{V}O_{2\max}$ in healthy individuals after 16 weeks of physical training and reported that ~50% of the increase in $\dot{V}O_{2\max}$ with training could be explained by an increase in cardiac output. The increase in cardiac output was, in turn, explained by an increase in SV.

It has been proposed that SV alone can explain most of the range observed in $\dot{V}O_{2\max}$ (Ekblom and Hermansen, 1968, Coyle, 1999). During incremental exercise, SV has been reported to plateau, particularly in untrained individuals, at approximately 40% of $\dot{V}O_{2\max}$ whereas in the trained individual, SV has been reported to continually increase up to $\dot{V}O_{2\max}$ (Vella and Robergs, 2005). Gledhill and colleagues (Gledhill et al., 1994) were the first to report this phenomenon after comparing competitive endurance cyclists and normally active males (matched for heart rates ranging from 90 to 190 $\text{b}\cdot\text{min}^{-1}$) during incremental cycle ergometry exercise. The authors reported a progressive increase in SV in the endurance trained group, whilst the normally active group demonstrated a plateau at an average heart rate of 120 $\text{b}\cdot\text{min}^{-1}$, equivalent to 40% $\dot{V}O_{2\max}$. In addition, the SV of the endurance trained group were significantly larger than their untrained counterparts at all heart rates.

Using an alternative approach, studies have also assessed the cardiac output limitation on $\dot{V}O_{2\max}$ by decreasing cardiac output, artificially, with the use of beta-blockers to reduce HR, and to a lesser extent, SV (Tesch, 1985). Using this approach, maximal cardiac output was reduced by ~18%, which in turn reduced $\dot{V}O_{2\max}$ by 5 to 15% (Tesch, 1985).

These authors reported that the smaller reduction in $\dot{V}O_{2\max}$, when compared to the reduction cardiac output, was primarily due to a compensatory increase in arterio-venous oxygen difference (a-vO₂) with some individuals showing a greater compensatory effect than others. Overall, studies demonstrate that the decline in $\dot{V}O_{2\max}$, as a result of cardio-selective beta-blockers, is primarily caused by a reduction in blood flow and O₂ delivery to exercising muscles.

Other intervention studies have also assessed the effect of mechanical limitations, associated with the heart, on cardiac function. Evidence from animal pericardiectomy studies has demonstrated the importance of the heart pericardium on cardiac function and $\dot{V}O_{2\max}$ (Stray-Gundersen et al., 1986, Hammond et al., 1992, Shabetai et al., 1992). For example, Stray-Gundersen and colleagues (Stray-Gundersen et al., 1986) demonstrated that the removal or opening of the restrictive pericardium resulted in higher $\dot{V}O_{2\max}$ measurements in dogs. Oxygen uptake, cardiac output, HR and SV measurements were taken during submaximal and maximal exercise, before and after the removal of the pericardium. At maximal exercise intensity, the removal of the pericardium increased cardiac output by 19.8%, SV by 16.9% and $\dot{V}O_{2\max}$ by 7.6%. Although these results were obtained using untrained dogs, this does provide proof-of-principle that experimental manipulation to increase SV and cardiac output also increases $\dot{V}O_{2\max}$, highlighting the central limiting role of SV and cardiac output in $\dot{V}O_{2\max}$ determination.

Further evidence is provided by studies that have also assessed the effect of training separate parts of the body on $\dot{V}O_2$. For example, Clausen and colleagues (Clausen et al., 1973) assessed changes in central and peripheral blood circulation after training of the arms or legs. After leg training, the authors reported an increase in $\dot{V}O_{2\max}$ during exercise in non-trained arms, clearly indicating a training effect related to the heart, blood flow and O₂ delivery. Similarly, in 13 healthy male subjects, Saltin and colleagues (Saltin et al., 1976) assessed and compared the effects of a control (i.e. no training), one and two-legged cycling performance, after training, at a work intensity of 70% of $\dot{V}O_{2\max}$. The authors reported a ~6%, ~22% and ~10% change in $\dot{V}O_{2\max}$, respectively. Thus, an improvement of ~22% was reported in one-legged $\dot{V}O_{2\max}$ after endurance training of two-legs, however, this was reduced to ~10% when participants were assessed during two-legged exercise at 70% $\dot{V}O_{2\max}$. Although cardiac output was not measured, the smaller increase in $\dot{V}O_{2\max}$ during two-legged exercise is indicative of central limitations as oxygenated blood was having to be shared between the legs, thus reducing oxygenated blood flow and

respective O₂ delivery to exercising muscles. Therefore, cardiac output distribution remained as the main limiting factor.

More recently, Trinity and colleagues (Trinity et al., 2012) have reported a reduction in the rate of increase in cardiac output for a given increase in $\dot{V}O_2$ from submaximal (40 to 70% $\dot{V}O_{2max}$) to maximal exercise intensities (70 to 100% $\dot{V}O_{2max}$). This was reported in ten well-trained male cyclists where reductions in cardiac output were underpinned by reductions in SV. The authors concluded a plateau in cardiac output prior the attainment of $\dot{V}O_{2max}$, as a result of compromised cardiovascular function, primarily caused by a decline in SV as HR increased in a linear manner during incremental exercise to maximal exertion.

In summary, the standpoint of the cardiovascular approach is one where the heart is identified as the key limitation to $\dot{V}O_{2max}$. Thus, the key characteristic that enables elite endurance athletes to run fast over a prolonged period of time is an enlarged, compliant heart where the pericardium elasticity can accommodate a large volume of blood, very quickly, thus enhancing the Starling mechanism of the which, in turn, generates a large SV.

1.4.2.2 Oxygen Carrying Capacity

Along with cardiac output, the O₂ carrying capacity of blood is the other key determinant of the amount of O₂ made available to exercising muscle. Thus, this is also a cardiovascular determinant of $\dot{V}O_{2max}$. The O₂ carrying capacity of blood influences the arterio-venous O₂ difference of the Fick equation and is primarily determined by haemoglobin concentration [Hb].

It is well documented that altering the O₂ carrying capacity of blood by increasing [Hb], via autologous infusion of blood, increases $\dot{V}O_{2max}$ (Ekblom et al., 1976, Berglund and Ekblom, 1991, Gledhill, 1982, Gledhill, 1985). For example, the re-infusion of 900 to 1350 ml of blood has been reported to increase $\dot{V}O_{2max}$ by 4-9% (Gledhill, 1982, Gledhill, 1985) and this due to the artificial increase in total red blood cells which, in turn, increases the total O₂ carrying capacity of blood. This increases central O₂ delivery to peripheral muscles due to more oxy-haemoglobin complexes being formed, as well as by increasing cardiac output due to the increase in blood volume. Strong correlations ($r = 0.97$) have been demonstrated between total haemoglobin mass measures (tHb) (i.e. total O₂ carrying capacity of blood) and $\dot{V}O_{2max}$ (Figure 1.5) (Schmidt and Prommer, 2010). Thus, manipulating the total O₂ carrying capacity of blood, by increasing [Hb], increases $\dot{V}O_{2max}$.

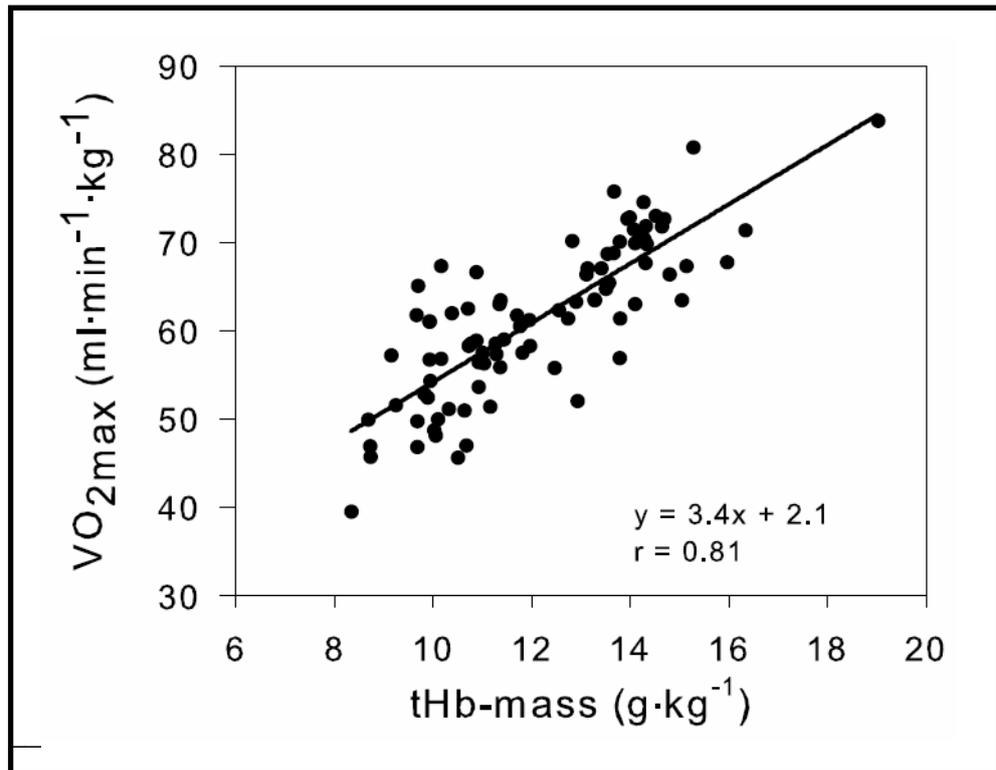


Figure 1.5: Relationship between tHb mass and $\dot{V}O_{2\max}$ (Schmidt and Prommer, 2010).

1.5 An Integrated O₂ Delivery System

It is clear that the transportation of O₂ from the atmosphere to the mitochondrion incorporates several sequential steps (as indicated in Figure 1.2). As a result, an integrated model has also been proposed where, it has been suggested, impediments on O₂ delivery occur at different points or steps. This complex transport pathway (Weibel, 1994) can also be described as an integrated system (Wagner, 1996a) where limitations exist, in series (a ‘bucket brigade’), at each component associated with O₂ delivery. The integrated components, each, contribute to limiting overall O₂ availability to exercising muscles which, in turn, collectively limit $\dot{V}O_{2\max}$. As indicated in Figure 1.2, the principal components of the integrated O₂ transport pathway, its structures and major functions can be summarised in 4 steps:

- 1) Ventilation – a convective process where inspired air is transported to the pulmonary alveoli.
- 2) Diffusion in the lungs – the movement of oxygen from alveolar gas into pulmonary capillary blood.

- 3) Perfusion – movement of oxygenated blood from capillaries to pulmonary veins to the left ventricle of the heart to body tissues.
- 4) Diffusion in the tissues – release of oxygen from carrier Hb, in red blood cells, to body tissue followed by diffusion of oxygen to mitochondria for ATP production.

It is argued that as the above steps are linked in series to each other, each step has, therefore, the ability to affect the whole O₂ delivery system. As a result, Wagner argues that O₂ delivery is the key limitation, but there is no single ‘limiting factor’ for $\dot{V}O_{2\max}$ (Wagner, 2008, Wagner, 1996a). Wagner goes further by proposing that the enhanced function of just one step has a relatively minor effect on overall O₂ delivery capability. Conversely, however, any reduced function in any one step of O₂ delivery (i.e. ventilation, pulmonary diffusion, blood circulation and tissue O₂ diffusion) can have a considerable negative effect on $\dot{V}O_2$ as illustrated in Figure 1.6 (Wagner, 1996b). This is effectively depicted when considering the relationship between $\dot{V}O_2$ and mitochondrial PO₂ *in vitro* (Figure 1.7) (Wilson et al., 1977). Wilson and colleagues demonstrated, *in vitro*, the importance of O₂ delivery to muscle where any limitations, associated with the steps prior O₂ delivery to mitochondria that cause a decline in mitochondrial PO₂ below 1 mmHg, will seriously decelerate $\dot{V}O_2$.

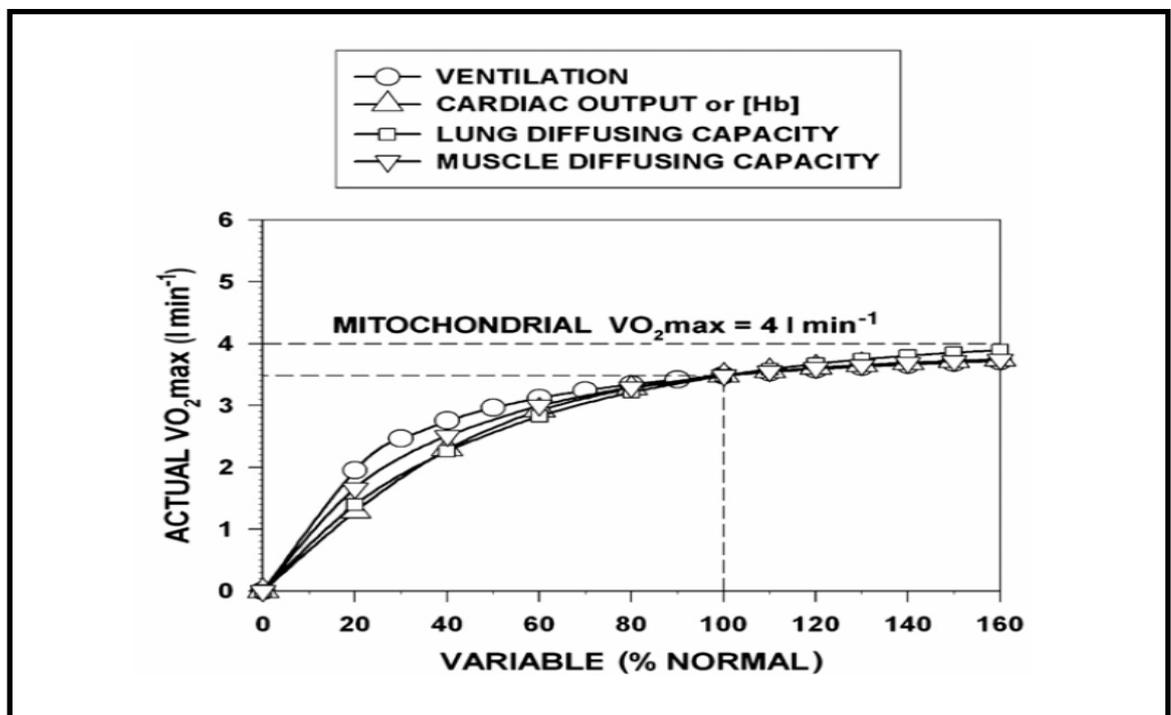


Figure 1.6: Calculations of the sensitivity of $\dot{V}O_{2\max}$ to each of the transport system components when maximal mitochondrial capacity to consume O₂ is taken to be 4 l min⁻¹. Each component shows relatively similar sensitivity. When one component has diminished function, $\dot{V}O_{2\max}$ can fall significantly (Wagner, 2008).

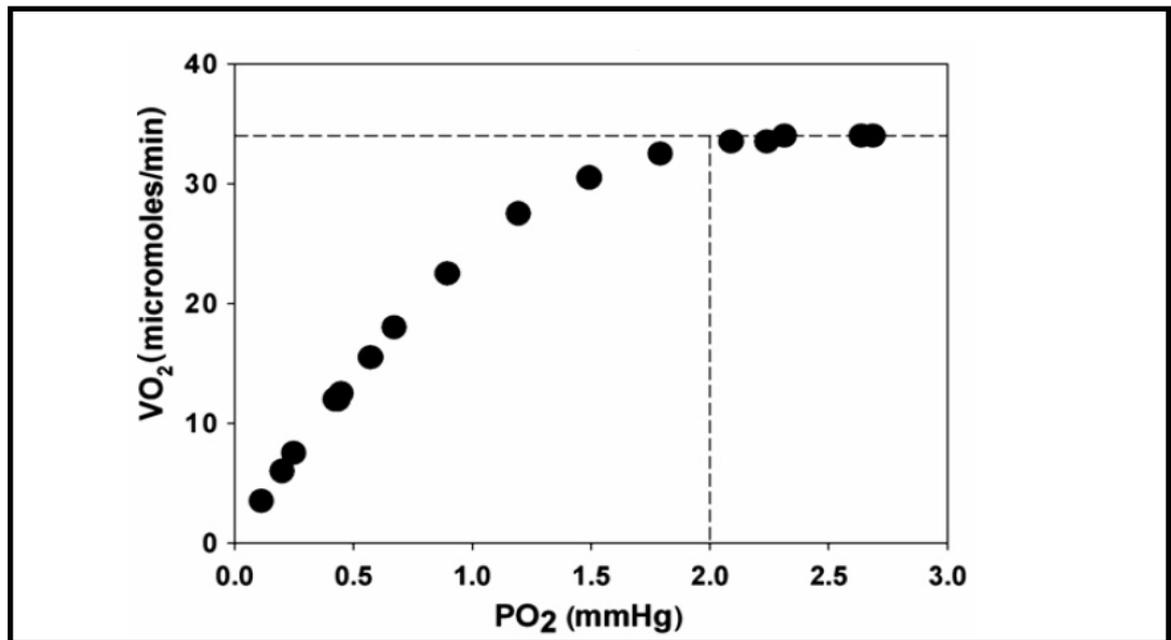


Figure 1.7: *In vitro* mitochondrial respiration curve showing dependence of O₂ consumption on mitochondrial PO₂. Maximal O₂ consumption occurs at a PO₂ of only 2 mmHg (Wilson et al., 1977).

Wagner uses the Fick equation to describe the determining mechanism of $\dot{V}O_2$ as one limited primarily by O₂ delivery; however, he also acknowledges the potential influence of peripheral musculature mechanisms associated with determining $CaO_2 - CvO_2$. For example, it has been demonstrated that, at rest, when blood flow is reduced by 73%, there is a compensatory 94% increase in O₂ extraction in order to maintain $\dot{V}O_2$ (Wagner, 2008). The same compensatory mechanisms can also be seen during exercise, albeit to a lesser degree. For example, as previously highlighted by Tesch and colleagues (Tesch, 1985), the use of cardio-selective beta-blockers to limit muscle blood flow, by reducing cardiac output, can be somewhat compensated for by a relatively small increase in $CaO_2 - CvO_2$.

Thus, the evidence clearly supports the assertion that the key limitation is in O₂ delivery. However, it is evident that this is regulated by a complex integrated system which collectively determines the effectiveness of O₂ delivery and, in turn, collectively limits $\dot{V}O_{2max}$.

1.6 Peripheral System Limitations

The ability of exercising muscles to extract O₂ from blood, delivered by the cardiovascular system, is associated with the $CaO_2 - CvO_2$ part of the Fick equation. The ability of exercising muscles to extract O₂ is determined by an interaction of several key factors: 1)

peripheral diffusion gradients, 2) capillary density and 3) muscle metabolism. As part of these key factors, key processes include 1) the dissociation of O₂ from haemoglobin, 2) the diffusion of O₂ from red blood cells to muscle cells and 3) the diffusion and transport of O₂ to the mitochondria within muscle.

1.6.1 Peripheral Diffusion Gradients

The transportation of O₂ from capillaries to muscle is largely dependent on a diffusion gradient between red blood cells in capillaries and muscle tissue. The transport of O₂ is thus facilitated by a low PO₂ in mitochondria, in comparison to the higher PO₂ within capillaries, encouraging the diffusion of O₂ into muscles. In addition, myoglobin – the oxygen binding protein in muscle – has a stronger affinity for O₂ than haemoglobin does, which also acts to facilitate O₂ transfer into muscle. Furthermore, muscles during exercise contribute to creating a diffusion gradient by using O₂ which consequently decreases muscle PO₂ (Honig et al., 1992). Exercise at $\dot{V}O_{2\max}$ elicits a maximal potential gradient difference and thus maximises the potential for O₂ diffusion into exercising muscles. Honig and colleagues demonstrated this by assessing blood flow to exercising muscle in isolation and during whole body exercise. Increased cardiovascular blood flow was effective in increasing $\dot{V}O_2$ only if mitochondria concomitantly consumed O₂ in order to draw down mitochondrial PO₂ to maintain a diffusion gradient (Honig et al., 1992).

The extraction of O₂ by exercising muscle, however, is never absolute (Shephard, 1970) and this may be, in part, as a result of distally decreasing PO₂ levels which reduces the pressure gradient between distally located capillaries and muscle tissue. Another explanation involves a ‘carrier-free region’, a short distance between the interior of the red blood cell surface and muscle tissue sarcolemma, where a decrease in PO₂ has been reported. The resulting decrease in PO₂ hinders O₂ diffusion capacity by reducing the diffusion gradient between red blood cells and muscle tissue (Honig et al., 1992).

Thus, the evidence indicates that while $\dot{V}O_{2\max}$ is dependent on O₂ blood delivery, maintaining a peripheral diffusion gradient is also necessary to draw O₂ into exercising musculature. There is general agreement with regards to the importance of a peripheral O₂ diffusion gradient. However, as this model is an integrated one (i.e. an interaction between cardiovascular and peripheral PO₂), peripheral diffusion gradients alone cannot be the sole factor that limits $\dot{V}O_{2\max}$.

1.6.2 Capillary Density

The importance of O₂ blood delivery and the maintenance of a peripheral diffusion gradient on $\dot{V}O_{2\max}$ have been discussed. Not surprisingly as a result, studies have investigated whether $\dot{V}O_{2\max}$ can be improved via training adaptations that could influence the O₂ blood supply and/or the peripheral diffusion gradient and thus improve overall O₂ delivery to exercising muscles.

One such adaptation of the periphery, as a result of training, is an increase in capillary density (Andersen and Henriksson, 1977) and this has been explored as to its potential to improve $\dot{V}O_{2\max}$. Overall, an increase in capillary density has been reported to improve redistribution of cardiac output which in turn assists to increase O₂ delivery to exercising muscles (Evans, 1985). In addition, just as the red blood cell transit time limitation located centrally, an increase in capillary density has been reported to extend mean blood flow transit time within muscle itself. This, in turn, has been reported to enhance muscle O₂ delivery by helping maintain a sufficient $CaO_2 - CvO_2$, particularly at high blood flow rates. Indeed, the beneficial effects of an increase in capillary density have been reported in several studies where, for example a 16% increase in $\dot{V}O_{2\max}$ has been reported with a concomitant 20% increase in capillary density (Andersen and Henriksson, 1977). However, any resulting increase in $\dot{V}O_{2\max}$, as a result of an increased capillary density, is one that is secondary to cardiovascular adaptations as, for example, the redistribution of cardiac output can only occur once cardiac output improvements have initially occurred at the heart. Thus, the role of capillary density must be viewed as a means to help improve endurance performance, but not as a key limiting factor on $\dot{V}O_{2\max}$.

1.6.3 Muscle Metabolism

Oxygen reaches its final destination in mitochondria, located in muscle, where it is utilised in the final steps of the electron transport chain in order to resynthesise ATP. Muscle metabolism of O₂ contributes to the arterio-venous O₂ difference (i.e. $CaO_2 - CvO_2$), as indicated in the Fick equation, where muscle O₂ consumption determines CvO_2 .

Several muscle metabolic mechanisms can undermine muscular performance (Westerblad and Allen, 2002, Westerblad et al., 2002) which, in turn, can be considered as a potential limitation on $\dot{V}O_{2\max}$. As previously discussed, during prolonged and high intensity exercise, the energy consumption of skeletal muscle cells can increase, one hundred-fold, when compared to rest (Westerblad and Allen, 2002, Westerblad et al., 2002) and so there

is an inevitable eventual reliance on energy regeneration via anaerobic energy systems in attempt to supply ATP from muscle mitochondria to exercising muscle fibres.

Several consequences arise as a result of this change and includes the production of inorganic acids such as hydrogen ions as a result of the disassociation of lactic acid (Posterino et al., 2001), which also increases muscle acidosis levels, and this then interferes with the muscle contractility. In addition, the hydrolysis of muscle PCr to Cr and Pi results in an increase Pi (Allen et al., 2008) which has also been reported to interfere with muscle filament cross-bridge formation by reducing myofibrillar Ca^{2+} sensitivity (Dahlstedt and Westerblad, 2001) and sarcoplasmic reticulum Ca^{2+} reuptake and release (Patel et al., 2000).

Given the key role of mitochondria, as the site for ATP regeneration, an individual with superior O_2 delivery adaptations may still be limited in his/her ability to regenerate and supply ATP to exercising muscle fibres. Theoretically, it has been suggested that low mitochondrial concentrations may limit the rate of ATP supply to exercising muscles and so studies have assessed the effects of increasing muscle mitochondrion concentrations on $\dot{\text{V}}\text{O}_{2\text{max}}$ via mechanisms associated with improved capacity to increase ATP supply. Some studies, in humans, have indeed shown increases of between 20 to 30% in $\dot{\text{V}}\text{O}_{2\text{max}}$ after ~2 fold increases in mitochondrial enzymes (Saltin et al., 1977). In addition, Costill and colleagues (Costill et al., 1976) reported succinate dehydrogenase activity (a respiratory enzyme located in mitochondrion) in elite distance and middle distance endurance male runners was 3.4- and 2.8-fold higher, respectively, compared to levels found in untrained males. However, the overall evidence indicates mitochondrial concentrations to play a minor role in limiting $\dot{\text{V}}\text{O}_{2\text{max}}$ and this is indicated in studies that have revealed that individuals, with nearly identical $\dot{\text{V}}\text{O}_{2\text{max}}$ values, can have up to a two-fold difference in mitochondrial enzyme levels and *vice versa* (Hartgens et al., 2004, Alen et al., 1984, Kuipers et al., 1993).

Thus, the coupling of a high O_2 transport capacity with an equally effective cellular O_2 utilisation capability is important for overall endurance performance and, in agreement with several authors (Jacobs et al., 2011) (Hepple, 2002, Hepple et al., 2002), peripheral components cannot be regarded as being key on limiting $\dot{\text{V}}\text{O}_{2\text{max}}$, but are important in improving endurance performance. Thus, the effects described do indeed contribute to causing fatigue at $\dot{\text{V}}\text{O}_{2\text{max}}$; however, these effects are a consequence of cardiovascular limitations associated with O_2 delivery (primarily a cardiac output limitation) and not the

initial cause. Thus, improving muscle metabolism (e.g. by increasing mitochondrial enzymes) is beneficial for improving general endurance performance, but cannot be regarded as the key factor limiting $\dot{V}O_{2\max}$.

1.7 Factors other than $\dot{V}O_{2\max}$ Influencing Endurance Performance

Despite the clear evidence that $\dot{V}O_{2\max}$ is a good predictor of endurance performance in a heterogeneous group (see Section 1.3), this is not the case when homogenous groups of endurance athletes with relatively high $\dot{V}O_{2\max}$ values are studied. For example, Conley and Krahenbuhl reported that $\dot{V}O_{2\max}$ explained only 1.4% of the variance in 10 km performance in elite distance runners (Conley and Krahenbuhl, 1980). Similarly, Weston and colleagues (Weston et al., 2000) compared physiological characteristics of sub-elite African and Caucasian runners, and reported that African runners had $\dot{V}O_{2\max}$ values ~14% lower than the Caucasians, but 10 km race times for the two groups were very similar (2% slower in Africans). This is further illustrated in a case-study of an Olympic athlete, whose 3000 m race performance time improved by 8% over a five-year period, despite $\dot{V}O_{2\max}$ decreasing by 9.6% over the same period (Jones, 1998). Thus, while a high $\dot{V}O_{2\max}$ is a necessary condition for elite endurance performance, it is not sufficient, and other factors also play a key role.

Beyond durations of a few minutes, all endurance exercise is sub-maximal (i.e. conducted at intensities below $\dot{V}O_{2\max}$). Thus a key determinant of performance in endurance events is the highest $\dot{V}O_2$ that can be sustained for a prolonged period. While improvements in $\dot{V}O_{2\max}$ plateau within a few months of endurance training, the maximal sustainable $\dot{V}O_2$ continues to improve beyond this (Astrand and Rodahl, 1970). A key determinant of maximal sustainable $\dot{V}O_2$ is the lactate threshold.

1.7.1 Lactate Threshold

The lactate threshold (LT) can be defined as the point where lactic acid starts to accumulate in blood faster than it can be removed and is indicative of an inability of the aerobic energy system to regenerate ATP at the required rate (Jones and Doust, 1998). Thus, the LT influences the maximal sustainable $\dot{V}O_2$ and, therefore, the maximal sustainable pace that can be maintained throughout an endurance event.

A common technique for LT determination involves the visual inspection of the relationship between work rate or $\dot{V}O_2$ and [La] to identify the initial 'deflection' point

where [La] increases consistently above baseline concentrations (Wasserman et al., 1973) (Figure 1.8). Studies have also indicated a second ‘deflection’ point, termed the lactate turnpoint (LTP), where blood [La] increases at a faster and consistent rate (Smith and Jones, 2001). Alternatively, researchers have also utilised a fixed point that is a standardised pre-determined [La]. For example, a [La] of 4 mmol/l, in blood (Figure 1.8), has been proposed (Coyle et al., 1983) and this has been termed the onset of blood lactate accumulation (OBLA) (Kindermann et al., 1979, Sjodin and Jacobs, 1981). More recently, the determination of the ‘maximal lactate steady state’ (MLSS) has also been proposed for profiling lactate response during submaximal exercise. The maximal lactate steady state represents the highest work intensity that can be sustained whilst a constant [La] (i.e. no increase in [La] greater than 1 mmol/l) is maintained over the final 20 minutes of a 30 minute constant work rate exercise test (Pringle and Jones, 2002, LaFontaine et al., 1981) (Figure 1.9).

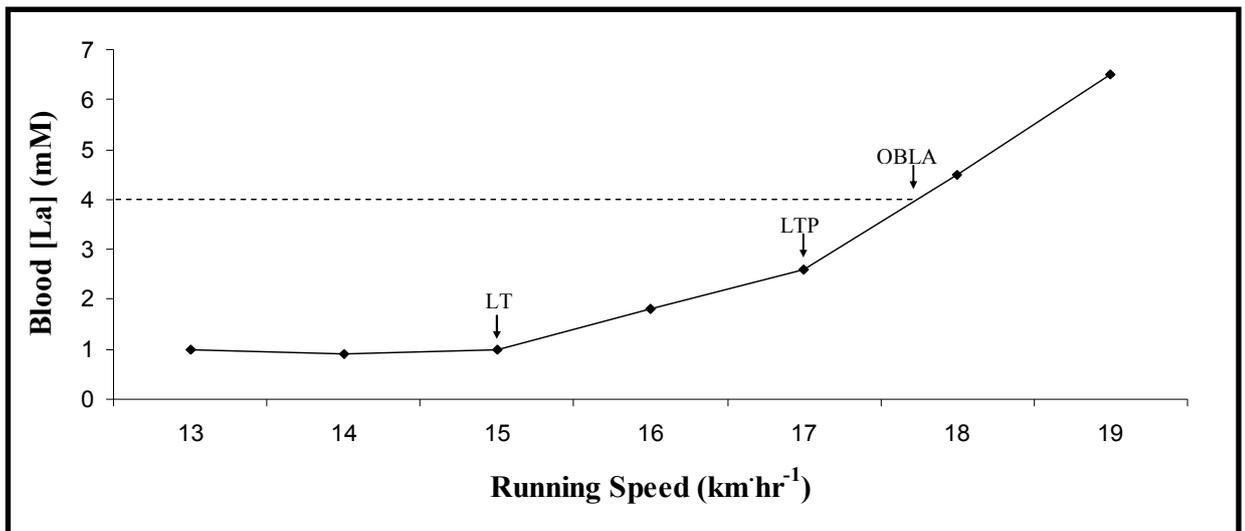


Figure 1.8: Illustration of a typical blood [La] response to incremental exercise. LT is defined as the initial ‘deflection’ point where [La] increases consistently above baseline concentrations. LTP is defined as the second ‘deflection’ point where blood [La] increases at a faster and consistent rate OBLA is defined as the point where [La] reaches 4 mmol/l.

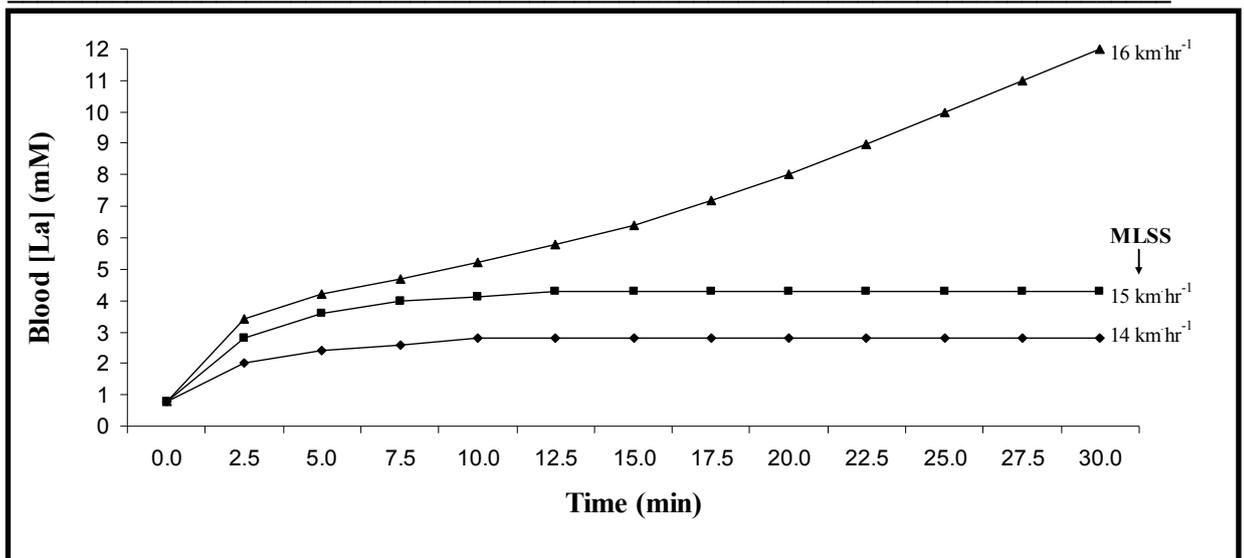


Figure 1.9: An example for determining MLSS in an endurance athlete. The individual completed several treadmill runs, up to 30 minutes in duration, at 14, 15 and 16 km·h⁻¹ running speeds. 15 km·h⁻¹ running speed is identified as MLSS as a higher yet still constant blood [La] was reached.

Lactate threshold, OBLA, MLSS and LTP are highly correlated with each other although the work rates/speeds and $\dot{V}O_2$ values at which they occur within an individual differ (Aunola and Rusko, 1992). The importance of LT and related concepts (i.e. LT, LTP OBLA, MLSS) on endurance performance has been clearly demonstrated. For example, in a homogenous group of endurance athletes ($\dot{V}O_{2max}$ range of 4.6 to 5.0 l·min⁻¹), the percentage $\dot{V}O_{2max}$ achieved at OBLA was a strong determinant of time-to-fatigue at 88% of $\dot{V}O_{2max}$ (Coyle et al., 1988). In another example, LT was found to be the best predictor for distance covered in 12 minutes in untrained female participants (Yoshida, 1987).

Whilst the LT is an important predictor of endurance performance capability, a number of studies have shown that the corresponding velocity or power output, at which the LT occurs, is a better predictor (Farrell et al., 1979, Hagberg and Coyle, 1983, Sjodin and Jacobs, 1981, Karlsson and Jacobs, 1982). This is a function of both the $\dot{V}O_2$ achieved at LT, and the velocity that can be achieved in that individual for that given $\dot{V}O_2$. Thus velocity at LT brings a third major determinant of performance into play – running economy (RE).

1.7.2 Running Economy

It has been well documented that a linear relationship exists between submaximal running velocities and $\dot{V}O_2$. However, the amount of O_2 required to run at a particular running velocity varies quite considerably between individuals (Bransford and Howley, 1977, Morgan et al., 1995). Thus, RE can be defined as the steady-state $\dot{V}O_2$ for a given running velocity (Morgan et al., 1989).

Studies have assessed the variance in oxygen cost for a given speed between differently trained groups of individuals, from untrained to elite. Overall, elite runners are the most economical and even within each training group category (i.e. untrained to elite), up to a 20% difference in $\dot{V}O_2$, at a given speed, between the least and the most economical can be observed (Morgan et al., 1995). The physiological importance of RE has also been illustrated by Conley and Krahenbuhl (Conley and Krahenbuhl, 1980) who assessed the top 19 finishers of a prominent 10 km race and reported a strong relationship ($r = 0.79-0.83$; $P < 0.01$) between RE and 10 km race times. Furthermore, Daniels and Daniels (Daniels and Daniels, 1992) demonstrated how the interaction between $\dot{V}O_{2max}$ and running economy can affect running velocity at $\dot{V}O_{2max}$ between genders. The authors demonstrated that, on average, woman displayed a lower $\dot{V}O_{2max}$ by 14% and that this also translated into a 14% lower velocity at $\dot{V}O_{2max}$ (when compared to male counterparts with a similar running economy profile). Such findings where RE is lower in women than men, of similar training status (Daniels and Daniels, 1992), has partly been explained by woman naturally possessing higher percentage body fat (which is additional economy impairing carried weight). Such explanations do, in part, explain the lower performance in endurance events of women in comparison to male counterparts.

While it is clear that RE influences endurance performance capability, explanations for differences in economy between individuals have not been fully elucidated. It appears that both genetic and environmental factors contribute. For example, contrasts have been made between African and Caucasian runners where Africans have been reported to exhibit better RE in comparison to the Caucasian comparisons (47.3 ± 3.2 vs. 49.9 ± 2.4 ml·kg⁻¹·min⁻¹ at 16 km·h⁻¹, respectively; $P < 0.05$) (Weston et al., 2000). Several explanation have been suggested in an attempt to explain such a phenomena and these have included: smaller body mass in African individuals (thus reducing the oxygen cost at a given running speed), a better ability to sustain a higher percentage of $\dot{V}O_{2max}$ (over a given distance, e.g. 10 km) in African individuals and the ability to accumulate less plasma lactate in

comparison to Caucasian counterparts (Weston et al., 2000). The authors suggest that such explanations, in part, help explain the dominance of African runners on the world-stage.

Conversely, Jones (Jones, 2006) physiologically assessed the current female world record holder for the marathon distance, Paula Radcliffe, over a 7-year period and reported a 15% improvement in RE over this period (but no change in $\dot{V}O_{2\max}$). Jones (Jones, 1998) also assessed the effect of long-term endurance training and its impact on 3000 m running performance and reported improvements in velocity at LT (from 15 to 18 km·h⁻¹) as well as an improved RE (from 53 to 48 ml·kg⁻¹·min⁻¹ at 16 km·h⁻¹). Strength and power training have also been shown to improve RE and, consequentially, endurance performance (Hickson et al., 1988, Millet et al., 2002, Spurrs et al., 2003, Paavolainen et al., 1999).

Further, in addition to training and the application of the above interventions, Saunders et al. (Saunders et al., 2004a) have also described several physiological and biomechanical factors, related to improved RE in runners. Such factors include increased mitochondrion and oxidative enzyme levels within exercising muscles as well as the ability to generate low ground reaction forces (Saunders et al., 2004a). Detailed discussion of the determinants of RE is beyond the scope of this Chapter and interested readers are directed to a review by Saunders and colleagues (Saunders et al., 2004a) for further information.

1.7.3 Integrated Effects of $\dot{V}O_{2\max}$, LT and Running Economy on Endurance Performance

To summarise, $\dot{V}O_{2\max}$ determines the ‘upper limit’ for oxidative ATP regeneration and $\dot{V}O_{2\max}$ and LT together determine the maximal sustainable $\dot{V}O_2$ in endurance events (Bassett and Howley, 2000). The velocity achieved for this $\dot{V}O_2$ is determined by RE. Thus, $\dot{V}O_{2\max}$, LT and RE integrate to determine endurance performance. Key cardiovascular limitations on $\dot{V}O_{2\max}$ and key factors that influence endurance performance capability have been discussed and these are summarised in Figure 1.10. However, while the factors described here are strong predictors of endurance performance, they do not explain 100% of its variance. Thus, other factors must also play a role in determining endurance performance. A number of researchers have investigated how factors other than those illustrated in Figure 1.10 may influence endurance performance. The most prominent alternative/complementary paradigm to the cardiovascular model is the Central Governor Model. This will be discussed in the following section.

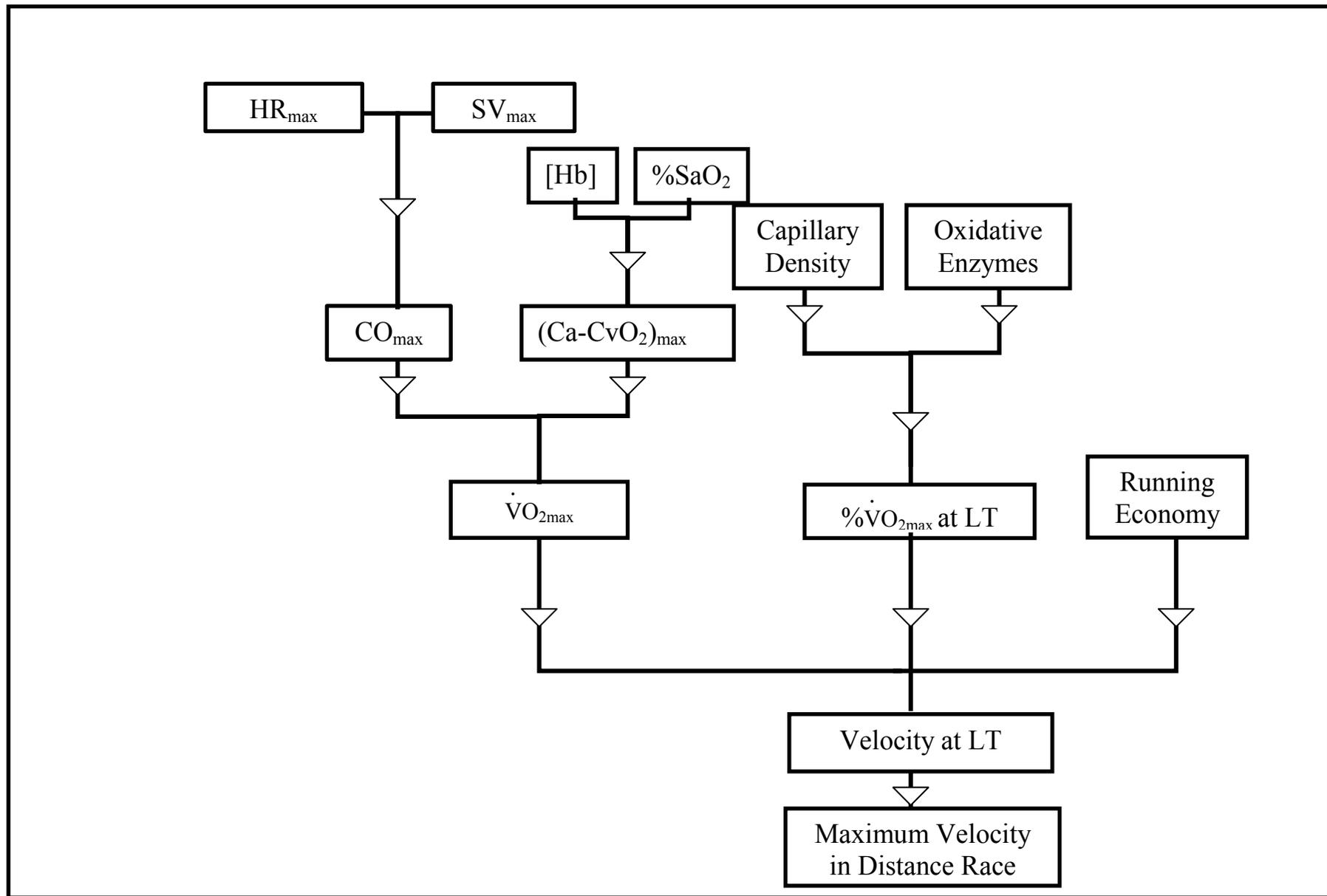


Figure 1.10: Summary of the major cardiovascular variables related to $\dot{V}O_{2max}$ and the maximal velocity that can be maintained in a distance race. Adapted from (Bassett and Howley, 2000). HR_{max} = maximal heart rate; SV_{max} = maximal stroke volume; [Hb] = haemoglobin concentration; %SaO₂ = percent oxygen saturation; CO_{max} = maximal cardiac output; $(Ca-CvO_2)_{max}$ = maximal arterial-venous difference; $\dot{V}O_{2max}$ = maximal oxygen uptake; % $\dot{V}O_{2max}$ at LT = percentage of maximal oxygen uptake at lactate threshold.

1.8 The Central Governor Model

An alternative to the cardiovascular model exists which emphasises the inclusion of the central nervous system (CNS) as the ‘central’ contributor to the regulation of endurance performance capability and $\dot{V}O_{2max}$. The importance of assessing the potential role of the CNS on endurance performance, which is not considered in the cardiovascular model, seems logical as without the brain all other factors become redundant. In the context of the Central Governor Model (CGM), the CNS determines the muscles ability to generate force (Noakes et al., 2004, Noakes et al., 2005).

Thus, the Central Governor Model (CGM) proposes an ‘integrative central neural regulation of effort and fatigue during exercise in humans’ (Noakes et al., 2004, Noakes et al., 2005). The Model’s key proponent, Professor Tim Noakes, describes exercise regulation as ‘using feed forward control in response to afferent feedback from different physiological systems, and the extent of skeletal muscle recruitment is controlled as part of a continuously altering pacing strategy with the sensation of fatigue being the conscious interpretation of these homoeostatic, central governor control mechanism’ (Noakes, 2011). It is important to highlight, at this stage, that one must be careful when considering what limits $\dot{V}O_{2max}$ and what limits endurance performance. These are two different questions, but have often been conflated in academic debates on limitations to human performance (Spurway et al., 2012).

There are areas of disagreement between the cardiovascular model and the CGM, notably on the factors limiting $\dot{V}O_{2max}$, with the CGM questioning the cardiovascular model assertion that cardiac output is the key limiter of $\dot{V}O_{2max}$. The CGM proposes that $\dot{V}O_{2max}$, during maximal exercise, is indeed a result preceded by a plateau in cardiac output but that this is secondary to a plateau in coronary blood flow (Figure 1.11) (Noakes, 1998). At this point, the CGM proponents argue, if exercise were to continue, the heart would be exposed to myocardial ischaemia, thus the plateau in cardiac output is a protective mechanism (Noakes, 1998) (Figure 1.12). Accordingly, the coronary flow plateau leads to a feed-forward neuronal mechanism, from the brain to the peripheral exercising muscles, reducing the recruitment of muscle fibres in order elicit a reduction in workload which, in turn, preserves the heart from experiencing myocardial ischaemia –a life threatening condition (Digenio et al., 1999). Furthermore, endurance training increases maximal coronary blood flow. Proponents of the CGM argue that it is this change which enables the training-

induced increases in cardiac output and muscle O₂ perfusion capability to occur (Noakes, 2000).

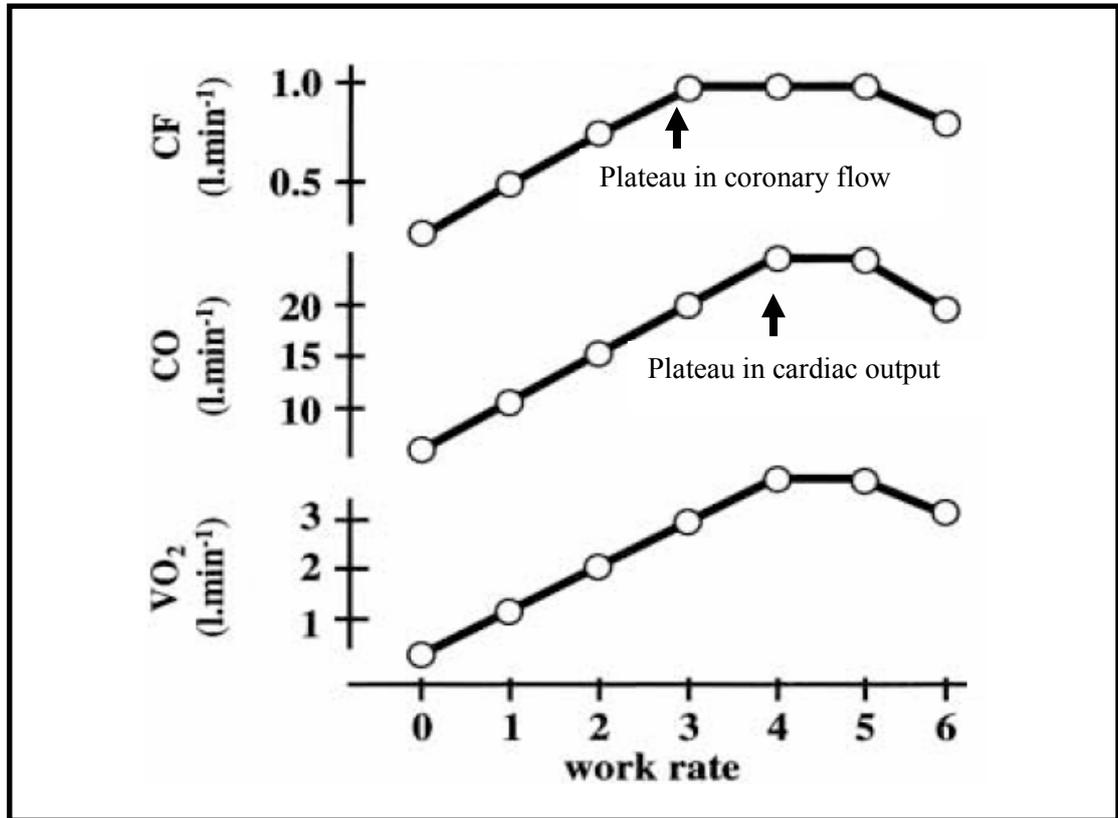


Figure 1.11: According to the CGM, the first variable to plateau during incremental exercise to exhaustion is coronary flow. Peak coronary flow (indicated with black arrow) then induces a plateau in cardiac output (CO) as a result of progressive myocardial ischaemia (Noakes, 1998).

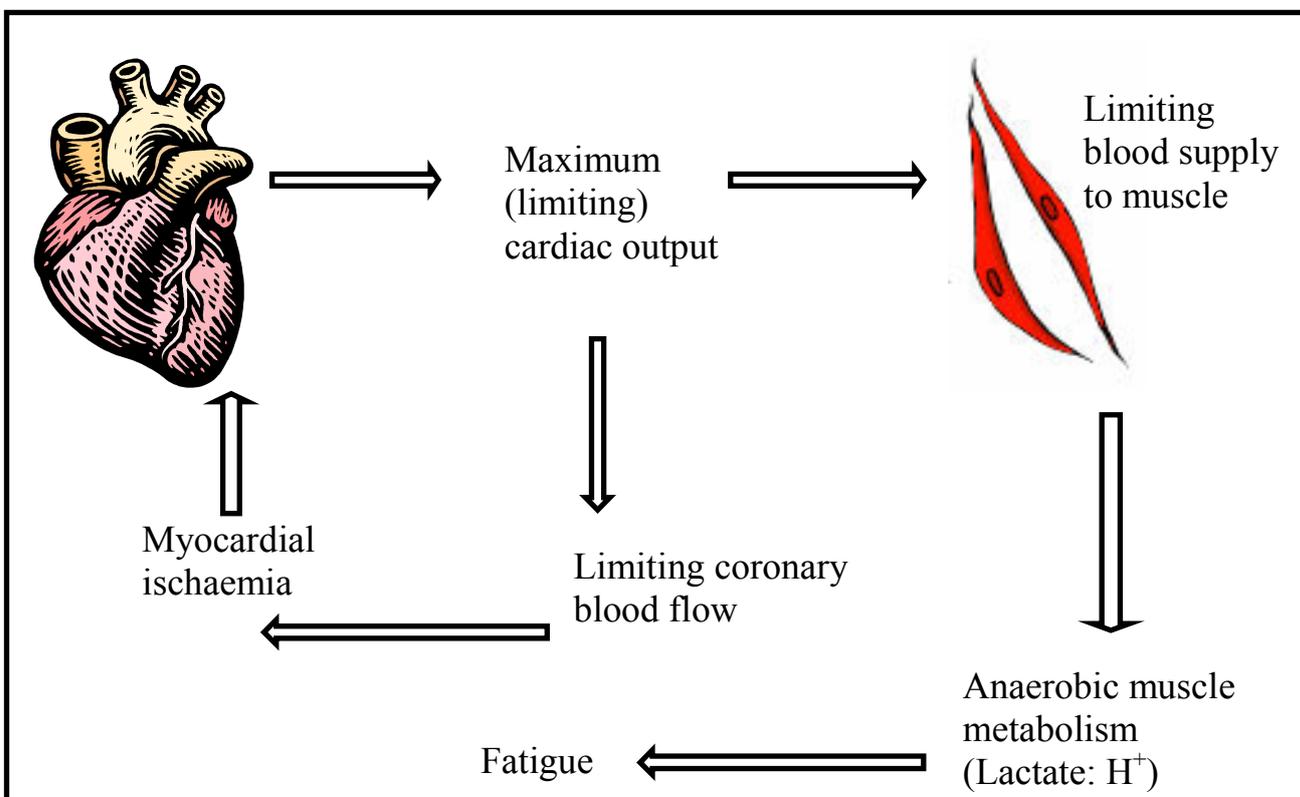


Figure 1.12: The weakness of the cardiovascular approach, according to the CGM model, is the failure to recognise that the attainment of maximal cardiac output has more serious consequences for the heart than skeletal muscles. Continuing to exercise at maximal cardiac output would cause myocardial ischaemia. The heart, unable to increase coronary flow (as dependent on an increase in cardiac output), thus limits the hearts ability to meet myocardial oxygen demand caused by increased work rates. Taken from (Noakes, 1998).

Beltrami and colleagues (Beltrami et al., 2012) have recently provided new evidence challenging the cardiovascular system's maximal capacity to transport O₂ in a study where subjects were randomised to conventional or reverse $\dot{V}O_{2\max}$ test protocols. The conventional group carried out several standard incremental $\dot{V}O_{2\max}$ tests, whilst the reverse group carried out: 1) an incremental uphill running test plus verification test, 2) a decremental running test where the same starting speed was used as during the verification, but was reduced progressively and 3) a final incremental test. The results revealed that $\dot{V}O_{2\max}$ values were 4.4% higher in the decremental tests when compared to the incremental test (63.9 ± 3.8 vs. 61.2 ± 4.8 ml·kg⁻¹·min⁻¹, respectively, $P = 0.004$). Reasons as to why $\dot{V}O_{2\max}$ was higher in the reverse group remains to be elucidated, but the authors concluded subjects must have terminated the conventional incremental test with a cardiorespiratory reserve (as indicated by a higher $\dot{V}O_{2\max}$ during the decremental protocol). Thus, termination of exercise was caused by factors other than those associated with the cardiovascular system.

When discussing CGM tenets with regards to how endurance performance is regulated (rather and not what limits $\dot{V}O_{2\max}$) recent studies have reported an interaction between the CNS and peripheral systems. The CGM proposes that peripheral fatigue influences the rate of central fatigue via neural feedback pathways from the working muscle to motor control areas of the CNS (Amann et al. 2006, 2007; Amann and Dempsey 2008; Dempsey et al. 2008). Furthermore, it has also been suggested that peripheral muscular fatigue is tightly regulated during exercise, and may act as a protective mechanism, where central motor control is adjusted by the performer in order to prevent peripheral muscular fatigue from rising above a 'critical threshold' (Amann and Dempsey 2008). It is argued that beyond this 'critical threshold', the level of sensory input (i.e. demand for work or training duration or training intensity) would not be tolerated (Gandevia, 2001). For example, Amman and Dempsey (Amann and Dempsey 2008) demonstrated that despite eliciting significant differences in quadriceps muscle fatigue immediately prior the start of self-paced 5 km cycling time trials, the level of peripheral muscle fatigue at the end of the trial was identical (as determined by quadriceps twitch force using electromyography).

The evidence supporting the interaction of central and peripheral components has been highlighted; however, studies have also focused solely on the brain and exercise regulation alone. One approach, to assess this, has been via the manipulation of brain temperature on exercise performance (Nybo, 2012). Experiments involving manipulations of brain temperature, independent of core body temperature, in exercising goats have shown

debilitating effects on motor performance under excessive brain hyperthermia. For example, Caputa and colleagues (Caputa et al., 1986) demonstrated this using goats where head and trunk temperatures were independently manipulated in order to assess the effect on a treadmill based exercise performance test. The authors reported that blood lactate increased concomitantly with rising brain and trunk temperatures; however, hyperthermic brain temperatures, alone, of 42 to 42.9°C reduced performance in 25% of cases independent of body core temperature (Caputa et al., 1986).

