The importance of haemoglobin mass for cycling performance

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Bachelor of Science (Hons) in Sport and Exercise Science

University of Birmingham

A thesis submitted in total fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Exercise Physiology

Faculty of Education, Humanities, Law and Theology

Flinders University

Physiology

Australian Institute of Sport

Cycling Australia

February 23rd, 2011

Dedication

This thesis is dedicated to Professor Robert (Bob) Withers and Dr Aldo Sassi – two wonderful scientists who leave behind them a long lasting legacy for excellence in sport and exercise science.

THESIS CONTENTS

PUBLICATIONS

The work in this thesis has been presented at scientific meetings and / or published in peer reviewed journals as listed below:

International Journals

- Seasonal variation of haemoglobin mass in internationally competitive female road cyclists. **Garvican LA**, Martin DT, McDonald W, Gore CJ. Eur J Appl Physiol. 2010 May; 109(2):221-31.
- Stability of hemoglobin mass during a 6-day UCI ProTour cycling race. **Garvican LA**, Eastwood A, Martin DT, Ross ML, Gripper A, Gore CJ. Clin J Sport Med. 2010 May; 20(3):200-4
- Time course of the hemoglobin mass response to natural altitude training in elite endurance cyclists. **Garvican L**, Martin D, Quod M, Stephens B, Sassi A, Gore C. Scand J Med Sci Sports. 2010 Jun 18. [Epub ahead of print]
- Carbon monoxide uptake kinetics of arterial, venous and capillary blood during CO rebreathing. **Garvican LA**, Burge CM, Cox AJ, Clark SA, Martin DT, Gore CJ. Exp Physiol. 2010 Dec; 95(12):1156-66.
- The contribution of haemoglobin mass to increases in cycling performance induced by simulated LHTL. **Garvican LA**, Pottgiesser T, Martin DT, Schumacher YO, Barras M, Gore CJ. Eur J Appl Physiol. 2010 Nov 27. [Epub ahead of print]
- Haemoglobin mass in an anaemic endurance athlete before and after iron supplementati**on. Garvican LA**, Lobigs L, Telford R, Fallon K, Gore CJ.

Accepted for publicatio*n in International Journal of Sports Physiology and Performance, June 2011*

National / International Conferences

- **Garvican LA**, Martin DT, Clark MA, Quod MJ, Stephens B, Prommer N, Schmidt W, Impellizzeri FM, Rampinini E, Sassi A, Gore CJ The Time Course of the Erythropoietic Response to Natural Altitude Training in Elite Endurance Cyclists. *55th Annual meeting of the American College of Sports Medicine 2008*

- Martin DT, Quod MJ, **Garvican LA**, Etxebarria N, Stephens B, Impellizzeri FM, Rampinini E, Sassi A, Gore CJ Cycling Economy Following a 3-wk Natural Altitude Training Camp (~2700m) in Nationally Competitive Cyclists. *55th Annual meeting of the American College of Sports Medicine 2008*

- **Garvican LA**, Martin DT, Clark MA, Quod MJ, Stephens B, Prommer N, Schmidt W, Impellizzeri FM, Rampinini E, Sassi A, Gore CJ. Australian Cyclists living at Passo Stelvio: Physiological adaptations to a 3 week altitude training camp (~2700m). *Science of Cycling: Pre World Championships Congress, Varese 2008*

- **Garvican LA**, Martin DT, Eastwood A, Ross MLR, Abbiss CR, Gripper A, Zorzoli M, Schmidt W, Gore CJ. Haemoglobin Mass, Hct and [Hb] throughout a 6d UCI ProTour cycling race. *14th Annual Congress of the European College of Sports Science 2009*

- **Garvican LA**, Pottgiesser T, Martin DT, Schumacher YO, Fallon K, Barras M, Gore CJ. "The importance of hemoglobin mass for altitude-induced

increases in cycling performance" *15th Annual Congress of the European College of Sports Science 2010*

Awards

2010 recipient of the *Robert T Withers AIS Award* for excellence in sports physiology - Awarded for exercise physiology research that has had a substantial impact, or the potential to have a strong impact, on Australian sport.

