

Beyond the $\dot{V}O_{2\max}$ plateau: a new approach for measuring maximal oxygen uptake

By

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Declaration

I, Fernando Gabe Beltrami, do hereby declare that this thesis is my own effort and work. Where other sources of information have been used, they have been properly acknowledged. The work presented here has not been submitted in any previous application for higher a degree. The study presented in **Chapter 4** of this thesis has already been published in a peer-reviewed journal, and the appropriate changes have been made to adapt the manuscript to the styling of this thesis. My participation, as well as that from the secondary authors from each chapter, is listed below:

Chapter 2

F.G. Beltrami, C. Froyd, A. Mamen and T.D. Noakes (2010) *The validity of the Moxus Modular Metabolic System during incremental exercise tests: impacts on detection of small changes in oxygen consumption.*

I personally devised the study design, drafted the research proposal and applied for ethical clearance. I was responsible for collecting the data during the study, with the assistance of C. Froyd and A. Mamen. I was responsible for analyzing the data and personally drafted the chapter, which was reviewed by the other authors.

Chapter 3

F.G. Beltrami, D. Wong, and T.D. Noakes (2011) *High prevalence of false-positive plateau phenomena during $\dot{V}O_{2max}$ testing in adolescents.*

I personally devised the study design, drafted the research proposal and applied for ethical clearance. I was responsible for reviewing the current literature on the topic and for deciding which mathematical analysis would be used in the data set. I personally analyzed all the data sets, which were provided by Dr. D. Wong. I personally drafted the chapter, which was reviewed by the other authors.

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F.G. Beltrami, C. Froyd, A.R. Mauger, A.J. Metcalfe, F. Marino and T.D. Noakes (2012) *Conventional testing methods produce sub-maximal values of maximum oxygen consumption in humans. British Journal of Sports Medicine, 45:85-90.*

I personally devised the study design, drafted the research proposal and applied for ethical clearance. I was responsible for reviewing the current literature on the topic and for collecting the data for the study, in which I was assisted by C. Froyd, Dr. A.R. Mauger, A.J. Metcalfe and Dr. F. Marino. I personally analyzed all the data sets and drafted the published manuscript, which was reviewed by the other authors.

Chapter 5

F.G. Beltrami and T.D. Noakes (2012) *Conventional testing methods produce sub-maximal values of maximum oxygen consumption in elite runners.*

I personally devised the study design and drafted the research proposal and applied for ethical clearance. I was responsible for reviewing the current literature on the topic and for collecting the data for the study. I personally analyzed all the data sets and drafted the chapter, which was reviewed by my supervisor.

Chapter 6

F.G. Beltrami, A.R. Mauger, C. Froyd, A.J. Metcalfe and T.D. Noakes (2012) *Comparison of physiological responses and muscle activity during incremental and decremental cycling exercise.*

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Chapter 7

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I personally devised the study design, drafted the research proposal and applied for ethical clearance, in which I was assisted by my co-supervisor, Dr. P.D. Wagner. I was responsible for reviewing the current literature on the topic and for collecting the data for the study, in which I was assisted by T. Simonson, J. Fine, H. Wagner and Dr. P.D. Wagner. I personally analyzed all the data sets and drafted the chapter, which was reviewed by the other authors.

All co-authors have allowed Fernando Gabe Beltrami to use the above-mentioned works for his degree of Doctor of Philosophy in Exercise Science at the University of Cape Town, Department of Human Biology.

I hereby grant the University of Cape Town free license to reproduce the above thesis in whole or in part, for the purpose of research.

This publication based thesis is presented for examination in fulfillment of the requirements for the degree of Doctor of Philosophy in Exercise Science at the University of Cape Town.

Signature:

Signed by candidate

Cape Town, 23rd of March 2013

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University of Cape Town

Thesis Abstract

University of Cape Town

Beyond the $\dot{V}O_{2\max}$ plateau: a new approach for measuring maximal uptake

Fernando Gabe Beltrami

Maximal oxygen consumption ($\dot{V}O_{2\max}$) is typically defined by the presence of the “plateau phenomenon” during incremental exercise tests, in which O_2 consumption levels-off in spite of increases in workload. This is thought to reflect a limitation in the capacity of the heart to pump O_2 -enriched blood to the exercising limbs. Some researchers however question not only the biological validity of the “plateau phenomenon”, but also mathematical and methodological issues, but whether O_2 delivery to the exercising muscles indeed sets the upper limit for $\dot{V}O_{2\max}$. This thesis aimed to address some of these controversies.

While **Chapters 2 and 3** studied the theoretical and practical issues that arise in relation to the measurement of $\dot{V}O_{2\max}$; in **Chapters 4-6** the $\dot{V}O_{2\max}$ measured during an incremental exercise test was compared to that achieved during a new protocol, based on decremental workloads. In **Chapter 7** the different terms used to describe $\dot{V}O_{2\max}$ measured during an incremental and a decremental exercise tests were compared.

Results from **Chapters 2 and 3** indicated that although simple in concept, the “plateau phenomenon” is in fact difficult to measure, partly because modern gas analyzers are not accurate enough to detect the small changes in O_2 consumption that define the plateau and partly because the prevalence of the plateau phenomenon greatly varies according to some mathematical and methodological choices necessary to define it. Furthermore in **Chapters 4-6** it was consistently shown that the

decremental protocol produces higher $\dot{V}O_{2\max}$ values than an incremental test even in the presence of the “plateau phenomenon” during the latter. In **Chapter 7**, it was demonstrated that the differences in $\dot{V}O_{2\max}$ between the two protocols can be explained by higher cardiac output and higher O_2 extraction by the exercising limbs, contrary to traditional views on the limitations of $\dot{V}O_{2\max}$.

Taken together, the findings of this thesis suggest that the biological significance attributed to the “plateau phenomenon” as an indicator of a true physiological symbol should be re-investigated. The applicability of the decremental exercise test as a substitute for the traditional incremental test approach however still needs to be evaluated.

List of common nomenclature

Abbreviation	Description
$\Delta \dot{V}O_2$	Changes in oxygen uptake
[La]	Blood lactate concentration
ATP	Adenosine triphosphate
BF	<i>Biceps femoralis</i>
BTPS	Body temperature, ambient pressure, saturated
CaO ₂ / CvO ₂	arterial (a) or venous (v) concentration of oxygen
CO ₂	Carbon dioxide
DBM	Douglas bag method
DEC	Decremental exercise test
EMG	electromyography
FeCO ₂	Fraction of expired carbon dioxide
FeO ₂	Fraction of expired oxygen
GM	<i>Gastrocnemius Medialis</i>
Hb	Hemoglobin
HR	Heart rate

INC	Incremental exercise test
LOA	Limits of agréments
O ₂	Oxygen
PaO ₂ / PvO ₂	arterial (a) or venous (v) Partial pressure of oxygen
PaCO ₂ / PvCO ₂	arterial (a) or venous (v) Partial pressure of carbon dioxide
$\dot{Q}$	Cardiac output
$\dot{Q}_{\text{LEG}}$	Leg blood flow
RER	Respiratory exchange ratio
RF	<i>Vastus Medialis</i>
STPD	Standard temperature and pressure, dry
SaO ₂ / SvO ₂	arterial (a) or venous (v) Saturation of oxygen
SV	Stroke volume
TEM	Technical error of measurement
V. $\dot{V}O_{2\text{max}}$	Speed at maximal oxygen uptake
$\dot{V}_E$	Pulmonar ventilation
VER	Verification test
VL	<i>Vastus lateralis</i>
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_{2\text{max}}$	Maximal oxygen uptake
$\dot{V}O_{2\text{peak}}$	Peak oxygen uptake
V-TEST	Exercise test with V-shaped workload profile

Chapter I

Testing for maximal oxygen uptake

An Introduction

INTRODUCTION

Oxygen consumption ($\dot{V}O_2$) represents the process by which an organism extracts oxygen (O_2) from the ambient air and transports it into the mitochondria where it has an important role in the synthesis of adenosine triphosphate (ATP) for energy production through oxidative phosphorylation (McArdle *et al.*, 2010). The maximal rate at which an organism is able to sustain this process is referred to as $\dot{V}O_{2max}$. In other words, $\dot{V}O_{2max}$ measures the ability of an individual to oxidize metabolic substrates for energy production (Rowell, 1974). Because it represents the complete integration of the respiratory, circulatory and muscular systems, $\dot{V}O_{2max}$ is believed to be a measure of cardiovascular function and fitness (Mitchell & Blomqvist, 1971; Arena & Sietsema, 2011), and has a range of applications, from predicting athletic ability (Jacobs *et al.*, 2011) to assessing cardiovascular health and mortality risk (Myers *et al.*, 2002).

The term $\dot{V}O_{2max}$ was originally used to describe the “true” maximal capacity to use oxygen during exercise in optimal conditions, which included employing at least 50% of the muscle mass during exercise (Rowell, 1974). The term $\dot{V}O_{2peak}$ was used to describe the highest $\dot{V}O_2$ achievable in a specific (but not truly maximal) situation, for instance during maximal arm ergometry (Rowell, 1974). Currently however $\dot{V}O_{2max}$ is more often used to refer to the specific situation where at the end of maximal incremental exercise test $\dot{V}O_2$ levels-off and reaches a ceiling despite further increases in work rate. If such pre-conditions are not met, and $\dot{V}O_2$ reaches its maximum level without levelling-off, the term $\dot{V}O_{2peak}$ has been suggested as more appropriate (Meyer *et al.*, 2005). Whether $\dot{V}O_{2max}$ and $\dot{V}O_{2peak}$ actually represent two distinct biological events is still debated (Alpert, 1992; Day *et al.*, 2003; Doherty *et al.*, 2003), and although some authors clearly distinguish between $\dot{V}O_{2max}$ and $\dot{V}O_{2peak}$ (Meyer *et al.*, 2005; Arena & Sietsema, 2011) there is compelling evidence from supra-maximal tests suggesting that $\dot{V}O_{2peak}$ is indeed equal in value – if not in concept – to $\dot{V}O_{2max}$ in the vast majority of individuals (Day *et al.*, 2003; Carter *et al.*, 2006; Brink-Elfegoun *et al.*,

2007b; Foster *et al.*, 2007; Tucker & Noakes, 2009; Midgley & Carroll, 2009; Elliott *et al.*, 2013).

Origin of the concept

The very first description of an exercise test of increasing intensity in which a plateau in oxygen consumption despite increasing work rates was noted can be traced to the work of Liljestrand & Stenström (1920). They attributed the phenomenon to increased economy in running efficiency with increasing speed. Three years later, $\dot{V}O_{2\max}$ as it is understood today was described in a series of papers by Nobel laureate AV Hill and colleagues (1923; 1924a). The authors concluded that $\dot{V}O_2$ rose linearly with increasing running speeds, until an intensity was reached where “however much the speed be increased beyond this point, no further increase in oxygen intake can occur: the heart, lungs, circulation and the diffusion of oxygen to the active muscle-fibers have attained their maximum activity”.

It was the authors’ belief that at very high intensities of exercise, oxygen tension in the coronary blood supply would fall due to the limited transit time of the blood in the capillaries of the lungs, which would lead to incomplete saturation of red blood cell hemoglobin. This would work as a self-regulating process, in which a reduction in the O_2 supply to the heart would decrease the output of the heart itself, thereby increasing the transit time of the blood in the lungs and restoring oxygen tension (Hill & Lupton, 1923). Under this paradigm, it was a logical conclusion that $\dot{V}O_{2\max}$ failed to increase during exercise “not because more oxygen is not required, but because the limiting capacity of the circulatory-respiratory system has been attained” (Hill *et al.*, 1924a). Accordingly, Hill and colleagues (1924a) concluded that “a heart, adequate in every other way, might fail to allow its owner to undertake severe continued effort, simply because of the imperfect arrangements of its own supply of blood”.

An interesting prediction from the Hill model was that breathing oxygen rich mixtures would in turn raise the O_2 tension of arterial blood, thus increasing cardiac output ($\dot{Q}$) and $\dot{V}O_{2\max}$ proportionately, until arterial desaturation again prevented $\dot{Q}$

from rising further. Thus “we suggest that in the body (either in the heart muscle itself or the central nervous system), there is some mechanism which causes a slowing of the circulation as soon as a serious degree of unsaturation occurs, and *vice versa*” (Hill *et al.*, 1924a).

At the time, it was understood that this “exhaustion” of the heart capacity during severe exercise was of the same nature as in pathological conditions (Clark-Kennedy & Owen, 1927), and the work of Clark-Kennedy & Owen (1927) was one of the few to question this assumption. The authors noted the absence of symptoms following maximal exercise, and more importantly that the lack of edema of the extremities of the lower limbs during maximal exercise meant that the heart was fully able to cope with the venous return at all times, as opposed to what should happen if there was ever a primary failure in the circulatory system. Thus they concluded that “an essential difference exists between the symptoms of extreme muscular exhaustion in the normal man, and those of congestive cardiac failure” (Clark-Kennedy & Owen, 1927). An alternative hypothesis was proposed, in which although the heart could be unable to supply enough O₂-enriched blood to active tissues, it was always able to cope with venous return (and therefore able to supply itself). The limitation of voluntary effort in turn would be set by “eventual failure of the cardio-respiratory system as a whole; and simultaneous failure in oxygen intake and carbon dioxide output, to prevent rise of hydrogen-ion concentration to the point where at which voluntary effort ceases” (Clark-Kennedy & Owen, 1927). Interestingly, the work of Clark-Kennedy & Owen, published only three years after the series of studies by AV Hill and colleagues (1923; 1924a; 1924b; 1924c) was ignored by subsequent generations of exercise scientists and to this day is still conveniently ignored in the published literature by proponents of the cardiovascular model (Noakes & St Clair Gibson, 2004).

Techniques for measuring $\dot{V}O_2$

Due to technological limitations, the first works to measure $\dot{V}O_2$ during exercise used very laborious and time consuming methods, to the extent that a single exercise test needed to be carried out over many days (Taylor *et al.*, 1955). In the early experiments of Hill and colleagues (1923; 1924a), the participants ran around a track

carrying meteorological balloons which were used to collect the expired air, now called the Douglas Bags. The air stored in these bags was later analyzed for O₂ and CO₂ fractions using the techniques described by Haldane (1898). Modern improvements in the techniques for measuring $\dot{V}O_2$ during exercise include the use of mass spectrometers, flow meters and metabolic carts (Wilmore *et al.*, 1976) that allow the measurement of pulmonary ventilation ($\dot{V}_E$) and the fractions of expired O₂ and CO₂ on a breath-to-breath basis (Auchincloss, Jr. *et al.*, 1971). In spite of all the latest technological advances, the use of the Douglas Bags is still considered the gold standard technique for measuring $\dot{V}O_2$. Analysis of gas fractions however are routinely performed using automated analyzers instead of the Haldane procedures, even when using the Douglas Bags (Bassett *et al.*, 2001; Duffield *et al.*, 2004; Faulkner & Eston, 2007).

Although the advent of fully automated gas analyzers became widespread in exercise physiology and clinical testing, some researchers have expressed their concern over the use of such equipment (Howley *et al.*, 1995; Macfarlane, 2001), mostly because this equipment is seen as a “black-box”, so that experimenters are often unaware of the calculations and assumptions made by the equipment software when calculating gas exchange data. Another particular reason for concern arising from studies using high-resolution gas analyzers to measure $\dot{V}O_2$ is that the smaller the sampling time the larger the room for measurement error (Howley *et al.*, 1995). Working on breath-by-breath conditions for example might lead to incorrect temporal alignment between the gas flow and gas fraction measurements (Hodges *et al.*, 2005), a problem that does not exist when using the Douglas Bag system. Therefore it is extremely important that all gas analyzers used in research and clinical settings are tested for their validity and reproducibility. Although there is a large body of literature in this area, most studies performed so far limited themselves to low exercise intensities and the use of long averaging intervals (Bassett *et al.*, 2001; Macfarlane & Wu, 2012), which does not reflect conditions present during maximal exercise testing (Foss & Hallen, 2004). Moreover, in biological variables, errors tend to be heteroscedastic so that their absolute size increases in proportion with the value of the measurement itself (Nevill & Atkinson, 1997), which further emphasizes the need for equipment to

be tested over the full range of values normally found during exercise testing. The measurement of $\dot{V}O_{2\max}$ and the “plateau phenomenon” (see section below) demand very high precision from analyzers working at very high values of pulmonary ventilation, and it is currently not known whether such equipment is able to meet these requirements.

Accordingly, **Chapter 2** of this thesis aims to validate an automated gas analyzer during high-intensity exercise when using short data sampling intervals. The resulting errors in measurement are discussed with those found in the literature and the practical implications of these measurement errors when determining $\dot{V}O_{2\max}$ are also explored.

$\dot{V}O_{2\max}$ and the plateau phenomenon

The influential works of Taylor and colleagues (1955) and Mitchell and colleagues (1958) were the first to describe the “plateau phenomenon” during standardized incremental exercise tests, which afterwards became the norm for defining $\dot{V}O_{2\max}$ (Levine, 2008). Taylor *et al.* (1955) developed a test protocol consisting of a series of discontinuous 3-minute running intervals, during which speed was fixed at 7 miles per hour ($\sim 11.3 \text{ km}\cdot\text{h}^{-1}$) but gradient increased by 2.5% at each interval. This procedure was repeated until the participant could not complete a 3-minute stage at the given workload. From this test, the authors reported that the 2.5% change in gradient produced an average change in $\dot{V}O_2$ of $299.3 \pm 86.5 \text{ ml}\cdot\text{min}^{-1}$ (range 159 – 470 $\text{ml}\cdot\text{min}^{-1}$) during sub-maximal stages, and therefore decided that “if one insists that two consecutive determinations separated by a grade of 2.5% differed by less than 150 cc/min or 2.1 cc/kg, there is a small chance of making an error in deciding that the maximal oxygen intake had been reached” (Taylor *et al.*, 1955). This study formed the basis of what today is defined as the $\dot{V}O_{2\max}$, which is the maximal value for $\dot{V}O_2$ measured during an incremental exercise test that ends with the “plateau phenomenon”.

Interestingly, the work of Mitchell *et al.* (1958), published not long after the study of Taylor and colleagues, found a much smaller “normal” change in $\dot{V}O_2$ during

sub-maximal workloads ($142 \pm 44 \text{ ml}\cdot\text{min}^{-1}$), in spite of using similar change in workload between exercise bouts. The authors decided that changes in $\dot{V}\text{O}_2$ equivalent to the expected changes less two times its standard deviation ($142 - 44 - 44 = 54 \text{ ml}\cdot\text{min}^{-1}$) would be representative of the “plateau phenomenon”, clearly a much more strict criterion for the levelling-off of $\dot{V}\text{O}_2$. Nowhere in these two works however was there mention that these criteria used to define the “plateau phenomenon” should be adopted by other investigators. In fact, in subsequent years from these authors on how to appropriately measure $\dot{V}\text{O}_{2\text{max}}$, although it was emphasized that a plateau in $\dot{V}\text{O}_2$ should be present during maximal exercise tests, there were no recommendations on how this plateau should be quantified (Wyndham *et al.*, 1959; Taylor *et al.*, 1963; Mitchell & Blomqvist, 1971).

Nonetheless the plateau criterion described by Taylor *et al.* (1955) gained widespread popularity and currently authors use the “change smaller than $2.1 \text{ ml}\cdot\text{kg}\cdot\text{min}^{-1}$ or $150 \text{ ml}\cdot\text{min}^{-1}$ ” criterion to define the presence of the plateau phenomenon, regardless of the rate of increments in workload used during the exercise protocol (Freedson *et al.*, 1986; Stachenfeld *et al.*, 1992; Gordon *et al.*, 2011; Gordon *et al.*, 2012). This is a serious issue since it can lead to an over- or underestimation of the plateau phenomenon during exercise tests (Howley *et al.*, 1995). Additionally, not only the same criterion is used for many different exercise protocols, today a large variety of plateau criteria to define $\dot{V}\text{O}_{2\text{max}}$ are available in the literature (Noakes & St Clair Gibson, 2004), and there is little agreement on which method should be preferred (Astorino *et al.*, 2000; Astorino, 2009).

Finally, even the biological meaning of the “plateau phenomenon” has been questioned. Some researchers argue that it might actually be a measurement artifact rather than a true physiological event, owing to the fact that $\dot{V}\text{O}_2$ “plateau phenomena” can be found at sub-maximal $\dot{V}\text{O}_2$ values during incremental exercise tests (Myers *et al.*, 1989; Myers *et al.*, 1990). It has also been demonstrated that participants who either do or do not exhibit the plateau phenomenon during exercise tests have similar physiological characteristics (Doherty *et al.*, 2003; Astorino *et al.*, 2005), although some evidence exists showing that individuals who exhibit the plateau phenomenon are better trained and have greater anaerobic capacity (Lacour *et al.*,

2007). Furthermore, manipulating the data sampling interval (Astorino, 2009) or performing “priming” bouts of heavy exercise prior to an incremental test (Gordon *et al.*, 2011) also greatly affect the prevalence of the “plateau phenomenon”.

In order to better understand how factors related to the methodological choices used to define a plateau in $\dot{V}O_{2\max}$ affect the prevalence of the “plateau phenomenon” during exercise testing, in **Chapter 3** of this thesis a data set comprising 63 maximal exercise tests is analyzed using six combinations of plateau criteria with three different sampling intervals each for the $\dot{V}O_2$ data. The effects each of these methodological choices is then discussed with regards to their assumptions, implications and limitations.

Classical views on the limitations of $\dot{V}O_{2\max}$

The original model developed nearly 90 years ago by Hill and colleagues (1924a), which proposes that $\dot{V}O_{2\max}$ is ultimately limited by the heart’s capacity to deliver O_2 -enriched blood to the exercising muscles, still prevails (Bassett & Howley, 1997; Levine, 2008), although it has been slightly modified over time. The claim that the heart “fails” during maximal exercise has been abandoned (Brink-Elfegoun *et al.*, 2007b; Spurway *et al.*, 2012), and the prediction that a “governor” would regulate cardiac output by sensing the O_2 tension in the coronary arteries has also been dismissed. Although coronary flow is rapidly affected by changes in arterial O_2 tension (Hilton & Eichholtz, 1925), numerous studies have shown that breathing hyperoxic mixtures increases $\dot{V}O_{2\max}$ without however affecting cardiac output ($\dot{Q}$) (Knight *et al.*, 1993; Peltonen *et al.*, 2001), as originally predicted by AV Hill.

Because arterial O_2 concentration hardly falls during maximal exercise (Mortensen *et al.*, 2005; Mortensen *et al.*, 2008; Duncker & Bache, 2008), the limits of O_2 delivery to the exercising muscles are usually thought to fall upon $\dot{Q}$, specifically stroke volume (SV), especially since manipulating heart rate (HR) alone does not affect $\dot{Q}$ (Bada *et al.*, 2012). Evidence supporting the idea that it is SV which limits $\dot{V}O_{2\max}$ comes mostly from animal studies which have shown that pericardiectomy in dogs (Stray-Gundersen *et al.*, 1986) and pigs (Hammond *et al.*, 1992) increases SV during

maximal exercise, and $\dot{V}O_{2\max}$ rises proportionally (Stray-Gundersen *et al.*, 1986; Hammond *et al.*, 1992). Furthermore it has been demonstrated (Astrand & Saltin, 1961; Brink-Elfegoun *et al.*, 2007a) that while upper limbs exercise can elicit up to 70% of leg $\dot{V}O_{2\max}$, performing combined arm and leg exercise does not achieve higher values than leg exercise alone (Bassett & Howley, 1997). In fact, exercise with both upper and lower limbs tends to reduce blood flow to the lower limbs, while maintaining blood pressure (by increased vasoconstriction), suggesting a central cardiac limitation (Secher & Volianitis, 2006) in maintaining blood flow. A similar situation is seen when one-leg exercise is compared to two-legs exercise (Saltin, 1985), which has been interpreted as definitive proof of central limitation to $\dot{V}O_{2\max}$ during whole-body exercise (Bassett & Howley, 2000). It has also been suggested that SV must be the limiting factor for $\dot{V}O_{2\max}$ because muscle O_2 extraction is already very close to 100% (Bassett & Howley, 1997). This latter claim however is not supported by recent studies (Calbet *et al.*, 2003; Mortensen *et al.*, 2005; Mortensen *et al.*, 2008), which show that figures for O_2 extraction during maximal exercise in fact range between 85-90%.

In summary, it is now widely believed that the heart reaches a physical limitation (possibly imposed by the size of the pericardium) in its capacity to increase SV and thus $\dot{Q}$ and O_2 delivery to the exercising muscles. This view is compatible with a range of experiments manipulating blood flow and arterial O_2 concentration during maximal exercise (Bassett & Howley, 1997; Calbet *et al.*, 2004; Mortensen *et al.*, 2005; Calbet *et al.*, 2006; Calbet *et al.*, 2007; Mortensen *et al.*, 2008; Bada *et al.*, 2012).

Challenging views on the limitations of $\dot{V}O_{2\max}$

The conclusion that in healthy subjects the $\dot{V}O_{2\max}$ plateau is caused by a plateau in $\dot{Q}$ is, however, not universally accepted (Wagner, 2006; Spurway *et al.*, 2012). Wagner (1982; 1992; 1993; 2006) points out that the chain for O_2 transport from the ambient air into the mitochondria can be seen as an *in series* system in which each step has potentially the same importance in determining O_2 transport (Spurway *et al.*, 2012). In this process, the diffusion of O_2 from the blood and into the mitochondria has been suggested to play a significant role in determining $\dot{V}O_{2\max}$ both

experimentally (Roca *et al.*, 1989; Roca *et al.*, 1992; Richardson *et al.*, 1995) and theoretically (Wagner, 1992; Wagner, 1993; Wagner, 1996). Further evidence of the importance of the muscle diffusing capacity comes from studies in which higher $\dot{V}O_{2\max}$ values were achieved without altering O_2 delivery to the muscles, either by adjusting blood flow and O_2 partial pressure (Hogan *et al.*, 1989) or by changing the O_2 affinity of hemoglobin ([Hb]) (Richardson *et al.*, 1998) through allosteric modifications. Thus it has been demonstrated that by decreasing flow and increasing O_2 pressure to match O_2 delivery (Hogan *et al.*, 1989) or by shifting the [Hb]- O_2 dissociation curve to the right without however changing $\dot{Q}$ or O_2 partial pressure (PO_2) (Richardson *et al.*, 1998) it is possible to increase O_2 extraction and also $\dot{V}O_{2\max}$ without any changes in O_2 delivery.

Alternatively, other researchers question the very existence of a “true” – biological – plateau in $\dot{V}O_{2\max}$ during incremental exercise tests. Even though some earlier studies had already questioned the validity of the “ $\dot{V}O_{2\max}$ plateau” concept (Froelicher *et al.*, 1974; Myers *et al.*, 1989; Myers *et al.*, 1990), Noakes (1988; 1997) was the first to directly challenge this concept, enumerating several methodological or logical flaws in the experiments and arguments developed by Hill in his series of works on $\dot{V}O_{2\max}$ (Hill & Lupton, 1923; Hill *et al.*, 1924a; Hill *et al.*, 1924b; Hill *et al.*, 1924c). Noakes argued that the plateau in $\dot{V}O_{2\max}$ during the early studies (Taylor *et al.*, 1955; Mitchell *et al.*, 1958), which used discontinuous protocols, could be the result from increased mechanical efficiency from running on a treadmill on multiple occasions (Froelicher *et al.*, 1974) or simply the fact that higher intensities were not tested (Noakes, 1988). Furthermore these studies typically measured $\dot{V}O_2$ only once at each intensity level, and $\dot{V}O_2$ could have been higher (thus not leveling-off) if measurements were performed at a different time-point (Noakes, 1997). Duncan *et al.* tested this hypothesis and found that only 60% of tests ended in a plateau when the exact protocol described by Taylor was repeated using continuous measurement of gas exchange (Duncan *et al.*, 1997). Additionally, the studies of Myers and colleagues (1989; 1990) demonstrated that sub-maximal “plateau phenomena” also occur during continuous exercise tests, which further questions the biological meaning of the plateau at the end of exercise tests.

Noakes (1998) later developed his arguments stating that if the presence of the plateau results from the plateau in $\dot{Q}$, then the opposite must also be true, that the absence of the plateau means that $\dot{Q}$ is not limited at $\dot{V}O_{2\max}$. This is in direct opposition with the view that “maximal cardiac output is limited by the maximal rate of depolarization of the sino-atrial node and the structural limits of the ventricle” (Bassett & Howley, 2000). In a series of subsequent publications (St Clair & Noakes, 2004; Noakes & St Clair Gibson, 2004; Noakes *et al.*, 2005; Noakes, 2008a; Noakes, 2008b; Noakes & Marino, 2009), Noakes and colleagues have continuously argued against the existence of the $\dot{V}O_{2\max}$ plateau as AV Hill described it, and instead propose that $\dot{V}O_{2\max}$ is regulated by the brain through muscle recruitment, to insure that no “catastrophic failure” of homeostasis can occur, including the myocardial blood “unsaturation” which would lead to a decrease in $\dot{Q}$.

In this thesis, after the validity and biological significance of measuring a plateau in $\dot{V}O_{2\max}$ is explored in **chapters 2 and 3**, **Chapters 4-7** focus on whether $\dot{V}O_{2\max}$ measured during a standard incremental test in normoxia is indeed representative of the maximal cardiovascular capacity. In **Chapter 4**, a new exercise protocol is presented, which despite imposing workloads in a decremental fashion, is hypothesized to produce higher values of $\dot{V}O_{2\max}$ compared to a standard incremental test. **Chapters 5 and 6** further explore this new decremental protocol, expanding it to different populations and exercise modalities. Finally, **Chapter 7** attempts to investigate how the different determinants of $\dot{V}O_{2\max}$ are affected during the decremental exercise test. **Chapter 8** discusses the findings from the different studies in this thesis in an integrated manner, and perspectives for future researches are also presented.

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Chapter 2

Validity of an automated gas analyzer and its effects on the detection of small changes in oxygen consumption

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ABSTRACT

Introduction

Automated gas analyzers are often validated during low intensity exercise and using long sampling intervals. This study aimed to investigate whether Moxus Modular Metabolic System (MOXUS) was able to correctly measure oxygen consumption ($\dot{V}O_2$) during high-intensity exercise using small sampling intervals, and correctly detect small changes in $\dot{V}O_2$ between consecutive measurements.

Methods

Twelve trained male runners performed two maximal incremental tests on a treadmill while gas exchange was analyzed simultaneously by the MOXUS and a Douglas Bags system (DBM), with systems connected in series. Gas exchange data were analyzed for four consecutive intervals of 30 s on each test, and comparisons between equipment were performed for $\dot{V}O_2$, ventilation ($\dot{V}_E$), fractions of expired O_2 (FeO_2) and CO_2 ($FeCO_2$) and $\Delta\dot{V}O_2$ between two consecutive measurements.

Results

The MOXUS produced significant higher readings for $\dot{V}O_2$ ($80 \pm 200 \text{ ml}\cdot\text{min}^{-1}$, $p < 0.01$) and $\dot{V}_E$ ($2.88 \pm 4.22 \text{ l}\cdot\text{min}^{-1}$, $p < 0.0001$), but not FeO_2 (-0.01 ± 0.09). Absolute and Log-transformed (in brackets) 95% limits of agreement for readings between methods were $-310 - 470 \text{ ml}\cdot\text{min}^{-1}$ (94 – 110%) for $\dot{V}O_2$, $-5.4 - 11.2 \text{ l}\cdot\text{min}^{-1}$ (97 – 108%) for $\dot{V}_E$ and $-0.2 - 0.2$ percentage-points (99 – 101%) for FeO_2 . Although there were no differences in $\Delta\dot{V}O_2$ for two consecutive measurements between systems (120 ± 110 vs. $90 \pm 190 \text{ ml}\cdot\text{min}^{-1}$ for MOXUS and DBM respectively, $p = 0.26$), agreement between methods was very low ($r = 0.253$, $p = 0.1245$).

Discussion

The MOXUS performed within the range of accuracy for automated analyzers reported in the literature, even though it was tested during high-intensity exercise and using short sampling interval. Most of the differences in $\dot{V}O_2$ between methods were due to differences in $\dot{V}_E$. Detecting changes in $\dot{V}O_2$ during an incremental test with small changes in workload however might be beyond the equipment's accuracy, as shown by the small agreement in $\Delta\dot{V}O_2$ between systems.

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INTRODUCTION

The first measurements of oxygen consumption ($\dot{V}O_2$) during exercise date back to the 1920s, and the concept of maximal oxygen consumption ($\dot{V}O_{2max}$) is one of the traditional cornerstones of exercise physiology (Taylor *et al.*, 1955; Hodges *et al.*, 2005; Astorino *et al.*, 2005). $\dot{V}O_{2max}$ can be defined as the maximal rate at which the body is able to utilize oxygen during exercise and is thought to be the best measure of cardiorespiratory fitness (Astorino *et al.*, 2000; Meyer *et al.*, 2005). It is also believed that $\dot{V}O_{2max}$ is reached when the $\dot{V}O_2$ “plateaus” despite a continually increasing workload (Taylor *et al.*, 1955).

The first studies to report a plateau used discontinuous protocols, in which numerous successive stages of increased fixed intensity exercise were performed, with large changes in O_2 requirement between stages ($\sim 300 \text{ ml} \cdot \text{min}^{-1}$) (Taylor *et al.*, 1955; Taylor *et al.*, 1963). During these trials, expired gas was collected in meteorological balloons (now termed the Douglas Bag) and subsequently analyzed with very laborious and time-consuming methods (Mitchell *et al.*, 1958). Over time, the $\dot{V}O_{2max}$ test protocol evolved (from a discontinuous to a continuous test with shorter stages) and the Douglas Bag method (DBM) was replaced by automated gas analyzers, but nonetheless it is still considered the gold standard for $\dot{V}O_2$ measurement. The use of higher resolution gas analyzers combined with continuous ramp-like protocols has significantly decreased the incidence of the plateau phenomenon during maximal exercise tests (Doherty *et al.*, 2003; Astorino, 2009).

The ability to correctly detect a plateau in $\dot{V}O_2$ during a single incremental test with short stages and small increments in workload depends largely on the capacity of the equipment used to measure the different gas exchange components of $\dot{V}O_2$. While numerous studies have validated gas analyzers from different manufacturers (La Mere *et al.*, 1993; Bassett *et al.*, 2001; Rietjens *et al.*, 2001; Medbo *et al.*, 2002; Duffield *et al.*, 2004; Crouter *et al.*, 2006), these studies are often performed at low to moderate exercise intensities (Bassett *et al.*, 2001; Macfarlane & Wu, 2012) with long averaging intervals (Bassett *et al.*, 2001; Crouter *et al.*, 2006; Macfarlane & Wu, 2012). This

differs from the way in which the analyzers are used in practice. For example, these systems may be unable to measure accurately pulmonary ventilation ($\dot{V}_E$) at high rates and to match those high rates with rapidly changing fractions of expired O_2 and CO_2 (Gore *et al.*, 2003). Other problems with validation studies include the lack of consensus as to what is acceptable in terms of measurement error for $\dot{V}O_2$ (Macfarlane, 2001) and the use of inadequate statistics to evaluate agreement between systems (Atkinson & Nevill, 1998; Atkinson *et al.*, 2005).

For example, Bassett *et al.* (2001) found significant differences in $\dot{V}O_2$ readings between a gas analysis system and the DBM ($18 \text{ ml.kg}^{-1}.\text{min}^{-1}$) in spite of very small 95% Limits of Agreement (LOA), while others (Wilmore *et al.*, 1976) found no significant difference between different sets of equipment despite standard deviations of $\pm 9\%$, which would produce very large LOA (Auchincloss *et al.*, 1971). Although the *t*-test results could at first indicate that the latter study showed better agreement, in fact the Parvo Medics equipment used by Bassett *et al.* (2001) had much better accuracy, as shown by the very small systematic bias and narrow LOA. When only indicators of systematic bias such as *t*-test or analysis of variance (Wilmore *et al.*, 1976; La Mere *et al.*, 1993) are reported, little is known about the range of errors between equipment. Therefore, the accuracy of gas analyzer systems should always be tested including some measurement of error, such as the Technological Error of Measurement (TEM) or LOA. LOA are now widely reported in validation studies, though the technique still causes some confusion. A method can be considered valid if the range determined by the LOA is of no clinical significance (Bland & Altman, 1986; Bland & Altman, 1999; Choudhary & Nagaraja, 2005), not if 95% of all the data fall within the LOA, as it has been interpreted (Duffield *et al.*, 2004).