Studies have also carried out manipulations of CNS neurotransmitter levels (e.g. dopamine and serotonin) on exercise performance (Roelands and Meeusen, 2010). Concentrations of serotonin and dopamine, neurotransmitters involved in signal transduction between neurons in the brain, have been associated with several factors in humans including sleep-wakefulness, emotion and appetite (Meeusen et al., 2006). The identified associations with sleep-wakefulness (e.g. increased serotonin levels associated with fatigue) (Meeusen et al., 2001) have been investigated to see if manipulations can influence characteristics such as motivation during exercise, for example. Under normal ambient conditions, an interaction between serotonin/dopamine have been suggested to influence CNS fatigue, with low and high ratios reported to improve (by improving motivation) and decrease performance (by decreasing motivation and augmenting lethargy), respectively (Davis and Bailey, 1997). Exercise has also been reported to induce CNS increases in the concentrations of tryptophan (a precursor of serotonin) and the resulting increase in brain concentrations, via movement across the blood-brain barrier, have been reported to increase serotonin concentrations (Meeusen et al., 2006). This has been associated with debilitating effects on exercise performance (Meeusen et al., 2001). Overall however, studies manipulating neurotransmitter brain concentrations have been inconclusive with either no change (Strachan et al., 2004, Roelands et al., 2009, Strachan et al., 2005) or an improvement in performance (Bridge et al., 2003, Roelands et al., 2008, Watson et al., 2005) being reported.

In support of the CGM model, Tim Noakes has also recently cited 21 studies (Noakes, 2011a) to support the notion that exercise performance can be modified by varying ‘centrally acting performance modifiers’ including music (Lim et al., 2009, Barwood, 2009), placebos (Trojian and Beedie, 2008), self-belief (Micklewright et al., 2010), time deception (Morton, 2009), prior experience (Mauger et al., 2009) and mental fatigue (Marcora et al., 2009). Noakes argues, more directly against the ‘cardiovascularists’,

where he describes their ‘brainless’ (Noakes, 2011b) approach as being incapable of explaining how such interventions can influence the performance of the heart.

On closer inspection, not all of the cited studies are fully supportive of the CGM. For example, Lim and colleagues (Lim et al., 2009) assessed the effects of music introduced and removed during 10 km cycling time trial performance. Male participants completed a 10 km time trial under 3 conditions: 1) no music, 2) music played initially then removed and 3) music introduced for 5 to 10 km distance. Although differences in pacing approaches were identified (e.g. participants in group 3 where music was introduced produced faster starting speeds in the first 5 km), there was no significant difference in overall completion times ($P = 0.10$). In a similar example, Micklewright and colleagues (Micklewright et al., 2010) assessed the influence of self-belief on exercise performance. The authors reported higher than usual power outputs and speeds (i.e. a change in pacing strategy) during the first 5 km when cyclists were led to believe, via false feedback, that they were capable of producing a better performance. However, 20 km completion times did not statistically differ between groups given true or false feedback. Thus, whilst manipulations of central factors altered pacing, it did not influence overall performance.

There are a few examples, however, cited by Noakes (Noakes, 2011b) that do warrant further consideration. Morton and colleagues (Morton, 2009) assessed the influence of time deception on exercise performance. In this study, a subject feedback clock was manipulated to test the effect on maximal effort during endurance cycle ergometry exercise where male and female subjects cycled at 90 rpm at a work intensity of 300 W and 250 W, respectively, for as long as possible. In a double-blinded fashion, participants took part in 3 conditions: 1) clock normally calibrated, 2) clock calibrated 10% faster and 3) clock calibrated 10% slower. Results indicated a significant clock calibration effect on real endurance times where times were longer during the slower clock condition when compared to normal (by 18.3%) and faster (by 20.5%) conditions. Furthermore, in a separate example, Marcora and colleagues (Marcora et al., 2009) reported that time to exhaustion at 80% of peak power output was significantly reduced by ~15% after 90 minutes of a demanding cognitive task. Thus, there is some evidence to suggest that mental fatigue can limit tolerance to exercise via mechanism other than those associated with cardiovascular system.

Further, it must be noted that the mind or ‘psyche’ must also play a key role on performance outcome as the desired exercising output may only be attempted if the ‘mind’

is motivated to do so. Psychological approaches are often criticised due to methodologies lacking enough true validity (i.e. not very well controlled); nevertheless, the psychological/motivational model essentially describes that a conscious effort is required in order to sustain prolonged exercise (Davis and Bailey, 1997).

Finally, Marino (Marino, 2008), a firm believer of the CGM concedes that, “the evidence for the governor is scarce in comparison to that supporting the classic view” [in reference to the cardiovascular model], however, “evidence for the governor will come in due course”. Figure 1.13 illustrates how the CGM may interact with variables, as proposed by the traditional ‘central dogma’ approach, which determine endurance performance.

In summary, the standpoint of the CGM is one where the brain and CNS carefully regulates exercise in a manner not to compromise body homeostasis and, in particular, the potentially life-threatening event of myocardial ischaemia from occurring during strenuous exercise. The central governor achieves this via neural control where the reduction of muscle fibre recruitment leads to the sensation of fatigue, in turn, causing a reduction in work rate.

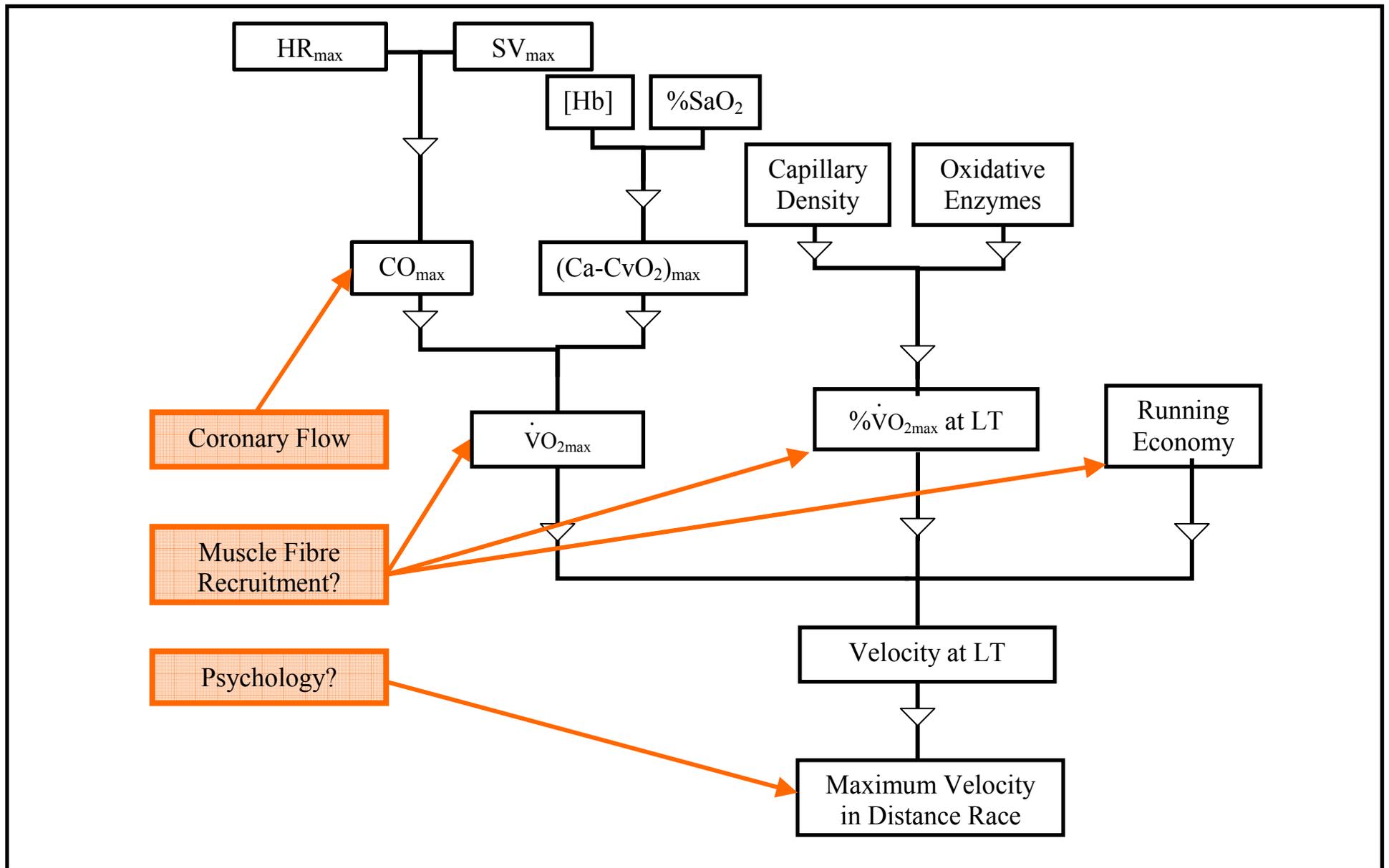


Figure 1.13: Summary of the major cardiovascular variables (black lines), with the addition of the Central Governor Model (CGM) (orange lines), related to $\dot{V}O_{2max}$ and the maximal velocity that can be maintained in a distance race. Adapted from (Bassett and Howley, 2000). HR_{max} = maximal heart rate; SV_{max} = maximal stroke volume; $[Hb]$ = haemoglobin concentration; $\%SaO_2$ = percent oxygen saturation; CO_{max} = maximal cardiac output; $(Ca-CvO_2)_{max}$ = maximal arterial-venous difference; $\dot{V}O_{2max}$ = maximal oxygen uptake; $\% \dot{V}O_{2max} \text{ at LT}$ = percentage of maximal oxygen uptake at lactate threshold.

1.9 Using Performance Enhancing Drugs to Understand Endurance Performance Limitations

It is vital that research continues in order to better understand what and how endurance performance is limited. One way of approaching this is by using performance enhancing drugs and investigating their potential to improve endurance performance. The World Anti-doping Agency (WADA) is responsible for preparing and publishing the 'Prohibited List', on an annual basis, and a simplified illustration is provided in Box 1.1. This list identifies substances and methods prohibited in sport and these are categorised based on their ergogenic effect or purpose. Different banned substances are prohibited in-competition or out-of-competition, or both, and can be banned for particular sports.

Box 1.1: Substances and Methods Prohibited In- and Out-Of-Competition

- Substances and Methods Prohibited In-and Out of Competition Only:

- S0: Non-Approved Substances
- S1: Anabolic Agents
- S2: Peptide Hormones, Growth Factors and Related Substances
- S3. Beta-2 Agonists
- S4. Hormone and Metabolic Modulators
- S5. Diuretics and Other Masking Agents
- M1. Enhancement of Oxygen Transfer
- M2. Chemical and Physical Manipulation
- M3. Gene Doping

- Substances and Methods Prohibited In-Competition Only:

- S6. Stimulants
- S7. Narcotics
- S8. Cannabinoids
- S9. Glucocorticosteroids

- Substances and Methods Prohibited In Particular Sports:

- P1. Alcohol
- P2. Beta-Blockers

Simplified version of the WADA Prohibited List (Full list available at:
<http://list.wada-ama.org/>)

There are reports of increasing use of performance enhancing drugs in both elite (Waddington et al., 2005, Pitsch, 2011, Weiss, 2012) and recreational level sport (Waddington et al., 2005, Weiss, 2012, Bojsen-Moller and Christiansen, 2010). This has been recently illustrated in the case of Lance Armstrong (United States Anti-doping Agency (USADA), 2012). As a result, recent years has seen a growing body of research assessing the performance and health implications of taking such substances (Bahrke et al., 1998, Dawson, 2001). Different classes of drugs are influencing exercise performance in different ways; from improving power by stimulating muscle hypertrophy for sprint events to increasing the carrying O₂ capacity of blood to enhance endurance performance for prolonged events.

For example, use of androgenic-anabolic steroids – synthetically produced derivatives of the male hormone testosterone – have been shown to induce strength gains up to 20% (Hartgens and Kuipers, 2004), mainly attributable to muscle hypertrophy (Hartgens and Kuipers, 2004); and stimulants, such as amphetamines and caffeine, which work on the CNS (Avois et al., 2006), have been shown to improve strength and endurance performance (Chandler and Blair, 1980, Doherty and Smith, 2004). However, it is drugs which increase O₂ delivery which have the largest potential to influence endurance performance.

One such drug that improves O₂ delivery and uptake is r-HuEpo (also more commonly known as ‘EPO’) where the performance benefits of increased [Hb] and haematocrit (Hct), often associated with living at altitude, can be replicated at sea-level with a series of r-HuEpo injections. The use of r-HuEpo falls under category M1 (Substances and Methods Prohibited In- and Out-Of-Competition) on the WADA Prohibited List. However, if used appropriately and only for research purposes, it can further our understanding on endurance performance capability.

1.9.1 Erythropoietin

Erythropoietin is an glycoprotein based hormone which, endogenously, is primarily secreted by the kidneys with a small amount also synthesised in the liver (Fisher, 2003). Endogenously present erythropoietin is the main regulator of erythropoiesis via the stimulation of pluripotent stem cell proliferation in bone marrow into reticulocytes (Ret) which enter the circulatory system and mature into red blood cells (RBC) (Fisher, 2003). The pharmaceutically engineered recombinant form of erythropoietin, r-HuEpo, was first developed in the 1980s and is now used in clinical applications; including the

administration to critically ill patients suffering from illnesses such as anaemia due to chronic renal failure (Macdougall et al., 1996) and cancer patients undergoing chemotherapy (Corwin, 2007). For example, in those anaemic patients who suffer from a significant reduction in the number of blood cells in circulation, r-HuEpo administration has been shown to increase red blood cell count. This effectively counter-acts the anaemic effect by mainly increasing total oxygen carrying capacity of blood and does not alter the oxygen saturation properties of haemoglobin (in relation to the oxy-haemoglobin dissociation curve as saturation is maintained, if at sea level, at ~96%). Research studies have also demonstrated that r-HuEpo administration, in healthy individuals, also increases Hct and therefore O₂ carrying capacity often improving the endurance performance of athletes as explained predominantly by the cardiovascular model (Audran et al., 1999, Ashenden et al., 2001, Ashenden et al., 2006, Lundby et al., 2007). As previously discussed (please see section 1.4.2.2), an enhancing blood O₂ carrying capacity simply increases the amount of O₂ that is made available to exercising muscle. Recombinant erythropoietin administration increases the O₂ carrying capacity of blood (in-turn increasing arterio-venous O₂ difference, as part of the Fick equation) and this is primarily caused by an increase in [Hb]. In addition to more oxy-haemoglobin complexes being formed, this enhancement of central O₂ delivery to peripheral musculature is also coupled with an increase in cardiac output (due to potential increases in blood volume) (please see section 1.4.2.1). As previously discussed, such enhancements (e.g. total O₂ carrying capacity of blood determined by total haemoglobin mass measures) are strongly correlated with enhancement in performance measures such as $\dot{V}O_{2\max}$, for example (Figure 1.5).

The regulation of erythropoietin, in humans, has been reported to involve a feed-back mechanism based on the level of blood oxygenation involving erythropoietin transcription factors known as hypoxia-inducible factors (Jelkmann, 2007) which bind to erythropoietin receptors located on surfaces including red blood cells, bone marrow cells and central/peripheral nerve cells (Reid and Mohandas, 2004). Interestingly, r-HuEpo administration has also been reported as having beneficial effects on patients suffering from specific neurological diseases such as schizophrenia (Ehrenreich et al., 2004) and cerebral malaria (Core et al., 2011). Currently, the evidence indicates that r-HuEpo use can improve limitations associated with the cardiovascular system; however, some evidence has also suggested r-HuEpo administration to have potential effects on the CNS.

Table 1.2 shows studies, to date, which have assessed r-HuEpo administration on exercise performance. The administration of r-HuEpo for 4 to 15 weeks has been widely reported

to increase [Hb] and Hct by up to approximately 10-20% with an associated increases in $\dot{V}O_{2\max}$ by approximately 7 to 13% (Russell et al., 2002b, Berglund and Ekblom, 1991, Parisotto et al., 2000b, Lundby et al., 2007), (Birkeland et al., 2000, Audran et al., 1999, Thomsen et al., 2007, Lundby et al., 2007, Robach et al., 2008). Recombinant erythropoietin administration has been reported not to change cardiac output, blood pressure, heart rate, ventilation and blood lactate concentrations during maximal running exercise (Berglund and Ekblom, 1991). The magnitude of change in [Hb], Hct and $\dot{V}O_{2\max}$ have been reported to be similar to red blood cell reinfusion (Ekblom and Berglund, 1991).

Table 1.2 Previous literature assessing r-HuEpo administration for haematological measures and $\dot{V}O_{2max}$

Study	Participants	r-HuEpo Dose (IU/Kg/Week)	Treatment Period (Weeks)	Hct (% Δ)	[Hb] (% Δ)	tHb (% Δ)	$\dot{V}O_{2max}$ (% Δ)
Ekblom and Berglund (1991)	15 Healthy	20-40	6	11.7	11.2	-	8.0
Audran et al. (1999)	9 Trained	350	4	11.5	9.3	-	9.3
Birkeland et al. (2000)	10 Well-trained	207	4	19.0	14.5	-	7.1
Parisotto et al. (2000)	8 Trained	150	4	10.1	10.7	12	6.9
Russel et al. (2002)	9 Recreational	94	8	14.3	-	-	7.0
Ashenden et al. (2006)	2 Well-trained	348	5	-	18.1	-	-
Thomsen et al. (2007)	16 Healthy	61	13	10.8	11.1	-	12.6
Lundby et al. (2008)	8 Healthy	120	4	12.0	11.6	-	7.6
Robach et al. (2008)	8 Healthy	60-181	15	7.0	11.1	-	7.2

r-HuEpo: Recombinant Human Erythropoietin; Hct: Haematocrit; Hb: Haemoglobin; tHb: Total Haemoglobin Mass; $\dot{V}O_{2max}$: Maximal Oxygen Uptake.

In addition to increasing red cell mass, administration of r-HuEpo also reduces plasma volume, magnifying the effects on [Hb] (Lundby et al., 2007) (Lundby et al., 2008a) (Olsen et al., 2010). This is reported to occur due to a down-regulation of proximal renal tubular reabsorption in the kidneys which consequently causes a decrease in glomerular filtration rate (Olsen et al., 2010).

Stopping r-HuEpo administration leads to the decrease in [Hb]. This may be mediated by the apoptosis of r-HuEpo induced newly formed red cells (Chang et al., 2009). However, clear evidence indicates that the initial reduction in [Hb], post r-HuEpo administration is as a result of a normalisation of plasma volume which, in turn, reduces [Hb] (Lundby et al., 2007). This has been supported by direct measures which show that red cell mass is maintained for ~2 weeks following the cessation of r-HuEpo despite the drop in [Hb] (Olsen et al., 2010).

The effects of r-HuEpo administration on skeletal muscle have also been assessed. Micro RNA levels of several muscular components remained largely unchanged but with minor changes in myoglobin, transferrin receptor mRNA level and MRF4 reported (Lundby et al., 2008b). As a result, r-HuEpo may indeed directly influence peripheral musculature; however, the physiological importance of this effect is presently thought to be very limited with no changes in muscle composition including capillary density, fibre type distribution and muscle fibre cross-sectional area (Lundby et al., 2008b).

More recent studies have also attempted to assess the effect of r-HuEpo on central mechanisms (i.e. brain and CNS). Recently, Rasmussen and colleagues (Rasmussen et al., 2010) assessed 15 healthy young men who received 3 days of high doses of r-HuEpo in a double-blinded placebo controlled study design. Exercise capacity, transcranial ultrasonography-derived middle cerebral artery blood velocity and arterial-internal jugular venous concentration differences of glucose and lactate were assessed. The authors reported an increase in cerebrospinal fluid concentrations of EPO as well as increased glucose and lactate concentrations ($P < 0.05$). However, this had no impact on cognition or voluntary muscle fibre recruitment. However, total work capacity increased by ~15% ($P < 0.01$). The authors concluded that the improvement in exercise capacity was solely explained by mechanisms associated with the cardiovascular system; primarily an improved O_2 carrying capacity of blood and, therefore, improved O_2 delivery to exercising muscles.

Interestingly, non-exercise studies have shown the potential for r-HuEpo mediated central effects. For example, Miskowiak and colleagues (Miskowiak et al., 2008a) assessed potential r-HuEpo mediated central effects on cognitive function after high doses (40000 IU) of r-HuEpo administration. The authors reported improved neural processing and cognitive function, in healthy subjects, during a verbal fluency test as a result of r-HuEpo administration. Similar improvements in neural processing have been reported in patients suffering from neuropsychiatric diseases (Miskowiak et al., 2008b) and patients with schizophrenia have also shown improvements in cognitive function after similar doses of r-HuEpo (Ehrenreich et al., 2004).

As a result of an r-HuEpo administration period, improvements in exercise capacity are well documented (Audran et al., 1999, Ashenden et al., 2001, Ashenden et al., 2006, Lundby et al., 2007). The majority of studies have assessed the effects by determining the impacts on $\dot{V}O_{2\max}$ and these are indicated in Table 1.2; however, some studies have also demonstrated the potential of r-HuEpo to improve exercise capacity at submaximal intensities. For example, Thomsen and colleagues (Thomsen et al., 2007) assessed 16 male volunteers who carried out time to exhaustion cycle tests where increase time to exhaustion at 80% of $\dot{V}O_{2\max}$. In this study, 5000 IU r-HuEpo was administered every two days for 14 days followed by a weekly dose of 5000 IU for 10 weeks. The authors reported that r-HuEpo administration improved time to exhaustion at 80% of $\dot{V}O_{2\max}$ by 54 and 54.3% after 4 and 11 weeks of r-HuEpo administration, respectively. This change was greater than the improvement in $\dot{V}O_{2\max}$ (12.6 and 11.6%, at week 4 and 11; $P < 0.05$, respectively).

To summarise, the administration of r-HuEpo on endurance exercise performance strongly indicates the primary effect as being one on the cardiovascular system by improving O_2 carrying capacity of blood and, therefore, O_2 delivery to exercising muscles. However, as evidence out with the exercise field suggests a potential central effect of r-HuEpo (Miskowiak et al., 2008a). Therefore, it is possible that the drug may also influence exercise performance by central mechanism. Figure 1.14 illustrates proven and potential r-HuEpo influences on human endurance performance. Further, as part of central mechanisms, the administration of r-HuEpo may also work via a placebo effect. This will be discussed in the following section.

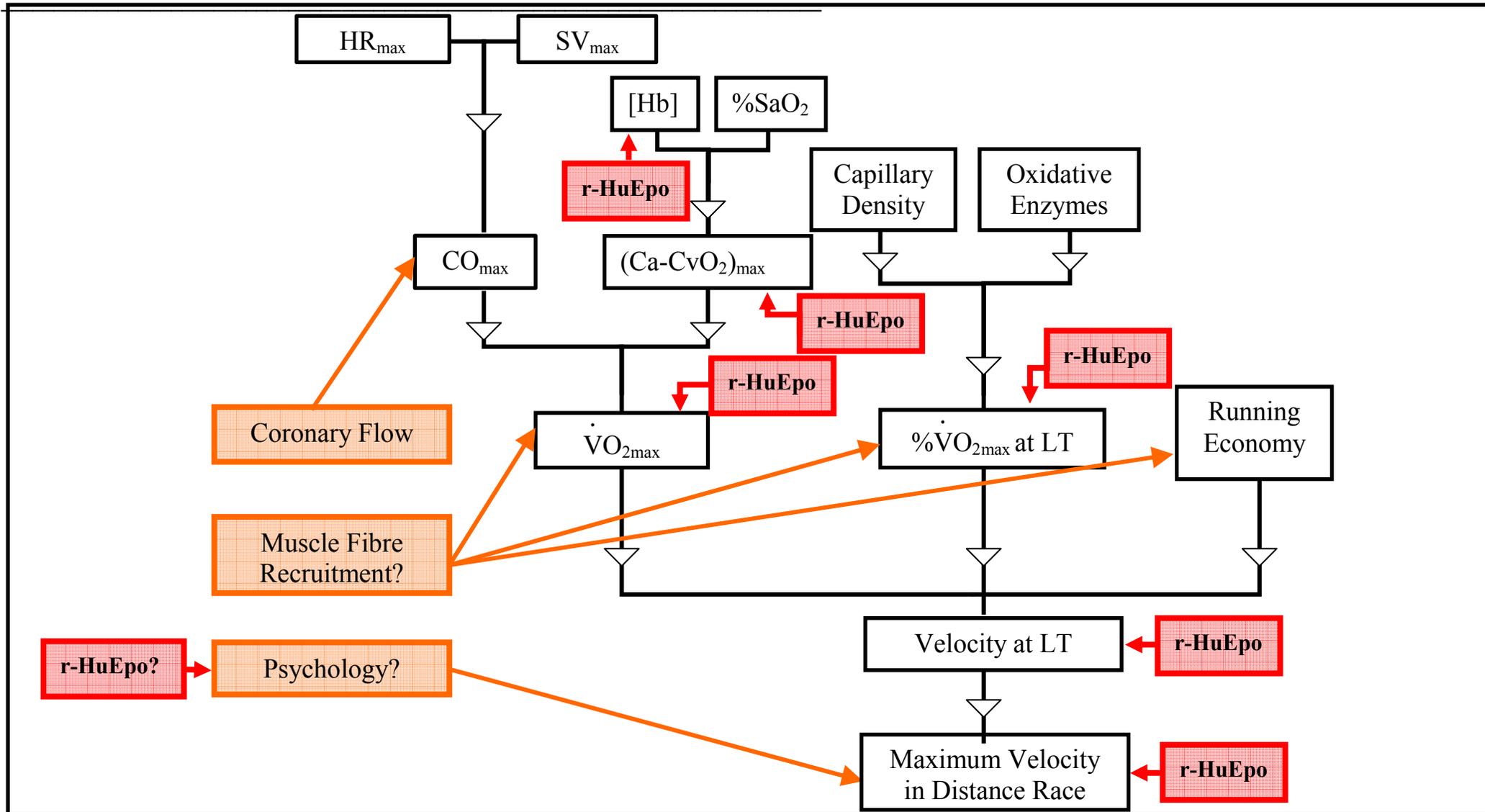


Figure 1.14: Summary of the major cardiovascular variables (black lines), with the addition of the Central Governor Model (CGM) (orange lines), related to $\dot{V}O_{2max}$ and the maximal velocity that can be maintained in distance races. The documented (red arrows) and potential effects (red arrows with question mark) of r-HuEpo are also included. HR_{max} = maximal heart rate; SV_{max} = maximal stroke volume; $[Hb]$ = haemoglobin concentration; $\%SaO_2$ = percent oxygen saturation; CO_{max} = maximal cardiac output; $(Ca-CvO_2)_{max}$ = maximal arterial-venous difference; $\dot{V}O_{2max}$ = maximal oxygen uptake; $\% \dot{V}O_{2max}$ at LT = percentage of maximal oxygen uptake at lactate threshold.

1.10 Using the Placebo Effect to Understand Endurance Performance Limitations

1.10.1 The Placebo Effect in Medicine and Sports Performance

The 'placebo' (Latin for *'I shall please'*) effect is one where a desired outcome effect is achieved via the subscription of an inert or ineffective treatment (Gensini et al., 2005). The placebo effect dates back centuries and is widely acknowledged as a key factor in medical research (Hrobjartsson and Gotzsche, 2003, Hrobjartsson and Norup, 2003). Placebos can be administered in varying forms from inactive drugs, sham surgery and other procedures involving sham protocols or the provision of false information (Lanotte et al., 2005). As a consequence, its effect has been controlled for in clinical trials for over 50 years (Beedie and Foad, 2009).

The evidence also suggests that the performance enhancing effect of any drug could partly be due to a placebo effect. This effect has been recognised in the context of sports medicine where researchers have reported placebo mediated changes of similar magnitude to those found in 'real' interventions (Price et al., 2008, Beedie and Foad, 2009). Furthermore, in an electronic survey, 97% of respondents believed that the placebo effect can positively influence sporting performance (Beedie, 2007b).

Clearly, the benefits of placebo cannot be explained in the context of the cardiovascular model and must be centrally mediated. The manipulations of belief systems, underpinned by expectations and conditioning, is often what placebo effects are reliant on. In other words the belief of change can result in real or tangible change (Kirsch, 2004). For example, a study examining the effect of bedside manner of doctors in interactions with patients found that the effectiveness of a placebo treatment increased from 44% to 62% when an extra effort was made by the doctor to be warm, attentive and confident, as opposed to being neutral (Kaptchuk et al., 2008). Furthermore, the brain and spinal cord, either subconsciously or consciously, have been proposed to regulate a response to override an undesired effect (e.g. pain) or to stimulate an enhancement effect of a process (Benedetti et al., 2005, Wager and Nitschke, 2005, Eippert et al., 2009). There is evidence of a direct cerebral effect of placebo in clinical studies. For example, Petrovic and colleagues (Petrovic et al., 2002) examined the effect of no intervention, placebo injection or analgesic injection on brain responses to a brief un-harmful experience. Positron emission tomography scans indicated activation of the rostral anterior cingulate cortex for

both the placebo and analgesic treatment, although the injected analgesic did provide more pain relief (Petrovic et al., 2002).

While there is a substantial body of research of the placebo in clinical settings, the study of the effects of placebo on sporting performance is still in its infancy. Furthermore, even less is understood about the placebo effect in real competition scenarios, as opposed to tests confined to the laboratory. Laboratory studies have reported positive effects associated with a placebo intervention on the enhancement of endurance, sprint and strength performance (Ariel and Saville, 1972, Maganaris et al., 2000, Foster et al., 2004b, Beedie and Foad, 2009). Studies evaluating the effect of placebo on endurance performance are shown in Table 1.3.

Studies have shown that an orally administered placebo can improve endurance performance by 1.1% to 8% (Clark et al., 2000, Foster et al., 2004b, Beedie et al., 2006, McClung and Collins, 2007, Foad et al., 2008, Wright et al., 2009) and Table 1.3 provides a summary of studies that have been carried out to assess the effects of a placebo intervention of endurance exercise performance.

Table 1.3. Summary of studies assessing the placebo effect on endurance exercise performance

Study	Participants	Study Design	Performance Measure	Intervention Informed	Intervention Received	% Change
Clark et al. (2000)	43 Sub-elite cyclists	Balanced repeated measures, 6 cell Latin square design. After baseline time trial (water), subjects split into 6 treatment groups in total: two subgroups (given carbohydrate, given placebo) for each of three main groups (told carbohydrate, told placebo, not told)	Mean power over 40 km cycling power distance	CHO	Placebo	7.8%
					CHO	1.5%
				Placebo	Placebo	-0.3
					CHO	0.2
				Not told (i.e. 50/50 chance of receiving CHO or placebo)	Placebo	-1.0
					CHO	0.1

Foster et al. (2004)	16 Sub-elite runners	Within subjects design. Subjects performed random- ordered 5 km time trials after consuming either water (and told water) or water falsely purporting to contain the ergogenic aid (and told this).	5 km running time	New ergogenic aid	Placebo	1.1
Porcari et al. (2006)	32 Sub-elite runners	Between-subjects design. 1 x habituation and 2 x counterbalanced experimental where participants informed either water (and given water) or super-oxygenated water (and given water)	5 km running time	Super-oxygenated water	Placebo	8.0
Beedie et al. (2006)	6 Sub-elite cyclists	Within-subjects design. 1 x habituation, 1 x baseline and 3 experimental trials	10 km cycling power	0mg/kg caffeine	Placebo	-1.4
				4.5 mg/kg caffeine	Placebo	1.3
				9.0 mg/kg caffeine	Placebo	3.1

McClung and Collins (2007)	16 Sub-elite endurance athletes	Within-subjects, Latin square/balanced placebo design (4-cell). 5 x 1000-m time trials (1 x habituation and 1 x trial per counterbalanced condition)	1000 m running time	Sodium bicarbonate	Sodium bicarbonate	1.7
				Sodium bicarbonate	Placebo	1.5
				Placebo	Sodium bicarbonate	-0.3
				Placebo	Placebo	0.0
Foad et al. (2008)	14 Sub-elite cyclists	Within-subjects, Latin square/balanced placebo design (4-cell). Subjects performed 2 x 40 km time trials, before and after, in each of four experimental conditions	40 km cycling power	Caffeine	Caffeine	2.3
				Caffeine	Placebo	0.1
				Placebo	Caffeine	2.9
				Placebo	No treatment	-1.9
Wright et al. (2009) #	32 runners	Within-subjects design. 1 x control and 1 x placebo where participants informed either	5 km running time	Super-oxygenated water	Placebo	6.5

water (and given water) or
super-oxygenated water (and
given water)

Subject descriptions have been taken from original papers. ^ Magnitude-based inference. CHO = carbohydrates. # Part 1 of a 3 part study.

In one example, Foster and colleagues (Foster et al., 2004b) assessed the effects of a ‘new’ ergogenic aid on 5 km running performance in sixteen well-trained runners ($\dot{V}O_{2\text{peak}}$ 58 ± 8 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). The authors reported a competitively meaningful improvement in 5 km time trial performance, although this was not statistically significant, after participants had ingested the ergogenic aid when compared to control (21.54 vs 21.40 minutes, respectively; $P = 0.11$). Interestingly, no differences were reported between conditions in RPE (8.2 ± 1.0 vs 8.4 ± 1.2), peak heart rate (177 ± 5 vs 177 ± 6 $\text{b}\cdot\text{min}^{-1}$) or blood lactate (12.2 ± 3.2 vs 11.4 ± 2.2 $\text{mmol}\cdot\text{l}^{-1}$). In another example, Porcari and colleagues (Porcari et al., 2006) assessed the effect of a placebo intervention on 5 km running performance, but in this instance the placebo was ‘super-oxygenated’ water and the control group was ‘control water’. Thirty-two experienced runners ($\dot{V}O_{2\text{max}}$ 60.8 ± 8.2 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) completed three 5 km time trials on an indoor 200 m track (1 x habituation and 2 x counterbalanced experimental). Significant differences were reported between the two conditions for 5 km total completion time (control water: 21.04 ± 3.34 minutes; placebo water: 19.41 ± 2.32 minutes; $P < 0.05$). Similarly, as with the previous study however, no significant differences were found in HR, RPE and blood lactate ($P > 0.05$). Interestingly, in this study the largest improvements reported after placebo ingestion were the in less accomplished runners in comparison to the more experienced runners (142 second vs. 28 second improvement, for less accomplished and more accomplished runners, respectively).

Caffeine supplementation has been a popular means in attempt to improve performance, as previously discussed. Thus, several studies have assessed the potential of a placebo effect in caffeine’s capability to influence endurance performance. A study by Foad and colleagues (Foad et al., 2008) assessed the effects of caffeine and placebo interventions on 40 km cycling performance in fourteen well-trained competitive cyclists. The authors found the effects of caffeine not significantly different from placebo on endurance performance. The study by Foad assessed the interaction between performance enhancing beliefs and expectations in relation to the administration of caffeine or a placebo. Performance did not improve when subjects were not given caffeine. However, performance was enhanced similarly when subjects were given caffeine irrespective of whether or not they believed caffeine had been administered. Interestingly, the authors reported a nocebo effect when participants were informed that no caffeine would be administered and caffeine was not administered (Table 1.3). The authors highlighted the presence of key interactions between pharmacology and psychology which they propose warrants further investigation.

Thus a number of studies have shown that improvements in endurance performance can be elicited by the use of various placebo interventions purporting to be established or novel ergogenic aids. However, all the above mentioned studies administered the placebo orally. Studies from clinical medicine have shown that the route of placebo administration can influence the magnitude of the placebo effect. Thus, a limitation in the present literature is that no study has assessed the effects of administering a placebo, by other routes, on endurance sports performance.

1.10.2 The Placebo Effect and Route of Delivery

A key feature of r-HuEpo administration is that it is given by injection. Evidence from clinical medicine indicates that the route of delivery is a key mediator of the placebo effect size, with placebos administered by injection inducing a larger effect than placebos administered orally (Zhang et al., 2008). For example, Zhang and colleagues (Zhang et al., 2008) carried out a meta-analysis on the studies examining determinants of the magnitude of the placebo effect in the treatment of osteoarthritis. One hundred and eighty eight trials with 193 placebo groups (16364 patients) and 14 untreated control groups (1167 patients) were included in the analysis. The trials involved varying treatment strategies from non-pharmacological to pharmacological to surgical interventions. Overall, the authors reported a significant placebo effect for pain relief when compared to untreated controls (placebo effect size: 0.51, 95% CI 0.46 to 0.55 vs. untreated control effect size: 0.03, 95% CI -0.13 to 0.18; $P = 0.020$). Furthermore, the effect size for placebos administered by injection/needles (acupuncture: 0.71, 95% 0.53 to 0.90, intra-articular hyaluronan (0.73, 95% CI 0.56 to 0.91) was significantly greater than those orally administered. Other studies have reported similar findings, for example, Kaptchuk and colleagues (Kaptchuk et al., 2006) reported that sham acupuncture (i.e. a needle placebo) was superior to a tablet, administered orally, in patients with arm pain.

Furthermore, Benedetti and colleagues (Benedetti, 2009) have highlighted the importance of the “open-hidden” paradigm. In the “open” approach a patient receives a treatment in the normal clinical “open” manner where the patient is fully aware of the treatment. Conversely, in a “hidden” manner, the patient is unaware that the treatment is being administered. The “open” administration approach has been reported as being at least 50% more effective than “hidden” for pain relief (Benedetti et al., 2004) and this is speculated to be due to higher therapeutic expectations when administration is observed. This may play a role in the performance benefits observed with the administration of r-HuEpo.

The application of placebo injections, in the context of sports performance, has only been assessed in one study which assessed the effects of a placebo administered injection on pain tolerance (Benedetti et al., 2007). Benedetti and colleagues assessed the effects of placebo mediated analgesia via use of morphine on a pain endurance test designed to stimulate sport competition (forearm spring exercise to maximum capacity whilst restricted with a tourniquet wrapped around the forearm). Forty recreationally active male participants took part in the study where morphine or placebo (saline or naloxone) was administered in a randomised and double-blinded fashion. Participants were split in two 4 groups: a) no pharmacological substance during training and received no treatment during competition, b) no pharmacological substance given in training and placebo given 1 hour prior competition, c) trained with morphine and given placebo morphine 1 hour prior competition and d) trained with morphine and received placebo yet told morphine was administered, but naloxone (i.e. an opiate antagonist that abolishes the morphine preconditioning effect) was actually administered prior competition. The authors reported the greatest change in tolerance times, on the day of competition, in group C (baseline: 13.8 ± 2.7 vs. competition: 20.8 ± 3.3 minutes; $P < 0.001$) in comparison to group B (baseline: 14.2 ± 3.2 vs. competition: 16.7 ± 2.5 minutes; $P < 0.01$), group A (baseline: 14.6 ± 2.9 vs. competition: 15.7 ± 1.7 minutes; $P > 0.05$) and group D (baseline: 14.5 ± 3.2 vs. competition: 15.4 ± 2.9 minutes; $P > 0.05$).

Thus, as only one study has incorporated an injection/needle element to understand the impacts of the placebo in 'sporting' context, there is a clear need to assess the route of which r-HuEpo is administration and its impact on endurance performance. Figure 1.15 illustrates how an injected r-HuEpo placebo may influence variables associated with endurance performance.

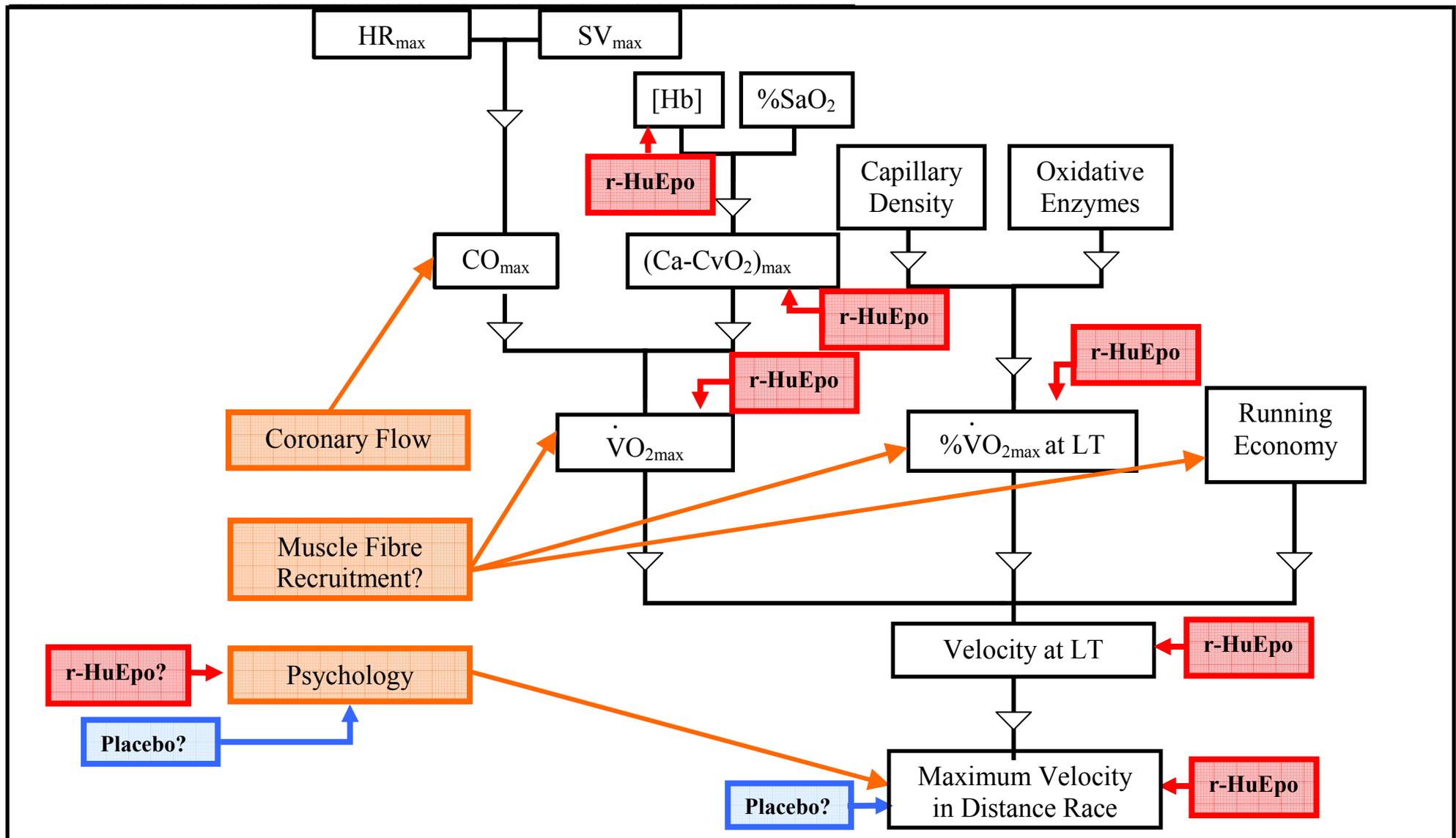


Figure 1.15: Summary of the major cardiovascular variables (black lines) related to $\dot{V}O_{2max}$ and the maximal velocity that can be maintained in distance races with the addition of the Central Governor Model (CGM) (orange lines), the known (red arrows) and potential (red arrows with question mark) pathways that r-HuEpo and an r-HuEpo placebo injection (blow arrows with question mark) may influence. HR_{max} = maximal heart rate; SV_{max} = maximal stroke volume; $[Hb]$ = haemoglobin concentration; $\%SaO_2$ = percent oxygen saturation; CO_{max} = maximal cardiac output; $(Ca-CvO_2)_{max}$ = maximal arterial-venous difference; $\dot{V}O_{2max}$ = maximal oxygen uptake; $\% \dot{V}O_{2max} \text{ at LT}$ = percentage of maximal oxygen uptake at lactate threshold. Adapted from (Bassett and Howley, 2000).

Given the clinical significance of injected/needle mediated placebo effects, the fact that r-HuEpo is administered by this route and that no study has assessed the implication of this in the context of endurance sporting performance there is a clear need for such a study to be undertaken.

Furthermore, it is vital that such studies assess any such intervention in an environment truly representative of a real-life sporting scenario. This would ensure that any potential findings are ecologically valid. For example, time to exhaustion protocols are commonly employed to investigate endurance performance; however it is generally acknowledged that such tests can often have a large coefficient of variation as compared to alternative testing methodologies like time trials (Jeukendrup et al., 1996). Thus, this can often be a problem when attempting to deduce performance enhancing effects of an ergogenic aid. In addition, laboratory tests are not realistic reflections of the experience of a real life performance situation (e.g. a race). No study (to the best of the author's knowledge), has assessed the effect of an r-HuEpo placebo administration phase on time trial performance in the field and in a competitive environment. Thus, field-based performance assessments are vital in order to truly understand, and further validate, the reported performance enhancing capabilities of r-HuEpo administration. Thus, the importance of ecologically valid assessments for meaningful measures will be discussed in the following section.

1.11 Measuring True Performance

Many tests evaluating the effects of interventions on performance measures are performed under laboratory conditions. However, studies have shown that care must be taken when extrapolating results obtained in a laboratory setting to the field, as the former may not provide an accurate representation of 'real life' events. Thus, in order to elicit a truly valid human sporting performance response exercise protocols must replicate, as closely as possible, real life conditions so that a true performance response is measured.

Studies have assessed various factors between laboratory and field-based conditions and their relative impacts on various performance outcome measures. Studies have often questioned the transferability of laboratory results to the field. For example, Kunduracioglu and colleagues (Kunduracioglu et al., 2007) reported a significantly higher running velocity during the employed field-based test in comparison to a laboratory test when running at a fixed blood lactate of 4 mmol/l (i.e. OBLA) ($P < 0.01$). Similarly, Di Michele and colleagues (Di Michele et al., 2009) compared blood lactate concentrations

during running tests carried out on a treadmill, natural grass and synthetic turf. The authors reported elevated blood lactate concentrations, by a minimum of $0.6 \text{ mmol}\cdot\text{l}^{-1}$ for a given speed (in this case at 8, 10, 12 and $14 \text{ km}\cdot\text{h}^{-1}$), on the synthetic surface versus both natural grass and treadmill conditions ($P < 0.05$) and running speed at $4 \text{ mmol}\cdot\text{l}^{-1}$ (i.e. OBLA) was significantly lower ($P < 0.05$) during running on the synthetic surface ($13.1 \pm 1.1 \text{ km}\cdot\text{h}^{-1}$) than when compared to natural grass ($13.9 \pm 1.2 \text{ km}\cdot\text{h}^{-1}$) and treadmill ($14.4 \pm 1.3 \text{ km}\cdot\text{h}^{-1}$). The authors concluded that the results are most likely explained by altered biomechanics during running upon the various surfaces and as a result stressed the importance of testing soccer players on specific services used during training.

Interestingly, differences in physiological characteristics between laboratory and field-based assessments may offer some explanation as to the reasons behind these findings. For example, Palatini (Palatini, 1997) assessed the effects of exercise on haemodynamics during athletic based field activities and laboratory tests and reported that blood pressure variations, recorded in the field, were not accurately reproduced in the laboratory tests where the latter often yielded lower values. This, therefore, raises further questions as to cardiovascular function during laboratory and field-based assessment protocols where the latter may indeed be able to induce a truer fatigue response.

Another indication that highlights limitations in laboratory assessment approaches is the inability of such assessments to differentiate between calibres of athlete. For example, in a recent study, Wells and colleagues (Wells et al., 2012) reported that sports-specific fitness testing was effective for differentiating between professional and amateur soccer players whereas $\dot{V}O_{2\text{max}}$ and $\dot{V}O_2$ kinetics were not. Thirty-six men (18 professional and 18 amateur) participated in the study in which both groups were similar for $\dot{V}O_{2\text{max}}$ (professional $56.5 \pm 2.9 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; amateur $55.7 \pm 3.5 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; $P = 0.484$). However, amateurs were out-performed by the professionals in both the employed field-based yo-yo intermittent recovery (professional $966 \pm 153 \text{ m}$; amateur $840 \pm 156 \text{ m}$; $P = 0.034$) and repeated sprint tests (best time, professional $6.46 \pm 0.27 \text{ s}$; amateur $6.84 \pm 0.24 \text{ s}$; $P = 0.012$).

As a result of the potential limitations associated with the ability of laboratory based assessment protocols to elicit a true fatigue and/or exercise performance response, it is essential that exercise assessment protocols include a field-based running assessment protocol. In addition, the effect of competition (i.e. in a form of a race with runners

competing against one another) is also an important consideration for the same reasons and will now be discussed.

Several studies have assessed the effect of competition on exercise performance-related variables (Pierce et al., 1976, Matlina et al., 1979, Booth et al., 1989, Filaire et al., 2001) where numerous approaches have been employed to compare and contrast potential differences in physiological and psychological variables between the laboratory, training and competition environments. Such studies also provide vital insights into the transferability of data, collected under non-competitive scenarios, to competitive situations.

The assessment of catecholamine levels (e.g. adrenaline, noradrenaline and dopamine), which are released by the adrenal glands as part of the fight-or-flight response (Jansen et al., 1995), has been one approach to assess potential physiological differences in exercising individuals in differing environments (Passelergue and Lac, 1999, Gonzalez-Bono et al., 1999, Suay et al., 1999, Elloumi et al., 2003, Filaire et al., 2009). For example, studies have reported significantly higher levels of adrenaline during competition when compared to training in various sports ($P < 0.01$) (i.e. in intercollegiate basketball and track and field teams) (Pierce et al., 1976). In this particular study, the track and field athletes who were attempting qualification for an international team also exhibited significantly higher ($P < 0.01$) adrenaline levels when compared to other members in the team. The assessment of testosterone has also revealed higher levels immediately prior competitive matches and higher levels demonstrated the most improvement in positive mood (Booth et al., 1989) – a potential influencing factor on performance.

Interestingly, more recent studies have suggested the importance of combining such physiological measures with psychological measures. For example, Filaire and colleagues (Filaire et al., 2001) suggested that the concomitant use of salivary cortisol (a measure of 'stress') and measures of anxiety may provide more meaningful results. Filaire and colleagues (Filaire et al., 2001) reported, in twelve male judo competitors prior judo competitions at regional and interregional levels, higher cortisol levels (by ~2.5 fold) when compared to resting non-competition values ($P < 0.05$) in both groups. In addition, cognitive and somatic anxiety levels were higher in interregional championships compared to regional championships whereas self-confidence was significantly lower in the latter. Thus, there is evidence to suggest that performance outcome, as a result of underlying physiological and psychological factors, may indeed vary depending on the testing

environment and, although limited research has been carried out in this area, studies have shown the effects on performance outcome.

Wilmore and colleagues (Wilmore, 1968), for example, assessed 22 males during total work capacity cycle ergometry exercise under three conditions in the order: 1) control conditions with no competition (i.e. cycling alone), 2) with competition introduced and 3) a repeat control condition with no competition (i.e. cycling alone). Competition was introduced by pairing participants based on their control values for work output. The authors reported greater work output measures and total cycling time when competition was introduced then when compared to both control conditions (work output control 1: 8760 ± 318 kJ; competition: 11136 ± 4199 kJ; control 2: 9798 ± 3484 kJ; $P < 0.05$ and total cycling time control 1: 332 ± 160 seconds; competition: 457 ± 233 seconds; control 2: 379 ± 180 seconds; $P < 0.05$). Interestingly, however, no differences ($P > 0.05$) in physiological measures, \dot{V}_E , $\dot{V}O_{2\max}$ and HR, were identified between conditions. The authors suggested that the improvements reported, with competition introduced, were likely due to improved anaerobic metabolism and may also involve an enhanced tolerance to exercise induced pain. Unfortunately, the authors did not assess circulating metabolites (e.g. lactate concentrations) or markers for pain to support their suggestions. Nevertheless, competition significantly influenced exercise performance.

More recently, Corbett and colleagues (Corbett et al., 2012) assessed the influence of believing that one is competing against another individual, in a head-to-head manner, on performance during a 2000 metre cycling time trial. The authors reported that participants completed the 2000 metre cycling time trial faster when they believed they were competing, in an head-to-head manner, than when completing the time trial alone (familiarisation time: 187.7 ± 8 seconds; time trial alone: 188.3 ± 9.5 seconds; and head-to-head competition: 184.6 ± 6.2 seconds). The head-to-head competition time trial time was reported as significantly faster than either familiarisation ($P = 0.003$) or time trial alone ($P = 0.021$). The authors also concluded that this was a result of, primarily, an enhanced anaerobic energy yield.

Interestingly, in a recent study by Viru and colleagues (Viru et al., 2010) the introduction of competition not only resulted in the improvement (when compared to non-competition) of exercise performance during incremental treadmill running to exhaustion (by 4.2%; +49 seconds; $P < 0.05$), but this was in addition to noticeable differences in the physiological measure of $\dot{V}O_{2\text{peak}}$ (by 4.4%; $+2.5 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; $P < 0.05$). The authors concluded that

in competitive situations, the added motivation experienced by athletes can enhance exercise performance and leads to an increase in $\dot{V}O_{2\text{peak}}$. Further, an enhancement in sympatho-adrenal system activation has been suggested to occur under such conditions which in turn may be a contributor to enhancing performance (Virtanen et al., 2010).

Thus, it is clear that to truly elucidate what limits exercise capacity or endurance performance, as previously described, research has to replicate scenarios of reality. That is, moving away from laboratory based tests to assessing performance as it is truly carried out in the field, where possible, in a competitive environment. This is a scenario where true maximal effort will most likely be given resulting in a truer fatigue response. As previously highlighted, there is convincing evidence to support this notion where differences between field and laboratory based assessments and, in addition to, the impacts of competition on performance outcome have been indicated. However, a challenge with attempting measurements in the field is the potential restriction based on available technologies, and with available technologies, the ability to obtain satisfactory accuracy in the measurements that are obtained.

A modern technological development in portable metabolic systems is one area which has enabled traditional laboratory based practices to be taken in to the field. However, there are few published studies examining the performance of such commercially available devices during real life, field-based activities. For example, there is no published research on the validity of one of the most popular models currently available, the Cosmed K4b2 portable breath-by-breath system (K4b2, COSMED s.r.l., Rome, Italy) (Figure 1.16), for use during running outdoors. Therefore, the assessment of such devices particularly during real life field-based activities is of key importance. The K4b2 is one of several commercially available systems and has been previously described (McLaughlin et al., 2001), but the assessment of its performance in the field is very limited as highlighted in Table 1.4. Overall, studies have found the validity of the K4b2 to be quite variable often dependent on the exercise protocol employed.

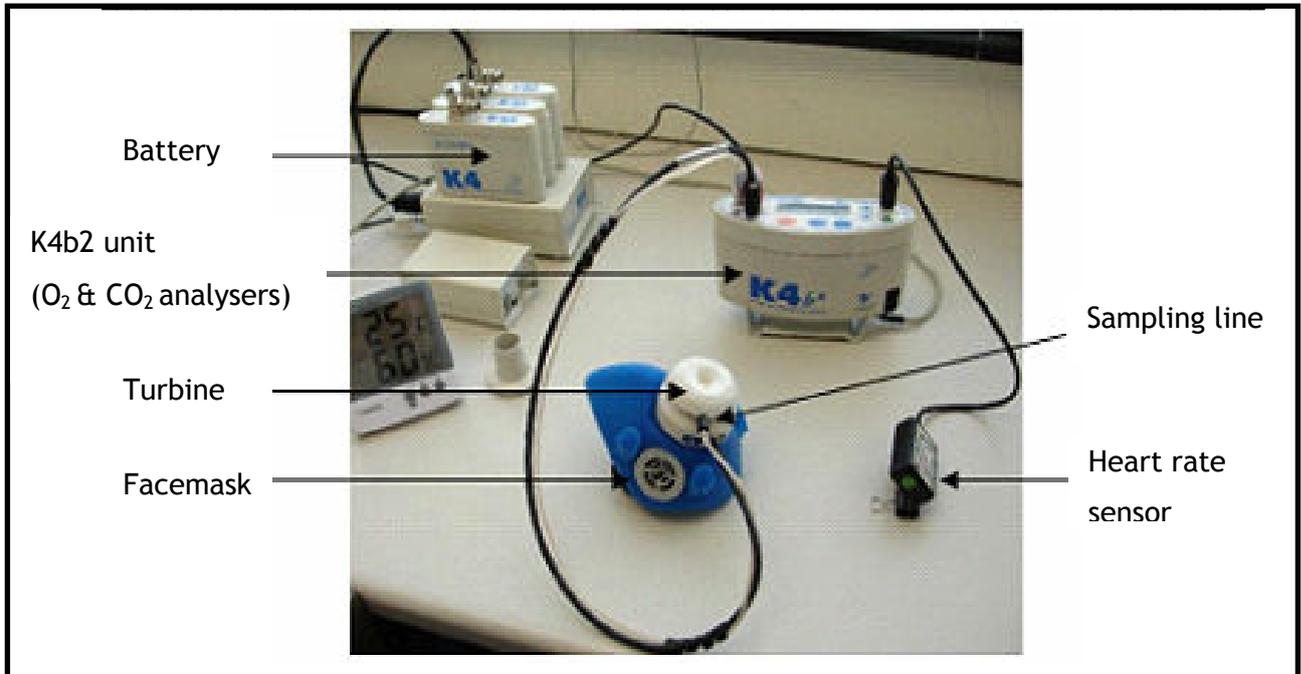


Figure 1.16: The K4b2 breath-by-breath gas with description of components

Table 1.4: Summary of studies assessing Cosmed K4b2 validity and/or reliability

Study	Participants Description	Environment & Comparison Method	Exercise Protocol	Study Summary Results & Conclusions
McLaughlin et al., 2000	10 healthy males (age: 27.6 ± 6.4 y; height: 180.9 ± 6.2 cm; weight: 75.3 ± 5.6 kg; mean \pm SD)	Indoor laboratory. Douglas Bag method.	Cycle ergometry exercise at 50W, 100W, 150W, 200W and 250W	No significant differences ($P > 0.05$) in $\dot{V}O_2$ between K4b2 and DB at rest and 250W. K4b2 values significantly higher ($P < 0.05$) than DB values at 50, 100, 150, and 200W. Magnitude of differences small (0.088, 0.092, 0.096, and 0.088 $l \cdot \text{min}^{-1}$, respectively).
Parr et al., 2001	7 healthy males (age: 27.5 ± 6.5 y; height: 181.8 ± 3.3 cm; weight: 74.9 ± 7.1 kg; mean \pm SD)	Indoor laboratory. Douglas Bag method.	Cycle ergometry exercise at 50W, 100W, 150W, 200W and 250W	No significant differences ($P > 0.05$) for $\dot{V}O_2$, $\dot{V}CO_2$, \dot{V}_E , and R at work rates from rest to 200W despite significant differences in FEO_2 ($P < 0.01$) and $FECO_2$ ($P < 0.05$).