FIGURES

TABLES

ABSTRACT

Delivery of oxygen during exercise is critically important for endurance athletes. Elite endurance athletes possess superior amounts of haemoglobin versus untrained counterparts, which enables a high rate of oxygen delivery during exercise. Haemoglobin mass (Hb_{mass}) can be measured via carbon monoxide (CO) rebreathing. The research in this thesis utilizes the recently 'optimised' method for the measurement of Hb_{mass} in elite endurance athletes, predominantly cyclists, with the aim of determining the importance of Hb_{mass} for endurance performance by: 1. examining factors which influence changes in Hb_{mass} in athletes, 2. the time course and magnitude of such changes and 3. the importance of changes in Hb_{mass} for cycling performance as reflected by maximal mean power (MMP).

The ability to accurately measure small changes in Hb_{mass} is essential; thus the first part of this thesis is concerned with the methodology underpinning Hb_{mass} measurement. Specifically, the uptake kinetics of CO during CO-rebreathing were investigated to identify potential sources of error associated with measurement in athletes. Compared with the conventional CO-rebreathing method, inhalation of a CO bolus during the optimised method resulted in faster carboxy-haemoglobin uptake, but did not shorten the time required for CO to mix completely throughout the circulation. Individual differences in circulatory mixing time and alterations of CO loss to extra-vascular compartments can confound the estimated Hb_{mass}.

The influence of stage-racing, iron supplementation, training load and altitude on Hb_{mass} was examined. Hb_{mass} remained stable throughout a 6-day pro-cycling stage race, despite plasma volume induced reductions in haemoglobin concentration and haematocrit. Iron supplementation of an anaemic female athlete had rapid and

marked effects on Hb_{mass} - increasing 49% within 2-weeks of supplementation and continuing to increase for 15-weeks. Hb_{mass} varied by ~3% throughout a competitive season in female cyclists, and was related to chronic training load. Additionally, changes in Hb_{mass} were associated with changes in MMP during training and racing.

The time course of the Hb_{mass} response during 3-weeks of natural altitude exposure (2760m) was determined, with a substantial increase (3%) in Hb_{mass} observed within 10-days. The time course was consistent with the hourly rate of increase previously documented for simulated altitude. Hb_{mass} and erythropoietin decreased on descent to sea level whilst ferritin increased (possibly indicative of neocytolysis), raising doubts as to the role of an enhanced Hb_{mass} for subsequent performance benefits at sea level. Therefore, in the final part of the thesis, the importance of hypoxia-induced increases in Hb_{mass} on cycling performance was examined. Using a unique study design, increases in Hb_{mass} induced by altitude exposure were removed, effectively 'clamping' the Hb_{mass} response. MMP_{4min} increased by ~4%, despite blocking a ~5% increase in Hb_{mass} suggesting that accelerated erythropoiesis is not the sole mechanism by which hypoxia improves performance. However, increases in Hb_{mass} appeared to influence the aerobic contribution to high-intensity exercise which may be important for subsequent high-intensity efforts. Overall this thesis confirms some existing observations regarding the influence of various external factors on Hb_{mass}, but challenges other notions regarding the importance of Hb_{mass} for traditional measures of endurance performance.

DECLARATION

I certify that this thesis does not incorporate, without acknowledgement, any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

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Laura Anne Garvican

We believe that this thesis is properly presented, conforms to the specifications for the thesis and is of sufficient standard to be worthy of examination.

....

Professor Christopher J Gore **Doctor David T Martin**

ACKNOWLEDGEMENTS

For me, this thesis encompasses far more than a series of research papers. This thesis has been an incredible journey, spanning many corners of the globe - from sea level to 2800 m, involving 21 airports, 4 continents and 8 trips around the world. What has made it even more memorable is that I was not alone, and I would like to extend my utmost appreciation to the many people and organisations who accompanied me along the way. Thanks for the ride!

To my supervisors, Chris Gore and David Martin, thank you for showing me the world of cycling, altitude training and Hb_{mass}! Thank you for the many hours of your time you have committed to me during this thesis, the thought processes, the comments, the heated debates – it was not possible without your faith, guidance and support. You have helped shape me into the sport scientist I am today. And to the late Bob Withers, thank you for having faith in me from the beginning and for ensuring that I stay true to my love of tea and the English language. I hope I have done you proud.