Therefore, part of the inconsistent observations of the plateau phenomenon may result not only from different biological responses (Doherty *et al.*, 2003; Lacour *et al.*, 2007) and the manner in which the respiratory data are analyzed (Rossiter *et al.*, 2006; Yoon *et al.*, 2007; Midgley & Carroll, 2009), but also as a consequence of the inability of automated gas analyzers to detect small changes in $\dot{V}O_2$ at very high $\dot{V}_E$ rates. To test this possibility, the purpose of this investigation was to compare the respiratory data values obtained using the DBM to those obtained simultaneously

using an automated gas analyzer during a short-stage incremental test to exhaustion and to evaluate the practical implications of those measurement errors in the calculation of stage-to-stage differences in $\dot{V}O_2$.

METHODS

Subjects

Twelve trained male runners (age: 25.5 ± 8.3 years; height: 179.9 ± 6.6 cm; body mass: 75.1 ± 5.4 kg) were recruited for this investigation. All participants were injury-free at the time of the study and were able to exercise to their personal best on a treadmill. A minimum running training volume of at least 40 km per week during the three months prior to the study was considered as an inclusion criterion for participation. The subjects were fully informed, verbally and in print, of the risks, benefits and requirements of participation, and were required to sign an informed consent form before taking part in the study, which was approved by the Research and Ethics Committee of the Faculty of Health Sciences of the University of Cape Town, in accordance to the Declaration of Helsinki. All participants were also clearly informed that they could withdraw from the study at any time if they so wished.

Study design

Participants visited the laboratory on two different occasions, separated by two to three days. On day one, after signing the consent form, participants underwent anthropometric measurements (weight and height) and subsequently performed a maximal incremental exercise test on the treadmill (see description below). On day two, participants again performed an incremental exercise test on a treadmill. During both days, gas exchange variables were continuously monitored throughout the test using an automated gas analyzer (MOXUS Modular Metabolic System, AEI Technologies, IL, USA). During a two-minute segment of the test expired gas was also collected into Plastic Bags (Douglas Bags) for later analysis of gas volume and composition. The DBM system was attached in series at the exit port of the Moxus

System mixing chamber, so that simultaneous analysis of gas exchange parameters could be performed.

Exercise test

On both days, the participants performed an incremental exercise test to exhaustion on a motor-driven treadmill (PPM 55, Woodway GmbH, Germany). Prior to the test, participants were requested to warm-up by running three 5-minute stages at 8 km.h⁻¹, 10 km.h⁻¹ and 12 km.h⁻¹ respectively. After the third 5-minute stage, the participants rested for 3 minutes before beginning the maximal test. For the first stage of the test, subjects ran at 12 km.h⁻¹ for 1 minute. Thereafter the speed was increased by 0.5 km.h⁻¹ every 30 seconds, until subjects reached volitional exhaustion. The treadmill grade was kept at 5% or 10% during the entire test, depending on the fitness level of each participant.

Data Collection and Analysis

The automated gas analyzer, Moxus Modular Metabolic System, consists of a mask incorporating a turbine for determination of ventilation volume. The turbine is attached on the inspiratory side of the valve, thus avoiding problems related to condensation of water vapour and accumulation of saliva (Bassett *et al.*, 2001). The mask covered the subject's nose and mouth so that they inhale the room air. All the expired gas travelled through a tube from the mask into a 4.2 L mixing chamber. Samples were constantly drawn from the mixing chamber (flow regulated to 250 ml.min⁻¹), through a dual-stage nafion dryer and into the O₂ and CO₂ content analyzers (S-3A/I and CD-3A respectively, AEI Technologies, IL, USA). The analyzers use the zirconia and non-dispersive infrared methods for O₂ and CO₂ analysis. Data from the Moxus system were averaged for each 30-seconds stage. Prior to the start of each testing session, the automated gas analyzer system was calibrated for gas volume and composition according to the manufacturers' instructions. Volume calibration was performed using a high precision 3 L calibration syringe (Hans-Rudolph, Kansas City, MO, USA) and gas calibration was made using a two-point calibration curve, consisting of room air and a gas of known concentration (15.00% O₂, 6.00% CO₂, AGA Norgas,

Norway). The DBM system (Cranlea, UK) was attached in series with this equipment. This method has been used previously with good results (Medbo *et al.*, 2012), and does not impose the technical problems of increased resistance to breathing (Duffield *et al.*, 2004; Crouter *et al.*, 2006) and increased dead space (Medbo *et al.*, 2002) associated with connecting multiple breathing valves close to the mouth. All expired air for each 30 second stages was collected into separate bags and stored for subsequent analysis, which was done immediately after completion of the exercise test. Collection time was clocked to the closest 0.1 second, and the bags were opened and closed always at the end of expirations, to contain only full breathing cycles. Gas volumes in the DBM were measured with a Digital Dry Gas Meter (Harvard Apparatus, MA, US). When determining the volume of expired gas collected into each bag, the small volume of air diverted to the automated gas analyzer (approximately 125 ml per 30 seconds) was accounted for. Fractions of O₂ and CO₂ in the expired gas collected into the bags were analyzed using the analyzers from the Moxus system, with the air flowing through the analyzers inlet using a closed-loop method, so that the analyzed air returned into the bags.

While the Moxus system could continuously monitor gas exchange variables, the DBM could only collect four bags at a time. Therefore, on the first day the DBM was used during the initial stages of the exercise test, and on the second day the point at which gas samples collection began was determined by each subject's predicted test duration from day one, in order to collect data from the final two minutes of the test.

Statistics

All analyzed data are presented using means $\pm$ standard deviations, and respiratory variables are expressed in STPD units. Fractions of expired O₂ (FeO₂) and CO₂ (FeCO₂) are expressed as percentage-points.L⁻¹ to avoid confusion. In addition to comparing single $\dot{V}O_2$ data points, the $\Delta\dot{V}O_2$ between two consecutive stages measured by each system was also compared whenever such calculation was possible. Regression analyses between variables (Kannagi *et al.*, 1983) measured by the two different systems and residual analyses were performed using Prism 3.0 (Graphpad Software, La Jolla, CA, USA). Values measured by the two different equipment were

compared by dependent *t*-tests (Statistica 10; Statsoft, OK, USA) (Kannagi *et al.*, 1983). The range of recorded values was divided into thirds (lower, mid and higher range), and the differences between equipment for the different thirds compared using a one-way ANOVA with Tukey *Post-hoc* when necessary (Prism 3.0). Statistical significance was accepted when $p < 0.05$.

A variety of statistical procedures were used to facilitate comparison between our data and other studies, as has been recommended (Hodges *et al.*, 2005; Atkinson & Nevill, 1998; Macfarlane & Wu, 2012; Macfarlane & Wong, 2012). TEM was defined as $TEM = \frac{D}{N}$, where D is the difference between methods and N is the number of pair comparisons (Macfarlane & Wong, 2012), and expressed as a percent of the mean. Absolute percent difference (APE) was calculated as the difference between equipment divided by the DBM reading times 100 (Macfarlane & Wu, 2012). Mean Individual Coefficient of variation (Macfarlane & Wu, 2012) (expressed as the mean individual standard deviation between measurements/mean times 100), and effect sizes, being the ratio of the mean difference between equipment and their overall standard deviations, were also calculated (Macfarlane & Wu, 2012; Hopkins, 2012) using Excel (Microsoft, WA, US) spreadsheets. The agreement between the two systems was further evaluated using Bland-Altman Plots (Bland & Altman, 1986; Bland & Altman, 1999; Hodges *et al.*, 2005). Since heteroscedastic errors are the norm when analyzing physiological parameters (Nevill & Atkinson, 1997), data were also log-transformed for calculation of LOA as a ratio between measurements (Nevill & Atkinson, 1997; Rietjens *et al.*, 2001; Bland & Altman, 2007). Although adjustments in the Bland-Altman can be made to adjust for multiple observations per subject, this has been shown to be of little or no significance when the number of comparison pairs is much greater than the number of observations per subject, as in our study (Bland & Altman, 2007).

Data from three participants tested on the same days produced apparently unreliable values for $\dot{V}O_2$ and respiratory exchange ratio (RER). Upon investigation, it was discovered that the drying chamber from the Moxus system required maintenance, and therefore the data from these three participants were excluded from the study (Kannagi *et al.*, 1983). A separate analysis showed that inclusion of

these data would not significantly impact our results (not shown) (Salier *et al.*, 2012), but the data were excluded nonetheless (Hodges *et al.*, 2005; Choudhary & Nagaraja, 2005).

RESULTS

Comparison between the data measured simultaneously by the Moxus and DBM is presented in table 1. All variables presented high correlation coefficient between the DBM and the Moxus (figures 1-5), ranging from 0.929 ($\dot{V}O_2$) to 0.989 ($\dot{V}_E$). Overall, the Moxus produced significantly higher readings for $\dot{V}_E$, $\dot{V}O_2$, $FeCO_2$, CO_2 excretion ($\dot{V}CO_2$) and RER, but not for FeO_2 . FeO_2 also displayed the smaller mean CV (table 2) and mean APE between the two systems (0.3% and 0.4% respectively), whereas $FeCO_2$ showed the larger variation between systems (mean CV 2.75%, APE 3.9%). Although mean individual CV for $\dot{V}O_2$ was 2.5%, individual values ranged from 0 to 7.43%. The effect sizes of the differences between equipment (table 1) were deemed “trivial” (0 – 0.20) according to the Cohen scale, except for $\dot{V}O_2$ and $FeCO_2$, which were deemed “small” (0.21 – 0.60).

The analysis of variance performed to compare the differences in readings over the lower, middle and higher third of recorded values showed no significant differences for RER, $\dot{V}O_2$ and FeO_2 , suggesting that there was no increase in bias as values increased. Ventilation readings for the higher third of the analyzed range showed higher differences in reading between the two systems than both the lower and middle ranges of values (5.45 ± 5.44 vs. 1.34 ± 2.51 vs. 1.96 ± 3.28 l.min⁻¹ higher for the Moxus than the DBM, $p < 0.05$). Similar patterns were seen for $FeCO_2$ and VCO_2 (388 ± 286 vs. 117 ± 205 vs. 110 ± 159 ml CO₂.min⁻¹ for the higher, middle and lower range of values respectively, $p < 0.01$).

The mean bias and 95% LOA for the respiratory values are shown in table 2 and also in figures 1-5. While most variables showed very small systematic bias between systems, some of the respiratory parameters showed considerably large 95% LOA. The

larger or O_2 than all other variables, which is expected. The standard deviation of the error measurements from $\dot{V}_E$, FeCO_2 and FeO_2 .

Although there were no significant differences between equipment in the $\Delta \dot{V}_E$ between two consecutive 30-seconds intervals (0.12 ± 0.11 vs. $0.09 \pm 0.19 \text{ l.min}^{-1}$ for the Moxus and DBM respectively, $p = 0.26$), the standard deviation for the difference between the systems was several times greater than the mean difference itself ($0.03 \pm 0.197 \text{ l.min}^{-1}$). As shown in figure 6, the level of agreement between the two systems when measuring stage-to stage $\Delta \dot{V}_E$ was very low ($r=0.253$, $p = 0.1245$), and the slope for the regression line between the Moxus and the DBM was not different from zero, further indicating low agreement.

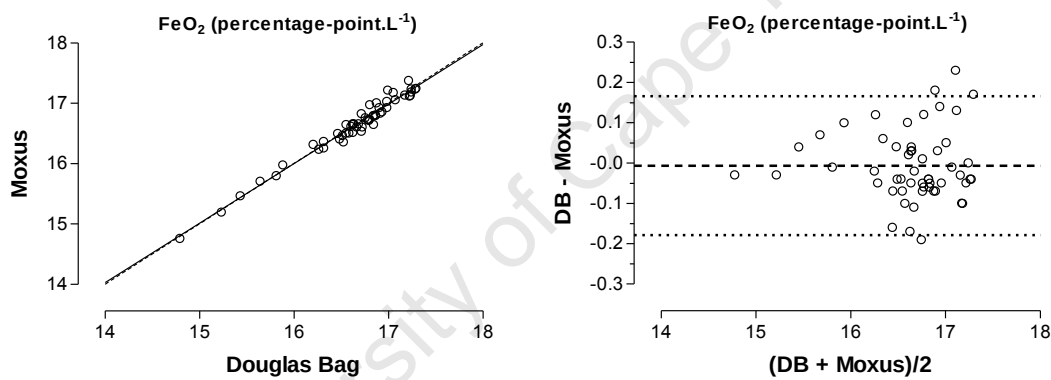


Figure 1: (left) Fraction of expired O_2 measured by the Moxus versus measured by the Douglas Bag (DB) System. The solid Line represents the identity line, and the dotted line represents the regression line. (Right) Bland-Altman plot of the error scores. Dashed line represents the mean bias and dotted lines represent the 95% limits of agreement.

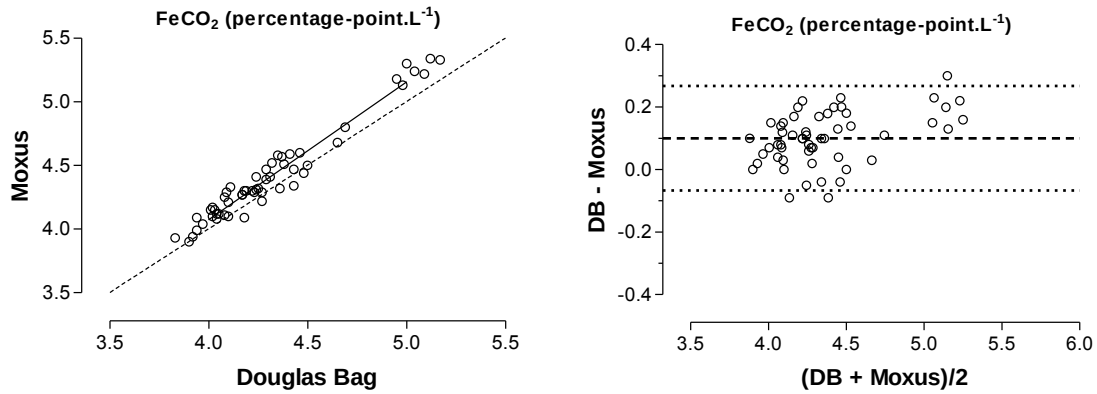


Figure 2: (left) Fraction of expired CO₂ measured by the Moxus versus measured by the Douglas Bag (DB) System. Solid Line represents the identity line and the dotted line represents the regression line. (Right) Bland-Altman plot of the error scores. Dashed line represents the mean bias and dotted lines represent the 95% limits of agreement.

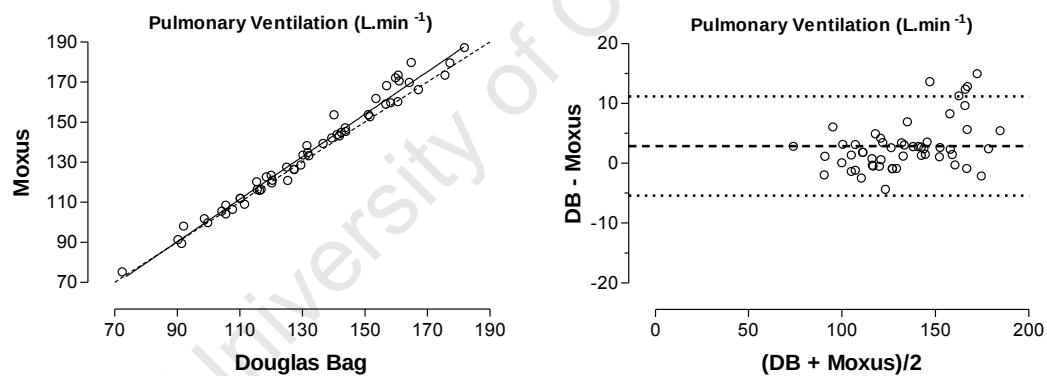


Figure 3: (left) Pulmonary ventilation measured by the Moxus versus measured by the Douglas Bag (DB) System. The solid Line represents the identity line and the dotted line represents the regression line. (Right) Bland-Altman plot of the error scores. Dashed line represents the mean bias and dotted lines represent the 95% limits of agreement.

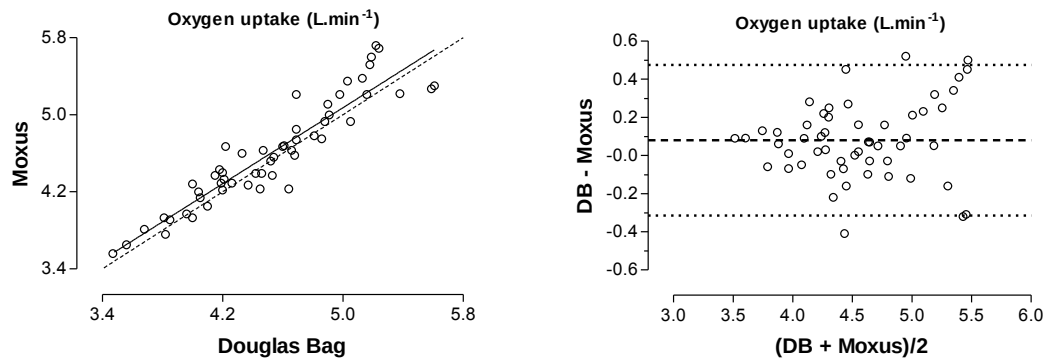


Figure 4: (left) Oxygen uptake measured by the Moxus versus measured by the Douglas Bag (DB) System. The solid Line represents the identity line and the dotted line represents the regression line. (Right) Bland-Altman plot of the error scores. Dashed line represents the mean bias and dotted lines represent the 95% limits of agreement.

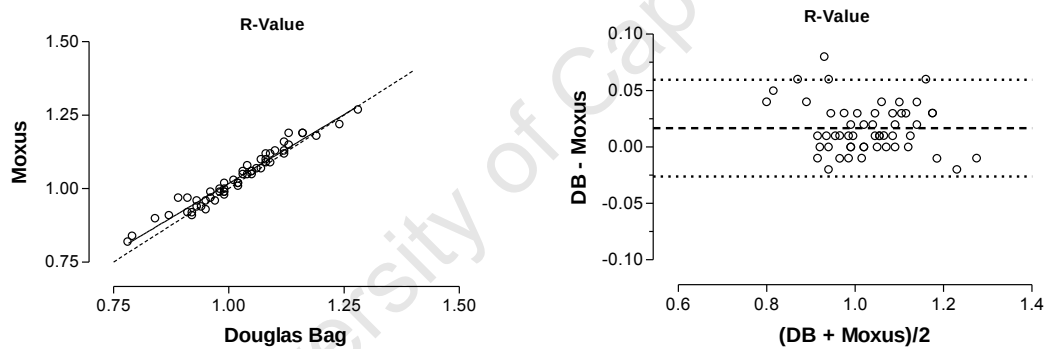


Figure 5: (left) R-value measured by the Moxus versus measured by the Douglas Bag (DB) System. The solid Line represents the identity line and the dotted line represents the regression line. (Right) Bland-Altman plot of the error scores. Dashed line represents the mean bias and dotted lines represent the 95% limits of agreement.

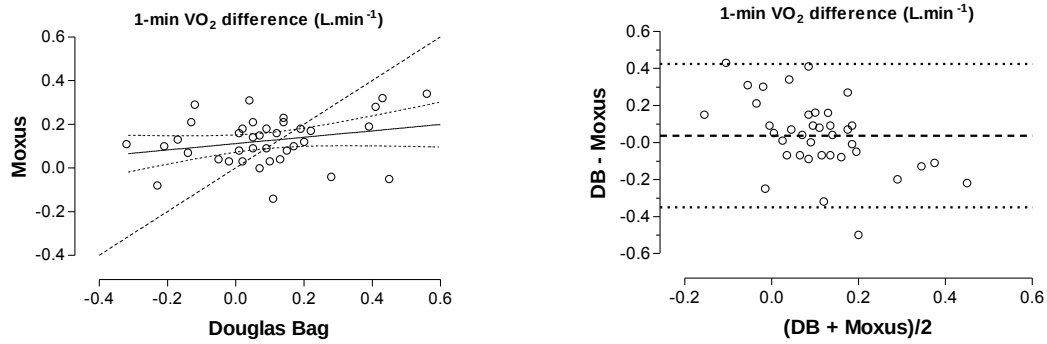


Figure 6: (left) $\Delta \dot{V}O_2$ between two consecutive 30-seconds intervals measured by the Moxus versus measured by the Douglas Bag (DB) System. The parameters for the regression line are as follows: $\{Y\} = -0.042 \{x\} + 0.072$; $s_b = 0.334$; $s_{y|x} = 0.109 \text{ l.min}^{-1}$; $r = 0.253$ ($p = 0.124$); $n = 38$. The solid Line represents the identity line and the dotted line represents the regression line. (Right) Bland-Altman plot of the error scores. Dashed line represents the mean bias and dotted lines represent the 95% limits of agreement.

Table 1: Regression equation between two systems simultaneously measuring gas exchange, Person's coefficient of correlation (r), standard error of estimate (SEE), mean $\pm$ SD values for the Douglas Bag (DB) and Moxus systems, absolute percentage differences (APE) and effect size

Variable	Regression Equation	r	SEE		Mean $\pm$ SD	Mean diff. $\pm$ SD (APE)	Effect Size
$\dot{V}O_2 (\text{l.min}^{-1})$	$\{Y\} = 0.986 \{x\} + 0.141$	0.929	0.203	DB	$4.53 \pm 0.51^*$	-0.08 ± 0.20	0.37
				Moxus	4.60 ± 0.54	(3.5)	
$\dot{V}CO_2 (\text{l.min}^{-1})$	$\{Y\} = 1.044 \{x\} - 0.043$	0.980	0.183	DB	$4.65 \pm 0.87^\dagger$	-0.16 ± 0.18	0.20
				Moxus	4.81 ± 0.93	(3.9)	
$\dot{V}_E (\text{l.min}^{-1})$	$\{Y\} = 1.061 \{x\} - 5.202$	0.989	3.974	DB	$132.08 \pm 25.13^\dagger$	-2.88 ± 4.22	0.15
				Moxus	134.96 ± 26.95	(2.6)	
$FeO_2 (\%)$	$\{Y\} = 0.986 \{x\} + 0.214$	0.985	0.088	DB	16.64 ± 0.51	0.01 ± 0.09	0.17
				Moxus	16.64 ± 0.51	(0.4)	
$FeCO_2 (\%)$	$\{Y\} = 1.084 \{x\} - 0.263$	0.977	0.081	DB	$4.31 \pm 0.39^*$	-0.10 ± 0.08	0.21
				Moxus	4.41 ± 0.38	(2.5)	
RER	$\{Y\} = 0.936 \{x\} + 0.082$	0.977	0.021	DB	$1.02 \pm 0.10^\dagger$	-0.02 ± 0.02	0.22
				Moxus	1.04 ± 0.10	(2.1)	

* $\dagger$ Significantly different means by paired t -tests at $*p < 0.01$; $\dagger p < 0.0001$

Table 2: Mean $\pm$ SD bias, 95% Confidence Interval of mean bias (95% CI), 95% Limits of agreement, technical error of measurement (TEM) and mean individual coefficient of variation (CV) between two systems measuring gas exchange simultaneously

Variable	Bland-Altman Bias Mean $\pm$ SD (95% CI)	95% LOA (log-transformed LOA)	Inter-Device TEM (%)	CV mean (range)
VO ₂ (l.min ⁻¹)	0.08 $\pm$ 0.201 (0.024, 0.134)	-0.31, 0.47 (0.94, 1.10)	3.33	2.5 (0, 7.43)
VCO ₂ (l.min ⁻¹)	0.16 $\pm$ 0.18 (0.11, 0.21)	-0.20, 0.53 (0.96, 1.11)	3.67	2.75 (0.02, 8.25)
VE (l.min ⁻¹)	2.88 $\pm$ 4.22 (1.73, 4.03)	-5.40, 11.16 (0.97, 1.08)	2.69	1.81 (0.07, 6.56)
FeO ₂ (%)	-0.08 $\pm$ 0.09 (-0.03, 0.017)	-0.18, 0.16 (0.99, 1.01)	0.37	0.30 (0.01, 0.96)
FeCO ₂ (%)	0.10 $\pm$ 0.08 (0.08, 0.12)	-0.07, 0.27 (0.99, 1.06)	2.11	1.03 (0, 4.05)
RER	0.02 $\pm$ 0.02 (0.01, 0.02)	-0.03, 0.06 (0.97, 1.06)	1.88	1.44 (0, 6.08)

DISCUSSION

To our knowledge, this is one of few studies testing the accuracy of a modern gas analyzer in more practical and challenging conditions (Meyer *et al.*, 2005). The first important finding of this investigation is that the Moxus performed similarly to several other commercially available systems and within recommended standards for validity of gas analysis systems. The second main finding is that even though the Moxus system performed similarly to other commercially available equipment, the errors of measurement are sufficiently large to affect the ability of the automated system to detect small changes in oxygen consumption, as required when searching for the $\dot{V}O_{2\max}$ plateau during incremental exercise tests.

Accuracy of the MOXUS against other systems

Although there is no consensus about what constitutes an acceptable error in measurement for automated gas analyzers, some standards have been proposed (Macfarlane, 2001; Atkinson *et al.*, 2005; Hodges *et al.*, 2005). Atkinson *et al.* (2005)

suggest that there is no practical impact on $\dot{V}O_2$ measurements if two methods disagree systematically by no more than $\pm 2 \text{ ml.kg}^{-1}.\text{min}^{-1}$, as judged by the 95% confidence interval of the mean bias. This was achieved in our study (-1.8 to -0.3 $\text{ml.kg}^{-1}.\text{min}^{-1}$). MacFarlane (Macfarlane, 2001) recommends that the technical error of measurement should be smaller than 3% for $\dot{V}O_2$ and 5% for $\dot{V}_E$, which is also supported by Hodges *et al.* (2005). Our data for $\dot{V}_E$ was within the recommended range (2.69%), but slightly off for $\dot{V}O_2$ (3.33%).

When comparing different methods to measure a given physiological variable, the total error in measurement can be split into technological errors and biological variation, the latter being the normal fluctuation of a variable over the time between measurements (Hopkins, 2012a). The technological error of measurement can be separated from the biological variations in $\dot{V}O_2$ by testing two systems working simultaneously, and this should be used whenever possible (Macfarlane, 2001; Atkinson *et al.*, 2005; Hopkins, 2012a). Since the measurement errors should be much smaller when systems work at the same time (Macfarlane & Wu, 2012), we will focus our discussion on studies that used similar methodology to ours. More detailed reviews on the performance of gas analyzers tested sequentially or over multiple days can be found elsewhere (Macfarlane, 2001; Atkinson *et al.*, 2005).

Validity studies comparing gas analyzers to the DBM have reported results for $\dot{V}O_2$ similar to ours. Rietjens and colleagues (2001) reported wider LOA than those in this study (ratio of DBM over Oxycon between 0.88 – 1.12 vs. 0.94 – 1.10 in our study) when validating the Oxycon Pro. Jakovljevic *et al.* (2008) found wider LOA for the Innocor system against the DBM than in our investigation for both $\dot{V}O_2$ and $\dot{V}_E$, though the authors interpreted their values as too wide to be acceptable for clinical testing. Reybrouck *et al.* (1992) presented LOA for $\dot{V}O_2$ in the range of $\pm 3.5 \text{ ml.kg.min}^{-1}$ and $\pm 3.0 \text{ l.min}^{-1}$ for $\dot{V}_E$ in a study validating an automated system, narrower values than those reported in the present investigation. It is important to note, however, that their rate of $\dot{V}_E$ did not exceed 40 l.min^{-1} , which is a very different range of exercise intensities than in the present investigation.

Crouter *et al.* (2006) reported 95% LOA for $\dot{V}O_2$ of $\pm 200 \text{ ml.min}^{-1}$ and $\dot{V}_E$ of $\pm 2.3 \text{ l.min}^{-1}$, but like the former study minute ventilation rarely exceeded 80 l.min^{-1} , and

data were averaged over two-minute intervals. The study by Gore *et al.* (2003) clearly demonstrates how differences between methods often grow proportionally to the size of the mean of measurements, indicating the importance of testing equipment over the full spectrum of physiological values normally measured. The presence of heteroscedastic errors in physiological variables has already been examined (Atkinson & Nevill, 1998) and is further shown by the larger difference between equipment for the higher third of measurements of $\dot{V}_E$, $\dot{V}CO_2$ FeO₂. In our study, $\dot{V}_E$ values ranged from ~70 to ~190 l.min⁻¹, while $\dot{V}O_2$ values ranged between ~3.3 – 5.5 l.min⁻¹, values much higher than often reported in validation studies (Reybrouck *et al.*, 1992; Bassett *et al.*, 2001; Macfarlane & Wu, 2012). A large study on the Oxycon Pro revealed that almost all the measurements of $\dot{V}_E$ above 120 l.min⁻¹ and $\dot{V}O_2$ above 3.8 l.min⁻¹, intensities that are seldom tested in validity studies.

Errors in gas exchange measurement

When measuring gas exchange parameters, it is important to consider the sources of error when using a dry gas meter (DBM) to measure $\dot{V}_E$ and $\dot{V}O_2$ (that is, a 1% change in $\dot{V}_E$ will change $\dot{V}O_2$ by 1%) (Kannagi *et al.*, 1983; Carter & Jeukendrup, 2002; Hodges *et al.*, 2005; Medbo *et al.*, 2012). Although the DBM is considered the gold standard for measuring $\dot{V}_E$, it has the potential for error, particularly at low $\dot{V}_E$. Carter and Jeukendrup (2002) used a similar dry gas meter than we used, and concluded that the equipment was slightly inaccurate, the DBM tended to result in lower $\dot{V}_E$ values than both an Oxycon Pro, an Oxycon Alpha and more importantly a metabolic simulator. This has also been the case in at least one more study (Rietjens *et al.*, 2001). Conversely, Macfarlane and Wong (2012) also used the same dry gas meter system and found similar $\dot{V}_E$ values for the Oxycon Pro and the DBM.

Another potential problem when measuring $\dot{V}O_2$ and $\dot{V}_E$ with the DBM is the timing for opening and closing the bags (Wilmore *et al.*, 1976; Rietjens *et al.*, 2001). In this regard, automated gas analyzers can measure the start and end of breathing cycles more accurately. This could have been minimized in our study by using longer sampling intervals; however this would have distracted from the main purposes of this

investigation (Bassett *et al.*, 2001), which was to test the accuracy of the analyzer using short sampling intervals at high exercise intensities, a situation that is common in clinical exercise testing but rare in validation studies (Hodges *et al.*, 2005). Another concern when working with the DBM is that the bags are made of PVC, and can be marginally permeable to gas leakage (Carter & Jeukendrup, 2002). In our study the bags were emptied within ~5 min of test completion so that gas leakage was of little consequence (Hodges *et al.*, 2005).

While errors in ventilation receive significant attention, errors in the measurement of gas fraction are often not considered in validation studies (Reybrouck *et al.*, 1992; Carter & Jeukendrup, 2002; Macfarlane & Wong, 2012). These will also have substantial effects on the calculation of $\dot{V}O_2$, particularly $\text{Fe}O_2$, and should be reported (Hodges *et al.*, 2005). When measuring $\text{Fe}O_2$ in the present investigation, the Moxus performance was better than some analyzers (Gore *et al.*, 2003; Crouter *et al.*, 2006; Jakovljevic *et al.*, 2008), but poorer than others (Bassett *et al.*, 2001; Foss & Hallen, 2004). The Moxus readings for $\text{Fe}O_2$ showed the best agreement with the DBM measurements from all variables, with a mean APE of only 0.4% and mean bias of 0.01 percentage-points. l^{-1} . Still, since each 0.01 percentage-point error in $\text{Fe}O_2$ amounts to an 0.13 mL O_2 error for each liter of $\dot{V}_E$, and considering our LOA for $\text{Fe}O_2$ (± 0.18 percentage-points. $\text{l}.\text{min}^{-1}$), $\text{Fe}O_2$ errors alone could explain differences of 350 ml in $\dot{V}O_2$ at a $\dot{V}_E$ of 150 $\text{l}.\text{min}^{-1}$.

Importance of detecting small changes in $\dot{V}O_2$

Errors in the measurement of gas exchange parameters can substantially affect the capacity to correctly detect small changes in $\dot{V}O_2$, as required by the different criteria for determining the occurrence of a plateau during incremental exercise tests. For this reason, researchers need to adopt plateau criteria that are the least likely to produce false-positive or false-negative results. The plateau criterion described by Mitchell and colleagues (1958), for example, was essentially an application of the LOA, in which values up to two standard deviation smaller than the mean stage-to-stage difference in $\dot{V}O_2$ were considered normal, so that only values outside the “normal” range (i.e., lower than two standard deviations) would be considered a plateau (Taylor

et al., 1955). Nowadays, however, the search for $\dot{V}O_{2\max}$ using automated gas analyzers has distorted this original criterion, and multiple plateau criteria have emerged (Noakes & St Clair Gibson, 2004). Measuring small differences ($< 50 \text{ ml}\cdot\text{min}^{-1}$) in $\dot{V}O_2$ over very short intervals (Astorino *et al.*, 2000; Lacour *et al.*, 2007; Gordon *et al.*, 2011; Gordon *et al.*, 2012) and high intensities might be beyond the accuracy of most analyzers. In the present study comparisons of $\dot{V}O_2$ between the Moxus and the DBM during high intensity running resulted in similar LOA as the $\Delta\dot{V}O_2$ between two consecutive 30-seconds intervals measured by both systems ($\pm 402 \text{ ml}\cdot\text{min}^{-1} \text{ O}_2$ vs. $\pm 386 \text{ ml}\cdot\text{min}^{-1}$ respectively). Thus, in this case, the level of uncertainty around the difference between two consecutive measurements was not greater than the uncertainty around each measurement alone, as it has been hypothesized (Howley *et al.*, 1995).

Nonetheless, these data indicate that if our exercise protocol was designed to test the incidence of the plateau phenomenon, larger increases in workloads should have been used together with longer collection intervals (Howley *et al.*, 1995). Both changes tend to minimize errors in measurements and allow the expected changes in $\dot{V}O_2$ to be greater than the LOA for the system accuracy in a particular condition. It has been demonstrated, however, that the time-averaging of gas exchange parameters does not affect the reproducibility of $\dot{V}O_{2\max}$ itself (Midgley *et al.*, 2007). Another possibility is the use of supra-maximal testing to confirm the attainment of $\dot{V}O_{2\max}$ (Day *et al.*, 2003), which has convincingly demonstrated that the occurrence of a plateau is not a pre-requisite for measuring $\dot{V}O_{2\max}$ (Howley, 2007).

Limitations

In our study, $\dot{V}_E$, $\dot{V}O_2$ and $\dot{V}CO_2$, were lower for the DBM than for the Moxus system. Howley and colleagues (1995) have already identified this possibility during high intensity exercise with short sampling intervals, but to our knowledge it had not been evaluated previously. Our Moxus system has already been tested against a different set of Douglas Bags and a different Dry Gas Meter under more stable conditions (Medbo *et al.*, 2012), with closer agreement between methods than the ones we reported here, in spite of equal systematic bias for $\dot{V}O_2$ ($\sim 80 \text{ ml}\cdot\text{min}^{-1}$). These

two studies were performed by the same researchers, and at the same time of the year, the only difference being the DBM equipment used, the averaging interval and the exercise protocols evaluated. Also, our Moxus system has been shown to be extremely reliable when measuring $\dot{V}O_{2\max}$ over different days (Beltrami *et al.*, 2012; Medbo *et al.*, 2012). Taken together, these suggest that our dry gas meter may have been slightly inaccurate (Carter & Jeukendrup, 2002), which is a limitation in the present investigation and underscores the uncertainty of the true value of measurements even when using “gold standard” techniques, as suggested by Bland and Altman (1986; 1995), but criticized by others (Medbo *et al.*, 2002). Unfortunately, a metabolic simulator (Carter & Jeukendrup, 2002) to test the accuracy of the dry gas meter was not available, and testing it by pumping air from the calibration syringe multiple times into the bags is not ideal. Another limitation of the current investigation is that although the Moxus system has a flow velocity controller for the air coming from the mixing chamber, this does not apply to the gas coming from the calibration bottles. O_2 analyzers are known to be highly dependent on pressure, or flow velocity, (Macfarlane, 2001) and to counteract this problem all calibrations were done by the same investigator. Finally, both systems used the same gas analyzers. Although this can affect the level of certainty about the values, the S-3A/I and CD-3A analyzers have been on the market for many years, and are often part of the DBM set-up for validation studies (Bassett *et al.*, 2001; Duffield *et al.*, 2004; Macfarlane & Wong, 2012).