Doyon et al., 2001	6 male and 2 female competitive cross-country skiers (subject demographic details not disclosed.)	Indoor laboratory and Field-based (640-m paved asphalt course). Mixing box system.	Incremental treadmill running test. Field test: 3 x roller ski maximal effort	No significant differences ($P < 0.05$) were found across the full range of $\dot{V}O_2$ measures.
Pinnington et al., 2001	12 males (age: 26.5 ± 11.2 y; height: 177.3 ± 5.5 cm; weight: 76.4 ± 15.3 kg). 8 females (age: 23.5 ± 4.5 ; height: 162.2 ± 4.5 cm; weight: 55.2 ± 4.8 ; mean \pm SD)	Indoor laboratory. CPX metabolic cart.	Treadmill running at self-selected speed for 12-15 mins to assess FEO_2 , $FECO_2$ and $\dot{V}E$.	K4b2 FEO_2 and $FECO_2$ measures were significantly lower ($P < 0.001$) than CPX metabolic cart.
Littlewood et al., 2002	4 males and 5 females (group average age: 28.2 ± 4.7 y; height: 168.7 ± 79.2 cm; weight: 73.2 ± 22.6 kg; mean \pm SD)	Indoor laboratory. Deltatrac II metabolic cart	Resting energy expenditure	Bland and Altman analysis revealed a mean bias for REE, RQ, $\dot{V}CO_2$, $\dot{V}O_2$ between K4b2 and Deltatrac II metabolic cart of 268 ± 702 kcal/day, -0.0 ± 0.2 , 26.4 ± 118.2 and 51.6 ± 126.5 ml min ⁻¹ , respectively. Variability between the two devices was very high and a degree of measurement error was detected.

Maiolo et al., 2002	9 healthy male footballers (age: 18.3 ± 2.2 y; height: 1.77 ± 0.57 m; weight: 67.3 ± 4.6 kg)	Indoor laboratory. Airspec QP9000 mass spectrometer.	Resting measures and treadmill incremental & exercise running test 8 km/h to 18 km h^{-1} in increments of 2 km h^{-1} .	No significant differences were found between $\dot{V}O_2$ ($P > 0.05$) and $\dot{V}CO_2$ ($P > 0.05$) at rest or during exercise.
Duffield et al., 2004	12 male athletes (age: 23.3 ± 3.2 y; height: 181.0 ± 5.6 cm; weight: 76.3 ± 7.6 kg; mean \pm SD)	Indoor laboratory. Metabolic cart.	Treadmill running: easy for 10 min, hard for 3 min and 1 min sprint.	The K4b2 measured significantly higher $\dot{V}O_2$ ($P < 0.05$) and $\dot{V}CO_2$ ($P < 0.05$).
McNaughton et al. 2005	8 males (age: 23.7 ± 1.1 y; height: 1.78 ± 0.01 m, weight: 74.4 ± 2.1 kg; mean \pm SD)	Indoor laboratory. Morgan EX670 mass spectrometer	Cycle ergometer based $\dot{V}O_{2\text{max}}$ test & submaximal exercise test at 150W, 200W, 250W and 300W	K4b2 significantly higher than Morgan EX 670 for both $\dot{V}O_2$ and $\dot{V}CO_2$ at 250 W ($P < 0.05$ and $P < 0.002$, respectively), and 300 W ($P < 0.002$ and $P < 0.005$, respectively). Unsystematic bias varied between 1% and 16% and systematic bias between 3% and 8%.

Schrack et al., 2010	10 men and 9 woman (group average age: 39.8 ± 13.8 y; height: 181.2 ± 4.8 cm, weight 86.90 ± 29.71 kg; mean \pm SD)	Indoor laboratory. Medgraphics D-Series stationary gas exchange system	Treadmill 400 m walk	The K4b2 not significantly different for $\dot{V}O_2$ ($P = 0.16$) and $\dot{V}CO_2$ ($P = 0.08$) during steady-state and submaximal exercise.
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Min: minutes; W: watts, ICC: intraclass correlation coefficient, $\dot{V}O_2$: oxygen uptake, $\dot{V}CO_2$: carbon dioxide production, REE: resting energy expenditure, RQ: respiratory quotient, R: respiratory exchange ratio, F_{EO_2} : fractional expired oxygen, F_{ECO_2} : fractional expired carbon dioxide, \dot{V}_E : expiratory ventilation, DB: Douglas bag method. Values are indicated as mean \pm SD.

In the only study to assess the K4b2 in the field, Doyon and colleagues (Doyon et al., 2001) compared the K4b2 in eight competitive cross-country skiers where each carried out one field- and one laboratory-based test to determine a number of variables including peak oxygen uptake ($\dot{V}O_{2peak}$). Field tests involved maximal roller skiing efforts on a straight 640 m long, with 2% gradient, paved asphalt course. Oxygen uptake was measured using the K4b2. The laboratory comparison test was carried out on a motorised treadmill where speed and gradient increased incrementally until exhaustion. Field K4b2 measures were compared to a standard laboratory mixing box system. The authors reported that $\dot{V}O_{2peak}$ was not significantly different during indoor treadmill running and outdoor diagonal stride roller skiing ($62 \pm 2.9 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and $60.4 \pm 2.8 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, respectively; $P > 0.05$). This sole study provides some indication as to performance of the K4b2 in an outdoor environment; however, the field test employed only took 2 to 3 minutes to complete. Further, the results are only truly applicable to roller skiing activity.

It is surprising that 364 studies (according to the K4b2 bibliography on the manufacturer website: http://www.cosmed.com/images/pdf/bibliography/k4b2_Bibliography.pdf [Accessed: 25th September 2012]) have been carried out using the K4b2, of which several were used for assessments in varying field-based environments e.g. (Devienne and Guezennec, 2000, Billat et al., 2003), yet only 1 study has attempted to assess its validity in the field. Furthermore, there are no published studies (to the best of knowledge) assessing the accuracy of the K4b2 during running activities, specifically, in outdoor environments. Therefore, there is a clear need for the K4b2 to be assessed during running exercise, at submaximal and maximal speeds, in the field. Having an accurate portable metabolic device for measuring gas exchange variables in the field would enable the assessment of endurance performance in a 'truer' environment (i.e. running on a track).

1.12 Psychosocial and Anti-doping Considerations in the use of Performance Enhancing Drugs

Psychosociology is the study of the interaction between psychological effects and social environments (Hemingway and Marmot, 1999). This has been a key approach in research assessing factors contributing to an individual's motivation and decision to engage in doping activity. Reports suggest that the motivation to dope can be triggered by a range of factors including: the growing cultural emphasis to 'win at all costs', the potential financial benefits of success, the lack of emphasis on sportsmanship and teamwork, the unwillingness to train hard over prolonged periods of time and the lacking of confidence in ones abilities (Petroczi, 2007) (Donovan et al., 2002). Such examples indicate a variety of

potential ‘triggers’ and emphasises the importance of understanding both psychological and social factors, if we are to obtain a full understanding of doping behaviours.

Several studies have provided useful insights into athlete viewpoints on drugs, sporting performance, doping in sport (Rossi and Botre, 2011, Waddington et al., 2005). Studies have also identified key psychosocial challenges associated with sports participation, competition and, consequently, the development of key coping strategies in order to improve how individuals deal with such psychosocial challenges (Reeves et al., 2011). In addition, researchers have attempted to assess, under laboratory conditions, the potential relationships between competition, performance enhancement and cheating (Schwieren and Weichselbaumer, 2008), where it has been reported that the ability of an individual to carry out a task, determined how they reacted to competition, with less skilled individuals being more likely to resort to cheating behaviours. Furthermore, the relationship between social support systems and use of performance enhancing drugs have also been discussed where, for example, steroid use has been reported as being more prevalent in users who had poorer relationships with their parents (Skarberg and Engstrom, 2007).

This information is important for improving anti-doping intervention strategies. However, two important limitations exist in the available evidence base. Firstly, the majority of data – generally from interviews and/or questionnaires – are obtained from users a number of months or years after drug use has taken place (Yesalis and Bahrke, 2000, Bahrke et al., 2000, Calfee and Fadale, 2006, News, 2005). Secondly, it is difficult to ascertain whether responses are truthful. For example, methodological problems have included the poor stability of results (Yesalis, 1988) as well as a high, yet unknown, degree of underestimation in relation to the use of doping substances (Pedersen, 2005).

One key problem of obtaining information ‘after the fact’ relates to the passage of time where, according to the Decay Theory, memories fade and the related information becomes harder to retrieve, with the accuracy of recall becoming less accurate (Berman et al., 2009). Furthermore, the Interference Theory suggests that stored memories are interfered with as a result of newly stored memories (Tomlinson et al., 2009). In addition, the importance of contemporaneous statements or notes in the court of law and in healthcare also provides evidence as to the quality of recall from memory (National Health Service, 2013). Regardless of the theory, however, it is clear that stored memories related to behaviour prior and during doping is likely to be distorted when asked to recall after a period of time/after the behaviour has taken place.

One obvious solution involves attempting to obtain information from users of performance enhancing substances while they are taking them. However, in reality, the task of trying to obtain information as doping behaviour is taking place is challenging, because doping practices are extremely secretive and dopers generally want to evade detection. Therefore, studies employing the use of performance enhancing drugs in a research context provide a unique opportunity to assess psychosocial factors associated with use of such substances contemporaneously (i.e. immediately taken down notes, audio recordings and interviews). Potential areas of interest may include: how participants behave when training with teammates and/or coaches (i.e. do they discuss their involvement in such a research study?) and if participants aren't sharing their experience with team mates do they feel guilty, particularly if they are performing better during training.

A final issue to consider is whether use of performance enhancing substances alters an individual's perception of fairness and cheating. Cheating is a word often heard in debates regarding doping, however, defining what constitutes as cheating can, at times, be complex. The *Oxford Dictionary* (Oxford Dictionaries, 2013) defines cheating as follows:

- act dishonestly or unfairly in order to gain an advantage: **she always *cheats at cards***
- gain an advantage over or deprive of something by using unfair or deceitful methods; defraud: **he had *cheated her out of everything she had***
- be sexually unfaithful: **his wife was *cheating on him***
- avoid (something undesirable) by luck or skill: **she cheated death in a spectacular crash**

The ethics behind the action of stealing provides an interesting parallel. Stealing is generally viewed as being a socially unacceptable act; however, this view can change if the act of stealing was perceived to have been performed for a 'good' reason (e.g. stealing to feed one's family). Such reasoning can also be applied in the context of drug taking in sport. There is evidence to suggest that cheating is often carried out by individuals because of the perception that other competitors are doing the practice and thus there is a need to 'level the playing field' (McCabe, 2009, McCabe, 1999). This notion of 'levelling the playing field' has increasingly been cited as justification for drug use by caught users of performance enhancing drugs. Examples include Dwain Chambers, Lance Armstrong and Tyler Hamilton (BBC, 2007, Guardian, 2013, CBS, 2013).

However, it is very difficult to determine whether such a justification was genuinely felt at the time of drug use or was a *post hoc* justification to mitigate their drug use after getting caught. Recent reports of widespread doping in Italy (Paoli and Donati, 2013), Australia (Australian Crime Commission, 2013) and the case of Lance Armstrong (BBC Sport Cycling, 2013) suggest that doping in sport may be more widespread than previously appreciated, adding weight to the ‘levelling the playing field’ argument.

Thus, there is a clear need for further research to understand the thought processes and more general experiences of individuals at the time that (or immediately after) performance enhancing substances are being taken.

1.13 Aims and Objectives

The aims of this PhD were therefore:

- i. To determine the validity of the K4b2 in the measurement of $\dot{V}O_2$ during outdoor running at submaximal and maximal intensities (Chapter 3).
- ii. To determine the effect of r-HuEpo administration on performance in a field-based setting and quantitative psychological factors, and to confirm its haematological effects (Chapter 4).
- iii. To determine the effect of r-HuEpo administration on psychosocial factors (Chapter 5).
- iv. To determine the effect of a subcutaneously injected placebo, purporting to be a legal substance with similar effects to r-HuEpo: on field-based performance, haematological measures, and psychological responses (Chapter 6).

2. General Methods

2.1 Participants

Participants were recruited using various methods and this depended on the experimental study. For the study in Chapter 3, posters were placed in appropriate advertising locations such community health centers, gymnasiums and sports clubs across Glasgow city. In addition, digital advertisements were circulated via emails and online forums of sports clubs, as well as within the University of Glasgow.

In Chapters 4, 5 and 6, such a relatively open public advertising approach was not pursued due to the sensitive nature associated, in the experimental studies, with the use of illegal and legal performance enhancing drugs. As a result, potential participants were identified either through Glasgow City Council (i.e. Sport and Events, Culture and Sport Glasgow), or by personal contact or direct advertisement. Athletes (with their respective coaches and/managers) were asked to meet with the investigators to discuss the project and to ascertain whether they would be suitable participants.

In all cases, an information sheet was also provided to all potential candidates (Chapter 3: Appendix A; Chapter 4: Appendix B, Chapter 5: Appendix B, Chapter 6: Appendix C). The information sheets provided a description of the purpose, procedures, potential risks and benefits of the study involved. All participants were encouraged to ask questions prior providing informed consent.

After informed consent was obtained (with the option to withdraw from participation at any point without reason), all recruited participants completed an exercise participation and health questionnaire (Appendix F). Participants were then recruited based on specific criteria for each experimental Chapter:

Chapter 3:

- i. Male aged 18-35 years
- ii. Endurance trained (i.e. more than 6 hours of planned endurance training per week)
- iii. Non-smokers

-
- iv. Non-diabetic
 - v. No previous history of established coronary heart disease
 - vi. No family history of early cardiac death

Chapters 4 and 5:

In this instance, the extent of any illnesses, medication or nutritional supplementation was noted, as well as the incidence of familial haemoglobinopathies (e.g. sickle cell trait) which could have a substantial effect on measured haematological parameters. All participants were then required to pass a comprehensive medical examination where the 'good health' of each participant was established prior starting the study by a qualified medical practitioner (Appendix H). Specifically, participants were recruited based on the following criteria:

- i. Male aged 18-35 years (addendum later submitted and approved for ages 18-38)
- ii. Endurance trained (i.e. more than 6 hours of planned endurance training per week)
- iii. Not involved in sporting competition for the duration of the study
- iv. No current illness
- v. No injury which impacts on the capacity to exercise
- vi. No disorders or history of disorders of the haematopoietic system
- vii. No family history of premature cardiovascular disease
- viii. No musculoskeletal conditions

Chapter 6:

After informed consent was obtained (with the option to withdraw from participation at any point without reason) all participants were instructed to complete a health and sports participation questionnaire (Appendix F) and have blood pressure measured. Participants were recruited based on the following criteria:

- i. Male aged 18-35 years
- ii. Endurance trained (i.e. more than 6 hours of planned endurance training per week)

-
- iii. Non-smokers
 - iv. Non-diabetic
 - v. Body mass index $< 35 \text{ kg}\cdot\text{m}^{-2}$
 - vi. No uncontrolled hypertension ($> 160/90$ mm Hg on anti-hypertensive medication)
 - vii. No previous history of established coronary heart disease (e.g. myocardial infarction, coronary artery bypass graft surgery, coronary angioplasty)
 - viii. No family history of early cardiac death (< 40 years)
 - ix. All participants in good health at the time of testing

2.2 Anthropometric Measures

2.2.1 Body Mass

In Chapters 3 and 4, body mass was measured to the nearest 0.05 kg using a beam balance scale (Avery, England). In Chapter 6, body mass was measured using a pre-calibrated standard digital weighing scale (SECA, Hamburg, Germany). On all occasions, participants were measured after voiding of the bladder and wore minimal clothing (i.e. generally light-weight shorts and t-shirt) with shoes and jewellery accessories (e.g. watches, heavy necklaces) removed. Participants stood on the scales with feet planted on the platform, facing forward and with arms relaxed to the sides of their body.

2.2.2 Height

Height was measured to the nearest 0.1 cm using a standard stadiometer (Invictus Plastics Ltd., Leicester, England). On all occasions, participants stood barefoot, with both feet alongside one another, and with their back against the vertical measuring rod. The head was positioned in a horizontal manner with the stadiometer headpiece rested on top of the individual's head and, when necessary, a slight downward pressure was applied to minimize the potential additive effect of hair. In order to account for daily variations in height, a slight upward pressure was applied to the head in an attempt to alleviate any potential

spinal compression. This was then immediately followed by recording the final measurement on the vertical measuring rod.

2.3 Respiratory Gas Exchange Analysis

2.3.1 Breath-by-Breath Exchange

Automated gas exchange systems were used in exercise protocols employed in Chapters 3 and 4 during. Chapter 3 featured a portable metabolic unit (K4b2, Cosmed, Italy) whilst Chapter 4 featured a laboratory based metabolic cart (Quark b2, Cosmed, Italy). Such systems enabled the determination of $\dot{V}O_2$, carbon dioxide output ($\dot{V}CO_2$) and minute ventilation (\dot{V}_E) during the maximal incremental exercise tests. The laboratory based Quark metabolic cart has been shown to be valid and reliable for use in indoor environments during submaximal and maximal exercise intensities (Bowles et al., 2011).

For both K4b2 and Quark systems, participants were required to wear a face mask connected to a 28 mm bi-directional turbine digital volume sensor and a capillary gas sampling line. These were held in place via a facemask which provides a lower resistance to breathing and allows for more natural breathing (i.e. allows participants to breathe through the nose and mouth) as opposed to when using a mouthpiece. The turbine features impellers (as part of the rotor makeup) which measure inspired and expired flow and volume by the recording of the number of impeller interruptions of an infra-red laser beam located within the turbine. The frequency of interruptions determines impeller velocity which is then used to determine both inspiratory and expiratory flow rates as impeller velocity and gas flow are proportional to each other. The turbine used, in both the K4b2 and Quark, featured low resistance characteristics with a resistance of $<0.07 \text{ kPa} \cdot \text{L}^{-1} \cdot \text{s}^{-1}$ at a flow rate of $14 \text{ L} \cdot \text{s}^{-1}$.

The capillary sample line used in the K4b2 and Quark systems, extracts a small sample of gas ($20 \text{ ml} \cdot \text{min}^{-1}$) into a vacuum, within the respective units, where then concentrations of O_2 and CO_2 are then measured. Extracted samples are measured within the vacuum every 20 ms by the respective O_2 and CO_2 analysers.

The O_2 analyser type differs between the K4b and Quark systems in that the K4b2 has an electro-galvanic fuel cell sensor whilst the Quark has a paramagnetic sensor. The electro-galvanic sensor involves a chemical reaction between potassium hydroxide (located inside the fuel cell) and oxygen which, in turn, creates an electric current between the anode and

cathode. The current produced is proportional to the concentration of oxygen present. Alternatively, the paramagnetic sensor utilizes the paramagnetic properties of oxygen in order to distinguish it from other gases. Oxygen molecules are attracted to the strong magnetic field and this exhibits a force which is proportional to the oxygen content of the surrounding gases. The CO₂ infrared analyser is based on infrared absorption where higher concentrations of CO₂ results in higher infrared absorption rates. Thus, the resulting infrared absorption rate is related to concentrations of CO₂ which is measured electro-optically. Once fractional concentrations of O₂ and CO₂ are analysed and determined by the sensors, using specified manufacturer algorithms, $\dot{V}O_2$ and $\dot{V}CO_2$ are calculated, respectively. In addition, an algorithm is also used to calculate \dot{V}_E .

2.3.1.1 Calibration of Breath-by-Breath Systems

For all tests involving the K4b2 and Quark systems, the device was warmed up for one hour prior the pre-test calibration procedure (conducted according to manufacturer guidelines) which was then started approximately 15 to 30 minutes prior each test. Both systems were calibrated according to manufacturer guidelines which involved a 3-step calibration process:

1. **Calibration to ambient conditions.**
2. **Flow-volume sensor calibration.** This involves the connection of a 3 litre volume syringe (Hans Rudolph Inc., Kansas City, MO, USA) where this known volume is pumped through the turbine mimicking inspiratory and expiratory flow. A series of 6 complete pumps were carried out and values were accepted within a range of 2.9 to 3.1 litres.
3. **Gas analyser and time-delay calibration.** This automated process involves the analysis of room air and calibration tank gases (16% O₂ and 5% CO₂) in order sample a range of concentrations that would be observed during exercise. The time-delay calibration component involves the delay between volume and gas concentration signals by each respective analyser.

2.3.1.2 Data analysis and Data Reduction

All data collected using the K4b2 and Quark systems was checked and edited with the exclusion of breath-by-breath $\dot{V}O_2$ values that exceeded four standard deviations from the average value for the last 30 seconds of each work rate. Values excluded indicated 'false' or 'artifactual' breaths; typically caused by swallows, sighing, coughing, or any other

reason that would end a breath prematurely (Beaver et al., 1973, Lamarra et al., 1987). The use of four deviations from the mean (i.e. 99.9% confidence interval) provides a 0.005 probability that the identified ‘artifactual’ or ‘false’ breath is in fact a genuine breath (Lamarra et al., 1987). The typical error of measurement was also calculated in order to assess the mean difference error for the measure of $\dot{V}O_2$ (i.e. the typical error in values from measurement to measurement). The typical error of measurement was calculated by dividing the standard deviation, for the data set, by the square root of two (Hopkins, 2000)

2.3.2 Douglas Bag Method

As Chapter 3 involved the validation of the K4b2 unit, thus the Douglas bag method was also used as the gold standard comparison. Expired gases were collected via a mouthpiece connected to a Hans-Rudolph 2700 series large 2-way valve (Hans-Rudolph Inc., Kansas City, MO, USA) and wide-bore Falconia tubing. Expired gas was collected over the last 30 sec of each 3 minute running increment. Each Douglas bag was analysed within 10 minutes for expired volume in litres (Harvard dry gas meter, Harvard appliances Ltd, UK), F_{EO_2} and F_{ECO_2} (Servomex 4100 Gas purity analyser, Servomex, UK). The gas analyser was calibrated prior to each test with known reference gases (i.e. room air calibration, reference 16% O_2 and 5% CO_2) and the gas meter calibrated with a known volume (i.e. 3L Hans Rudolph calibration syringe, Kansas City, MO, USA). Barometric pressure was measured using a standard mercury barometer and the volume, temperature and flow rate of the sample to the Servomex were noted. These were used along with fractional expired oxygen (F_{EO_2}) and carbon-dioxide concentrations (F_{ECO_2}) to calculate oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$), respectively, and ventilation (\dot{V}_E) according to the Haldane transformation (Parr et al., 2001). As an example, the calculation for $\dot{V}O_2$, via the Douglas bag method, is provided:

$$\begin{aligned}\dot{V}O_2 &= (\dot{V}_I \times F_{IO_2}) - (\dot{V}_E \times F_{EO_2}) \\ \dot{V}O_2 &= (33.9 \text{ l min}^{-1} \times 0.2093) - (33.8 \text{ l min}^{-1} \times 0.1658) \\ \dot{V}O_2 &= 1.49 \text{ l min}^{-1}\end{aligned}$$

2.4 Exercise Tests

Depending on the experimental Chapter, participants performed exercise tests as follows:

1. Chapter 3 – Manually controlled treadmill (Woodway PPS55 Med, Weilam Rhein, Germany) and on an outdoor 400 m running track (Bellahouston Park, Glasgow, UK)
2. Chapter 4 - Manually controlled treadmill (Woodway PPS55 Med, Weilam Rhein, Germany) and on an indoor 200 m running track (Kelvin Hall Arena, Glasgow, U.K)
3. Chapter 6 - Indoor 200 m running track (Kelvin Hall Arena, Glasgow, U.K)

2.4.1 Maximal Incremental Exercise Testing

Maximal incremental exercise tests were used in Chapters 3 and 4 and were manually controlled using a treadmill (Woodway PPS55 Med, Weilam Rhein, Germany). However, the protocol varied slightly between the experimental Chapters 3 and 4.

Chapter 3 featured two indoor and one outdoor continuous maximal incremental test per participant. The indoor tests comprised participants running on a treadmill set at 1% gradient with 2 km·h⁻¹ continual incremental increases in speed, every 3 minutes, starting from 8 km·h⁻¹ to the individual's maximal attainable speed (Figure 2.2) (Appendix I). A 1% gradient was used during treadmill tests as it has previously been demonstrated that this most accurately reflects the energetic cost of running outdoors (Cureton et al., 1978). Once the participant reached maximal capacity, the speed was reduced according to participant preference for a cool down for a minimum duration of 5 minutes.

The outdoor test was similar except participants were requested to run on a running track (Figure 2.1) where velocities were set by an experimenter cycling a road bike fitted with a calibrated speedometer (Cateye Velo 5, Osaka JAPAN). The participant was strictly told to run alongside the middle of the bike in line with the experimenter's leg. The experiment was stopped by the cycling experimenter once the participant could no longer run as instructed. This, in turn, was used as a marker for the end of the test.

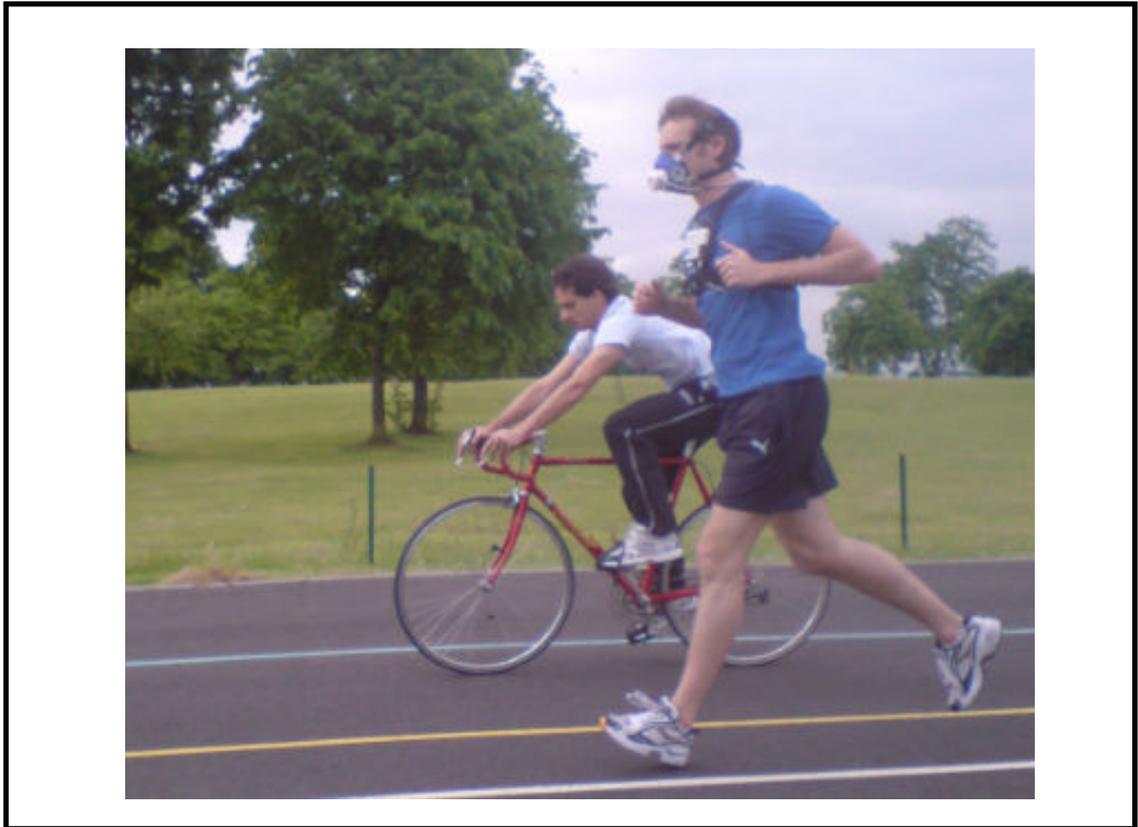


Figure 2.1: Participant undergoing the K4b2 outdoor test

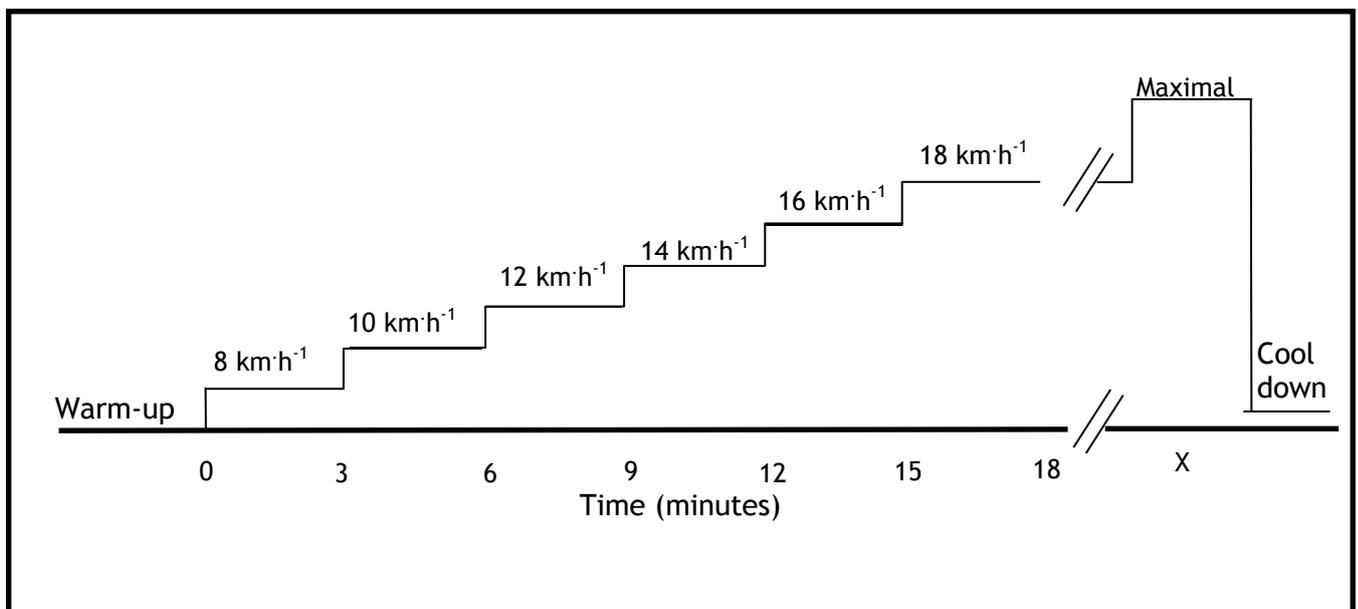


Figure 2.2: Continual maximal incremental running protocol

Chapter 4 featured four discontinuous maximal incremental treadmill tests per participant. Similar to the test employed in Chapter 3, participants were required to run on a treadmill set at 1% gradient with 2 km·h⁻¹ incremental increases in speed every 3 minutes (between running speeds of 8 and 12 km·h⁻¹) and, from then onwards, with 1.5 km·h⁻¹ incremental increases, every 3 minutes, to the individual's maximal attainable speed. However, in this instance, every increment was separated by up to 3 minutes at walking pace (i.e. 3-4 km·h⁻¹) in order to obtain a capillary blood sample for the determination of blood lactate concentration (see section 2.5.2 below). Essentially, the aim was to obtain the sample whilst minimising the break time. Such breaks were employed to facilitate 'clean' blood sampling and to ensure that lactate analysis was complete prior moving on to the next intensity level. Such breaks have been shown not to have a significant influence on the blood lactate curve (Gullstrand et al., 1994). Once the participant reached maximal capacity, the speed was reduced according to participant preference for a cool down for a minimum duration of 5 minutes (Figure 2.3).

A verification test was then carried out and this involved participants running at a supramaximal constant speed (i.e. 1 stage higher than what was achieved during the incremental phase) to exhaustion, after completion of the incremental phase (Midgley et al., 2007). Establishing $\dot{V}O_{2max} / \dot{V}O_{2peak}$ initially involved the removal of non-breaths as previously described in section 2.3.1.2. The $\dot{V}O_2$ values of the final 30 seconds of the incremental test were averaged to determine the maximal or peak value. If there was less than 2% difference between this value and the $\dot{V}O_{2peak}$, obtained in the verification test, then the incremental value was accepted as a $\dot{V}O_{2max}$ value (Midgley et al., 2007).

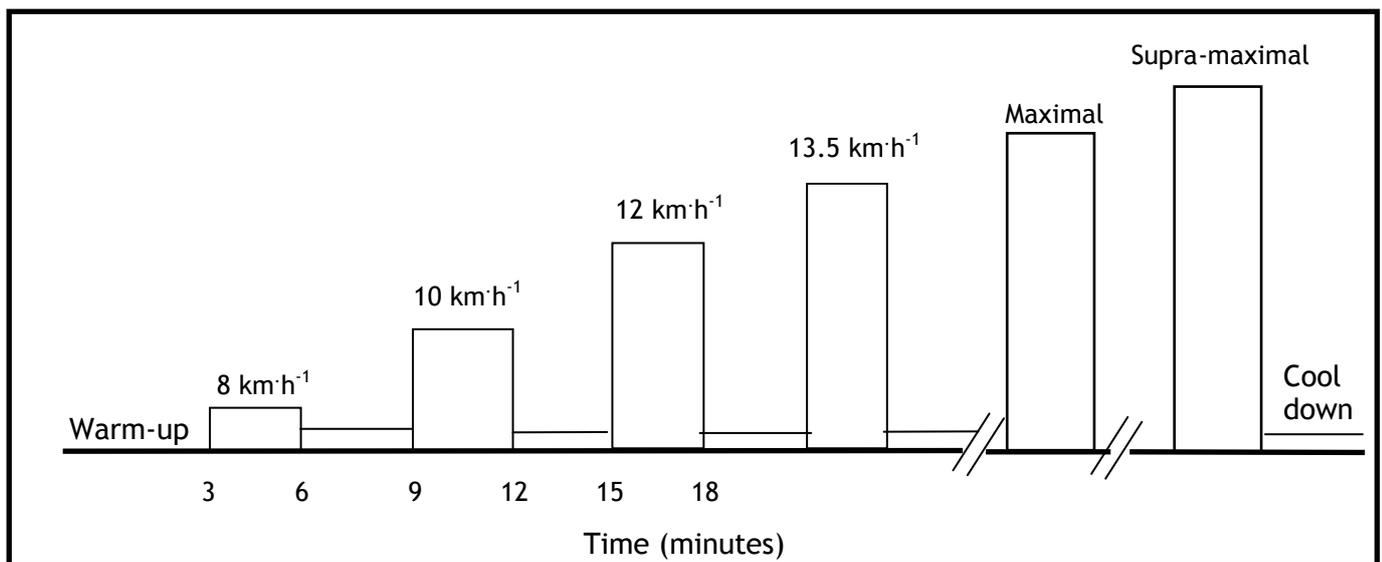


Figure 2.3: Discontinuous maximal incremental running protocol followed by a verification test

2.4.1.1 Criteria for determination of $\dot{V}O_{2\max}$ / $\dot{V}O_{2\text{peak}}$

Maximal oxygen uptake is defined as the highest rate at which O_2 can be taken up and used by the body during severe exercise (Bassett and Howley, 2000). Researchers in the first half of the 20th century first observed that a high $\dot{V}O_{2\max}$ was a key physiological characteristic of elite endurance athletes (Hill and Lupton, 1923, Robinson et al., 1937). Traditionally, a widely used criterion in order to identify whether or not $\dot{V}O_{2\max}$ has been achieved involves the attainment of two or more of the following criteria:

1. A plateau in $\dot{V}O_2$ – no change in $\dot{V}O_2$ over 250 ml
2. A respiratory exchange ratio of 1.15 at the point of exhaustion
3. A peak heart rate within $10 \text{ b}\cdot\text{min}^{-1}$ of the age predicted maximum which is calculated by subtracting age from 220
4. A minimum blood lactate concentration of $8 \text{ mmol}\cdot\text{l}^{-1}$ at the point of exhaustion

Recent issues have, however, been identified surrounding the use of such criteria for the identification of $\dot{V}O_{2\max}$ (Rossiter et al., 2006) and some authors have even deemed it unnecessary (Poole et al., 2008). For example, Rossiter and colleagues (Rossiter et al., 2006) have shown that not all individuals achieve a plateau in $\dot{V}O_2$ and when such individuals then carried out a following supramaximal exercise (i.e. at a higher intensity than what was achieved in the incremental test), no differences in $\dot{V}O_2$ were identified between tests. Thus, in order to ensure the determination of $\dot{V}O_{2\max}$, the use of such criteria was used in addition to a supramaximal verification test (as previously described in section 2.4.1) immediately post each maximal incremental test in Chapter 4.

2.4.2 Assessment of Running Economy

As previously discussed in section 1.7.2, running economy related to the amount of energy expended at a given submaximal running velocity, can be expressed in $\dot{V}O_2$ either in litres per minute or according to body mass (i.e. $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). This was assessed in Chapter 4. For reliable measures of running economy, it has been demonstrated that $\dot{V}O_2$ values obtained during running speeds below 85% of $\dot{V}O_{2\max}$ should be utilised for running economy comparison purposes (Saunders et al., 2004a). Accordingly, for the group of

participants recruited in Chapter 4, a running speed of $12 \text{ km}\cdot\text{h}^{-1}$ was used as this ensured that all participants were below 85% of $\dot{V}\text{O}_{2\text{max}}$.

2.4.3 Time Trial Testing

In Chapters 4 and 6, as part of the exercise testing protocol, field-based 3 km time trials were performed by participants on a 200 m indoor running track at the Kelvin Hall Arena, Glasgow.

Participants refrained from alcohol, caffeine and strenuous exercise on the day before testing. For the measurement of heart rate during each 3 km time trial, in Chapter 4, a Garmin watch (Garmin Forerunner 405, Garmin, Kansas, USA) and heart rate belt were worn by participants. During the time trial itself, participants were only informed about the number of laps remaining and were asked not to look at the watch for timing information. In order to ensure this, after the participant started the watch they were instructed to flip the watch round their wrist in order to hide the front display. Individual lap times and total times were taken by one of either two trained experimenters and were noted on each occasion (Appendix J).

Chapter 6 also featured 3 km time trials carried out on the same 200 m indoor running track, however, the protocol varied slightly in comparison to Chapter 4. The key difference involved ensuring that the 3 km races were competitive, thus, participant starting times were handicapped based on the times achieved during the individual 3 km time trial familiarisation runs (i.e. a participant with an achieved 3 km time of 11:00 would start 30 seconds before a participant with a predicted 3 km time of 10:30). In this instance, lap completion times were taken by experimenters for each participant during each competition run using standard stopwatches. Heart rate measurements were also recorded and monitored on a real time basis using Fitpulse technology (Fitpulse, TT Sport S.R.L, Italy). Competition prizes (maximum value £35) were provided depending on overall competition finishing positions (i.e. on completion of all four competition runs). This was added to encourage competition between competitors. All participants were positively motivated during each competition run and only received information on the number of laps remaining. Participants were not given any further information or results until completion of the study.

2.5 Blood Collection

2.5.1 Venous Blood Sample Collection

Chapters 4 and 6 involved the collection and analysis of venous blood. Prior any blood samples being extracted, either a cannula (Vasofix, Braun, Melsungen, Germany) was inserted or a venipuncture (BD Vacutainer, Becton Dickinson, USA) carried out into the antecubital vein of the inner forearm. Following a 10-minute resting period, in a supine position, a 10 ml sample was extracted into an ethylenediamine tetra-acetic acid (EDTA) containing vacutainer (BD Vacutainer, Plymouth, UK) with the vacutainer gently mixed for 2 minutes. Where several blood samples were required, this involved an adapter (BD Vacutainer Multiple Sample Luer, Plymouth, UK) being connected to a three-way valve which pierced the EDTA vacutainer to allow for direct and quick blood collection. Once the required amount of blood was obtained, a 0.9% sterile sodium chloride solution (B. Braun, Melsungen, Germany) was inserted and flushed through the cannula in order to keep it clean and free from blood clots. During the venepuncture procedure, an EDTA tube was directly punctured when the hypodermic needle was attached to a vacutainer (Bectin Dickinson, Helsinborg, Sweden), and blood was directly collected upon insertion of the needle into the antecubital vein of the inner forearm. In both blood collection methodologies, upon the completion of blood sampling, the needle was removed and pressure was applied at the insertion site for a minimum of 3 minutes in order to minimise any potential bruising. All venous blood samples were collected by the author or another qualified phlebotomist.

2.5.2 Capillary Blood Sample Collection

Chapter 4 involved the determination of lactate concentrations during the discontinuous maximal incremental exercise protocol (Bentley et al., 2007) as previously described in section 2.4.1. The collection of capillary blood samples involved the disinfection (using an alcohol wipe) of one of the participant's fingertips which was then punctured with a lancet (Accu-Check Safe-T-Pro Plus, Roche, Mannheim, Germany). The first drop of blood was discarded and capillary blood samples were collected in pre-heparinized glass capillary tubes (Clinitubes, Radiometer, Copenhagen, Denmark) and were filled in under 10 seconds. A magnetic rod was then placed inside the blood-containing capillary tube as this allowed for the sample to be mixed with the use of an external magnet before being analysed for lactate concentration using the Radiometer ABL 725 analyser.

2.6 Blood Analysis

Blood samples were analysed using flow cytometric erythrocyte analysis performed on a portable Sysmex XT-2000i (Sysmex, Norderstedt, Germany) haematological analyser. Flow cytometry involves a laser beam, of a single wavelength, being directed on a hydrodynamically focused (i.e. cells are forced to pass through a specific 'tunnel' or pathway) stream of liquid of which a sample of blood is suspended. Each suspended particle (i.e. located in the blood sample), from 0.2 to 150 micrometers, is then hydrodynamically focused and moved through and perpendicular to the laser beam. This results in the formation of scattered and fluorescent lights of varying wavelengths, due to the excitation of suspended particles, which is then picked up by detectors and analysed for physical and chemical properties (Duffield et al., 2004). In Chapter 4, samples were immediately analysed (in-house) after mixing within 10 minutes, whilst in Chapter 6 samples were analysed within 30 minutes at the Western Infirmary Hospital in Glasgow city.

In both Chapters 4 and 6, direct erythrocyte measurements included red cell count (RBC), haemoglobin concentration [Hb], haematocrit Hct, mean cell volume (MCV) and percentage reticulocyte (%retic). The analyser was calibrated against Sysmex™ diagnostics reference materials, before samples were processed, according to manufacturer guidelines.

2.6.1 Capillary Blood Sample - Lactate Analysis

The Radiometer ABL 725 measures lactate concentrations using amperometric electrodes with enzymatic membranes. This has an accuracy bias of -2.0% when compared to the reference method of spectrophotometry using a lactate dehydrogenase (LDH) method measured on serum (Radiometer Medical ApS., 2009). The amperometric electrodes with enzymatic membranes measurement is based on an enzyme that recognises blood based lactate which results in a biochemical reaction. This, in turn, results in flow current changes (i.e. electrochemical changes) which is then transduced into a concentration signal (Thompson and Krull, 1991). Lactate threshold values were determined, by two investigators, using the visual inspection method (Wasserman et al., 1973) where the lactate threshold was defined as the work rate beyond which the lactate concentration increased more rapidly from baseline measures by 1 mmol·l⁻¹ (Goodwin et al., 2007). The criteria used for OBLA was the work rate which corresponded to a lactate concentration of

4 mmol·l⁻¹, and these were also visually determined and independently verified by two investigators (Goodwin et al., 2007).

The determination of MLSS, as previously described in section 1.7.1, was not directly assessed in experimental Chapter 4. However, studies have shown the LTP, as previously described in section 1.7.1, as providing a good approximation of the work rate at MLSS (Pringle and Jones, 2002). The authors however do indicate that the blood lactate concentration measure, at LTP, is typically under-estimated when compared to those obtained at MLSS.

2.6.2 Optimised Carbon Monoxide (CO)-rebreathing method

The optimised CO-rebreathing is an easy to handle and minimally invasive laboratory method to determine total haemoglobin mass. The accuracy and reliability of this method is similar to the well-established Chromium release (⁵¹Cr) method, which is considered to be the gold standard by the International Committee for Standardization in Haematology (Saunders et al., 2004a). According to published recommendations and via personal correspondence with the Jon Werhlin research group (Swiss Federal Institute of Sport), who are current experts in the optimised CO-rebreathing method, the following operating procedures were employed.

2.6.2.1 Carbon Monoxide Dose Determination

As part of the optimised CO-rebreathing method, participants were instructed to inhale a bolus of chemically pure CO (99.97%) (BOC Gases, Surrey, UK), during an initial inspiration, from a custom-made spirometer (University of Glasgow, UK) made according to previous instructions (Schmidt and Prommer, 2005a). A dose of 1.0 ml·kg⁻¹ (at room temperature and pressure) was administered and has been shown to effectively elicit a ~6.5% change in carboxy-haemoglobin (Δ HbCO), in turn, ensuring a good degree of sensitivity (i.e. 1.5% of Hb mass) whilst not compromising safety (Schmidt and Prommer, 2005a, Nilsson et al., 2002). To emphasise the uncompromised approach to safety, even increasing the CO dose beyond the state dose (e.g. up to 100 ml·kg⁻¹) should not cause any substantial undesirable effects (Schmidt and Prommer, 2005a) and so a wide safety margin is present. Nevertheless, participants were constantly monitored for any signs of early CO toxicity such as weakness, confusion, headache, nausea, disorientation and for any visual disturbances (Opie et al., 1976).

2.6.2.2 Optimised Carbon Monoxide -rebreathing Procedure

Prior the measurement of tHb, the Radiometer ABL 725 was calibrated and made ready for the measurement of carboxy-haemoglobin (HbCO) (Radiometer Medical ApS., 2009). The Radiometer ABL 725 measures HbCO using principles of visible absorption spectroscopy. Visible absorption spectroscopy involves the interaction between light, from the visible electromagnetic spectrum (i.e. wavelengths of 380 to 720 nm), and its absorption by a solution. The Beer-Lambert Law states that the absorbance of light, by a solution, is directly proportional to the concentration of the absorbing species in the solution (Kocsis et al., 2006). The Radiometer ABL 725 has an HbCo measurement range of 0 to 100% with a +0.03 bias in measurement, when compared to the reference gas chromatography method, for determining HbCO (Radiometer Medical ApS., 2009).

Following this, the netting bag of the spirometer was filled with 10 g of soda lime in order to absorb excess carbon dioxide, the rebreathing bag was filled with 4 litres of pure oxygen and carbon monoxide analysers (Fluke CO-220, Fluke, Norwich, UK and Draeger Safety, Northumberland, UK) were switched on. The participant's body weight was measured and the required CO dose was calculated based on $1 \text{ ml} \cdot \text{kg}^{-1}$ and filled into a 100 ml syringe. Ambient barometric pressure and temperature was then noted. A baseline venous blood sample was collected as instructed in section 2.5.1 and analysed using the Radiometer ABL 725. Participant's end-tidal CO concentration was then analysed using the Draeger Pac7000 CO analyser. The Pac 7000 Draeger CO-analyser was connected to the specific adapter and mouthpiece provided by Draeger Safety using a piece of tubing (~9 cm long). The participant was then, whilst wearing a nose-clip, instructed to hold their breath for 20 seconds followed by exhalation into the mouthpiece over a 20 second period after which the maximal value of end-tidal carbon monoxide observed was recorded. Measurements of HbCO were made in triplicates with baseline values not exceeding a < 2% difference.

During the tHb measurement, the participant was then instructed to exhale; initially out with the spirometer, but completing the exhalation in the spirometer beyond residual volume (i.e. cannot exhale any further). Only once the participant has exhaled beyond residual volume, were they instructed to open the rebreathing bag by turning a specified valve. At the same instance, the individualised CO dose was administered via the pre-filled 100 ml syringe, a standard digital stopwatch was started and the individual inhaled deeply and held the breath for 10 seconds. The participant was then instructed to breathe normally (i.e. neither too deeply nor too superficially) into the spirometer for 1 minute and 50 seconds. At the end of the 2 minute period, the participant was instructed to exhale to

residual volume and to then try to fully inflate the rebreathing bag. Following this the participant was disconnected from the spirometer by letting go of the mouthpiece and questions were asked, by the experimenter, regarding potential signs of early CO toxicity (e.g. headache, dizziness). Once no sign of CO toxicity has been established, the end-tidal CO concentration at minute 4 was repeated and a venous blood sample was taken at minute 8 and immediately measured for HbCO as previously described. The rebreathing bag was then measured in order to assess any potential remaining CO using the Draeger Pac7000 CO analyser.

Prior to actual testing each participant was familiarised with the rebreathing procedure where the CO dose was replaced with an equivalent dose of ambient air. In addition, during actually rebreathing measurements a portable CO analyser (Fluke CO-220, Fluke, Norwich, UK) with parts-per-million sensitivity, was placed beside the mouth piece and nose-clip to monitor any potential gas leakages. If this did occur, noted values were used to correct calculated values. Further, the reproducibility of the employed method was quantified by calculating the typical error of measurement (Hopkins, 2000). Precision was good, with a typical error <2%, for the determination of total haemoglobin mass and blood volume performed on consecutive days (Durussel, 2011).

2.6.2.3 Optimised Carbon Monoxide (CO)-rebreathing Calculations

Total haemoglobin mass was calculated as:

$$tHb (g) = K \times MCO \times 100 \times (\Delta HbCO \times 1.39)^{-1}$$

where $K = (\text{current barometric pressure (mmHg)} \times 273.15^{\circ}\text{K}) \times (\text{current temperature (}^{\circ}\text{K)} \times 760 \text{ mmHg})$; $\Delta HbCO\%$ = the difference between basal haemoglobin carbon monoxide percentage (HbCO%) and HbCO% in blood samples after CO administration; 1.39 = Hufner's number of CO binding capacity for Hb ($\text{ml CO} \cdot \text{Hb}^{-1}$) (Gorelov, 2004);

Myoglobin carbon monoxide content (MCO) (ml), the volume of CO bound to Hb = volume of CO administered (ml) - (the amount of CO remaining in the spirometer and lung after disconnection (ml)) + the amount of CO exhaled after rebreathing until blood sampling (ml) + the amount of CO diffused to myoglobin (ml)).

The amount of CO remaining in the spirometer and the lung (ml) = $[\text{CO}]$ in the rebreathing bag (ppm) \times (spirometer volume (ml) + lung residual volume (ml)) $\times 10^{-6}$. The lung

residual volume was estimated according to a prediction equation (Withers and Ball, 1988). Estimated instead of measured residual volume was considered sufficient since an error of 500 ml would lead only to a ~2 g deviation of tHb for a given subject with 900 g of tHb and inhaling 70 ml of CO. The amount of CO exhaled after disconnection (ml) = difference between end-tidal [CO] measured before and after the rebreathing procedure (ppm) x alveolar ventilation (ml·s⁻¹) x time (s) x 10⁻⁶.

The amount of CO diffused to myoglobin from the vascular bed (ml) = 0.3% x volume of CO administered (ml) x time (min) (Nilsson et al., 2002). As myoglobin is another potential binding site for CO, this amount has to be considered to avoid overestimating tHb (Garvican et al., 2010, Nilsson et al., 2002). The calculation of the amount of CO lost to myoglobin, derived by Prommer and Schmidt (2007), was deemed sufficient as a ±10 kg change of lean body mass and therewith myoglobin content would lead only to a ~3 g error of tHb for a given subject with 950 g of tHb.

Finally, blood volume (BV), erythrocyte volume (EV) and plasma volume (PV) were derived from tHb, [Hb] and Hct measures via formulae:

$$\begin{aligned} BV_A (l) &= tHb (g) \times [Hb]^{-1} (g\ dl^{-1}) \times 10^{-1} \\ EV_A (l) &= BVA (l) \times Hct (\%) \times 0.91 \times 10^{-2} \\ PV_A (l) &= BVA (l) - EVA (l) \end{aligned}$$

These formulae have been frequently used in previous research (Christensen et al., 1993, Saunders et al., 2004a, Pottgiesser et al., 2007, Schumacher et al., 2008, Thomsen et al., 1991).

2.7 Blood Pressure

When blood pressure measurements were taken participants were instructed to lay in a supine position for at least 10 minutes prior the automated measurement being taken (Omron HEM705 CP, Omron Healthcare UK Ltd, Milton Keynes, UK). This automated blood pressure monitor has been validated according to the European Society of Hypertension International Protocol (El Assaad et al., 2003). Each occasion involved three measurements taken, of which values were averaged and then used for analysis (Appendix G). Participants were familiarized with the blood pressure taking protocol during the initial screening process.

2.8 Recombinant Erythropoietin Storage, Preparation, Self-administration and Placebo Self-administration

2.8.1 Recombinant Erythropoietin Storage

The r-HuEpo (Neorecormon, Basel, Switzerland) was stored in a refrigerator between 2°C – 8°C (Roche, 2009) and this was monitored and logged on a daily basis. Care was taken to ensure that the reconstituted solution was made in an ambient temperature below 25°C and away from sunlight.

2.8.2 Recombinant Erythropoietin Preparation

The experimenter first washed their hands and wore protective gloves in order to minimise the chance of contamination. The lyophilisate vial was removed from the packing and the date of reconstitution and expiry were written on the label (expiry is 1 month after reconstitution). The protective plastic cap was then removed from the lyophilisate vial and the rubber seal was disinfected using an alcohol wipe. The reconstitution solution and withdrawal device (which allows sterile air exchange) was taken out and the protective cover from the spike was removed. The withdrawal device was attached to the vial until the snap lock clicked into place. A needle and syringe, contained in the packaging, was then lifted and the needle cover removed. The solvent containing One-Point-Cut ampoule was then shook/tapped in order to get any fluid, in the stem, into the body of the ampoule. The stem was then snapped off away from the experimenter. All the solvent was withdrawn into the syringe. The rubber seal of the withdrawal device was then disinfected using an alcohol wipe. The seal was penetrated with the needle, to a depth of about 1 cm, and the solvent was slowly injected into the vial. The syringe (with needle) was then disconnected from the withdrawal device. The reconstituted vial was swirled gently until the lyophilisate dissolved. The solution was then checked to ensure that the solution was clear, colourless and free of particles. If this was not the case, the injection was discarded.

2.8.3 Recombinant Erythropoietin Self-administration Instructions

Participants were instructed that they were to self-administer the injection subcutaneously at the abdomen. The injection was warmed, by the experimenter, by rolling the filled syringe between the hands for a few minutes. This was a comfort measure for the person receiving the injection. The injection site was then exposed by the participant and the area

was clean in a circular motion with an alcohol swab, beginning at the centre and moving outwards. The area was then allowed to dry for approximately ten seconds.

Participants were instructed to remove the needle cap and hold the syringe, as though holding a pencil, and were instructed to pinch the skin where the injection is to be made with the other hand. Participants were instructed to hold the syringe at a 45-90 degree angle and about two inches from the skin surface. Participants were instructed to insert the needle with a quick jab with the needle going all the way into the skin. If no blood was identified in the syringe, the participant was then instructed to slowly push down on the plunger until all the solution was injected. A clean alcohol swab was then placed over the needle and the skin site, without pressing down, and the needle was pulled out. Pressure was then applied to the injection site. Participants were instructed to remove the swab after five to ten seconds and an adhesive bandage or plaster was placed over the site in case of any bleeding.

The exact same procedure was followed in Chapter 6 for the self-administration of placebo except r-HuEpo was replaced by saline.

2.8.4 Recombinant Erythropoietin Dosages and Iron Supplementation

In Chapter 4, each participant received $50 \text{ U}\cdot\text{kg}^{-1}$ body mass subcutaneous injections of r-HuEpo (Neorecormon, Roche, Switzerland) every 2 days over a 4-week period and this is in line with previous studies (Parisotto et al. 2000). Participants also received daily iron tablets providing approximately 105 mg of elemental iron derived from 350 mg of dried ferrous sulphate (Abbott Ltd, Maidenhead, UK). This was carried out as studies have shown oral iron supplementation to be more effective than intravenous in assisting with red blood formation and development (Parisotto et al., 2000a).