To all at Cycling Australia, particularly Shayne Bannan, Warren McDonald, Martin Barras, Brian Stephens and Dave McPartland, thank you for welcoming me into the cycling family and allowing me to share your passion for this amazing sport.

To Elsa Lepore, I don't know how you do what you do, but you make it easier for us all. Thank you.

To the other support staff: Beth Duryea, Christine O'Connor, Wayne Nichols and James Nitis, thank you for the good times (and the tough times) on tour. Now I know what hard work really is.

To Walter Schmidt and Nicole Prommer, thank you for ‘optimising’ the CO-rebreathing technique. Who would have thought carbon monoxide could take me so many places?

To AIS Performance Research, thank you for investing in the Sports Based PhD program. I hope you consider it as worthwhile as I.

To those who had gone before me, Tammie Ebert and Marc Quod – you could have warned me! Thank you for your wisdom, SRM lessons and the dos and don’ts. You ensured I would not be labelled an NTTA!

To all those in Physiology, past and present - what a fantastic place to work, thank you. You are the reason we are able to produce world leading research like nowhere else in the world. And to Marilyn Dickson (Maz) - everyday you continue to make me smile. Thank you for looking out for me!

To all those at Mapei, my Italian home, thank you for your hard work and hospitality. One day I will repay you. To Aldo, you will not be forgotten.

To all the Australian Cyclists, thank you for putting up with the capillary bloods, the broken cables and the nights with ‘no air.’

To Amanda Spratt, you are an inspiration and the most valuable and rewarding part of my PhD experience. I will see you at the movies.

To Megan Ross, we did it! Thank you for being a fantastic friend, but please come home soon – I’ll put the kettle on.

To Mum and Dad, thank you for the travel bug and inquisitive genes. Not the most conventional of PhD’s but it has certainly taken me places, given you something to write about in the Christmas letter and ensured another Garvican is listed on Pubmed.

And last, but of course no means least, to Shaun, thank you for your love, support and patience. We got there in the end.

Thank you.

LIST OF ABBREVIATIONS

AIS	Australian Institute of Sport
BLa	blood lactate
bpm	beats per minute
BV	blood volume
CL	confidence limit
CO	carbon monoxide
Δ	change
∑7	sum of seven (skinfold thicknesses)
EPO	erythropoietin
g	gram
Hb	haemoglobin
[Hb]	haemoglobin concentration
Hb_{mass}	haemoglobin mass
HbCO	carboxy-haemoglobin
Hct	haematocrit
HIF-1α	hypoxic inducible factor 1-alpha
HR	heart rate
kg	kilogram
L	litre
$L.min^{-1}$	litres per minute
LHTL	Live high: Train Low
Mb	myoglobin
$ml.kg.min^{-1}$	millilitres per kilogram of body mass per minute

$mmol.L^{-1}$	millimole per litre
MMP	maximum mean power output
min	minute
PV	plasma volume
Retics	reticulocytes
RBC	red blood cell
RCV	red cell volume
RPE	rating of perceived exertion
rpm	revolutions per minute
s	second
SD	standard deviation
SL	sea level
SRM	Schoeberer Resistance Measurement
SWC	smallest worthwhile change
TE	typical error
T_{lim}	ride time to exhaustion
T_{mix}	circulatory mixing time
VO_2peak	peak oxygen consumption
VO_2max	maximal oxygen consumption
vs.	versus
W	watt
$W.kg^{-1}$	watts per kilogram of body mass (power to mass ratio)
U23	Under 23 years of age
UCI	Union Cycliste Internationale

Introduction

Road cycling is an endurance sport, with success often accompanied by superior endurance physiology (Coyle, Coggan et al. 1988). Years of endurance training, combined with a favourable genetic disposition, result in a series of physiological adaptations, all designed to maximise endurance performance by increasing the amount of oxygen (O_2) which can be delivered and utilised by working muscles (Hawley and Stepto 2001). These adaptations can be broadly divided into two – those that occur *peripherally* such as increased vascularisation, increased mitochondrial density and increased enzyme activity, which enable O_2 to be extracted and metabolised at a high rate; and those that occur *centrally*, involving the cardiovascular system, and affecting the rate at which O_2 can be delivered around the body. Endurance training enhances this system via adaptations to heart contractility and function, blood volume and O_2 carrying capacity.