Summary

In summary, our data show that despite good agreement between the DBM and the Moxus system, within recommended ranges of accuracy, there are large discrepancies in the $\Delta\dot{V}O_2$ measured between two consecutive 30-seconds intervals by the two sets of equipment. Gas analyzers with this range of accuracy are unable to accurately detect the small changes in $\dot{V}O_2$ required to identify the plateau phenomenon as it has been described during discontinuous tests (Taylor *et al.*, 1955; Mitchell *et al.*, 1958). While the use of ramp incremental tests with small load increments over time is widely promoted (Meyer *et al.*, 2005), our results support the

idea that larger increments in workload or longer sampling intervals should be used (Howley *et al.*, 1995). Researchers should be aware of the accuracy of their equipment before establishing a protocol for an incremental test, and choose load increments and sampling intervals that are large enough to minimize random errors and so to allow small changes in $\dot{V}O_2$ (Alpert, 1992; Beltrami *et al.*, 2012) to be correctly detected.

Further testing of gas analyzers during high intensity exercise is also encouraged to determine the true ability of modern equipment to measure the occurrence or not of the plateau phenomenon during maximal incremental exercise tests.

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Chapter 3

High-prevalence of false-positive plateau phenomena during maximal exercise testing in adolescents

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ABSTRACT

Introduction

It is believed that a plateau in oxygen consumption ($\dot{V}O_2$) at the end of an incremental exercise test identifies the upper limits of cardiovascular capacity to supply oxygen to the exercising muscles. We investigated how different mathematical criteria influenced the frequency with which the “plateau phenomenon” is detected and the prevalence at which “false” plateau phenomena occur during sub-maximal exercise.

Methods

Six different criteria, each using three different sampling intervals, were used to identify the “plateau phenomenon” from a single data set comprising 63 incremental exercise tests along with secondary criteria based on target heart rate (HR) and respiratory exchange ratio (RER). A single criterion (defining the cut-off to determine a plateau as changes in $\dot{V}O_2$ between two consecutive workloads smaller than half of the expected difference using 30 s intervals, HALF) was also used to detect the occurrence of “plateau phenomena” during sub-maximal stages of each test.

Results

The plateau phenomenon was detected in 16-82% of the tests depending on the criteria used, mostly as a result of the different sampling intervals. HALF identified 103 “plateau phenomena” but 73 (70.9%) of these occurred during sub-maximal exercise and so were “false”. “False” plateaus were verified by at least one secondary criterion in 27% of cases. Subjects reached the HR and RER targets after 83.6 ± 11.7 % and 81.9 ± 18.1 % of total test duration, respectively.

Discussion

The wide range in the percentage of plateau phenomena detected by different criteria plus the high rate (71%) of “false” plateaus during sub-maximal exercise could indicate that this phenomenon is a calculation artifact rather than an indicator of true physiological events. Secondary criteria can be reached early in exercise and thus identify “false” plateau phenomena as “true”.

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INTRODUCTION

Maximal oxygen consumption ($\dot{V}O_{2\max}$) was first measured in the early 1920s (Hill & Lupton, 1923; Hill *et al.*, 1924a; Hill *et al.*, 1924b); currently it is perhaps the most measured variable in exercise physiology. Those early experiments developed the concept that oxygen consumption ($\dot{V}O_2$) reaches a ceiling during exercise beyond which any further increase in work rate does not produce a further increase in $\dot{V}O_2$ (Taylor *et al.*, 1955; Taylor *et al.*, 1963; Mitchell & Blomqvist, 1971). The classic work of Taylor and colleagues (Taylor *et al.*, 1955) popularized what became known as the $\dot{V}O_2$ “plateau phenomenon”. The plateau phenomenon is believed to occur when some factor within the cardiorespiratory system reaches its limit preventing any further increase in $\dot{V}O_2$ even though the work rate, and consequently O_2 requirement, continues to increase (Calbet *et al.*, 2006).

Although these early studies reported the presence of the plateau phenomenon in almost all subjects that were tested (Taylor *et al.*, 1955; Taylor *et al.*, 1963; Doherty *et al.*, 2003), more recent studies employing automated gas analyzers and continuous exercise protocols have failed to show a clear plateau phenomenon in all or even most tests in human subjects (Day *et al.*, 2003; Noakes & St Clair Gibson, 2004; Rossiter *et al.*, 2006). Some suggest (Shephard, 2009) that the plateau phenomenon is more likely to occur in well trained and highly motivated subjects. However, the protocol used and the mathematical definition of what constitutes a “plateau” also influence the relative frequency with which this phenomenon is identified (Shephard, 2009). Several studies have reported the effects of the test protocol and $\dot{V}O_2$ sampling intervals on the frequency with which the plateau phenomenon can be detected (Armstrong *et al.*, 1996; Hawkins *et al.*, 2007; Yoon *et al.*, 2007). However, there is still no consensus of the optimum mathematical method to detect “true” plateau phenomena.

In the absence of such consensus, several additional criteria have been created to determine if a test is truly “maximal”. These additional criteria include maximum values for heart rate (HR), respiratory exchange ratio (RER) and blood lactate

concentration [La] (Poole *et al.*, 2008). These criteria however have been criticized and may be of limited value (Midgley *et al.*, 2009). This further emphasizes the need to standardize the criteria for a $\dot{V}O_{2\max}$ plateau.

The purpose of the present study was to examine the prevalence of the $\dot{V}O_2$ plateau phenomenon during a maximal incremental exercise test to exhaustion in teenage male soccer players. We employed different but established criteria to detect the presence of the plateau phenomenon in order to determine the influence of different specific criteria on the prevalence with which the plateau phenomenon can be detected in young individuals. We also wished to identify the relative frequency with which criteria for the plateau phenomenon are fulfilled during sub-maximal exercise. By definition such plateaus must be “false” (Myers *et al.*, 1989; Myers *et al.*, 1990) if the athlete is able to continue exercising to a higher $\dot{V}O_2$.

METHODS

Participants

Sixty-eight young male soccer players (age 14.3 ± 0.8 years, body mass 51.8 ± 9.5 kg, height 163 ± 9 cm) competing at the highest level for their age category in Hong Kong participated in the study. Written consent was obtained from each subject (legal guardian if applicable) and the study was conducted according to the Declaration of Helsinki. The protocol was fully approved by the Clinical Research Ethics Committee of Hong Kong Baptist University before testing began. All subjects were familiar with treadmill running including maximal exercise testing on the treadmill and underwent a familiarization trial before data collection began. Following a standardised warm-up consisting of static and dynamic stretching, each participant performed a single $\dot{V}O_{2\max}$ test. Testing was conducted immediately before the competitive season began. Data from five subjects were excluded due to non-physiologically fluctuations in $\dot{V}O_2$ values during the test (ie. over $30 \text{ ml.kg}^{-1}.\text{min}^{-1}$ between two consecutive five-second intervals), probably due to problems with the placement of the face mask causing leakage of expired air.

Incremental Test

Participants ran on a motorized treadmill (cos10198, h/p/cosmos, Germany), set at a slope of 5.5%, for 4 min at 7 km·h⁻¹; thereafter speed was increased by 1 km·h⁻¹ every minute until the subject voluntarily terminated the test (Chamari *et al.*, 2005b; Chamari *et al.*, 2005a). Participants were strongly encouraged to run for as long as possible during the test. The experimenter conducting the test was unaware of the nature of the study. Cardiorespiratory variables were measured using a calibrated breath-by-breath system (MetaMax 3B, Cortex, Germany). All breath-by-breath data were exported to an Excel spreadsheet for further analysis. A 25 µl sample of capillary blood was drawn from a fingertip 3.5 min after the incremental test ended. Blood lactate concentrations were subsequently measured on these samples using an enzymatic method (YSI 1500, Yellow Springs Instruments, USA).

Determination of $\dot{V}O_2$ plateau

Six different methods were employed to determine the occurrence of a plateau at the end of the test. Each of these methods has been described in the literature (Astorino *et al.*, 2000; Doherty *et al.*, 2003; Noakes & St Clair Gibson, 2004; Astorino, 2009). These methods fall into two main categories: fixed value methods and relative change methods. Furthermore, for each method the $\dot{V}O_2$ data were analyzed with three different sampling intervals: 15 s, 30 s or 1 min (Astorino *et al.*, 2000). When using 15 s or 30 s sampling times, the interval within the 60 seconds of each stage of the test within which the highest $\dot{V}O_2$ reading occurred was used for further comparison against $\dot{V}O_2$ measured in subsequent stages. This was done to ensure that all comparisons between $\dot{V}O_2$ values were performed between values from different workloads, as required by the definition of a plateau. For all three sampling intervals, data were considered valid until the last full data set during the test. For example, a subject with a time to exhaustion of 10:45 min would have his $\dot{V}O_2$ considered until 10:00 min for the 1 min sampling intervals, 10:30 min for the 30 s intervals and 10:45 min for the 15 s intervals.

For one criterion (the plateau defined as half of the expected difference in $\dot{V}O_2$ based on data from all subjects, see below), the prevalence of the plateau

phenomenon was quantified not only between the two final stages, but in all stage changes during the tests except the first four minutes of the test, workload was constant). If a participant demonstrated a plateau between two sub-maximal stages, but was able to increase workload to higher workloads and higher $\dot{V}O_2$ than the plateau was deemed “false”. Plateau phenomenon that happened at the end of the test (between the two stages) was deemed “true”.

Fixed value methods

(a) A plateau is deemed to exist when the difference between the highest $\dot{V}O_2$ values from the last and next to last completed stages is smaller than $2.1 \text{ ml.kg}^{-1}.\text{min}^{-1}$ (Armstrong *et al.*, 1996).

(b) A plateau is deemed to exist when the difference between the highest $\dot{V}O_2$ values during the last and next to last completed stage is smaller than $1.3 \text{ ml.kg}^{-1}.\text{min}^{-1}$ (Armstrong *et al.*, 1996; Midgley *et al.*, 2009; Astorino, 2009).

Relative change methods

(a) All subjects’ stage-to-stage difference $\dot{V}O_2$ method

The presence of a plateau was determined if the difference between the highest $\dot{V}O_2$ from the last and next to last stages with valid data was smaller than either half or one-third of the average stage-to-stage $\dot{V}O_2$ difference for all tested subjects during sub-maximal parts of the test, corrected for the number of individuals completing each stage (Taylor *et al.*, 1955). This method (using half of difference as the cut-off value) was also used to search for “false” plateau phenomenon.

(b) Subject stage to stage difference $\dot{V}O_2$ method

The method used as a plateau criterion used the subject’s stage-to-stage $\dot{V}O_2$ during the sub-maximal parts of the test. A plateau was confirmed if the difference $\dot{V}O_2$ for the final two stages was smaller than half or one-third of the average stage difference $\dot{V}O_2$ for that individual (Rossiter *et al.*, 2006).

For both methods based on relative change, the expected difference in $\dot{V}O_2$ was used to determine the stage-to-stage difference $\dot{V}O_2$ between the fifth and next to

last stage of the test, since during this part of the tests $\dot{V}O_2$ rose as a linear function of exercise duration (figure 1).

Secondary Criteria

Two secondary criteria were used to validate the presence of a plateau in $\dot{V}O_2$: a) RER higher than 1.1; b) HR equivalent to 90% of age-predicted maximum ($HR_{max} = 220 - \text{age}$). Post test [La] values were compared with the literature but no cut-off concentration was established.

Statistical Analysis

All data are presented as the mean $\pm$ standard deviation (SD). The prevalence of each plateau criterion was calculated as a percentage of the total tests. Rates of “false” and “true” plateau phenomena were calculated as a percentage of all plateau phenomena identified. All percentage calculations as well as linear regressions were calculated using Microsoft excel 2007 for Windows.

RESULTS

The subjects' mean time to termination of exercise during the $\dot{V}O_{2max}$ test was $09:34 \pm 01:25$ min (range 06:00 – 12:30 min) and the mean $\dot{V}O_{2max}$ of the group was 55.9 ± 6.9 ml $\cdot$ min $^{-1}\cdot$ kg $^{-1}$ (30 seconds sampling intervals, range 38.2 – 69.5). $\dot{V}O_2$ rose linearly as a function of speed ($r^2 = 0.961$, $p < 0.0001$), with no discernible “leveling off” of $\dot{V}O_2$ at the end of the test for the combined data from all subjects (figure 1) when data were expressed as a percentage of total test time. The maximal values for recorded HR, minute ventilation and RER during the tests were 199 ± 4 beats.min $^{-1}$, 111 ± 22 l.min $^{-1}$ and 1.19 ± 0.09 respectively. Blood lactate concentrations at the end of the test were 9.8 ± 2.2 Mmol.l $^{-1}$.

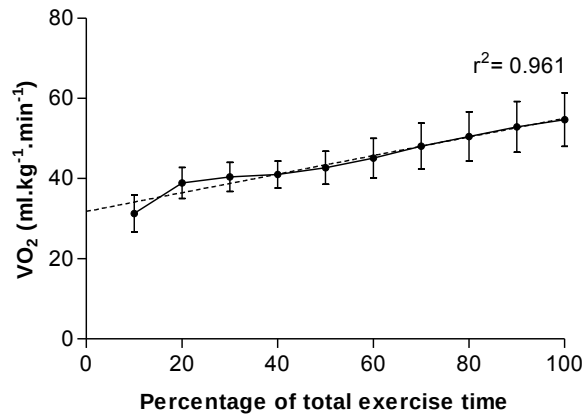


Figure 1: Mean ($\pm$ SD) $\dot{V}O_2$ for each 10% time interval for 63 subjects.

Depending on the method used, the incidence of the plateau phenomenon at the end of the $\dot{V}O_{2\max}$ test ranged from 16% to 82% (Table 1, Figure 2). In spite of this large spectrum, six methods identified a plateau in 44-49% of the participants. Six participants (9.5%) reached their $\dot{V}O_{2\max}$ in the next to last completed stage and therefore displayed a plateau regardless of the calculation method used.

Table 1: Prevalence of the $\dot{V}O_{2\max}$ plateau phenomenon using different criteria and data sampling intervals. The detection of the plateau phenomenon is performed between the final two stages of each individual test.

	Prevalence of $\dot{V}O_{2\max}$ plateau (%)		
	1 min	30 s	15 s
$\dot{V}O_2$ difference smaller than 2.1 ml.kg ⁻¹ .min ⁻¹	46.0%	69.8%	82.5%
$\dot{V}O_2$ difference smaller than 1.3 ml.kg ⁻¹ .min ⁻¹	23.8%	49.2%	63.5%
Half of expected $\dot{V}O_2$ difference from each subject	23.8%	47.6%	65.1%
Third of expected $\dot{V}O_2$ difference from each subject	15.9%	38.1%	52.4%
Half of expected $\dot{V}O_2$ difference from all subjects	25.4%	51.6%	61.9%
Third of expected $\dot{V}O_2$ difference from all subjects	17.5%	40.6%	49.2%

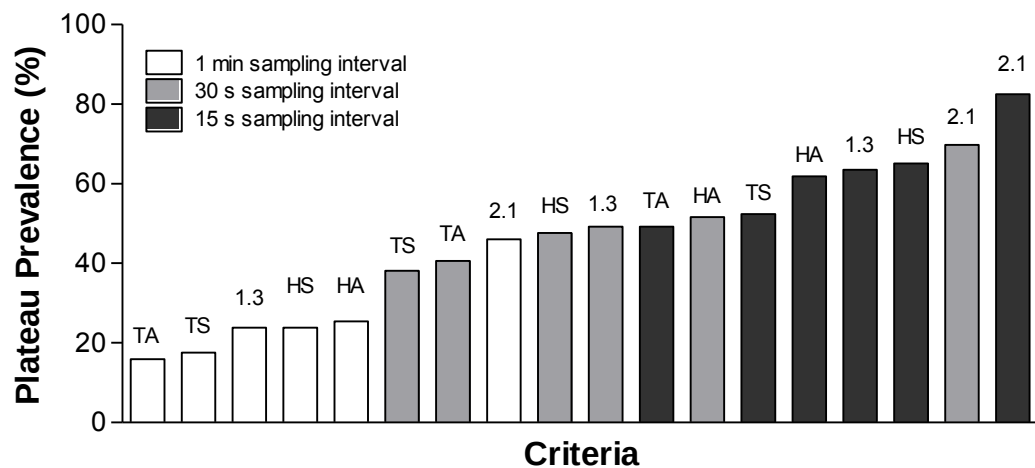


Figure 2: Percentage of “plateau phenomena” detected by different criteria and sampling intervals. TS, HS: plateau as a third (TS) or half (HS) the expected $\dot{V}O_2$ difference from each subject; TA, HA: plateau as a third (TA) or half (HA) of expected $\dot{V}O_2$ difference from all subjects; 2.1 and 1.3 refer to the rigid plateau criteria of 2.1 and 1.3 $\text{ml.kg}^{-1}.\text{min}^{-1}$, respectively.

Table 2 shows the total number of plateau phenomena identified by a single method (plateau defined as changes in $\dot{V}O_2$ smaller than half the average stage-to-stage $\dot{V}O_2$ difference from all subjects) during each stage change in all tests that were analyzed. These specific criteria identified 103 plateaus during the 379 workload changes in the total sample (table 2). However, 73 (70.9%) of these plateau phenomena were “false” by definition, since subjects continued exercising and reached higher $\dot{V}O_2$ on subsequent stages (table 2).

During the incremental tests, 68.9% of subjects achieved the criterion for HR, while 88.9% of participants attained the RER criterion. Both RER and HR cut-offs were generally achieved during the final portion of the tests ($81.9 \pm 18.1\%$ and $83.6 \pm 11.7\%$ of total time to exhaustion, respectively). However there were large deviations from the average and 19.0% of the subjects achieved the HR cut-off before 80% of their time to exhaustion, whereas the proportion for RER increased to 31.7% of participants achieving the cut-off before completing 80% of the exercise test. The efficacy of the secondary criteria used singly or in combination to confirm the presence of a plateau are presented in table 3, which shows the percentage of “false” and “true” plateau phenomena that were measured in combination with one, both or none of secondary

criteria used for confirming $\dot{V}O_{2\max}$. While most of the “false” plateau phenomena (73.3%) happened before the participants had achieved the secondary criteria for either HR or RER, most of the “true” (64.5%) plateau phenomena were measured when participants had already exceeded the secondary criteria thresholds.

Table 2: Rates of “false” and “true” plateau phenomena during the entire test.

	n	plateaus (n)	non plateaus (n)	end of tests plateaus (n)	“false” plateaus (n)	false-positive plateaus (%)	“true” plateaus (%)
stage 4 to 5	63	27	36	0	27	100,0%	0,0%
stage 5 to 6	63	6	57	0	6	100,0%	0,0%
stage 6 to 7	63	10	53	1	9	90,0%	10,0%
stage 7 to 8	62	14	48	3	11	78,6%	21,4%
stage 8 to 9	53	11	42	2	9	81,8%	18,2%
stage 9 to 10	42	20	22	11	9	45,0%	55,0%
stage 10 to 11	24	11	13	9	2	18,2%	81,8%
stage 11 to 12	8	3	5	3	0	0,0%	100,0%
stage 12 to 13	1	1	0	1	0	0,0%	100,0%
all	379	103	276	30	73	70.9%	29.1%

Plateau defined as changes in $\dot{V}O_2$ smaller than half the average stage-to-stage $\dot{V}O_2$ difference from all subjects. “False-positive” plateau means that the subject continued exercising to a higher $\dot{V}O_2$; “true” plateau means that the subject ended the test at the same time as the plateau phenomenon occurred.

Table 3: Percentage of “false” and “true” plateau phenomena which happened above the secondary criteria thresholds for heart rate (90% of age predicted maximum), and RER (>1.1).

	HR Only	RER Only	Both HR and RER	None
	[n (%)]	[n (%)]	[n (%)]	[n (%)]
False Plateau [n (%)]	3 (5.0%)	8 (13.3%)	5 (8.3%)	44 (73.3%)
True Plateau [n (%)]	2 (6.5%)	8 (25.8%)	20 (64.5%)	1 (3.2%)

HR, heart rate; RER, respiratory exchange ratio. The total number of plateau phenomena here (n=91) is lower here than the total of 103 reported in table 2 because some plateau phenomena lasted for more than two stages.

DISCUSSION

According to the model originally developed by Hill and colleagues (1924a), the heart eventually reaches its maximum pumping capacity during incremental exercise and becomes unable increase skeletal muscle blood flow. As a result an easily identified “plateau” should develop in which the $\dot{V}O_2$ reaches a clear maximum value without any further increase despite further increases in work rate. It is concluded that at that point oxygen delivery to the muscles becomes inadequate causing skeletal muscle hypoxia leading to a lactic acid acidosis, impaired skeletal muscle function and termination of exercise (Noakes, 1997; Noakes, 1998; Noakes & St Clair Gibson, 2004; Hawkins *et al.*, 2007; Levine, 2008; Noakes, 2008a; Noakes, 2008b; Noakes, 2011a; Noakes, 2011b). Indeed the iconic figure in the definitive article by Mitchell and Blomqvist (1971, figure 1) shows the presence of this absolute plateau for three consecutively higher workloads, each lasting 3 minute (Noakes, 2008a; Noakes, 2008b).

Accordingly, the first relevant finding of this study was that we identified the $\dot{V}O_2$ plateau phenomenon in as few as 16% or as many as 82% of the same tests depending on the criteria used to identify this phenomenon. This is in agreement with other published studies in which the reported incidence of the plateau phenomenon ranges from 35-76% in children (Armstrong *et al.*, 1996) and from 8-100% in adults

(Astorino *et al.*, 2000; Noakes & St Clair Gibson, 2004) depending on the calculation method and test protocol employed. It is important to note that whereas those different studies used a single method to analyze the results from a single data set, exploring at best the effects of different data sampling intervals, we used multiple methods to analyze the same data set. Thus to our knowledge, the data we present are unique.

Prevalence of the plateau phenomenon

The first study to define the plateau phenomenon mathematically (Taylor *et al.*, 1955) utilized the criterion that $\dot{V}O_2$ smaller than $150 \text{ ml} \cdot \text{min}^{-1}$ or $2.1 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ between two consecutive workloads were considered diagnostic of the plateau phenomenon. Although these rigid cut-off values have gained widespread popularity (Sidney & Shephard, 1977; Freedson *et al.*, 1986; Stachenfeld *et al.*, 1992; Rowland, 1993; Armstrong & Welsman, 1994; Sloniger *et al.*, 1996; Fielding *et al.*, 1997; Duncan *et al.*, 1997; Figueroa-Colon *et al.*, 2000; Karila *et al.*, 2001; Sargent *et al.*, 2002), it is clear that they were never intended to be a general criterion, but were instead meant to be specific to the participants in each particular study (Howley *et al.*, 1995). Of all the methods evaluated in the present investigation, the $2.1 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ criterion resulted in the highest prevalence of plateau phenomena and decreasing the sampling interval (from 60- to 30- to 15-second means) further increased the prevalence of the plateau phenomenon (figure 2). Higher sampling intervals increase the noise in the data (Howley *et al.*, 1995) so that the prevalence of the plateau phenomenon is greater when the sampling interval is smaller. The prevalence of the plateau phenomenon, according to the assumption that the measured $\dot{V}O_2$ is representative of a steady state period (Astorino *et al.*, 2000; Astorino *et al.*, 2005; Astorino, 2009). The standard deviation in $\dot{V}O_2$ increases substantially when smaller sampling intervals are used (Myers *et al.*, 1990) as $\dot{V}O_2$ measured over short time intervals does not change as a simple linear function of the work rate (Myers *et al.*, 1989; Myers *et al.*, 1990). Thus, even though $\dot{V}O_{2\text{max}}$ values show the same day-to-day variability independent of sampling interval (Midgley *et al.*, 2007a), smaller sampling intervals (breath-by-breath, up to 15 s) will result in a higher prevalence of the plateau due to larger variability between data points. Accordingly,

we suggest that sampling intervals between 30 s and 1 minute should be used when attempting to define the plateau phenomenon (Howley *et al.*, 1995).

Further evidence of the problem with generalizing the $2.1 \text{ ml.kg}^{-1}.\text{min}^{-1}$ or 150 ml.min^{-1} plateau criterion was provided by the work of Mitchell *et al.* (1958), who used similar load increments (2.5% increase in gradient) as in the original work of Taylor and colleagues (1955) but found much smaller “normal” increases in $\dot{V}\text{O}_2$ between stages ($142 \pm 44 \text{ ml.min}^{-1}$ versus $299 \pm 86.5 \text{ ml.min}^{-1}$ in the work of Taylor *et al.*). The plateau was determined as changes in $\dot{V}\text{O}_2$ smaller than the mean change during sub-maximal stages minus two standard deviations, which resulted in a cut-off value of 54 ml.min^{-1} . This is about a third of the value used by Taylor *et al.* (1955). More recent studies (Niemela *et al.*, 1980a; Niemela *et al.*, 1980b) using continuous protocols with shorter stages and smaller work increments suggest however that this method may lead to the over-identification of the plateau phenomenon. Currently a variety of plateau criteria are used by different authors (Noakes & St Clair Gibson, 2004). However, these usually do not take into account the changes in $\dot{V}\text{O}_2$ during sub-maximal stages for each participant of that specific study, rather relying on criteria published by others (Freedson *et al.*, 1986; Stachenfeld *et al.*, 1992; Gordon *et al.*, 2011; Gordon *et al.*, 2012). This may lead to criteria that are too strict or too loose, under- or over-estimating the proportion of tests that show the plateau phenomenon.

Prevalence of “false” plateau phenomenon

Indeed our second main finding was the high prevalence of plateau phenomena that occurred during the sub-maximal segments of the tests. We are not the first to identify this phenomenon (Myers *et al.*, 1989; Myers *et al.*, 1990; Duncan *et al.*, 1997; Foss & Hallen, 2004). Thus Myers *et al.* (1989; 1990) used a different evaluation method to show that the slope of the increase in $\dot{V}\text{O}_2$ during incremental exercise continually changes and at several times it was equal to zero (indicating the presence of the plateau phenomenon) during sub-maximal exercise. Due to a large variability in the slope of $\dot{V}\text{O}_2$ changes especially during sub-maximal exercise, the authors concluded that the plateau concept has limited application during exercise testing (Myers *et al.*, 1989).

The present investigation showed (table 2) that 71% of all plateau phenomena detected during exercise are “false-positives” since participants continued to exercise to higher work rates and $\dot{V}O_2$. The “plateau phenomenon” during sub-maximal exercise cannot be causally-linked to the biological events that cause the termination of exercise and must therefore be the result of poorly understood or measured phenomena (Howley *et al.*, 1995; Atkinson *et al.*, 2005) including biological variation (Macfarlane, 2001). Indeed these findings raise the interesting conundrum: currently the sole criterion determining whether or not a plateau phenomenon is “true” is the timing when it occurs. Thus it is assumed that a plateau phenomenon that occurs at the instant of exercise termination must be “true”. But such temporal association does not prove causation. Instead it might be that some “true” plateaus are in reality also artifactual as they are related temporarily but not causally to the termination of exercise.

However it is clear that the original mechanism invoked by Hill and colleagues to explain the appearance of the definitive plateau phenomenon – the “sensing” of coronary blood desaturation and consequently reduction of cardiac output (Noakes, 1997; Noakes & St Clair Gibson, 2004; Noakes, 2008a; Noakes, 2008b) – cannot be correct, since neither myocardial ischemia (Raskoff *et al.*, 1976; Noakes & St Clair Gibson, 2004; Calbet *et al.*, 2007; Brink-Elfegoun *et al.*, 2007) nor cardiac failure (Foster *et al.*, 1997; Calbet *et al.*, 2007; Brink-Elfegoun *et al.*, 2007) develops at the “plateau phenomenon”. It is clear that the exact biological events that cause both “true” and “false” plateau phenomena remain to be established (Myers *et al.*, 1989; Myers *et al.*, 1990).

Secondary criteria

It has been extensively demonstrated that the plateau is not a pre-requisite for the achievement of $\dot{V}O_{2max}$ (Duncan *et al.*, 1997; Hawkins *et al.*, 2007; Midgley *et al.*, 2007b; Midgley & Carroll, 2009), which is often termed $\dot{V}O_{2peak}$ (Day *et al.*, 2003). As a result the use of the plateau phenomenon as a criterion for determining maximal aerobic capacity has been criticized (Alpert, 1992; Noakes, 1998) and defended (Bergh *et al.*, 2000). On the basis that the plateau phenomenon is not a reliable criterion to

confirm the attainment of $\dot{V}O_{2\max}$, the use of secondary criteria based on cut-off values for HR, RER and blood lactate concentration has been suggested (Duncan *et al.*, 1997).

Accordingly, the third important finding of this investigation is that the use of secondary criteria to confirm the attainment of $\dot{V}O_{2\max}$ largely reduced the number of false-positive plateau phenomena (table 3). However secondary criteria were generally reached early in exercise, well before exhaustion. Thus their use as criteria for indicating maximal exercise would result in underestimation of $\dot{V}O_{2\max}$, as also noted by others (Duncan *et al.*, 1997; Poole *et al.*, 2008). Stachenfield *et al.* (1992) reported that most of their participants achieved RER values above 1.0 and 1.15 and HR above 85% of age-predicted. However these “maximal” values were also reached in tests in which no plateau was detected, so that these criteria are of little help to confirm the presence of a “true” plateau phenomenon. In the present investigation ~27% of all “false” plateau phenomena were confirmed by at least one secondary criteria, whereas only one (3.2%) “true” plateau occurred without fulfilment of any secondary criteria.

In the current investigation more participants reached the RER criterion for maximal exercise (>1.10) than achieved the HR criterion ($>90\%$ of age-predicted maximum), a finding reported by others (Freedson *et al.*, 1986; Baquet *et al.*, 2003) but not by all (Christie & Lock, 2009). This is probably a reflection of the high variability in maximal HR in the general population in contrast to age-predicted values. This difference is more pronounced in younger individuals (Baquet *et al.*, 2003). Alternatively, a minimum blood [La] of 8 mMol.l^{-1} achieved at exhaustion has been suggested as a better criterion for defining a maximal exercise test (Stachenfeld *et al.*, 1992). Comparing blood [La] data between studies however requires consideration of differences in [La] measurement methods, including the site of measurement and timing (Howley *et al.*, 1995). In the present study, most participants attained this threshold for blood lactate concentration.

Limitations and technical considerations

An important consideration is that this study was carried out analyzing data collected from adolescent boys, who can be characterized as circumpuberal (Baquet *et*

al., 2003). Boys and girls this age and younger have been shown to behave similarly to older participants during incremental exercise in terms of cardiovascular function (Rowland *et al.*, 1997; Armstrong & Welsman, 2001), though adaptation to aerobic training may be slightly smaller (Baquet *et al.*, 2003). Since this study was performed as a single test, this is of little significance. It has also been noted that children have limited anaerobic capacity compared to adults (Tolfrey & Armstrong, 1995), and this could be important when trying to extrapolate our results to the general population, since the anaerobic capacity is seen as an important pre-requisite for developing the plateau phenomenon (Lacour *et al.*, 2007; Shephard, 2009). The participants in the present study however reached maximal values for RER and blood lactate concentrations similar to those measured in adults during maximal exercise (Tolfrey & Armstrong, 1995). Thus we believe that until contradictory evidence becomes available our findings can likely be generalized to other populations.

Indeed, the existence of the plateau phenomenon has been described in a variety of populations (Sidney & Shephard, 1977; Armstrong *et al.*, 1991; Fielding *et al.*, 1997; Figueroa-Colon *et al.*, 2000; Armstrong & Welsman, 2001) in both the healthy (Boone *et al.*, 2010) and diseased (Moore *et al.*, 1993; Sargent *et al.*, 2002) of all ages (Sidney & Shephard, 1977; Rowland, 1985; Fielding *et al.*, 1997; Armstrong & Welsman, 2001). Different diseases are substantially different in the manner in which they affect exercise capacity (Moore *et al.*, 1993; Sargent *et al.*, 2002) so that the criteria for detecting the plateau phenomenon and secondary criteria used to judge maximal effort should be case and disease-specific. It is important that regardless of the population studied the criterion used for establishing a plateau in oxygen consumption, and thus a maximal effort, should consider expected changes in $\dot{V}O_2$ from sub-maximal workloads before establishing the cut-off values for defining the plateau phenomenon. Despite the evidence that criteria based either on fixed values (Mitchell *et al.*, 1958; Armstrong *et al.*, 1996; Hawkins *et al.*, 2007) or relative changes in $\dot{V}O_2$ (Armstrong *et al.*, 1995; Rossiter *et al.*, 2006) can result in similar prevalence of the plateau phenomenon (Armstrong *et al.*, 1995), we suggest that the relative change method (using each individual subject's change in $\dot{V}O_2$) should be preferred since it is more sensitive to individual variability and efficiency in $\dot{V}O_2$ with increases in work rate

(Boone *et al.*, 2010). However the present investigation confirms the findings of others (Myers *et al.*, 1989; Myers *et al.*, 1990; Rowland, 1993; Duncan *et al.*, 1997; Figueroa-Colon *et al.*, 2000; Doherty *et al.*, 2003) showing the limited value of trying to differentiate $\dot{V}O_{2peak}$ from $\dot{V}O_{2max}$ (Day *et al.*, 2003; Doherty *et al.*, 2003), as laboratory and field tests have unequivocally resulted in similar or overestimated $\dot{V}O_{2max}$, regardless of the presence of the plateau phenomenon (Rowland, 1993; Rossiter *et al.*, 2006; Midgley *et al.*, 2007b).

Summary

In summary, this study showed that the prevalence of the plateau phenomenon varied from 16-82% depending on the criteria used to detect the phenomenon. Therefore the criteria used to define the plateau phenomenon will be a major determinant of the frequency with which the plateau is detected. This does not make biological sense if the plateau phenomenon is as Hill and colleagues originally described it. We also showed that about 71% of the plateaus that developed during an incremental exercise test were “false positives” that occurred during sub-maximal exercise, despite being “confirmed” by secondary criteria based on target RER and HR values, confirming the original observation of Myers and colleagues (1989; 1990).

These data cast doubt on the use of the plateau phenomenon as the indicator of a “true” $\dot{V}O_{2max}$. The use of secondary criteria reduces the prevalence of the plateau but also leads to substantial under-estimation of $\dot{V}O_{2max}$.

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Chapter 4

Traditional testing produces sub-maximal values of oxygen consumption in humans

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ABSTRACT

Introduction

Uphill running incremental exercise tests are thought to produce the highest values of maximal oxygen uptake ($\dot{V}O_{2\max}$) in trained subjects. This study used a novel exercise protocol to test the hypothesis that a plateau in $\dot{V}O_{2\max}$ during incremental exercise testing to exhaustion represents the maximal capacity of the cardiovascular system to transport oxygen.

Methods

Twenty six subjects were randomly divided into two groups matched by their initial $\dot{V}O_{2\max}$. On separate days, the Reverse group performed (i) an incremental uphill running test on a treadmill (INC_1) followed by a verification test (VER) at a constant workload 1 km.h⁻¹ higher than the last completed stage in INC_1 ; (ii) a decremental test (DEC) in which the initial speed was the same as that in the VER but was reduced progressively thereafter; and (iii) a final incremental test (INC_F). The Control group performed the INC only on the same days that the Reverse group performed their tests.

Results

$\dot{V}O_{2\max}$ remained within 0.6 ml.kg⁻¹.min⁻¹ across the 3 trials for the Control group ($p = 0.93$) but was 4.4% higher during DEC compared to INC_1 (63.9 ± 3.8 vs. 61.2 ± 4.8 ml.kg⁻¹.min⁻¹ respectively, $p = 0.004$) in the Reverse group, even though speed at $\dot{V}O_{2\max}$ was lower (14.3 ± 1.1 vs. 16.2 ± 0.7 km.h⁻¹ for DEC and INC_1 respectively, $p = 0.0001$).