Saline (NaCl 0.9% BP, Baxter, Glasgow, UK) injections were substituted for r-HuEpo injections if a pre-determined haematocrit (Hct) limit of 55% was reached in response to r-HuEpo. Participants resumed the standard r-HuEpo regimen once Hct dropped below 55%. The American College of Sports Medicine position on Epo and blood doping (Sawka et al. 1996) refers to an Hct greater than 55% as “dangerous”. However, ceasing r-HuEpo injections if Hct reaches 55% is a medical safeguard which in-line with previous studies commissioned by the World Anti-doping Agency (WADA) and the International Olympic Committee (IOC) with no untoward events recorded (e.g. Parisotto et al. 2001). The dangers associated with elevated Hct values relates to blood viscosity and, in turn, the

increased risk of thrombosis and related events. There have been no reports of significant side effects as a result of r-HuEpo injections in normal healthy individuals and, in athletes, there has been one report of elevated systolic blood pressure (from 177 to 191 mmHg) during moderate-intensity exercise (compared with mild or low intensity exercise) following treatment with r-HuEpo at doses of ~30 units per kilogram body weight over 6-7 weeks. Other potential side effects include flu like symptoms (mild and of short duration), suppression of natural r-HuEpo production, a burning sensation associated with subcutaneous injection of r-HuEpo, joint pains, rashes, and one case of a hypotensive episode associated with r-HuEpo injection.

The management of side effects including allergy and arthralgia, and those listed above, included the cessation of r-HuEpo treatment and withdrawal from further participation in the study. It is important to highlight that higher doses of r-HuEpo per kilogram body weight per week have been safely used in previous studies (Audran et al., 1999). Further, athletes typically have Hct levels lower than those of non-exercising populations. With values around 40-45% (compared with “normal” values of 45-50%) and the lower Hct values have been associated with training-induced haemodilution (Szygula, 1990).

This effect of training should minimise the risks of hyperviscosity associated with r-HuEpo administration. In addition, throughout the study period, participants were encouraged to maintain normal hydration practices. Finally, the administration of the first r-HuEpo dose was carried out under medical supervision because of the very low but possible risk of anaphylaxis (Goodnough et al., 2000).

2.9 Psychological and Psychosocial Measures

Prior the use of psychological and psychosocial measures, an expert in the field was contacted (at Swansea University). Based on expert advice and on what quantitative scales and qualitative interview questions have been previously used in similar type of research, it was concluded that the following quantitative and qualitative measures should be carried out:

2.9.1 Quantitative measures

The profile of moods (POMS) and cognitive-state anxiety (CSAI-2) (Appendix D) questionnaires were used in Chapters 4 and 6. The POMS and CSAI-2 questionnaires contained 24 and 27 self-report items with a 4-point scale, respectively. Participants were

instructed to choose from 0 (not at all) to 4 (extremely/very much) as to how they felt in response to the question. The questionnaires took a combined time of approximately 3 to 7 minutes to complete. Participants were familiarised with each questionnaire prior experimental use.

2.9.2 Qualitative measures

In Chapters 4 and 6, participants individually took part in one and two semi-structured interviews, respectively. In the case of experimental Chapter 6, one interview was carried out prior the placebo deception being revealed and one after the placebo deception being revealed. In each case, the interviews were carried out on the final day of each respective experimental protocol (Appendix E). All interviews involved participants answering questions in relation to the trial. In Chapter 6, upon completion of interview one, subjects were given a scripted debriefing document to read after the initial interview was completed and this revealed that a deception had taken place with further information explaining the rationale behind the study.

3. The Evaluation of the Cosmed K4b2 Portable Metabolic System during Running Outdoors

3.1 Introduction

Modern technological developments in portable metabolic systems have enabled traditional laboratory based practices to be taken into the field and many research studies have used these systems (Strath et al., 2000, Billat et al., 2000, Sheel et al., 2003, Roels et al., 2005, Abel et al., 2008, Thornton et al., 2011, Bertuzzi et al., 2012). One widely used system is the Cosmed K4b2 system (K4b2, COSMED s.r.l., Rome, Italy).

Several studies have examined the validity and reliability of the K4b2 portable metabolic system (McLaughlin et al., 2001, Pinnington et al., 2001, Eisenmann et al., 2003, Duffield et al., 2004, Hodges et al., 2005, Schrack et al., 2010) however, all but one study have considered a static indoor testing environment (i.e. treadmill or cycling ergometer). The only study to investigate K4b2 accuracy in an outdoor environment used roller skiing exercise (Doyon et al., 2001). There have been no published studies (to the best of the author's knowledge) on the accuracy of the K4b2 during running activities in outdoor environments. Given the differences between the static laboratory setting and the often dynamic outdoor setting, it does not necessarily follow that validity will transfer to the latter.

The main potential problem that may be incurred during outdoor testing is interference of facing head winds with the K4b2 flow-meter and sampling line. The extent to which this theoretical factor may influence oxygen uptakes, calculated by the K4b2 during outdoor running is unclear and thus there is insufficient data available to determine whether the K4b2 is reliable and accurate for use during testing outdoors. Despite this, the K4b2 has been used for data collection in outdoor environments in a number of studies (Devienne and Guezennec, 2000, Billat et al., 2003).

The purpose of this study was therefore to determine the validity of the K4b2 for determining oxygen uptake during treadmill and outdoor running.

3.2 Methods

3.2.1 Participants

Nineteen trained male volunteers (age: 22.9 ± 1.0 years; weight: 74.1 ± 1.8 kg; height: 179.2 ± 1.4 cm; $\dot{V}O_{2\max}$ 59.4 ± 0.30 ml·kg⁻¹·min⁻¹ mean \pm SD) were recruited as described in section 2.1 with inclusion and exclusion criteria also described.

3.2.2 Experimental Design and Procedures

Each participant completed three continuous maximal incremental running tests in random order: K4b2 indoors, K4b2 outdoors and Douglas bag (DB), as previously described (see section 2.4.1). Cardiopulmonary measurements obtained using the K4b2 were compared to the DB.

K4b2 indoor tests were carried out in a mean ambient temperature of 21°C (Comark N8006, Hertfordshire, UK) and were performed on a motorised treadmill (Woodway PPS55 Med, Weilam Rhein, Germany). Douglas bag indoor tests were carried out under the same conditions. K4b2 outdoor tests were carried out on a 400 m concrete running track (Bellahouston Park, Glasgow, UK) with a mean ambient temperature of 18°C and with no to slight winds (<2 m·s⁻¹, Skymaster Wind Meter SM-28, Speedtech Instruments, Great Falls, VA, USA).

Running tests for each individual were separated by at least two days for appropriate recovery and were scheduled at approximately the same time of day to avoid the influence of diurnal variation on the metabolic response to exercise. Each participant completed the trial within a 2-week period. Participants were requested to wear the same athletic clothing and footwear for all tests.

Participants reported to the laboratory on the day of testing following a 3-hour fast and having refrained from alcohol, caffeine and strenuous exercise the day before. Participants' height and weight were measured at the start of the study and body weight (Avery, England, UK) and height (Invicta Plastics Ltd, Leicester, UK) were measured prior each test as previously described. Participants wore a heart rate transmitter belt (Polar NV, Polar Oy, Kempele, Finland) and were asked to perform their usual warm-up before commencing each test.

3.2.3 Statistical Analysis

Statistical analysis was completed using Statistica statistical software (Release 6.0, Statistica, StatSoft, Inc., Tulsa, OK, USA). Differences in $\dot{V}O_2$, $\dot{V}CO_2$, F_{EO_2} , F_{ECO_2} , \dot{V}_E and respiratory exchange ratio (RER) between conditions were compared by a 2-way Analysis of Variance (condition x running speed) with Tukey *post hoc* tests used to identify where differences lay. Linear regression analyses were used to determine the relationships between $\dot{V}O_2$ measured under the three different conditions. A limits of agreement approach (Bland and Altman, 1986, Bland and Altman, 1996) was used to determine agreement between $\dot{V}O_2$ measured under the three different conditions. All continuous data were checked for normality utilising the Anderson-Darling test and transformed where appropriate. All data are presented as mean \pm SEM unless otherwise stated.

3.3 Results

Figures 3.1, 3.2, 3.3, 3.4, 3.5, 3.6 and 3.7 show the data obtained for $\dot{V}O_2$, $\dot{V}CO_2$, F_{EO_2} , F_{ECO_2} , \dot{V}_E , RER and HR, respectively, for K4b2 indoors, K4b2 outdoors and Douglas bag method conditions.

Oxygen uptake, when measured by K4b2 indoors, was significantly higher than when measured using the Douglas bag method or K4b2 outdoors at all running speeds ($P = 0.03$) (Figure 3.1). No statistically significant differences were found between K4b2 outdoors and the Douglas bag method for $\dot{V}O_2$ at any running speed ($P > 0.05$). K4b2 indoors $\dot{V}CO_2$ measures were significantly higher than the Douglas bag method ($P = 0.03$) and K4b2 outdoors ($P = 0.02$) at a running speed of $12 \text{ km}\cdot\text{h}^{-1}$. No statistically significant differences were found between K4b2 outdoors and the Douglas bag method for $\dot{V}CO_2$ at any running speed except at $16 \text{ km}\cdot\text{h}^{-1}$ ($P = 0.01$) (Figure 3.2).

No statistically significant differences were found between K4b2 indoors and K4b2 outdoors for F_{EO_2} at any running speeds except at $8 \text{ km}\cdot\text{h}^{-1}$ ($P < 0.05$). K4b2 F_{EO_2} measures, both indoors and outdoors, were significantly lower when compared to the Douglas bag method at all running speed ($P < 0.05$) (Figure 3.3). No statistically significant differences were found between K4b2 indoors and K4b2 outdoors for F_{ECO_2} at any running speeds. The Douglas bag method was significantly lower for F_{ECO_2} when compared to K4b2 indoor and K4b2 outdoor measures at all running speed ($P < 0.05$) except at $16 \text{ km}\cdot\text{h}^{-1}$ (Figure 3.4).

No statistically significant differences were found between K4b2 indoors and the Douglas bag method for \dot{V}_E at any running speed. The K4b2 indoors was significantly higher, for \dot{V}_E , than the K4b2 outdoors at all running speeds ($P < 0.05$) except at $8 \text{ km}\cdot\text{h}^{-1}$ (Figure 3.5).

No statistically significant differences were found between the K4b2 indoors and K4b2 outdoors for RER at all running speed. The K4b2 indoors was statistically lower from the Douglas bag method for RER at all running speeds ($P < 0.05$) except at $10 \text{ km}\cdot\text{h}^{-1}$. The K4b2 outdoors was statistically lower from the Douglas bag method for RER at all running speeds ($P < 0.05$) except at 8 and $10 \text{ km}\cdot\text{h}^{-1}$ (Figure 3.6). Heart rate measurements were not significantly different between any conditions and at any running speeds (Figure 3.7).

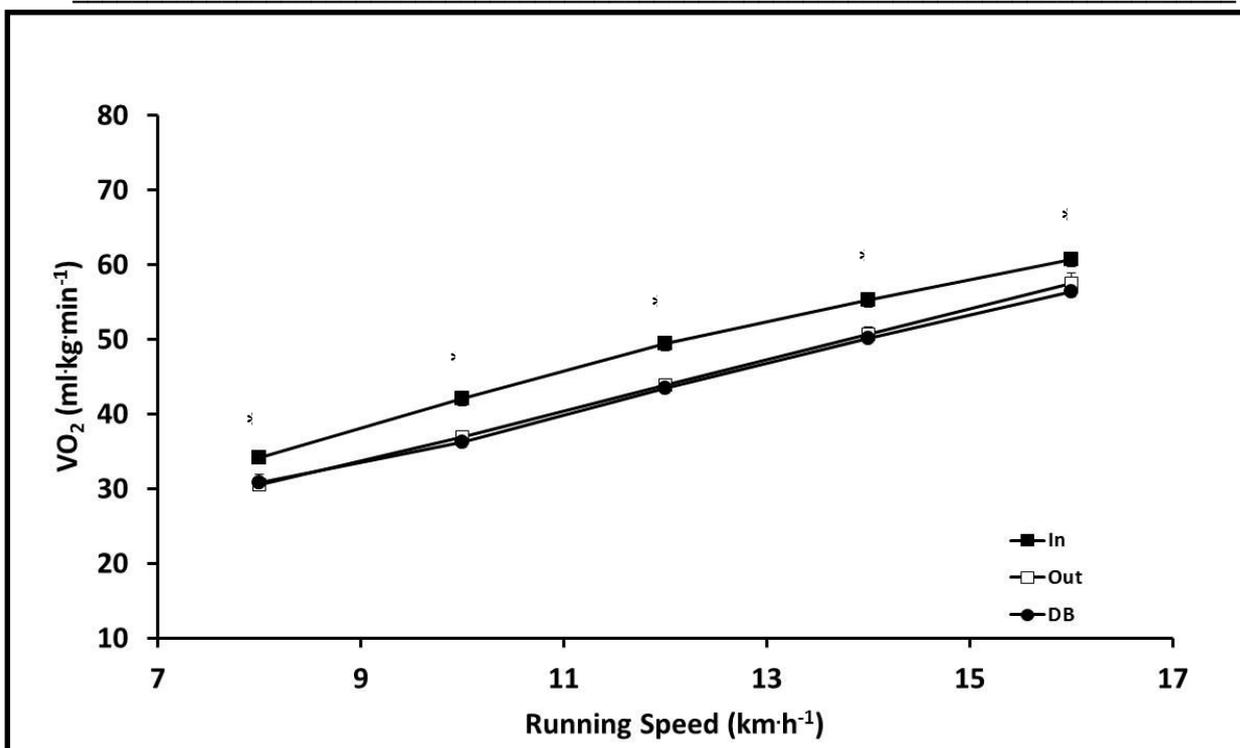


Figure 3.1: $\dot{V}O_2$ values at different running speeds for K4b2 indoors (In), K4b2 outdoors (Out) and Douglas Bag (DB) conditions. * K4b2 indoors significantly different ($P < 0.05$) from K4b2 outdoors and Douglas bag method.

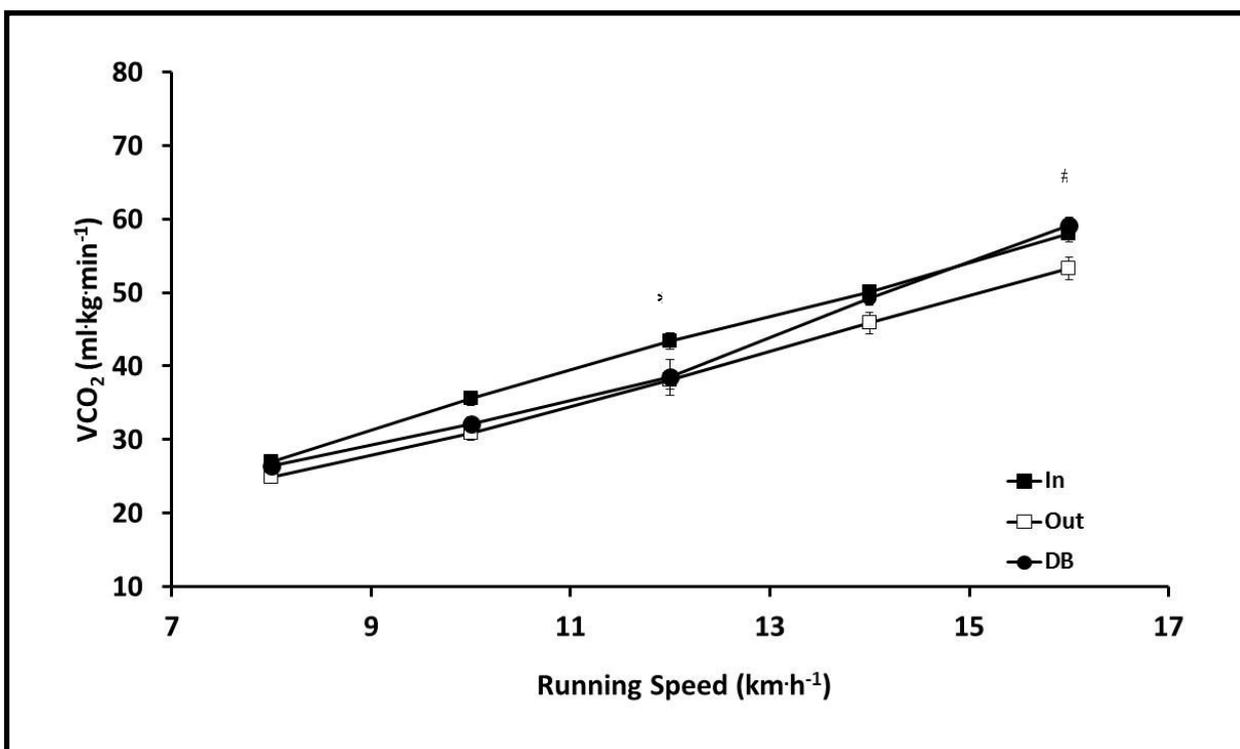


Figure 3.2: $\dot{V}CO_2$ values at different running speeds for K4b2 indoors (In), K4b2 outdoors (Out) and Douglas Bag (DB) conditions. * K4b2 indoors significantly different ($P < 0.05$) from K4b2 outdoors and Douglas bag method. # K4b2 outdoors significantly different ($P < 0.05$) from the Douglas bag method.

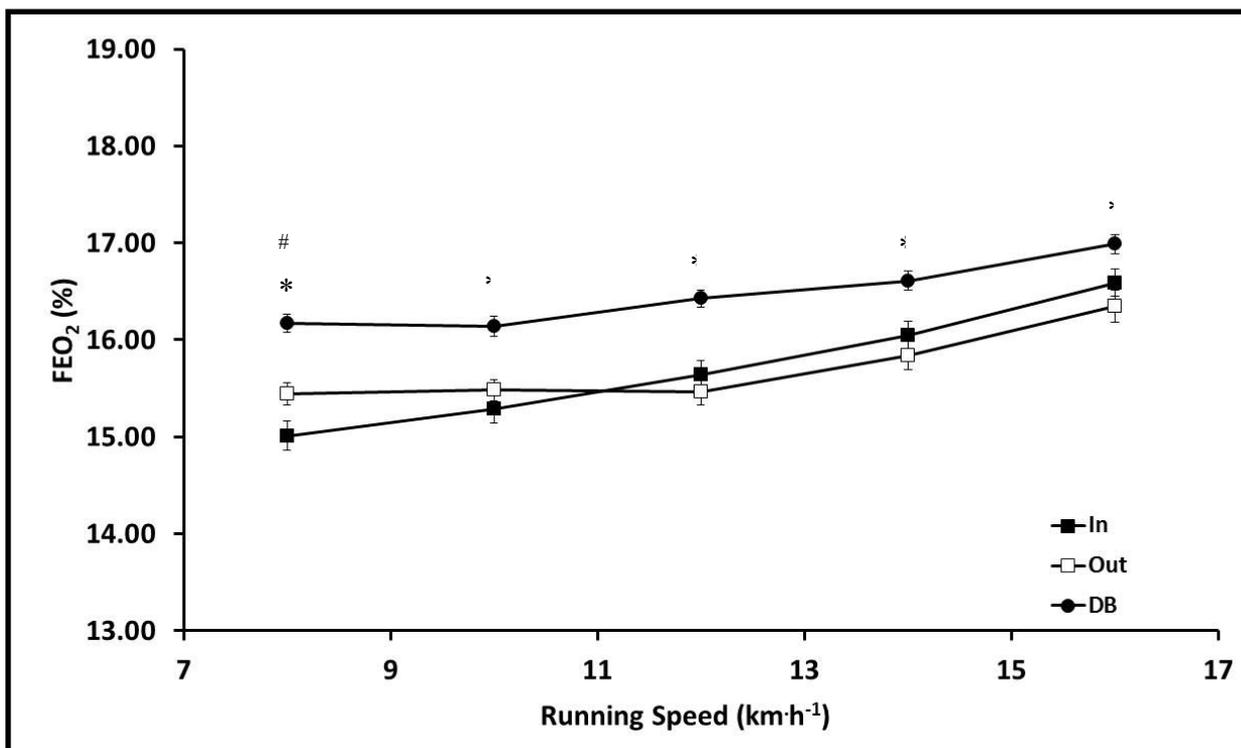


Figure 3.3: FEVO₂ values at different running speeds for K4b2 indoors (In), K4b2 outdoors (Out) and Douglas Bag (DB) conditions. * K4b2 indoors and K4b2 outdoors significantly different (P < 0.05) from Douglas bag method. # K4b2 indoors significantly different (P < 0.05) from K4b2 outdoors.

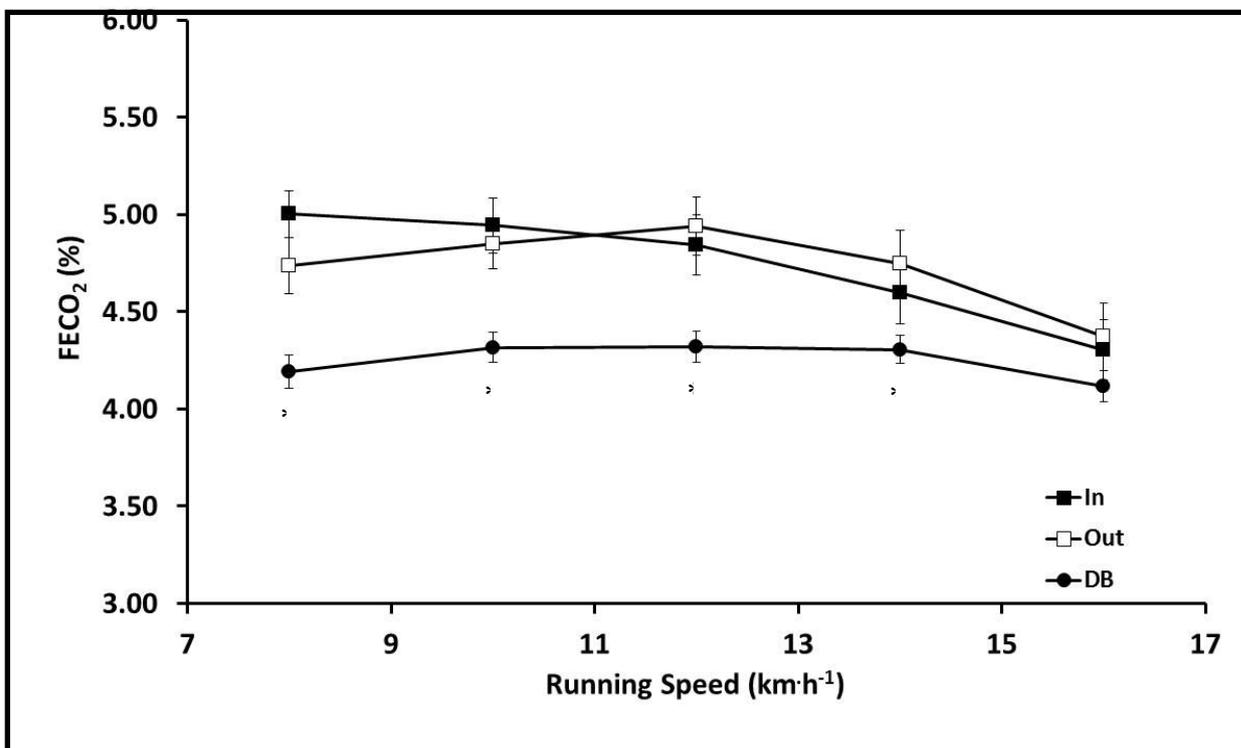


Figure 3.4: FEVC₀₂ values at different running speeds for K4b2 indoors (In), K4b2 outdoors (Out) and Douglas Bag (DB) conditions. * K4b2 indoors and K4b2 outdoors significantly different (P < 0.05) from Douglas bag method.

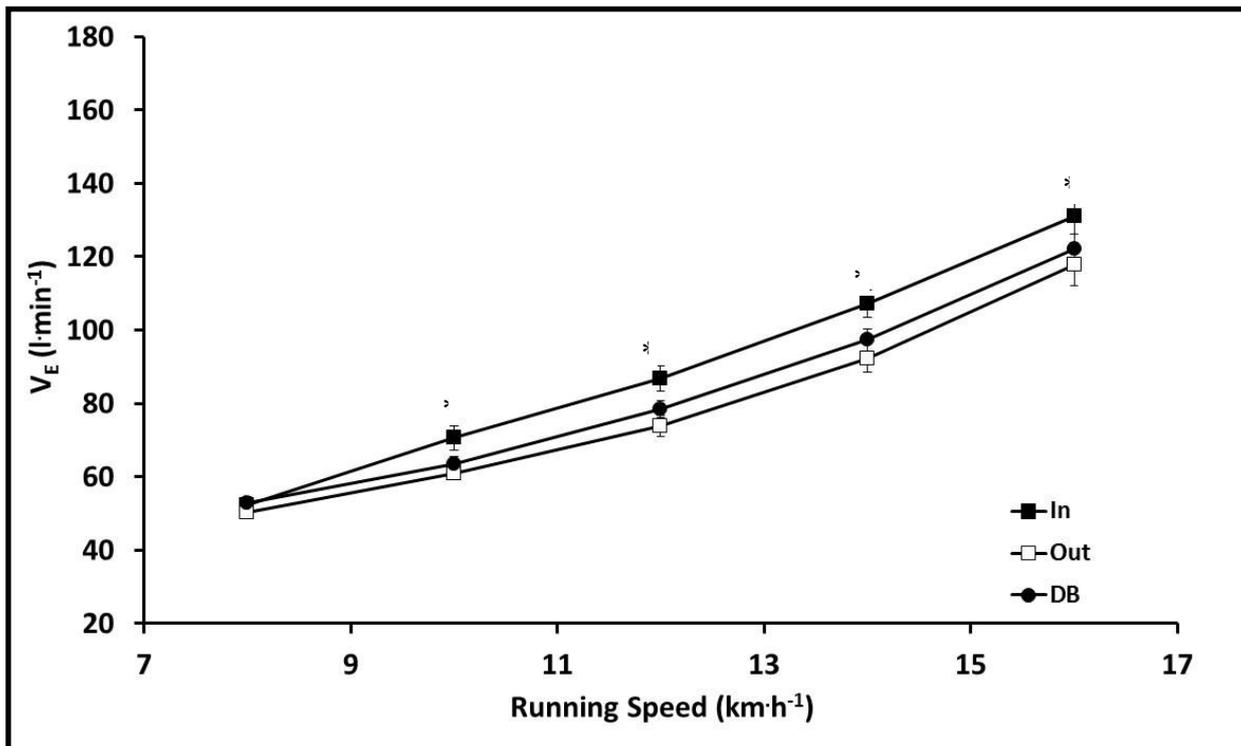


Figure 3.5: \dot{V}_E values at different running speeds for K4b2 indoors (In), K4b2 outdoors (Out) and Douglas Bag (DB) conditions. * K4b2 indoors significantly different ($P < 0.05$) from K4b2 outdoors.

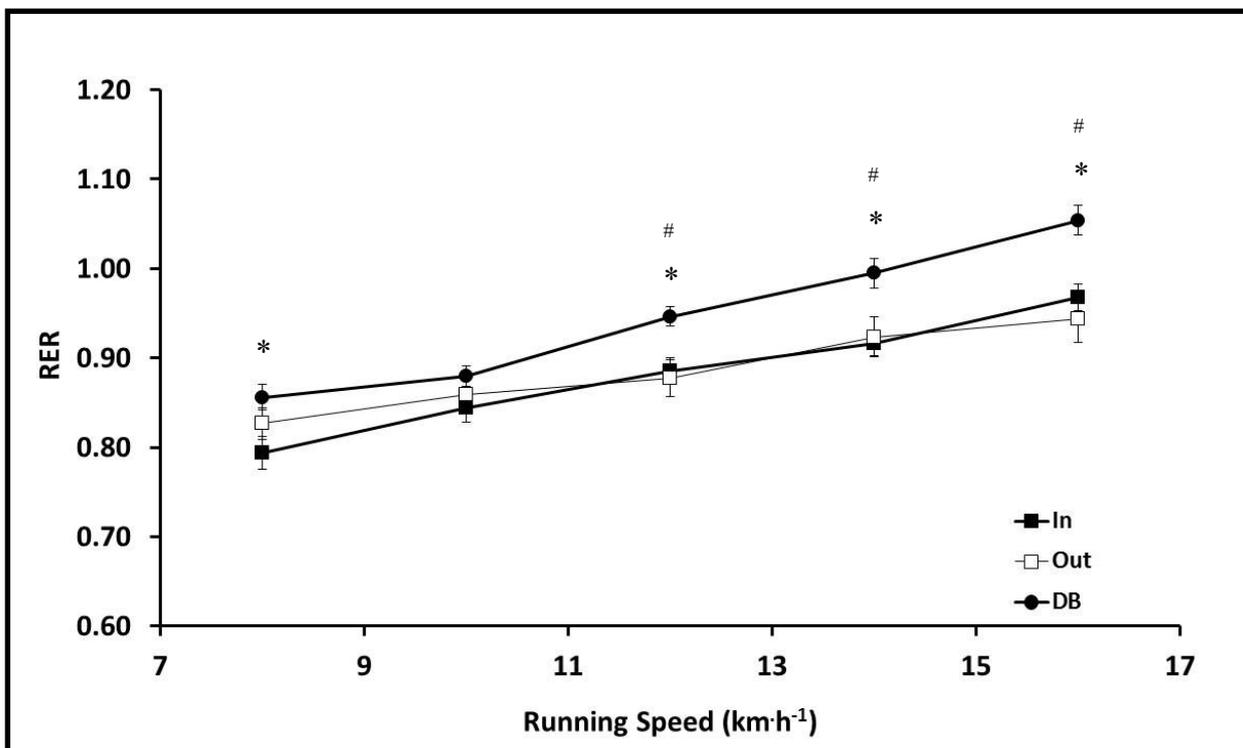


Figure 3.6: RER values at different running speeds for K4b2 indoors (In), K4b2 outdoors (Out) and Douglas Bag (DB) conditions. * K4b2 indoors significantly different ($P < 0.05$) from Douglas bag method. # K4b2 outdoors significantly different ($P < 0.05$) from Douglas bag method.

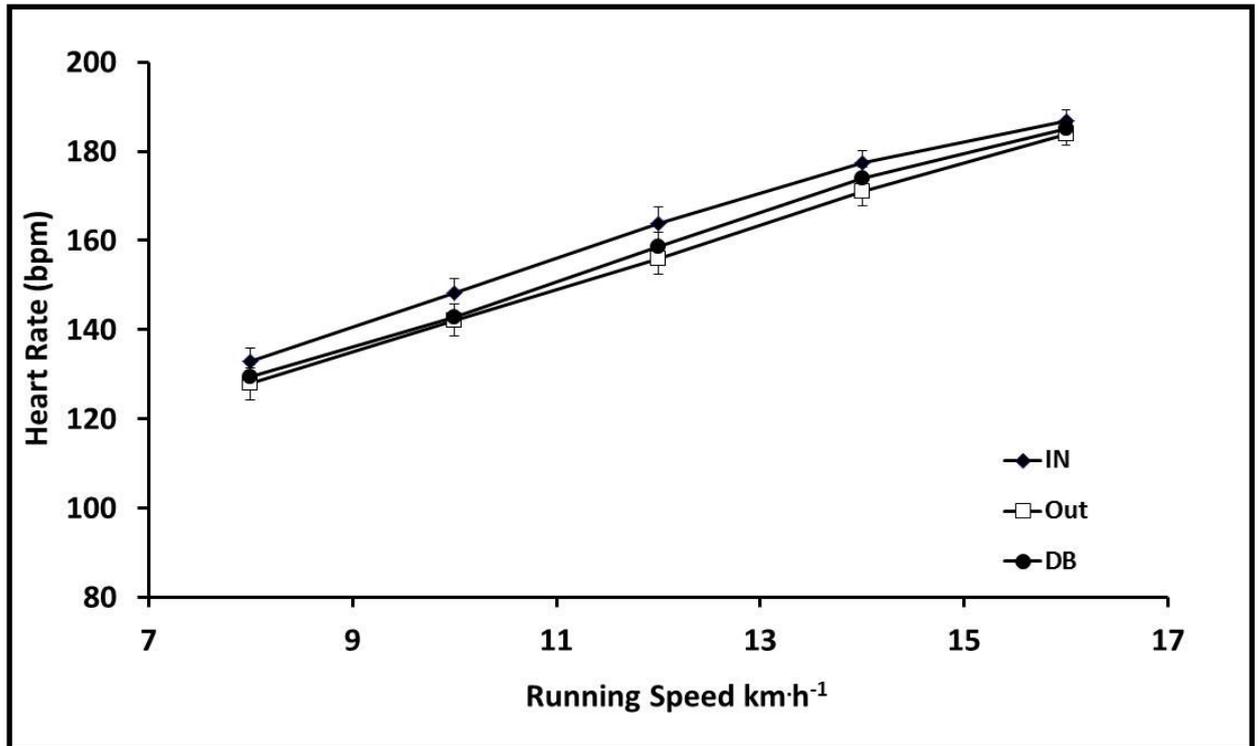


Figure 3.7: Heart rate values at different running speeds for K4b2 indoors (In), K4b2 outdoors (Out) and Douglas Bag (DB) conditions. No significant differences found between conditions at any running speed.

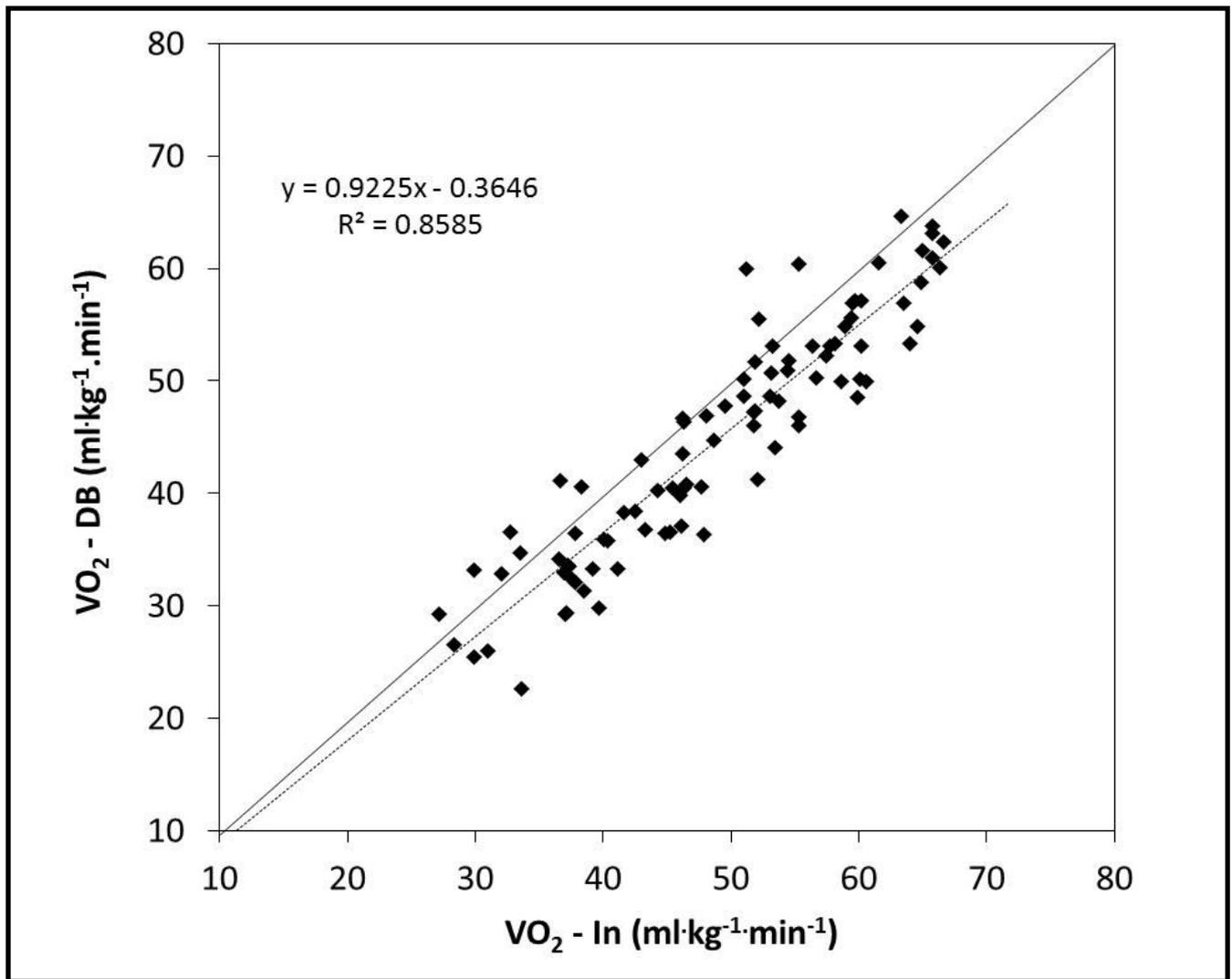


Figure 3.8: Scatterplot of K4b2 indoors (In) and Douglas bag method (DB) for $\dot{V}O_2$ (solid line: line of identity; dashed line: line of best fit).

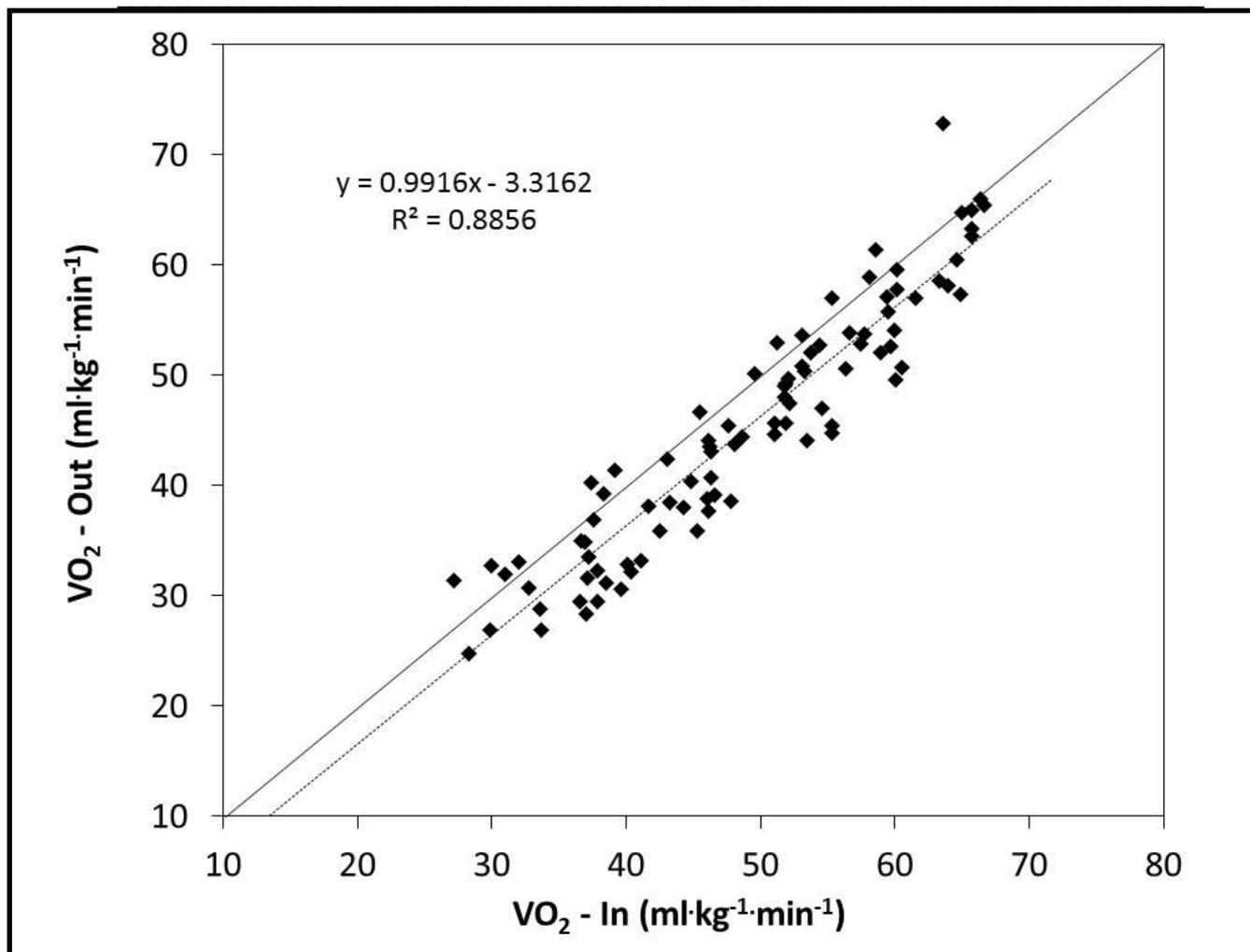


Figure 3.9: Scatterplot of K4b2 indoors (In) and K4b2 outdoors (Out) for $\dot{V}O_2$ (solid line: line of identity; dashed line: line of best fit).

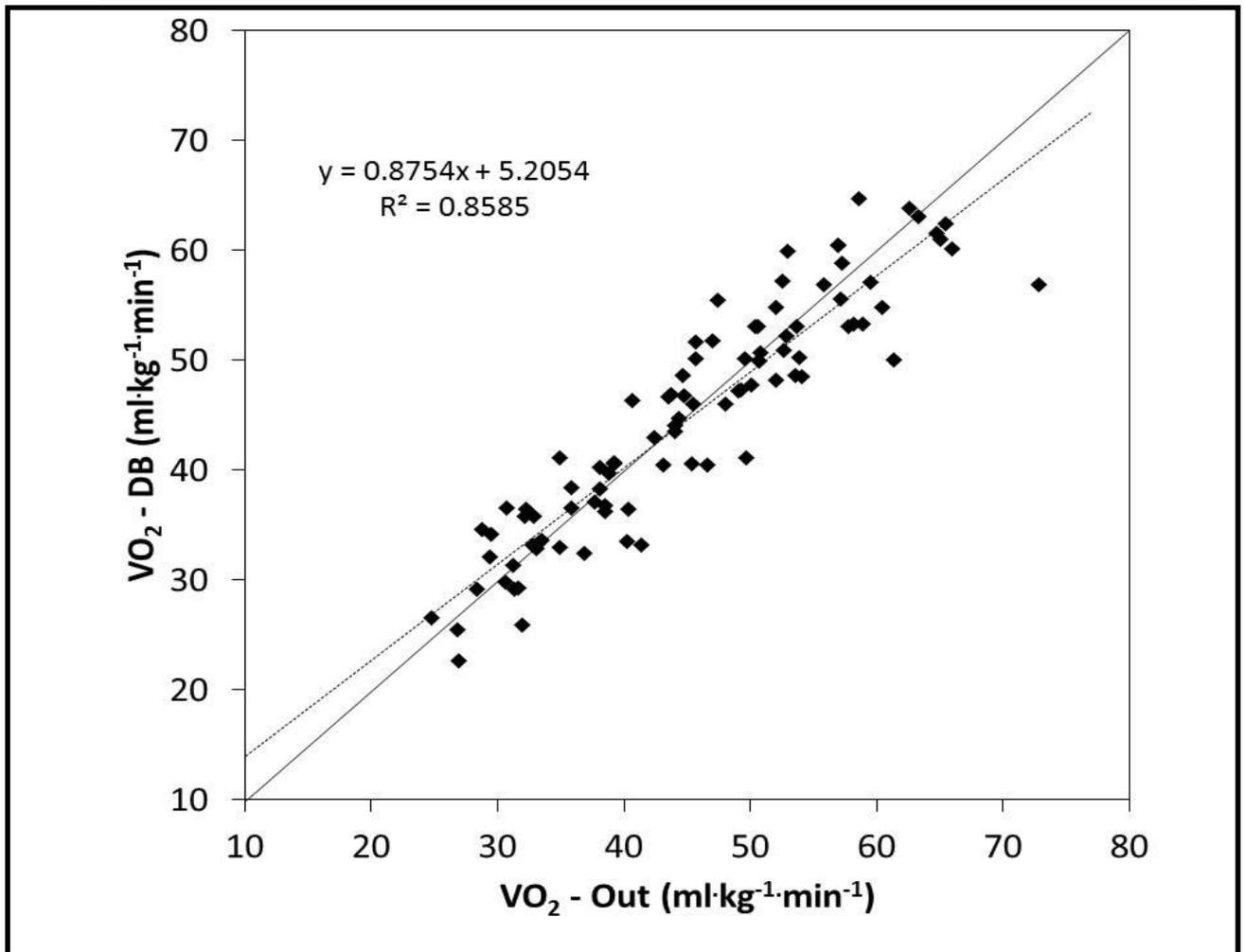


Figure 3.10: Scatterplot of K4b2 outdoors (Out) and Douglas bag method (DB) for $\dot{V}O_2$ (solid line: line of identity; dashed line: line of best fit).

Figure 3.8 shows the relationships between $\dot{V}O_2$ measured using the Douglas bag method and $\dot{V}O_2$ measured using the K4b2 indoors. The shared variance (r^2) between the Douglas bag method and K4b2 indoors was 85.9%. The gradient of the line was 0.92 with an intercept of $0.36 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. This indicates that the intercept was very close to zero (only a $0.36 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ offset), but the K4b2 indoors systematically overestimated $\dot{V}O_2$ when compared to Douglas bag by 8.4% (i.e. $1/0.9225$).

Figure 3.9 shows similar data for K4b2 indoors versus the K4b2 outdoors. The shared variance (r^2) between the two methods was 88.6%. In contrast to Figure 3.8, a substantial offset is shown between the two methodologies with the K4b2 outdoor intercept being systematically $3.32 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ lower than K4b2 indoor measurements. Furthermore the gradient of the line was very close to 1 (exactly 0.99) so there was only a 0.85% (i.e. $1/0.9916$) difference in the increment in $\dot{V}O_2$ between these two conditions. Thus, in contrast to the previous comparison, when comparing K4b2 indoors and K4b2 outdoors, the gradient was the essentially the same with a change in intercept.

Figure 3.10 shows the relationships between $\dot{V}O_2$ measured using the Douglas bag method and $\dot{V}O_2$ using the K4b2 outdoors. The shared variance between the two methods was 85.9%. The gradient of the line was 0.88 with an intercept of $5.2 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. This intercept indicates (i.e. a $5.2 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) a K4b2 outdoors negative offset by $5.2 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, but this was coupled with a K4b2 outdoors overestimation of $\dot{V}O_2$ by 14.2% (i.e. $1/0.8754$). The direction of these errors were opposite, thus for values towards the middle of the $\dot{V}O_2$ range these errors appear to cancel out.

This is further seen when we consider the Bland Altman plots (Figures 3.11-3.13) where mean biases and levels of agreement between conditions, for $\dot{V}O_2$, are also shown.

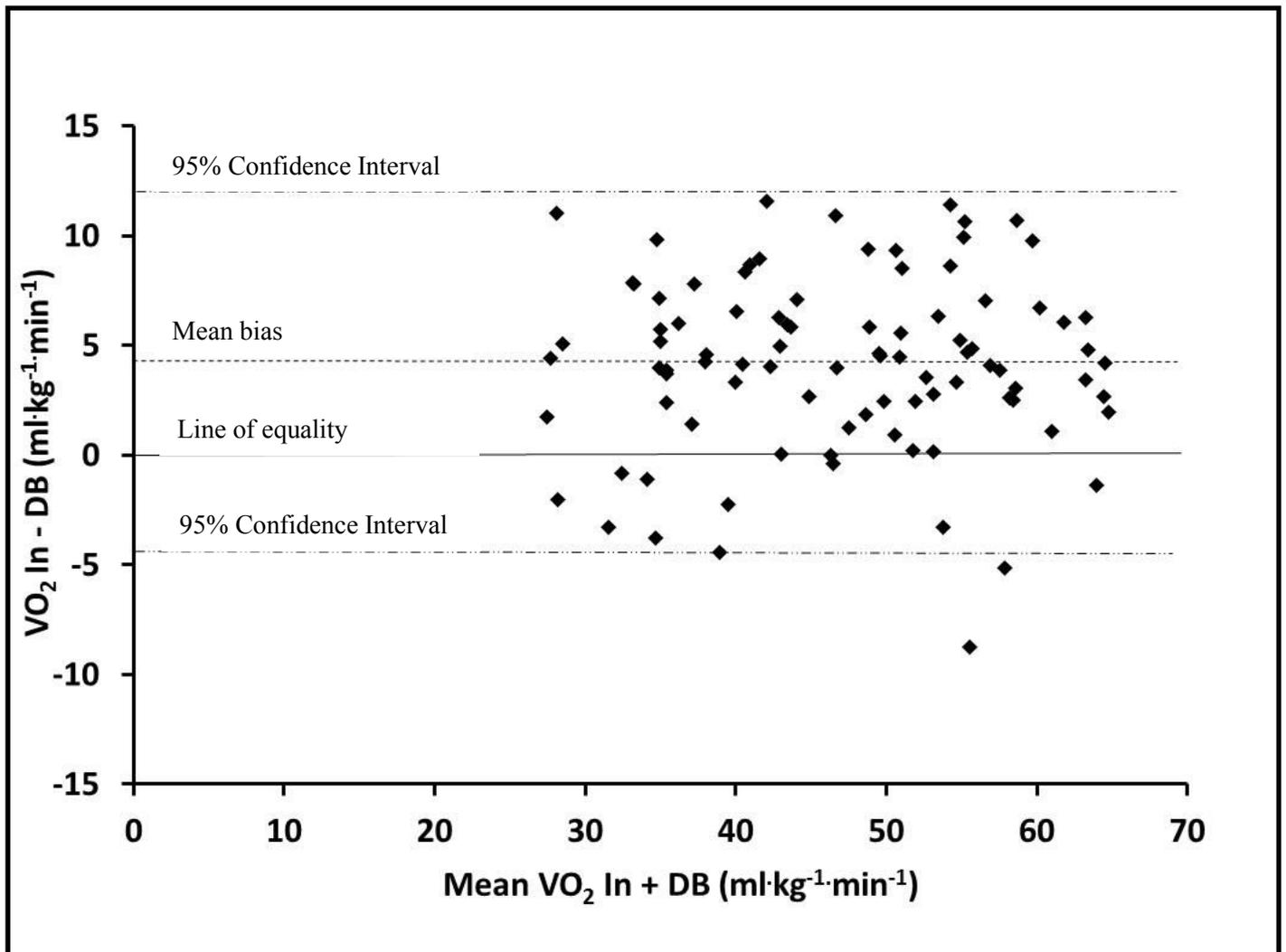


Figure 3.11: Bland and Altman plot of K4b2 indoors (In) and the Douglas bag method (DB).

Mean $\dot{V}O_2$ was $4.2 \text{ ml kg}^{-1} \cdot \text{min}^{-1}$ higher with the K4b2 indoors than when compared to the Douglas bag method. 95% confidence interval limits of agreement range from -3.7 and $12.1 \text{ ml kg}^{-1} \cdot \text{min}^{-1}$. This is equivalent to a typical measurement error of 6.7% (i.e. $\sqrt{2} \times \text{SD}$) or $2.9 \text{ ml kg}^{-1} \cdot \text{min}^{-1}$.

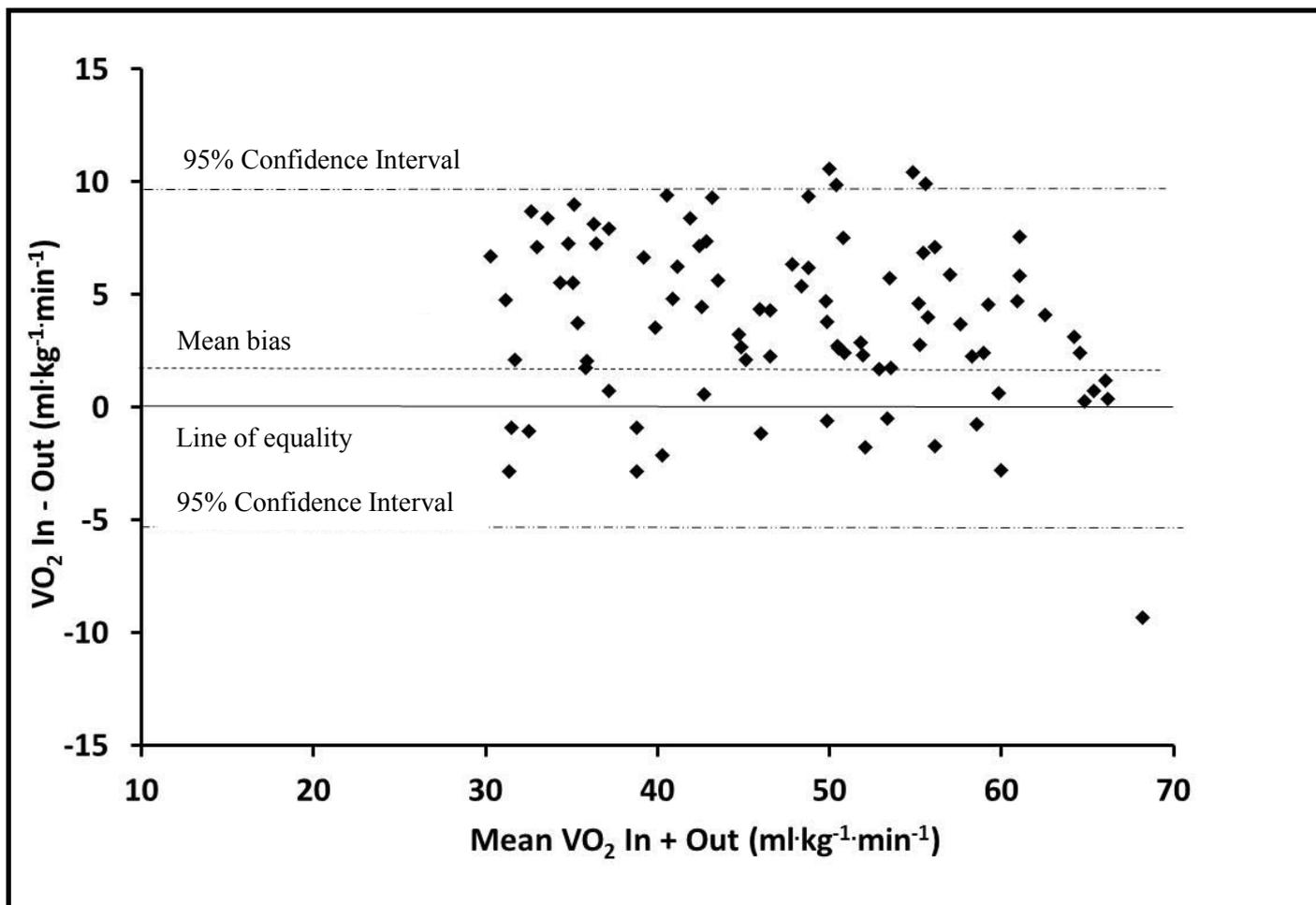


Figure 3.12: Bland and Altman plot of K4b2 indoors (In) and the K4b2 outdoors (Out).

Mean $\dot{V}O_2$ was $1.9 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ higher with the K4b2 indoors than when compared to the K4b2 outdoors. 95% confidence interval limits of agreement range from -5.7 and $9.4 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. This is equivalent to a typical measurement error of 6.3% (i.e. $\sqrt{2} \times \text{SD}$) or $2.7 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$.