When both the peripheral and central systems are highly adapted, as in the case of an elite endurance athlete, high rates of work can be achieved for extended durations. The maximal amount of O_2 which can be consumed during exercise, defined as the maximal aerobic power (VO_2max), is dependent on both the delivery and utilisation of O_2, and as such will be limited by whichever system is least adapted In elite endurance athletes it is generally believed that VO_2max is centrally limited, that is, by the rate of O_2 delivery (Wagner 1996). Therefore, alterations to the O_2 transport system have the potential to enhance VO_2max further (Schmidt and Prommer 2010).

Haemoglobin (Hb), the O_2 carrying protein contained in red blood cells, is therefore critically important for endurance exercise. Total haemoglobin mass (Hb_{mass}) refers

to the total amount of Hb within the body, irrespective of compartmental fluid volumes. Elite endurance athletes are characterised by a high Hb_{mass}, and indeed, there is a strong relationship between Hb_{mass} and VO_2max. A 1 g increase in Hb_{mass} results in an increase in VO_2max of approximately 4 $ml.min^{-1}$ (Schmidt and Prommer 2010). It follows, that Hb_{mass} may also be closely related to endurance *performance;* and it is for this reason, that the goal of the majority of blood doping practices in endurance sport is to increase Hb_{mass}, either by inducing erythropoiesis (e.g. recombinant human erythropoietin administration) or via direct infusion of whole blood. Such practices are banned by the World Anti-Doping Agency (WADA 2011) due to their overwhelming affect on O_2 transport.

Interestingly, whilst the magnitude of changes to Hb_{mass} incurred though blood-doping has a significant impact on endurance performance (Gledhill 1982), the Hb_{mass}-performance relationship under physiological conditions is not as clearly defined as that for VO_2max. What is the affect of a 10 g increase in Hb_{mass} on individual pursuit time or on the ability to sustain a high power output whilst climbing? Much of the difficulty in attempting to answer this question depends upon the ability to define and measure endurance performance; since performance itself can be influenced by a multitude of factors. In addition, the plasticity and scope for alterations to Hb_{mass} via natural means remains to be determined. Therefore the primary aim of the research contained in this thesis is to extend previous investigations of Hb_{mass} in elite endurance athletes, with a specific focus on road cycling performance. Specifically, this thesis aims to quantify the stability and plasticity of Hb_{mass} in elite cyclists as well as to determine the importance of any changes for subsequent cycling performance.

The ability to accurately measure small changes in Hb_{mass} is critically important for longitudinal monitoring; thus, the first part of this thesis is concerned with the methodology underpinning the measurement of Hb_{mass}. In recent years, measurement of Hb_{mass} via carbon monoxide (CO) rebreathing has become a popular research tool, largely due to the recent "optimisation" of Burge and Skinner's method (1995) by Schmidt and Prommer in 2005. The optimised methodology greatly reduces the time required to complete a single test; which, combined with its relative portability has enabled measurement of Hb_{mass} in elite athletes on a more regular basis. In addition to its use in a research setting, the optimised rebreathing method has been proposed as a potential tool for doping detection (Prommer, Sottas et al. 2008). However, in order to guarantee successful application in either instance, the method must be shown to be both accurate and reliable – being able to detect small changes in Hb_{mass} with a low degree of error. It is with this respect, that the methods of Burge & Skinner (1995) and Schmidt & Prommer (2005) were critically compared. Specifically, the uptake kinetics of CO into vascular and non-vascular compartments during the above two protocols of CO-rebreathing were investigated in order to identify the potential sources of error associated with measurement in athletic populations, and therein to make any modifications to the method if required. The data presented serve to demonstrate the validity of the Schmidt & Prommer (2005) method and its suitability for the measurement of small but worthwhile changes in Hb_{mass} of elite endurance athletes.