$\dot{V}O_{2\max}$ remained significantly higher during INC_F ($63.6 \pm 3.68 \text{ ml.kg}^{-1}.\text{min}^{-1}$, $p = 0.01$), despite an unchanged exercise time between INC_1 and INC_F . Heart rate, ventilation and respiratory exchange ratio at $\dot{V}O_{2\max}$ were the same in INC_1 , DEC and INC_F .

Discussion

These findings are not compatible with the original theory that a plateau in oxygen consumption measured during the classically-described INC and VER represents a systemic limitation in oxygen use. The reasons for the higher $\dot{V}O_2$ during INC_F following the DEC test are unclear.

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INTRODUCTION

In 1923 Nobel Laureate Archibald Hill and his colleagues (1923; 1924) proposed that the body has a limited capacity to consume oxygen during intense exercise. Subsequent studies (Taylor *et al.*, 1955; Taylor *et al.*, 1963; Mitchell & Blomqvist, 1971) refined the original testing methods and honed the theory that the occurrence of a plateau in oxygen intake ($\dot{V}O_2$) despite an increasing workload represents the maximal capacity of the cardiovascular system to transport oxygen to the exercising muscles (Mitchell *et al.*, 1958). Thus in 1971 Mitchell and Blomqvist (1971) proposed that: “there is a linear relationship between workload and oxygen uptake until the maximal oxygen uptake is reached. Heavier workloads can usually be achieved, but oxygen uptake levels off or may even decline” (p. 1018).

Considering that the human body is a closed system, there must be a finite capacity for extracting oxygen from the atmosphere and for it to be used by the exercising muscles. The debate however is whether the plateau phenomenon measured during incremental exercise tests to exhaustion accurately identifies this ceiling and, if so, what are the implications of this phenomenon. In the past 80 years no study has yet convincingly challenged the original conclusion that a plateau in $\dot{V}O_2$ ($\dot{V}O_{2max}$) measured with the conventional incremental exercise testing protocol (INC) represents the absolute true maximal capacity of the cardiovascular system to transport oxygen (Day *et al.*, 2003; Rossiter *et al.*, 2006; Hawkins *et al.*, 2007; Brink-Elfegoun *et al.*, 2007a; Duffield & Bishop, 2008). This finding is therefore interpreted in favour of the original (Hill) theory. One of the most recent scholarly review of the topic concludes: “athletes stop exercising at $\dot{V}O_{2max}$ because of severe functional alterations at the local muscle level due to what is ultimately a limitation in convective oxygen transport” (Levine, 2008) (p. 31).

However a few studies have indeed shown that higher $\dot{V}O_{2max}$ values can occasionally be achieved when either different incremental exercise protocols are used (Kindermann *et al.*, 1980; Fielding *et al.*, 1997; Yoon *et al.*, 2007) or during testing in the heat (Lafrenz *et al.*, 2008) or when subjects exercise at progressively increasing

rates of perceived exertion (RPE) (Mauger & Sculthorpe, 2012). Conversely some argue that a plateau in $\dot{V}O_2$ is an inconstant phenomenon, consistently difficult to demonstrate and which may also occur during sub-maximal exercise (Myers *et al.*, 1989; Myers *et al.*, 1990). Despite these contradictory findings, it is currently accepted that a true $\dot{V}O_{2max}$ is always achieved during uphill treadmill running tests as confirmed by the same or lower $\dot{V}O_{2max}$ during subsequent exercise at workloads higher than that achieved at $\dot{V}O_{2max}$ during the traditional INC (Brink-Elfegoun *et al.*, 2007a; Hawkins *et al.*, 2007).

While it is fair to conclude that higher-than-maximal, constant speed tests do not result in higher $\dot{V}O_{2max}$ values than those achieved during traditional INC (Rossiter *et al.*, 2006; Midgley & Carroll, 2009), it is somewhat surprising that sub-maximal decremental exercise protocols produce higher than expected $\dot{V}O_2$ when compared to a similar power output during an incremental protocol (Yano *et al.*, 2003a). One of the possible explanations for this finding is that the body starts “paying” the so-called “ O_2 deficit” as it moves from higher towards lower, more *aerobic* work rates (Yano *et al.*, 2003b), thus increasing oxygen consumption beyond predicted levels.

In light of the investigations that have attempted unsuccessfully to elicit higher-than-maximal $\dot{V}O_{2max}$ values during exercise at (supra-maximal) workloads greater than that at which the $\dot{V}O_{2max}$ was measured (Brink-Elfegoun *et al.*, 2007a; Hawkins *et al.*, 2007), we decided to evaluate the effects of a novel “reverse” testing protocol in which the exercise began at a high running speed and then slowed progressively. We reasoned that if subjects knew beforehand that the test would become progressively easier the longer it continued, the possibility was that any biological controls directing the termination of exercise (Noakes & Marino, 2009b; Noakes, 2011b) might be relaxed. This would allow the achievement of a $\dot{V}O_{2max}$ higher than that achieved with conventional INC testing. Here we report the results of $\dot{V}O_{2max}$ testing using this novel protocol.

METHODS

Subjects

Twenty six participants involved in regular running / cross country skiing training [23 male, 3 female, age 29.0 ± 10.0 years (range 17 – 47), body mass 73.7 ± 9.8 kg, height 177 ± 6 cm] were recruited. All participants were injury-free for the duration of the trials and gave their written informed consent (parental consent if applicable) to participate in this investigation, which was approved by the Research and Ethics Committee of the University of Cape Town and all other institutions where trials were performed. The trials were conducted in three different laboratories: Sogn og Fjordane University College (Norway, $n = 18$), University of Bedfordshire (UK, $n = 6$) and Charles Sturt University (Australia, $n = 2$). Collecting data from three different institutions partially prevented our data from possible experimenter or equipment bias.

Study Design

The participants visited the laboratory on five occasions (figure 1), each separated by at least 48 hour. After performing a maximal INC during the first two visits, the participants were matched for their $\dot{V}O_{2max}$ and randomly divided into two groups. While visit one served for familiarization purposes only, visit two was used to establish the $\dot{V}O_{2max}$ of the participants during an uphill running incremental test (INC₁). The Control group performed incremental tests to fatigue on the next three visits, while the Reverse group performed a familiarization decremental test on visit three, a tailored decremental test on their fourth visit (DEC), and a repeat incremental test on their fifth visit (INC_F) (figure 1).

The participants were instructed to avoid hard training sessions for the 24 hours preceding each visit and not to ingest caffeine for 6 hours before the tests. Tests were scheduled at the same time of the day and laboratory conditions were stable (temperature 20 ± 0.5 °C, humidity 46 ± 3 %) for the duration of the study. All tests were performed on a motor-driven treadmill, with a constant inclination of 5%. All trials were completed within three weeks per participant.

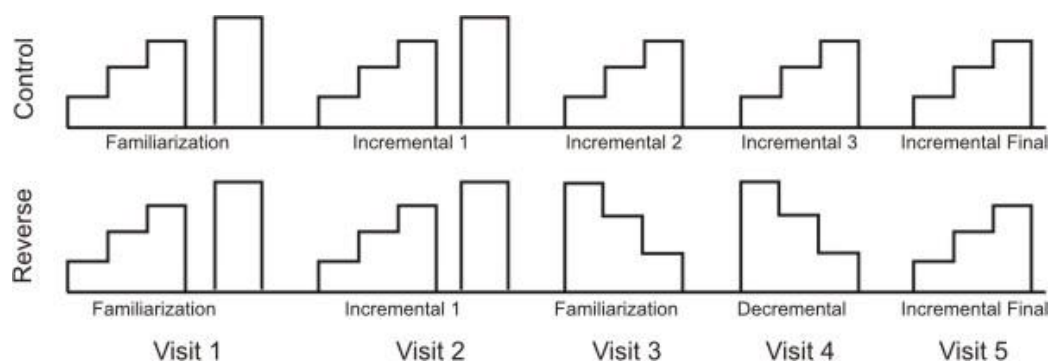


Figure 1: Diagram showing the timing of the different testing sessions. Sessions were separated by at least 48h.

Incremental test

All INC were preceded by a 10 minute warm-up (5 minutes at 10 km.h⁻¹, five minutes at 12 km.h⁻¹). The tests started at 9 km.h⁻¹ for men and 7 km.h⁻¹ for women, and the speed was increased by 1 km.h⁻¹ every minute (therefore one minute is equivalent to one stage) until subjects were unable to continue the test. On the first (familiarization) and second visit, a verification test (VER) was performed 15 minutes after the end of the incremental test. In between the two tests the participants were allowed to walk, jog or rest. The VER began at 10 km.h⁻¹ and 5% inclination for one minute. The speed was then increased to 1 km.h⁻¹ higher than the last stage completed by the participant during INC. Participants were instructed to run at that speed for as long as each could.

Decremental test

The protocol for the decremental (DEC) tests was established as a function of the performance from INC and VER. Following the same warm-up, the test started with a 1 minute run at 10 km.h⁻¹ and 5% inclination. Thereafter the treadmill speed was increased to that used during VER. Subjects ran at that speed for 60% of the time each had managed during the VER. After the initial stage, which usually lasted around one minute, the treadmill speed was decreased by 1 km.h⁻¹ and maintained for 30 seconds. This was followed by consecutive decrements of 0.5 km.h⁻¹ that were maintained for

30 s, 45 s, 60 s, 90 s and 120 s respectively (figure 3). This standard approach was used on visit 3 (familiarization with DEC). Depending on the reaction of the participant to the familiarization DEC protocol, the durations of the stages on visit 4 were modified by either shortening or lengthening so that each subject would require at least five minutes before becoming exhausted (figure 3). An extra 30 seconds warm-up stage ($12\text{--}13 \text{ km}\cdot\text{h}^{-1}$) was also included on visit 4 before the treadmill speed was increased to the starting speed for the test in order to diminish the gap in speed between the warm-up and the high-intensity start of the test.

Instruments and data handling

All respiratory variables were measured using automated gas analyzer systems (MOXUS Modular Metabolic System, AEI Technologies, IL, USA; $n = 18$; ParvoMedics, True2400, East Sandy, Utah, USA; $n = 2$; Cortex Metalyser 11R, Cortex GmbH, Leipzig, Germany; $n = 6$). Prior to the trials, both the analyzers and their respective softwares (only the Moxus requires this step) were calibrated strictly according to the manufacturers' recommendations. Ventilation measurements were calibrated using a 3 L calibration syringe (Hans-Rudolph, Kansas City, MO, USA).

Samples of expired air were continuously drawn into the analyzers to calculate the fractions of O_2 and CO_2 . The data were fed into a PC that calculated the results by programs developed by the manufacturers. Heart rate data were monitored continuously using a Polar S410 heart rate monitor (Polar Electro OY, Kempele, Finland), synchronized with the gas analyzer software.

All physiological data were collected breath-by-breath, exported from the analyzer software into Excel spreadsheets and calculated as consecutive 30 seconds intervals. During INC, data were considered until the end of the final completed stage. Data collected during VER and DEC were considered up to the time that a final complete 30 second sample had been collected. This was done to ensure that the comparison between INC and VER was done at two different workloads, as required to define a plateau in $\dot{\text{V}}\text{O}_2$. Further calculations revealed that this procedure had no impact on the $\dot{\text{V}}\text{O}_{2\text{max}}$ values for INC (data not shown). A plateau in $\dot{\text{V}}\text{O}_2$ during INC was accepted if the change in $\dot{\text{V}}\text{O}_2$ during the highest 30 second interval of the two final

stage the test was less than half of the normal stage-to-stage change in $\dot{V}O_2$ during the initial (linear) parts of the tests for each subject. The average stage to stage difference in $\dot{V}O_2$ for all participants was calculated as $246 \pm 37 \text{ ml}\cdot\text{min}^{-1}$, that plateau phenomenon was defined as a change in $\dot{V}O_2 < 123 \pm 18 \text{ ml}\cdot\text{min}^{-1}$ (or an average of $1.7 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, considering the average body mass of the participants) between the two final stages of the test. The same criterion was used to define plateau in $\dot{V}O_2$ between the peak $\dot{V}O_2$ values measured during both INC and VER, since VER was performed at one stage higher than the maximal stage completed during INC.

Statistics

All data are presented as mean $\pm$ SD. $\dot{V}O_{2\max}$ and time to fatigue values for groups Control and Reverse during INC₁ were compared using Student's *t*-test for independent samples. To investigate possible differences in the physiological variables during the trials, repeated measures ANOVA was performed, and a Tukey *post-hoc* test was used when appropriate. All calculations were performed using StatSoft Inc., Tulsa, OK, USA). The typical error in the day-to-day change in $\dot{V}O_2$ for the control group was calculated using an Excel spreadsheet (Hopkins, 2000). The effect-size difference was determined by calculating Cohen's *d* (defined as the difference between mean divided by the square root of the pooled squared standard deviations) when appropriated.

RESULTS

There were no differences in $\dot{V}O_{2\max}$ between the Reverse and Control groups (61.2 ± 4.9 vs. $61.3 \pm 7.8 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ respectively, $p = 1.000$) during INC₁, indicating a similar level of aerobic fitness between the groups. Although there was a small difference in time to exhaustion for INC₁ (8.71 ± 0.62 vs. 8.58 ± 1.52 minutes respectively, $p = 0.004$), this represented only 7.8 seconds of exercise and is likely of little or no biological significance. Moreover, Cohen's magnitude effect was this difference was only 0.11, which is interpreted as "trivial". Four participants in each

group did not develop a plateau in $\dot{V}O_2$ during INC₁. Only one participant in each group however did not demonstrate the plateau phenomenon in VER. Interestingly these two participants displayed a plateau during INC₁ alone. $\dot{V}O_{2max}$ during VER was 0.1 ± 2.3 ml.kg⁻¹.min⁻¹ lower for the Control group and 0.3 ± 1.2 ml.kg⁻¹.min⁻¹ higher for the Reverse group compared to the values measured in their respective INC₁.

All physiological variables measured at $\dot{V}O_{2max}$ are listed in table 1. $\dot{V}O_{2max}$ remained constant for the Control group over the different testing days, as did time to fatigue. The typical error for $\dot{V}O_{2max}$ in the Control group over the four INC was 1.6 ml.kg⁻¹.min⁻¹ (95% Confidence Interval 1.3 to 2.1 ml.kg⁻¹.min⁻¹). On the other hand, $\dot{V}O_{2max}$ for the Reverse group was 4.4% higher during DEC compared to INC₁ (63.9 ± 3.8 vs. 61.2 ± 4.8 ml.kg⁻¹.min⁻¹ respectively, $p = 0.004$; 0.62 or “moderate” effect using Cohen’s scale for effect magnitude) (table 1, figure 2). The difference in $\dot{V}O_{2max}$ between INC₁ and DEC was on average 2.7 ± 3.1 ml.kg⁻¹.min⁻¹ (~ 200 ml.min⁻¹), which is 60% higher than the threshold for a $\dot{V}O_2$ plateau during INC₁ (1.7 ml.kg⁻¹.min⁻¹, 123 ml.min⁻¹), even though the subjects were running at about 1.9 km.h⁻¹ slower during DEC compared to INC₁ (table 1). $\dot{V}O_{2max}$ remained significantly higher during INC_F than during INC₁ for the reverse group (63.6 ± 3.8 vs. 61.2 ± 4.8 ml.kg⁻¹.min⁻¹, $p = 0.015$), despite similar values for time to fatigue and speed at $\dot{V}O_{2max}$ during both trials.

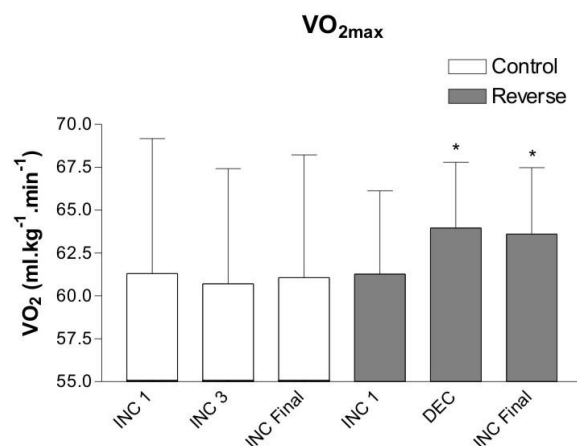


Figure 2: Mean $\pm$ SD for maximal oxygen consumption ($\dot{V}O_{2max}$) during the different trials for the Decremental (A) and Control (B) group. Dotted lines represent individual results. INC 1, Incremental test 1; INC 3, Incremental test 3; INC Final, Final Incremental test; DEC, Decremental test. * indicates significant different from respective INC 1 ($p < 0.05$).

There were no differences in values for ventilation, heart rate, breathing frequency, and respiratory exchange ratio measured at $\dot{V}O_{2\max}$ between the trials for any of the groups, except for ventilation for the control group between INC₁ and INC_F (157.0 ± 25.6 vs. 146.5 ± 24.0 L.min⁻¹, $p = 0.013$; Cohen's d 0.4 or “small”). A graphic illustration of the relationship between $\dot{V}O_2$ and speed in the different trials for one illustrative subject is presented in figure 3.

Table 1: Physiological variables for control and reverse group during the different trials.

	Control group			Reverse group		
	Incremental 1	Incremental 3	Incremental final	Incremental 1	Decremental	Incremental final
$\dot{V}O_{2\max}$	61.3 ± 7.8	60.7 ± 6.7	61.0 ± 7.1	61.2 ± 4.8	$63.9 \pm 3.8^*$	$63.6 \pm 3.8^*$
TTF	8.58 ± 1.52	8.53 ± 1.27	8.45 ± 1.27	$8.71 \pm 0.62^\dagger$	$6.08 \pm 0.52^*$	8.80 ± 0.93
$\dot{V}_E$	157.0 ± 25.6	149.2 ± 28.0	$146.5 \pm 24.0^*$	153.2 ± 26.2	154.5 ± 23.4	153.4 ± 23.7
HR	185.7 ± 11.0	182.1 ± 12.0	182.9 ± 13.2	182.9 ± 14.9	179.2 ± 13.1	183.7 ± 9.7
BR	53.4 ± 5.8	50.0 ± 7.2	51.1 ± 6.4	51.6 ± 6.5	51.3 ± 5.8	50.0 ± 7.8
RER	1.15 ± 0.07	1.12 ± 0.08	1.11 ± 0.07	1.15 ± 0.06	1.07 ± 0.06	1.09 ± 0.08
$V \cdot \dot{V}O_{2\max}$	15.7 ± 1.6	15.4 ± 1.2	15.6 ± 1.2	16.2 ± 0.9	$14.3 \pm 1.1^*$	16.3 ± 0.9

All data presented as mean $\pm$ SD. $\dot{V}O_{2\max}$, maximal oxygen consumption (ml.kg⁻¹.min⁻¹); TTF, time to fatigue (min); $\dot{V}_E$, ventilation (l.min⁻¹); HR, heart rate (beats.min⁻¹); BR, breathing rate (cycles.min⁻¹); RER, respiratory exchange ratio; $V \cdot \dot{V}O_{2\max}$, speed at $\dot{V}O_{2\max}$ (km.h⁻¹). * Different from respective Incremental 1 at $p < 0.05$. † Different from control group at the same time at $p < 0.05$.

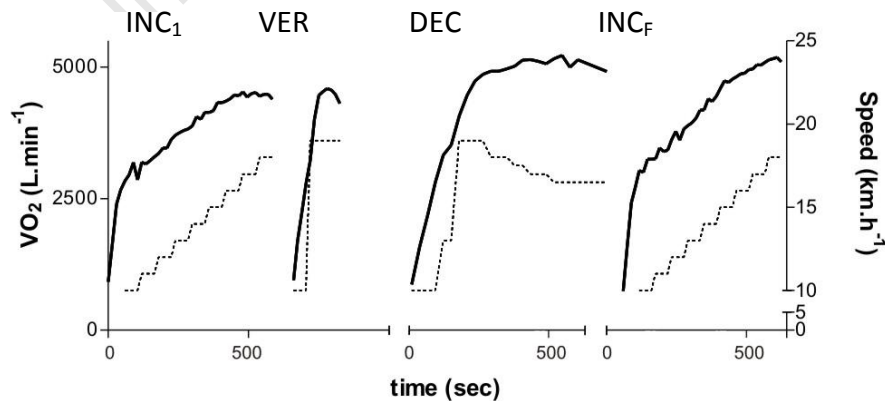


Figure 3: Oxygen consumption and treadmill speed during different trials for the participant with the second highest $\dot{V}O_{2\max}$ difference between INC₁ and DEC. The four trials presented are: INC₁, VER, DEC, INC_F. For this particular subject, $\dot{V}O_2$ was 70 ml.min⁻¹ (1.5 %) higher during VER, 683 ml.min⁻¹ (15.1 %)

higher during DEC and $640 \text{ ml}\cdot\text{min}^{-1}$ (14.2 %) higher during INC_F in comparison to INC₁. $\dot{V}\text{O}_2$ is represented by the solid lines, the dotted lines represent speed.

DISCUSSION

After its original conception in 1923 (Hill & Lupton, 1923; Hill *et al.*, 1924), the theory that a plateau in $\dot{V}\text{O}_2$ at the end of an incremental exercise test to exhaustion indicates an ultimate physiological ceiling in cardiovascular function was rapidly adopted (Taylor *et al.*, 1955; Mitchell *et al.*, 1958; Taylor *et al.*, 1963; Mitchell & Blomqvist, 1971). Only more recently has this view been challenged (Noakes, 1997; Doherty *et al.*, 2003; Noakes, 2008; Noakes & Marino, 2009a; Noakes & Marino, 2009b;) and vigorously defended (Bassett & Howley, 1997; Howley, 2007; Hawkins *et al.*, 2007; Brink-Elfegoun *et al.*, 2007b; Levine, 2008; Shephard, 2009). To counter some of this criticism, the so-called verification test (VER) emerged (Rowland, 1993; Day *et al.*, 2003; Rossiter *et al.*, 2006; Midgley *et al.*, 2007; Hawkins *et al.*, 2007; Foster *et al.*, 2007; Duffield & Bishop, 2008; Midgley *et al.*, 2009; Midgley & Carroll, 2009). This requires the participant to exercise for as long as possible at a higher power output than that achieved during the conventional INC. If the peak $\dot{V}\text{O}_2$ measured during VER is no higher than that achieved during INC, it is assumed that a true plateau in oxygen consumption had developed during INC and VER (Brink-Elfegoun *et al.*, 2007a; Brink-Elfegoun *et al.*, 2007b). The adoption of the VER method substantially increased the frequency with which a plateau in $\dot{V}\text{O}_2$ was detected and further strengthened the original theory on which the plateau concept is founded (Midgley *et al.*, 2007; Lafrenz *et al.*, 2008; Midgley & Carroll, 2009; Scharhag-Rosenberger *et al.*, 2011).

$\dot{V}\text{O}_{2\text{max}}$ during DEC

The first important finding of this study was that the $\dot{V}\text{O}_{2\text{max}}$ measured with the conventional INC is not the highest value of which each individual is capable, even when VER ensured that the original INC did indeed produce a “maximal” result. Instead the novel “reverse” protocol produced $\dot{V}\text{O}_{2\text{max}}$ values that were about 4.4% higher than those achieved with the conventional INC.

While studies using DEC have demonstrated an excess $\dot{V}O_2$ during sub-maximal exercise compared to values measured during INC (Yano *et al.*, 2000; Yano *et al.*, 2004; Yano *et al.*, 2007a; Yano *et al.*, 2007b; Arimitsu *et al.*, 2008), our study is the first to show this phenomenon during maximal exercise. The increase in $\dot{V}O_2$ measured during DEC (2.7 ml.kg⁻¹.min⁻¹ in this study) was substantially greater than the value (1.7 ml.kg⁻¹.min⁻¹) which defined the plateau threshold during INC and which was present in all but one subject in each group during the INC₁ (Rossiter *et al.*, 2006; Hawkins *et al.*, 2007). In fact, the increase in $\dot{V}O_2$ measured in this study is almost of the magnitude that should have enabled the runners to perform an additional stage during INC assuming that the increase in $\dot{V}O_2$ during that stage was a linear function of the increase in work rate during the sub-maximal portion of the test) (Taylor *et al.*, 1955; Doherty *et al.*, 2003). In addition, the 2.7 ml.kg⁻¹.min⁻¹ increase in $\dot{V}O_2$ was greater than the 2.1 ml.kg⁻¹.min⁻¹ upper limit of the 95% confidence interval of the typical error of measurement calculated for the participants in the control group. It was also greater than the difference in $\dot{V}O_{2max}$ between trials (Atkinson *et al.*, 2005; Midgley *et al.*, 2007). Therefore, the increase in $\dot{V}O_{2max}$ are accepted in the literature as indicators of improved aerobic capacity in elite alpine skiers (Gross *et al.*, 2009), elite runners after altitude training (Stray-Gundersen *et al.*, 2001), and well trained runners after "high-altitude" training (Levine & Stray-Gundersen, 1997).

This finding therefore convincingly shows that subjects terminated the conventional INC, appropriately conducted according to all required protocols (including the use of VER), with cardio-respiratory reserve and therefore before an absolute limitation in either oxygen delivery to, or its use by the exercising muscles had been reached (Noakes, 1997; Noakes & St Clair Gibson, 2004; Noakes, 2008). Also, it goes against the conventional belief (Mortensen *et al.*, 2005; Calbet *et al.*, 2007; Levine, 2008) and confirms our initial hypothesis that a plateau in $\dot{V}O_2$ during an uphill running INC does not provide the final proof that the body has reached an absolute limit in its capacity to consume oxygen.

Other studies have shown that the $\dot{V}O_{2max}$ measured during conventional INC may be less than that achieved when subjects are either free to choose their power outputs (Foster *et al.*, 1993; Foster *et al.*, 1997) or during an exercise test using the

ratings of perceived exertion to regulate the progressive increases in work rates (Mauger & Sculthorpe, 2012). Our study however differs from previous attempts in that we used subjects with high levels of aerobic capacity (Shephard, 2009), employed a VER to confirm the attainment of $\dot{V}O_{2max}$ during INC₁ (Rossiter *et al.*, 2006; Midgley *et al.*, 2009; Midgley & Carroll, 2009), and chose a graded treadmill running INC test that produces the higher $\dot{V}O_{2max}$ values (Kang *et al.*, 2001; Brink-Elfegoun *et al.*, 2007a; Hawkins *et al.*, 2007). More importantly, our subjects did not exercise at higher power outputs during the DEC than during INC or VER. This satisfies the issue of different muscle masses required to complete the different tests (Marcora & Staiano, 2010; Burnley, 2010; Allen & Westerblad, 2010; MacIntosh & Fletcher, 2011).

Effects of absolute exercise intensity on $\dot{V}O_{2max}$

Our second unexpected finding was that during the reverse protocol subjects reached their $\dot{V}O_{2max}$ when running at about 90% of the speed associated with $\dot{V}O_{2max}$ during INC. Several studies (Billat *et al.*, 1995; Billat *et al.*, 1998; Billat *et al.*, 1999; Billat *et al.*, 2000; Ozyener *et al.*, 2001) have investigated the physiological effects of running to exhaustion at constant speeds corresponding to ~90%-105% of the speed associated with $\dot{V}O_{2max}$. None found a higher $\dot{V}O_{2max}$ during these constant load trials compared to INC, even when a bout of intense exercise was performed prior to the constant load test (Ferguson *et al.*, 2007). This establishes that the higher $\dot{V}O_{2max}$ found during the reverse protocol is not an effect of the lower speed per se, but rather an effect of the protocol design.

The body's capacity to deliver oxygen to the muscles is usually thought to be the factor "limiting" the $\dot{V}O_{2max}$ (Stray-Gundersen *et al.*, 1986; Mortensen *et al.*, 2005; Mortensen *et al.*, 2008; Esposito *et al.*, 2010) since there is metabolic reserve in the mitochondrial capacity to use oxygen during maximal exercise (Boushel *et al.*, 2011) and the fact that simply requiring more work to be done by increasing the workload does not increase the $\dot{V}O_{2max}$ (Brink-Elfegoun *et al.*, 2007a; Hawkins *et al.*, 2007; Duffield & Bishop, 2008). The finding that heart rate, breathing rate and ventilation at $\dot{V}O_{2max}$ were not different between protocols (table 1) excludes the possibility that the

excess $\dot{V}O_2$ is the result simply of extra mechanical work done by the heart or respiratory muscles.

Determinants of $\dot{V}O_{2\max}$

To test the hypothesis of an O_2 delivery limited $\dot{V}O_{2\max}$, several attempts using different techniques to increase the $\dot{V}O_{2\max}$ by increasing oxygen delivery to the muscles have already been performed, with mixed results. Exposure to hyperoxia increases leg muscle oxygen uptake during knee extension exercise in both patients with chronic heart failure (Esposito *et al.*, 2010), and in well trained athletes (Richardson *et al.*, 1999) but not in healthy sedentary individuals (Esposito *et al.*, 2010). Infusion of adenosine and ATP to increase vasodilation during maximal exercise increases blood flow and oxygen delivery to the lower limbs; however this occurs without concomitant changes in $\dot{V}O_{2\max}$ (Calbet *et al.*, 2006; Barden *et al.*, 2007). On the other hand, treatment with recombinant human erythropoietin increases $\dot{V}O_{2\max}$ without augmenting skeletal muscle blood flow (Lundby *et al.*, 2008).

Acutely exposing skeletal muscle to hypoxic episodes is thought to facilitate redistribution of blood flow from inactive to active muscle fibers (de Groot *et al.*, 2010), therefore improving O_2 delivery. Both acute (de Groot *et al.*, 2010) and prolonged (Park *et al.*, 2010) ischemic conditioning of the lower limbs through blood flow occlusion can increase $\dot{V}O_{2\max}$ by the same magnitude as found in this study. Whereas pre-conditioning for two weeks is likely to produce more meaningful physiological changes (Park *et al.*, 2010), an acute bout of 30 minutes of blood flow occlusion may alter blood flow distribution during subsequent exercise (de Groot *et al.*, 2010) and in response to acute hypoxia (Wagner, 2008). Conceivably this mechanism might also have occurred during DEC in this study.

Emotional stress can affect blood flow during exercise (Berdina *et al.*, 1972; Pribram & McGuinness, 1975; Williamson, 2010) and stimulation of sympathetic cholinergic fibers are thought to promote not only arteriolar vasodilation but also to induce changes in metabolism, producing a switch from aerobic metabolism to increased oxygen-independent glycolytic pathways (Berdina *et al.*, 1972). The type of emotional stress faced during INC in which the participant knows that the load will

become progressively more difficult with each stage of a never ending test is likely different from the stress of DEC, in which the load is perceived as becoming progressively easier, especially during the stage changes. It has been shown that aerobic and anaerobic exercises produce markedly differently acute responses in the levels of several hormones, which will directly impact energy mobilization (Kindermann *et al.*, 1982). We propose the interesting possibility that an anticipatory difference in perception of the future workload might impact the sympathetic / parasympathetic drives and lead to differences in the metabolic response during exercise.

A final intriguing finding was that higher $\dot{V}O_{2max}$ values achieved with the “reverse” protocol were retained during INC_F, despite the fact that time to exhaustion did not change and subjects reached $\dot{V}O_{2max}$ at the same speeds during both INC₁ and INC_F. This finding was unexpected. One possible speculation is that the body retained the adaptation that caused it to use more oxygen during DEC, possibly an altered recruitment of different skeletal muscle fiber types, during the INC_F (Cannon *et al.*, 2011). Indeed, a recent study (Vanhatalo *et al.*, 2011) showed that a progressive increase in $\dot{V}O_2$, so called the slow-component phase, can occur during an all-out 3-min cycling protocol despite decreasing levels of muscle electromyographic activity and power output throughout the trial.

Limitations

It is important to stress that all our trials were conducted in the space of no more than three weeks per participant and $\dot{V}O_{2max}$ is known to be reproducible over this period in highly trained subjects (Froelicher *et al.*, 1974). Also, data from the Control group show that no training effect was produced by repetitive INC. This is also confirmed by the lack of improvement in the time to fatigue for the reverse group in INC_F despite a persistently higher $\dot{V}O_{2max}$. The protocol used in our study (incremental uphill running) as the standard INC has also been shown to elicit the highest $\dot{V}O_2$ responses in trained (Astrand & Saltin, 1961; Brink-Elfegoun *et al.*, 2007a; Hawkins *et al.*, 2007) and untrained subjects (Kang *et al.*, 2001; Rossiter *et al.*, 2006; Foster *et al.*, 2007; Midgley *et al.*, 2009). This protocol also fulfilled other criteria required to consider a test “maximal”, such as optimal duration (Yoon *et al.*, 2007) and the use of

participants with high levels of fitness and motivation (Shephard, 2009). We believe this establishes that the novel reverse protocol evaluated in this study produces significantly higher $\dot{V}O_2$ values that cannot be explained by measurement error or a training effect.

Summary

In conclusion, this study therefore is in direct conflict with the belief, firmly held and passionately defended (di Prampero & Ferretti, 1990; Levine, 2008; Christie & Lock, 2009; Shephard, 2009) for more than 80 years, that the plateau detected by conventional INC “beyond which, no further increase in oxygen intake can occur”(p. 156)” (Hill & Lupton, 1923) can be explained only by the development of a “limiting” oxygen delivery to the exercising muscles (Levine, 2008). This finding has important implications for the interpretation of factors that might limit maximal exercise performance in diseased, in healthy and in athletic populations. In particular it establishes that subjects terminate the conventional INC with cardio-respiratory reserve.

We understand that there must be an absolute $\dot{V}O_{2max}$ for all individuals. However our study shows that this limiting condition is not reached during the conventional INC. We further conclude that a plateau in $\dot{V}O_2$ cannot be used as an indicator that a limiting condition has been reached. The reasons why the conventional INC terminates at a sub-maximal “ $\dot{V}O_{2max}$ ” and at sub-maximal levels of skeletal muscle recruitment are however unclear. But our findings support the interpretation that maximal exercise during the conventional INC is terminated at the $\dot{V}O_{2max}$ by a complex regulated process (Noakes, 2011a; Noakes, 2011b) before there is a loss of homeostasis in one of more physiological system(s) (Noakes & St Clair Gibson, 2004).

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Chapter 5

Traditional testing produces sub-maximal values of oxygen consumption in elite runners

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ABSTRACT

Introduction

It has been demonstrated recently that trained individuals can reach higher values of maximal oxygen uptake ($\dot{V}O_{2\max}$) when performing a decremental exercise test in comparison to a traditional incremental test. This however might be an artifact of the physical ability of the participants and in particular their level of physical training. This study aimed to determine whether findings in less well-trained subjects could be reproduced in a sample of better trained elite athletes.

Methods

Nine male elite runners (age 25.8 ± 5.1 years, season best 10 km time $31:19 \pm 1:50$) performed three maximal uphill (5% grade) running exercise tests on different days, in fixed order: a) an incremental test (INC₁) in which speed increased by $0.5 \text{ km}\cdot\text{h}^{-1}$ every 30 s; b) a V-shape exercise test (V-TEST, in which speed started at $0.5 \text{ km}\cdot\text{h}^{-1}$ higher than the top stage finished during INC₁ and was slowly decreased over the next 5.5 minutes; when it was again increased as in the INC tests) and c) a final incremental test (INC₂) similar to INC₁.

Results

$\dot{V}O_{2\max}$ during the V-TEST was higher than during INC₁ (6.3 ± 3.0 % higher, range 1.5 – 10.6 %, $p = 0.01$), although running speed was lower (16.6 ± 1.7 vs. $17.9 \pm 1.6 \text{ km}\cdot\text{h}^{-1}$, $p = 0.01$). Performance was similar between INC₁ and INC₂, but $\dot{V}O_{2\max}$ during INC₂ was higher than during INC₁ ($p < 0.001$). During the V-TEST five participants reached the incremental part of the test, but $\dot{V}O_2$ did not increase ($\Delta\dot{V}O_2 = 52 \pm 259 \text{ ml}\cdot\text{min}^{-1}$, $p = 0.67$), despite increasing running speed ($\sim 1.1 \text{ km}\cdot\text{h}^{-1}$, $p < 0.01$). Heart rate, pulmonary ventilation, breathing rate or respiratory exchange ratio values measured at $\dot{V}O_{2\max}$ were not different between tests.

Discussion

This study confirms that a decremental exercise test of sufficient intensity produces higher $\dot{V}O_{2\max}$ values than a traditional incremental test, even in elite athletes, and that this effect is maintained during a subsequent incremental test is performed within a few days of the initial test. The reasons for this phenomenon remain unclear.