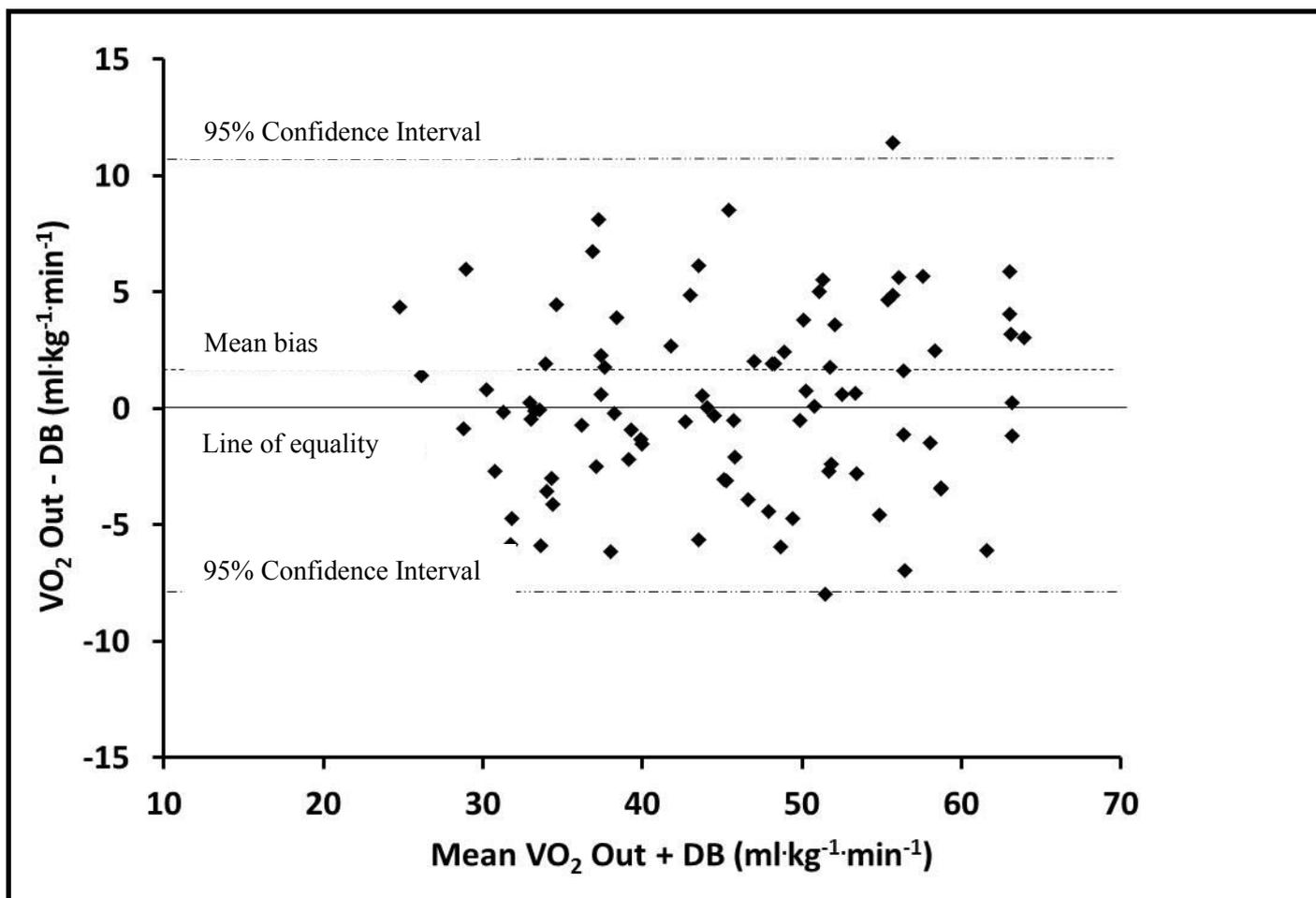


Figure 3.13: Bland and Altman plot of K4b2 indoors (In) and K4b2 outdoors (Out).

Mean $\dot{V}O_2$ was $2.3 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ higher with the K4b2 outdoors than when compared to the Douglas bag method. 95% confidence interval limits of agreement range from -6.1 and $10.7 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. This is equivalent to a typical measurement error of 6.5% (i.e. $\sqrt{2} \times \text{SD}$) or $3.0 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$.

3.4 Discussion

The primary purpose of this study was to assess the accuracy of the K4b2 in measuring $\dot{V}O_2$ during running in an outdoor environment. To the author's knowledge this is the first study to do so. In summary, $\dot{V}O_2$ measured using the K4b2 outdoors appeared to provide similar values to those obtained using the Douglas bag method. However, this apparent agreement was a consequence of two different errors which acted in opposite directions (i.e. a $5.2 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ negative offset in $\dot{V}O_2$ coupled with a 14.2% higher gradient in the relationship between the K4b2 outdoors and the Douglas bag method for measuring $\dot{V}O_2$).

This is further illustrated from the Bland Altman plot (Figure 3.13) which indicates a typical measurement error in $\dot{V}O_2$ between the K4b2 outdoors and the Douglas bag method of 6.5%. In other words, on average, values between the K4b2 outdoors and the Douglas bag method will differ by 6.5%. The 95% confidence interval range for agreement between these two methods was $16.8 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. This degree of error is substantial and thus limits the potential for using the K4b2 for the measurement of potentially subtle differences in performance measures such as $\dot{V}O_{2\text{max}}$, $\dot{V}O_2$ at lactate threshold or running economy.

As there are two methodological changes when moving from the Douglas bag method to the K4b2 outdoors (i.e. change in device and change in condition), it is logical to consider these two aspects separately in order to further assess the sources of these observed errors.

The first aspect to consider is the difference between the Douglas bag method and K4b2 when both were used under the indoor treadmill condition. In this instance, one methodological difference is the added $\sim 1.5 \text{ kg}$ weight that wearing the K4b2 device adds. For the average person in the group (i.e. weighing 74.1 kg), this represents a 2% increase in weight, which would in turn be expected to increase $\dot{V}O_2$ by 2%. Thus, the added $\sim 1.5 \text{ kg}$ weight that the K4b2 indoors contributes cannot solely explain the 8.4% difference in $\dot{V}O_2$ found. Although not statistically different, heart rate values during K4b2 indoor tests were numerically higher by an average of 3% when compared to both the Douglas bag method. This may indicate a small additional workload during the K4b2 indoor condition. This could conceivably reflect the additional weight and potentially breathing resistance from the K4b2 mask. Ventilation rates were also numerically higher during K4b2 indoor tests when compared to the Douglas bag method by an average of 6.2%. Interestingly, in previous literature examining K4b2 measured $\dot{V}O_2$ with various reference methods, indoor

cycling ergometer based assessments have, more often, shown good agreement (McLaughlin et al., 2000, Parr et al., 2001). However, there seems to be discrepancies where treadmill running is involved with at least one study (Duffield et al., 2004) showing an over-estimation of $\dot{V}O_2$ by the K4b2 device; similar to the findings reported here. A key difference between cycling and running exercise is that the exercising individual does not need to support their own body weight in cycling, thus the weight of the K4b2 should not influence $\dot{V}O_2$ values. The fact that the $\dot{V}O_2$, during running, was higher by more than what would be expected by the weight of the device suggests that wearing the K4b2 may have a negative impact on biomechanical efficiency which may, in turn, have reduced running economy. Another interesting point to consider would be the potential psychological impacts, on performance, of the device physically attached to the individual.

Looking at Figure 3.5, \dot{V}_E was on average 6.2% higher with K4b2 indoors than when compared to the Douglas bag method. Thus, given similar levels of F_{EO_2} , \dot{V}_E could explain approximately two-thirds of the difference in $\dot{V}O_2$ (8.4%) between the two methods. Thus, about a third of the difference must be explained by differences in oxygen extraction (i.e. F_{EO_2}). The average F_{EO_2} measures for the Douglas bag method and K4b2 indoor conditions was 16.47% and 15.72%, respectively. Assuming an ambient O_2 concentration of 20.93%, a 4.46% and 5.21% extraction of O_2 occurred with the Douglas bag method and K4b2 indoor conditions, respectively. Thus, the percentage difference (i.e. $((5.21-4.46) / 4.46)*100$) between these averages was 16.8% which is much higher than what is needed to explain the remaining difference (i.e. 2.2%) in $\dot{V}O_2$ between the two methodologies. Thus, due to such large F_{EO_2} discrepancies, it seems logical to assess potential differences in how F_{EO_2} is measured and used for the calculation of $\dot{V}O_2$ between the K4b2 device and the Douglas bag method.

Both the K4b2 and Servomex gas analysers (used during the Douglas bag method) were calibrated and checked prior use and were reading within their respective range of tolerance (both manufacturers claim an error of 0.02% for measured O_2 and 0.01% for measured CO_2). However, the K4b2 indoors still measured significantly lower F_{EO_2} at all running speeds when compared to the Douglas bag method by an average of 0.7%. For F_{ECO_2} , a similar pattern emerged, however in this instance; the K4b2 indoor values for F_{ECO_2} were significantly higher, by 0.5%, than those measured by the Douglas bag method.

One difference between the Douglas bag method and the K4b2 is that the latter excludes the initial 70 ml of each breath in order to reduce the occurrence of ‘double-breaths’ (i.e. where a single breath may be measured as two breaths as a result of an interruption to the breath). This results in the calculation of lower $F_{E}O_2$ and higher $F_{E}CO_2$ values (Parr et al., 2001) (i.e. only after 70 ml of air passes through the flow meter are air gas fractions measured). In contrast, the Douglas bag method includes each entire breath, including apparatus dead space, which also contributes to Douglas bag measures being inherently higher when compared to the K4b2. The difference between the two methods is illustrated in Figure 3.14.

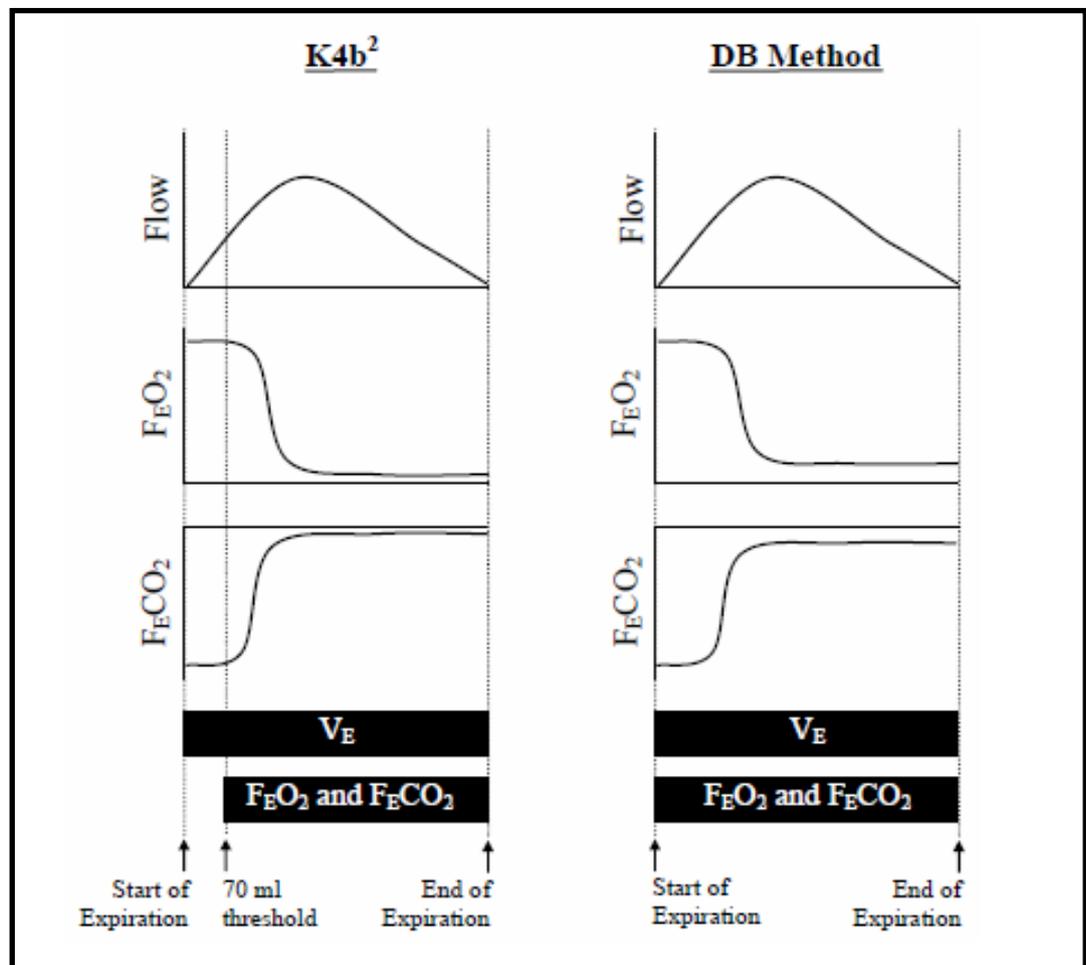


Figure 3.14: An illustration of the difference between the K4b2 and Douglas bag method in determining metabolic variables (extracted from Parr et al., 2001)

This special K4b2 characteristic, as well as differences in how $\dot{V}O_2$ is calculated by the K4b2 and Douglas Bag, has been previously described (Parr et al., 2001) using the following example formulae comparison:

Douglas Bag -

$$\dot{V}O_2 = (\dot{V}_I \times F_{I}O_2) - (\dot{V}_E \times F_{E}O_2)$$

$$\dot{V}O_2 = (33.9 \text{ L}\cdot\text{min}^{-1} \times 0.2093) - (33.8 \text{ L}\cdot\text{min}^{-1} \times 0.1658)$$

$$\dot{V}O_2 = 1.49 \text{ L}\cdot\text{min}^{-1}$$

K4b2 -

$$\dot{V}O_2 = (\dot{V}_I \times F_{I}O_2) - (\dot{V}_E \times F_{E}O_2) - (V_D \times (F_{I}O_2 - F_{ET}O_2))$$

$$\dot{V}O_2 = (33.9 \text{ L}\cdot\text{min}^{-1} \times 0.2093) - (33.8 \text{ L}\cdot\text{min}^{-1} \times 0.1618) - (2.0 \text{ L}\cdot\text{min}^{-1} \times (0.2093 - 0.1427))$$

$$\dot{V}O_2 = 1.49 \text{ L}\cdot\text{min}^{-1}$$

The K4b2 uses the equation, based on the above, where $\dot{V}O_2$ is calculated using the volume of O_2 inspired ($\dot{V}_I \times F_{I}O_2$) subtracted from the volume of O_2 expired ($\dot{V}_E \times F_{E}O_2$). This is similar to how $\dot{V}O_2$ is calculated by the Douglas Bag method. The key difference, however, is related to the expulsion of the initial 70 ml measured by the K4b2 which aims to reduce the occurrence of ‘double-breaths’. In addition, the 70 ml expulsion, in turn, corrects for the small amount of oxygen located in the dead space (V_D) of the breathing apparatus, at the start of inspiration and expiration ($V_D \times (F_{I}O_2 - F_{ET}O_2)$), as such dead-space is flushed out prior the calculation of air gas fractions. This mechanisms and/or correction, therefore, aim to improve K4b2 measures as to match measures by the Douglas bag method, despite K4b2 measuring lower $F_{E}O_2$ values. Similar findings have also been reported previously (McLaughlin et al., 2001, Pinnington et al., 2001, Mc Naughton et al., 2005). Although this algorithm seems logical, large discrepancies in $\dot{V}O_2$ measures were still present between the K4b2 indoor and the Douglas bag method. Thus, the fact that the difference was bigger than 3.3% (i.e. $\dot{V}O_2$), it is due to the way the K4b2 measures $F_{E}O_2$, which therefore is not comparable to the Douglas bag method due to the differences highlighted.

The next consideration is potential changes in $\dot{V}O_2$ when moving from an indoor to an outdoor running environment. One major difference between running outdoors and indoors involves facing air velocities encountered during running outdoors versus running on a treadmill.

One major difference between running indoors and outdoors involves facing air velocities and how this impacts the determination of \dot{V}_E and $F_{E}O_2$ (and $F_{E}CO_2$) at the sampling level. This is yet to be conclusively determined. Minute ventilation was lower in the K4b2 outdoor condition in comparison to the K4b2 indoor condition (Figure 3.5). This may be a consequence of head-winds which in turn impacts the rotation of the internal turbine, used to calculate \dot{V}_E , by the expiratory breaths. As a result of this finding, a potential dilution effect on $F_{E}O_2$ (and $F_{E}CO_2$) values, as a result of the interfering ambient air head winds, might also be expected. However, $F_{E}O_2$ values were not significantly different between K4b2 indoor and K4b2 outdoor conditions. Thus, an explanation as to the discrepancies in $\dot{V}O_2$ between the K4b2 indoors and outdoors remains to be elucidated, but the reduction found outdoors in \dot{V}_E will likely have contributed. Furthermore, it remains unclear as to how well the sampling line and turbine of the K4b2 are protected, from both head and environmental winds, when considering that participants ran in an angular fashion, round track bends, in the outdoor condition.

Another consideration may be related to the impact of the 1% gradient used during indoor tests as to better resemble the energetic costs of running outdoors (Cureton et al., 1978). The gradient contribution to $\dot{V}O_2$ was assessed using an American College of Sports Medicine formula for the calculation of $\dot{V}O_2$ during running indoors ($\text{speed} \times 3.334 + 0.15 \times \text{speed} \times \text{grade} + 3.5$) (American College of Sports Medicine, 2000). This may have been a contributing factor to the statistically higher K4b2 indoor values when compared to the K4b2 outdoors. The formula indicates a constant multiplied by speed which suggests a divergence in the relationship between speed and $\dot{V}O_2$ as speed is increased. However, this was not the case as indicated in Figure 3.9, where the lines of identity and best fit are parallel with one another (i.e. a K4b2 indoor systematic bias of $3.3 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Thus, there are a number of potential errors in the measurement of $\dot{V}O_2$ between the Douglas bag method and K4 outdoors. Taken together this leads to a typical error of $3 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ and 95% confidence interval range of -6.1 and $10.7 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. This is substantial and is not suitable for measuring potential subtle differences in performance measures (equivalent to typical measurement error of 6.5%).

Another point of concern is in relation to K4b2 derived RER values. Statistically significant differences were found for RER values between both K4b2 conditions and the Douglas bag method at various running speeds (Figure 3.6). Furthermore, measurements made by the K4b2 both indoors and outdoors, during maximal running speeds, were unusually low when considering a frequently used criterion of $\text{RER} > 1.15$ for $\dot{V}O_{2\text{max}}$

(Rossiter et al., 2006). This may have been due to the numerically lower $\dot{V}CO_2$ measurements made by the K4b2 during maximal exercise where a greater plateau is present for $\dot{V}CO_2$ compared to $\dot{V}O_2$.

In summary, the degrees of error for the measurement of $\dot{V}O_2$, as reported in this experimental chapter, are unacceptable when considering the level of accuracy required for physiological measures such as $\dot{V}O_{2max}$ and running economy, amongst others. Such differences can have significant impacts on data interpretation and conclusions in research studies. Overall, it is clear that no single factor is responsible for the results found in this experimental Chapter. It is most likely the case that several errors found in various sub-component variables (i.e. F_{EO_2} and \dot{V}_E), from which $\dot{V}O_2$ is derived, as well as the potential negative impact wearing the K4b2 may have on running economy, all contributed to the physiologically meaningful differences reported. It is acknowledged that portable metabolic devices for use in the field will always have an inherently higher error in comparison to laboratory based technologies. However, it is also clear that improvements are required to improve the accuracy of field based measures in order for such technologies to be more readily employed with confidence in the results obtained.

3.4.2 Study Limitations

Theoretically, the ideal experimental design would have been to use both the K4b2 and the Douglas bag method simultaneously (Atkinson et al., 2005). However, previous reports have found that simultaneous use proved too problematic as a result of interferences between the two measuring devices (McLaughlin et al., 2001). This, alongside practicality issues (i.e. using the Douglas Bag outdoors), warranted the design employed in this study. As a result, testing on different days did incur a 48 hour resting period between tests and so a day-to-day variation in $\dot{V}O_2$ was expected to be in the region of 4% (Armstrong and Costill, 1985). To add, although experimenters did their utmost best to ensure that speeds during the outdoor trials were maintained as closely as possible, this could potentially be a source for variation in measured variables.

3.5 Conclusion

The present investigation (to the best of the author's knowledge) is the first to attempt the assessment of the K4b2 device during submaximal and maximal running velocities outdoors. The K4b2 metabolic device appears valid for measuring $\dot{V}O_2$ during

submaximal and maximal running velocities in an outdoor environment. However, a K4b2 outdoors mean bias of $2.3 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ with a 95% confidence interval limits of agreement range from -6.1 and $10.7 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (equivalent to a measurement typical error of 6.5%) indicates unacceptable degree of error. Furthermore, the K4b2 significantly overestimated $\dot{V}\text{O}_2$ indoors when compared to the Douglas bag method. As a result, users must seriously consider the level of measurement accuracy required prior using the K4b2 portable device.

4. The Effects of Human Recombinant Erythropoietin on Exercise Performance, Haematological and Psychological Variables

4.1 Introduction

The use of r-HuEpo in clinical practice is common and includes the administration to anaemic patients due to chronic renal failure (Eschbach et al., 1989) and to cancer patients undergoing chemotherapy (Platanias et al., 1991). The administration of r-HuEpo has also been widely reported to improve athletic performance (Ashenden et al., 2001, Ninot et al., 2006, Ashenden et al., 2006, Lundby et al., 2007, Robach et al., 2008, Lundby et al., 2008b, Boning et al., 2008, Lundby, 2008) and such improvements are largely as a result of changes in the haematological profile resulting in improvements in aerobic fitness. Some studies have also shown improvements in physical strength scores immediately post r-HuEpo treatment (Ninot et al., 2006). As a result, the use of r-HuEpo is speculated to frequently occur amongst the athlete population and high profile convictions such as Lance Armstrong have shown that this may indeed be the case (United States Anti-doping Agency (USADA), 2012).

Endogenously present erythropoietin, primarily produced by the kidneys, is the main regulator of erythropoiesis in the human body and is regulated by the renin-angiotensin system (Dunn et al., 2007). The administration of additional recombinant erythropoietin, exogenously, directly targets red blood cell progenitors and precursors which further stimulates pluripotent stem cell proliferation, differentiation and maturation of red blood cells as they enter the circulatory system (Jelkmann and Wolff, 1991, Ng et al., 2003). It is by this mechanism that r-HuEpo administration can increase blood [Hb] and blood Hct, in-turn increasing blood oxygen-carrying capacity. This can lead to enhancements in key endurance performance determinants, such as $\dot{V}O_{2max}$, and such improvements have been shown to improve endurance performance (Egrie et al., 1986, Ashenden et al., 2001).

Several clinical studies have also examined the psychological effects of r-HuEpo administration and clinical outcomes have included improvements in cognitive function (Nissenson, 1990), decreases in the fatigue (Nissenson, 1990, Johansen et al., 2011) and improvements in perceived physical conditioning (Ninot et al., 2006). However, there is still a lack of research assessing the effects of repeated r-HuEpo injections on

psychological measures in well-trained endurance individuals and how this may contribute to changes in exercise performance.

The assessment of psychological changes in athletes is often problematic. Under normal circumstances (i.e. assessing well-trained endurance individuals pursuing normal training approaches), detecting psychological changes can be problematic due to potential changes being very small (due to the high initial physical fitness levels) (Ninot et al., 2006). In addition, any potential psychological changes associated with training will require relatively long periods of time in order for training adaptations to occur (Fox, 2000). The use of r-HuEpo, however, can overcome such limitations as it induces noticeable performance enhancing effects over a substantially shorter timeframe (Lundby et al., 2007). As a result, this may trigger significant changes in psychological measures related to motivation, such as mood and anxiety, particularly during and immediately post the r-HuEpo period of administration. This may, in turn, contribute to the positive performance enhancing effect which previous studies have solely explained from a physiological standpoint (Ashenden et al., 2001, Ninot et al., 2006, Ashenden et al., 2006, Lundby et al., 2007, Robach et al., 2008, Lundby et al., 2008b, Boning et al., 2008, Lundby, 2008). In addition, this is the first study to assess whether results obtained from laboratory based performance tests, after the administration of r-HuEpo, are transferable to the field via 3 km time trial assessments.

Therefore, the aims of this study were to:

1. Confirm the erythropoietic effect of r-HuEpo administration on haematological measures.
2. Determine the effects of r-HuEpo administration on performance in an outdoor field setting.
3. Determine which endurance performance-related physiological factors are changed by r-HuEpo administration.
4. Determine whether r-HuEpo administration influences psychological measures, such as mood and anxiety, which may be attributable to potential changes in running performance.

Figure 4.1 illustrates the key factors that influence endurance performance and how r-HuEpo administration may influence such factors with those measured, in Chapter 4, also indicated.

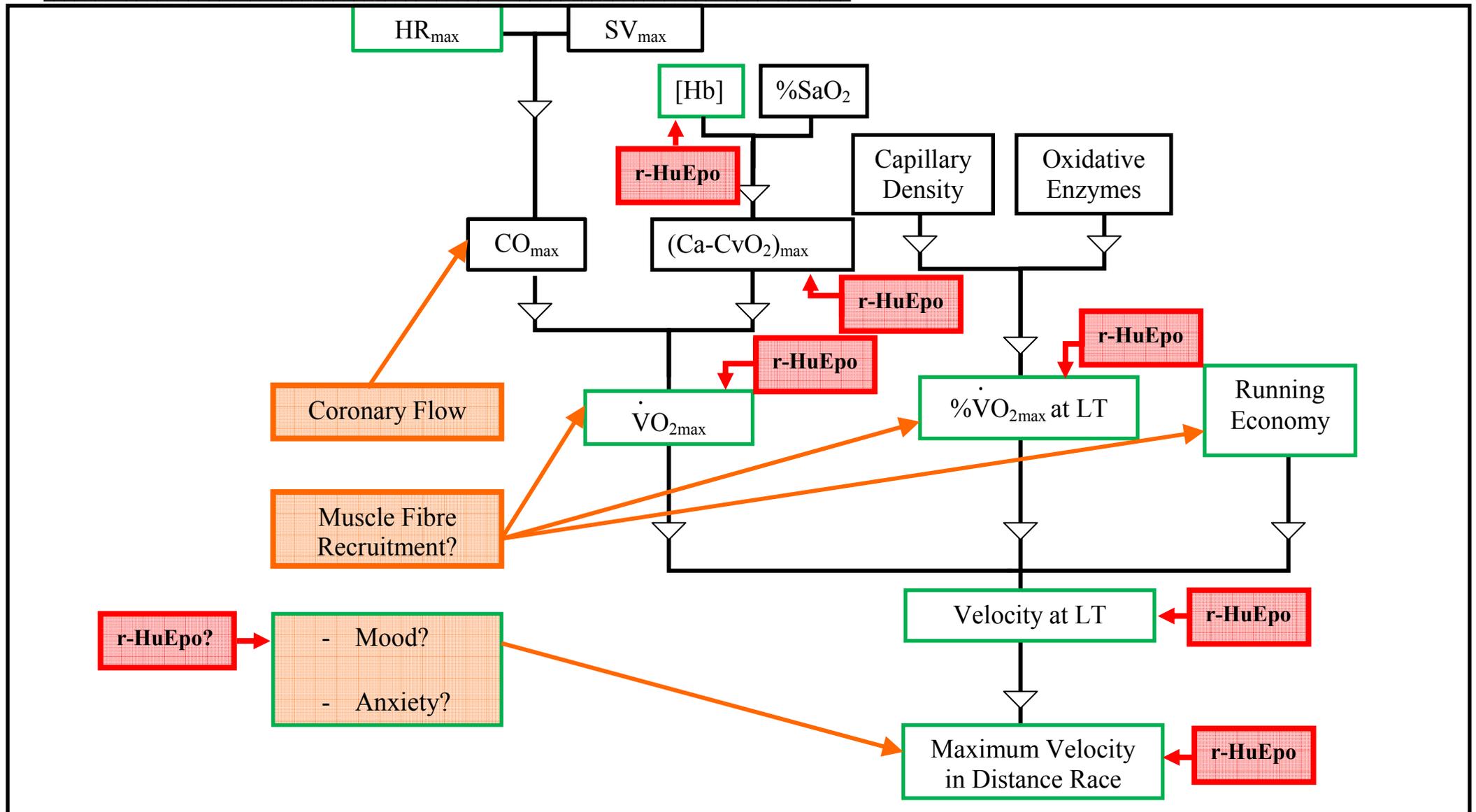


Figure 4.1: Summary of the major cardiovascular variables (black lines), with the addition of the Central Governor Model (CGM) (orange lines), related to $\dot{V}O_{2max}$ and the maximal velocity that can be maintained in distance races. The documented (red arrows) and potential effects (red arrows with question mark) of r-HuEpo are also included. Variables measured in Chapter 4 (green border). HR_{max} = maximal heart rate; SV_{max} = maximal stroke volume; $[Hb]$ = haemoglobin concentration; $\%SaO_2$ = percent oxygen saturation; CO_{max} = maximal cardiac output; $(Ca-CvO_2)_{max}$ = maximal arterial-venous difference; $\dot{V}O_{2max}$ = maximal oxygen uptake; $\% \dot{V}O_{2max}$ at LT = percentage of maximal oxygen uptake at lactate threshold.

4.2 Methods

4.2.1 Participants

Seven men (age: 25.7 ± 2.2 years; BMI 22.5 ± 0.7 kg m⁻²; $\dot{V}O_{2\max}$: 57.7 ± 2.9 ml kg⁻¹·min⁻¹; mean \pm SD) participated in the study. Inclusion and exclusion criteria are described in section 2.1.

All participants had been training regularly for several years, and during the study period were asked to continue their normal training activities (actual training durations of 8-18 h·wk⁻¹). Participant 10 km running personal best (PB) times (average for this group was 36.0 ± 1.8 min; mean \pm SD) were directly obtained from the individual and, where possible, was cross-checked using an official athlete profiling website approved by United Kingdom Athletics (<http://www.thepowerof10.info/>). For two participants a 10 km time was not available, therefore a 10 km completion time equivalence was calculated using one participant's 3 km PB and another's 5 km PB via an online running distance converter (<http://www.runningtimes.com>).

All participants came from middle-class socioeconomic backgrounds and none were facing or had faced major negative life events that would have affected psychological responses over the study period. Haematological measures were obtained for all seven participants. One participant's exercise performance data was excluded from the study due to an illness not associated with r-HuEpo administration. Thus, maximal oxygen uptake, running economy, ratings of perceived exertion, maximal respiratory exchange ratio (RER_{\max}) and maximal heart rate measures (HR_{\max}) were obtained for six participants during the $\dot{V}O_{2\max}$ tests. Running speed at LT, OBLA, LTP and % $\dot{V}O_{2\max}$ at LT were obtained for five participants at all time points (i.e. at weeks 2, 6 and 10), with one participant's data not collected at the immediately post r-HuEpo time point due to technical problems. Psychological measures, CSAI-2 and POMS, were obtained for six participants. Three kilometre time trial results were obtained for six participants.

This study was approved by the University of Glasgow and performed according to the World Medical Association (Declaration of Helsinki) code of ethics.

4.2.2 Experimental Design

Participants took part in a 10-week protocol divided into three phases; baseline (2 weeks), r-HuEpo administration (4 weeks) and post administration (4 weeks) (Appendix K). Fifty $\text{IU}\cdot\text{kg}^{-1}$ subcutaneous injections of r-HuEpo were administered every two days during the 4 week administration phase and this was supplemented by daily oral iron tablets (Parisotto et al., 2001) (~100 mg of elemental iron, Ferrous Sulphate, Almus, Barnstaple, UK.) as previously described in section 2.8. If [Hct] rose above 55%, the administration of r-HuEpo would have been stopped and replaced by the administration of saline (0.9% NaCl).

4.2.3 Haematological Measures

Standard haematological analyses were made using a haematology analyser (Sysmex XT-2000i, Norderstedt, Germany), as described in section 2.6, and measures included [Hb], Hct and %ret. Samples were collected and immediately analysed at baseline (2 weeks), 4 weeks during r-HuEpo administration and 4 weeks post r-HuEpo administration as described in section 2.8. The haematology analyser was calibrated against diagnostic reference materials prior the measurement of collected samples as described in section 2.6. Total haemoglobin mass was also determined once a week using the optimised carbon monoxide rebreathing method (Schmidt and Prommer, 2005a, Schmidt and Prommer, 2005b, Gore et al., 2006) as described in section 2.6.2.2.

4.2.3.1 Blood Lactate Determination

Capillary blood samples were taken from the fingers of participants during recovery phases of the discontinuous incremental exercise protocol, as previously described in section 2.5.2. Capillary blood samples were immediately analysed upon collection (Radiometer ABL 700, Copenhagen, Denmark) for blood [Lac] and measures were consequently used to assess any changes in running speed at the points of LT, OBLA, LTP and $\dot{V}\text{O}_{2\text{max}}$ at LT (as previously described in section 2.6.1).

4.2.4 $\dot{V}\text{O}_{2\text{max}}$ and 3 km Time Trials

Maximal oxygen uptake and 3 km time trial performance was assessed at baseline (2 weeks), immediately post (6 weeks) and 4 weeks post the r-HuEpo administration phase (10 weeks) (see section 2.4.1). All 3 km time trial assessments were carried out on an

indoor 200 m running track located at the Kelvin Hall Arena (Glasgow, U.K) in a mean ambient temperature and humidity of $21.5 \pm 1.8^{\circ}\text{C}$ and $48.1 \pm 8.0\%$ (Comark, UK), respectively (Figure 4.2).

4.2.5 Psychological Measures

The CSAI-2 and POMS were used to assess anxiety and mood, respectively, at baseline (2 weeks), immediately post (6 weeks) and 4 weeks post (10 weeks) the r-HuEpo administration phase (see section 2.9). The CSAI-2 questionnaires were administered approximately 20 to 30 minutes (Craft et al, 2003) prior $\dot{V}\text{O}_{2\text{max}}$ and 3 km time trial performance tests (Appendix D).

4.2.6 Data Analysis

A one-way ANOVA for repeated measures with Tukey post hoc analysis was used to locate differences between all variables and time points. Statistical significance was accepted at $P < 0.05$. All values are reported as mean \pm SEM unless otherwise shown. Relationships between variables were determined using Pearson correlations.

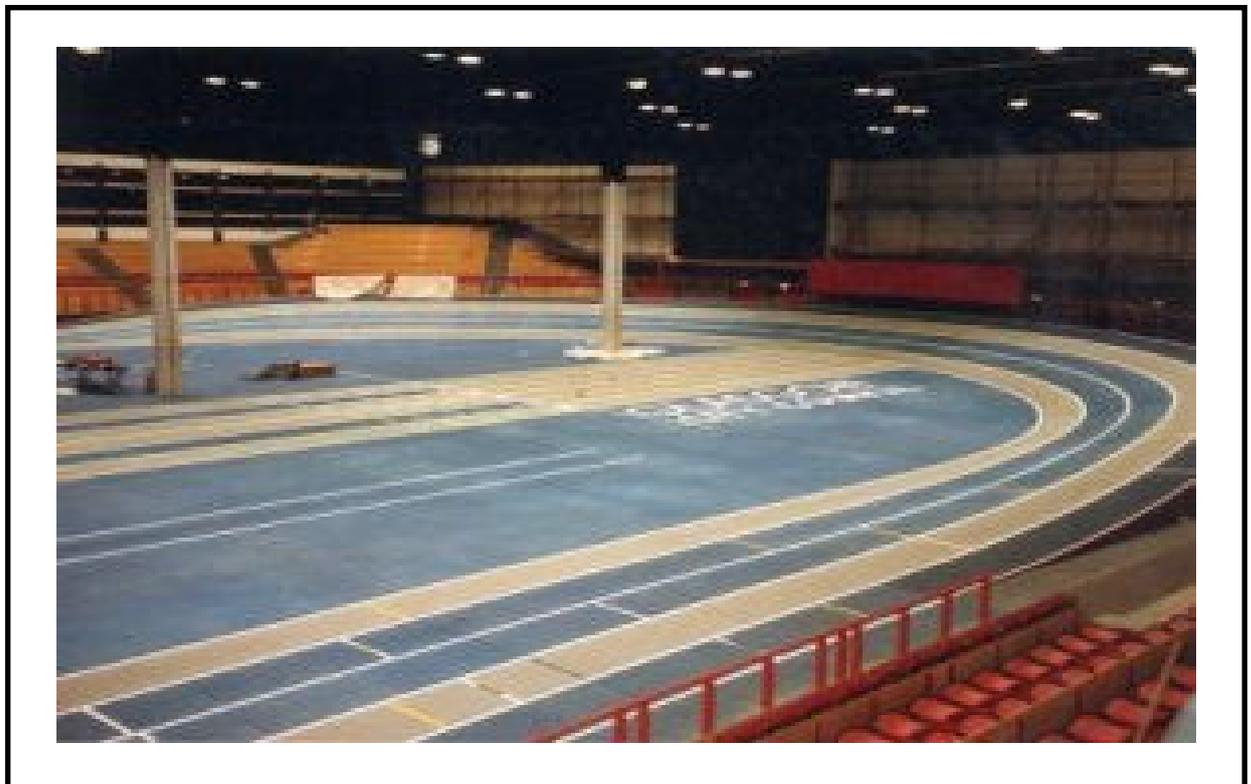


Figure 4.2: The Kelvin Hall Arena and Running Track

4.3 Results

4.3.1 Haematological Measures

The r-HuEpo administration was tolerated by all participants with no adverse effects reported and/or observed, and with no participants reaching an Hct of 55% or above. Table 4.1 shows haematological results at baseline (2 weeks), immediately post r-HuEpo administration (6 weeks), and 4 weeks post r-HuEpo administration (10 weeks). Immediately post r-HuEpo administration, [Hb], Hct, ret and tHb significantly increased ($P < 0.05$) by 21.2%, 21.3%, 75.0% and 11.7%, respectively. At 4 weeks post r-HuEpo administration, [Hb], Hct and tHb decreased compared to immediately post but remained significantly elevated compared to baseline by 7.9%, 9.3% and 6.9%, respectively ($P < 0.05$). Reticulocytes decreased to below baseline levels (by 41.7%) 4 weeks post r-HuEpo administration ($P < 0.05$).

Table 4.1: Haematological measures at Baseline, Immediately Post and 4 weeks Post r-HuEpo administration

Haematological measures (n = 7)	Baseline	Immediately Post	4 weeks Post
[Hb] (g/dl)	14.0 ± 0.3	16.9 ± 0.5*	15.1 ± 0.3* [#]
Hct (%)	40.8 ± 0.6	49.5 ± 1.0*	44.6 ± 0.8* [#]
Ret (%)	1.2 ± 0.1	2.1 ± 0.3*	0.7 ± 0.1 * [#]
tHb (g)	1003.1 ± 40.7	1120.0 ± 43.7*	1072.2 ± 39.9*

Values are mean ± SEM. Significant differences between groups ($P < 0.05$) are denoted as follows: * different from baseline, [#] different from immediately post

4.3.2 Performance Measures

Table 4.2 shows performance results at baseline, immediately post r-HuEpo administration, and 4 weeks post administration. Maximal oxygen uptake did not significantly change ($P = 0.07$) over the course of the protocol, but was numerically 5.2% higher immediately post r-HuEpo administration when compared to baseline.

Running speed at which the LT occurred was significantly higher by 6.4% ($P < 0.05$) immediately post r-HuEpo administration when compared to baseline. Running speed at OBLA was significantly higher immediately post r-HuEpo administration when compared to baseline by 5.9% ($P < 0.05$) and this remained elevated 4 weeks post, by 5.3%, when compared to baseline ($P < 0.05$). The $\% \dot{V}O_{2\max}$ at LT was significantly higher immediately post r-HuEpo administration when compared to baseline by 6.6% ($P < 0.05$). Lactate turn-point values were also significantly higher ($P < 0.05$) immediately post r-HuEpo administration when compared to baseline and 4 weeks post by 9.6 and 7.6%, respectively. Running economy and maximal heart rate measures were not significantly different between any of the time-points ($P > 0.05$).

Three kilometre time trial performance significantly improved immediately post r-HuEpo administration by 4.9% ($P < 0.05$) when compared to baseline. Faster times were sustained 4 weeks post r-HuEpo administration, when compared to baseline, by 3.9% ($P < 0.05$).

Table 4.3 shows correlations, using the difference between measured values at baseline and immediately post r-HuEpo administration, between measured haematological variables (i.e. [Hb], Hct, Ret and tHb), physiological performance-related variables (i.e. LT running speed, OBLA running speed, LTP running speed, $\% \dot{V}O_{2\max}$ at LT and Running Economy) with performance related outcome measures (i.e. $\dot{V}O_{2\max}$ and 3 km time trial completion times). Changes in [Hb] significantly correlated with changes in $\dot{V}O_{2\max}$ ($r = 0.985$; $P = 0.016$) (Figure 4.3). There was a tendency for changes in Hct to correlate with changes in $\dot{V}O_{2\max}$ ($r = 0.807$; $P = 0.053$) (Figure 4.4).

No significant relationships were found between any changes in haematological variables and changes in 3 km time trial performance (Table 4.3). No significant relationships were found between changes in any of the other measured variables (i.e. $\dot{V}O_{2\max}$, LT running speed, OBLA running speed, LTP running speed, $\% \dot{V}O_{2\max}$ at LT and Running Economy) and 3 km time trial performance (Table 4.3). No significant relationships were found

between changes in haematological variables and changes in LT, LTP, OBLA running speeds or with running economy (Table 4.3). However, a significant *negative* relationship was found between changes in $\dot{V}O_{2\max}$ at LT with changes in [Hb] ($r = -0.882$; $P = 0.048$) and Hct ($r = -0.926$; $P = 0.024$) immediately post r-HuEpo administration.

Table 4.2: Performance measures at Baseline, Immediately Post and 4 weeks Post r-HuEpo administration

Performance measures (n = 6)	Baseline	Immediately Post	4 weeks Post
$\dot{V}O_{2\max}$ ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	57.9 ± 2.9	60.9 ± 3.0	59.7 ± 1.8
LT Running Speed ($\text{km}\cdot\text{h}^{-1}$) [^]	13.8 ± 1.0	$14.7 \pm 0.8^*$	14.0 ± 0.7
OBLA Running Speed ($\text{km}\cdot\text{h}^{-1}$) [^]	15.2 ± 1.0	$16.1 \pm 1.0^*$	$16.0 \pm 1.0^*$
LTP Running Speed ($\text{km}\cdot\text{h}^{-1}$) [^]	15.6 ± 0.6	$17.1 \pm 0.8^{*\#}$	15.9 ± 0.9
$\% \dot{V}O_{2\max}$ at LT [^]	70.8 ± 2.6	$75.5 \pm 2.2^*$	68.0 ± 4.7
Running Economy - $\dot{V}O_2$ at 12 $\text{km}\cdot\text{h}^{-1}$ ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	37.5 ± 1.0	39.8 ± 1.3	38.5 ± 0.9
RPE _{max}	19.2 ± 0.2	19.2 ± 0.2	19.3 ± 0.2
RER _{max}	1.1 ± 0.0	1.1 ± 0.0	1.1 ± 0.0
HR _{max} (bpm)	188.3 ± 1.7	189.8 ± 1.8	186.8 ± 1.4
3 km Time Trial (s)	607.8 ± 28.5	$577.9 \pm 25.4^{*\#}$	$583.8 \pm 26.7^*$

Values are mean \pm SEM. Significant differences between groups ($p < 0.05$) are denoted as follows: * different from baseline. # different from 4 weeks post. LT = lactate threshold. OBLA = onset of blood lactate. LTP = lactate turn-point. RPE_{max} = maximal rate of perceived exertion. RER_{max} = maximal respiratory exchange rate. HR_{max} = maximal heart rate. [^] n = 5.

Table 4.3: Pearson correlations showing relationships between change in $\dot{V}O_{2\max}$ and change in 3 km time trial performance with changes in haematological and physiological variables from baseline to immediately post r-HuEpo administration

	$\dot{V}O_{2\max}$ ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	3 km Time Trial (s)
Hb (g/dl)	0.895*	-0.544
Hct (%)	0.807	-0.574
Ret (%)	0.101	-0.187
tHb (g)	0.050	0.509
LT Running Speed ($\text{km}\cdot\text{h}^{-1}$)	0.272	-0.380
OBLA Running Speed ($\text{km}\cdot\text{h}^{-1}$)	-0.031	0.219
LTP Running Speed ($\text{km}\cdot\text{h}^{-1}$)	-0.363	0.183
% $\dot{V}O_{2\max}$ at LT	0.610	-0.129
Running Economy - $\dot{V}O_2$ at 12 $\text{km}\cdot\text{h}^{-1}$ ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	0.512	-0.055
$\dot{V}O_{2\max}$ ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)		-0.532

LT = lactate threshold. OBLA = onset of blood lactate. LTP = lactate turn-point.

* $P < 0.05$.

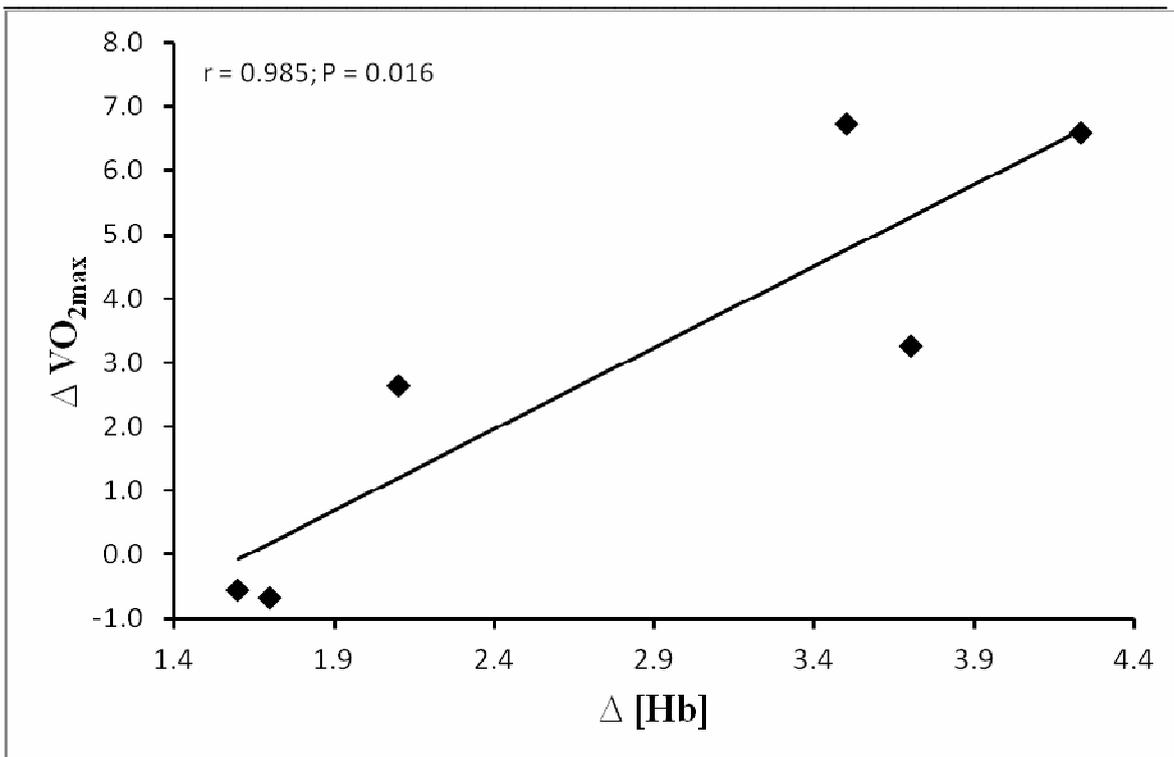


Figure 4.3: Pearson correlation showing a strong positive relationship between $\Delta\dot{V}O_{2max}$ and Δ [Hb] from baseline to immediately post r-HuEpo administration. $r = 0.985$; $P = 0.016$.

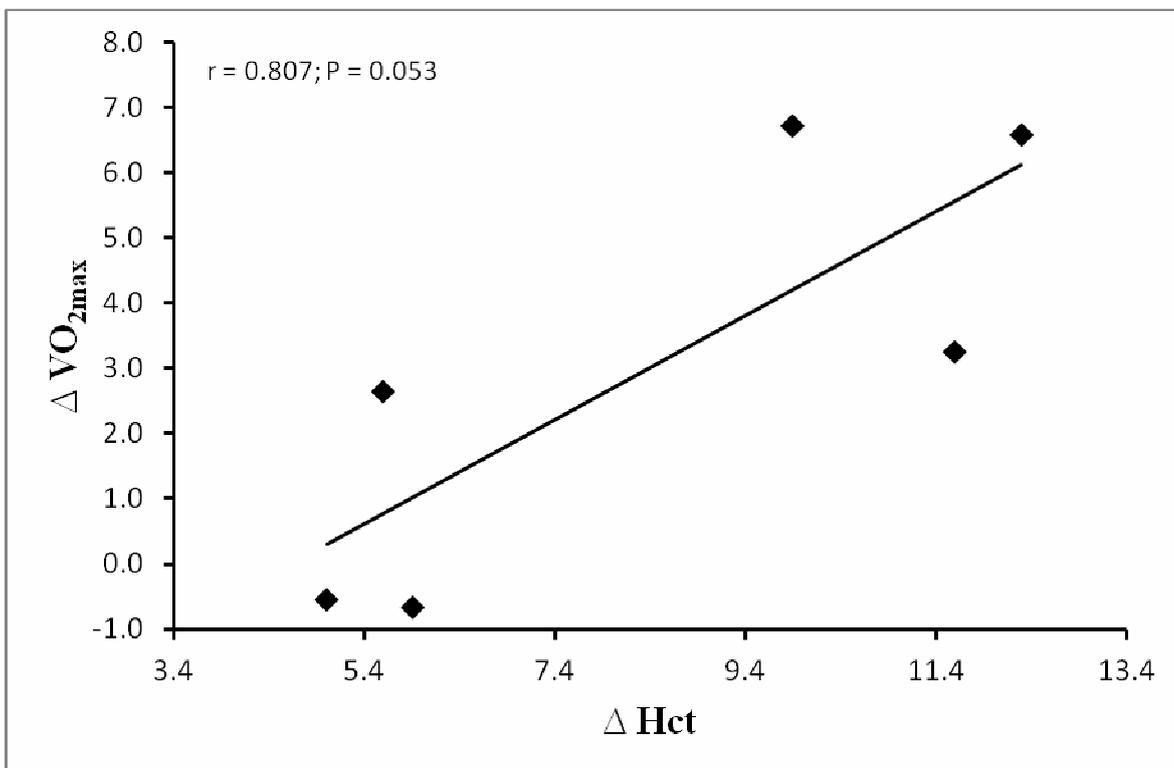


Figure 4.4: Pearson correlation showing a positive correlation tendency between $\Delta\dot{V}O_{2max}$ and Δ Hct from baseline to immediately post r-HuEpo administration. $r = 0.807$; $P = 0.053$.

4.3.3 Psychological Measures

Table 4.4 shows psychological results at baseline, immediately post r-HuEpo administration and 4 weeks post administration. The CSAI-2 cognitive subscale decreased significantly immediately post r-HuEpo administration by 18% ($P < 0.05$) compared to baseline. The POMS tension subscale decreased significantly immediately post r-HuEpo administration compared to baseline by 61% and this was also sustained 4 weeks post r-HuEpo administration by 50% ($P < 0.05$). Tables 4.5 and 4.6 show correlations, using the difference between measured values at baseline and immediately post r-HuEpo administration, between POMS and CSAI-2 sub-scales with performance related outcome measures (i.e. $\dot{V}O_{2\max}$ and 3 km time trial completion times). A significant relationship was found between the POMS confusion sub-scale and $\dot{V}O_{2\max}$ ($r = 0.894$; $P = 0.016$). There was a tendency for changes in CSAI-2 somatic anxiety sub-scale and $\dot{V}O_{2\max}$ ($r = 0.800$; $P = 0.058$).

Table 4.4: Psychological measures at Baseline, Immediately Post and 4 weeks Post r-HuEpo

Psychological measures (n = 6)	Subscales	Baseline	Immediately Post	4 weeks Post
CSAI-2	Cognitive	21.6 ± 1.7	17.7 ± 1.9* [#]	19.2 ± 1.8
	Somatic	15.1 ± 1.0	13.9 ± 0.8	14.2 ± 1.7
	Self-confidence	21.3 ± 0.9	20.9 ± 1.8	21.0 ± 2.0
POMS	Tension	2.8 ± 0.7	1.1 ± 0.7* [#]	1.4 ± 0.4* [#]
	Depression	0.4 ± 0.3	0.7 ± 0.5	0.7 ± 0.4
	Anger	1.2 ± 0.7	0.5 ± 0.3	0.7 ± 0.5
	Vigour	8.8 ± 1.3	9.2 ± 1.5	8.9 ± 1.7
	Fatigue	4.1 ± 1.0	2.0 ± 0.7	3.1 ± 0.7
	Confusion	0.9 ± 0.5	0.4 ± 0.3	0.7 ± 0.4

Values are mean ± SEM. Significant differences between groups ($P < 0.05$) are denoted as follows: * different from baseline. [#] considered as positive changes.

Subscale considerations: Decreases in: cognitive, somatic, tension, depression, anger, fatigue and confusion = positive change. Increase in: self-confidence and vigour = positive change.

Table 4.5: Pearson correlations showing relationships between change in $\dot{V}O_{2\max}$ and change in 3 km time trial performance with changes in POMS sub-scales from baseline to immediately post r-HuEpo administration

	$\dot{V}O_{2\max}$ ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	3 km Time Trial (s)
Tension	0.124	0.217
Depression	0.544	-0.123
Anger	0.625	-0.489
Vigour	-0.262	0.723
Fatigue	0.522	-0.358
Confusion	0.894*	-0.319

$\dot{V}O_{2\max}$ = maximal oxygen uptake. * $P < 0.05$.

Table 4.6: Pearson correlations showing relationships between change in $\dot{V}O_{2\max}$ and change in 3 km time trial performance with changes in CSAI-2 sub-scales from baseline to immediately post r-HuEpo administration

	$\dot{V}O_{2\max}$ ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	3 km Time Trial (s)
Cognitive	0.118	-0.360
Somatic	0.800	-0.008
Self-confidence	-0.179	0.564

$\dot{V}O_{2\max}$ = maximal oxygen uptake. * $P < 0.05$.

4.4 Discussion

All variables found to be significantly influenced, immediately post r-HuEpo administration, are indicated in Figure 4.5. Furthermore, variables found to be related to changes in haematological factors, as well as changes in 3 km time trial performance, are also indicated. In line with previous studies (Audran et al., 1999, Parisotto et al., 2000b, Birkeland et al., 2000, Ashenden et al., 2001, Russell et al., 2002a, Ashenden et al., 2006) [Hb], Hct, ret and tHb increased (by 21.2%, 21.3%, 75.0% and 11.7%, respectively) and $\dot{V}O_{2\max}$ values were numerically higher immediately post r-HuEpo administration (by 5.2%). This change in $\dot{V}O_{2\max}$ was lower than values reported in previous studies, however the present findings confirmed that changes in [Hb] and Hct, elicited by r-HuEpo administration, are associated with changes in $\dot{V}O_{2\max}$ as indicated in Figure 4.3 and Figure 4.4, respectively. This has previously been reported elsewhere (Audran et al., 1999, Parisotto et al., 2000b, Birkeland et al., 2000, Ashenden et al., 2001, Russell et al., 2002a, Ashenden et al., 2006, Ninot et al., 2006).

Interestingly, treadmill running speeds at LT, OBLA and LTP were significantly higher immediately post r-HuEpo administration when compared to baseline by 6.4%, 5.9% and 9.6%, respectively (Table 4.2). Furthermore, $\% \dot{V}O_{2\max}$ at LT were also significantly higher immediately post r-HuEpo administration when compared to baseline by 6.6% (Table 4.2). In addition to the change reported in $\dot{V}O_{2\max}$, it would be logical to suggest that such changes would contribute to the 4.9% improvement found in 3 km time trial performance immediately post r-HuEpo administration. However, there was no significant relationship between changes in 3 km time trial performance and changes in [Hb] ($r = -0.544$; $P = 0.265$) or Hct ($r = -0.574$; $P = 0.234$). Furthermore, no significant relationships were found between changes in 3 km time trial performance and changes in $\dot{V}O_{2\max}$ ($r = -0.532$; $P = 0.278$), LT ($r = -0.380$; $P = 0.528$), OBLA ($r = 0.219$; $P = 0.723$), LTP ($r = 0.183$; $P = 0.769$) or running economy ($r = -0.055$; $P = 0.918$). This may be the consequence of a type 2 statistical error as the small sample size in the present study limits the ability to detect significant relationships between variables. Indeed, with $n = 5$ – as was the case with many of the variables measured due to missing data – only correlations of $r = 0.81$ or higher would have achieved statistical significance. Thus it is very likely that real relationships between variables affecting performance were missed in this study. Nevertheless, it is also possible that the performance improvements observed in this study were not mediated by effects of r-HuEpo on haematological variables, as is classically reported, and this warrants further investigation.

The assessment of haematological variables with other performance related variables revealed no significant relationships between changes in [Hb] and changes in LT ($r = 0.438$; $P = 0.460$), LTP ($r = -0.159$; $P = 0.798$), OBLA ($r = 0.300$; $P = 0.624$) or running economy ($r = 0.453$; $P = 0.367$). In addition, no significant relationships were observed between changes in Hct and changes in LT ($r = 0.456$; $P = 0.440$), LTP ($r = -0.063$; $P = 0.920$), OBLA ($r = 0.353$; $P = 0.560$) or running economy ($r = 0.389$; $P = 0.446$). Interestingly, however, a significant *negative* relationship was found between changes in $\% \dot{V}O_{2\max}$ at LT and changes in [Hb] ($r = -0.882$; $P = 0.048$) and Hct ($r = -0.926$; $P = 0.024$). In other words, those who experienced the greatest increases in [Hb] and Hct experienced the smallest changes in $\% \dot{V}O_{2\max}$ at LT. Closer observation of the data reveals that a negative (albeit not statistically significant) relationship between changes in $\% \dot{V}O_{2\max}$ at LT and changes in $\dot{V}O_{2\max}$ ($r = -0.610$; $P = 0.274$) which would have been expected given the closer associations between [Hb], Hct and $\dot{V}O_{2\max}$, than between [Hb], Hct and LT. Thus, increases in $\% \dot{V}O_{2\max}$ at LT increased $\dot{V}O_{2\max}$ more than LT, leading to a fall in $\% \dot{V}O_{2\max}$ at LT in those with the biggest [Hb] and Hct changes. Additional considerations could be made with regards to the potential use of non-parametric based statistics (e.g. Spearman rank correlation). However, parametric statistics were solely preferred as clear numerical interpretations were required to be made. Also, when considering the small sample size present, larger sample sizes are often required (when using non-parametric statistics) to draw conclusion with the same degree of confidence (in comparison to parametric statistics).