Owing to the methodology previously available, there is a lack of longitudinal data obtained from elite endurance athletes, and as a result, the stability and plasticity of Hb_{mass} in this population has not been clearly defined. This thesis presents data showing the effect of periods of intense exercise (stage-racing), natural altitude

exposure, and iron supplementation on Hb_{mass} of elite endurance athletes. These data are important for an overall understanding of natural variations in Hb_{mass} which in turn, is an essential prerequisite for its potential application in the fight against blood doping in endurance sport. In addition, understanding the natural rhythms of the Hb_{mass} protein may assist in designing training programs for optimal aerobic adaptation. In this context, longitudinal data are presented which detail the variability of Hb_{mass} throughout a 6-month training and competition season in internationally competitive female cyclists. Changes in Hb_{mass} are analysed in relation to changes in training load and indices of performance, in an attempt to determine the relationship between Hb_{mass} and performance during training and racing.

Based on the current literature, the greatest potential for increasing Hb_{mass} by natural means in a healthy athlete arises from hypoxic exposure. Hypoxia, be it simulated or terrestrial altitude, is a popular training strategy amongst endurance athletes aiming to improve their performance at sea level. The mechanisms responsible for performance enhancements are still debated; however the most popular theory is that increases in VO_2max arising from an enhanced Hb_{mass} are responsible. Therefore, in the final part of the thesis, the importance of hypoxia-induced increases in Hb_{mass} on cycling performance will be examined. Specifically, using a unique study design, the role of Hb_{mass} following simulated-altitude training will be isolated in an attempt to establish the relative contribution of Hb_{mass} vs. non-haematological adaptations for cycling performance.

Overall, this thesis provides insight into the importance of Hb_{mass} for cycling performance – information which may be useful in the optimisation of athletic performance, as well as in the fight against blood – doping in endurance sport.

AIMS

The primary aim of this thesis was to determine the importance of Hb_{mass} for endurance performance by addressing the following key questions:

1. What factors influence changes in Hb_{mass} in elite endurance athletes (primarily cyclists), and what is the time course and magnitude of such changes?
2. How important are changes in Hb_{mass} for cycling performance as reflected by maximal mean power (MMP) for a fixed duration?

Specifically, the aims of each of the studies contained in this thesis are outlined below:

Study 1: CO uptake kinetics of arterial, venous and capillary blood during CO-rebreathing

1. Compare the uptake and distribution of CO throughout the circulatory system during two different methods of CO-rebreathing.
2. Determine the impact of differences in circulatory mixing time (t_{mix}), CO diffusion to myglobin (Mb), and CO wash-out following rebreathing on Hb_{mass}.

Study 2: Stability of haemoglobin mass during a 6 day UCI ProTour cycling race

1. Quantify the stability of Hb_{mass} throughout a UCI ProTour event in professional cyclists.
2. Assess the reliability of the method in a race setting and its viability as a potential anti-doping detection tool.

Study 3: Case study - Haemoglobin mass in an anaemic female middle –distance runner before and after iron supplementation

1. Document changes in Hb_{mass} following iron supplementation in a female endurance athlete diagnosed with iron-deficient anaemia.

Study 4: Time course of the haemoglobin mass response to natural altitude training in elite endurance cyclists

1. Determine the time course of Hb_{mass} changes to natural moderate altitude during and for 10 days after a 3 week altitude training camp in elite endurance athletes.

Study 5: Seasonal variation of haemoglobin mass in internationally competitive female road cyclists

1. Quantify the seasonal variation of Hb_{mass} in a group of internationally-competitive female endurance cyclists.
2. Examine the relationship between changes in training load and changes in Hb_{mass}.
3. Model the relationship between changes in Hb_{mass} with changes in road cycling performance, estimated from mean maximal power measured during training or racing.

Study 6: The importance of haemoglobin mass for increases in cycling performance induced by simulated LHTL

1. Investigate the importance of increases in Hb_{mass} for cycling performance following simulated normobaric LHTL, by removing any Hb_{mass} gained

throughout the period of hypoxic exposure and, thereby, effectively "clamping" the Hb_{mass} response.