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INTRODUCTION

A high ($> 80 \text{ ml.kg}^{-1}.\text{min}^{-1}$) maximal oxygen uptake ($\dot{V}\text{O}_{2\text{max}}$) is considered a pre-requisite for success in endurance sport (Levine, 2008), and also an important indicator of cardiovascular health and overall fitness (Myers *et al.*, 2002). $\dot{V}\text{O}_{2\text{max}}$ is typically measured during incremental exercise tests, in which there is a linear increase in work rate and in oxygen consumption ($\dot{V}\text{O}_2$) over time. The original concept was that subjects would always develop a maximum level of oxygen consumption that would level off despite further increases in work rate, which became known as the $\dot{V}\text{O}_{2\text{max}}$ plateau phenomenon (Hill *et al.*, 1924; Taylor *et al.*, 1955; Mitchell *et al.*, 1958). Although the plateau phenomenon is believed to represent the ceiling of the cardiovascular system's ability to deliver oxygen to the exercising muscles, there is still no consensus on how to define the occurrence of the plateau phenomenon during incremental exercise tests (Alpert, 1992; Astorino, 2009). This controversy has led some researchers to use the term $\dot{V}\text{O}_{2\text{max}}$ when a plateau is present at the end of an incremental test, preferring this term over tests which do not without discernible $\dot{V}\text{O}_2$ plateau (Meyer *et al.*, 2005).

To confirm that $\dot{V}\text{O}_{2\text{peak}}$ is actually the $\dot{V}\text{O}_{2\text{max}}$, the so-called "verification test" has been introduced (Billat *et al.*, 1995; Day *et al.*, 2003). In this test, for the $\dot{V}\text{O}_{2\text{max}}$ test, the subject is required to perform a constant load test to exhaustion at a higher intensity than the highest one achieved during the incremental test (Billat *et al.*, 1995; Day *et al.*, 2003; Hawkins *et al.*, 2007; Duffield & Bishop, 2008; Midgley *et al.*, 2009; Midgley & Carroll, 2009). The verification test has consistently demonstrated that, regardless of the protocol, $\dot{V}\text{O}_2$ is never higher during supra-maximal exercise than during incremental exercise, thereby confirming that the continuous incremental test does indeed measure the $\dot{V}\text{O}_{2\text{max}}$, so that the term " $\dot{V}\text{O}_{2\text{max}}$ " and " $\dot{V}\text{O}_{2\text{peak}}$ " are interchangeable, at least during whole-body exercise.

A more recent challenge to the $\dot{V}\text{O}_{2\text{max}}$ concept has come from a series of experiments showing that different protocols produce $\dot{V}\text{O}_{2\text{max}}$ values higher than

those measured with the traditional incremental test (Foster *et al.*, 1993; Moore *et al.*, 1993; Foster *et al.*, 1997; Mauger & Sculthorpe, 2012) in populations including cardiac patients (Foster *et al.*, 1993), active individuals (Mauger & Sculthorpe, 2012) as well trained athletes (Foster *et al.*, 1997; Moore *et al.*, 1993). Also, Beltrami and colleagues (Beltrami *et al.*, 2012) showed that a test in which the work rate was originally very high but then reduced rapidly – a “decremental test” – produced higher $\dot{V}O_{2\max}$ values than did the conventional incremental test.

The success of an incremental exercise test in truly determining $\dot{V}O_{2\max}$ is partly attributed to the fitness levels of the participants (Lucia *et al.*, 2006; Levine, 2008), their pain tolerances (Shephard, 2009) and anaerobic capacities (Lacour *et al.*, 2007). For example, the novel testing protocol created by Mauger and Sculthorpe (2012) and which measured higher $\dot{V}O_{2\max}$ values in untrained subjects did not result in higher $\dot{V}O_{2\max}$ values when tested in trained individuals (Chidnok *et al.*, 2012), although some other discrepancies between protocols in the two studies were present (Mauger, 2013). This raises the possibility that the higher $\dot{V}O_{2\max}$ obtained by Beltrami and colleagues (2012) using a decremental protocol were also a consequence of the fitness state of their participants.

Therefore, the aim of this investigation was to compare the values for $\dot{V}O_{2\max}$ obtained by elite runners during an incremental and a decremental protocols used for measuring $\dot{V}O_{2\max}$. We hypothesized that these trained subjects would exhibit a similar response to those less good athletes studied by Beltrami *et al.* (2012) and would also achieve higher $\dot{V}O_{2\max}$ values during the decremental protocol than during the traditional incremental exercise test.

METHODS

Participants

Nine male elite runners (age 25.8 ± 5.1 years, body mass 57.4 ± 10.5 kg, height 170 ± 8 cm, season best 10 km time $31:19 \pm 1:50$) volunteered to participate in this

investigation. All participants visited the laboratory on five occasions, each separated by 2-3 days, so that all participants completed the study within eight days. For each participant, tests were always performed at the same time of the day.

Sample size was calculated using G*Power (version 3.2.1, Kiel, Germany) and indicated that seven participants would be required to reach a statistical power of 80% when $\alpha = 0.05$, assuming an effect size (standard deviation/change in mean) of 0.25 and correlation between repeated measurements of 0.9, based on data from control subjects performing incremental tests on multiple occasions (Beltrami *et al.*, 2012).

General design

On the first visit, participants were fully informed of the aims and procedures of this study, which was approved by the Ethics and Research Committee of the Faculty of Health Sciences of the University of Cape Town. Upon signing an informed consent form, participants undertook anthropometric measurements and performed a familiarization incremental running test on a motor-driven treadmill (Viasys LE 500CE, h/p/cosmos Sports & Medical GmbH, Germany), which was used in all subsequent trials. The incremental test was repeated on visit two (INC₁). On visit three the participants performed a familiarization test with the decremental exercise test, which was repeated on visit four (V-TEST). On visit four the protocol was adapted so that the rate at which the load was decreased was reduced, thereby increasing the total work load sustained by the subjects. On visit five, participants once again performed the normal incremental test (INC₂) to detect any training effects resulting from the multiple testing sessions. Data from visits two (INC₁), four (V-TEST) and five (INC₂) were used for subsequent analysis. All exercise tests were preceded by 10 minutes of light running at a self-selected pace. Participants were paid the equivalent to US\$ 20.00 for each visit, but that was not related in any way to their performance during the exercise testing.

Incremental exercise test

The same protocol was used on the first (familiarization), second (INC₁) and fifth (INC₂) testing sessions. The incremental exercise test was performed at a constant

5% gradient. The first minute of the test was run at 9 km.h^{-1} and afterwards the treadmill speed was increased by 0.5 km.h^{-1} every 30 seconds. Participants were strongly encouraged to keep running for as long as they could. After completion of the incremental test, participants were allowed 10 minutes of rest and were then requested to perform a verification test. The verification tests consisted of a square-wave protocol in which participants ran to exhaustion at a speed 0.5 km.h^{-1} higher than the last completed stage of the incremental test. Prior to the verification test participants were allowed to jog for 5 minutes plus one minute running at an intermediate speed between the warm-up and the test speed. An examiner stood behind the treadmill to ensure that no participant fell from the ergometer during the final minutes of all tests. After the first session, a board with all their performance data for was shown to all participants before the tests, to stimulate competition and maximal effort during each test.

Decremental Exercise Test

The decremental exercise test on visit 3 started with one minute of light jogging, followed by 30 seconds of gradual increase in treadmill speed and gradient until it matched that achieved during the verification test performed on visit 2. Participants then ran at the verification test speed for 60 seconds, after which the treadmill speed was decreased by 0.5 km.h^{-1} at each subsequent stage, as follows: four stages of 30 seconds, two stages of 60 seconds each and two stages of 90 seconds each, for a total of eight minutes of exercise. If by the end of the eighth minute the participant was not exhausted, the treadmill was again increased by 0.5 km.h^{-1} every 30 seconds, until exhaustion. This trial was used to familiarize participants with the decremental exercise protocol.

The test on visit 4 again started at the same speed at which the verification test was performed, but the initial stage lasted for 60% of the participant's time to exhaustion during that test. For example, a participant who had run for two minutes at 20 km.h^{-1} during his verification test would start the decremental protocol by running at 20 km.h^{-1} for 72 seconds. After the first stage, the treadmill speed was decreased by 1 km.h^{-1} which was maintained for 30 seconds. This was followed by decrements of 0.5

km.h⁻¹, which were maintained for 30, 45, 60 and 90 seconds, thus totaling ~5.5 minutes of decremental exercise. If a participant was able to reach the end of the final 90 seconds stage, the treadmill speed was again raised by 0.5 km.h⁻¹ every 30 seconds, until the participant could no longer continue running.

Instruments and data handling

During all the testing sessions expired gas samples were collected for determination of oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$) and minute ventilation ($\dot{V}_E$) using an automated, non-invasive, online breath-by-breath gas analyzer (Jaeger, Oxycon Pro, Viassis, Hoechberg, Germany). Prior to each trial, both the analyzers and the software were calibrated using calibration gases of known concentrations according to the manufacturers' instructions. The gas analyzer flowmeter was calibrated using a 3 L calibration syringe (Hans-Rudolph, Kansas City, MO, USA). Heart rate data were continuously monitored using a Polar S410 heart rate monitor (Polar Electro OY, Kempele, Finland), synchronized with the gas analyzer software.

All physiological data were collected breath-by-breath, exported from the analyzer software into excel spreadsheets and calculated as consecutive 30-second intervals. During all tests, data were considered until the last full 30-second interval collected during exercise. $\dot{V}O_{2max}$ was defined as the highest value measured during any 30-second interval. All other gas exchange variables and heart rate data used for comparisons between the different protocols were recorded during the 30-second period that $\dot{V}O_{2max}$ was recorded. A plateau in $\dot{V}O_2$ during INC₁ was defined as a change in $\dot{V}O_2$ between $\dot{V}O_{2max}$ and the previous data point smaller than half of the mean $\dot{V}O_2$ difference for that participant during the linear (incremental) sections of the test. The verification test was used to confirm the presence of a $\dot{V}O_{2max}$ regardless of whether or not the plateau phenomenon occurred during the incremental test.

During the course of the study, one participant suffered a bout of influenza, which severely affected his exercise capacity. His data were excluded from the study. Therefore, our sample was reduced to n = 8.

Statistics

All data are presented as mean $\pm$ standard deviation. Differences in time to exhaustion and speed at $\dot{V}O_{2\max}$ between INC₁ and INC₂ were compared using Student's *t*-test for paired samples. After checking for data normality, comparisons of physiological data between the different tests were performed using one-way repeated measures ANOVA, with Tukey post-hoc analysis whenever a main effect was found. Repeated measures ANOVA were also used to compare the change in $\dot{V}O_2$ over time between INC₁, INC₂ and the incremental portion of V-TEST. Significance level was set at $p < 0.05$. All calculations were performed using Prism 5 (GraphPad Software, La Jolla, CA, USA).

RESULTS

During INC₁, the participants exercised at $\dot{V}O_{2\max}$ of $64.7 \pm 9.4 \text{ ml.kg}^{-1}.\text{min}^{-1}$ while running at $17.9 \pm 1.6 \text{ km.h}^{-1}$ (5% grade). Only two participants did not demonstrate the plateau phenomenon during INC₁. $\dot{V}O_{2\max}$ during the verification phase was $94.7 \pm 4.9\%$ of that achieved during the INC₁, and not higher than the incremental test value reached by the participants. $\dot{V}O_{2\max}$ was $87.4 \pm 4.9\%$ of that achieved during the INC₁, and not higher than the incremental test value reached by the participants. $\dot{V}O_{2\max}$ during the verification phase was $94.7 \pm 4.9\%$ of that achieved during the INC₁, and not higher than the incremental test value reached by the participants. $\dot{V}O_{2\max}$ during the verification phase was $94.7 \pm 4.9\%$ of that achieved during the INC₁, and not higher than the incremental test value reached by the participants.

During INC₂, the participants exercised at $\dot{V}O_{2\max}$ of $63.0 \pm 3.0 \text{ ml.kg}^{-1}.\text{min}^{-1}$ while running at $16.6 \pm 1.7 \text{ km.h}^{-1}$ (5% grade). $\dot{V}O_{2\max}$ during the verification phase was $94.7 \pm 4.9\%$ of that achieved during the INC₂, and not higher than the incremental test value reached by the participants. $\dot{V}O_{2\max}$ during the verification phase was $94.7 \pm 4.9\%$ of that achieved during the INC₂, and not higher than the incremental test value reached by the participants. $\dot{V}O_{2\max}$ during the verification phase was $94.7 \pm 4.9\%$ of that achieved during the INC₂, and not higher than the incremental test value reached by the participants.

All participants were able to run into the incremental phase of the V-TEST. The highest $\dot{V}O_2$ value achieved during the final incremental part of the test was not significantly higher than that of the decremental phase of the test ($\Delta\dot{V}O_2 = 52 \pm 259$).

ml.min⁻¹, p = 0.67), but occurred at a significantly higher speed (~1.1 km.h⁻¹ higher, p = 0.006). For these five participants the average change in $\dot{V}O_2$ between consecutive 30-second intervals was significantly smaller than when the participants ran at the same speeds during both INC₁ and INC₂ (29.7 ± 75.3 vs. 102.0 ± 61.6 vs. 124.9 ± 66.7 ml O₂.min⁻¹ for the incremental part of the V-TEST, INC₁ and INC₂ respectively, p < 0.01).

On INC₂, although there were no differences in time to exhaustion or speed at $\dot{V}O_{2max}$ in comparison to INC₁ (17.9 ± 1.6 vs. 17.8 ± 1.7 km.h.⁻¹ respectively, p = 0.87), $\dot{V}O_{2max}$ was higher (3,972 ± 269 vs. 3,626 ± 268 ml.min⁻¹, p < 0.001), although it was not different from that achieved during the V-TEST (table 1, figure 1).

Table 1: Physiological variables measured at $\dot{V}O_{2max}$.

	INC ₁	V-TEST	INC ₂
$\dot{V}O_{2max}$	3,626 ± 268	3,854 ± 293*	3,973 ± 270†
$\dot{V}_E$	117.8 ± 14.3	122.4 ± 14.4	119.0 ± 15.3
HR	193 ± 10	185 ± 7	188 ± 4
BR	60.1 ± 10.7	61.5 ± 9.2	62.4 ± 7.2
RER	1.16 ± 0.04	1.10 ± 0.07	1.17 ± 0.06
V. $\dot{V}O_{2max}$	17.9 ± 1.6	16.6 ± 1.7*‡	17.8 ± 1.7

All data presented as mean ± SD. $\dot{V}O_{2max}$, maximal oxygen consumption (ml.min⁻¹); $\dot{V}_E$, pulmonary ventilation (l.min⁻¹, Btps); HR, heart rate (beats.min⁻¹); BR, breathing rate (cycles.min⁻¹); RER, respiratory exchange ratio; V. $\dot{V}O_{2max}$, speed at $\dot{V}O_{2max}$ (km.h⁻¹). * Different from INC₁ at p < 0.001. † Different from INC₁ at p < 0.0001. ‡ Different from INC₂ at p < 0.001

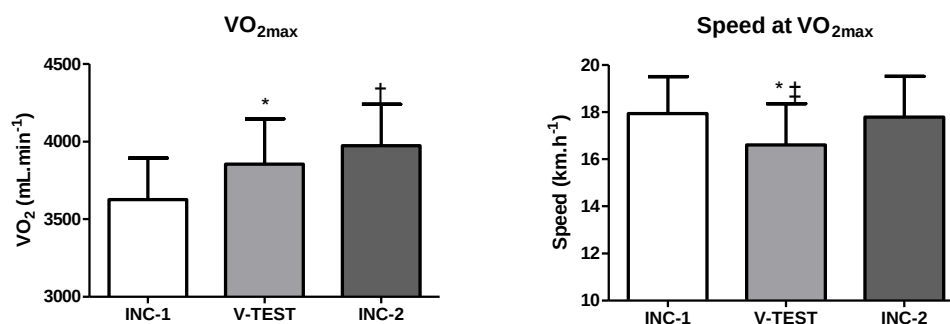


Figure 1: Maximal oxygen consumption ($\dot{V}O_{2max}$) (left panel) and speed at $\dot{V}O_{2max}$ for the different trials. * Different from INC₁ at p < 0.001. † Different from INC₁ at p < 0.0001. ‡ Different from INC₂ at p < 0.001.

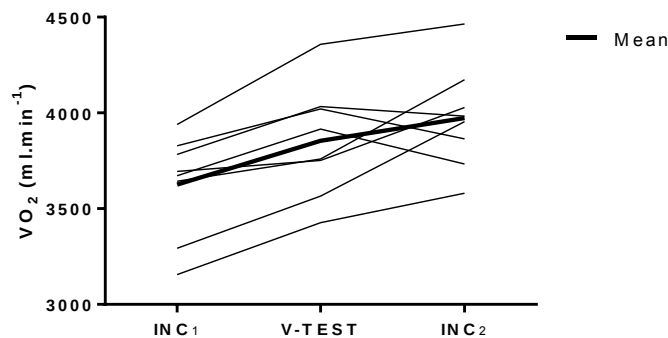


Figure 2: Individual (thin lines) and mean (thick line) $\dot{V}O_{2\max}$ responses to the different trials.

DISCUSSION

This investigation was designed to test whether the findings reported by Beltrami *et al.* (2012) in well-trained subjects were reproducible in a set of elite athletes, who are thought to exercise closer to their physiological limits during $\dot{V}O_{2\max}$ testing (Shephard, 2009; Levine, 2008). Accordingly, the major findings of this study were i) that elite runners achieved higher $\dot{V}O_{2\max}$ values during a decremental exercise test than during an incremental test despite running at a lower speed; and ii) that these higher $\dot{V}O_{2\max}$ values were retained during a subsequent incremental exercise test, even though this occurred without any changes in maximal exercise performance. These findings confirm those previously described in well-trained but not elite individuals (Beltrami *et al.*, 2012).

Apart from the training status of the participants, a major difference between the present study and that of Beltrami *et al.* (2012) was that the decremental protocol in this study was designed with a V-shape, allowing participants to continue running at the end of the decremental protocol whereafter the work rate was again increased. This was done to allow room for load correction in case of a load miscalculation during the decremental phase of the protocol. If the starting load is too low or the rate of decrease is too steep, $\dot{V}O_2$ will fall soon after the start of the protocol, and although still higher for a given workload than that measured during the incremental test,

$\dot{V}O_{2\max}$ will not be achieved. By raising the workload after a series of speed decrements, we hypothesized that the demand for O_2 from the exercising muscles would create a second opportunity for participants to reach $\dot{V}O_{2\max}$. Interestingly however $\dot{V}O_{2\max}$ was similar for the two phases of the V-TEST in spite of a significant difference in speed at which $\dot{V}O_{2\max}$ was measured.

The biological explanation for the higher $\dot{V}O_{2\max}$ during the V-TEST remains unclear, since none of the other measured variables were different between tests. It has been proposed that during fatiguing exercise that causes a reduction in power output, the additional recruitment of type IIx muscle fibers, which are less efficient in generating ATP, explains the excess $\dot{V}O_2$ that is measured (Vanhatalo *et al.*, 2011). However, the additional recruitment of type IIx muscle fibers is a possibility in both the incremental and decremental tests and currently there are no studies comparing the pattern of muscle recruitment between these two tests. Another explanation is that during decremental exercise the extra O_2 uptake might be used to “pay” the oxygen deficit created at the start of the exercise bout (Yano *et al.*, 2004; Yano *et al.*, 2000). This extra O_2 might be used in non-active areas such as inactive muscle tissue and the liver. It has already been shown that subjects breathing hyperoxic mixtures reach disproportionately higher $\dot{V}O_{2\max}$ values relative to changes in peak power output, suggesting that the increased O_2 consumption occurs in “non-exercising” tissues (Nielsen, 2003). If true, both explanations require that either O_2 delivery or O_2 extraction must be increased during the V-TEST, and although current evidence suggests that O_2 delivery capacity is a more likely candidate (Mortensen *et al.*, 2005; Calbet *et al.*, 2007; Levine, 2008), this remains to be tested.

Our results may provide some further insight into this matter. An interesting finding was the much lower increase in $\dot{V}O_2$ between stages during the incremental phase of the V-TEST. Changes in $\dot{V}O_2$ between stages in this phase of the V-TEST averaged only $29.7 \text{ ml}\cdot\text{min}^{-1}$, though individual responses were highly variable. In contrast, running at the equivalent speeds during the incremental test produced much higher changes in $\dot{V}O_2$ between stages ($102.0 \text{ ml}\cdot\text{min}^{-1}$). If the decremental test causes a shift in O_2 use from active muscles to non-active tissues and vice-versa for incremental exercise, this could explain how the demand for oxygen at the different

workloads could be met without it being reflected by changes in the pulmonary $\dot{V}O_2$. This possibility however is highly speculative, and further studies are required to test whether this is actually a physiological mechanism.

The marked change in the $\dot{V}O_2$ x speed relationship during the incremental phase of the V-TEST has another important implication. The decremental protocol originally proposed by Beltrami and colleagues (2012) hypothesized that if participants knew that the load was going to decrease (instead of the traditional increase), they might be more likely to use their full metabolic capacity, regardless of whether this is a conscious or subconscious process (Noakes, 2008b; Noakes & Marino, 2009; Noakes, 2011b; Beltrami *et al.*, 2012). Our finding that five subjects could still exercise at higher intensities having completed the decremental part of the test is at odds with a conscious decision to preserve the metabolic milieu, since in many cases participants were very close to their previous $\dot{V}O_{2max}$ or even higher at the time that they started the incremental part of the V-shaped decremental protocol.

Critics of the incremental test paradigm have proposed that $\dot{V}O_{2max}$ might not be limited by the cardiovascular system during incremental exercise, but rather it might be regulated by the central nervous system (Noakes, 2008a; Noakes, 2008b; Noakes & Marino, 2009; Noakes, 2011a). While our results do not directly argue in favor of this theory, they do provide evidence that during normal incremental exercise the cardiovascular system is not working at full capacity, as previously shown (Brink-Elfegoun *et al.*, 2007b) since a higher $\dot{V}O_{2max}$ was achieved without externally manipulating the variables thought to have direct influence on the limits of $\dot{V}O_{2max}$, such as inspired fraction of O_2 (Peltonen *et al.*, 2001) or exercise modality (Brink-Elfegoun *et al.*, 2007a; Brink-Elfegoun *et al.*, 2007b) and maximal power output (Kang *et al.*, 2001; Alexander & Mier, 2011).

The $\dot{V}O_{2max}$ paradigm based on incremental exercise tests has been challenged before, mainly on the grounds that incremental exercise is not a common way of exercising for humans, and thus lacks ecological validity (Foster *et al.*, 1993; Foster *et al.*, 1997; Foster *et al.*, 2007; Noakes, 2008b; Mauger & Sculthorpe, 2012). Uncertainty about the endpoint of a task, as during an open-loop test to determine $\dot{V}O_{2max}$, causes participants to hold back part of their metabolic capacity, at least in time trials (Swart

et al., 2009). Different tests based on self-paced exercise produced higher $\dot{V}O_{2\max}$ values than the incremental test (Foster *et al.*, 1993; Foster *et al.*, 1997; Mauger & Sculthorpe, 2012), but none of these has been consistently reproduced. For example, Foster and colleagues found that well trained cyclists (Foster *et al.*, 1997) as well as cardiac patients (Foster *et al.*, 1993) were able to reach higher $\dot{V}O_{2\max}$ values during a 5-km time trial than during an incremental test however were unable to replicate their results when they tested runners (Crouter *et al.*, 2001). Running, especially uphill, is believed to produce higher values of $\dot{V}O_{2\max}$ than cycling, the exception being in highly trained cyclists, because of the greater muscle mass employed and circulatory responses during upright exercise (Brink-Elfegoun *et al.*, 2007a; Brink-Elfegoun *et al.*, 2007b). Cycling, therefore, does not make full use of the cardiovascular capacity, and thus different protocols are more likely to produce different results.

Limitations

A limitation of the present study is that we did not have a control group to account for training effects that might have occurred as a result of the multiple testing sessions. However, our participants were elite athletes and it is very unlikely that the stimulus provided by a testing session (~2-3 min of intense running) would be enough to raise their $\dot{V}O_{2\max}$ by 6% as a result of any training stimuli. This hypothesis is confirmed by other studies with designs of similar complexity and none of which found a training effect on $\dot{V}O_{2\max}$ as a result of repeated INC testing (Beltrami *et al.*, 2012; Brink-Elfegoun *et al.*, 2007a). Finally, the fact that time to exhaustion was similar between INC₁ and INC₂ also indicates that no training effects took place over the duration of the study, and therefore does not explain the increase in $\dot{V}O_{2\max}$ found during V-TEST. The increase in $\dot{V}O_{2\max}$ found on the final incremental test, however, is puzzling, but was not due to an increased capacity to reach a higher peak work rate.

However, this finding is not completely unexpected. Several studies indicate the existence of “luxurious” oxygen consumption, greater than required to achieve a given workload, in a variety of interventions. Studies using hyperoxia to increase $\dot{V}O_{2\max}$ have found that the increases in $\dot{V}O_{2\max}$ are accompanied by much smaller, if any, increases in performance or peak workload (Nielsen *et al.*, 1998; Peltonen *et al.*,

2001). Five weeks of recombinant erythropoietin treatment resulted in a 5.8% higher pulmonary $\dot{V}O_{2\max}$ without measurable changes in power output (Robach *et al.*, 2008), which was further reflected by higher $\dot{V}O_2$ at the same sub-maximal power output following EPO administration. Finally, Baden *et al.* (2005) demonstrated that expectation about task duration can affect $\dot{V}O_2$, so that a longer trial causes the body to use less oxygen at a given speed, similar to what has been shown in muscle activity (Vidacek & Wishner, 1971). $\dot{V}O_{2\max}$ seems to be more susceptible to changes than exercise performance, which also would be in line with the explanation that the extra O_2 uptake may be occurring in non-exercising tissues.

Summary

In summary, this study showed that elite athletes achieve higher $\dot{V}O_{2\max}$ when performing a V-shaped decremental protocol than during traditional incremental exercise test or during incremental test. Remarkably the higher $\dot{V}O_{2\max}$ was retained for at least 48h, even when tested with the conventional incremental test. This demonstrates that traditional test does not fully utilize $\dot{V}O_{2\max}$ during incremental exercise testing represents the upper ceiling of the capacity of the cardiovascular system to provide oxygen to the maximally exercising muscles.

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Chapter 6

Comparison of physiological responses and muscle activity during incremental and decremental cycling exercise

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ABSTRACT

Introduction

A decremental exercise test produces higher values of maximal oxygen uptake ($\dot{V}O_{2\max}$) compared to a standard incremental test using uphill running. This study aimed to test whether these results could be reproduced in cycling and to establish whether the tests produce different electromyographical activity (EMG) in the exercising muscles of the lower limbs at $\dot{V}O_{2\max}$.

Methods

Nineteen participants performed a maximal incremental cycling test (INC) and a decremental cycling test (DEC), separated by approximately 60 min, after being familiarized with both trials (n=8) or with the incremental test only (n=11). During INC the load was increased by 20 W.min⁻¹ until exhaustion. During DEC the load started at 20 W higher than the peak load achieved during INC and was progressively decreased during the test. Gas exchange and EMG activity (n=11) from four lower limb muscles were monitored throughout the trials.

Results

$\dot{V}O_{2\max}$ during the DEC was $3.2 \pm 5.4\%$ higher than during INC (range 0.94 – 1.16; $p = 0.01$), in spite of a lower power output (-21 ± 20 W, $p = 0.004$). Pulmonary ventilation ($p = 0.004$) and breathing rate ($p = 0.01$) were also higher during DEC in comparison to INC. Performing a specific familiarization trial for the DEC test did not change the gas exchange results ($p = 0.49$). EMG activity increased over time for all analyzed muscles during INC, but values measured at $\dot{V}O_{2\max}$ were not different between trials. EMG activity did not change significantly throughout DEC despite progressive reduction in workload.

Discussion

When appropriately performed, a decremental exercise test produces higher $\dot{V}O_{2\max}$ values in cycling compared to a standard incremental test. This is accompanied by higher pulmonary ventilation. Part of the additional oxygen uptake measured during the decremental test in cyclists might be related to extra work performed by the respiratory muscles. The decremental tests might reduce the efficiency of the lower limbs.

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INTRODUCTION

Maximal oxygen uptake ($\dot{V}O_{2\max}$) in exercising humans has been a topic of interest and controversy (Noakes, 1997; Bassett & Howley, 1997) since the concept was first introduced, in the 1920s (Hill *et al.*, 1924; Hill & Lupton, 1923). Since those early experiments, it was noted that oxygen consumption ($\dot{V}O_2$) increases linearly with increasing workloads, until a point is reached where no further increase in $\dot{V}O_2$ is seen despite further increases in workload, in what is termed the $\dot{V}O_{2\max}$ plateau phenomenon (Taylor *et al.*, 1963; Hunter *et al.*, 2002).

Because $\dot{V}O_{2\max}$ represents the highest possible value that can be measured during exercise for a given modality (Astrand & Saltin, 1961; Faulkner *et al.*, 1971), an extensive body of research has been devoted to finding the best way to correctly measure $\dot{V}O_{2\max}$ and to identify the plateau phenomenon (Astrand & Saltin, 1961; Kang *et al.*, 2001; Meyer *et al.*, 2005; Yoon *et al.*, 2007; Alexander & Mier, 2011). Most of these studies operate within the paradigm of incremental exercise for optimum measurement of $\dot{V}O_{2\max}$, focusing on topics such as load increments (Kang *et al.*, 2001; Alexander & Mier, 2011), test duration (Yoon *et al.*, 2007), sampling interval (Astorino, 2009), and “supra-maximal” tests to confirm the attainment of $\dot{V}O_{2\max}$ (Rossiter *et al.*, 2006) using the conventional incremental testing method.

Only a few studies have considered alternatives to this incremental test paradigm and have evaluated protocols that are not based solely on progressive load increments until the achievement of volitional fatigue. Surprisingly, some of these new protocols have produced higher $\dot{V}O_{2\max}$ values than the traditional incremental protocol (Foster *et al.*, 1993; Foster *et al.*, 1997; Mauger & Sculthorpe, 2012; Beltrami *et al.*, 2012). For example, a test where load increments are based on the participant’s perceived exertion produced higher $\dot{V}O_{2\max}$ values compared to a traditional incremental test in which the load increments are set by the experimenter (Mauger & Sculthorpe, 2012). This finding, however, has not yet been successfully reproduced (Chidnok *et al.*, 2012; Mauger, 2013). Similarly, a cycling time-trial approach has been shown to produce higher $\dot{V}O_{2\max}$ values than an incremental test in different

populations (Foster *et al.*, 1993; Foster *et al.*, 1997), but this was not found when running was used as the exercise mode (Crouter *et al.*, 2001). Finally, a decremental exercise test produces higher $\dot{V}O_{2\max}$ during uphill running in both trained individuals (Beltrami *et al.*, 2012) and elite athletes (**Chapter 5**). The physiological reasons why higher $\dot{V}O_{2\max}$ values are achieved with some of these protocols remain speculative however.

One common feature of these studies is that either through pacing (Foster *et al.*, 1993; Foster *et al.*, 1997; Mauger & Sculthorpe, 2012) or by test design (Beltrami *et al.*, 2012) the protocol in these different studies allowed the power output to decrease during the tests, contrary to what happens during incremental exercise. Decreasing power output during the exercise tests when participants reach $\dot{V}O_{2\max}$ extends the duration of the $\dot{V}O_{2\max}$ plateau phenomenon (Petot *et al.*, 2012; Billat *et al.*, 2013) and also produces “excess” $\dot{V}O_2$ during sub-maximal power outputs (Yano *et al.*, 2000; Yano *et al.*, 2004). Thus it is possible that it is the reduction in power output during exercise that allows the body to elicit and sustain a very high level of O_2 uptake. Research on sub-maximal decremental exercise has attributed the “excess” $\dot{V}O_2$ to oxygen consumption by “non-exercising” tissues (Yano *et al.*, 2000; Yano *et al.*, 2004). On the other hand, it is also suggested that during all-out exercise in which power output falls due to reduced muscle contractile function, there is also a loss of muscle efficiency in the exercising limbs so that $\dot{V}O_2$ is inappropriately high for the achieved power output (Vanhatalo *et al.*, 2011).

In order to determine whether the excess oxygen consumption achieved during decremental exercise occurs as a result of increased recruitment of the active muscles, we compared muscle activity in the incremental and decremental $\dot{V}O_{2\max}$ tests with the use of electromyography (EMG). Although direct measurement of the various determinants of $\dot{V}O_{2\max}$ would provide a direct answer to this question (**Chapter 8**), this requires exercise to be performed on a cycle ergometer, due to safety and methodological issues. Although the presence of the higher $\dot{V}O_{2\max}$ during decremental exercise has already been confirmed in well-trained (Beltrami *et al.*, 2012) and elite athletes (**Chapter 5**), both studies used uphill running, and results cannot be generalized to cycling (Foster *et al.*, 1997; Crouter *et al.*, 2001). $\dot{V}O_{2\max}$ is not only

specific to a given exercise modality (Faulkner *et al.*, 1971; Kohrt *et al.*, 1987; Basset & Boulay, 2000), but also varies according to subjects' training background (Millet *et al.*, 2009).

Therefore, the purpose of this study was to test whether the finding of higher $\dot{V}O_{2\max}$ during decremental exercise tests originally reported in well-trained individuals (Beltrami *et al.*, 2012) and elite runners (Chapter 5) could also be present in a group of well-trained cyclists. In addition, the study aimed to determine whether muscle activity of the lower limbs would be different at the time that $\dot{V}O_{2\max}$ was achieved in both tests. Two decremental protocol designs were used to compare the physiological response between an incremental and a decremental exercise test. It was hypothesized that a higher $\dot{V}O_{2\max}$ would be present during the decremental exercise test; and that in spite of a lower power output at $\dot{V}O_{2\max}$ during the decremental test, EMG activity would be higher indicating the greater muscular effort. Higher relative muscle mass would then explain the greater $\dot{V}O_{2\max}$ during the decremental test.

METHODS

Participants and General Design

This study was divided in two parts, as shown in figure 1. In part A, 11 participants (ten male and one female, age 29.0 ± 7.4 years, body mass 75.2 ± 7.8 kg, height 181 ± 9 cm), all accustomed to high-intensity cycling, visited the laboratory of the Sogn og Fjordane University College (Sogndal, Norway) on two occasions. On their first visit, participants were briefed in detail about the methods, aims and risks of the investigation. After they signed an informed consent form, the participants performed a maximal incremental exercise test on a cycle ergometer (INC_{fam}), followed 10 minutes later by a verification test to confirm the attainment of $\dot{V}O_{2\max}$. The tests performed on visit one served for familiarization purposes. On visit two, the participants performed three exercise tests, each separated by approximately one hour. Firstly, they performed an incremental test (INC_1), following which a decremental (DEC) and another incremental test (INC) were performed in randomized,

counter-balanced order. During the trials on visit two, gas exchange data, heart rate and EMG activity of four leg muscles (*vastus lateralis* (VL), *biceps femoralis* (BF), *vastus medialis* (VM) and *gastrocnemius medialis* (GM)) were continuously monitored.

In part B, eight male participants (age 23.8 ± 5.5 years, body mass 69.8 ± 8.1 kg, height 179 ± 4 cm), all accustomed to high-intensity cycling, visited the laboratory on three occasions (University of Bedfordshire, Bedford, UK, n=3; University of Kent, Kent, UK, n=5) each separated by 2-3 days. The first visit was similar to that described in part A. After signing an informed consent form the participants performed a maximal incremental cycling test (INC_{fam}) followed 30 minutes later by a constant load verification test. On the second visit, the participants performed a decremental exercise test (DEC_{fam}). Both the first and second visits were used for familiarization purposes. On their third visit, the participants repeated both the INC and DEC exercise tests in randomized, counter-balanced order, separated by at least 60 minutes of rest. Gas exchange data, heart rate and power output were monitored throughout the tests.

All studies were approved by the Ethics and Research Committee of the Faculty of Health Sciences of the University of Cape Town and the Institutional Review Boards from all institutions where data were acquired. Participants were clearly informed about the risks and procedures involved in this investigation and were assured that they could withdraw from the study at any time. All procedures performed were in accordance with the latest revision of the declaration of Helsinki (World Medical Association, 2008).