Regardless of the associations reported in this study, however, it is clear that 3 km time trial performance significantly improved immediately post r-HuEpo administration by 4.9% and faster times were sustained for 4 weeks post r-HuEpo administration (by 4.0% compared to baseline). It is not clear, however, as to what factors likely caused this and to what degree. Potential differences in physical effort (i.e. HR_{\max} and RPE_{\max}) did not contribute to this effect as measures were not significantly different between any of the time-points. However, this study does show r-HuEpo induced changes in [Hb] and Hct that were only strongly related to $\dot{V}O_{2\max}$ with no clear association between changes in [Hb], Hct or $\dot{V}O_{2\max}$ and changes in 3 km time trial performance. This suggests that, subject to statistical caveats outlined above, factors other than [Hb], Hct and $\dot{V}O_{2\max}$ changes are likely to have influenced 3 km time trial performance. Furthermore, this is the first study (to the best of the author's knowledge) to demonstrate the sustained nature of improvement in a field-based performance assessment following r-HuEpo administration. Three km running performance remained 4% faster than baseline 4-weeks (a decline of

~1% from immediately post-administration). In contrast the increase in [Hb] and Hct had declined by more than 50% from the immediately post-administration time-point over this 4-week period, again suggesting a disconnect between the haematological and performance-related changes seen in this study.

4.4.1 Psychological Effects

A significant decrease in cognitive anxiety, by 18.3%, was found immediately post r-HuEpo administration when compared to baseline (Table 4.4). This suggests that the mental component of anxiety, caused by negative expectations about success or by negative self-evaluation (Martens, 1971), was reduced immediately post r-HuEpo administration. However, none of these sub-scales were related to changes in either $\dot{V}O_{2max}$ or 3 km time trial performance (Table 4.6). A previous meta-analysis on CSAI-2 and sport performance (Craft, 2003) reported mixed results for the relationship between CSAI-2 subscales and sport performance. However, cognitive and somatic anxiety were reported as being more influential in individual rather than team sports; possibly due to the greater pressures inflicted onto a single person as opposed to a team. In contrast, Jones and colleagues (Jones et al., 1993) reported no significant group differences in cognitive anxiety, state anxiety or self-confidence in 48 gymnasts who were categorised into poor and good performance groups. Interestingly, these authors reported that the good performance group viewed cognitive anxiety to be more facilitative than debilitating when compared to the poor performance group. The 3 km time trial performance tests carried out in this study were on an individual basis and, in contrast to previous literature, it appears that a reduction in cognitive anxiety may have facilitated, not debilitated, the improvement found immediately post r-HuEpo administration in 3 km time trial performance.

The POMS tension (i.e. a form of stress) subscale was also significantly lower, by 61%, immediately post r-HuEpo administration when compared to baseline and this remained lower by 50% for 4 weeks post r-HuEpo administration (Table 4.4). However, this was not related to changes in either $\dot{V}O_{2max}$ or 3 km time trial performance (Table 4.5). A POMS and sport performance meta-analysis reported tension and anger as having small performance debilitating effects and is also associated with both positive and negative performance outcomes (Beedie, 2000). This present study would suggest that a reduction in tension may have facilitated the improvement found immediately post r-HuEpo administration in 3 km time trial performance. As to why only tension and cognitive

anxiety proved significant in comparison to other subscales such as self-confidence (which did not) remains to be elucidated. Furthermore, the relationships found between POMS confusion sub-scale and CSAI-2 somatic anxiety subscale with $\dot{V}O_{2\max}$ also remains to be elucidated.

The notion that r-HuEpo may influence central components (i.e. the brain) and, in turn, influence exercise performance outcome is supported by recent evidence which suggests possible r-HuEpo mediated effects on the CNS (Ehrenreich et al., 2004, Core et al., 2011). The findings reported are also in line with the fact that no clear associations were found between [Hb], Hct or $\dot{V}O_{2\max}$ and 3 km time trial performance suggesting, in turn, that factors other than [Hb], Hct and $\dot{V}O_{2\max}$ changes likely influenced 3 km time trial performance. Thus the endurance performance enhancing effect, of r-HuEpo administration, may not solely be due to peripheral physiological changes, but may indeed be more complex with central mechanisms, psychology (Noakes, 2007) and the placebo effect being key potential mediators (Zhang et al., 2008).

4.4.2 Anti-Doping Considerations

The r-HuEpo administration of 50 UI kg^{-1} three times a week over a 4-week period is in line with previous studies (Parisotto et al., 2000b, Parisotto et al., 2001). These authors have reported a continued elevation in [Hb] and Hct for 21 days after the cessation of r-HuEpo injections and a return to baseline for tHb within one month of the cessation of r-HuEpo injections. Similar findings are found here for the continued elevation in [Hb] and Hct. However this present study found that 4 weeks (i.e. 28 days) post r-HuEpo administration [Hb], Hct and tHb decreased compared to immediately post r-HuEpo administration, but these remained significantly elevated when compared to baseline measures.

This has important connotations for the field of anti-doping. It is acknowledged that alternative dosage protocols (Russell et al., 2002b, Ashenden et al., 2006), in contrast to moderate doses of r-HuEpo over a 4 week period, more likely reflect how r-HuEpo is utilised by athletes who choose to 'dope' in an attempt to evade detection. However, the haematological results are indeed very similar (Ninot et al., 2006). The variation in haematological measures post the cessation of r-HuEpo injections between this and previous studies (i.e. ~30 days return to baseline reported by Parisotto and colleagues (Parisotto et al., 2000b), in contrast to the 28-day continued significant elevation compared

to baseline reported in this study), further exemplifies this. In agreement with Parisotto and colleagues (Parisotto et al., 2000b), the analysis of blood data using a models based approach may indeed be a more effective deterrent than simply attempting to detect current r-HuEpo use.

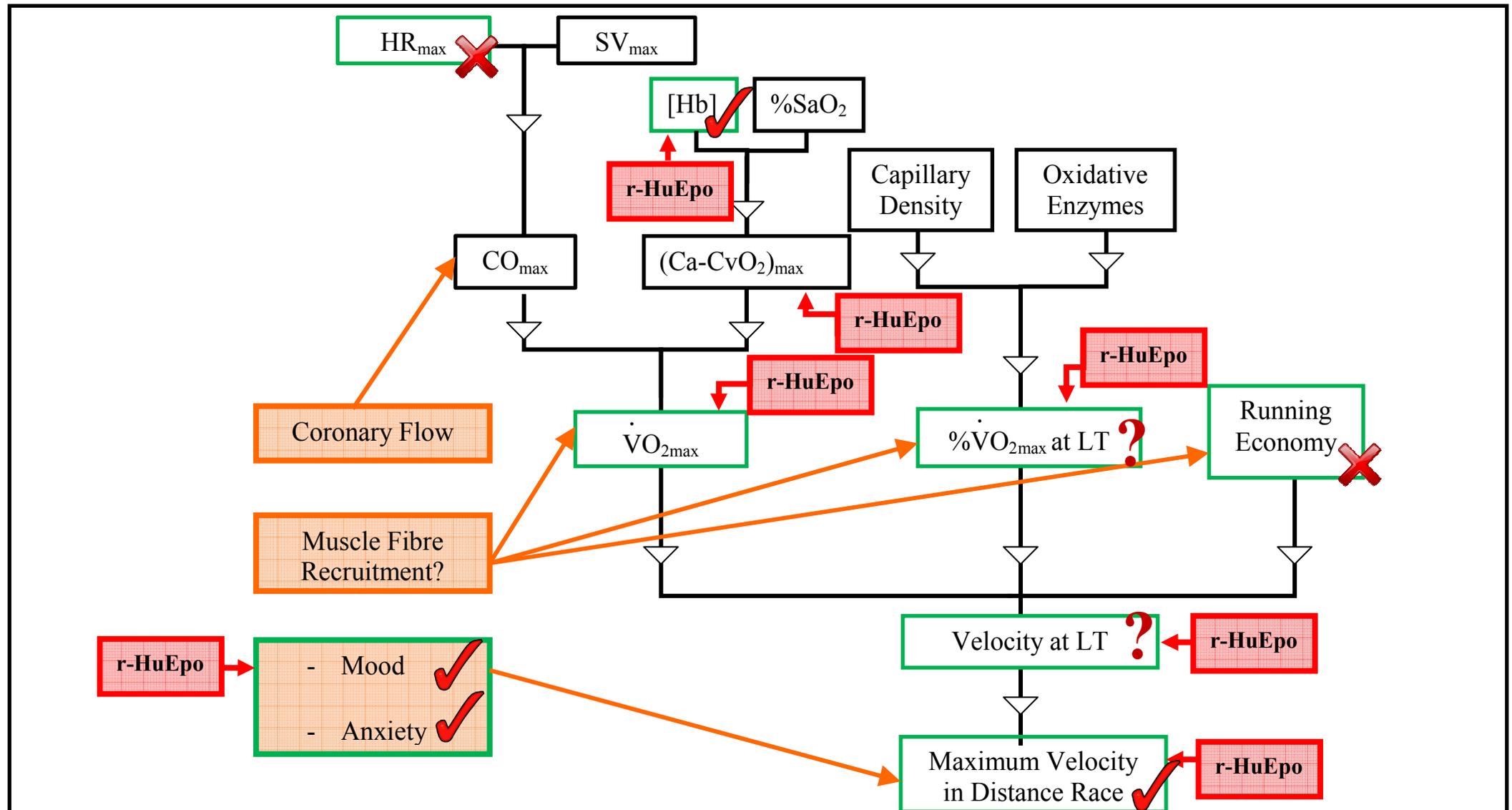


Figure 4.5: Summary of the major cardiovascular variables (black lines), with the addition of the Central Governor Model (CGM) (orange lines), related to $\dot{V}O_{2max}$ and the maximal velocity that can be maintained in distance races. The documented (red arrows) and potential effects (red arrows with question mark) of r-HuEpo are also included. Variables measured in Chapter 4 (green border). Variables found to be of significance immediately post r-HuEpo administration (red tick). Variables not found to be of significance immediately post r-HuEpo administration (red cross). No definitive evidence for effect immediately post r-HuEpo administration (red question mark). HR_{max} = maximal heart rate; SV_{max} = maximal stroke volume; $[Hb]$ = haemoglobin concentration; $\%SaO_2$ = percent oxygen saturation; CO_{max} = maximal cardiac output; $(Ca-CvO_2)_{max}$ = maximal arterial-venous difference; $\dot{V}O_{2max}$ = maximal oxygen uptake; $\% \dot{V}O_{2max}$ at LT = percentage of maximal oxygen uptake at lactate threshold.¹⁵⁵

4.4.3 Study Limitations and Future Considerations

This study has two important limitations. The first is the relatively small sample size, which limits statistical power and increases the chances of type 2 statistical error: this issue has been discussed above. Although several key variables were found to be significantly different after the period of r-HuEpo administration, $\dot{V}O_{2\max}$ was not found to be significant (very likely to be as a result of a type 2 statistical error given previous evidence). Based on a $\dot{V}O_{2\max}$ improvement of 5.2% (as present in this chapter) and an SD of 1.8%, 8 participants will provide 80% power to detect a significant effect at $p < 0.05$.

The second is the absence of a control group. As a result of this, it is not possible to discount the possibility of a placebo effect influencing the study findings. It is therefore imperative that further studies are carried out to assess the effect of placebo injections on running performance in individuals who believe (i.e. deceptive studies) they are receiving r-HuEpo or equivalent injections. A final consideration is that the effect of training was not strictly controlled across all phases of the study – participants were asked to maintain their usual training programmes. Although these athletes were well-trained and followed strict training regimes, this lack of tight control may have influenced the performance results to some small degree.

4.5 Conclusions

This study confirmed r-HuEpo increases [Hb] and Hct, and these were associated with improvements in $\dot{V}O_{2\max}$. Three kilometre time trial performance improved immediately post r-HuEpo administration by 4.9%. However, no clear association was found between [Hb], Hct or $\dot{V}O_{2\max}$ and 3 km performance, suggesting that factors other than changes in [Hb], Hct and $\dot{V}O_{2\max}$ are likely to have influenced running performance. Furthermore, the improvement in 3 km time trial performance persisted for 4 weeks post r-HuEpo administration, despite the r-HuEpo mediated changes in [Hb] and Hct declining by more than 50% over this interval. Positive changes were also observed in anxiety and tension which warrant further investigation as a potential contributor to enhancing performance. Finally, this study is the first (to the best of the author's knowledge) to demonstrate performance enhancing effects r-HuEpo administration, both, in laboratory and in a field-based performance test.

5. Psychosocial and Doping Effects: Viewpoints from Well-trained Individuals after using an Illegal Performance Enhancer

5.1 Introduction

He won numerous medals on the international stage including winning bronze in the 1999 World Championships (www.iaaf.org). He is one of Europe's fastest ever over the 100 m distance holding the second fastest time in British history (www.iaaf.org). In October 2002 he received a two-year ban from athletics as well as a life ban from competing in the Olympics for taking an anabolic steroid derivative - tetrahydrogestrinone (aka THG).

In an in-depth interview on BBC radio in 2008, he was asked, ‘...but when you took drugs for the first time, when people came up to you afterwards and said that you had run brilliantly, didn't you feel just so incredibly guilty?’ He responded, “when I got home, not during, you've got to put on a brave face. Again, that was pretty hard for me to deal with, but eh, you know, we're going back a long time, it's difficult for me to be open about those views...I wish I'd never done it”, Dwain Chambers responded. He was then asked, “...how would you have felt if you had gone through and fulfilled yourself and won an Olympic title? How would you have felt knowing that it wasn't really true?” Dwain Chambers responded: “...it would have been bitter sweet...there would have been a part of me, that yes, I would have got my goals, but at the same time I knew I would have been cheating myself and others...”.

The use of substances to enhance sporting performance has been prevalent in sport for decades, with some reports dating the practice back to the 1890s where cyclists were supplemented with caffeine, cocaine and sometimes even strychnine (Bloodworth and McNamee, 2010). Many studies have attempted to measure athlete viewpoints on drugs and sport as well as attempt to measure the prevalence of doping in sport (Rossi and Botre, 2011, Waddington et al., 2005). Varying methodologies have been employed and include observational studies, interview based studies and questionnaire based studies; each possessing limitations (Laure et al., 2004).

A common problem that is incurred with such methodologies is that one can never ensure the honesty of participants with regards to their own personal experiences concerning illegal substances. For example, review articles, predominantly in North American studies,

have reported that 3 to 12% of male and 1 to 2% of female adolescents as having used anabolic-androgenic steroids at some point in their lives and, of these individuals, 40% were not involved in competitive sport (Yesalis and Bahrke, 2000, Bahrke et al., 2000, Calfee and Fadale, 2006). The fact that this study suggests that 60% were actually involved in competitive sport and admitted their interactions with illegal performance enhancers remains of serious concern. Further, one could realistically suggest that these figures may indeed be under-estimating the true prevalence of drug use in sport as it is likely that some participants, in such studies, may simply be providing researchers with false information either intentionally or unintentionally.

In a recent publication, Lentillon-Kaestner and Ohl (Lentillon-Kaestner and Ohl, 2011) suggested that in order to obtain more reliable data on the prevalence of doping in sport, it is important to utilise various methods including: using various ways of questioning athletes, observing the usage of substances, cross checking data collected, taking into account the aim of substances used, as well as utilising various definitions on what constitutes as doping. Thus, the same approach should also be used when attempting to identify and understand, not only, the underlying reasons behind doping behaviour, but also when obtaining and assessing athlete views on doping (past and present). This is also in addition to approaches on the psychosocial factors and consequences that may be involved and experienced.

In a previous study by Bloodworth & McNamee (Bloodworth and McNamee, 2010) the authors carried out several focus groups on a total of 40 talented male and female British athletes and these sessions were intended to investigate athletes' attitudes towards doping. They reported that, in general, 'the athletes embraced those values promoted in anti-doping educational programmes, although there were some notable exceptions'. Further, it was reported that the social emotion of 'shame' was the most significant deterrent not to dope. Although there were a few notable exceptions identified in this study, a major limitation in such studies is that it is unknown if some viewpoints were actually being given by an individual who is potentially on or who has previously taken an illegal performance enhancing substance. As the authors indicated, the most likely reason being to avoid capture or the reported shame associated with the practice of doping (Bloodworth and McNamee, 2010).

The 2008 BBC interview with Dwain Chambers is useful and unique as it sheds further light on the psychosocial reasons not only behind doping, but also, the psychosocial effects

of carrying out 'doping'. The difficulties associated with this type of research is acknowledged; however the assessment of individuals that have utilised a substance that is illegal in competitive sport (in the confines of research) and who have experienced the associated effects of the substance on sporting performance, as well as on psychosocial factors, would further enhance our understanding in this field.

In this unique study, participants participated in private, individualised semi-structured interviews after completing a trial involving the use of the illegal performance enhancing drug r-HuEpo (according to the WADA code). This WADA approved study (as part of a larger study) assessed the effect of r-HuEpo use on psychological and social (i.e. psychosocial) factors associated with the practice.

5.2 Methods

5.2.1 Participants

The present qualitative study involved six well-trained men (age: 26 ± 2 y; BMI: 22 ± 1 ; $\dot{V}O_2\text{max}$: 58 ± 3 ml·kg⁻¹·min⁻¹) who individually participated in semi-structured interviews (Appendix E) after completing a research trial involving the administration of r-HuEpo by subcutaneous injection for 4 weeks. The 4 weeks of r-HuEpo administration was preceded by a 2 week baseline period and was followed by a 4 week post r-HuEpo administration period. Participants individually participated in semi-structured interviews which were carried out within 1 week of completing the study.

5.2.2 Experimental Procedures

The semi-structured interviews consisted of a series of questions (Appendix E) designed to assess participant psychosocial experiences during the trial as well as their viewpoints on doping and anti-doping prior and after completing the trial. The use of focus groups (Bloodworth et al., 2012) has been suggested to be a more attractive method for participants as it may induce a more relaxed environment as opposed to the more intimidating one-to-one interview. Although focus groups may potentially be a useful means to extract more truthful and open responses, when discussing sensitive issues such as these (Kitzinger, 1995, Bloodworth and McNamee, 2010), it was decided that private interviews would be carried out as confidence and trust was obtained between participants and researchers throughout the 10 week trial period prior the interview stage. In all cases participants knew and interacted with the interviewee for a prolonged period (i.e. over 10

weeks). In all cases, it was made clear and understood by both parties that the contents of the discussion would only be made public in an appropriately anonymous form.

Participants were happy to discuss their psychosocial experiences during the trial and any issues relating to doping and anti-doping practices. All participants signed a declaration stating that they would not take part in any sporting competitions whilst participating in the trial. It is acknowledged that a degree of caution must always be taken when assessing participant responses, however the development of an atmosphere of trust was crucial, and the researchers believed that this was achieved.

5.2.3 Data Analysis

The semi-structured interviews were recorded and transcribed. Transcriptions were manually analysed and coded (Express Scribe v 5.45, NCH Software and Microsoft Office Excel 2003). For consistency, the interviewee also carried out the transcription (exactly as spoken) and coding processes.

5.3 Results

5.3.1 Overall Thoughts on r-HuEpo Trial Participation

All the participants were happy about taking part in the study. The general consensus was that it was an enjoyable and informative experience and participants particularly enjoyed the learning of new aspects on their physiology and psychology. Two individuals made more exceptional comments:

Participant 1: *‘...interesting for myself to go through these 10 weeks, try something that’s banned. Definitely yes’.*

Participant 2: *“...it was really interesting to see what potential I have whilst on EPO”.*

In general, all participants were intrigued to experience the effect of r-HuEpo administration on exercise performance. All participants showed a belief that r-HuEpo administration would elicit a positive change, and in some cases reveal ones ‘true ability’ if

they were to pursue their training further with a legal alternative to doping, for example, altitude training.

5.3.2 Thoughts, Expectations, Anticipations, Anxieties Prior r-HuEpo Administration

Participants were asked how they came to their final decision to participate in the trial and how they evaluated the potential risks. All participants understood the potential risks involved in the trial as described in the trial information sheet (Appendix B) and from discussions held prior study participation. However, it became apparent that the environmental setting of a research facility was what ultimately persuaded and comforted all the participants to participate. Interestingly, no participants commented on any potential health risks. A notable response was:

Participant 3: *“I was quite excited by it to be honest. Em... the other stuff was obviously legal [in reference to other substances that this individual has previously used], haha, whereas this stuff could get you banned...yeah I was excited. To do it in a controlled environment such as this was a good opportunity...comfortable with it here...I’ve read the horror stories of cyclists dying, but I trusted that you would do a good job.”*

There was one individual who showed more concern prior deciding to participate in the trial. However this was not in relation to potential health implications, but more associated with the fear that others would ‘find out’ and the resulting potential social stigma that may occur even after completing the trial and being ‘clean’ again:

Participant 2: *“Yeah, I remember at the start being a bit unsure and I needed a couple of weeks to think about it, but at the end of the day I wouldn’t take it professionally...nah...nah. I totally trust you and everything else, it was more the fact that if somebody found out about it, they would tell everybody and people at a race would say oh he’s done that. Just because some, well most...won’t understand this, so...”*

When participants were asked about their anticipated feelings when taking r-HuEpo, all six participants believed that r-HuEpo would improve their endurance exercise capability;

however, five participants were unsure as to how and what feelings would manifest. A representative response was:

Participant 3: *“I don’t know what I expected really...”*

In contrast to this feeling, one individual felt the opposite and was more specific with regards to how r-HuEpo would make the individual feel:

Participant 1 *“...I was really interested to see how EPO would affect, eh, how I will run. I thought yeah, I thought maybe I’d find something completely different when I would be running...to see if it would be easier or if I would be stronger”.*

When participants were asked whether or not they had any anxieties about taking such a substance (in reference to the point where participants were about to start self-administering r-HuEpo), 5 participants developed a concern closer to the action of self-administering r-HuEpo. These individuals started to mention, more adamantly, how they wanted their participation in the trial to be kept private. Most participants told nobody outwith the testing environment, whilst those who regularly trained with a trusted coach informed their coach of their participation. Participants were not asked specifically how they felt with regards to needles and self-injecting the substance; however, one individual commented:

Participant 5: *“...I think the only doubt was the injection really...never had a problem with needles before, but it was maybe the self-injection thing...that was the only part I was worried about. Yeah, the fact that I wasn’t a trained professional, me and my chubby fingers and that...”*

More notably one participant commented on the initial concern regarding the self-injections, but elaborated further how quickly this became a non-issue:

Participant 3: *“beforehand it was quite...I was quite excited, but also apprehensive about it, ‘cause I have never taken any performance enhancer like that before, there was some apprehension, but when I started, I was...felt quite strange about giving myself an injection, it was I*

don't know, there's a kind of a stigma attached to that. But I was surprised how quickly I just slipped in... just how quickly it became normal".

Further, although this aspect was not mentioned by all participants during the final interview stage, it was clear that prior starting the trial, all participants sought comfort with regards to the self-injection process as none had self-injected any substance before. It was clear to the researcher, who observed participants self-inject, that participants showed notable anxiety during the first injection but this anxiety was not present during the remaining injections. Participants quickly became less concerned with the action of self-injecting.

5.3.3 Experiences of Effects during r-HuEpo Administration

All participants expected to experience a physiological and/or psychological change whilst taking r-HuEpo; although the majority were unsure in what form exactly. Participants were asked questions with regards to how they felt during r-HuEpo administration in general, but also how they felt during training / performing / running. More specifically, participants were then asked whether recovery was different in any way. Participants were also asked to what degree they felt any changes, that may have occurred, were down to the substance itself or down to the individuals own hard work.

All participants described feeling different or commented that taking the substance had an effect; however the magnitude of the perceived effect varied between participants. Five of the participants described an achievement that they have never managed before and this ranged from maintaining greater work rates during training, for longer periods, to achieving certain fitness targets at a much quicker rate. Notable comments include:

Participant 4: *"One day, just, I was running with the same people I would normally, they would finish the run and I would be the back of the group, but half way through the run they were running fast, and as they were running fast they were saying 'you know we are running pretty fast' and I was with them. They started to struggle and then I took off and left them, it was ridiculous, I got quite embarrassed, and you know it was like where the hell did that come from. I thought that must be the EPO. You could see people in the group when they came in at the end, they are like 'how you doing?' I was like, 'I'm off' [laughs]. I was embarrassed. I felt*

like that was...but then again, since then, I have had experiences where I have run as well, so I don't know of that was a special day, or were they struggling...but that was one occasion where I took off".

Participant 3: *"... certainly I would say looking at the times I was running, I mean that was certainly what I would expect from a much longer period of training. So something's done something for sure".*

With regards to recovery, 5 participants referred to recovery periods either in between training intervals or between training days as being either 'quicker' or 'better'. For example:

Participant 3: *"...and the recovery was something that I noticed, the recovery sessions felt so much better...quicker".*

More specifically, all participants commented on how they felt peripheral components of fatigue (i.e. legs) were limiting factors, whilst respiratory components felt fine. One individual commented on a notable occasion during training:

Participant 2: *"That was the day, when I came in, my heart rate fell down quickly, massively, within a couple of minutes, I probably was thinking that must be the EPO kicking in. I think yeah, anytime I did run like that, I must have thought yeah that must be the EPO".*

Conversely, one participant mentioned feeling more fatigued; one throughout the 4 week r-HuEpo administration period, and the other more specifically at the end of the r-HuEpo administration phase.

Participant 4: *"I found that I became quite tired at the end of EPO, I don't know, maybe 'cause I was trying to domaybe I knew I was on EPO, so I thought I could do much more so I went out hard, but at the end of it was taking more days to recover. I just remember, getting quite tired".*

Participants were then asked to what degree were any of the performance enhancing effects down to the substance itself versus the individuals own hard work. In addition, they were

also asked if there was a possibility of a placebo effect. Two participants were sure that it was solely down to the substance itself. A notable response included:

Participant 6: *“I would say that was down to the substance, like, feeling floored for a week and then running a PB. I can still remember being float-like during the whole 15 laps and, like, it was pretty weird...”*

Participant 6 continued to describe how the substance itself had impacted on his feelings as well as how the substance had changed his behaviour with no reference to a potential placebo effect:

Participant 6: *“I think that when you’re training so hard and then still be able to recover and then still train really quick that your confidence is high so your confidence plus EPO has a big boost and then the fact that you are willing to risk in training, like running faster, ‘cause you think during training, I’m on this drug and going to get better, so I’m going faster...let’s take more risks, let’s run ‘til you drop... See where this will take you, so maybe you feel a bit more I would say ‘immortal’ in training. You would feel as if like, I felt, there sessions where I could feel I could run so fast where I would generate no lactate, but didn’t know why, but at the end of the sessions you would feel lactate, but during the first few reps you’re still running really quick. And you got lots of confidence, your running going like you can’t feel the burn yet –brilliant! And people behind you chasing you. Gives you a massive boost psychologically...that’s something that I’ve not got in my locker usually. I’m not such a confident person normally, I feel quite a lot of athletes are better than me and that’s the reason why I train really hard to become better than them. People say the hard way isn’t the right way all the time, but when on EPO I was training hard and running fast, felt like nobody could stop me. I had something that I’ve never had before”.*

One participant was less adamant and suggested that it was a combination of both substance and hard work, but with no reference to a placebo effect:

Participant 5: *“...I would say to a good degree the substance, but there’s been a lot of effort into that”.*

The remaining participants showed uncertainty in response to this question. An example response included:

Participant 4: *“Placebo side... I don’t know, I think knowing that I had it in me, should be able to do something, and I must have enough energy to do more. It sometimes pushed me out when I didn’t feel too keen; I went out because I thought I would be fine due to having it in me”.*

Interestingly, participant 4 also commented specifically that he felt more of a performance enhancing effect on the training days that coincided, specifically, with the days of r-HuEpo administration.

Participant 4: *“But I think I was just, I don’t know, once I really noticed that I felt wow, it was at the running club, 7 mile run, 8 miles we were running, during the EPO phase, and I think I also found that, I think, I performed better the day I took EPO rather than the next day” [EPO was administered every second day].*

5.3.4 Viewpoints After r-HuEpo Administration

Participants were asked a series of questions regarding their feelings since the cessation of r-HuEpo administration 4 to 5 weeks after the last r-HuEpo injection. All participants acknowledged their performances had slowed over the 3 km distance performance measure employed in the trial 4 weeks after the final r-HuEpo injection. Three participants provided a more in-depth description:

Participant 3: *“...it was quite exciting the whole way through to see what kind of changes would take place, and then afterwards I don’t know, I suffered a mental withdrawal....I need more!”*

Participant 4: *“...and EPO to post, post was the slowest, yeah, so yeah, you know good in the sense that how the training has gone. As long as I’ve, even though I was feeling horrific here [in reference to post phase 3 km time trial], so even though that’s a bit slower than this, I’m happy, ‘cause I felt really bad then [post EPO]. I’m happy that I was faster than*

the pre-race. From pre to post, a 3% difference...interesting, so training must have been going well too”.

Participant 6: “...now getting further away from taking EPO, my body, it’s getting more fatigued, more easily, but could be many factors...it’s a wee [i.e. little] bit frustrating, I could and now can’t. I would be lying in saying it’s me just going back to being normal, but no I do feel a bit frustrated that I’ve not got that same leg turnover and I’m no [i.e. not] just now, I’m not so confident as what I was...because I’m no running, I’m almost running the same times though the constant training, but I’m no running the same as on EPO. I’m finding it harder to achieve things now”.

5.3.5 Viewpoints on whether athletes should be able to use such a substance as a component of their training

All participants generally showed an anti-doping sentiment, however some more convincingly than others. Three participants demonstrated outright objection and associated sporting performance with one’s natural ability and hard work:

Participant 4: “I think anything [in relation to substance abuse] that’s going to enhance your performance, I think should be banned, I think, there’s, I’d rather see everyone play on the same playing field...I guess. The best ultra-runner in GB would run in the Chamonix race, and has to work a full-time job as opposed to others who don’t. Would it be okay for semi-pro Scottish athletes to take EPO to catch full timers up? I think it’s up to the individual to make time, like people in my running club, to make time to fit it, or change your lifestyle according to what is priority. I’d rather, I think it’s, what else could they use? It would go out of control; if you’re allowed to use EPO then people would find another drug to make people go faster. I don’t know, I would say, I think it’s very interesting, but at the same time one of the reasons as well that I took part in this, when you said you would be also looking at detecting methods, that’s probably one of the reasons why I participated in something to clamp down on drug use. I was a part of this effort, say 10 years down the line, I was a part of this work. Great! One of my main reasons probably”.

Participant 1: *“...I still don’t think so, because you should put limits and boundaries for everybody and I do think taking injections like this shouldn’t be allowed. Saying this, we know that current tests are not very, I would say, efficient. We have to find a solution...other solutions must be found”*.

Participant 2: *“I’m still very much against it, it is just cheating...I just, it is cheating, like, you are using something that’s not naturally made from your body, it’s not right. It should be all the athlete’s work. People say that there’s not much difference between this and altitude training, but at altitude you’re still putting in the work, it’s not just putting in EPO into your body. If I was, if I was at that level, and like everybody else was doing it, I would probably, but yeah I can see the sort of draw to it for athletes. Like I said when I was training it did feel amazing, just to be honest. I can appreciate it a wee bit more why people would do it, but I don’t really think it...my view hasn’t really changed on it. If somebody else was taking it to try and cheat, I would still be against them”*.

The other three participants described the situation as ‘complex’ or ‘difficult’. Particular reference was made to what was perceived as their competitors, from countries such as Kenya, who live in conditions more favourable. More specifically, references were made with regards to the benefits of living at high altitude for endurance athletes. These participants perceived that r-HuEpo use essentially provided similar performance enhancing properties to training models utilising high altitude as a component. Notable comments include:

Participant 3: *“It’s a tough one because, I mean, I have had this conversation before with others where athletes are fortunate enough to live at altitude and they get similar results, eh, whereas in this country we don’t have that kind of advantage. I don’t know, I think my views have changed since being involved in the trial. If these guys are allowed to create these results just by sleeping in a certain place, just by taking advantage of their surroundings, I think we are disadvantaged in this country [i.e. UK] simply because the place is the way it is, and if... I don’t know...where can you draw the line. I don’t know...if you can sleep in a*

tent to give you the same results? Is that cheating? Is it cheating when you introduce a needle, or not? It's a tough one, as you are achieving the same results, yet artificially".

Participant 5: *"...well, it's hard to say because there are so many illegal substances out there that could maybe do similar things, but I don't think would give you such a great improvement. But, if you took a proper athlete and it did the similar effect, that's un-heard off. How do you eradicate drugs? I mean, what's the difference between coffee and EPO you know....really deep question. A similar reaction will be brought about by blood transfusions...emm....ehhh...no, very much against drugs in sport, but I think they've got a tough job on their hands"[in reference to anti-doping agencies].*

Participant 6: *"...tough question, personally you would go yeah because I would then be allowed to take it and train even better, but could be many factors. As you've said, doesn't mean just 'cause blood profiles go up, doesn't mean your performance will go up. Yeah, like what happens if you've got a higher haematocrit level then the guy standing next to you in the line, but he can utilise it better then you. So if everybody could use it on a level playing field then maybe, but that wouldn't happen due to money, it's really expensive, like the poorer and going to run bad and the rich going to run well. Like poor runners in European countries, not Kenya and that. Like the well-off families are going to be able to buy this substance and run well, em...then you've got your poor runners in Kenya that have altitude, so they're going to run well anyway. It would kind of off-set the imbalance. I would say, keep it banned, there's so many like, you want people to be running races and showing excitement because well they've done hard work to get there...people think that somebody that has run fast might be on drugs, thinking on my god, that's amazing, but like what's really going on.....a big report comes out saying they've taken drugs...oh that's how he's run fast. Too much to lose kinda, so if you can train hard and run fast clean, you've got people that will look up to you and follow you, it's so much more. Taking drugs takes a bit of pride away you know. I'd say keep it banned, but the effects are good, but I think keep it banned".*

5.4 Discussion

The information obtained in this experimental chapter has overcome two important limitations which exist in currently available literature, regarding psychosocial insights and experiences felt by users whilst ‘doping’. Firstly, issues regarding the truthfulness and accuracy of responses have been overcome as participants were given a platform on which they could discuss issues open and freely, without risk, whilst using a banned substance. Furthermore, this study also provided useful insights into athlete viewpoints on drugs (as well as potential contrasts before and after using a banned substance), impacts on sporting performance as well as doping in sport. This, in turn, has also provided potentially useful insights for anti-doping strategies.

Overall, all participants reported experiencing a performance enhancing effect as a result of r-HuEpo administration and participants described an achievement that they have never managed before, ranging from being able to maintain greater work rates during training for longer periods to achieving certain fitness targets at a much quicker rate. All but one participant referred to recovery periods either in between training intervals or between training days as being either ‘quicker’ or ‘better’. All participants commented on how they felt peripheral components of fatigue (i.e. legs) were limiting factors, whilst respiratory components felt fine. All participants generally maintained an anti-doping sentiment, however some more convincingly than others especially when comparing to those deemed to be privileged with living at altitude.

Interestingly, a key psychosocial implication that emerged involved the feelings and/or emotions of guilt, shame and/or embarrassment. The difference between the two emotions of shame and guilt as well their relationship has long been debated (Tangney et al., 2011). Typically however, shame can be described as an emotion where the social norm is not adhered to whereas guilt is an emotion experienced after carrying out an act against socially accepted rules (Morris, 1971). One could argue that participants were protected from committing a shameful act due to participation in the name of science, however as previously highlighted; all participants refrained from telling peers and those who did choose to tell anyone, told a very small number of individuals. This was due to two factors, firstly the fear that public perception would not believe the individuals true motive for being part of the trial involving an illegal substance, and secondly, that the person did not want to be perceived as willing to carry out a shameful act.

Five participants showed a degree of guilt or in one case ‘embarrassment’ as result of the performance enhancing effects of r-HuEpo.

Participant 3: *“I didn’t tell anybody at my club that I was doing this, one thing that I hadn’t considered is that we all gauge our fitness on other people. I didn’t realise that it could have a negative effect on other people when they see how quickly I am improving. They are doing the same training and same sessions, but they aren’t getting the same results. On a couple of occasions people did say like they just weren’t improving the way they should be...that kind of thing. Obviously it was noticed, it was noticed by coaches in club...you’ve gained shape quite quickly, but I didn’t say anything, although, it is kind of funny from my point of view...it must be quite dispiriting for others. I just attributed to the miles of running...yeah never even considered it until I was in the situation. Maybe not ashamed, but I felt bad for those kinds of people...”*

Participant 4: *“One day, just, I was running with the same people I would normally, they would finish the run and I would be the back of the group, but half way through the run they were running fast, and they were running fast they were saying you know we are running pretty fast and I was with them. They started to struggle and then I took off and left them, it was ridiculous, I got quite embarrassed...you know it was like where the hell did that come from. I thought that must be the EPO. You could see people in the group when they came in at the end, they are like how you doing? I was like, I’m off. I was embarrassed”.*

Participant 2: *“Just mentally, sometimes I think eh, one of the sessions I thought about...I was running with a guy and coach, and was told to tuck in behind, but in the last straight, even between reps...my legs and breathing felt amazing and I flew past this guy on the last stretch. Wasn’t even funny how easy it was. It was 4 x 600 reps, 800 pace, towards the second week of EPO. When I finished that, I thought it was, I felt I was like cheating...that’s when I thought there’s no way I could do this properly, to cheat people, if I was racing...just didn’t feel right, felt like an unfair advantage”.*

However, one particular individual clearly stated that no guilt was experienced and entertained the idea of using r-HuEpo again and/or alternative performance enhancing methods:

Participant 6: *“Even though it was illegal, I didn’t really have that guilt or anything, no anxiety, at the same time without taking it I was running really well. I was like well...aye [i.e. yes], like that’s probably maybe my personality but sometimes while, quite a lot of the time I care a lot about what people think. I did keep it to just a very, very, very few people that I knew. EPO is illegal, and I would say that if say... I’ll be really honest...I would do your trial again to go back on it. I would make sure I was out of competition, in a way I’m still cheating, but I’m not competing, so one way I’m not allowed to compete, but I’m still taking a banned substance which I was fine in doing during the trial and I would still do it again...just to boost my performance. I know that there’s a certain time limit or time where it would go out the system, so I would do the trial and take EPO and then wait till it was out, carry on training hard, keep working through a hard training phase which I’m sure this is what some athletes do through their phase of [being] on EPO and wait till it’s out their system. I’ve actually looked into altitude generators that you can get, like you can get the tent over your bed, it’s a lot of money....like just under £3500”.*

Not only does limited research exist assessing psychosocial factors, attitudes and values of athletes towards performance enhancing drugs, but even less exists examining those who have been truly known to ‘cheat’ or those who are in the process. The reasons for this are obvious. However, assessments of those individuals who have used illegal means for enhancing performance would enable us to further understand more factually, as opposed to most studies which are often hypothetical, about the beliefs and complications of doping practices and also the likely psychosocial implications of doing so.

Although alternative methods, such as questionnaires and/or focus groups, could have been employed, the quality and depth of the data obtained as a result of one-to-one interviews suggests that this method is effective when a strong rapport has been established between the interviewer and volunteer. In this case, the interviewer had closely interacted (physically, and via email and telephone) with all participants on a very regular basis to ensure a trusting and open relationship was established.

It is acknowledged that, due to the small number of participants in this study, one must be careful to extrapolate views as being general to the greater population. Further, it could be argued that these individuals are already of a certain ‘personality type’ as they have willingly put themselves forward to participate in such a trial. However, even when taking these points into consideration, the data still shows varied responses to questions which, in itself, indicate the complexity behind human attitudes and personalities. There may be certain attributes that may make an individual more likely to dope; for example an individual who is less susceptible to the emotion of guilt as may be the case with one participant in this study. However, in this case for example, if the environmental setting was outwith the confines of a ‘controlled’ and ‘safe’ research facility and more associated with the ‘underground’ or ‘black market’, this individual may have felt differently about participating. This may be because the health risks, associated with inappropriate use of the substance, would have become more possible or real in that situation.

In five cases, the individual’s personal performance enhancement expectations were generally fulfilled, but to varying degrees, as a result of r-HuEpo administration. However, in one case, although there was a measured performance improvement from baseline to immediately after r-HuEpo administration, this particular individual’s expectations were not fulfilled; this individual expected ‘more’ of an effect. Interestingly, this particular individual showed the least enthusiasm or excitement that r-HuEpo would elicit a great change in performance throughout the trial.

Five of the participants did not change their stance with regards to doping in sport and, of them, one individual entertained the idea that doping should be allowed, and four individuals stood by their initial stance of ‘drugs should be banned in sport’. These five individuals provided a range of reasons against the use of such substances for performance enhancing purposes upon varied emotions from guilt and shame to the unfair advantage that may be obtained against fellow competitors. Interestingly, participant 6 suggested that he had changed since being involved in the trial stating:

Participant 6: *“I think we are disadvantaged in this country simply because the place is the way it is” [referring to Great Britain].*

This individual perceived the geographical advantages in certain other countries, where altitude can be more readily and more effectively incorporated into training, as simply

being an unfair advantage. However, this particular individual did not condone the use of substances such as r-HuEpo as long as they were still categorised as being illegal.

The highlighted psychosocial consequences of using an illegal performance enhancing drug, as previously indicated, may also assist in acting as a deterrent to doping. If the public are made more aware of such potential psychosocial implications, which could potentially seriously affect relationships and psychological well-being, then those tempted by doping may be encouraged to think twice.

5.5 Further studies

This study adds useful insights into athlete viewpoints on drugs (as well as potential contrasts before and after using a banned substance), impacts on sporting performance as well as doping in sport. In agreement with previous authors (Reeves et al., 2011), these are useful for incorporation into the development of key coping strategies in order to improve how individuals deal with such related psychosocial challenges. Further research is needed to assess how such psychosocial impacts can be dealt with in order to help athletes better cope with the many pressure associated with elite sport (e.g. to educate athletes on the psychosocial consequences of doping and also provide coping strategies in order to deal with scenarios where athletes might be encouraged to dope).

5.6 Conclusions

All participants reported experiencing a performance enhancing effect as a result of r-HuEpo administration and participants described an achievement that they have never managed before, ranging from being able to maintain greater work rates during training for longer periods to achieving certain fitness targets at a much quicker rate. All but one participant referred to recovery periods either in between training intervals or between training days as being either 'quicker' or 'better'. All participants commented on how they felt peripheral components of fatigue (i.e. legs) were limiting factors, whilst respiratory components felt fine. Psychosocial implications included the feelings and/or emotions of guilt, shame and/or embarrassment and these were prominent themes that emerged. These feelings and/or emotions may also, in turn, be an effective deterrent to doping. All participants generally maintained an anti-doping sentiment, however some more convincingly than others especially when comparing to those deemed to be privileged with living at altitude.

6. The Effects of an Injected Placebo on Endurance Running Performance in Simulated Race Conditions

6.1 Introduction

The placebo effect is acknowledged as a key factor in medical research and, as a consequence, its effect has been controlled in clinical trials for over 50 years (Price et al., 2008). The placebo effect has also been recognised in the context of sports performance, where researchers have reported statistically significant performance improvements with placebo interventions (Price et al., 2008, Beedie and Foad, 2009). Studies, mostly in laboratory conditions, have shown the positive effects a placebo intervention can have on physical performance where athletes have been shown to improve performance by either exceeding performance limitations and/or by diminishing the perception of fatigue/pain (Ariel and Saville, 1972, Maganaris et al., 2000, Foster et al., 2004b, Beedie et al., 2006, Beedie, 2007a, Beedie and Foad, 2009). However there are few studies which have assessed how the placebo effect impacts on self-paced running performance, during field-based head-to-head competition scenarios, as opposed to tests confined to the laboratory. Such an approach is likely to provide a better measure, than can be determined from lab-based studies, of the likely magnitude of the placebo effect on sporting performance in real competition environments (Wilmore, 1968).

There are reports of increasing use of performance enhancing drugs in both elite and recreational level sport (Waddington et al., 2005, Carpenter, 2007, Pitsch, 2009, Holt et al., 2009, Cakic, 2009, Rossi and Botre, 2011) and in recent years a growing body of research has been undertaken into the health and performance implications of taking such substances (Wagner, 1991, Bahrke et al., 1998, Goldberg et al., 2000, Dawson, 2001). One such drug is r-HuEpo, a recombinant form of the endogenously present glycoprotein based hormone primarily secreted by the kidneys in humans (Egrie et al., 1986). Pharmaceutically engineered r-HuEpo was first developed in the 1980s and is now used in clinical applications; including the administration to patients suffering from anaemia due to chronic renal failure (Eschbach et al., 1989) and to cancer patients undergoing chemotherapy (Platanias et al., 1991). Research studies have also demonstrated that r-HuEpo administration increases Hct and oxygen-carrying capacity which often results in the improvement of endurance performance in athletes (Egrie et al., 1986, Ashenden et al., 2001). However, r-HuEpo is banned for use in sport by the World Anti-Doping Code and in recent years a number of elite athletes have received bans for its use.

A key feature of r-HuEpo administration is that it is given by injection, and there is clear evidence from clinical medicine that the route of delivery is a key mediator of the size of a placebo effect. Further, placebos administered by injection have been shown to induce a larger effect than placebos administered orally (Zhang et al., 2008). In the context of sports performance, one study has demonstrated a greater placebo effect on pain tolerance with use of intramuscular injections compared to oral administration (Benedetti, 2009). However, to the authors' knowledge, no study has assessed the effect of a placebo injection on endurance running performance. Furthermore, carrying out qualitative interviews to assess the effects of the perceived r-HuEpo injections on physical self-perceptions is also important to further our understanding on how such an intervention may influence endurance running performance. As previously highlighted in section 1.10, Figure 6.1 illustrates the key factors that influence endurance performance and how an injected placebo effect may influence endurance performance. In addition, the key variables which were used to assess placebo influences, during this study (Chapter 6), are also highlighted.

The aim of this study was therefore to determine whether an injectable placebo (0.9% saline injected subcutaneously) claiming to be a legal substance (OxyRBX), with similar effects to r-HuEpo, would improve endurance running performance in simulated race conditions. This study also assessed whether injecting a placebo elicited psychological changes associated with a positive performance response. These effects were assessed under real-life competition scenarios as it has been suggested that in such conditions the effect of a treatment may be substantially different when compared to a laboratory setting (Foster et al., 2004a).

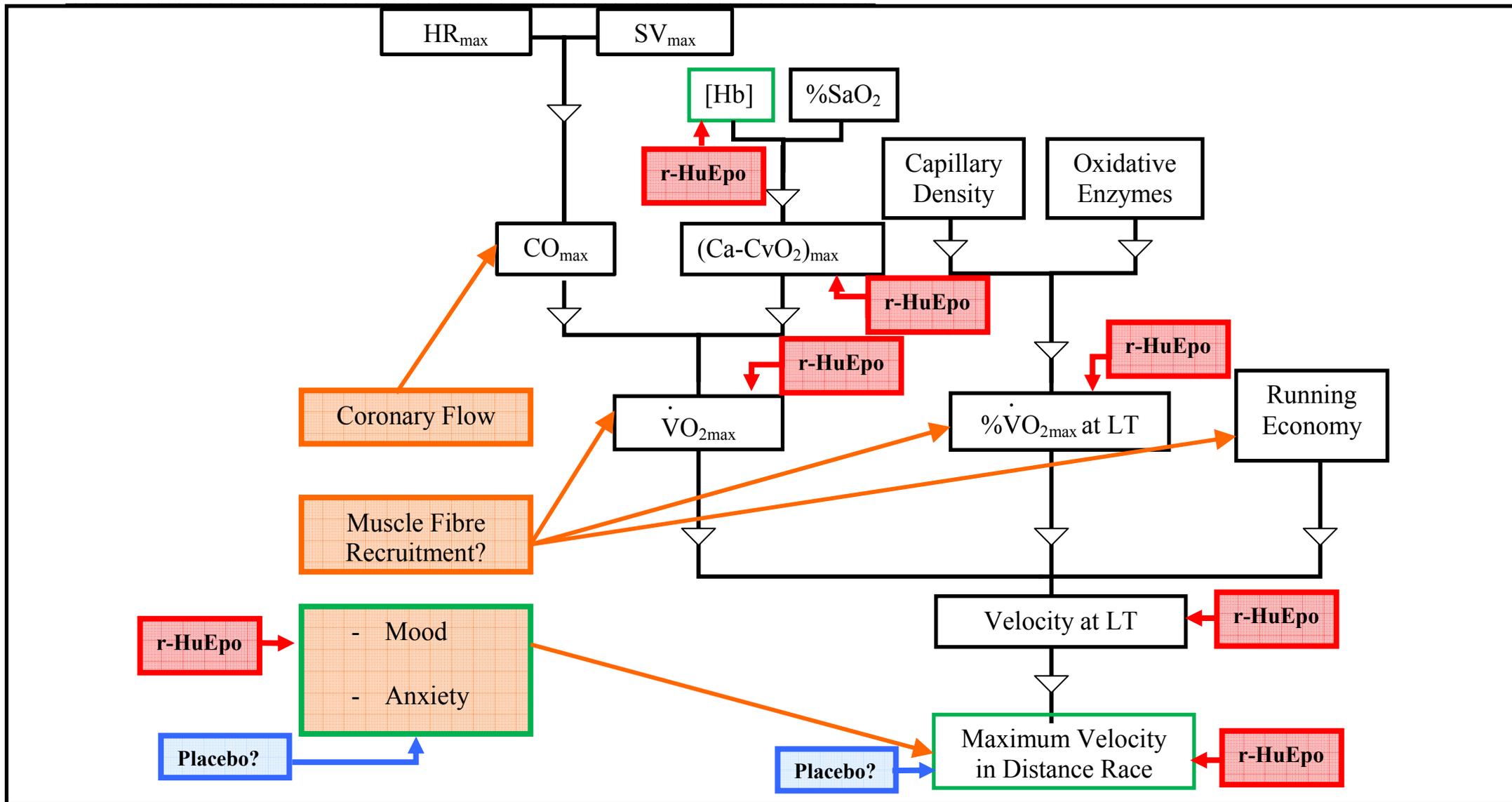


Figure 6.1: Summary of the major cardiovascular variables (black lines) related to $\dot{V}O_{2max}$ and the maximal velocity that can be maintained in distance races with the addition of the Central Governor Model (CGM) (orange lines), the known (red arrows) and potential (red arrows with question mark) pathways that r-HuEpo and an r-HuEpo placebo injection (blow arrows with question mark) may influence. Variables measured in Chapter 5 (green border). HR_{max} = maximal heart rate; SV_{max} = maximal stroke volume; [Hb] = haemoglobin concentration; %SaO₂ = percent oxygen saturation; CO_{max} = maximal cardiac output; $(Ca-CvO_2)_{max}$ = maximal arterial-venous difference; $\dot{V}O_{2max}$ = maximal oxygen uptake; % $\dot{V}O_{2max}$ at LT = percentage of maximal oxygen uptake at lactate threshold. Adapted from (Bassett and Howley, 2000).

6.2 Methods

6.2.1 Participants

Nineteen endurance-trained male volunteers were initially recruited to take part in the study. Four participants dropped out of the study prior the commencement of ‘OxyRBX’ administration. Three of the four drop-outs mentioned ‘fear’ of the possible complications (e.g. blood clotting) associated with OxyRBX use. One individual did not give a reason for dropping-out. Thus, fifteen participants (age 27.5 ± 6.8 years, height 1.79 ± 0.05 m, body mass 73.4 ± 7.6 kg and BMI 22.9 ± 2.0 kg m⁻²; mean \pm SD) successfully completed the experimental protocol. The fifteen participants were well-trained club level athletes, who reported engaging in 213 ± 129 minutes of endurance-based training and 50 ± 58 minutes of resistance training per week, with personal best times for running 10 km of 39.3 ± 4.4 min (mean \pm SD). All participants were apparently healthy and none were taking any medications at the start of the study. Participants provided written informed consent on the basis that they were undertaking a trial to investigate the effects of the legal EPO-like substance ‘OxyRBX’ on sporting performance, rather than a trial of the placebo effect (Appendix C). This deception was essential for the study to be successfully undertaken and is standard practice in published studies of the placebo effect (Beedie and Foad, 2009). Participants were fully debriefed about the true nature of the study, on completion, in a final exit interview. The study was approved by the research ethics committee of the College of Medical, Veterinary and Life Sciences at the University of Glasgow and was performed according to the World Medical Association (Declaration of Helsinki) code of ethics.

6.2.2 Preliminary Study Brief

Prior the commencement of the performance trials, the effects of r-HuEpo administration on exercise performance were described to participants and discussions took place concerning its alleged use particularly amongst elite cyclists. Following this, participants were informed about purported benefits of ‘OxyRBX’ on performance which was described as being a legal EPO-like substance, which had been shown to induce similar benefits to EPO in animal studies and which extensive testing had shown to be safe to use in humans. Potential side effects of OxyRBX (e.g. rash; hives; itching; difficulty breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue) were also described, but it was emphasised that these were extremely rare (less than one case in a million). This

priming process was carried out in order to reinforce beliefs about the effects of OxyRBX on exercise performance and the similarity of these effects to those of r-HuEPO. The effectiveness of this reinforcement procedure was confirmed in two post-study interviews.

6.2.3 Experimental Design

The experimental design is outlined in Figure 6.2. Participants initially underwent an individual familiarisation 3 km time-trial run. This was carried out to familiarise participants to the track and racing over the 3 km distance. With participants unaware, these individual 3 km completion times were used for handicapping purposes for the future 3 km competition runs. Participants then followed a randomised cross-over study design where each participant underwent tests before and after a 7 day 'control' phase, during which no intervention was given, and before and after a 7 day 'placebo' phase, in which participants received daily subcutaneous saline injections (0.5 ml sterile 0.9% NaCl). Participants were informed that these injections contained OxyRBX, which should elicit similar effects as r-HuEpo. Eight participants underwent the control phase before the placebo phase, with the other seven undergoing the placebo phase before the control phase. There was a 2 week 'washout period' between the two study phases and participants were told that this was to ensure a suitable OxyRBX 'washout period' for the participants who started with the OxyRBX administration phase. Participants were regularly informed not to discuss which group they have been assigned and, as far as possible, were monitored to ensure that no such discussions took place.

At the start and end of each 7-day phase of the study (i.e. 7 days of control and 7 days of placebo) each participant's body weight was measured and a blood sample was collected for a full blood count assessment (as described in section 2.6). In addition, participants completed a CSAI-2 and POMS questionnaire (Appendix D) which was then followed by a competitive 3 km race.

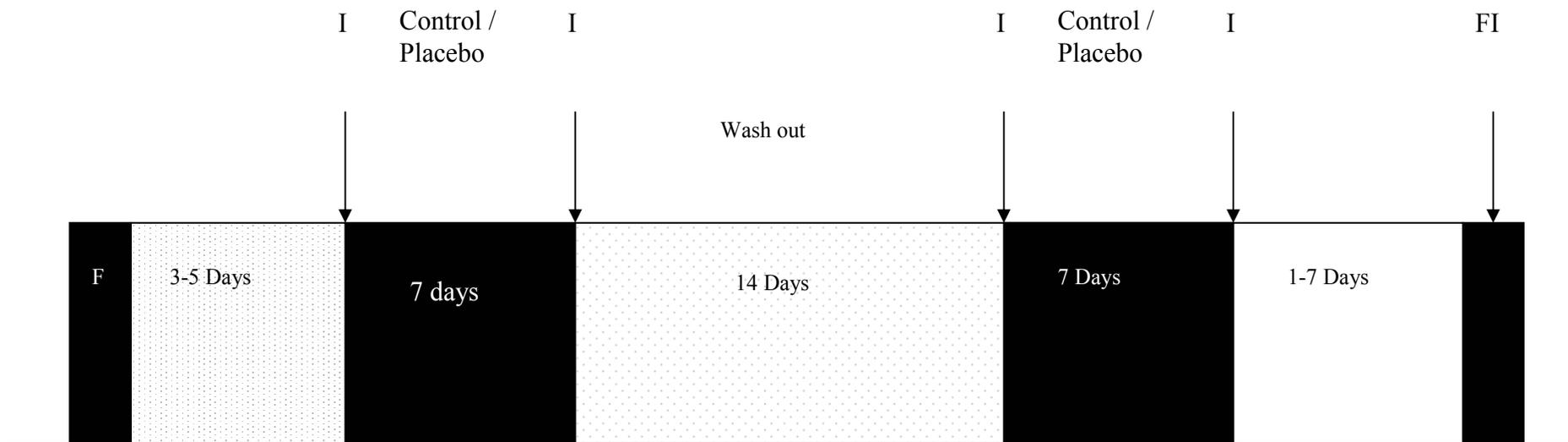


Figure 6.2: Study design. F: Familiarisation; I: Interview; FI: Final interview, in two phases, prior to and post revealing deception. Vertical arrows indicate data collection days (i.e. demographics, blood, questionnaires and 3 km time trials). Placebo injections were self-administered on each day of the placebo phase.

6.2.4 3 km Race Competitions

Each participant in each cohort undertook four performance tests as a competitive 3 km race on an indoor 200 m running track located at the Kelvin Hall Arena (Glasgow, U.K). The competitive 3 km races were carried out in a mean ambient temperature and humidity of $17.9 \pm 0.8^{\circ}\text{C}$ and $39.6 \pm 3.4\%$, respectively. To ensure that the 3 km races were competitive, participant starting times were handicapped based on the times achieved during the individual 3 km time trial familiarisation runs (i.e. a participant with an achieved 3 km time of 11:00 would start 30 seconds before a participant with a predicted 3 km time of 10:30). Lap completion times were taken by experimenters for each participant during each competition run using standard stopwatches. Participants were reminded to prepare as they would normally for competition and were instructed to refrain from consuming alcohol 48 hours preceding each competition run. Furthermore, in addition to handicapping competitor times to ensure a maximal competitive effort during each competition run, heart rate measurements were also recorded and monitored on a real time basis (Fitpulse, TT Sport S.R.L, Italy) and competition prizes (maximum value £35) were provided depending on overall competition finishing positions (i.e. on completion of all four competition runs). All participants were positively encouraged during each competition run and only received information on the number of laps remaining. Participants were not given any further information or results until completion of the experimental protocol.

6.2.5 Haematological Measures

Standard haematological analyses were made using a haematology analyser (Sysmex XT-2000i, Norderstedt, Germany), as described in section 2.6, and measures included [Hb], Hct and ret. Samples were collected and immediately analysed prior and post control/placebo phases (i.e. 7 days). The haematology analyser was calibrated against diagnostics reference materials prior the measurement of collected samples as described in section 2.6.

6.2.6 Training and Diet

Participants were instructed to maintain their normal training and diet patterns throughout the study and were asked to record all training sessions in a diary provided. Participants were asked to replicate training and food intake for the one week and two days,

respectively, leading up to each competition run. Endurance-based and resistance-based training were quantified in terms of duration for each week prior and during, both, control and placebo conditions.

6.2.7 Study Interviews

After every 3 km competition race, participants were briefly interviewed on an individual basis for feedback on the particular race they had just completed. On completion of the study, participants also underwent two semi-structured interviews (Appendix E) with one interview prior to and one after the deception being revealed. Personal results relating to the study were revealed to participants during the first of the final interviews.

6.2.8 Data Analysis

Statistical analyses were conducted using Statistica 6.0 (Tulsa, OK). Data are presented as mean \pm SEM. Two-way repeated measures ANOVA was used to locate differences between conditions for heart rate, 3 km competition runs, RPE, [Hb], Hct, red blood cell count (RBC), mean cell volume (MCV), mean corpuscular volume (MCH), POMS and CSAI-2, endurance-based and resistance-based training durations. Statistical significance was accepted at $P < 0.05$.

6.3 Results

Figures 6.3a and 6.3b illustrate the group average and individual 3 km race completion time differences (i.e. post subtracted from pre) in both control and placebo conditions, respectively. Three km running performance improved by 9.73 ± 1.96 seconds in response to placebo and by 1.82 ± 1.94 seconds in response to the control condition ($p < 0.05$) (Figure 6.3a). Eleven participants improved performance more in response to placebo than in response to control; change in performance was similar between conditions for one participant; and three participants had greater performance increases in the control condition (Figure 6.3b). No significant differences were found between control and placebo conditions for average HR, RPE and haematological variables (Table 6.1). No significant differences were found in POMS (Table 6.2) or CSAI-2 (Table 6.3) subscales.

Time spent on endurance-based training and resistance-based training were not significantly different in the lead up to (i.e. 1 week prior) and during both control and

placebo conditions (Pre-control endurance-based training: 192 ± 33 mins; Control endurance-based training: 223 ± 72 mins; Pre-control resistance training: 44 ± 24 mins; Control resistance training: 43 ± 9 mins; Pre-placebo endurance-based training: 230 ± 51 mins; Placebo endurance-based training: 206 ± 31 mins; Pre-placebo resistance training: 62 ± 21 mins; Placebo resistance training: 50 ± 22 mins; $P > 0.05$).

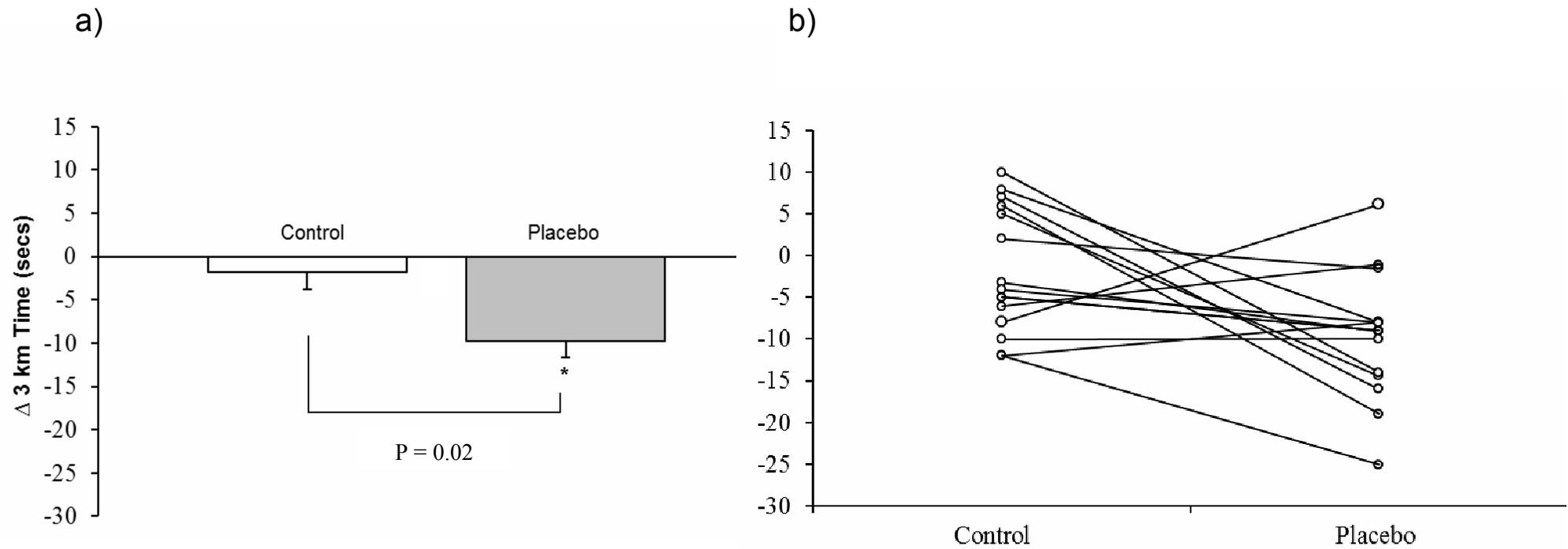


Figure 6.3: a) Average 3 km Completion Time Difference and b) Individual 3 km Completion Time Differences (post-pre) (Secs). * $P < 0.05$. Values shown as mean \pm SEM.

Table 6.1: The effects of an injected placebo on 3 km race time, HR, RPE and haematological variables

	Control		Placebo	
	Pre	Post	Pre	Post
3 km Time (s)	665.9 ± 13.8	664.0 ± 13.6	662.0 ± 12.2	652.4 ± 13.1*
HR (b·min ⁻¹)	174.3 ± 0.7	174.6 ± 0.5	177.1 ± 0.4	177.1 ± 0.5
RPE	18.5 ± 0.4	19.3 ± 0.2	18.9 ± 0.2	19.5 ± 0.2
Hb (g·dl ⁻¹)	14.3 ± 0.2	14.5 ± 0.2	14.3 ± 0.2	14.4 ± 0.1
Hct (%)	43.2 ± 0.5	43.9 ± 0.6	43.2 ± 0.7	43.0 ± 0.3
RBC (10 ⁹ ·l ⁻¹)	4.8 ± 0.1	4.9 ± 0.1	4.8 ± 0.1	4.8 ± 0.0
MCV (fL)	90.8 ± 0.8	90.3 ± 0.7	90.3 ± 0.9	90.4 ± 0.7
MCH (pg)	30.0 ± 0.3	29.9 ± 0.3	29.9 ± 0.2	30.0 ± 0.2

HR: Heart rate; RPE: Rate of perceived exertion; Hb Haemoglobin; Hct: Haematocrit; RBC: Red blood cell count; MCV: Mean cell volume; MCH: Mean corpuscular haemoglobin. Each value is the mean ± SEM. * Different from pre-control, post control and pre-placebo. * P < 0.05.

Table 6.2: The effects of an injected placebo on psychological POMS sub-scales

POMS		Tension	Depression	Anger	Vigour	Fatigue	Confusion
Control	Pre	2.93 ± 0.59	0.20 ± 0.14	0.13 ± 0.09	7.33 ± 0.99	3.40 ± 0.87	0.47 ± 0.27
	Post	2.53 ± 0.64	1.07 ± 0.93	0.53 ± 0.27	7.93 ± 1.02	3.27 ± 0.79	1.20 ± 0.94
Placebo	Pre	3.13 ± 0.48	0.53 ± 0.53	0.33 ± 0.21	8.47 ± 1.12	3.47 ± 0.56	0.60 ± 0.35
	Post	2.53 ± 0.64	0.33 ± 0.21	0.53 ± 0.41	8.47 ± 1.19	2.40 ± 0.48	0.00 ± 0.00

POMS: Profile of mood states; CSAI-2: Cognitive state anxiety inventory-2. Each value is mean ± SEM. * P < 0.05.

Table 6.3: The effects of an injected placebo on psychological CSAI-2 sub-scales

CSAI-2		Cognitive	Somatic	Self- confidence
Control	Pre	15.73 ± 1.08	14.93 ± 0.85	23.13 ± 0.99
	Post	14.73 ± 1.17	13.27 ± 0.78	23.07 ± 1.13
Placebo	Pre	15.87 ± 1.12	14.00 ± 0.75	22.33 ± 1.19
	Post	15.8 ± 1.07	14.13 ± 0.79	23.93 ± 1.15

POMS: Profile of mood states; CSAI-2: Cognitive state anxiety inventory-2. Each value is mean ± SEM. * P < 0.05.

6.4 Discussion

The aim of this study was to determine whether an injectable placebo, claiming to be a legal substance with similar effects to r-HuEpo, would improve endurance running performance. Effects on psychological changes which may be conducive to improving running performance, in the form of 3 km completion times under simulated race conditions, were also assessed. The principal finding was that participants completed the 3 km distance 1.2% faster in the post-placebo condition when compared to the post-control condition – a difference that was statistically significant and of relevance in a sporting context.

The 1.2% faster running performance reported post-placebo is an important finding particularly, as a <1.0% difference in events ranging from 1000 to 4000 m is likely to mean the difference between a gold and silver medal in major sporting events (Foster et al., 1994). The findings reported in this study complement studies previously published where a placebo has deceptively been administered whilst claiming to be a potential performance enhancer (Beedie and Foad, 2009). A recent study by Stone and colleagues (Stone et al., 2011) was the first to present the beneficial effects of deceptive feedback on previously achieved time trial performance during self-paced cycling in trained individuals. These authors reported that, with deceptive feedback, participants improved (i.e. achieved faster times) by 1.7% compared to baseline and by 1.0% compared to the accurate feedback condition. However, the findings were inconclusive as to whether or not this performance effect was solely down to deception due to a study design limitation being the lack of a correctly informed deception group. As a result, the authors could not elucidate the potential effect of competition in this particular case.

The present study is the first (to the best of the author's knowledge) to report an improved running performance, in simulated race conditions, with an injected placebo claiming to have similar performance enhancing effects to r-HuEpo in trained participants. An explanation as to these findings is difficult as all other measured variables such as heart rate, haematological indices and psychological subscales did not differ significantly between control and placebo conditions (Tables 6.1, 6.2 and 6.3, respectively). Some authors, however, have previously proposed a potential explanation as the presence of a dissociative task during performance assessment (Sporer and McKenzie, 2007, Stone et al., 2011). Self-paced exercise has been suggested to be regulated within the brain involving complex interactions between peripheral sensory feedback and comprehension of exercise

duration (Amann and Dempsey, 2008a, Swart et al., 2009a). More specifically, there has been speculation that afferent sensory information related to body tissue homeostasis involves the dorsal posterior insula of the brain which is generated into a sensation by the right anterior insula of the brain (Hettinga et al., 2011). A dissociative task can take the form of competition (Foster et al., 2004a) which can become the focus of one's attention, in turn, restricting one's ability to process distressing cues from sensory afferents. This, therefore, distracts the ability to perceive cues related to fatigue (Sporer and McKenzie, 2007). However, for this present study, the dissociative task of 'competition' is not a possible explanation for the improved running performance found post placebo as 'competition' was a feature of every race and was therefore controlled. Furthermore, endurance-based and resistance-based training was similar between control and placebo conditions and is therefore unlikely to explain the faster running performance found in response to the placebo condition.

Thus, it is unclear why placebo improved performance in the present study. However, the results shown in this study may also be related to task dissociation, but in a different form to that previously described. It is possible that the dissociative task in this case may have been internal thought processes in relation to the belief that a performance enhancing drug has been administered. This, as a result, may have limited the participants' ability to pick up distressing cues during the race and this may have potentially diminished the sensation of fatigue during the run itself. The fact that RPE ratings did not differ significantly between conditions, despite participants running faster in the placebo condition, is consistent with this hypothesis; although this does not provide unequivocal evidence in support.

Qualitative assessments of participants immediately post each 3 km competition race provides some insight into how participants felt during each race. When interviewed immediately after each 3 km competition race, eleven of the fifteen participants described feeling different and/or commented that taking OxyRBX had an effect on performance. Effects of recovery were also mentioned. The magnitude of effects reported did vary between participants and notable examples include:

Participant 2: *"...better today [in reference to the race immediately post the placebo week] than last week, felt like I could put in more effort. Feel fresher, don't know why as training was identical. Definitely felt quicker this week than last...partly due to the substance. Felt like I took*

advantage of the drug week, was easier to talk when training and people mentioned that I looked more comfortable than usual. Felt thirsty more than usual though...possibly due to the drug or training harder?”

Participant 6: *“...felt pretty good [in reference to the race immediately post the placebo week]. Felt like I started quicker and maintained a better pace...more of an adrenaline rush this time [in contrast to the control race condition]. I believe I have done better than last week [in reference to immediately post control week]. Felt quicker and felt that I was more able to stick it out...certainly gave me the confidence to go for it harder”.*

Participant 9: *“I was nervous before the race [in reference to prior the placebo race], but I wasn't bothered about who was on the drug or not through. I tried to focus whilst going down to the track. During the run, I just ran according to how my body felt. This week [in reference to placebo week] I felt like I could work harder, especially during the last 3 laps of the race. Muscles felt good...drug has definitely had an effect, you know, you've got this drug in your body...so let's push, push. I felt like I recovered quicker too, same RPE, but recovered faster than last week [in reference to immediately post control week]”.*

Furthermore, when interviewed on completion of the study, the same eleven out of the fifteen participants re-affirmed these feelings. Three notable examples include:

Participant 2: *“...the effect of the substance just made everything quicker. I have to admit when I was on the substance I was on the verge of injury...I kept pushing myself, probably too hard...just because I felt I could. I was taking something...that's why. Maybe it was all in my head, although I am sure I did notice a difference in breathing. Then again I could have put new running shoes on and felt lighter or something ...could be psychological? It certainly boosts self-confidence”.*

Participant 3: *“...the finishes of the races stood out for me throughout the trial...I really enjoyed every race. During the drug week training and recovery felt better, but unsure whether it could just be psychological? My second competition race, post drug, the final laps felt faster...”.*

Participant 14: *“Wow...my fastest run was post drug [after being shown results in the first interview]. Yeah, that correlates with how I felt. I certainly felt like the first run [in reference to the control race] was the slowest. I felt tired during that run...physically. The fastest run [in reference to post placebo race] surprises me actually, wow, that’s faster than what I expected...although I knew it was my fastest race. Wow, pretty consistent splits as well. I knew it...I felt that was my best race. Certainly faster than what I thought I could do...I am elated. I have exceeded my expectations. I mean, it was hard, but training was normal...if I just trained without some external influence [in reference to drug] I doubt I would have achieved that time. So, it does make you think, maybe there is a little element being me, but 90% must be a result of the injections to be honest”.*

These qualitative assessment examples suggest that participants, overall, believed that taking a performance enhancing drug would improve performance and may have specifically done so by helping participants disassociate from picking up cues related to fatigue.

Studies assessing pacing strategies and exercise performance have proposed that the brain may act to regulate exercise output in order to prevent full depletion of aerobic energy reserve (Amann and Dempsey, 2008b, Swart et al., 2009a, Swart et al., 2009b). This mechanism of action has been proposed to occur in order to insure that exercise can be carried out safely without a catastrophic biological failure (Swart et al., 2009b). If this holds true, one could infer that during self-paced exercise the brain, via specific neural control mechanisms (Noakes, 2007), essentially determines a work intensity relative to maximal but below absolute capacity (Amann and Dempsey, 2008b, Amann and Dempsey, 2008a, Swart et al., 2009a, Swart et al., 2009b). This may be subconsciously done in an attempt to avoid the negative complications of severe metabolite build up or biological failure which may, in turn, suggest the potential existence of a metabolic and/or central reserve (Stone et al., 2011, Noakes et al., 2004, Noakes, 2007, Tucker and Noakes, 2009, Tucker, 2009, Swart et al., 2009b). Discovering new ways and understanding their respective underlying mechanisms in order to attenuate one’s ability to detect cues related to fatigue will further improve endurance performance by accessing and ‘pushing’ these central and/or metabolic reserves to their own safe hypothetical limits – if they do indeed exist.

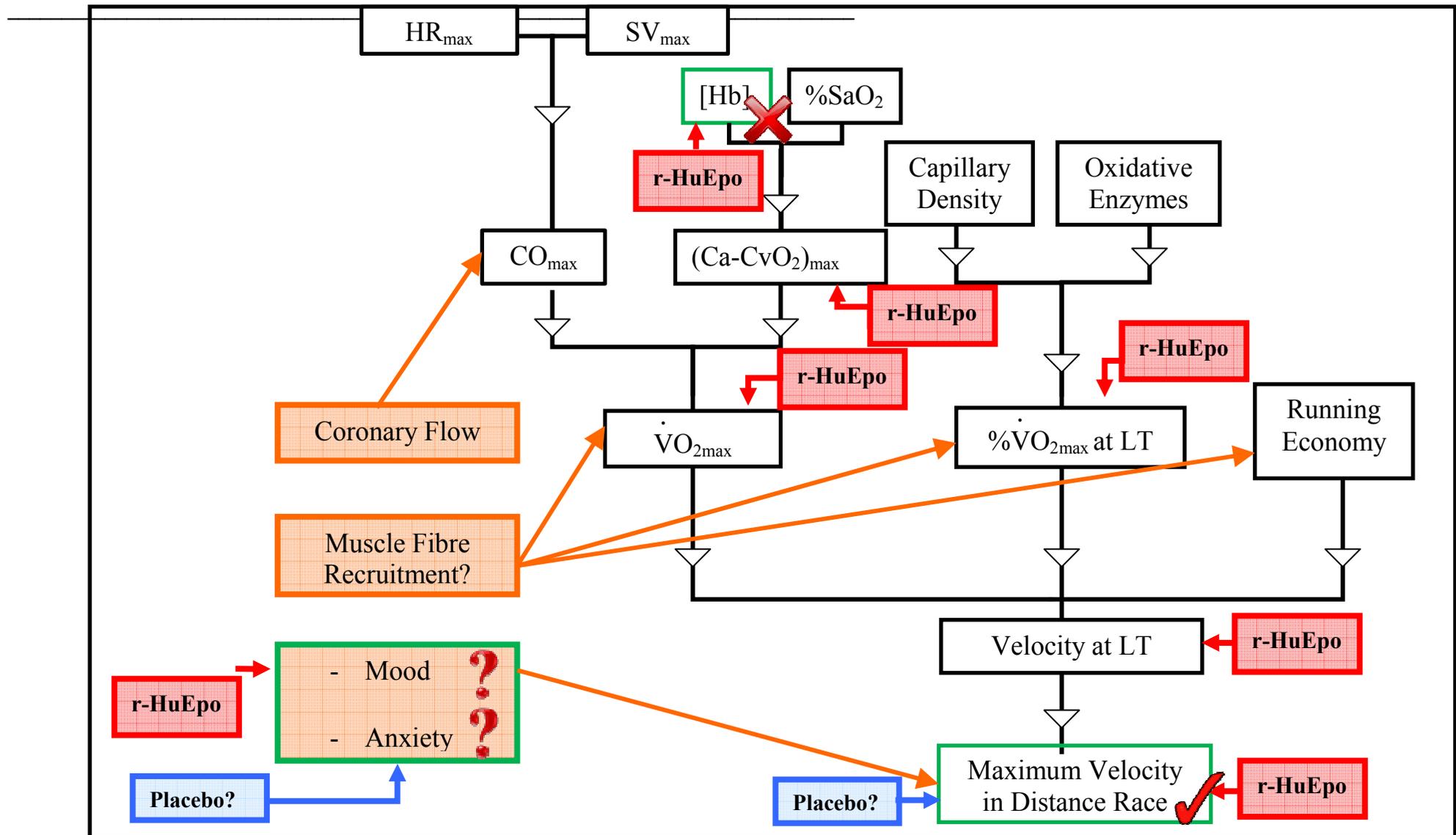


Figure 6.4: Summary of the major cardiovascular variables related to $\dot{V}O_{2max}$ and the maximal running.

This diagram shows the major variables which determine $\dot{V}O_{2max}$ and the maximum sustained running velocity. The known interactions are shown as black lines.

The known interactions with the Central Governor Model (CGM) are shown as orange lines.

The known influences of $r-HuEpo$ injections are shown by red arrows. Potential influences of placebo injections are shown by blue arrows with question mark.

Red ticks mark variables found to be of significance immediately post placebo administration and red crosses mark variables which were not found to be of significance immediately post placebo administration. HR_{max} = maximal heart rate; SV_{max} = maximal stroke volume; $[Hb]$ = haemoglobin concentration; $\%SaO_2$ = percent oxygen saturation; CO_{max} = maximal cardiac output; $(Ca-CvO_2)_{max}$ = maximal arterial-venous difference; $\dot{V}O_{2max}$ = maximal oxygen uptake; $\% \dot{V}O_{2max}\ at\ LT$ = percentage of maximal oxygen uptake at lactate threshold. This figure is adapted from (Bassett and Howley, 2000).

6.5 Conclusions

To summarise, participants completed the 3 km race distance significantly faster following the placebo condition compared to the control condition. The magnitude of the effect of placebo on performance (i.e. 1.2%) is highly relevant in terms of sporting competition. It is not clear why placebo improved performance to this extent, as measured heart rate, haematological variables and psychological subscales did not differ significantly between control and placebo conditions. It is possible that the belief that a performance enhancing drug had been administered may have caused participants to disassociate cues related to fatigue during running. Limiting one's ability to detect distressing cues during running may have assisted participants to diminish or attenuate the perception of fatigue which, in turn, has likely encouraged a faster running performance. Further study is needed to confirm whether this mechanism contributed to the observed performance-enhancing placebo effect.

7. General Discussion

A summary of the main findings found in each chapter are provided below:

- i) Chapter 3 was the first study (to the best of the author's knowledge) to assess the K4b2 portable metabolic device during submaximal and maximal running velocities outdoors. Initially, the K4b2 metabolic device appeared to be valid for the measurement of $\dot{V}O_2$ during submaximal and maximal running velocities in an outdoor environment. Upon further investigation, however, this apparent agreement was a consequence of two different errors which acted in opposite directions (i.e. a $5.2 \text{ ml kg}^{-1} \text{ min}^{-1}$ negative offset in $\dot{V}O_2$ coupled with a 14.2% higher gradient in the relationship between the K4b2 outdoors and the Douglas bag method for measuring $\dot{V}O_2$). Furthermore, a K4b2 outdoors mean bias of $2.3 \text{ ml kg}^{-1} \text{ min}^{-1}$ with a 95% confidence interval limits of agreement range from -6.1 and $10.7 \text{ ml kg}^{-1} \text{ min}^{-1}$ (equivalent to a measurement typical error of 6.5%) was found which indicates unacceptable degree of error. The K4b2 device also significantly overestimated $\dot{V}O_2$ indoors when compared to the Douglas bag method. As a result of these findings, unfortunately, the K4b2 could not be considered for use in further experimental Chapters due to the sensitive physiological measurements required. Thus, the level of measurement accuracy required by users must seriously considered prior using the K4b2 portable device.
- ii) Chapter 4 assessed the effect of r-HuEpo administration on haematological, psychological and exercise performance measures to provide further insight into how this powerful perturbation may impact those systems that, as previously described, limit endurance exercise capability. According to the author's knowledge, this experimental study is the first to assess r-HuEpo mediated performance effects in both laboratory and field conditions. Chapter 4 confirmed the erythropoietic effect of r-HuEpo administration with increases in [Hb] and Hct associated with improvements in $\dot{V}O_{2\text{max}}$. Three km time trial performance also improved immediately post r-HuEpo administration by 4.9%. However, no clear associations were found between [Hb], Hct or $\dot{V}O_{2\text{max}}$ and 3 km performance which suggested that factors other than changes in [Hb], Hct and $\dot{V}O_{2\text{max}}$ are likely to have influenced running performance. Furthermore, the improvement in 3 km time trial performance persisted for 4 weeks post r-HuEpo administration, despite the r-HuEpo mediated changes in [Hb] and Hct declining by more than 50% over this

interval. Finally, changes in psychological measures (i.e. anxiety and tension) were found which warrant further investigation as a potential contributor to enhancing performance.

- iii) Chapter 5 qualitatively assessed participants (i.e. via semi-structured interviews) on the associated effects of r-HuEpo administration on endurance performance as well as on psychosocial factors and consequences associated with the practice. This study also offered unique insights into athlete viewpoints on drugs (as well as potential contrasts before and after using a banned substance, i.e. r-HuEpo), impacts on sporting performance and on doping in sport. The study found that all participants reported experiencing a performance enhancing effect as a result of r-HuEpo administration. The majority of participants (i.e. 83% or 5 out of 6 participants) referred to an enhancement in recovery periods either in between training intervals or between training days. However, with these 'positive' enhancement effects were also psychosocial implications such as feelings of guilt, shame and/or embarrassment. From an anti-doping perspective, all participants generally maintained an anti-doping sentiment; however some more convincingly than others especially when comparing to those deemed to be privileged with living at altitude.
- iv) Chapter 6 determined whether an injectable placebo, claiming to be a legal substance with similar effects to r-HuEpo, would improve endurance running performance in simulated race conditions. Exercise performance was assessed in the field (i.e. 3 km track time trial), but with the element of competition introduced (i.e. racing opponents). Results were compared to those obtained from Chapter 4 and previous literature assessing the placebo effect on endurance performance. Participants completed the 3 km race distance significantly faster following the placebo condition compared to the control condition by 1.2%. This magnitude of change is highly relevant in terms of sporting competition. However, it was not clear as to what caused this enhancement in performance as measured heart rate, haematological variables and psychological subscales did not differ significantly between control and placebo conditions. It may be possible that the belief that a performance enhancing drug had been administered may have caused participants to disassociate cues (or limit one's ability to detect distressing cues) related to fatigue during running. Further study is needed to confirm whether this mechanism contributed to the observed performance-enhancing placebo effect.

Figure 7.1 illustrates the measured variables in order to assess the effects of r-HuEpo administration and r-HuEpo placebo injections on endurance performance and, of these, which have been found to significantly change immediately post r-HuEpo administration according to the experimental chapters.

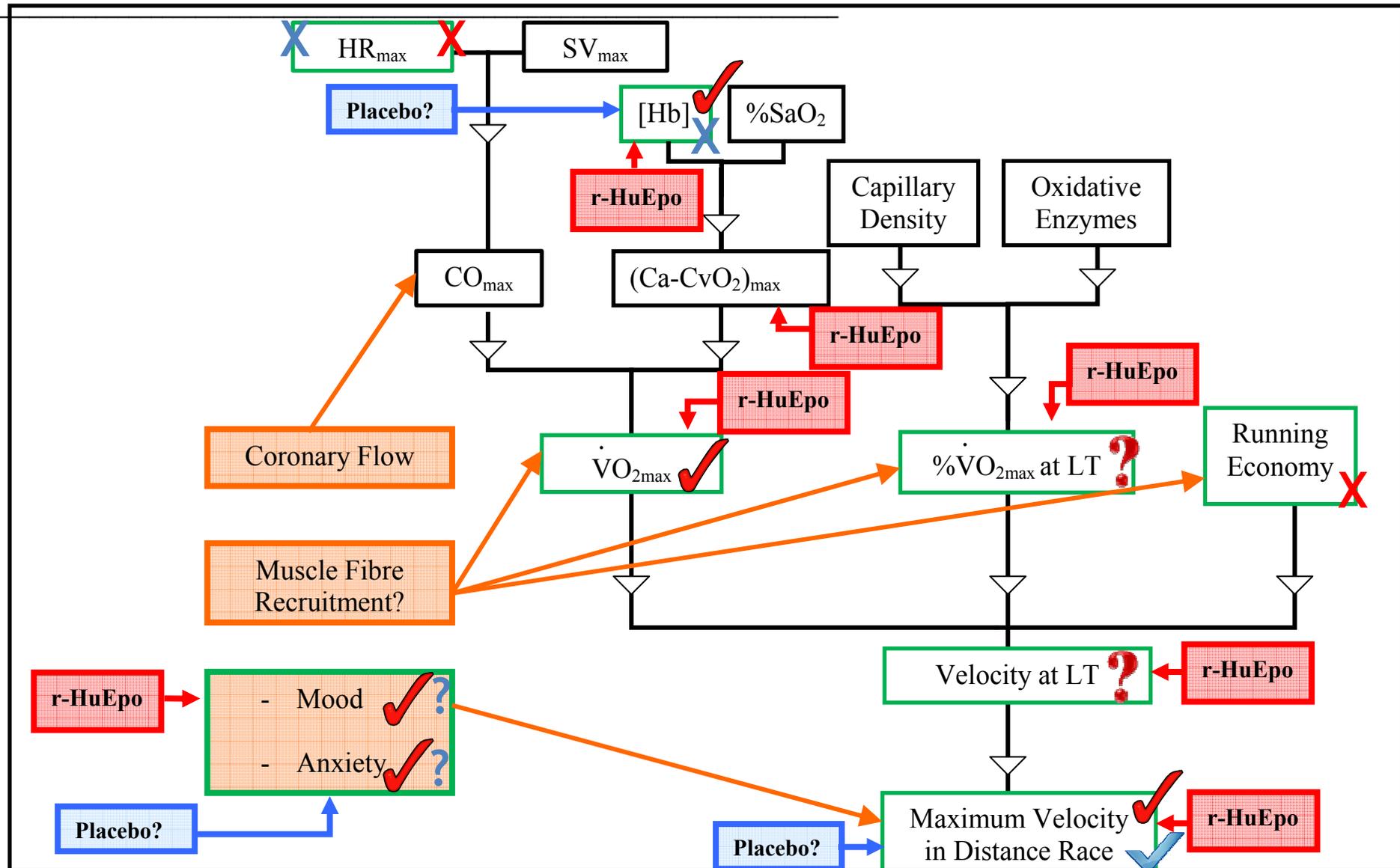


Figure 7.1: Summary of determined (ticks) and potential effects (question mark) of r-HuEpo (red colour) and injected r-HuEpo placebo (blue colour) immediately post administration on endurance performance capability.

HR_{max} = maximal heart rate; SV_{max} = maximal stroke volume; $[Hb]$ = haemoglobin concentration; $\%SaO_2$ = percent oxygen saturation; CO_{max} = maximal cardiac output; $(Ca-CvO_2)_{max}$ = maximal arterial-venous difference; $\dot{V}O_{2max}$ = maximal oxygen uptake; $\% \dot{V}O_{2max}$ at LT = percentage of maximal oxygen uptake at lactate threshold. Adapted from (Bassett and Howley, 2000).

7.1 The K4b2 Portable Metabolic Device and Associated Limitations

Unfortunately, due to the high level of accuracy required in subsequent studies (i.e. experimental Chapters 5 and 6), it was ultimately concluded that the K4b2 would not provide accurate enough measures to justify its further use. In Chapter 3, the K4b2 metabolic device initially appeared to be valid for the measurement of $\dot{V}O_2$ during submaximal and maximal running velocities in an outdoor environment. However, with a K4b2 outdoors mean bias of $2.3 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ with a 95% confidence interval limits of agreement range from -6.1 and $10.7 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (equivalent to a measurement typical error of 6.5%), the device showed an unacceptable degree of error. This, in addition to unusual performances during indoor conditions, warrants caution when collecting and interpreting data collected by the device. Overall, no single factor was responsible for the inaccuracies reported and it was most likely the cause of several errors found in various sub-component variables (i.e. $F_{E}O_2$ and \dot{V}_E), used to derive $\dot{V}O_2$, as well as the potential negative impact of wearing the K4b2 on running economy.

Even when taking into account such problems, however, field-based portable metabolic devices are still vital in order to be able to assess exercise performance in ‘truer’ environments. This is essential in order to improve ecological validity for health/exercise/sports performance related studies. It is clear that improvements are required in order to improve the accuracy of the K4b2 measures in order for it, and other similar technologies, to be more readily used with confidence in the result obtained. However, in addition to the required technological improvements required, there must also be a general consensus with regards to how metabolic validity studies are carried out, both under laboratory and field-based conditions.

Authors have previously highlighted the lack of standardisation in how validity and reliability between portable metabolic analysers and criterion methods are assessed and reported (Atkinson et al., 2005). Since Atkinson and colleagues first reported this in 2005, little has changed and even less has been done to standardise field-based validity and reliability studies. This is peculiar given the importance ‘ecological validity’ is given in science. Furthermore, different data analysis approaches have also been used in different studies to assess validity and/or reliability. For example, the previous study assessing K4b2 validity by Pinnington et al. (Pinnington et al., 2001) merely reported Pearson correlation coefficients between the K4b2 device and metabolic cart measurements ($F_{E}O_2$: $r = 0.971$; $F_{E}CO_2$: $r = 0.925$; \dot{V}_E : $r = 0.982$). It is now well established that utilising such methods alone, is not sufficient for comparisons between methods as they do not assess

systematic bias (Bland and Altman, 1986, Bland and Altman, 1996). In another study, McLaughlin et al. (McLaughlin et al., 2001) may have taken this into consideration as these authors used Bland-Altman plots to compare the K4b2 and Douglas Bag. However, in this instance, these authors only reported sample mean differences and did not report 95% limits of agreement which is a better indicator of likely differences in population means (Atkinson et al., 2005).

Chapter 3 offers useful insights on the validity of the K4b2 portable device using a variety of unique comparison exercise tests with runners. The authors believe it to be novel in that it provides both K4b2 indoor and K4b2 outdoor comparisons against a criterion Douglas bag system. In turn, Chapter 3 is an important contribution to existing literature examining the validity of portable metabolic systems for use in the field, during indoor sporting activities and other general activities. Furthermore, this experimental chapter offered a unique solution for assessing portable metabolic devices outdoors without the necessity of wind tunnels (which are not easily accessible and are more costly). Fundamentally, however, to confirm or refute the findings, future key investigations must assess the effects of facing head winds under controlled laboratory conditions using industrial-sized fans or a wind tunnel.

From another perspective, this general discussion section provides an opportunity to discuss the wider relevance of portable metabolic systems in our world today. It is clear that there is a growing global prominence of portable metabolic analysers as solutions to various problems from global health issues to space flight. The personalised portable metabolic analysis market is ever growing with various industries including the National Aeronautics and Space Administration (NASA) (e.g. the development of the portable unit for metabolic analysis –‘PUMA’) and commercial fitness and health industries (e.g. Breezing –currently the world’s only metabolic assessment tool that links to your mobile phone) showing increasing interest. This is no surprise given that such devices can be targeted for a variety of applications including:

- The determination of personalised caloric requirements for daily living
- The determination and manipulation of energy profiles through the development of personalised dietary/nutritional/weight-loss/exercise programs
- Field testing, training and coaching of athletes for performance assessments and training programs
- The determination of the metabolic cost of activities related to various situations for the development of occupational health/fitness strategies

- Various nutritional applications from food-metabolism related assessments in normal and in clinical situations.

Furthermore, the BBC's 'The Health Show' (BBC News, 2013) recently featured the ever growing relevance of personalised medicine by showing the latest advances in a variety of fields, including biotechnologies, in order to help combat global health challenges. The development of unique portable metabolic devices, such as 'Breezing' (<http://breezing.co/>), as a potential solution to global obesity, for example, will likely prove increasingly more and more important in order to engage the world population in solving such issues. The global obesity pandemic example is a key global health issue and recent reports by World Health Organisation, stating that obesity has doubled since 1980 (World Health Organisation, 2013), clearly demonstrates this to be the case. As portable metabolic devices seem to be playing an ever increasing role in global health solutions, the relevance of accurate and reliable measures will become increasingly more important as this industry continues to grow. In order for this to occur, interested parties must establish standards to allow for more direct comparisons to be made between validation/reliability studies, on relevant devices, under both laboratory and field conditions.

To summarise, the degrees of error for the measurement of $\dot{V}O_2$, as reported in experimental Chapter 3, are unacceptable when considering the level of accuracy required for physiological measures such as $\dot{V}O_{2max}$ and running economy, amongst others. Such differences can have significant impacts on data interpretation and conclusions in research studies. Thus, it was concluded that potential users must seriously consider the level of measurement accuracy required prior using the K4b2 portable device.

7.2 The Effect of Recombinant Erythropoietin on Endurance Performance

The investigation in Chapter 4 demonstrated the positive ergogenic effect r-HuEpo administration has on endurance performance, not only, in a laboratory setting but also in the field. The r-HuEpo mediated changes in haematological measures (Table 4.1) and psychological measures (Table 4.3), associated with improvements in performance measures (Table 4.2), demonstrate the mechanistic complexities behind the impacts of r-HuEpo administration on those systems that limit endurance performance capability. To the author's knowledge, this is the first study to assess the effect of r-HuEpo administration on psychological, haematological and performance measures and is the first to assess the transferability of r-HuEpo mediated endurance performance enhancement from the laboratory to the field.

The administration of r-HuEpo improved endurance exercise capacity (i.e. $\dot{V}O_{2max}$) and exercise performance (i.e. 3 km time trial performance) (Table 4.2). Maximal oxygen uptake did not change significantly over the course of the protocol, but was numerically 5.2% higher immediately post r-HuEpo administration when compared to baseline. This percentage change was in the right direction and is comparable to previous literature (Table 7.1). In reference to Table 7.1, the average reported change in $\dot{V}O_{2max}$ was 7.6% (with a range of 6.6 to 9.3% depending on the r-HuEpo administration employed) and this was achieved using a minimum of 8 participants. Thus, if it were possible to achieve a similar number of participants for Chapter 4, the average change in $\dot{V}O_{2max}$ would very likely have not suffered from a lack of statistical power. Interestingly however, no significant relationships were found between changes in $\dot{V}O_{2max}$ and 3 km time trial performance (Table 4.3) which is likely to be a spurious finding given previous evidence (Hawley and Noakes, 1992, Ramsbottom et al., 1988, Coyle et al., 1988).

Fundamentally, Chapter 4 reported a significant improvement in 3 km time trial performance immediately post r-HuEpo administration by 4.9% and this enhancement was sustained for 4 weeks post r-HuEpo administration (by 4.0% compared to baseline). Interestingly, no significant relationships were also found between changes in 3 km time trial performance with changes in other performance related measures including $\dot{V}O_{2max}$ ($r = -0.532$; $P = 0.278$), LT ($r = -0.380$; $P = 0.528$), OBLA ($r = 0.219$; $P = 0.723$), LTP ($r = 0.183$; $P = 0.769$) or running economy ($r = -0.055$; $P = 0.918$). Even when taking into account the potential effect of a type 2 statistical error (i.e. small sample size), it is possible that the improvements found in 3 km time trial performance were due to both

haematological and psychological underpinning mechanisms. This could also be described as r-HuEpo potentially attenuating the onset of fatigue by alleviating those limitations associated with such systems. In addition to what has already been discussed previously, other potential effects/mechanisms will now be explored further.

In line with previous studies (Audran et al., 1999, Parisotto et al., 2000b, Birkeland et al., 2000, Ashenden et al., 2001, Russell et al., 2002a, Ashenden et al., 2006) [Hb], Hct, ret and tHb increased immediately post r-HuEpo administration when compared to baseline measures. The collective evidence, as a result, strongly suggests r-HuEpo administration to have a significant effect on the cardiovascular system by improving its ability to deliver O₂ to the exercising muscles. The increases in [Hb], Hct, ret and, in particular tHb, may mean that this improvement is, in part, underpinned by an improved cardiac output and O₂ carrying capacity (Bassett and Howley, 2000). Although cardiac output was not directly measured (Brink-Elfegoun et al., 2007), tHb was determined using the optimised carbon monoxide rebreathing method (Schmidt and Prommer, 2005a), which can also be used to calculate blood volume – a contributing factor of cardiac output.

Determining tHb provides an accurate and reliable measure of tHb (Durussel, 2011) which is not influenced by haemodilution and/or heamoconcentration as a result of fluid ingestion, fluid loss or infusion of plasma expanders, as examples (Schmidt and Prommer, 2005a). The tHb values increased from baseline to a maximum measure, immediately post the 4 week r-HuEpo treatment period, by 11.9% (Table 4.1). Interestingly, although this increase was found, blood volume remained relatively stable (baseline: 7044 ml, immediately post r-HuEpo administration: 6639 ml). This may appear surprising as one could likely assume a concomitant increase in blood volume with the reported increase in [Hb] which would, therefore, provide some evidence for an improved cardiac output in addition to O₂ carrying capacity. However, the reported blood volumes found in Chapter 4 are in line with previous literature and an explanation is likely to involve the depression of plasma volume (Lundby et al., 2007) – a potentially debilitating paradoxical effect of r-HuEpo administration.

Lundby and colleagues have reported a ~300 ml increase in red blood cell volume with a, concomitant, ~400 ml reduction in plasma volume (Lundby et al., 2007). These authors also reported reduced plasma rennin activity and aldosterone concentrations resulting in blood volume remaining relatively unchanged, but with a slight transient decrease when compared to baseline (Lundby et al., 2007). Chapter 4 is in agreement where r-HuEpo administration induced an increase in [Hb] by increasing red blood cell volume, but by also

potentially decreasing plasma volume. This may mean, therefore, that r-HuEpo administration improves endurance performance capability by improving arterial O₂ content (i.e. improving O₂ carrying capacity in the body), but cardiac output may remain relatively unchanged.

The potentially debilitating ‘paradoxical’ effect of plasma volume depression, associated with r-HuEpo administration, is an interesting factor to consider. A paradoxical comparison can be made to the ‘athlete paradox’ or ‘sports anaemia’ where exercise training has been associated with post-exercise plasma expansion, intensified haemolysis during physical efforts, iron deficiency, losses of erythrocytes via digestive and urinary tracts and potential disturbances in erythropoiesis (Szygula, 1990). Similarly, in a paradoxical sense, although the depression of plasma volume is likely to contribute to a ‘performance enhancing’ increase in [Hb], this may also concomitantly causes an increase in blood viscosity. Without careful monitoring and control, increased blood viscosity can be life threatening and this is clearly a potentially ‘performance debilitating’ effect. This is particularly the case if Hct levels reach >50% which is more often associated with endurance sports due to natural dehydration (Scott, 1990, Jenkins, 2002).

Interestingly, anecdotal verbal feedback indicated in Chapter 4 suggested that some participants did indeed feel more thirsty than normal throughout the r-HuEpo administration phase. Although this was naturally counteracted by drinking more water, the potential dehydrating effects, particularly after strenuous exercise, may have also contributed to increasing [Hb]. Furthermore, with plasma depression comes potential consequences including high blood pressure and thrombosis, associated with increased blood viscosity and decreased cardiac output (Gauthier, 2001). Stroke and myocardial infarction have also been associated with a rapid rate of rise in haemoglobin (Smith et al., 2003). Thankfully, none of these consequences were observed in the study presented in Chapter 4.

As previously highlighted, the haematological and performance findings also have importance in relation to anti-doping. As indicated in Chapter 4, the results highlight the variability in the duration of r-HuEpo induced haematological changes from baseline measures which, in this instance, remained for 28 days after the cessation of r-HuEpo (in contrast to, for example, 21 days (Parisotto et al., 2000b, Parisotto et al., 2001)). It is acknowledged that these findings may only likely apply to an r-HuEpo administration protocol of 50 UI·kg⁻¹ three times a week over a 4-week period, although alternative regimens have produced similar haematological profiles (Ninot et al., 2006). This provides

support to WADA's 'Athlete Biological Passport' as such measures (e.g. haematological measures) can be monitored over time on an individual bases, thus indirectly indicating any potential doping effect. This removes any limitations (e.g. drug wash-out times) associated with the more traditional approach of 'drug detection'.

The administration of r-HuEpo clearly improved exercise endurance performance. A likely contribution to this effect is a cardiovascular adaptation from which peripheral systems are likely to have benefited. However, there were also indications of possible psychological effects as significant psychological measures were also identified (Table 6.3). Both the CSAI-2 'cognitive' subscale and the POMS 'tension' subscale decreased significantly compared to baseline, immediately post r-HuEpo administration, by 18.3% and 61% ($P < 0.05$), respectively. Although no relationships were found between such changes and changes in performance, previous studies have suggested the contrary (Martens, 1971, Craft, 2003). For example, there have been reports of r-HuEpo administration eliciting improved mood (Miskowiak et al., 2008b) and physical conditioning (Ninot et al., 2006) in healthy individuals. It must also be acknowledged, however, that recent studies, using healthy participants, have also concluded no attenuation in central fatigue or in cognitive performance strategy (Rasmussen et al., 2010). This study found this based on, in part, increased RPE with no changes in cognitive function following the administration of high-doses of r-HuEpo (Rasmussen et al., 2010). These authors do acknowledge, however, that the employed cognitive tests may have not been sensitive enough to detect a change in cognitive function in healthy participants – as opposed to reported effects within patients with pre-existing cerebral dysfunction administered with r-HuEpo (Moore et al., 2011).

Rasmussen and colleagues (Rasmussen et al., 2010) also attempted to demonstrate central fatigue via the assessment of central motor drive during activation of the elbow flexor. The administration of high (30,000 IU/day) and low (5,000 IU/day) doses of r-HuEpo, over a 3 day and 3 month period, respectively, indicated no discrepancies in central motor drive between the two conditions. The authors argue that the inability to fully activate the elbow flexor as a result of high doses of r-HuEpo, which has been reported to cross the blood brain barrier (Xenocostas et al., 2005), is indicative of a lack of influence on central systems. This suggests that r-HuEpo enhances endurance capability solely via improving O₂ carrying capacity.

Fundamentally, however, conflicting evidence still remains. Whether the psychological changes, reported in Chapter 4, are directly as a result of r-HuEpo administration remains to be elucidated. These findings do warrant further investigation as these potential changes may indeed be r-HuEpo induced psychological changes, as determined by POMS and CSAI-2 subscales, or may simply be reflections of greater central effects – as recent evidence also suggests (Core et al., 2011). In this example, Core and colleagues (Core et al., 2011) investigated r-HuEpo’s neuroprotective effects, for cerebral malaria (involves the occlusion of blood vessels located in the brain), which includes the activation of endogenous neural stem cells. The authors reported a rapid increase in neural stem cells, which was r-HuEpo dependent, during cerebral malaria pathology. The connotations of such research are tremendous and similar novel applications must be carried out on whether such effects play a role on r-HuEpo-mediated performance enhancing effects. Furthermore, there also still remains a requirement to attempt to quantitatively explain the qualitative measures often associated with the performance enhancing effects of performance enhancing beliefs. Such research will also further enhance our understanding on how r-HuEpo mediates its effects, but will also provide possible answers to those factors that fundamentally limit human physicality.

Table 7.1: Results comparison with previous literature assessing r-HuEpo administration for haematological measures and $\dot{V}O_{2max}$

Study	Participants	r-HuEpo Dose (IU/Kg/Week)	Treatment Period (Weeks)	Hct (%Δ)	[Hb] (%Δ)	tHb (%Δ)	$\dot{V}O_{2max}$ (%Δ)
Lundby et al. (2008)	8 Healthy	120	4	12.0	-	10.2	6.6
Thomsen et al. (2007)	16 Healthy	180	13	9.2	9.4	8.3	12.6
Russel et al. (2002)	9 Recreational	94	8	6.0	-	-	7.0
Audran et al. (1999)	9 Trained	350	-	11.5	9.3	-	9.3
Parisotto et al. (2000)	8 Trained	150	4	10.1	10.7	12	6.9
Ashenden et al. (2006)	2 Well-trained	348	5	-	18.1	-	-
Birkeland et al. (2000)	10 Well-trained	207	4	19.0	14.5	-	7.6
Ross et al. (2012)	7 (6) Trained	150	4	8.7	21.2	12	5.4

r-HuEpo: Recombinant Human Erythropoietin; Hct: Haematocrit; Hb: Haemoglobin; tHb: Total Haemoglobin Mass; $\dot{V}O_{2max}$: Maximal Oxygen Uptake. Ross et al. (2012): haematological variables (n=7), $\dot{V}O_{2max}$ (n=6). All % Δ values represent baseline to maximum change for each study.

7.3 Viewpoints from Well-trained Individuals during and after r-HuEpo and Placebo Trial Participation: Performance Effects

According to the majority of evidence to date, there is no doubt that r-HuEpo assists in improving endurance performance capability by impacting the cardiovascular system (e.g. by increasing O₂ carrying capacity in the human body), but this is not exclusively. In addition to physiological changes, Chapters 4 and 6 also provided evidence of participants verbally reporting benefits during and immediately after, both, r-HuEpo and placebo administration. The similarities between comments made by participants, from both r-HuEpo and placebo trials, provide evidence for this. Example comparisons include:

Comparison A:

Participant 2 (r-HuEpo trial): *“That was the day when I came in, my heart rate fell down quickly, massively, within a couple of minutes. I probably was thinking that must be the EPO kicking in. I think yeah, anytime I did run like that, I must have thought yeah that must be the EPO”.*

Participant 4 (r-HuEpo trial): *“I found that I became quite tired at the end of EPO, I don’t know, maybe ‘cause I was trying to domaybe I knew I was on EPO, so I thought I could do much more so I went out hard, but at the end it was taking more days to recover. I just remember, getting quite tired”.*

Participant 2 (Placebo trial): *“Definitely felt quicker this week than last...partly due to the substance. Felt like I took advantage of the drug week, was easier to talk when training and people mentioned that I looked more comfortable than usual...”*

Comparison B:

Participant 6 (r-HuEpo trial): *“I think that when you’re training so hard and then still be able to recover and then still train really quick that your confidence is high so your confidence plus EPO has a big boost. And then the fact that you are willing to risk in training, like running faster, ‘cause you think during training, I’m on this drug and going to get better, so I’m going faster...let’s take more risks, let’s run till you drop... See where this will take you, so maybe you feel a bit more I would say ‘immortal’ in training. You would feel as if like, I felt, there were sessions where I could*

feel I could run so fast where I would generate no lactate, but didn't know why, but at the end of the sessions you would feel lactate, but during the first few reps your still running really quick. And you got lots of confidence, your running going like you can't feel the burn yet... brilliant! And people behind you chasing you. Gives you a massive boost psychologically...that's something that I've not got in my locker usually".

Participant 3 (r-HuEpo trial): *"... certainly I would say looking at the times I was running, I mean that was certainly what I would expect from a much longer period of training. So something's done something for sure...and the recovery was something that I noticed, the recovery sessions felt so much better...quicker".*

Participant 2 (Placebo trial): *"...the effect of the substance just made everything quicker. I have to admit when I was on the substance I was on the verge of injury...I kept pushing myself, probably too hard...just because I felt I could. I was taking something...that's why. Maybe it was all in my head, although I am sure I did notice a difference in breathing. Then again I could have put new running shoes on and felt lighter or something ...could be psychological? It certainly boosts self-confidence".*

Participant 3 (Placebo trial): *"...the finishes of the races stood out for me throughout the trial...I really enjoyed every race. During the drug week training and recovery felt better, but unsure whether it could just be psychological? My second competition race, post drug, the final laps felt faster".*

Comparison C:

Participant 4 (r-HuEpo trial): *"One day, just, I was running with the same people I would normally...they started to struggle and then I took off and left them, it was ridiculous, I got quite embarrassed, and you know it was like where the hell did that come from. I thought that must be the EPO.*

Participant 14 (Placebo trial): *"Wow...my fastest run was post drug [after being shown results in the first interview]. Yeah, that correlates with how I felt. I certainly felt like the first run [in reference to the control race] was the slowest. I felt tired during that run...physically. The fastest run*

[in reference to post placebo race] surprises me actually, wow, that's faster than what I expected... although I knew it was my fastest race. Wow, pretty consistent splits as well. I knew it; I felt that was my best race. Certainly faster than what I thought I could do...I am elated. I have exceeded my expectations. I mean, it was hard, but training was normal... if I just trained without some external influence [in reference to drug] I doubt I would have achieved that time. So, it does make you think, maybe there is a little element being me, but 90% must be a result of the injections to be honest”.

An important element that is often associated with all r-HuEpo regimes, employed in studies assessing the effects of this powerful perturbation on exercise capability, is the belief, by participants, that a performance outcome should improve. In other words, the belief of change can often result in real or tangible change (Kirsch, 2004) where the CNS, either subconsciously or consciously, may regulate a response to stimulate an enhancement effect of a specific process or several underlying processes (Benedetti et al., 2005, Wager and Nitschke, 2005, Eippert et al., 2009). Indeed, participant blinded studies are designed to control for this. However, there remains a possibility that the simple action of injecting a substance believed to be r-HuEpo may alone contribute to a performance enhancing effect. This can also be inferred as being psychological and/or central effects.

Furthermore, previous studies have reported the route of delivery, in which interventions are carried out, as being influential on measured outcomes with, for example, injected placebos inducing a larger effect than placebos administered orally (Zhang et al., 2008). As result, the study in Chapter 6 aimed to make study findings in Chapter 4 more robust by assessing a ‘control’ comparative group who believed the saline received was r-HuEpo. In addition, Chapter 6 also aimed at assessing participant’s belief system, which was further facilitated with the process of having to self-inject, on endurance performance capability due to reasons as previously described. Experimental Chapters 4 and 6 outcomes are also summarised in Figure 7.2. It is acknowledged that a potential third group could have consisted of participants who were aware they were receiving a placebo. However, the difficulties associated with recruiting further trained/training participants into an r-HuEpo administration trial did prove challenging.

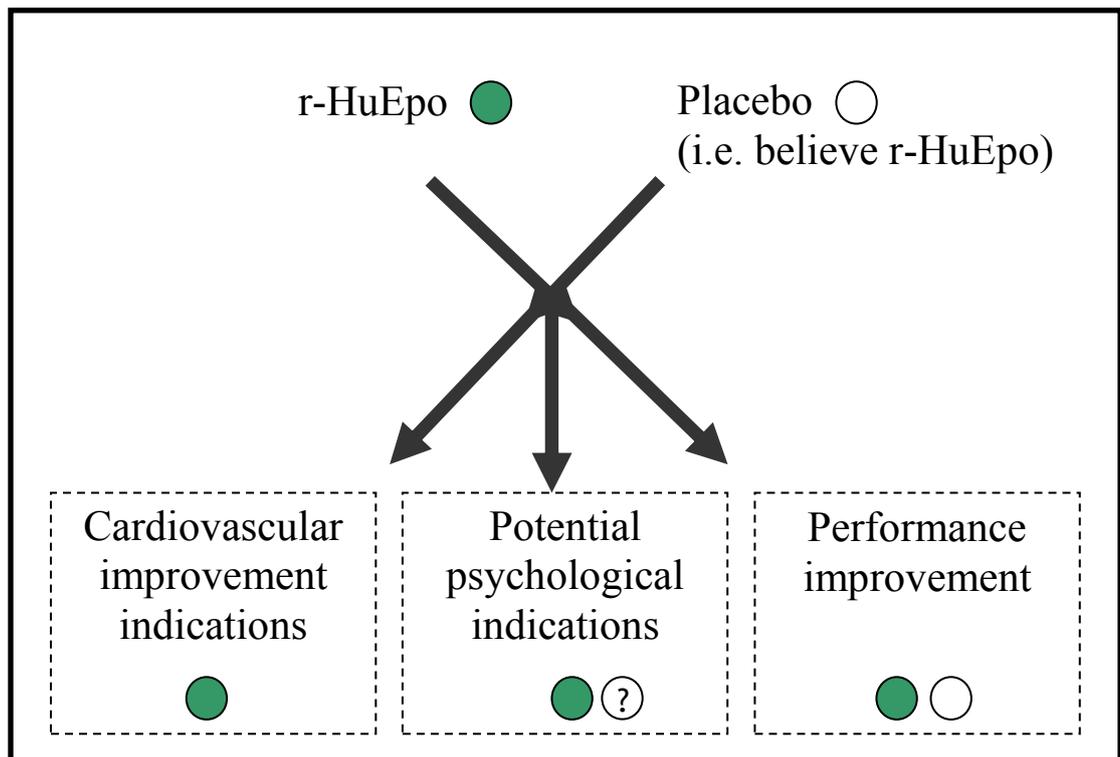


Figure 7.2: Simplified summary of selected outcomes associated with r-HuEpo administration and believing r-HuEpo was being administered

The major finding, in the study described in Chapter 6, was a 1.2% improvement in 3 km performance in the placebo condition (652.4 ± 13.1 ; $p < 0.05$) when compared to control (664.0 ± 13.6 ; $p < 0.05$). Consequently, however, potential explanations could not be identified in measured variables (i.e. HR of each competing individual during each 3 km race, RPE ratings immediately upon completing 3 km distance, haematological variables, and psychological measures) aimed at providing some mechanistic reasoning as to the reported improvement in performance. It is speculated, however, that the performance improvement may be associated with the disassociation of cues related to fatigue, during running, as a result of believing that a potent performance enhancing drug was administered. Comments obtained during the study discussed in Chapter 6 provide some support for this, as previously highlighted.