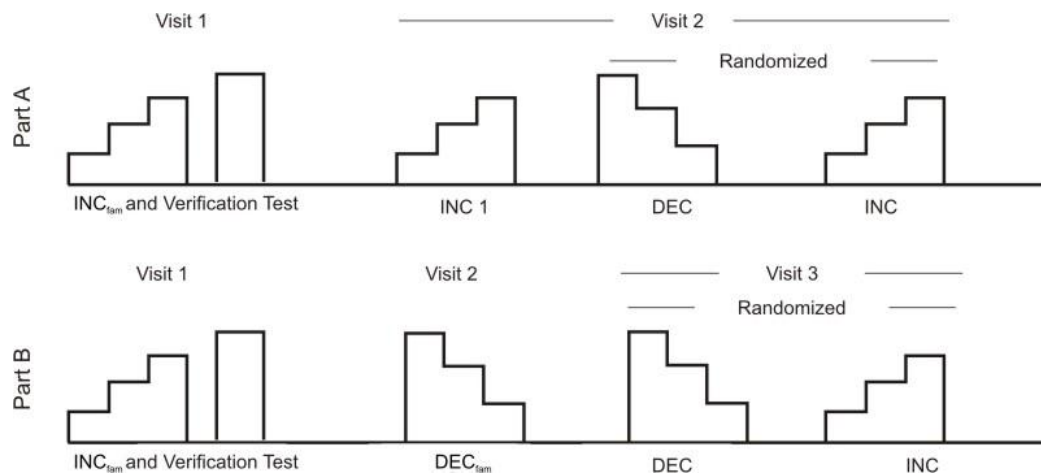


Figure 1: Study design diagram. Visits were separated by 2-3 days. See text for detailed description of each testing protocol.

Incremental Exercise test

The INC protocol was the same for both parts A and B of this investigation. Prior to each trial, the participants warmed-up for 10 minutes at a self-selected cadence and power output. The INC test started at 200 W for men and 160 W for women, with increments of 20 W applied every 60 seconds thereafter, until the participant was unable to maintain the pedaling cadence above 60 revolutions per minute (rpm). A screen showing real-time cadence was placed in front of the participant, and strong verbal encouragement was given whenever the pedaling cadence approached the 60 rpm mark.

Verification Test

The verification test consisted of a square-wave test to exhaustion performed at one stage higher (20 W) than the last completed stage during INC_{fam}. Immediately prior to the test, the participants cycled for three minutes at 150 W (120 W for women). The load was then increased to the desired power output within 20 seconds, and participants were instructed to cycle for as long as they could, while maintaining their pedaling cadence above 60 rpm.

Decremental Exercise Test

Following the standard 10 min warm-up, the DEC test started with three minutes of light cycling (150 W for men, 120 W for women), following which the load was increased within 20 seconds to that equivalent to the load used during the verification test (that is, 20 W higher than the load of the last completed stage during INC_{fam}). The initial load was maintained for 60% of each individual's time to task failure (TTF) recorded during the verification phase test. The average duration for the first stage of DEC was 90 seconds (range 75-105 seconds). Following the first stage, the load was decreased by 20 W and maintained for 30 seconds. Subsequent decrements of 10 W were maintained for 45, 60, 90 and 120 seconds, thus totalizing ~7 min 15 s of test.

If this protocol proved too demanding for the subject (TTF < 3 minutes during DEC_{fam}, part B only, figure 1), on the second occasion participants performed the decremental protocol (DEC, Part B, figure 1), the first decrement in workload was increased from 20 to 30 W. Participants in Part A of the study performed the decremental protocol only once, so these adjustments were not possible.

Instruments and Data Handling

All exercise trials were performed on an electronically braked cycle ergometer (Lode Excalibur Sport, Lode, the Netherlands), and seat and handlebar position were recorded for each subject and maintained constant throughout the trials. During part A of the study, gas exchange was continuously monitored using an automated gas analyzer system (MOXUS Modular Metabolic System, AEI Technologies, IL, USA). In part B, a different on-line gas analyzer was used (Cortex Metalyser, 3B, Biophysik, Leipzig, Germany). Prior to each exercise trials, both the analyzers for O₂ and CO₂ were calibrated against room air and high-precision gas bottles of known concentrations, which spanned the range of gas fractions measured during maximal exercise. The Moxus system required an additional software calibration step, which was also performed before each trial. Ventilation measurements for both analyzers were performed using a 3 L calibration syringe (Hans-Rudolph, Kansas City, MO, USA) at different pumping rates, including those commonly measured during intense exercise. Heart rate data were monitored continuously using a Polar S410 (part A) or a Polar T31

(part B) heart rate monitor (Polar Electro OY, Kempele, Finland), synchronized with the gas analyzer software.

All gas exchange and heart rate data were collected as consecutive 5-seconds means and exported in Excel spreadsheets, and each 5-second window contained only full breaths. Data from the exercise tests were averaged as consecutive 1-minute intervals (Howley *et al.*, 1995). $\dot{V}O_{2\max}$ was defined as the highest value measured during a test and all other physiological variables were recorded at the same time as $\dot{V}O_{2\max}$.

During part A of the study, the EMG signals from the VL, VM, GM and BF of the right leg were continuously recorded using DE-2.1 single differential surface sensors with 10 mm inter-electrode distance (Delsys Inc., Boston, MA, USA). SENIAM (Hermens *et al.*, 2000) and Delsys recommendations for the placement of the sensors on the skin were followed. Before electrode placement, the skin was shaved to remove excessive hair and wiped with isopropyl alcohol. A reference electrode was applied over the patella. The EMG signals were sampled at 2000 Hz and amplified using Bagnoli-8 (Delsys Inc). The EMG signals were transferred into Power Lab (ADInstruments, Colorado Springs, CO, US) and filtered using a 15-500 Hz band pass filter in Lab Chart Pro software (ADInstruments).

Root mean square (RMS) EMG activity was averaged over the final 20 seconds of each minute of exercise. EMG activity was expressed as a percentage of the RMS activity recorded during a maximal 10 s sprint performed immediately prior to the first exercise trial. Prior to the sprint, the participants cycled at a low power output for two minutes. The cycle ergometer was then set to the linear mode in which power output increases in proportion to the cycling cadence. The subjects were instructed to cycle as fast as they could for 10 s, and the 10 cycles with higher RMS values during this period were used as reference for all other EMG activity recorded.

Statistics

All data are presented as mean $\pm$ standard deviation, unless stated otherwise. After checking for data normality, the physiological responses from the participants in

both parts of the study was pooled together and the physiological responses between the DEC and INC were analyzed using Student's paired t-tests for dependent samples.

The effect of adding a specific familiarization trial for the decremental test (part B vs. part A) was analyzed by comparing the DEC/INC $\dot{V}O_{2\max}$ ratio in parts A and B of the study, using Student's t-test for independent samples.

The reproducibility of $\dot{V}O_{2\max}$ and EMG variables between INC₁ (performed before the experimental trials) and INC (performed in randomized order with DEC) was determined by using Student's *t*-test for dependent samples and by calculating the intraclass correlation coefficient (ICC), the typical error of measurement (defined as the standard deviation of the difference between measurements divided by the square root of two) (Hopkins, 2012) and the individual coefficient of variation (defined as the standard deviation of measurements divided by their mean) (Hopkins, 2000).

EMG activity between the INC and DEC was compared using a two-way ANOVA (using test and time as factors), comparing muscle activity at the start (first minute), 50% of TTF and at the final minute of exercise, with Bonferroni post-tests when necessary. Furthermore, a comparison of $\dot{V}O_{2\max}$ between the tests was performed using repeated-measures ANOVA, with Tukey *post-hoc* when necessary. Significance level was set at $p < 0.05$, and all statistical procedures were calculated using Prism 5 (Graphpad, La Jolla, CA, US) or dedicated Excel spreadsheets (Hopkins, 2012).

RESULTS

Reproducibility of variables and the effect of familiarization

On the experimental day of Part A of the study, $\dot{V}O_{2\max}$ values were very similar between INC₁ and INC ($4,335 \pm 543$ vs. $4,333 \pm 492$ ml.min⁻¹ respectively, $p = 0.96$). Intra-class coefficient for $\dot{V}O_{2\max}$ was 0.96, and the typical error of measurement was 111 ml.min⁻¹. For the EMG variables, reliability data for each muscle between INC₁ and

INC are shown in table 1. Overall, all four analyzed muscles showed a good degree of reliability.

Table 1: Reproducibility of normalized RMS activity (%RMS_{max}) for four lower limbs muscles during a maximal incremental cycling test.

	ICC	CV (%)	Typical error (percentage points)
VL (%RMS _{max})	0.92	8.9	5.1
VM (%RMS _{max})	0.94	7.1	3.6
BF (%RMS _{max})	0.96	5.6	3.2
GM (%RMS _{max})	0.95	10.6	4.6

ICC, Intraclass correlation coefficient; CV, coefficient of variation; VL, *Vastus Lateralis*; VM *Vastus Medialis*; BF, *Biceps Femoris*; GM, *Gastrocnemius Medialis*.

Adding a familiarization trial in Part B did not result in different DEC/INC $\dot{V}O_{2max}$ ratios between participants from the two parts of the study (1.024 ± 0.037 vs. 1.042 ± 0.073 for parts A and B respectively, $p = 0.49$). Furthermore, there were no differences in $\dot{V}O_{2max}$ between the INC_{fam} and INC ($4,120 \pm 795$ vs. $4,090 \pm 660$ ml.min⁻¹ respectively, $n=19$, $p = 0.73$) or between DEC_{fam} and DEC ($3,889 \pm 700$ vs. $3,904 \pm 743$ respectively, $n=8$, $p = 0.80$).

Physiological measurements at $\dot{V}O_{2max}$

During INC, participants were able to exercise for 8.3 ± 1.7 minutes reaching a $\dot{V}O_{2max}$ of 55.8 ± 8.2 ml.kg⁻¹.min⁻¹. During DEC, participants reached $\dot{V}O_{2max}$ values on average 3.2% higher than during INC ($4,215 \pm 0.698$ vs. $4,090 \pm 0,660$ ml.min⁻¹ respectively, $p = 0.0184$, figure 2), although they were exercising at a significantly lower power output when $\dot{V}O_{2max}$ was achieved (312 ± 43 vs. 333 ± 47 W for DEC and INC respectively, $p = 0.0004$). All physiological data measured during the exercise test are shown in table 2. During DEC, participants also reached 7% higher ventilation rates in comparison to INC ($p = 0.004$), mostly due to an increased breathing rate (BR) (52.6 ± 6.4 vs. 46.8 ± 5.8 breaths.min⁻¹ for DEC and INC respectively, $p = 0.01$). Respiratory exchange ratio, carbon dioxide output and heart rate were not different between the two exercise tests (table 2).

Table 2: Physiological data at $\dot{V}O_{2\max}$ and P value for Student's *t*-tests between an incremental (INC) and a decremental (DEC) exercise tests.

	INC	DEC	P value
$\dot{V}O_{2\max}$ (ml.min ⁻¹)	4,090 ± 660	4,215 ± 680	0.01
HR (beats.min ⁻¹)	178 ± 9	177 ± 11	NS
$\dot{V}_E$ (l.min ⁻¹)	148.2 ± 23.8	158.7 ± 29.0	0.004
RER	1.09 ± 0.11	1.11 ± 0.12	NS
$\dot{V}CO_2$ (ml.min ⁻¹)	4,508 ± 689	4,653 ± 777	NS
BR (breaths.min ⁻¹)	46.9 ± 5.8	52.6 ± 6.4	0.01

$\dot{V}O_{2\max}$, maximal oxygen consumption; HR, heart rate; $\dot{V}_E$, ventilation (BTPS); RER, respiratory exchange ratio; $\dot{V}CO_2$, carbon dioxide output; BR, breathing rate.

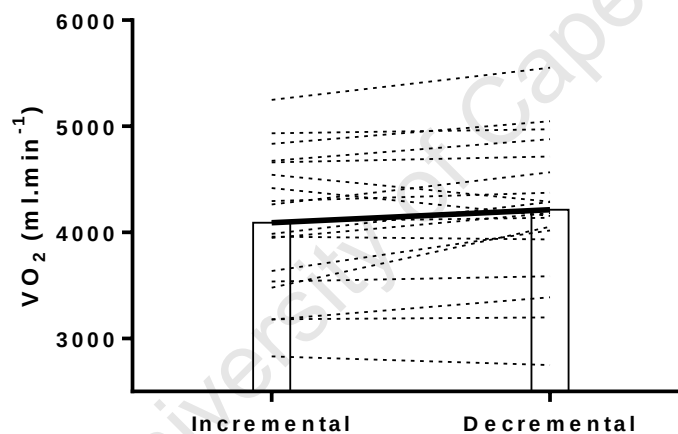


Figure 2: Individual (dotted lines) and mean (thick solid line and columns) $\dot{V}O_{2\max}$ responses during an incremental and a decremental exercise test.

Muscle Activity

Muscle activity increased for all analyzed muscles during INC (Figure 3). For VL and VM, EMG activity during INC was higher at 100% of TTF than both at the Start or 50% of TTF. For GM and BF EMG activity during INC at 100% of TTF was higher than the values measured at the Start only. During DEC, however, there was no differences in EMG activity between the different parts of the test (figure 4), for any of the analyzed muscles. Two-way repeated measures ANOVA did not show any Test effects for EMG,

and although there was significant interaction between test and time (start, 50% and 100%) for all four muscles, Bonferroni *post hoc*s did not identify any differences between tests at any time.

There were no differences in mean EMG activity measured at $\dot{V}O_{2\max}$ between INC and DEC for any of the analyzed muscle groups (figure 4).

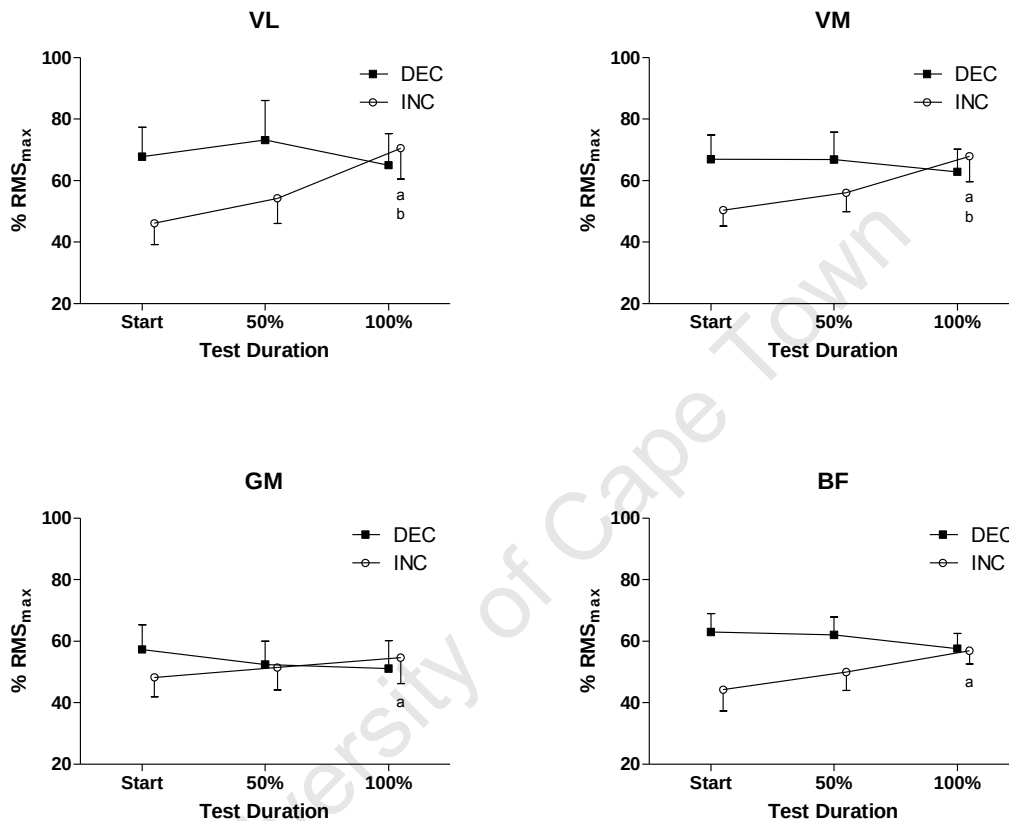


Figure 3: Mean RMS of EMG activity (mean $\pm$ SE) during an incremental exercise test (open circles) and a decremental exercise test (solid squares). *Vastus Lateralis* (VL), *Vastus Medialis* (VM), *Biceps Femoris* (BF), *Gastrocnemius Medialis* (GM). ^a Different from respective Start ($p < 0.05$); ^b Different from respective 50% (<0.05).

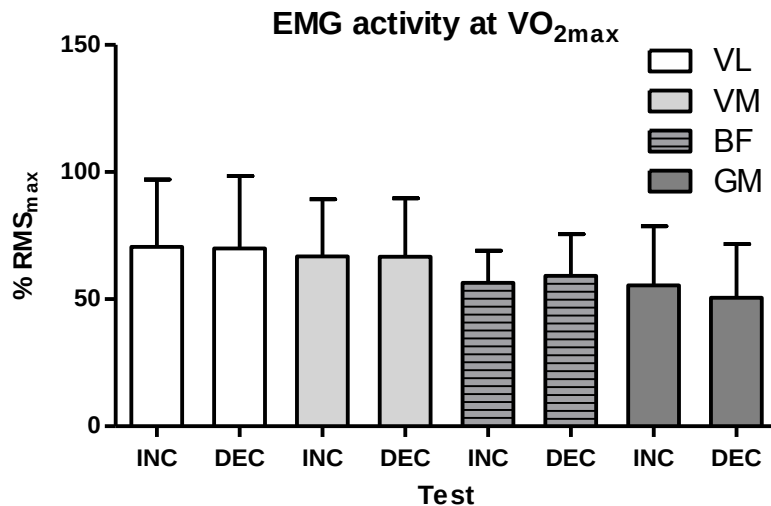


Figure 4: Mean RMS electromyographical (EMG) activity for four lower limbs muscles at $\dot{V}O_{2max}$ during an incremental (INC) and a decremental (DEC) exercise tests. *Vastus Lateralis* (VL), *Vastus Medialis* (VM), *Biceps Femoris* (BF), *Gastrocnemius Medialis* (GM).

DISCUSSION

This study was designed to test whether trained individuals could reach higher $\dot{V}O_{2max}$ values in cycling exercise during a decremental protocol in comparison to the traditional incremental exercise test. Furthermore, we wished to investigate if muscle activity in the lower limbs was different between tests. Accordingly, the main findings from this study were that: i) trained subjects achieved higher $\dot{V}O_{2max}$ values during DEC in comparison to INC; ii) the increase in $\dot{V}O_{2max}$ during DEC was accompanied by higher values for $\dot{V}_E$ in comparison to INC; iii) EMG activity for four lower limbs muscles was similar at $\dot{V}O_{2max}$ in both testing protocols; and iv) the order of the tests or previous familiarization with DEC did not affect the $\dot{V}O_{2max}$ results. Although the higher $\dot{V}O_{2max}$ during DEC was in line with our initial hypothesis, the differences in $\dot{V}_E$ and similar EMG activity at $\dot{V}O_{2max}$ between tests were unexpected findings.

Gas Exchange

The results from the present study extend the findings originally reported during uphill running (Beltrami *et al.*, 2012), showing that ~3% higher $\dot{V}O_{2max}$ values

can be achieved during DEC in comparison to INC when exercise is performed on a cycle ergometer. Since incremental exercise protocols have long been the norm for measuring $\dot{V}O_{2\max}$, every new protocol proposed in the literature must be compared to that traditional method (Foster *et al.*, 1993; Foster *et al.*, 1997; Alexander & Mier, 2011; Chidnok *et al.*, 2012; Beltrami *et al.*, 2012). These comparisons need to be performed across a range of different exercise modalities, since responses to an exercise protocol are not necessarily the same (Crouter *et al.*, 2001) for different modalities. $\dot{V}O_{2\max}$ is known to vary depending on the type of activity performed, and this is thought to reflect the different amounts of muscle mass that can be activated by different exercise modalities (Astrand & Saltin, 1961). It is generally accepted that untrained individuals reach 5-10% lower $\dot{V}O_{2\max}$ in cycling compared to running (Hermansen & Saltin, 1969; Kamon & Pandolf, 1972; Miyamura & Honda, 1972) and this difference decreases as the participant's training level in cycling increases (Basset & Boulay, 2000), to the point that $\dot{V}O_{2\max}$ can be higher in cycling than in running in well-trained cyclists (Millet *et al.*, 2009; Pecher *et al.*, 1974).

In the present study, both $\dot{V}_E$ and BR were higher during DEC in comparison to INC at the time of $\dot{V}O_{2\max}$. This was unexpected, since it was not found in previous studies in runners (Beltrami *et al.*, 2012, **Chapter 4**). It is a possibility that, at least in cycling, part of the difference in $\dot{V}O_{2\max}$ measured between tests is related to the additional O_2 cost of breathing. During maximal exercise, the work done by the respiratory muscles is thought to account for 8-10% of whole-body $\dot{V}O_2$ (Shephard, 1966; Aaron *et al.*, 1992; Harms *et al.*, 1998). The $10.0 \text{ l}\cdot\text{min}^{-1}$ difference in $\dot{V}_E$ measured between DEC and INC could raise $\dot{V}O_2$ by approximately $30\text{-}45 \text{ ml}\cdot\text{min}^{-1}$ according to different estimates of the O_2 cost of breathing during strenuous exercise (Shephard, 1966; Aaron *et al.*, 1992). This means that between 25-33% of the difference in $\dot{V}O_{2\max}$ measured between DEC and INC in cycling ($125 \text{ ml}\cdot\text{min}^{-1}$) could be attributed to the extra work performed by the respiratory muscles.

Additional oxygen uptake, be it in the respiratory or exercising muscles, would need either additional blood supply or increased oxygen extraction by the active muscles which would have increased the arterio-venous O_2 difference for a similar cardiac output. But it is assumed that all these variables are already at their maximum

values during incremental exercise tests, so it is not immediately clear how a higher $\dot{V}O_{2\max}$ can be achieved by further increases in these already maximal values. Moreover, simply forcing the respiratory muscles to perform more work does not increase whole-body $\dot{V}O_{2\max}$ or cardiac output (Harms *et al.*, 2000), similar to what happens when an additional (supra-maximal) load is imposed to the muscles of the exercising limbs (Billat *et al.*, 1995; Day *et al.*, 2003; Brink-Elfegoun *et al.*, 2007).

EMG activity

In spite of the lower power output at $\dot{V}O_{2\max}$ during DEC, the third main finding of this study was that EMG activity was similar between DEC and INC at the $\dot{V}O_{2\max}$. Vanhatalo and colleagues (2011) proposed that the $\dot{V}O_2$ during all-out exercise tests (where $\dot{V}O_2$ increases up to $\dot{V}O_{2\max}$ despite decreased power output and activity) is a “sustained” phenomenon, resulting from the recruitment of type IIx fibers. According to this theory, $\dot{V}O_2$ increases during exercise because less efficient, type IIx fibers are activated, increasing the $\dot{V}O_2$ cost for force production per fiber (Vanhatalo *et al.*, 2011). In the present study there was no difference in EMG between DEC and INC in spite of different power output, which indicates that a similar muscle mass was employed to produce less force, thus implying reduced efficiency. An alternative explanation proposed by Mauger (2013) is that while an initial bout of very intense exercise creates the demand for $\dot{V}O_2$, a subsequent decrease in power output might improve limb blood flow and optimize oxygen transit time. The lower power output would allow the activation of type I fibers, creating optimal physiological conditions for $\dot{V}O_2$ utilization (Mauger, 2013).

Noakes (2011; see also Spurway *et al.*, 2012) have proposed that $\dot{V}O_{2\max}$ is not limited by cardiovascular or peripheral factors, but rather is regulated by the central nervous system (CNS) during strenuous exercise, in order to prevent physiological catastrophe, such as myocardial ischemia. According to this theory, the CNS can regulate $\dot{V}O_2$ uptake by varying the amount of muscle mass recruited during activity. If this is correct, protocols that elicit higher $\dot{V}O_{2\max}$ should present a higher degree of muscle activity if the additional $\dot{V}O_2$ is used by the exercising muscles. This is clearly not the case in the current study. It has already been noted that muscle activity

can be higher during “supra-maximal” then during maximal exercise without any increases in $\dot{V}O_{2\max}$ (Brink-Elfegoun *et al.*, 2007; Chidnok *et al.*, 2012). Therefore, it seems that $\dot{V}O_{2\max}$ can be achieved with differences in the EMG activity of the exercising limbs, which make it difficult to define the contribution of different extent of muscle activation in determining the absolute $\dot{V}O_{2\max}$ values that are achieved. However it is noticeable that $\dot{V}O_{2\max}$ is achieved at sub-maximal levels of skeletal muscle recruitment (figure 4), confirming that the CNS determines the peak workload during the $\dot{V}O_{2\max}$ test (Noakes *et al.*, 2005), which in turn would set the demand for O_2 during exercise. Another complication is that different muscles can exhibit different patterns during maximal exercise, with some increasing mean EMG activity, while others remain unaltered or even showing decreased EMG activity (Dorel *et al.*, 2009). These alterations are thought to result from changes in coordination and pedal force application (Dorel *et al.*, 2009). Nonetheless the range of changes in EMG activity measured in the present study is compatible to that available in the literature for incremental exercise tests (Chidnok *et al.* 2012), and we believe that the lack of differences in EMG activity at $\dot{V}O_{2\max}$ between the exercise trials is a real phenomenon, rather than a measurement error.

Effects of familiarization and order of trials

The fourth main finding from this study is that the $\dot{V}O_{2\max}$ differences between DEC and INC were independent of whether participants were allowed a specific familiarization trial for DEC or not, and were also independent of the order in which INC and DEC were performed. This contrasts with previous findings (Beltrami *et al.*, 2012, **Chapter 5**), in which following DEC participants retained the higher $\dot{V}O_{2\max}$ on a subsequent INC, despite an unchanged performance in the final incremental test. Should the same have happened in the present study, $\dot{V}O_{2\max}$ would have increased from INC_{fam} to INC (Part B), since participants performed the DEC_{fam} trial between these two tests. Also, if participants had retained the higher $\dot{V}O_{2\max}$ values following DEC, the $\dot{V}O_{2\max}$ differences between INC and DEC would have been blunted for all participants who performed INC after DEC. But this did not happen. Although these findings do not help to explain why the running studies showed a persistent increased

$\dot{V}O_{2\max}$ following the DEC protocol, they show that future research using cycling exercise can be performed using a familiarization trial for both INC and DEC. Although our results suggest that there is little if any effect of a familiarization trial on $\dot{V}O_{2\max}$ itself during INC or DEC, in contrast to what is seen for time-trials (Swart *et al.*, 2009) and sub-maximal exercise (Shephard, 1966), it is still important to ensure that participants are familiar with all the exercise procedures involved in a study.

Limitations

A possible limitation of this study was the pooling of data from the Parts A and B of the investigation, which had slightly different designs. However, it is clear that the inclusion of INC₁ from Part A of the study and DEC_{fam} from Part B had little effect on the data used for analysis. This is probably because our participants were all accustomed to high-intensity training, thus had little to benefit from a familiarization trial with high-intensity exercise, and were also able to tolerate an additional bout of high-intensity exercise if they were allowed an appropriate rest period. A more serious concern arises from the use of electromyography to quantify muscle activation during cycling. All EMG data were normalized to a maximal sprint, which has been recommended as one of the best ways to normalize the EMG signals since it eliminates the problems associated with the muscle belly displacements under the skin when normalization to an isometric contraction is used (Jacobs *et al.*, 2011). Moreover, the two incremental tests presented levels of reliability similar to those found by others (Larsson *et al.*, 1999; Laplaud *et al.*, 2006), confirming the quality of these data. Nonetheless, the differences in power output between DEC and INC at $\dot{V}O_{2\max}$ were relatively small (21 W) and probably beyond the detection limits of the EMG. Thus, even if our results suggest a loss of efficiency in producing mechanical work, as shown by a higher $\dot{V}O_2$ for a similar EMG activity, it is difficult to determine whether the measured difference in $\dot{V}O_2$ ($\sim 125 \text{ ml}\cdot\text{min}^{-1}$) could be related to any change in muscle efficiency, or if in addition to changes in efficiency there was also a shift in O_2 uptake to non-exercising tissues (Yano *et al.*, 2004; Yano *et al.*, 2000).

The participants of the present study were all accustomed to high-intensity cycling, but although they presented a similar response between the DEC and INC

protocols as previously demonstrated in runners (Beltrami *et al.*, 2012, **Chapter 5**), it is not known whether the $\dot{V}O_{2\max}$ measured in DEC would be similar to or higher than their $\dot{V}O_{2\max}$ measured during running. Since it is expected that a maximum cycling effort might terminate with greater cardiovascular “reserve” in comparison to uphill running (Hermansen *et al.*, 1970; Miyamura & Honda, 1972), future research could be directed to evaluating whether a decremental cycling protocol decreases the $\dot{V}O_{2\max}$ difference observed between cycling and running. This could potentially benefit the detection of “true” $\dot{V}O_{2\max}$ in unfit individuals, who have significantly lower $\dot{V}O_{2\max}$ during cycling (Hermansen *et al.*, 1970; Miyamura & Honda, 1972).

Summary

In summary, this study showed that trained individuals achieve higher $\dot{V}O_{2\max}$ values during a decremental cycling protocol in comparison to a traditional incremental exercise test. The additional $\dot{V}O_2$ can partly be attributed to the extra work performed by the respiratory muscles during DEC, and future studies can be designed in order to compare the activation of the respiratory muscles between the DEC and INC protocols. Muscle activation of the lower limbs was similar in both test protocols in spite of the different power outputs at $\dot{V}O_{2\max}$, suggesting a loss of efficiency from the exercising muscles, which would also increase their demand for oxygen.

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Chapter 7

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ABSTRACT

Introduction

It has been shown that during a decremental exercise protocol higher values of maximal oxygen uptake ($\dot{V}O_{2max}$) can be achieved compared to a traditional incremental exercise test. The aim of this investigation was to compare central and peripheral hemodynamics, hematological and gas exchange variables at the time of $\dot{V}O_{2max}$ between these two exercise protocols to better understand why $\dot{V}O_{2max}$ differs between them.

Methods

After determining peak power output (PPO) required to achieve $\dot{V}O_{2max}$ in a standard incremental cycling protocol, nine trained participants performed an incremental cycling exercise test (INC) followed by a decremental cycling exercise test (DEC). During INC power output was adjusted to 20% of PPO for 5 min, 50% for 3 min, and 80, 90 and 100% for 2 min each. After a 45 min rest, DEC started at 20 W higher than the PPO during INC for ~1.5 min and afterwards load was decreased by 20-30W and held for 30 seconds, and then reduced in 10 W steps lasting, in sequence, 45, 60, 90 and 120 seconds.

Results

Participants reached higher $\dot{V}O_{2max}$ ($p = 0.04$) despite lower power output at the time ($p < 0.05$). Supporting this, cardiac output ($\dot{Q}$) ($p = 0.02$), O_2 extraction ($p < 0.0001$) and minute ventilation ($p = 0.02$) were all higher during DEC, while leg blood flow and leg $\dot{V}O_2$ were numerically but not significantly greater ($p = 0.11$, 0.12 respectively, likely due to high variance). DEC also resulted in considerably higher arterial blood lactate levels ($p < 0.0001$) and lower blood pH ($p < 0.0001$).

Discussion

Higher $\dot{V}O_{2\max}$ during DEC has three likely contributors: 1) greater O_2 extraction 2) higher $\dot{Q}$, enabling greater muscle blood flow (although confirmation of this was inconclusive due to high variance in leg flow measurements) and 3) higher work of breathing due to greater ventilation. Higher O_2 extraction during DEC was facilitated by the lower blood pH and higher blood temperature, which shifted the Hb- O_2 dissociation curve rightwards; there were no difference in calculated muscle O_2 diffusional conductance.

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INTRODUCTION

Maximal oxygen uptake ($\dot{V}O_{2\max}$) is typically characterized by the presence of the “plateau phenomenon” at the end of maximal incremental exercise tests, where oxygen consumption ($\dot{V}O_2$) levels off in spite of increases in workloads (Taylor *et al.*, 1955; Mitchell *et al.*, 1958). This phenomenon is believed to represent a limitation in the cardiovascular capacity to deliver blood to the exercising muscles (Levine, 2008). Plateauing of $\dot{V}O_2$ however does not always happen at the same absolute value for O_2 consumption or power output - for example, when O_2 supply is acutely manipulated by changing the fraction of oxygen in the inspired air (FIO_2) (Knight *et al.*, 1993; Peltonen *et al.*, 2001; Wehrlin & Hallen, 2006). In such circumstances, maximal cardiac output does not differ with FIO_2 , implying that oxygenation of the arterial blood and not just blood flow can affect the plateau value of $\dot{V}O_2$.

The pathway for O_2 transport from the ambient air into the exercising muscles' mitochondria can be seen as a cascade of *in series* resistances, and each step of this pathway can affect $\dot{V}O_{2\max}$ (Wagner, 1996). Manipulating each of the variables involved in the O_2 transport pathway allows identification of the role of each step in limiting $\dot{V}O_2$ during maximal exercise. Thus reducing the fraction of O_2 in the inspired air (Peltonen *et al.*, 2001) will reduce $\dot{V}O_{2\max}$ due to decreased O_2 delivery to the exercising muscles, while increasing red blood cell content (Lundby *et al.*, 2008; Robach *et al.*, 2008) or increasing the fraction of O_2 in the inspired air (Knight *et al.*, 1993) increase O_2 delivery and $\dot{V}O_{2\max}$. Alternatively, $\dot{V}O_{2\max}$ can be increased by increasing O_2 extraction at the muscle via induced changes in the affinity of O_2 binding to hemoglobin, specifically without changes in O_2 delivery by the circulation (Hogan *et al.*, 1989; Richardson *et al.*, 1998). It is thus clear that each step of the O_2 transport pathway has the power to affect $\dot{V}O_{2\max}$.

$\dot{V}O_{2\max}$ can also be altered without direct manipulation of the variables within the O_2 transport system described above. A recent study by Beltrami and colleagues (2012) demonstrated that a decremental exercise test led to a higher $\dot{V}O_{2\max}$ than that achieved during a standard incremental maximal exercise testing the same subjects.

The authors however were unable to determine if the additional $\dot{V}O_2$ was taken up by the exercising limbs or other organs such as the brain, liver or respiratory muscles (Nielsen, 2003; Beltrami *et al.*, 2012). Even so, there must have been an underlying difference between the two exercise protocols in one or more components of the O_2 transport chain to explain the difference in $\dot{V}O_{2max}$, for it is clear that simply requiring the active limbs (Brink-Elfegoun *et al.*, 2007b) arms (Brink-Elfegoun *et al.*, 2007a) or respiratory muscles (Harms *et al.*, 1998) to perform additional work at maximal exercise does not in itself increase $\dot{V}O_{2max}$. This is often attributed to the belief that $\dot{V}O_{2max}$ is limited by O_2 delivery, not O_2 demand, so that once the heart reaches its limiting pumping capacity $\dot{V}O_{2max}$ will plateau regardless of how much, or even at which site of the body, the demand for O_2 is increased (Brink-Elfegoun *et al.*, 2007a, Brink-Elfegoun *et al.*, 2007b).

Nonetheless, the unusual situation created in the protocol devised by Beltrami *et al.*, (2012) provides a possibility to approach the question of what limits $\dot{V}O_{2max}$ from a different perspective: instead of directly manipulating variables related to O_2 delivery to test whether it produces noticeable changes in $\dot{V}O_{2max}$, it is possible to compare two situations that produce different $\dot{V}O_{2max}$ values to determine which, if any, of the $\dot{V}O_{2max}$ determinants are different between the two tests.