It is clear that the reported performance improvement of 1.2% is a significant finding, as for example, Foster and colleagues (Foster et al., 1994) have indicated a ~1.0% difference in events ranging from 1000 to 4000 m as being a difference between a gold and silver medal. Furthermore, when assessing the positional time differences in the Beijing Olympics 2008 and London Olympics 2012 for the men's 10,000 m race, the time difference between 1st and 7th position was 0.43% and 0.24%, respectively, and Figure 7.3

provides an interesting perspective on this. It is theoretically possible, therefore, that the individual who finished 7th (and actually several positions beyond) in these races, could have potentially finished 1st – assuming all other factors remained constant. .

It is acknowledged that care must be taken when extrapolating performance results from well-trained individuals to world-class individuals. Myburgh (Myburgh, 2003), for example, raised questions as to the applicability of exercise science research findings as often these are carried out on sub-elite individuals. Sub-elite individuals are a different calibre of athlete in comparison to the world-class individual as the adaptations to consistent training and experiences is achieved over many years in the latter (Verkhoshansky, 1998). In agreement, Laursen and Jenkins (Laursen and Jenkins, 2002) also support this notion; commenting that results of training studies on participants who are previously untrained, or who are recreationally trained, are not applicable to elite athletes. Assuming that results obtained from well-trained individuals are applicable to the world-class individuals may, indeed, prove problematic. However, an important additional feature of the study discussed in Chapter 6 is the inclusion of handicapped group competition races in the field-based 3 km exercise performance assessment protocol. In addition to small monetary prizes and having heart rate measured real-time, this competitive handicapping element assisted in ensuring that all competing individuals were carrying out their best efforts and being truly competitive.

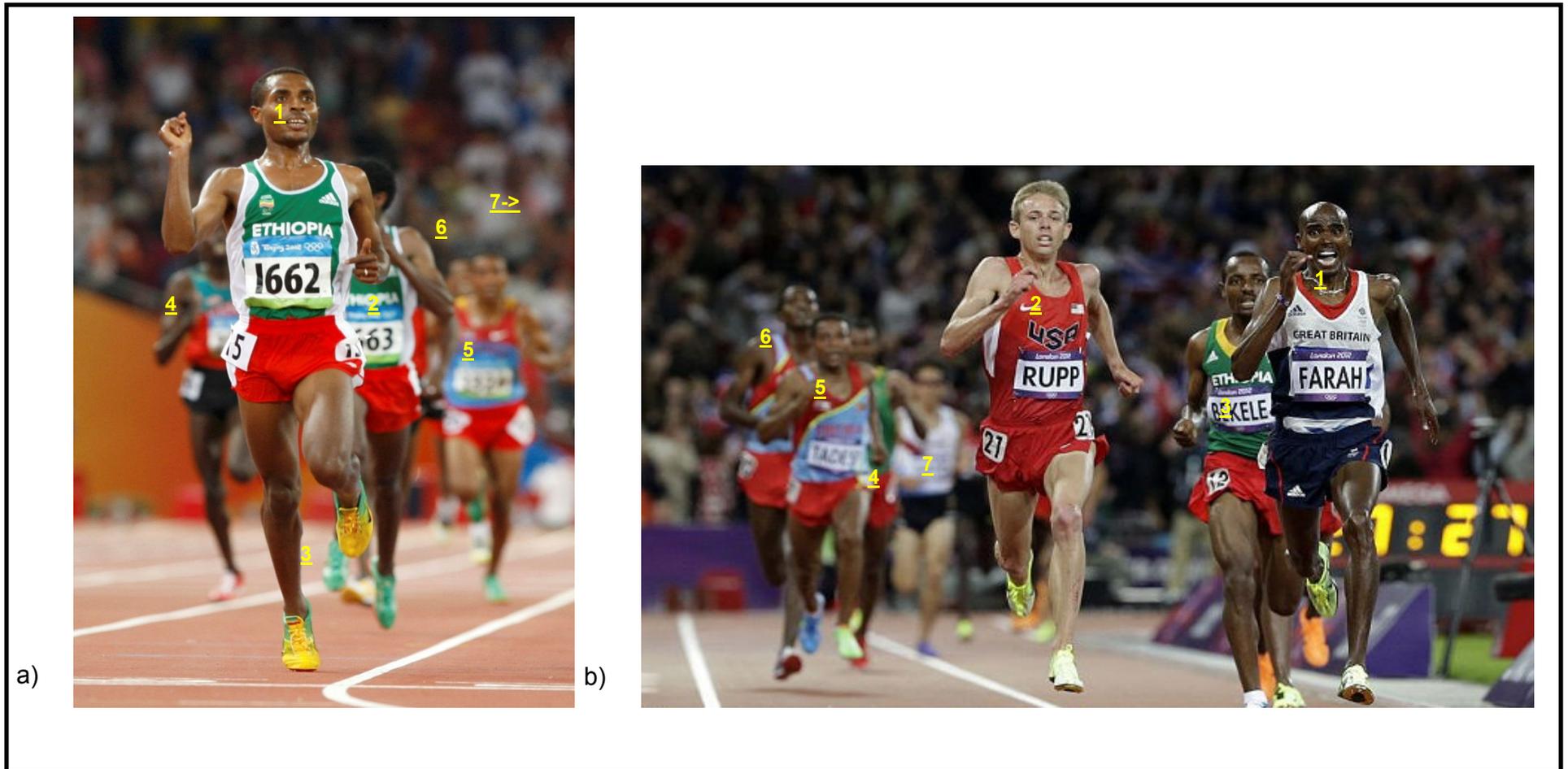


Figure 7.3: Finishing line photo shot at a) Beijing Olympics 2008 and b) London Olympics 2012

Competitiveness should therefore, theoretically, be the same regardless of the calibre of athlete. Thus, competitiveness may instead be an association between personality types and how these varying types interact with the placebo effect and racing in a competitive environment. It is therefore possible that a specific personality type may have supplemented the enhancement in endurance performance under such conditions. From another perspective, only 44% of athletes indicated good understanding of the placebo effect (and how it can be used) in a study including 96 national-level Australian athletes (Brooling et al., 2008). This further suggests that over 50% of athletes may be suggestible in this instance. Interestingly, there have also been anecdotal reports of under-performing world-class sportsmen being encouraged, by team managers, to take illegal substances (e.g. growth hormone) in attempt to turn their performance around. Instead the athlete is actually administered with a placebo, believing an illegal substance has been used. Not only discovering the mechanistic but also discussing the ethical complexities behind such a situation, if an athlete were to report such activities to the authorities, is a very interesting topic, but is one that is also outwith the remit of this thesis.

Realistically, being able to carry out a similar study using world-class individuals will likely be impossible, for obvious reasons. Therefore research in the area of performance enhancement will likely, more often than not, be constrained by this limitation. Fundamentally however, Chapter 6 offers unique insights into how the Central Governor may work in order to attenuate fatigue and improve performance. In reference to previous literature (Benedetti et al., 2005, Wager and Nitschke, 2005, Eippert et al., 2009), it appears that this improvement in performance was achieved subconsciously. Interestingly, previous studies have proposed that the brain (i.e. in a possible subconscious nature) may act to regulate exercise output in order to prevent full depletion of aerobic energy reserve (Amann and Dempsey 2008). This mechanism of action has been proposed to occur in order to insure that exercise can be carried out safely without a catastrophic biological failure (Swart et al., 2009b). If this holds true, one could infer that during self-paced exercise the brain, via specific neural control mechanisms, essentially determines a work intensity relative to maximal but below absolute capacity (Amann and Dempsey 2008). This may have been subconsciously done (as demonstrated in Chapter 6) in an attempt to avoid the negative complications of severe metabolite build up or biological failure which may, in turn, suggest the potential existence of a metabolic and/or central reserve. Furthermore, although competitors, on average, ran faster post-placebo they did not perceive that they were working harder to achieve this (as indicated by no significant changes in RPE measures). This also suggests that the competitors may not have been

aware that a greater work output was being produced and so this is likely to be of a sub-conscious mechanistic nature which may have enabled individuals to access and use slightly more of their a metabolic and/or central reserve. Thus, Chapter 6 provides unique insights into the effects of an injected placebo, perceived to be a known powerful performance enhancing agent, on endurance performance.

Discovering new ways to assess current models on exercise fatigue is important to further our understanding in this field. Furthermore, attempting to understanding underlying mechanism that enabled the performance improvement, possibly explained by being able to access and ‘push’ central and/or metabolic reserves to their own safe hypothetical limits, (if they do indeed exist), remains of importance. Thus, a very fundamental necessity remains- the necessity to discover the mechanisms behind such a subconscious effect. Such findings certainly support the potential role of the Central Governor (as opposed those physiological measures that were assessed), but mechanistic explanations are required in order to explain such findings.

Fundamentally, however, the administration of r-HuEpo did elicit a greater improvement in endurance performance capability, in the field, when compared to the placebo (4.9% vs. 1.2%, respectively) with a net improvement of 3.7%. This supports the notion that physiological impacts outweigh psychological (or central governor) ones. However, the potential impact of the placebo effect, particularly on psychology, should never be underestimated

7.4 Viewpoints from Well-trained Individuals during and after r-HuEpo and Placebo Trial Participation: Psychosocial Considerations

As previously highlighted, utilising powerful perturbations such as illegal performance enhancing drugs, in a legal setting, provides a unique opportunity to assess the associated psychosocial issues and experiences. The uniqueness is further exemplified by the minimising, or even possible removal, of the social desirability effect of dishonesty due to the nature of which experimental Chapters 4 and 6 were carried out - a factor often overlooked by doping research (Petroczi et al., 2011, Petróczi, 2012). Ethical reasons, indeed, prohibited the use of competitive athletes and so participants abstained from sporting competition for the duration of both the r-HuEpo and placebo trials. In the case of Chapter 4, participants refrained from competition for a further 28 days after receiving the

final r-HuEpo injection. However, all other activities, including training environment for example, continued as normal. This, in turn, provided unique insights into the psychosocial consequences of using such substances/techniques, which can often improve performance over relatively short period of time, on psychological well-being and social interaction effects.

As the below quotes from Chapter 4 indicate, however, psychosocial impacts are likely to include a sense of embarrassment, shame and/or guilt (Bloodworth and McNamee, 2010):

Participant 3: *“I didn’t tell anybody at my club that I was doing this, one thing that I hadn’t considered, we all gauge our fitness on other people... didn’t realise that it could have a negative effect on other people when they see how quickly I am improving. They are doing the same training and same sessions, but they aren’t getting the same results. On a couple of occasions people did say like they just weren’t improving the way they should be...that kind of thing. Obviously it was noticed, it was noticed by coaches in club... ‘you’ve gained shape quite quickly’, but I didn’t say anything, although, it is kind of funny from my point of view...it must be quite dispiriting for others. I just attributed to the miles of running... yeah... never even considered it until I was in the situation. Maybe not ashamed, but I felt bad for those kinds of people...”*

Participant 4: *“One day, just, I was running with the same people I would normally, they would finish the run and I would be the back of the group, but half way through the run they were running fast, and they were running fast they were saying ‘you know we are running pretty fast’ and I was with them. They started to struggle and then I took off and left them... it was ridiculous, I got quite embarrassed, and you know it was like where the hell did that come from. I thought that must be the EPO. You could see people in the group when they came in at the end, they are like ‘how you doing?’ I was like, ‘I’m off’. I was embarrassed. I felt like that was...but then again, since then, I have had experiences where I have run as well, so I don’t know if that was a special day, or were they struggling? But that was one occasion where I took off”.*

The fact that participants told nobody, outwith the testing environment, of their participation in a perfectly legal trial (except those who regularly trained with a trusted coach who they then only informed), is a further indication of the, potentially, very dark underworld of doping.

The consequences may also help to persuade those who are tempted by doping to avoid such a path as, alongside potential sporting success, there will likely be serious moral anxiety, stress, guilt and shame. Such consequences, for some, will likely be a strain on mental well-being and maybe even affect quality of life in general. For example, Lucidi and colleagues (Lucidi et al., 2008) assessed the longitudinal effects of social-cognitive mechanisms on the self-reported use of doping substances and supplements amongst Italian high school students. These authors reported stronger intentions and moral disengagement as contributors to increased use of doping substances (Lucidi et al., 2008). If increased moral disengagement, in particular, is associated with increased doping substance use, the reverse effect is also possible where further doping substance use may lead to further moral disengagement. This is likely to have psychosocial complications – most likely negative ones. There were hints of this and potential doping ‘addictiveness’ (in Chapter 5) which, like taking any ‘feel good’ drug, can contribute to psychosocial harm. For example, participant comments made via interviews carried out in Chapter 4 included:

Participant 3: “...it was quite exciting the whole way through to see what kind of changes would take place, and then afterwards, I don’t know, I suffered a mental withdrawal....I need more!”

Participant 6: “...I’m no [i.e. not] so confident as what I was. I’m almost running the same times through the constant training, but I’m no running the same as on EPO. I’m finding it harder to achieve things now”.

Another point for consideration is that with potential addiction (due to r-HuEpo mediated performance enhancing effects) may also come resentment against those deemed privileged with geographical advantages of altitude, as in the case of participant 6 in Chapter 6:

Participant 6: “I think we are disadvantaged in this country simply because the place is the way it is” [referring to altitude of Great Britain in comparison to East Africa].

It is important to reiterate that this particular individual did not condone the use of substances such as r-HuEpo as long as they were still categorised as being illegal. However, with the ever increasing pressures involved in elite sport and the ever growing rewards, it is unclear as to whether such individuals will remain 'clean' or whether the temptation, at some point, will cause a change in attitude.

As a result of such 'psycho-social' issues, anti-doping research (e.g. the WADA Social Science Research Grant Program) has started to, only more recently, focus on what the agency have termed 'tertiary prevention' schemes. The 'tertiary prevention' schemes have a particular focus on decreasing doping relapse in athletes who have been sanctioned and this is likely an indication of possible problems associated with 'doping addiction'. This is a vital consideration; however, it is also vital that such research focuses on identifying factors (e.g. personality traits), situations (e.g. geographical disadvantages) and/or circumstances (e.g. competitors known to be cheating and who are not caught) that could turn those 'clean' athletes, who feel 'disadvantaged', into becoming 'cheats' themselves.

Regarding the research presented in Chapters 4 and 6, however, none of the participants recruited indicated serious signs or desires to pursue continuing the use of r-HuEpo illegally, even though positive performance enhancing effects were experienced. It might possibly be the case that participants, who took part in Chapter 4, will have likely realised the level of care required when using such a substance. This, in turn, may have hopefully encouraged any potential 'doper' not to pursue the path as 'black market' drug taking will certainly not come with the level of care and attention that was provided in this study.

7.5 Future directions

Chapter 3 assessed the K4b2 portable metabolic device during submaximal and maximal running velocities outdoors. According to the author's knowledge this is the first study to do so. Assessing portable metabolic analysers, in conditions that they were designed and often used, both in the field and laboratory, is clearly of great importance to insure data integrity. Future work should encompass the testing of reliability between differing K4b2 devices (with the same specifications) in order to investigate general K4b2 device comparability. The K4b2 should also be tested further, in the field, under varying environmental conditions (e.g. high ambient temperatures, humidity and varying levels of

pollution) as well as during different work modes (e.g. skill based games, skiing, cycling). Such studies are essential in order to truly assess the K4b2 and, indeed, other similar devices.

Chapters 4 and 6 reported improvements in endurance performance after both r-HuEpo and placebo interventions, respectively. Future work should attempt to assess, more directly, the effects of such interventions on the varying endurance performance limiting factors, as previously described, similar to what has previously been attempted (Rasmussen et al., 2010). The incorporation of new technologies, as they become available, is a vital process as past technologies may not be sensitive enough to detect potential changes. This is particularly relevant to studies assessing CNS activity as very recently highlighted by Tanaka and Watanabe (Tanaka and Watanabe, 2012) where, for example, the authors suggest advances in neuroimaging are required to further evaluate the inhibitory and facilitative systems as part of the CNS. More specifically, as in the case with the Rasmussen et al. study (Rasmussen et al., 2010) which assessed the effects of r-HuEpo on cerebral metabolism and exercise capacity, developing methodologies that can detect potential changes in CNS activity more directly in the areas involved in exercise (e.g. quadriceps activation during cycling/running, as opposed to assessments of non-exercising areas such as the elbow flexor) will help to more concretely assess CNS activity and fatigue during endurance exercise activities in both drug and placebo trials. For example, for running based activities, future studies could assess effects of such interventions on lower leg muscle EMG pre-activation activity and stiffness (e.g. via foot contact duration) where improved leg stiffness has been associated with improved running performance capability (Jones, 2002, Hobara et al., 2010).

The r-HuEpo mediated performance enhancing ‘paradoxical’ effect of depressing plasma volume to assist in increasing [Hb] should also be explored further. For example, further studies could assess the concomitant effects of administering r-HuEpo whilst also carefully expanding plasma volume (e.g. infusion of saline) on physiological variables including, in particular, cardiac output (due to a potentially improved afterload) on endurance performance capability.

Finally, assessing training activities in more detail in terms of ‘quality’ of session could also provide further insights into potentially behavioural changes associated with taking an actual and/or believed performance enhancing drug.

From a wider perspective in regards to this area of research, it seems reasonable to suggest that proposed theories and models (e.g. cardiovascular versus central governor) actually work together in order determine performance outcome or ‘hold back’ together in order to cause/induce fatigue. However, further evidence is necessary to refine how much mechanisms contribute to the overall outcome effect. This is particularly the case for the central governor model as mechanistic evidence is often lacking. Therefore, researchers should aim to assess novel ways to study the central governor model and its relative importance to a measured outcome. Specific research approaches of particularly potential should involve blood manipulations (e.g. increasing red blood cell count), but then removing this effect (i.e. by removing the ‘added’ red blood cell volume) and testing performance outcome. Another approach could be the use of carbon-monoxide where the effect of enhancing oxygen carrying capacity (by r-HuEpo administration or blood transfusion) could be calculated to cancel out the enhanced oxygen carrying capacity effect. Performance outcome can then be assessed to test this effect of such an intervention. The findings of such studies would be of great interest to the field, however, researchers must try to ensure that potential mechanism can be identified and explained if findings of significance are reported. In agreement with Tanaka and Watanabe (Tanaka and Watanabe, 2012), measurement tools must be carefully selected and also improved in order to detect potentially very sensitive changes.

7.6 General Conclusions

Preventing and/or attenuating fatigue experienced during endurance exercise in order to improve human performance, for any calibre of individual, is a considerable topic of investigation and is continually debated to this day. Understanding the mechanism behind prolonged exercise induced fatigue is even more complex and this is demonstrated on a number of levels throughout the previous chapters.

Chapter 3 revealed, initially, that the K4b2 metabolic device appeared to be valid for the measurement of $\dot{V}O_2$ during submaximal and maximal running velocities in the field (i.e. outdoors). However, upon further investigation, this initial apparent agreement was a consequence of two different errors which acted in opposite directions (i.e. a $5.2 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ negative offset in $\dot{V}O_2$ coupled with a 14.2% higher gradient in the relationship between the K4b2 outdoors and the Douglas bag method for measuring $\dot{V}O_2$). The K4b2 device also significantly overestimated $\dot{V}O_2$ indoors when compared to the Douglas bag method. As a result of these findings, unfortunately, the K4b2 could not be considered for use in further experimental chapters due to the sensitive physiological measurements required. Outwith the purposes of this thesis, potential users of the K4b2 device must seriously consider the level of measurement accuracy required prior use.

Chapter 4 provided confirmation on the erythropoietic effect of r-HuEpo administration where increases in [Hb] and Hct were associated with improvements in $\dot{V}O_{2\text{max}}$. Three km time trial performance also improved, immediately post r-HuEpo administration, by 4.9%. Interestingly, no clear associations were found between [Hb], Hct or $\dot{V}O_{2\text{max}}$ and 3 km time trial performance. This suggests that factors, other than changes in [Hb], Hct and $\dot{V}O_{2\text{max}}$, are likely to have influenced 3 km time trial running performance. Furthermore, the improvement in 3 km time trial performance remained for 4 weeks post r-HuEpo administration. This was despite [Hb] and Hct declining by more than 50% over the 4 week post r-HuEpo administration phase. Finally, changes in psychological measures (i.e. anxiety and tension) were found which warrant further investigation as a potential contributor to enhancing performance.

Chapter 5 offered unique insights into athlete viewpoints on drugs, contrasts before and after using r-HuEpo, impacts on sporting performance and on doping in sport. The study found that all participants reported experiencing a performance enhancing effect as a result of r-HuEpo administration. The majority of participants commented on an enhancement in

recovery periods either in between training intervals or between training days. However, alongside these ‘positive’ enhancement effects, psychosocial implications such as feelings of guilt, shame and/or embarrassment were also present. From an anti-doping perspective, all participants generally maintained an anti-doping sentiment; however some more convincingly than others, especially when participants considered ‘geographical privileges’ (i.e. living and training with the use of altitude).

Chapter 6 found that participants completed the 3 km race distance significantly faster following the injected placebo condition (perceived to be a form of r-HuEpo) compared to the control condition (saline injection) by 1.2%. This magnitude of change has been found to be highly relevant in terms of the potential sporting success in competition. However, it was not clear as to what caused this enhancement in performance as measured heart rate, haematological variables and psychological subscales did not differ significantly between control and placebo conditions. Furthermore, no relationships were found between changes in any of these factors with 3 km time trial performance. It may be possible that the belief that a performance enhancing drug had been administered may have caused participants to disassociate cues (or limit one’s ability to detect distressing cues) related to fatigue during running. Further study is needed to confirm whether this mechanism contributed to the observed performance-enhancing placebo effect.

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Appendices

Appendix A: Participant Information Sheet

University of Glasgow Faculty of Biomedical & Life Sciences

INFORMATION SHEET

Study Title: To assess the accuracy of a pioneering portable metabolic measurement system (K4b2) only previously done during sub-maximal exercise.

You are being invited to take part in a research study. Before you decide whether to with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

What is the purpose of the study? Modern technological developments in human gas analysis have helped improve laboratory practices enabling researchers to carry out the analysis of human gas, in a portable manner, out on the field. The development in measures of performance not only allows for the interpretations of cardiovascular health, but can potentially have even greater impacts on valid performance testing when attempting to measure endurance capacity, running economy and energy expenditure, as examples. The key aspect in this study involves the concept of performance testing as this is key in training programmes and athlete development and despite performance analysis via means of gas analysis being widely accepted as being a useful indicator of performance in a wide range of activities, methodological limitations (e.g. the constraints of the laboratory setting) still exist that hinder the obtaining of data that reflects ‘true’ exercise performance.

Why have I been chosen? You have been selected as a possible participant in this investigation because you regularly take part in endurance activity and you are in good health. Twenty volunteers are being sought.

Do I have to take part? It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form and fill in a lifestyle questionnaire. If you decide to take part you are still free to withdraw at any time and without giving reason.

What will happen to me if I take part? You will be asked to visit the laboratory on two occasions and a running track once, where a series of assessments will be carried out. All experimental trials should last no longer than 2 hours. On your initial visit to the laboratory you will be medically examined by a qualified doctor. The first test will take place in the laboratory on a motorised treadmill at a fixed 1% gradient. During this test you will be instructed to run continuously at 10, 12, 14, 16 and 18 km·h⁻¹ (or until volitional exhaustion) for 3 minutes at each speed. This will be done using a ‘Gold standard’ method. The second test is exactly the same,

except it will be conducted using the K4b2 unit. The final third test will be conducted outdoors on a 400m running track; an investigator will cycle next to you to monitor and control the running speed.

Heart rate and expired gas will be recorded throughout all tests via a heart rate monitor and a flexible rubber mouthpiece, respectively. Your height and weight will also be measured on each visit to the lab. Finally, you will not be able to perform any exercise 24 hours prior to testing or consume any alcohol 48 hours prior to each lab visit. You will be excluded from participating in this study if you take drugs (recreational or performance enhancing drugs).

What are the side effects of taking part? There are none.

What are the possible disadvantages and risks of taking part? Exercise has a negligible risk in healthy adults, although maximal exercise has a small risk of myocardial infarction (“heart attack”). The primary symptom of myocardial infarction is chest pain on exertion. If you experience any unusual sensations in your chest during the experiment, you should cease exercising immediately.

You will breathe through a rubber mouthpiece during the tests, in order for us to collect the air you breathe out. You will also wear a noseclip during the first testing session. You may experience difficulty swallowing while breathing through a mouthpiece and wearing a noseclip, due to some pressure in the ears. In addition some participants experience increased salivation when breathing through a mouthpiece.

What are the possible benefits of taking part? We hope to find out whether or not this pioneering device can be used out on the field with full confidence that it serves its purpose. The information collected will help us to decide whether the device is reliable and accurate or if changes are required. This testing will also allow us to provide you with feedback with regards to your physiology which would include a $\dot{V}O_{2\max}$ value.

What if something goes wrong? If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone’s negligence, then you may have grounds for a legal action but you may have to pay for it. The principal investigators, although not medically qualified, are fully trained in Advanced Life Support. In the event of an untoward incident, the principal investigator(s) will provide basic life support including chest compressions and ventilation until emergency medical staff are on hand. You may want to consult your GP if you are experiencing any side effects from taking part in the study and should also inform the principal investigator.

Will my taking part in this study be kept confidential? All information about you that is collected during the course of the research will be kept strictly confidential.

What will happen to the results of the research study? Results will be published in a peer-reviewed scientific journal once the study is completed. You will automatically be sent a copy of the full publication. You will not be identified in any publication.

If you wish to find out more about this investigation, you can contact:

Ramzy Ross
Institute of Biomedical & Life Sciences
West Medical Building
University of Glasgow
Glasgow, G12 8QQ
E-mail: ramzyross5@gmail.com

Dr Yannis P. Pitsiladis
Institute of Biomedical & Life Sciences
West Medical Building
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Glasgow, G12 8QQ
E-mail: Y.Pitsiladis@bio.gla.ac.uk

CONSENT

Title of Investigation: Evaluation of the Cosmed K4b2 Portable Metabolic System during Running Outdoors

I

Give my consent to the research procedures which are outlined above, the aim, procedures and possible consequences of which have been outlined to me.

Signature

Date

Appendix B: Participant Information Sheet

University of Glasgow
Institute of Biomedical and Life Sciences
University of Glasgow

INFORMATION SHEET

Study title: A Gene-Microarray Based Approach to the Detection of Recombinant Human Erythropoietin Doping in Endurance Athletes

You are being invited to take part in a research study. Before you decide whether to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. This project is being financed and supported by the World Anti-Doping Agency.

Thank you for reading this.

What is the purpose of the study? The project is aimed at validating a number of blood tests that can be used to detect erythropoietin (Epo) abuse. Presently, Epo abuse is detected indirectly in blood or directly in urine samples. The objective of this study is to build on the work already performed in the area of blood testing to detect Epo abuse and potentially provide alternative and robust gene based testing methods in the battle against drugs in sport, and in particular, Epo abuse. This will benefit athletes who believe in drug free sport.

Why have I been chosen? You have been selected as a possible participant in this investigation either because of your status as an athlete, who is indigenous to sea-level (Caucasian) or to moderate altitude (east African) to provide a comparison for findings on gene expression profiles following altitude training or Epo administration. 40 volunteers are being sought.

Do I have to take part? It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

What will happen to me if I take part? Initially, you will be asked to undergo a medical examination to establish your suitability as a participant in the study.

If selected, you will receive 50 Ukg⁻¹ body mass subcutaneous injections of r-HuEpo every two days over a 4-week period. You will also receive daily iron tablets providing ~105 mg of iron. Saline injections will substitute r-HuEpo injections when a pre-determined blood concentration is reached in response to r-HuEpo. You will then resume the standard r-HuEpo regimen. We would like to take a small amount of blood from an intravenous line in the back of your hand pre, during (days 1, 3, 10, 15, 17, 22, 26, 28) and for 4 weeks after r-HuEpo administration (days 5, 7, 12, 14, 19, 21, 26, 28).

On your first visit to the lab you will be asked to complete two confidential questionnaires; the first will allow us to obtain information related to your general health; and the second will allow us to quantify your past exercise/activity involvement.

Your height, weight and percentage body fat will also be measured on each visit to the lab. Your percentage body fat will be estimated by a bioelectrical impedance technique, which involves placing slightly adhesive small patches (“electrodes”) on your right hand and foot and introducing a very small and imperceptible electrical current between these.

In order to estimate your nutritional intake and energy expenditure, we may ask you to record your normal food and drink intake for 24 hour prior to each test and to keep a diary of your physical activity.

Finally, you will not be able to consume any alcohol 48 hours prior to each lab visit. You will be excluded from participating in this study if you take drugs (recreational or performance enhancing drugs).

What are the possible side effects, disadvantages and risks of taking part?

There are only minimal risks associated with the blood sampling procedure. Slight bruising may occur around the site of collection, but this can be minimised by applying pressure at the site for a minimum of 3 minutes. Blood will be collected by either, a qualified phlebotomist and/or a medically qualified individual.

There have been no reports of significant side effects as a result of Epo injections in normal healthy individuals. In athletes, there has been one report of elevated systolic blood pressure (from 177 to 191 mmHg) during moderate-intensity exercise (compared with mild- or low-intensity exercise) following treatment with Epo at doses of ~30 units per kilogram body weight over 6-7 weeks. Other side effects reported include flu like symptoms (mild and of short duration), suppression of natural Epo production, a burning sensation associated with subcutaneous injection of Epo, joint pains, rashes, and one case of a hypotensive episode associated with Epo injection. There is also an increased risk of seizures and thrombo-embolic events. The management of side effects including allergy and arthralgia, and those listed above, will include cessation of treatment and withdrawal from further participation in the study. Doses of Epo have been used up to 1200 units per kilogram body weight per week, far beyond those in the present study.

Mild gastrointestinal discomfort (i.e. loose bowel, constipation) may result from oral iron supplementation, which is relieved upon cessation of the supplementation.

What are the possible benefits of taking part? It is envisaged that this research will result in the formulation of new genetic method(s) with improved discriminatory power relative to current haemoglobin and haematocrit detection protocols and in doing so significantly reduce (hopefully eliminate) the possibility of false positives due to athletes living and/or training at altitude and false negatives due to inadequate detection.

What if something goes wrong? If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. The principal investigators, although not medically qualified, are

fully trained in Advanced Life Support. In the event of an untoward incident, the principal investigator(s) will provide basic life support including chest compressions and ventilation until emergency medical staff is on hand. You may want to consult your GP if you are experiencing any side effects from taking part in the study and should also inform the Principal Investigator.

Will my taking part in this study be kept confidential? All information about you that is collected during the course of the research will be kept strictly confidential

What will happen to the results of the research study? Results will be published in a peer-reviewed scientific journal once the study is completed. You will automatically be sent a copy of the full publication. You will not be identified in any publication.

If you wish to find out more about this investigation, you can contact:

Dr Yannis Pitsiladis
Reader, Faculty of Biomedical and Life Sciences
West Medical Building
University of Glasgow
Glasgow, G12 8QQ
Phone: 0141 330 3858
e-mail: Y.Pitsiladis@bio.gla.ac.uk

INFORMED CONSENT (Participant 's copy/Investigator's copy)

PRE-STUDY VISIT

I, _____ hereby (give permission to have my child) consent to take part as a volunteer in the research project entitled "A Gene-Microarray Based Approach To the Detection of Recombinant Human Erythropoietin Doping in Endurance Athletes".

- I acknowledge that I have read the Information Sheet in relation to the above-mentioned project. I have had the project, so far as it affects me, fully explained to my satisfaction by the research worker(s). My consent is given freely.
- I understand that the purpose of this project is to determine blood and genetic indicators of Epo doping in athletes.
- I understand that this study will aid in the further development of tests for detecting abuse of Epo doping in athletes.
- I have had all medical risks explained to me.
- I have been informed that I (my child) will not be identified and my (child's) personal results will not be divulged while information is gained during the study for the purpose of publication and/or presentation.
- I understand that I am (my child is) free to withdraw from the project at any time.
- I have retained a copy of this consent form and the information sheet.

Signature of Participant : _____ Date: _____

Signature of Witness/Parent/

Guardian of Minor: _____ Date: _____

I, the undersigned, was present when the study was explained to the participant /s in detail and to the best of my knowledge and belief it was understood.

Signature of Researcher: _____ Date: _____

Appendix C: Participant Information Sheet



University of Glasgow | College of Medical,
Veterinary & Life Sciences

VOLUNTEER INFORMATION SHEET

A study to assess the effects of OxyRBX on sporting performance in well trained individuals

You are being invited to take part in a research study. Before you decide whether to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

What is the purpose of the study? Erythropoietin (EPO) plays an important role in the creation of red blood cells. An increase in red blood cells results in increases in several blood factors including haematocrit which essentially allows you to increase your ability to carry and transport oxygen around your body. This can often enhance sporting performance. However, the use of EPO for performance enhancement is illegal as indicated by the 'World Anti-Doping Agency' (WADA) code. A new **legal** substance, called OxyRBX, has been developed which has similar effects to EPO on the blood profile. We are testing the effects of OxyRBX on sporting performance.

Why have I been chosen? You have been selected as a possible participant in this investigation because of your status as a well-trained individual who has lived at sea-level for the majority of your life. A total of 16 volunteers are being sought.

Do I have to take part? It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part? Initially, you will be asked to undergo a screening procedure to establish your suitability as a participant in the study. This will involve:

- a discussion regarding your health, family history, and physical activity levels
- a blood pressure measurement
- your height and weight being measured
- an opportunity for you to ask any relevant questions

You will undergo either a 'control phase' (7 days) or 'OxyRBX' intervention phase' (7 days) followed by the other separated by a 14 day 'wash out' period. Please see Figure 1 below:

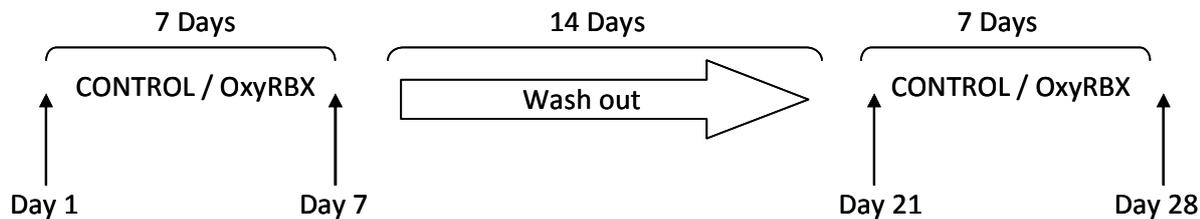


Figure 1: Schematic plan of the study design. Arrows indicate days when data collection (height, weight, blood, questionnaires and 3km time trials) will be carried out. OxyRBX injections will be self-administered on each day of the OxyRBX phase.

You will be asked to attend the laboratory on 9 occasions as follows:

- A screening visit during which you will be provided with written and oral information about the study and given the opportunity to ask questions. You will also be asked to complete questionnaires related to health and sports participation; have your blood pressure measured; and provide written informed consent.
- A blood sample, blood pressure measurement, questionnaires and 3 km time trial performance test will be carried out on Day 1 and Day 7 of both control and OxyRBX phases.
- During the OxyRBX phase, on each day (days 1-7) an injection containing OxyRBX (0.5 ml, about a tenth of a teaspoon) will be provided to you to self-inject into your tummy.
- During the control phase no intervention will be carried out between days 1-7.
- Finally, on the final day of the trial, you will be interviewed and asked questions concerning the trial. This will be an opportunity to discuss your results which will include blood results, 3 km time trial performance results, and questionnaire results.

Psychological questionnaires

The questionnaires used in this study contain a number of questions. You will be asked to choose from 0 (not at all) to 4/5 (extremely/very much) for each question. Each questionnaire takes approximately 5 minutes to complete.

Blood sampling

A small needle will be inserted into your arm and a small blood sample (10ml, approximately 2 teaspoons full) will be taken.

3 km time trial

You will carry out a 3 km running time trial on days 1, 7, 21 and 28 and this will be carried out on a 200 m indoor running track at the Kelvin Hall Arena, Glasgow. These time trials will be carried out as a competition, so you will meet other participants.

Training, diet and general monitoring

You will receive a diary to log your diet and training activities both during the total 28 day trial period and also 1 week prior starting each phase (i.e. control/ OxyRBX phases). Please do inform us if you suffer from any illness, or have any significant change in lifestyle (e.g. you start taking a certain medication).

Finally, you will not be able to consume any alcohol 48 hours prior to each lab visit. You will be excluded from participating in this study if you take drugs (recreational or performance enhancing drugs). This protocol includes 15 visits per participant.

What are the possible side effects, disadvantages and risks of taking part?

- There are only minimal risks associated with the blood sampling procedure. Slight bruising may occur around the site of collection, but this can be minimised by applying pressure at the site for a minimum of 3 minutes. Blood will be collected by a qualified phlebotomist and/or a medically qualified individual.
- There have been no reports of significant side effects as a result of OxyRBX injections in normal healthy individuals. However rare (less than one in ten thousand) side effects might include: allergic reactions (rash; hives; itching; difficulty breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue). In these circumstances stopping OxyRBX use results in complete resolution of symptoms within 24 hours.
- Doses of OxyRBX up to 10 times higher than the dose used in the present study have been safely used in previous studies.
- Exercise testing will be at a maximal level and so there is a possibility, very occasionally, that certain changes may occur during or shortly after the exercise performance test. This might include abnormal blood pressure, fainting or a change in the normal rhythm of the heartbeat.
- There is a small possibility that taking part in this study will reveal a health problem that you already have, such as high blood pressure. If such a problem is revealed, we will seek your permission to inform your GP to ensure that you receive the appropriate treatment.

What are the possible benefits of taking part?

There may be no direct benefits to you, but as a result of taking part in this study you will receive health and fitness information about yourself including blood measurements, blood pressure, and exercise performance tests as well as information on your psychological performance. Further, this research will provide further insight into what effect OxyRBX has on sporting performance.

What if something goes wrong?

If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. The principal investigators, although not medically qualified are fully trained in Advanced Life Support. In the event of an untoward incident, the principal investigator(s) will provide basic life support including chest compressions and ventilation until emergency medical staff is on hand. You may want to consult your GP if you are experiencing any side effects from taking part in the study and you should also inform the Principal Investigator.

Will my taking part in this study be kept confidential?

All information about you that is collected during the course of the research will be kept strictly confidential. Any information about you which leaves the University of Glasgow will have your name and address removed so that you cannot be recognised.

What will happen to the results of the research study?

Results will be published in a peer-reviewed scientific journal once the study is completed. You will automatically be sent a copy of the full publication. You will not be identified in any publication.

Who has reviewed this research study?

This study has been reviewed and approved by the College of Medical, Veterinary and Life Sciences (Institute of Cardiovascular & Medical Sciences) Ethics committee at the University of Glasgow.

If you wish to find out more about this investigation, you can contact:

Ramzy Ross on 0141 330 3475 (Office) or 07799197099 (Mobile) (email: r.ross.1@research.gla.ac.uk)

Dr. Jason Gill (email: Jason.Gill@glasgow.ac.uk)

You will be given a copy of this information sheet and signed consent form to keep for you records.

INFORMED CONSENT (Participant 's copy)

I, _____ hereby consent to take part as a volunteer in the research project entitled, '**A study to assess the effects of OxyRBX on sporting performance in well trained individuals**'.

- I acknowledge that I have read the Information Sheet in relation to the above-mentioned project. I have had the project, so far as it affects me, fully explained to my satisfaction by the research worker(s). My consent is given freely.
- I understand that the purpose of this project is to assess the effects the effects of OxyRBX on sporting performance in well trained individuals.
- I have had all medical risks explained to me.
- I have been informed that I will not be identified and my personal results will not be divulged while information is gained during the study for the purpose of publication and/or presentation.
- I understand that I am free to withdraw from the project at any time.
- I have retained a copy of this consent form and the information sheet.

I, the undersigned, was present when the study was explained to the participant /s in detail and to the best of my knowledge and belief it was understood.

Signature of Participant : _____ Date: _____

Signature of Researcher: _____ Date: _____

INFORMED CONSENT (Investigator's copy)

I, _____ hereby consent to take part as a volunteer in the research project entitled, '**A study to assess the effects of OxyRBX on sporting performance in well trained individuals**'.

- I acknowledge that I have read the Information Sheet in relation to the above-mentioned project. I have had the project, so far as it affects me, fully explained to my satisfaction by the research worker(s). My consent is given freely.
- I understand that the purpose of this project is to assess the effects the effects of OxyRBX on sporting performance in well trained individuals.
- I have had all medical risks explained to me.
- I have been informed that I will not be identified and my personal results will not be divulged while information is gained during the study for the purpose of publication and/or presentation.
- I understand that I am free to withdraw from the project at any time.
- I have retained a copy of this consent form and the information sheet.

I, the undersigned, was present when the study was explained to the participant /s in detail and to the best of my knowledge and belief it was understood.

Signature of Participant : _____ Date: _____

Signature of Researcher: _____ Date: _____

Appendix D: Questionnaires**CSAI-2 (Prior Performance Trial)**

Name: _____ Date: _____

Directions: A number of statements that athletes have used to describe their feelings before competition are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate *how you feel right now* - at this moment. There are no right or wrong answers. Do not spend too much time on any one statement, but choose the answer which describes your feelings *right now*.

	Not At All	Somewhat	Moderately So	Very Much So
1. I am concerned about this competition	1	2	3	4
2. I feel nervous	1	2	3	4
3. I feel at ease	1	2	3	4
4. I have self-doubts	1	2	3	4
5. I feel jittery	1	2	3	4
6. I feel comfortable	1	2	3	4
7. I am concerned that I may not do as well in this competition as I could	1	2	3	4
8. My body feels tense	1	2	3	4
9. I feel self-confident	1	2	3	4
10. I am concerned about losing	1	2	3	4
11. I feel tense in my stomach	1	2	3	4
12. I feel secure	1	2	3	4
13. I am concerned about choking under pressure	1	2	3	4
14. My body feels relaxed	1	2	3	4
15. I'm confident I can meet the challenge	1	2	3	4
16. I'm concerned about performing poorly	1	2	3	4
17. My heart is racing	1	2	3	4
18. I'm confident about performing well	1	2	3	4
19. I'm concerned about reaching my goal	1	2	3	4
20. I feel my stomach sinking	1	2	3	4
21. I feel mentally relaxed	1	2	3	4
22. I'm concerned that others will be disappointed with my performance	1	2	3	4
23. My hands are clammy	1	2	3	4
24. I'm confident because I mentally picture myself reaching my goal	1	2	3	4
25. I'm concerned I won't be able to concentrate	1	2	3	4
26. My body feels tight	1	2	3	4
27. I'm confident of coming though under pressure	1	2	3	4

POMS SCALE

Name: _____

Age _____ years

Below is a list of words that describe feelings people have. Please read each one carefully. Then tick the answer that best describes HOW YOU FEEL RIGHT NOW. Make sure you answer every question.

	Not at all	A little	Moderately	Quite a bit	Extremely
1. Panicky	0	1	2	3	4
2. Lively	0	1	2	3	4
3. Confused	0	1	2	3	4
4. Worn out	0	1	2	3	4
5. Depressed	0	1	2	3	4
6. Downhearted	0	1	2	3	4
7. Annoyed	0	1	2	3	4
8. Exhausted	0	1	2	3	4
9. Mixed- up	0	1	2	3	4
10.Sleepy	0	1	2	3	4
11.Bitter	0	1	2	3	4
12.Unhappy	0	1	2	3	4
13.Anxious	0	1	2	3	4
14.Worried	0	1	2	3	4
15.Energetic	0	1	2	3	4
16.Miserable	0	1	2	3	4
17.Muddled	0	1	2	3	4
18.Nervous	0	1	2	3	4
19.Angry	0	1	2	3	4
20.Active	0	1	2	3	4
21.Tired	0	1	2	3	4
22.Bad tempered	0	1	2	3	4
23.Alert	0	1	2	3	4
24.Uncertain	0	1	2	3	4

Appendix E: Interview Schedule



University of Glasgow | College of Medical,
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**INTERVIEW SCHEDULE FOR R-HuEpo (Chapter 4) and PLACEBO
(Chapter 6)**

(Prior Deception Revealed for Placebo study- Chapter 6)

Part 1 – Ice Breakers

1. You have undertaken the research project. Was it a worthwhile and interesting experience?
2. How did you feel about it before hand? Did you understand the risks of participating? How did you evaluate these?
3. Did you anticipate any effects taking r-HuEpo / OxyRBX?
4. Are you glad you took part in the trial or was it burdensome?

Part 2 – Experience of effects during the trial

1. Did you have any anxieties about taking such a substance?
2. Whether you did or did not, did you ‘kind of’ relish the thought of taking such a substance legitimately?
3. Did you imagine it would alter your performance at all/significantly?
4. Can you describe how you felt, if differently at all, during the experiment?
5. How did training/performing/running feel during the experiment?
6. Was your recovery any different? Did you change any aspects of your training regime in a way that might have affected things?
7. Did you note any side effects whether positive or negative?

Part 3 – Post Data

Introduce data here

(All data revealed apart from blood results)

1. Do you understand the data that I have shared with you? Do you want to ask any questions about them?
2. Is it what you expected or are you surprised in any way?

3. How do you feel about it? (Important Question – Help prompt if struggling: happy, elated, guilty, ashamed, anxious about returning to competition, unable to look fellow club athletes in the eye? And so on...)
4. (If performance has improved) To what extent do you feel this is a response to your own hard work; that the substance just allowed you to work harder?
5. Do you think athletes should be able to use such a substance as a component of their training?

Part 4 – Post Data (Deception Revealed for placebo study Chapter 6 and blood results now shown)

1. Now that you have had time to read the debriefing document and reflect, how do you feel now having learnt that a placebo was being administered?
2. Did you ever have any suspicion that this may be the case throughout the study? If so, when?

Introduce data here

(All data revealed again, including blood results now)

3. Do you understand the data that I have shared with you? Do you want to ask any questions about them?
4. Is it what you expected or are you surprised in any way?
5. How do you feel about it?
6. Do you think athletes should be able to use such techniques (e.g. Pre-conditioning) as a component of their training?
7. Now that the deception has been revealed. Was taking part in this study a worthwhile or interesting experience?
8. Is there anything else you would like to say or ask me?

Appendix F: Health Screening and Physical Activity Questionnaire

**UNIVERSITY OF GLASGOW
INSTITUTE OF BIOMEDICAL AND LIFE SCIENCES**

**PARTICIPANT 'S QUESTIONNAIRE AND ASSENT FORM FOR
HIGH-INTENSITY EXERCISE TESTING**

If you feel unwell on the day of a proposed test, or have been feeling poorly over the preceding day or two, DO NOT TAKE PART in a high-intensity exercise test.

The considerations which follow apply to people who are feeling well at the time.

Name:

Sex (M/F) _____ Age _____ (yrs) Height _____ (m)
Weight _____ (kg)

Exercise Lifestyle

- a) What kind(s) of exercise do you regularly do (20 + min/session)? (*Please circle*)

Number of times per average week

Walking	1	2	3	4	5
Running	1	2	3	4	5
Cycling	1	2	3	4	5
Swimming	1	2	3	4	5
Skiing	1	2	3	4	5
Rowing	1	2	3	4	5
Gymnastics	1	2	3	4	5
Martial arts	1	2	3	4	5
Tune Up	1	2	3	4	5
Pop mobility	1	2	3	4	5
Sweat Session	1	2	3	4	5
Field athletics	1	2	3	4	5
Weight training	1	2	3	4	5
Racquet sports	1	2	3	4	5
Rugby/soccer/hockey	1	2	3	4	5
Other(s) *	1	2	3	4	5

* (*Please specify*) _____

- b) How long have you been exercising at least twice/week for at least 20 min/session?

Smoking*(Please tick one)*

Never smoked _____
 Not for > 6 months _____
 Smoke <10 per day _____
 Smoke > 10 per day _____

IllnessesHave you ever had ...? *(Please circle Yes or No)*

Asthma	YES	NO
Diabetes	YES	NO
Epilepsy	YES	NO
Heart Disease	YES	NO
High Blood Pressure	YES	NO

Any other illness that could affect your safety in performing maximal exercise

YES NO

(If YES, please specify) _____**Symptoms**Have you ever had any of the following symptoms to a significant degree?
i.e., have you had to consult a physician relating to any of the following?*(Please circle Yes or No)*

Breathlessness	YES	NO
Chest Pain	YES	NO
Dizzy fits / Fainting	YES	NO
Heart Murmurs	YES	NO
Palpitations	YES	NO

Muscle or joint injury***Do you have / or have had any muscle or joint injury which could affect your safety in performing maximal exercise or strength testing or strength training?***

YES NO

Medication

Are you currently taking any medication? YES NO

*(Please circle Yes or No)**(If Yes, please specify)* _____**Signature** _____**Date** _____

Signed _____ Date _____ (Proposer of
research)

Where the proposal is from a student, the Supervisor is asked to certify the
accuracy of the above account.

Signed _____ Date _____ (Supervisor of
student)

Email the completed form to: S.Morrison@bio.gla.ac.uk

And send the signed hard copy to:

Stuart Morrison
Faculty Research Office
Faculty of Biomedical & Life Sciences
West Medical Building
University of Glasgow
Gilmorehill
Glasgow
G12 8QQ

Appendix G: Demographic Data Collection Sheet

Subject Surname _____ _____	First Name _____
Subject Number _____	Date ___/___/___

DEMOGRAPHICS

Height: _____cm Weight: _____kg

BLOOD COLLECTION

Was a blood sample taken? Yes No

Sampling Time ____:____ am/pm

Have you had a training session in the last 24 hours? Yes No

If YES, when was the most recent session? Today
Yesterday

If YES, how long did you train? _____ (hs)

If YES, what time did you finish training? ____:____ am/pm

Comments on sample collection

BLOOD PRESSURE

Have you consumed tobacco, caffeine or alcohol in the last hour? Yes
No

Measurement 1 of the right arm: _____

Measurement 2 of the right arm: _____

Measurement 3 of the right arm: _____

Mean of the right arm: _____

Comments on blood pressure measurements

Signature of Investigator _____	Date: ___/___/___
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Appendix H: Clinician Medical Examination Form**EPO STUDY SUBJECT MEDICAL ASSESSMENT****NAME:****D.O.B./AGE:****HOSPITAL NO.:****SYSTEMIC ASSESSMENT:**

CVS: CHEST PAIN / PALPITATIONS / ORTHOPNOEA

RESP: SOB / COUGH / SPUTUM / HAEMOPTYSIS

GU: DYSURIA / HAEMATURIA / FREQ.

GI: NAUSEA / VOMITING / DIARRHOEA / MALAENA / HAEMATEMESIS

NEURO: HEADACHE / WEAKNESS / ALTERED SENS. / VISUAL DIST.

OTHER:

PAST MEDICAL HISTORY

IHD MI CVA EPILEPSY ASTHMA DIABETES

THYROID JAUNDICE PREV. TB DU RHEUM ARTH

CON. HEART DISEASE CLOTTING DISORDER

OTHER:

ALLERGIES:**CURRENT MEDICATION (INCL. NON-PRESCRIPTION):****SOCIAL HISTORY:**

SMOKING: YES / NO / EX (____/DAY)

ALCOHOL:

____UNITS/WEEK

FAMILY HISTORY:**EXAMINATION:****GENERAL APPEARANCE:****CVS:**

Peripheries
JVP
Heart sounds
Oedema

RESP:

Cyanosis
Trachea
Percussion
Breath sounds

ABDO:

Masses
Bowel sounds

NEURO:

GCS / 15
Pupils
Eye movements
Limbs
Plantars

INITIAL OBSERVATIONS:

BP / H Sats RR Temp

INITIAL BLOOD RESULTS:

Hb	Na	Bil
MCV	K	AST
WCC	Cl	ALT
Neut	Bic	AlkP
Plts	Ur	GGT
	Cr	TotPr
	eGFR	Alb

Signatures:

(Consultant)

(Volunteer)

Date (D/M/Y):

Appendix I: Maximal Incremental Exercise Checklist

VO2MAX DATA COLLECTION SHEET:					
Subject:					
Date:					
Speed (km.hr-1)	Time (mins)	Time (if change) (mins)	RPE	HR	Blood Sample Present (Y/N)
6	0-3				
3	3-6				
8	6-9				
3	9-12				
10	12-15				
3	15-18				
12	18-21				
3	21-24				
13.5	24-27				
3	27-30				
15	30-33				
3	33-36				
16.5	36-39				
3	39-42				
18	42-45				
3	45-48				
19.5	48-51				
3	51-54				
21	54-57				
3	57-60				
22.5	60-63				
3	63-66				
24	66-69				
Recovery Samples	Time Post ex.(if change) (mins)			Blood Sample Present (Y/N)	
Post ex. min 1	x		x		
Post ex. min 2	x		x		
Post ex. min 5	x		x		
Post ex. min 10	x		x		
Notes / Comments:					

Subject Surname _____		First Name _____	
Subject Number _____		Date --/-- -- Time --/--	
Experimenter: Time Trial			
Name			
Time start		Temperature [°C]	
		Humidity [%]	
	Distance [m]	Comments: <div style="border: 1px solid black; height: 200px; width: 100%; margin-top: 5px;"></div>	
Time lap 1	200		
Time lap 2	400		
Time lap 3	600		
Time lap 4	800		
Time lap 5	1000		
Time lap 6	1200		
Time lap 7	1400		
Time lap 8	1600		
Time lap 9	1800		
Time lap 10	2000		
Time lap 11	2200		
Time lap 12	2400		
Time lap 13	2600		
Time lap 14	2800		
Time lap 15	3000		
	min	sec	csec
Final time			
		RPE score	

Appendix K: Chapter 4 – Participant Schedule Sample

Subject _____

	PRE							TREATMENT														POST																							
Day	-xx	-14	-11	-8	-6	-4	-1			0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	29	30	31	34	35	41	43	48	50	52	55	57	59								
Protocol planned	Md	B S U R M	T U R M	B S U R M	T U R M	V	B S U R M			E	B S U R M	B S U R M	B S U R M	E	B S U R M	B S U R M	B S U R M	B S U R M	E	B S U R M	B S U R M	E	B S U R M	B S U R M	T	B S U R M	T	V	B S U R M	B S U R M	B S U R M	B S U R M	T	T	B S U R M	V									
Scheduled dates		24 May 2011	27 May 2011	30 May 2011	01 June 2011	03 June 2011	06 June 2011			07 June 2011	09 June 2011	11 June 2011	13 June 2011	15 June 2011	17 June 2011	19 June 2011	21 June 2011	23 June 2011	25 June 2011	27 June 2011	29 June 2011	01 July 2011	03 July 2011	05 July 2011	06 July 2011	07 July 2011	08 July 2011	11 July 2011	12 July 2011	18 July 2011	20 July 2011	25 July 2011	27 July 2011	29 July 2011	01 August 2011	03 August 2011	05 August 2011								
		Tuesday	Friday	Monday	Wednesday	Friday	Monday			Tuesday	Thursday	Saturday	Monday	Wednesday	Friday	Sunday	Tuesday	Thursday	Saturday	Monday	Wednesday	Friday	Sunday	Tuesday	Wednesday	Thursday	Friday	Monday	Tuesday	Monday	Wednesday	Monday	Wednesday	Friday	Monday	Wednesday	Friday	Monday	Wednesday	Friday					
Protocol carried out																																													
Date																																													
Comments									START Iron																STOP Iron																				

Md=Medical visit B=Blood M=Metabolomics S=Saliva U=Urine R=Rebreathing (tHb) V=VO₂max E=HuEPO injection