Therefore, the aim of this study was to compare variables related to O_2 transport and uptake measured at $\dot{V}O_{2max}$ between an incremental and a decremental exercise tests in the same subjects. We hypothesized that participants would achieve higher $\dot{V}O_{2max}$ values during the decremental exercise test, and that this would occur mainly due to improved extraction of O_2 from the arterial blood by the exercising muscles. While blood flow in the exercising limbs during incremental tests might be limited by vasoconstrictor sympathetic activity (Calbet *et al.*, 2006), acute increases in VO_2 through ischemic preconditioning have been related to improvements in the distribution of blood flow within active areas of the muscle (de Groot *et al.*, 2010; Park *et al.*, 2010). Thus, the $\dot{V}O_2$ difference between the tests could be explained by the recruitment of a larger muscle microvascular surface area for diffusive unloading of O_2 during the decremental exercise, increasing extraction of O_2 from the arterial blood by

the exercising muscles. This would in turn be manifest as a higher muscle O₂ diffusional conductance.

METHODS

Subjects

Nine participants (7 male and 2 female, age 23.7 ± 3.4 years, height 174 ± 6 cm, body mass 70.7 ± 9.5 Kg) volunteered to take part in this investigation. The participants were involved in cycling (n=4) and triathlon (n=5) for at least three years, and were used to high-intensity cycling training. Before taking part in the study, the participants were fully informed about the risks and procedures involved, and signed an informed consent form. This study was performed in accordance with the latest revision of the Declaration of Helsinki (World Medical Association, 2008), and was approved by the Ethics and Research Committee of the Faculty of Health Sciences of the University of Cape Town and the Human Research Protection Program at the University of California San Diego.

General Design

Participants visited the laboratory on two occasions, separated by 2-3 days. The first day (Visit #1) served to familiarize them with the exercise protocols and establish conventional $\dot{V}O_{2max}$ and peak power output (PPO) in an incremental test, and on the second day (Visit #2) the main experiments described below were performed. Participants were instructed to eat a light breakfast before reporting to the laboratory on the morning of the main experiment and were also requested to refrain from alcohol, caffeine and exercise for the 24h before the experiments.

All exercise tests were performed on an electronically braked cycle ergometer (Lode Excalibur Sport, Lode, the Netherlands), and the seat and handlebar position were adjusted for each subject as they wished, and then maintained constant throughout the trials. During the exercise trials, participants were clearly told that they

and not stand the odds, this subject recruitment, hemodynamic parameters, and $\dot{V}O_2$.

Preliminary Testing

On Visit #1, upon signing an informed consent form, the participants provided their medical history and underwent a physical examination, which included hemoglobin count (and pregnancy test for females). Participants then performed three tests, in the following order, each separated by 45 min rest: Incremental Test to $\dot{V}O_{2max}$, $\dot{V}O_{2max}$ Verification Test and Decremental $\dot{V}O_{2max}$ Test. These are each described below.

Prior to each test, participants were allowed to warm-up for 10 min by cycling at a self-selected power output. During the incremental test, power output after warm-up was increased to 50% of predicted peak power output, and was increased by 20 watts (W) every minute, until participants were unable to maintain the cycling cadence above 60 rpm despite strong verbal encouragement (task failure). The Verification Test consisted of a square-wave protocol, where, immediately after warm-up, participants were required to cycle until task failure at 20 W (one stage) higher than the last completed stage of the earlier incremental test. The Decremental Test started, after warm-up, at the same power output as that achieved in the Verification Test. The first stage of this test lasted for 60% of the time the participant had sustained before task failure at the power output used during the Verification Test. Power output was then decreased by 20 W for 30 seconds, and subsequent decreases of 10 W were applied over the next 45, 60, 90 and 120 s (Beltrami *et al.*, 2012).

Participant preparation

Upon their arrival on Visit #2 participants rested for approximately 10 min. The femoral vein was cannulated percutaneously about 1" below the inguinal ligament with a 16 gauge 20 cm venous catheter (Arrow AK-04301, Teleflex Medical, NC, USA). The catheter was introduced in sterile fashion under local (1% lidocaine) anaesthesia in

the distal direction, and was kept flushed, bubble free and firmly fixed to the skin to prevent dislodgement. Its tip was advanced about 8 cm from the skin to assure its location within the vein. Next, a sterile 0.5 mm diameter Physitemp IT-19 Teflon-coated thermistor wire (Physitemp Instruments Inc., Clifton, NJ, USA) was inserted into the same femoral vein in a proximal direction. The thermistor was situated at the tip, and the wire was advanced about 10-12 cm proximally from the skin to ensure its intravenous location.

The radial artery of the non-dominant hand was cannulated percutaneously using a 20 gauge 1.16" Angiocath cannula. This was connected to a pressure transducer to record arterial blood pressure for both scientific and safety purposes for the remainder of the study except when used to take blood samples.

Main Experiments

Following the satisfactory placements of catheters, participants performed three cycling tests, in fixed order: an Incremental Test to $\dot{V}O_{2\max}$ (INC₁), a Decremental Test (DEC) and, in those who agreed, a second Incremental Test to $\dot{V}O_{2\max}$ (INC₂). Each test was separated by approximately 60 min of rest.

Based on the tests from Visit #1, both INC₁ and INC₂ were designed to elicit 20% of individual peak power for 5 min, 50% for 3 min, and 80, 90 and 100% of peak power for 2 min each. Additional increases equivalent to 10% of peak power were to be applied for any participant able to continue the test after the 100% stage. However this did not happen for any subject.

Prior to the DEC test, participants cycled for 5 min at 20% and for 3 min at 50% of PPO as a warm-up. Then power output was raised over 20 s to the power used in the decremental test performed on Visit #1 and held for approximately 1.75 min (range 1.25 – 2.25 min) after which power was reduced stepwise in the same manner as in the decremental test on visit #1 described above.

Even though the fixed order in which the tests of the main experiments were performed could potentially affect our results, we are unaware of any evidence showing that repeating tests on a single day leads to increased values on the second or

third trial. If anything, values could be expected to drop if participants were not able to fully recover between trials. Furthermore, data from **Chapter 6** showed no order effect on $\dot{V}O_{2\max}$ values when an incremental test was performed before or after a decremental one.

Gas exchange

During all tests, participants breathed through a non-rebreathing valve (model 2700, Hans Rudolph, Kansas City, MO, USA) connected to a mouthpiece. Expired gas flow was measured using a pneumotach (Hans Rudolph 3813, Hans Rudolph, Kansas City, MO, USA) and gas fractions were measured using a TrueOne 2400 Gas Analyzer (ParvoMedics, Salt Lake City, UT, USA) for determination of minute ventilation ($\dot{V}_E$), $\dot{V}O_2$, carbon dioxide excretion ($\dot{V}CO_2$) and respiratory exchange ratio (RER). The equipment was calibrated for both ventilation and gas fractions, using reference values that spanned those found during the maximal exercise tests. Prior to the study, the TrueOne 2400 Gas Analyzer was validated against a mass spectrometer and a different TrueOne 2400 Gas Analyzer system (data not shown). All respiratory data were collected as 15 s averages containing only full breaths and subsequently averaged over consecutive 1 min intervals for analysis. The minute containing the highest value for $\dot{V}O_2$ was considered as $\dot{V}O_{2\max}$.

Cardiac output

Cardiac output ($\dot{Q}$) and heart rate (HR) were measured non-invasively using thoracic bio-impedance (Physioflow PF07 Enduro, Manatec Biomedical, France), and stroke volume (SV) thereby calculated. The equipment measures the change in impedance across the thorax between a pair of electrodes positioned on the neck and another pair on the xiphoid process. A third pair of electrodes is positioned in the chest area to detect the ECG signal.

After cleaning and drying the skin, all six electrodes (Skintact, Innsbruck, Austria) were positioned strictly according to the manufacturer's guidelines. Once the cables were connected to the electrodes, an elastic chest net was used to secure the electrodes and their connections during exercise. The system was calibrated prior to

INC₁ while participants rested on the bicycle. This calibration was used for the two subsequent trials (DEC and INC₂). Data were recorded continuously, with signals averaged over each 10 s interval. For analysis, we subsequently averaged the results over consecutive 1 min intervals and synchronized them with the gas exchange variables.

Leg blood flow

Leg blood flow ($\dot{Q}_{\text{LEG}}$) during exercise was determined using the thermodilution technique (Ganz & Swan, 1972; Andersen & Saltin, 1985). Venous blood temperature was measured immediately before, during and after a short-term (~20 s) steady state infusion of iced (2-4°C) 0.9% sterile saline. The rate of saline infusion was controlled by a pump (Masterflex, model no. 7533-70, Cole Parmer Instrument Company, IL, US) and was adjusted for each exercise intensity to result in a ~1 °C drop in blood temperature. Femoral blood temperature, saline infusion temperature and rate were continuously recorded and displayed on a personal computer using the Acqknowledge data acquisition system (Biopac Systems Inc., Goleta, CA, USA). Leg blood flow was calculated using the heat conservation equation described by Ganz & Swan (1972), as follows:

$$F_B = F_I \times 1.08 \times \left(\frac{T_B - T_I}{T_B - T_M} - 1 \right)$$

where F_B is the rate of blood flow (in l.min⁻¹), F_I is the rate of infusion (in l.min⁻¹), 1.08 represents the ratio between the specific heat and gravity of blood to that of the saline infusion, T_B is the temperature of the blood prior to the infusion, T_I is the infusion temperature and T_M is the steady state temperature of the blood and saline mixture (in degrees °C). $\dot{Q}_{\text{LEG}}$ was measured in duplicate for each exercise level during INC₁ and INC₂ whenever possible, with both measurements performed in the final 90 s of each stage. In between measurements, the collection of blood samples (see below) was performed. During DEC, $\dot{Q}_{\text{LEG}}$ was measured after 60 s of heavy exercise (following the initial 5 min of light and 3 min of moderate cycling), and thereafter measurements were continuously alternated with the collection of blood samples until the end of the test. The values for $\dot{Q}_{\text{LEG}}$ during DEC are the average from two consecutive

measurements, to replicate the duplicate approach used during the incremental tests. All values for leg blood flow were multiplied by two so that values represent both legs.

Blood Samples

Samples (2 ml) of arterial and femoral venous blood were taken anaerobically (using sterile syringes) from the venous and arterial catheters at rest and at each exercise level during both INC₁ and INC₂. As mentioned, during DEC the blood samples were collected after every measurement of $\dot{Q}_{LEG}$, which resulted in around one sample per minute. Samples were immediately cleared of bubbles, placed in ice slurry and analyzed immediately after the end of each test for the following: haematocrit (%), pH, O₂ and CO₂ partial pressures (PO₂ and PCO₂, respectively, in mmHg), O₂ saturation (SO₂, in %) and hemoglobin concentration ([Hb], in g.dl⁻¹) (IL GEM Premier 3000; IL 682 Co-Oxymeter System, Instrumentation Laboratories, Lexington, MA, USA). Values for PO₂, PCO₂ and pH were corrected to the femoral blood temperature measured during the corresponding exercise step. Blood lactate concentration ([La], in mmol.l⁻¹) was measured using an YSI 2300 STAT Plus blood lactate analyzer (YSI Life Sciences, OH, USA).

Calculations

Oxygen content (ml/l) in arterial (a) and venous (v) blood was calculated as O₂ content = [Hb] × 1.39 × O₂ saturation + (PO₂ × 0.003), where [Hb] is the hemoglobin concentration (g.l⁻¹), O₂ saturation is expressed as a fraction and PO₂ is the partial pressure of oxygen in mmHg. Systemic and leg O₂ delivery (in ml.min⁻¹) were calculated as O₂ content × blood flow, where O₂ content is expressed in ml.l⁻¹ and blood flow ($\dot{Q}$ or $\dot{Q}_{LEG}$, in l.min⁻¹). Two-leg $\dot{V}O_2$ (in ml.min⁻¹) was calculated using the Fick principle ($\dot{V}O_2 = \dot{Q}_{LEG} \times (\text{arterial-venous O}_2 \text{ content})$), where $\dot{Q}_{LEG}$ is two-leg blood flow (l.min⁻¹) and O₂ content in ml.l⁻¹. Percentage O₂ extraction by the exercising muscles was calculated as 100 times the difference between arterial and venous O₂ content divided by the arterial O₂ content. Alveolar ventilation ($\dot{V}A$, in l.min⁻¹) was calculated as $\dot{V}A = (\dot{V}CO_2 \times 0.863)/PaCO_2$, where $\dot{V}CO_2$ (in ml.min⁻¹) represents the pulmonary excretion of CO₂ and PaCO₂ is the arterial CO₂ pressure (in mmHg). Alveolar O₂ pressure (PAO₂, in

mmHg), was calculated as $PAO_2 = 149.23 - PaCO_2 \times (0.2093 + (0.7907/RER))$, where $PaCO_2$ represents the arterial pressure of CO_2 (in mmHg) and RER represents the respiratory exchange ratio ($\dot{V}CO_2/\dot{V}O_2$). Alveolar-arterial difference in O_2 pressure ($AaDO_2$, in mmHg) was calculated as the difference between PAO_2 and PaO_2 .

Muscle diffusion capacity (D_{MO_2}) was calculated performing the Bohr integration (Roca *et al.*, 1989; Roca *et al.*, 1992), in which the D_{MO_2} required to achieve the measured levels of PvO_2 are calculated by a forward integration procedure based on the actual values for limb blood flow and arterial PO_2 , PCO_2 and pH measured during exercise. A more detailed explanation of the procedures and assumptions in this calculation can be found elsewhere (Roca *et al.*, 1989).

In order to understand the relative contributions of factors known to influence the Hb- O_2 affinity curve (changes in arterial pH, blood temperature and $PaCO_2$) the Bohr integration was first performed with the actual data collected at $\dot{V}O_{2max}$ and subsequently repeated after normalizing each of the above mentioned variables, one at a time, to the respective standard values (7.40, 37 °C and 40 mmHg).

Statistics

All data are presented as mean $\pm$ SD unless stated otherwise. Comparisons between tests for all collected variables were performed during the minute in which $\dot{V}O_{2max}$ was recorded. All comparisons between INC_1 and DEC were performed using Student's paired t-test for dependent samples. Analysis of variables over time within the exercise tests was performed using repeated-measures ANOVA, with Tukey *post hoc* analysis whenever a main effect was detected. All calculations were performed using Prism 5.0 (GraphPad Software, La Jolla, CA, USA), and statistical significance was set at $p < 0.05$.

During the analysis process, it was discovered that [Hb] levels dropped by ~7.5% between INC_1 and INC_2 ($p < 0.001$). This difference was probably due to plasma expansion from the saline infusions used to measure leg blood flow, which alone could lead to significant differences in hemodynamics and possibly also in $\dot{V}O_{2max}$ during exercise (Schaffartzik *et al.*, 1993; Stray-Gundersen *et al.*, 2001), and therefore the

data from the INC₂ test were excluded from the comparisons between tests in the present study.

ally, two participants failed to achieve at least 98% of $\dot{V}O_{2\max}$ from the INC₁ test during DEC (“non-responders”). The data from these participants are shown separately in the results table but were not included in the statistical analysis and figures because the objective of the present study was to understand the basis for the higher $\dot{V}O_{2\max}$ during the decremental test whenever this was observed – not to determine whether a decremental test raises plateau $\dot{V}O_2$, which has already been established (Beltrami *et al.*, 2012). Their response to the DEC test is further explored in the discussion section.

RESULTS

Systemic and leg O₂ uptake, gas exchange and exercise performance

All physiological variables collected at $\dot{V}O_{2\max}$ in the two tests are listed in table 1. During INC₁ participants were able to exercise for 13.0 ± 0.6 min, and only one participant was not able to complete at least one minute at 100% of the peak power output measured during the familiarization trial. During DEC, five participants were able to complete the entire exercise protocol and the other two were “non-responders”. During INC₁ $\dot{V}O_2$ increased progressively from 20% to 80 % of peak power (figure 1A, table 1) and peaked at a power output of 284 ± 72 W. During DEC, $\dot{V}O_2$ increased from the first to the third minute of the test (figure 1A) but did not change thereafter. $\dot{V}O_{2\max}$ during DEC was significantly higher than during INC₁ ($\sim 2.9\%$, $p = 0.04$), in spite of a significantly lower power output at which $\dot{V}O_{2\max}$ occurred during DEC (284 ± 63 W, $p = 0.004$).

CO₂ excretion at $\dot{V}O_{2\max}$ was also higher during DEC (figure 2D, $p = 0.04$) and as a result RER values were similar between tests (figure 2E). Both $\dot{V}O_2$ and RER however decreased towards the end of DEC.

$\dot{V}_E$ increased continually during INC₁ until 90% of peak power but values measured at $\dot{V}O_{2max}$ during DEC were still 9.8% higher than in INC₁ (figure 2A, $p = 0.02$). Similarly, $\dot{V}A$ was 14.8% higher during DEC at $\dot{V}O_{2max}$ compared to INC₁ ($p = 0.002$, figure 2F) but AaDO₂ at $\dot{V}O_{2max}$ was similar between tests (table 1). Both $\dot{V}_E$ and $\dot{V}A$ increased from the second to the third minute of DEC, and stabilized thereafter.

Leg $\dot{V}O_2$ rose linearly during INC₁ ($r^2 = 0.94$, $p = 0.005$, figure 1B, 4D). During DEC, there was no difference in $\dot{V}O_2$ values between the different times measured. Leg $\dot{V}O_2$ at $\dot{V}O_{2max}$ during DEC was numerically higher (18%) than during INC₁, but the difference did not reach statistical significance ($p = 0.12$, table 1).

Cardiac output, leg blood flow and oxygen delivery

During INC₁, $\dot{Q}$ increased linearly throughout the test ($r^2 = 0.967$, $p = 0.002$) (figure 1C, 4E and 5). During DEC however, $\dot{Q}$ was constant at each step of the test. $\dot{Q}$ at $\dot{V}O_{2max}$ was on average 4.9% higher during DEC in comparison to INC₁ ($p = 0.008$, table 1). The differences in $\dot{Q}$ between the two tests were due to higher HR during DEC in comparison to INC₁ (5.6 ± 2.8 beats.min⁻¹, $p = 0.001$) since SV was similar at $\dot{V}O_{2max}$ between the two tests ($p = 0.25$).

During INC₁, SV increased only until 50% of peak power (figure 1I). Thereafter $\dot{Q}$ rose due to higher HR, which increased continuously during the test (figure 1G). During DEC, HR increased until the third minute of the test and remained stable thereafter, whereas SV was constant throughout the trial.

Similarly to $\dot{Q}$, systemic O₂ delivery also increased continuously during INC₁ ($r^2 = 0.963$, $p = 0.003$, figures 1E, 4A). During DEC, no changes were detected throughout the test. Although $\dot{Q}$ values measured at $\dot{V}O_{2max}$ were significantly higher for DEC, there was no difference between tests in systemic O₂ delivery ($p = 0.21$, table 1).

Two-leg blood flow ($\dot{Q}_{LEG}$) increased in proportion to $\dot{Q}$ during INC₁ (figure 1F, 5). During DEC, $\dot{Q}_{LEG}$ was constant throughout the test, and although values at $\dot{V}O_{2max}$ were 17.6% higher than those during INC₁, they only approached statistical significance ($p = 0.11$, figure 1D, table 1). Leg O₂ delivery measured at $\dot{V}O_{2max}$ during INC₁ was also

numerically higher (15%) for DEC but this difference was not statistically significant ($p = 0.18$, table 1, figure 1F).

Lactate, pH and blood gases

Despite arterial lactate concentration $[la]$ increasing continuously during INC_1 (figure 2B), values measured during DEC at $\dot{V}O_{2max}$ were even higher ($p = 0.0001$). Conversely, arterial pH dropped continuously during INC_1 but the decrease was more pronounced during DEC ($p = 0.001$, figure 2C). Values for arterial $[la]$ and pH during DEC were significantly different between each pair of measurements within the test, except for the final two values (90% and $\dot{V}O_{2max}$ for INC_1 and $\dot{V}O_{2max}$ and last values for DEC).

Both SaO_2 and temperature-corrected PaO_2 decreased by small amounts during INC_1 so that values at $\dot{V}O_{2max}$ were significantly reduced only when compared to 20% of peak power (figure 3C, E). The small changes in SaO_2 and PaO_2 however were insufficient to significantly affect CaO_2 during the different stages of INC_1 (figure 3A). During DEC, PaO_2 did not change but SaO_2 fell towards the end of the test, which decreased CaO_2 ($p < 0.001$ for values at $\dot{V}O_{2max}$ and final value versus first and second measurements). As a result, both CaO_2 and SaO_2 were lower at $\dot{V}O_{2max}$ in DEC compared to INC_1 (figure 3A, C).

Femoral venous $[O_2]$ (CvO_2) was also lower at $\dot{V}O_{2max}$ during DEC ($p = 0.006$, figure 3B), mostly due to lower SvO_2 in comparison to INC_1 (figure 3D). As a result, leg a-v O_2 difference was similar between the two tests at $\dot{V}O_{2max}$ ($p = 0.57$, figure 1H, 4C), despite higher leg O_2 extraction during DEC ($p = 0.003$, figure 1J, 4B, table 1).

To understand the individual roles of exercised-induced changes in pH, temperature and PCO_2 on O_2 extraction as a result of shifts in the $[Hb]-O_2$ dissociation curve, we used a forward numerical integration procedure (Bohr Integration) to calculate expected extraction values, all other factors held constant. During INC_1 , when O_2 extraction was calculated after raising arterial pH to resting values (which resulted in a leftward shift of the $[Hb]-O_2$ dissociation curve), the computed value of O_2 extraction decreased from 93.1 to 84.2 % and further decreased to 82.2% when blood

temperature was also changed to resting values. Normalizing to resting PaCO_2 values had no additional effect.

During DEC, theoretical O_2 extraction decreased from 94.7 to 82.7% after pH normalization, and further decreased to 79.5% when blood temperature was also normalized. Normalizing to resting PaCO_2 values again did not affect theoretical O_2 extraction.

The average $\text{D}_{\text{M}\text{O}_2}$ required to match the measured PvO_2 (calculated using the Bohr integration using actual pH, PCO_2 and temperature in each case) was numerically 7.8% higher at $\dot{\text{V}}\text{O}_{2\text{max}}$ during DEC (table 1), but this difference failed to reach statistical significance ($p = 0.36$).

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Table 1: Comparison of physiological data measured at $\dot{V}O_{2\max}$ during the incremental (INC₁) and decremental (DEC) exercise tests.

	Responders (n=7)		P	Non-responders (n=2)	
	INC ₁	DEC		INC ₁	DEC
[Hb] (g.l ⁻¹)	143 ± 11	141 ± 11	0.21	153 ± 8	147 ± 13
Hematocrit (%)	39.6 ± 3.3	39.6 ± 3.6	0.86	41.5 ± 1.3	41.3 ± 2.6
$\dot{V}O_{2\max}$ (l.min ⁻¹)	3.42 ± 0.80	3.51 ± 0.80	0.04*	3.90 ± 0.14	3.70 ± 0.00
Leg $\dot{V}O_2$ (l.min ⁻¹)	2.81 ± 0.66	3.28 ± 0.93	0.12	3.50 ± 0.26	3.41 ± 0.06
$\dot{Q}$ (l.min ⁻¹)	20.1 ± 2.6	21.1 ± 2.6	0.007*	21.6 ± 2.8	21.7 ± 2.6
Heart Rate (beats.min ⁻¹)	172 ± 6	177 ± 6	0.001*	178 ± 5	174 ± 3
Stroke volume (ml.beat ⁻¹)	117 ± 15	119 ± 15	0.25	122 ± 19	125 ± 17
RER	1.06 ± 0.04	1.07 ± 0.08	0.56	1.10 ± 0.06	0.96 ± 0.03
$\dot{V}_E$ (l.min ⁻¹ , btps)	122 ± 24	134 ± 27	0.02*	166 ± 2	133 ± 23
$\dot{V}CO_2$ (l.min ⁻¹)	3.65 ± 0.86	3.78 ± 0.92	0.04*	4.34 ± 0.42	3.58 ± 0.13
$\dot{V}A$ (l.min ⁻¹)	90 ± 20	103 ± 20	0.002*	110 ± 4	98 ± 6
PAO ₂ (mmHg)	116 ± 3	119 ± 3	0.001*	118 ± 3	117 ± 2
AaDO ₂ (mmHg)	25 ± 5	27 ± 5	0.21	20 ± 9	18 ± 17
Syst. O ₂ delivery (l.min ⁻¹)	3.93 ± 0.70	4.03 ± 0.67	0.21	4.52 ± 0.36	4.36 ± 0.09
$\dot{Q}_{LEG}$ (l.min ⁻¹)	15.7 ± 3.3	18.5 ± 4.7	0.11	20.7 ± 2.6	19.8 ± 1.6
Leg O ₂ delivery (l.min ⁻¹)	3.02 ± 0.69	3.46 ± 0.99	0.18	4.02 ± 0.04	3.79 ± 0.32
a-v O ₂ difference (ml.l ⁻¹)	180 ± 13	179 ± 11	0.57	172 ± 6	179 ± 9
Extraction (%)	92.2 ± 2.1	93.9 ± 2.9	0.003*	86.6 ± 6.6	89.1 ± 4.5
D _M O ₂ (ml.min ⁻¹ .mmHg ⁻¹)	84.4 ± 20.5	91.1 ± 23.9	0.36	96.7 ± 24.3	78.6 ± 28.5
O ₂ concentration (ml.l ⁻¹)	a 195 ± 14	191 ± 15	0.09	209 ± 1	202 ± 2
	v 15 ± 5	12 ± 7	<0.006*	27 ± 16	20 ± 14
[la] (mmol.l ⁻¹)	a 9.3 ± 1.7	13.4 ± 1.7	0.0001*	9.5 ± 0.0	10.7 ± 0.9
	v 10.8 ± 2.1	14.5 ± 2.1	0.0002*	10.5 ± 0.9	11.3 ± 0.1
pH	a 7.21 ± 0.04	7.10 ± 0.06	0.0003*	7.21 ± 0.01	7.18 ± 0.03
	v 7.00 ± 0.06	6.92 ± 0.07	0.0002*	7.05 ± 0.03	7.04 ± 0.00
PO ₂ (mmHg)	a 91 ± 7	93 ± 7	0.19	97 ± 7	98 ± 15
	v 11 ± 2	11 ± 2	0.34	14 ± 4	12 ± 5
O ₂ saturation (%)	a 96.7 ± 1.3	95.8 ± 1.8	0.009*	97.7 ± 0.0	97.2 ± 1.0
	v 7.5 ± 2.1	5.8 ± 2.3	0.003*	12.5 ± 7.2	9.5 ± 6.0
PCO ₂ (mmHg)	a 35 ± 3	32 ± 3	0.004*	34 ± 4	32 ± 1
	v 84 ± 10	82 ± 8	0.53	75 ± 5	70 ± 8
Venous temp. (°C)	37.8 ± 0.5	38.5 ± 0.4	0.0008*	38.2 ± 0.5	38.2 ± 0.6

P indicates the Student's *t*-test for dependent samples (2 tails) p value. [Hb], hemoglobin concentration; $\dot{V}O_{2\max}$, maximal oxygen uptake; $\dot{Q}$, cardiac output; RER, respiratory exchange ratio; $\dot{V}_E$, minute ventilation; $\dot{V}CO_2$, CO₂ excretion; $\dot{V}A$, alveolar ventilation; PAO₂, alveolar pressure of O₂; AaDO₂, alveolar-arterial difference in O₂ pressure; D_MO₂, muscle diffusing capacity; $\dot{Q}_{LEG}$, leg blood flow; a-v O₂ difference, arterio-venous difference of O₂; a, arterial; v, venous; [la], blood lactate concentration; PO₂, partial pressure of O₂; PCO₂, partial pressure of CO₂. * Significant difference between DEC and INC₁ at p<0.05

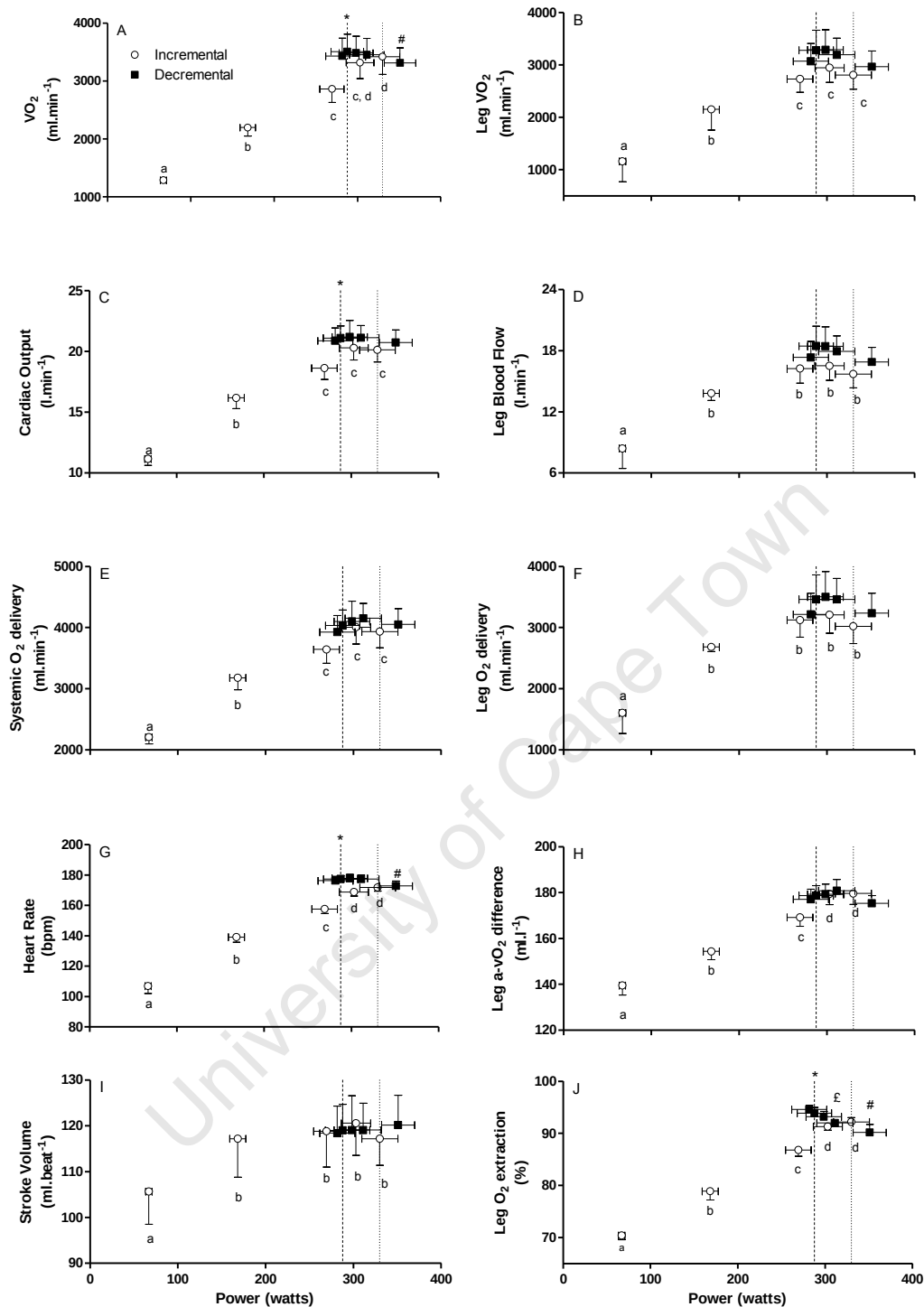


Figure 1: Physiological data (mean $\pm$ S.E.M.) during the INC₁ (○) and DEC (■) protocols. Data points (from left to right) for INC₁ are representative of 20, 50, 80 and 90% of peak power output and the value measured at $\dot{V}O_{2max}$. Data points (from right to left) for DEC represent the second, third and fourth minutes of the test, value collected at $\dot{V}O_{2max}$ and the final value measured during the test. Vertical dotted line (on the left) marks $\dot{V}O_{2max}$ during INC₁, vertical dashed line (on the right) marks $\dot{V}O_{2max}$ during DEC. For INC₁, different letters indicate statistical difference. * $p < 0.05$ between DEC and INC₁ at $\dot{V}O_{2max}$; # $p < 0.05$ vs. all other time points of the same test; £ $p < 0.05$ vs. the point at $\dot{V}O_{2max}$ and last point (DEC test).

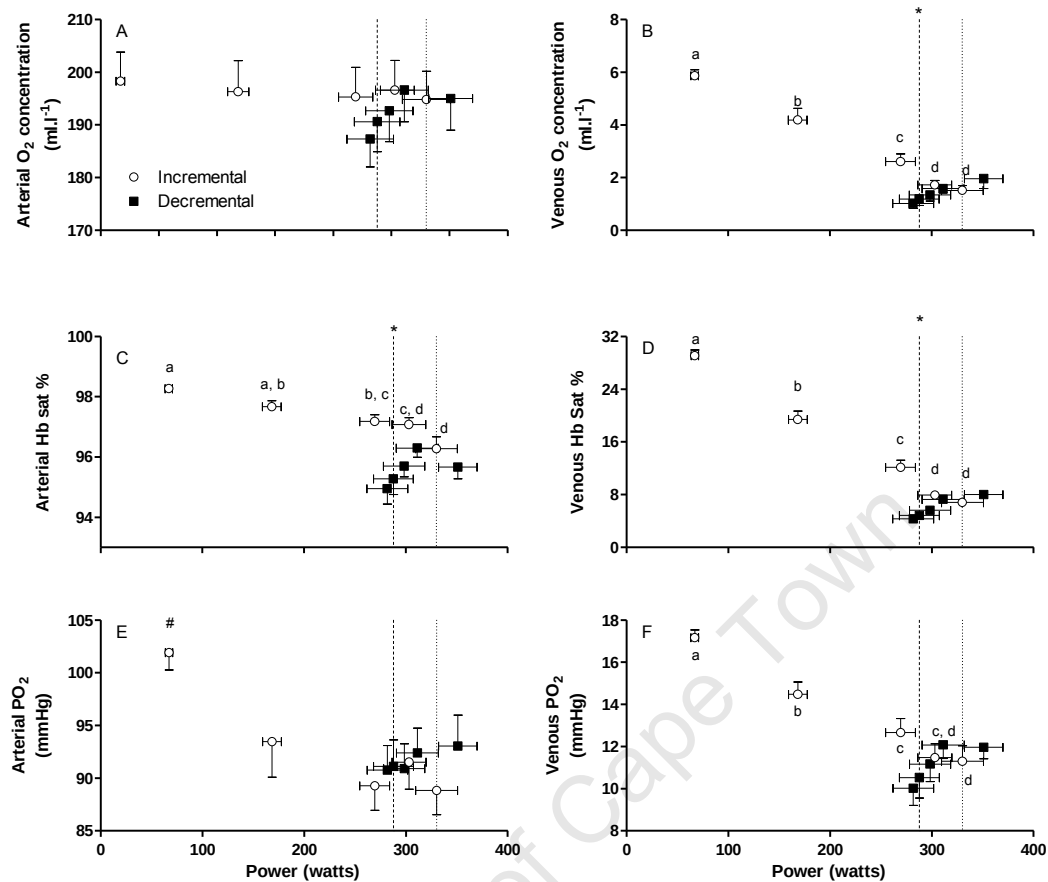


Figure 2: Mean $\pm$ S.E.M. for Ventilation (A), blood lactate concentration (B), arterial pH (C), CO₂ excretion (D), respiratory exchange ratio (RER, E) and alveolar ventilation (F) during the INC₁ (○) and DEC (■) protocols. Data points (from left to right) for INC₁ are representative of 20, 50, 80 and 90% of peak power output and the value measured at $\dot{V}O_{2max}$. Data points (from right to left) for DEC represent the second, third and fourth minutes of the test, value collected at $\dot{V}O_{2max}$ and the final value measured during the test. Vertical dotted line (on the right) marks $\dot{V}O_{2max}$ during INC₁, vertical dashed line (on the left) marks $\dot{V}O_{2max}$ during DEC. For INC₁, different letters indicate statistical difference. * $p < 0.05$ between DEC and INC₁ at $\dot{V}O_{2max}$. Within-test comparisons for DEC are not shown to improve clarity. See text for further details.

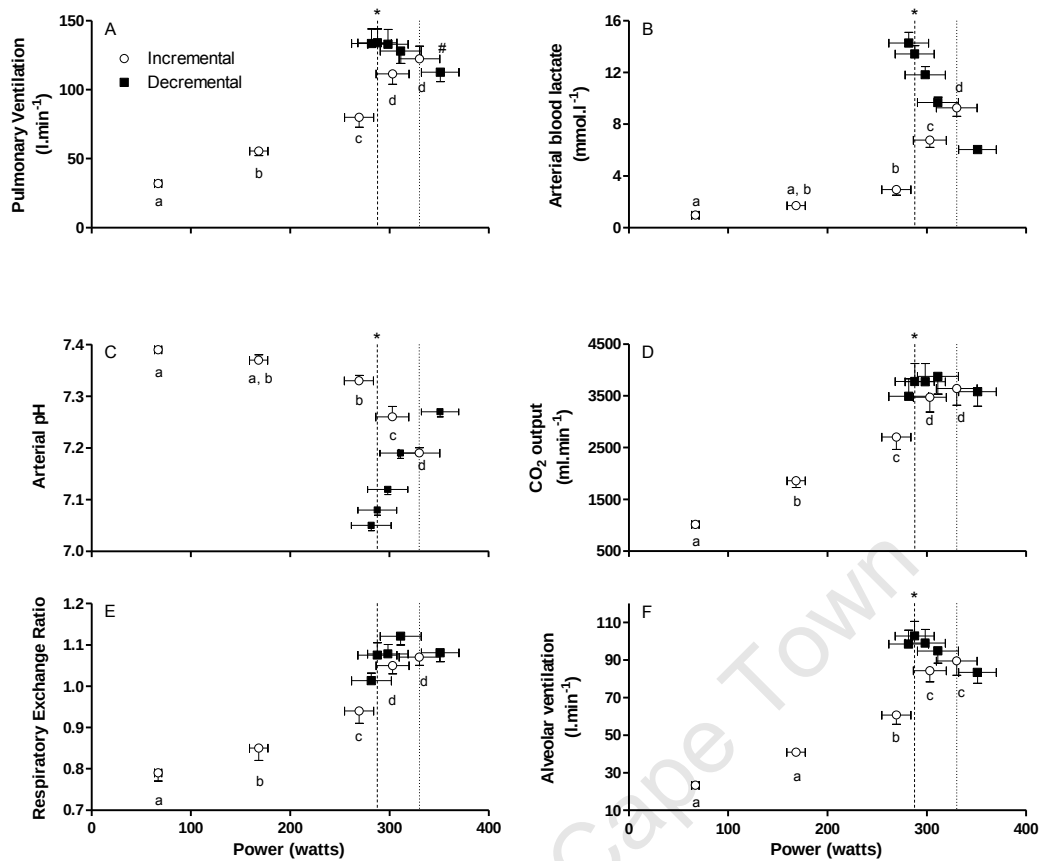


Figure 3: Mean $\pm$ S.E.M. for Arterial and venous O₂ concentration (top panels), arterial and venous [Hb] saturation (middle panels) and arterial of venous partial pressure of O₂ (PO₂, lower panels) during the INC₁ (○) and DEC (■) protocols. Data points (from left to right) for INC₁ are representative of 20, 50, 80 and 90% of peak power output and the value measured at $\dot{V}O_{2max}$. Data points (from right to left) for DEC represent the second, third and fourth minutes of the test, value collected at $\dot{V}O_{2max}$ and the final value measured during the test. Vertical dotted line (on the right) marks $\dot{V}O_{2max}$ during INC₁, vertical dashed line (on the left) marks $\dot{V}O_{2max}$ during DEC. For INC₁, different letter indicate statistical difference. * $p < 0.05$ between DEC and INC₁ at $\dot{V}O_{2max}$; # $p < 0.05$ vs. all other data points. Within-test comparisons for DEC are not shown to improve clarity. See text for further details.

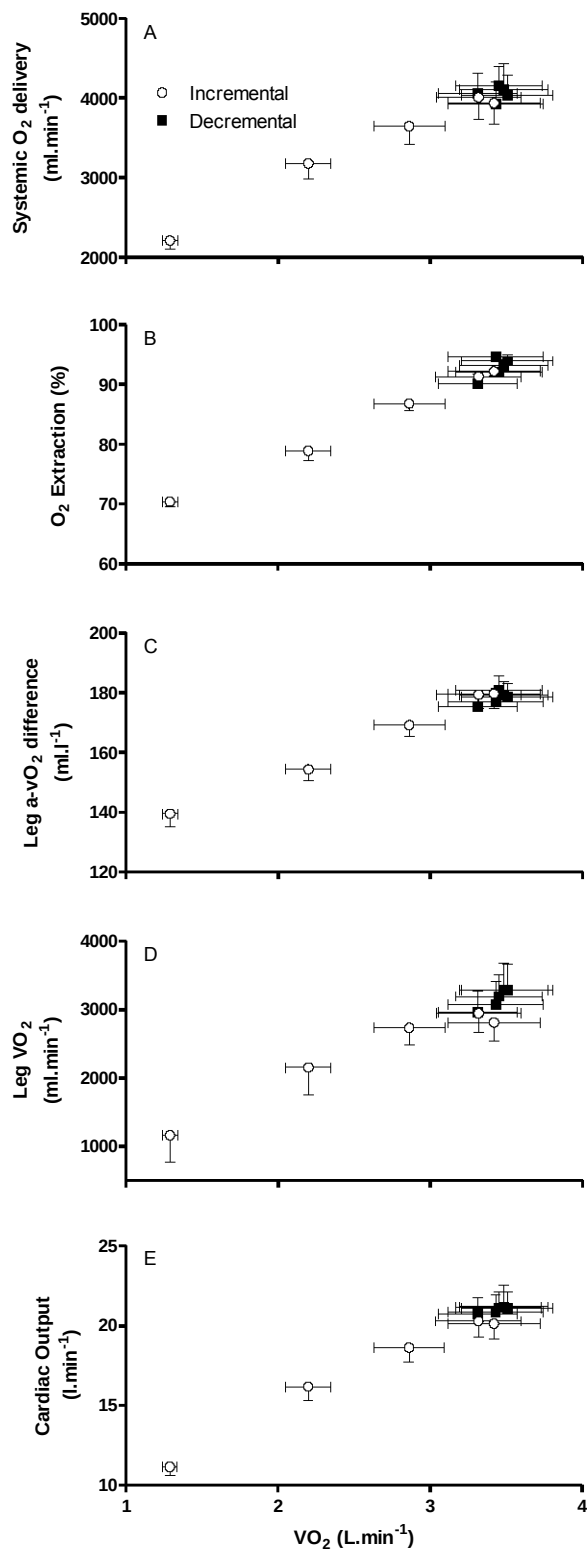


Figure 4: Mean $\pm$ S.E.M. data for systemic O₂ delivery (A), O₂ extraction (B), leg a-v O₂ difference (C), Leg $\dot{V}O_2$ (D) and cardiac output (E) expressed as a function of $\dot{V}O_2$ during the INC₁ (○) and DEC (■) protocols.

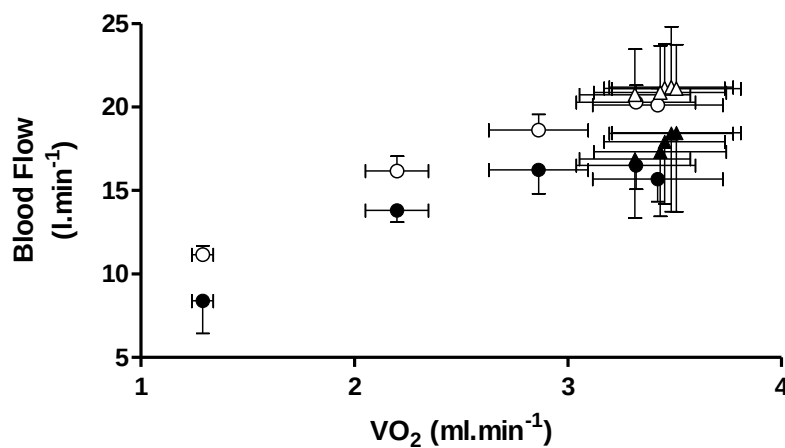


Figure 5: Mean $\pm$ S.E.M. data for cardiac output (open symbols) and Leg blood flow (solid symbols) during the INC₁ (circles) and DEC (triangles) protocols, expressed as a function of whole-body $\dot{V}O_2$.

DISCUSSION

The first main finding from this study was that, compared to INC₁, the higher $\dot{V}O_{2max}$ during DEC was achieved by two differences in O₂ transport: higher $\dot{Q}$ values, and, despite this, higher leg %O₂ extraction. The differences are small, but significant statistically, and suggest that the underlying basis of the higher VO₂ in DEC than INC₁ is multi-factorial, involving both “central” and “peripheral factors” as well as different contribution from the active limbs and respiratory muscle to the total O₂ uptake

The second important finding is that the higher O₂ extraction during DEC occurred in the presence of a higher blood temperature, lower blood pH and higher blood lactate values, (without differences in muscle O₂ diffusional conductance (D_{MO_2}), in contrast to our initial hypothesis). Finally, even though differences were not statistically significant likely due to relatively high inherent variability, the values of $\dot{Q}_{LEG}$, leg O₂ delivery and Leg $\dot{V}O_2$ were all on average numerically higher (by 15-18%) in DEC than INC₁. While it cannot be confirmed statistically, these results are consistent with the difference in O₂ uptake between the two tests being related to higher O₂ availability to, and consumption by, the legs in the DEC test.

Cardiac Output and systemic O₂ delivery

Participants achieved ~5% higher $\dot{V}O_{2\max}$ while $\dot{V}O_{2\max}$ was ~2.9% higher during DEC compared to INC₁. The smaller change in $\dot{V}O_{2\max}$ is in comparison to that assured with theoretical predictions on the impacts of increased $\dot{V}O_{2\max}$ (Wagner, 1993; Wagner, 1996). Wagner (Wagner, 1993) proposed that increasing $\dot{Q}$ at $\dot{V}O_{2\max}$ would simultaneously increase the capacity for mass transport of O₂ in the blood but would also decrease diffusional equilibration of O₂ in the lungs, so that percentage increases in $\dot{V}O_{2\max}$ would be substantially smaller than the observed $\dot{Q}$.

During exercise is believed to be limited by $\dot{V}$, due to the size of the pericardium (Stray-Gundersen *et al.*, 1986; Hammond *et al.*, 1992) and vasoconstriction of the exercising limbs, which reduce venous return (Mortensen *et al.*, 2008). Interestingly, the difference in $\dot{Q}$ between DEC and INC₁ resulted from higher SV while HR was unchanged. The reason why $\dot{Q}$ was higher during DEC compared to INC, however, is unclear. $\dot{Q}$ has been related to multiple afferent mechanisms (Amann *et al.*, 2011) and direct central command (Liang *et al.*, 2011), and the higher O₂ extraction during DEC might have provided input to increase HR during the test. Furthermore, reduced arterial pH and PCO₂ induced higher HR during DEC, which could potentially stimulate higher HR (Whipp & Ward, 1982).

In addition, higher $\dot{V}O_{2\max}$ during DEC, systemic O₂ delivery was similar between trials. Even though O₂ delivery to the heart muscle is expected to be higher during DEC, this limitation is attributed to restrictions in $\dot{Q}$, which was higher during the DEC test. Instead systemic O₂ delivery was not different between tests due to slightly (not significant) lower [Hb] coupled with lower SaO₂ during DEC. Although differences in SaO₂ at $\dot{V}O_{2\max}$ between tests were significant ($p = 0.009$), these amounted to only 0.9 percentage points lower during DEC, which would decrease systemic O₂ delivery at a $\dot{Q}$ of 20 l.min⁻¹ by only 35 ml O₂, so its biological importance should not be overstated (Nielsen, 2003).

AaDO₂ at $\dot{V}O_{2\max}$ was not different between DEC and INC₁, suggesting that differences in SaO₂ between tests cannot be explained by $\dot{V}A/\dot{Q}$ inequality (Nielsen, 2003) or alveolar-capillary diffusion capacity (Wagner *et al.*, 1986). Therefore the

reduced SaO_2 during DEC is probably the result of decreased [Hb]- O_2 affinity during DEC, caused by the differences in blood temperature and pH.

Nonetheless for $\dot{V}\text{O}_{2\text{max}}$ to be higher during DEC in comparison to INC_1 the flux of O_2 into the mitochondria must also have been higher. Since systemic O_2 delivery was not different between trials O_2 extraction must also have been higher during DEC, which argues against a purely central limitation of O_2 delivery in setting the limits for $\dot{V}\text{O}_{2\text{max}}$ (Wagner, 1993; Stainsby *et al.*, 1995; Richardson *et al.*, 1998).

Ventilation, blood gases and pH

In the present study, the higher $\dot{V}\text{O}_{2\text{max}}$ measured during DEC was coupled with higher $\dot{V}_E$ and $\dot{V}\text{CO}_2$ values in comparison to INC_1 , different to what has been reported in running (Beltrami *et al.*, 2012), but in accordance to what has been shown in cycling (**Chapter 6**). Even though both $\dot{V}_E$ and $\dot{V}\text{CO}_2$ at $\dot{V}\text{O}_{2\text{max}}$ were higher during DEC in comparison to INC_1 , PaCO_2 was in fact reduced during DEC. This implies the presence of relative hyperventilation during DEC in comparison to INC_1 , likely as a result from the greater blood acidosis. The lower blood pH also played a significant role in reducing the [Hb]- O_2 affinity, so that in spite of increased $\dot{V}_E$ (and also $\dot{V}_A$) during DEC there was a slight reduction in SaO_2 , as mentioned before. Therefore the higher $\dot{V}_E$ during DEC was not enough to maintain SaO_2 in the face of higher $\dot{Q}$ and right-shifted [Hb]- O_2 dissociation curve, suggesting that $\dot{V}_E$ did not directly affect the O_2 transport chain during DEC.

The higher $\dot{V}_E$ during DEC however likely caused a greater demand for O_2 from the respiratory muscles. The O_2 cost of breathing to raise $\dot{V}_E$ by $12 \text{ l}\cdot\text{min}^{-1}$ during high intensity exercise is approximately $36\text{-}54 \text{ ml } \text{O}_2\cdot\text{min}^{-1}$ (Aaron *et al.*, 1992) or about a third to one half of the measured difference in whole body $\dot{V}\text{O}_{2\text{max}}$ between tests. Therefore part, but not all, of the additional VO_2 measured during DEC can be accounted for by the extra work performed by the respiratory muscles (Aaron *et al.*, 1992). Although it is logical to assume that the higher $\dot{V}_E$ required additional O_2 supply to the respiratory muscle, it is important to note that imposing additional load to the respiratory muscles at maximal exercise does not increase $\dot{V}\text{O}_{2\text{max}}$ (Harms *et al.*, 1998),

and that protocols with differences in $\dot{V}_E$ but similar power outputs also do not result in different $\dot{V}O_{2\max}$ (Roach *et al.*, 1999; Robach *et al.*, 2008).

Interestingly, our two “non-responders” participants had much lower $\dot{V}_E$ during DEC compared to INC₁ ($\sim 33 \text{ l}\cdot\text{min}^{-1}$). Should their $\dot{V}_E$ have presented a similar pattern to our responders, $\dot{V}O_{2\max}$ could be expected to increase by $135 - 200 \text{ ml}\cdot\text{min}^{-1}$, which approximates the difference in $\dot{V}O_{2\max}$ between the two tests ($200 \text{ ml}\cdot\text{min}^{-1}$), emphasizing the importance of the work performed by the respiratory muscles in explaining at least part of the differences in pulmonary $\dot{V}O_2$ between tests. It must be noted however that several variables related to O_2 transport, which were different between tests for the “responders”, were not different between tests for these two subjects, so the unresponsive pattern is not simply the result of lower $\dot{V}_E$ values.

O_2 Diffusion from muscle microcirculation to mitochondria

$D_M O_2$ was numerically 9% higher during DEC in comparison to INC₁, but this difference did not reach significance ($p = 0.36$). Even so, any increase in $D_M O_2$ would facilitate flux of O_2 from the microcirculation to the cell interior (Richardson *et al.*, 1995). The increase in $D_M O_2$ was smaller than the increase in $\dot{Q}_{\text{LEG}}$ ($\sim 17\%$), implying that the increase in $D_M O_2$ was only partly able to off-set the deleterious effects from likely reduced capillary transit time caused by the increase in $\dot{Q}_{\text{LEG}}$ (Roca *et al.*, 1992). The results for $D_M O_2$ however were also affected by the high variability, so more conclusive data will require further investigations.

Contrary to our hypothesis, increased O_2 extraction did not seem to occur due to improved microvascular surface area for diffusive unloading of O_2 . Instead, the improved O_2 extraction during DEC can best be explained by the differences in blood pH and blood temperature between the exercise conditions. Lower pH and higher blood temperature are well known to cause a shift to the right in the [Hb]- O_2 dissociation curve, thereby increasing in vivo P50 values (the PO_2 at which 50% of the hemoglobin is saturated), in what is called the Bohr Effect (Briehl & Fishman, 1960). This facilitates O_2 unloading from the hemoglobin and hence will enhance diffusion into the muscle cells, so that $\dot{V}O_{2\max}$ will increase even if CaO_2 and perfusion remain constant (Richardson *et al.*, 1998).

In the present study, theoretical calculations showed that the drop in arterial pH from 7.40 to the measured value of 7.10 (Table 1) during DEC would by itself increase O_2 extraction by 12 percentage points (from 82.7 to 94.7%), while during INC_1 the increase based on the fall in pH from 7.40 to 7.21 (Table 1) would amount to 9 percentage points (from 84.2 to 93.1%). The effects of increased blood temperature (37.8 and 38.5 °C in INC_1 and DEC respectively) would be smaller (2.0 percentage points increase in INC_1 vs. 3.0 percentage points increase in DEC), but additional to those of pH. Thus O_2 extraction during DEC would have increased by 4 percentage points more than during INC_1 due to differences in arterial blood pH and temperature, with 75% of the difference attributed to the lower pH, and 25% to the higher temperature. The values for both arterial and venous [la] and blood pH measured during DEC are amongst the highest reported in the literature during tests leading to $\dot{\text{V}}\text{O}_{2\text{max}}$ (Knight *et al.*, 1993; Hussain *et al.*, 1996; Nielsen, 2003) in non-elite athletes or even during Wingate tests in trained subjects (Hussain *et al.*, 1996; Calbet *et al.*, 2003) and are consistent with the greater acidosis.

Leg Blood flow and oxygen uptake

Although relative differences for $\dot{\text{Q}}_{\text{LEG}}$ or leg $\dot{\text{V}}\text{O}_2$ at $\dot{\text{V}}\text{O}_{2\text{max}}$ between the two tests were 17-18% (Table 1), these were not statistically significantly different. One possible reason for this lack of statistical significance is the small sample size and high variability in $\dot{\text{Q}}_{\text{LEG}}$ measurements. The coefficient of variation (SD/mean) between duplicate measurements of $\dot{\text{Q}}_{\text{LEG}}$ during INC_1 however was 7.2% when all intensities were considered and 4.2% when only values at $\dot{\text{V}}\text{O}_{2\text{max}}$ were considered, in line with has been reported in the literature (Andersen & Saltin, 1985). This suggests that the variability was mostly inter-subject, and not intra-subject, in origin. Despite this likely type II statistical error, the significantly higher O_2 extraction by the legs coupled with the similar O_2 delivery suggests that there is a difference in Leg $\dot{\text{V}}\text{O}_2$ between tests.

Even though the difference in $\dot{\text{Q}}_{\text{LEG}}$ at $\dot{\text{V}}\text{O}_{2\text{max}}$ between DEC and INC_1 was not statistically significant, several factors known to have an effect on the distribution of blood flow during exercise were different between the two tests, all favouring higher $\dot{\text{Q}}_{\text{LEG}}$ during DEC. For example, blood flow is related to blood pH, so that lower pH

promotes sympatholysis-related vasodilation through decreased sensitivity to α -agonist vasoconstrictor, phenylephedrine (Ives *et al.*, 2013). Increases in temperature (Pearson *et al.*, 2011; Ives *et al.*, 2012) and reductions in SaO₂ (Jensen, 2009) or CaO₂ (Roach *et al.*, 1999), may each contribute to increased blood flow, and were differentially altered between INC₁ and DEC. It is likely that $\dot{V}_{O_{2\text{LEG}}}$ could have been increased during DEC through one or several of these mechanisms, although the regulation of blood flow is a complex process with multiple redundant mechanisms (Hellsten *et al.*, 2012). Regulation of blood flow appears to be highly specific to areas where O₂ demand is high since the use of ATP to promote vasodilation increases leg O₂ delivery without increasing $\dot{V}_{O_{2\text{max}}}$, possibly because the additional blood flow perfuses muscle areas that are inactive (Calbet *et al.*, 2006; Barden *et al.*, 2007).

Assuming that $\dot{V}_{O_2}$ was probably increased in the DEC trial, and in the face of lower power output, the bioenergetic explanation is currently unclear. One possible explanation is that the leg muscles became less efficient in using O₂ during DEC, which could be attributed to differences in muscle temperature (Brooks *et al.*, 1971; Willis & Jackman, 1994), muscle recruitment patterns (Bangsbo *et al.*, 2001; Ferguson *et al.*, 2002; Smith *et al.*, 2005) and muscle pH (Smith *et al.*, 2005), although experimental studies have shown that the latter has little or no effect on muscle efficiency (Bangsbo *et al.*, 2001; Krstrup *et al.*, 2003).

Mitochondrial phosphorous/oxygen ratio has been shown to decrease with increases in temperature (Brooks *et al.*, 1971; Willis & Jackman, 1994), but estimated to increase $\dot{V}_{O_2}$ by 10% for a 3-4 °C change (Willis & Jackman, 1994). In the present study the difference in temperature between tests was only 0.7 °C, so the impact of temperature at the mitochondria was probably small. Furthermore studies using heating during leg-exercise did not consistently find increased $\dot{V}_{O_2}$ during exercise at higher muscle temperature (Koga *et al.*, 1997; Ferguson *et al.*, 2002; Ferguson *et al.*, 2006), which can be attributed to simultaneous changes in mitochondrial efficiency but also in the efficiency-velocity curve of the muscle (Ferguson *et al.*, 2002).

Increased recruitment of type II fibers during exercise have also been linked to decreased muscle efficiency, because these fibers use O₂ less efficiently, partly due to

higher activity of α -glycerophosphate oxidase (Willis & Jackman, 1994). The $\dot{V}O_2/W$ relationship becomes steeper when type IIx fibers are recruited (Jones *et al.*, 2004), but changes in fiber type recruitment however are not consistently related to changes in the $\dot{V}O_2/W$ relationship (Zoladz *et al.*, 1995; Pedersen *et al.*, 2002). Nonetheless, the proportion of different fiber types activated during the different tests in the present study cannot be quantified, so this hypothesis needs further testing.

Limitations and technical considerations

Although $\dot{V}O_{2max}$ was significantly higher ($\sim 2.9\%$, $p = 0.04$) during DEC in comparison to INC₁, differences were smaller than we have previously reported (Beltrami *et al.*, 2012) and could lead to speculation of whether they are truly biological or simply the result of measurement error. Using the Fick equation ($\dot{V}O_{2max} = \dot{Q} \times a-v O_2$ difference) to calculate $\dot{V}O_{2max}$ yields similar results (3657 ± 667 vs. 3786 ± 653 ml.min⁻¹ for INC₁ and DEC respectively, $p = 0.02$) to the gas analyzer data, strengthening the original finding. Although this calculation used femoral venous blood as a surrogate for whole-body CvO_2 , there is no apparent reason that the ~ 5 l of $\dot{Q}$ that did not perfuse the legs during $\dot{V}O_{2max}$ would be systematically different in O_2 extraction between the two tests to affect these results, with exception of the blood perfusing the respiratory muscles, since $\dot{V}E$ and breathing frequency were higher during DEC. Nonetheless, O_2 cost of breathing and thus O_2 extraction by the respiratory muscle must have been higher during DEC, so correcting for this would only increase the difference in $\dot{V}O_{2max}$ between INC and DEC.

Additionally, two participants were excluded from the analysis due to lower $\dot{V}O_{2max}$ values measured the DEC test. This was done because the primary aim of the present study was not test whether or not $\dot{V}O_{2max}$ was different between tests, (which has already been established (Beltrami *et al.*, 2012)) but rather to understand why $\dot{V}O_{2max}$ is higher during DEC in subjects who do show this behaviour. There are various possibilities however as to why these two participants did not respond as expected during DEC. As demonstrated in table 1, these participants had more pronounced [Hb] differences between tests than the other subjects, which could interfere with their O_2 carrying capacity. More importantly, these two participants achieved $\dot{V}O_{2max}$ during

DEC at lower HR and much lower RER and $\dot{V}E$ than during INC₁, an opposite pattern to all the other participants. As mentioned earlier, the differences in O₂ cost of breathing between tests alone would have sufficed to raise their $\dot{V}O_{2max}$ to the same value as in INC₁. We therefore conclude that their poor response to the test was possibly due to load miscalculation, which resulted in $\dot{V}O_{2max}$ during DEC at an even lower power output compared to INC₁ (since starting at too low a power output or decreasing the load too rapidly will decrease the demand for O₂ from the onset of exercise), a problem that does not occur during incremental exercise testing.

In the present study we used non-invasive thoracic impedance to determine $\dot{Q}$. The Physioflow device has been tested frequently with mixed results (Welsman *et al.*, 2005; Bougault *et al.*, 2005). The $\dot{Q}$ data related very well with the $\dot{Q}_{LEG}$ measurements (figure 5), and combining it with the blood gas data for determination of $\dot{V}O_{2max}$ using the Fick equation also resulted in very similar values as those produced by the gas analysis system (above). In addition, plotting all available measurements of $\dot{Q}$ against $\dot{V}O_2$ values fitted well into a linear model ($r^2=0.87$; slope 4.54 and y intercept 5.19), which is what would be expected from an appropriate $\dot{Q}$ versus $\dot{V}O_2$ relationship (Whipp & Ward, 1982). Finally, differences between $\dot{Q}$ and leg $\dot{Q}_{LEG}$ and pulmonary $\dot{V}O_2$ and leg $\dot{V}O_2$ at $\dot{V}O_{2max}$ during INC₁ averaged 4.4 l.min⁻¹ and 600 ml O₂.min⁻¹ respectively, similar to values reported by Calbet *et al.* (2007) (~6.5 l.min⁻¹ and ~640 ml O₂.min⁻¹), indicating good agreement between methodologies.

Another limitation of the present study could be that the order in which the exercise protocols were performed (INC always first and DEC always second) was not randomized. Although this could lead to differences in exercise performance, participants were trained individuals and had over 45 min of rest between tests, which should have minimized any deleterious effects on performance. Furthermore, the fundamental observation under study is the *higher* $\dot{V}O_{2max}$ in the second (DEC) exercise test. The potential hemodilution from infusion of saline for the thermo-dilution measurements could also be a reason for concern, but even though [Hb] and haematocrit were lower during the final incremental test (INC₂, data not shown), values for both variables were similar between INC₁ and DEC (table 1), and thus had little or no impact in the results presented.

Conclusion

In summary, this study shows that when performing a high intensity decremental exercise test, most trained individuals reach slightly higher $\dot{V}O_{2\max}$ values than during a conventional incremental test. This is because of greater cardiac output (and likely greater exercising muscle perfusion), as well as greater muscle O_2 extraction. The latter is best explained by the greater acidosis and higher blood temperature observed during the decremental test causing a rightward shift in the O_2 -[Hb] dissociation curve, thereby facilitating O_2 unloading. However, additional studies are needed to determine unequivocally if the additional O_2 uptake occurs only in the exercising limb muscles or partially also in non-exercising tissues including the respiratory muscles. DEC appears to cause greater stress on the respiratory (higher $\dot{V}E$), cardiovascular (higher cardiac output) and limb muscular systems (higher blood lactate and lower pH). No matter the underlying mechanisms, neither of the two variables often thought to be the “bottleneck” for $\dot{V}O_{2\max}$, namely cardiac output and O_2 extraction, seem to be truly maximal at the end of conventional incremental exercise tests performed under standard sea-level conditions.

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Chapter 8

Integrated discussion

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INTRODUCTION

Since the concept of maximal oxygen uptake ($\dot{V}O_{2\max}$) in humans was first introduced in 1923 (Hill & Lupton, 1923), the question of what determines $\dot{V}O_{2\max}$ has received substantial attention from researchers (Bassett, Jr. & Howley, 1997; Noakes, 1997; Levine, 2008; Spurway *et al.*, 2012), because high $\dot{V}O_{2\max}$ values are considered beneficial for both athletic performance (Jacobs *et al.*, 2011) and cardiovascular health (Myers *et al.*, 2002; Arena & Sietsema, 2011). Hill and colleagues (1924) in

studied that $\dot{V}O_{2\max}$ is limited by the capacity of the heart to pump oxygen enriched blood through exercising muscles, but concluded that “it would be unwise for the heart to make an excessive effort if by doing so it merely produced a lower degree of arterial blood oxygen saturation” (Hill *et al.*, 1924). They therefore proposed the existence of a hypothetical control mechanism – “governor”, either the heart or the central nervous system – that would slow the circulation of blood whenever a dangerously low degree of arterial O_2 saturation (SaO_2) was reached (Hill *et al.*, 1924). Even though this mechanism for controlling the output of the heart was never found or even after its hypothetical existence has been abandoned by subsequent research, there has been further argument that $\dot{V}O_{2\max}$ is limited by the heart during whole-body exercise (Bassett, Jr. & Howley, 1997; Brink-Elfegoun *et al.*, 2007a; Levine, 2008).

While the works of Hill and colleagues established the physiological basis of the $\dot{V}O_{2\max}$ concept as it is understood today, the studies of Taylor and colleagues (1955) and Mitchell and colleagues (1958), provided the mathematical definition of what became known as the $\dot{V}O_{2\max}$ “plateau phenomenon”. It follows that when $\dot{V}O_2$ fails to increase in spite of increasing workloads (that is, $\dot{V}O_2$ “plateaus”), “true” $\dot{V}O_{2\max}$ is achieved. It is currently believed that subjects who fail to produce the plateau phenomenon during incremental exercise test employing sufficient muscle mass to raise oxygen uptake to its true maximum are either not pain tolerant enough, not sufficiently motivated (Shephard, 2009) or do not possess enough anaerobic capacity to keep exercising after $\dot{V}O_{2\max}$ is achieved (Lacour *et al.*, 2007).

In spite of its wide acceptance by modern exercise physiologists, recently the concept that a plateau in $\dot{V}O_{2\max}$ represents a true biological limitation in cardiac output and thus oxygen delivery and aerobic capacity has been criticized on multiple grounds (Noakes, 1997; Noakes, 1998; Noakes & St Clair Gibson, 2004; Noakes, 2006; Noakes, 2008b). These include that fact that the plateau phenomenon is a rather inconsistent finding (Duncan *et al.*, 1997), even when testing Olympic level athletes (Doherty *et al.*, 2003). Moreover, it appears that the “plateau phenomena” can also occur during sub-maximal exercise, an event which cannot be explained by cardiac limitation, but instead is more readily explained by the accuracy of gas analyzers used to measure oxygen consumption (Macfarlane, 2001) and the biological variation that naturally occurs in the variable during exercise testing (Myers *et al.*, 1989; Myers *et al.*, 1990). Finally, it has been shown that different exercise protocols can acutely increase $\dot{V}O_{2\max}$ without manipulation of variables directly associated with the O_2 transport pathway from the ambient air into the mitochondria (Foster *et al.*, 1997; Mauger & Sculthorpe, 2012). Combined, these different studies show that the biological interpretation of the $\dot{V}O_{2\max}$ “plateau phenomenon” needs to be re-evaluated.

Accordingly, this thesis aimed to investigate some of the existing concerns regarding the validity and adequacy of the “plateau phenomenon” as an indicator of true physiological limitation.

Is the “plateau phenomenon” a useful indicator of $\dot{V}O_{2\max}$?

Although the first works on the topic detected the “plateau phenomenon” in the vast majority of exercise tests, more recent studies failed to identify the plateau in most exercise tests evaluated (Duncan *et al.*, 1997), even though the exercise protocol used was exactly the same, only more modern techniques were used to monitor gas exchange continuously during the tests. This confirms that despite its simplicity in concept, it is in fact much more difficult to clearly define the “plateau phenomenon” mathematically (Wyndham *et al.*, 1959). While part of the difficulty can be attributed to the biological variation of $\dot{V}O_2$ during exercise tests (Wyndham *et al.*, 1959; Myers *et al.*, 1989; Myers *et al.*, 1990), the accuracy level required from modern gas analyzers to

detect small changes in O_2 uptake is also part of the problem (Macfarlane, 2001). Although fully automated gas analyzers are now widely used in research and clinical settings, this type of equipment is not considered the gold standard for measuring $\dot{V}\text{O}_2$, and most validity studies that have been performed have used exercise conditions which are different from maximal exercise tests (Bassett, Jr. *et al.*, 2001).

Data from **Chapter 2** revealed that even though modern gas analyzers may perform within the recommended range of accuracy (Atkinson *et al.*, 2005), they are still unable to correctly detect small changes in $\dot{V}\text{O}_2$ as required in most “plateau phenomenon” definitions, which typically range between 50 – 150 ml $\text{O}_2 \cdot \text{min}^{-1}$ (Noakes & St Clair Gibson, 2004). While the absolute size of errors did not increase from measuring single $\dot{V}\text{O}_2$ values to measuring the $\Delta \dot{V}\text{O}_2$ between two consecutive measurements, as it could potentially happen (Howley *et al.*, 1995), errors in either case are sufficiently large to significantly impact the capacity for detecting differences in $\dot{V}\text{O}_2$ within the range of the “plateau phenomenon”. In order to do so, differences in workload between stages would need to be sufficiently large so that the difference in O_2 requirement surpasses the size of the measurement errors. Unfortunately this is often not taken into account when designing studies specifically relating to the occurrence of the plateau phenomenon, which can lead to inconsistent results (Astorino *et al.*, 2005; Lacour *et al.*, 2007; Astorino, 2009; Gordon *et al.*, 2011; Gordon *et al.*, 2012).

Not only the accuracy of the gas analyser can substantially affect the prevalence of the plateau phenomenon during incremental exercise tests, the mathematical method of choice, and more importantly the time-averaging of the data will also greatly affect whether an incremental exercise test ends with a plateau or not (**Chapter 3**) independently of motivation, pain tolerance or anaerobic capacity. In the study performed in **Chapter 3** it was shown that nearly 75% of all “plateau phenomena” detected during incremental exercise tests were in fact “false-positive” phenomena, since they occurred at sub-maximal stages of the tests, and participants were able to continue exercising to higher levels of $\dot{V}\text{O}_{2\text{max}}$. Taken together, the results from **Chapters 2 and 3** indicate that the plateau phenomenon is not a good indicator of true biological events, but rather is more likely to be an artifactual finding caused by

the inaccuracy of the equipment used (**Chapter 2**), biological variation of the variable (**Chapter 3**) and inadequacy of mathematical formulae used by researchers (**Chapter 3**).

Nonetheless, in response to the criticism of the validity of the plateau phenomenon measured during continuous incremental exercise tests, the so-called “verification phase” procedure has emerged (Day *et al.*, 2003; Hawkins *et al.*, 2007; Midgley *et al.*, 2009; Midgley & Carroll, 2009). This procedure consists in testing participants that do not exhibit the “plateau phenomenon” during a continuous incremental test by adding a second exercise bout at a “supra-maximal” workload to confirm the attainment of $\dot{V}O_{2\max}$ (Midgley & Carroll, 2009). This procedure has consistently shown that the $\dot{V}O_{2\max}$ reached at the end of the continuous exercise test does not increase during this “supra-maximal” verification phase test, and this is further confirmed by the verification tests used in **Chapters 4-6** of this thesis.

Thus, it appears that even though the plateau phenomenon is not observed in most continuous exercise tests, a plateau can be created by adding a second bout of exercise at a higher intensity, which argues against the importance of identifying a clear “plateau phenomenon” during the continuous exercise test to indicate that a “true” $\dot{V}O_{2\max}$ has been achieved. Although this is suggested to be the same phenomenon as the original “plateau phenomenon” (Bassett, Jr. & Howley, 1997; Bassett, Jr. & Howley, 2000), this view is also disputed by some (Noakes, 1998; Spurway *et al.*, 2012), because the verification tests are necessarily of much shorter duration, due to the higher load, and $\dot{V}O_2$ might fail to increase simply because of its kinetics (Ozyener *et al.*, 2001), rather than due to a physiological upper ceiling being reached. However, it must be noted that adding additional bouts of constant workload tests to exhaustion is in fact no different to the protocols used by early researchers to measure $\dot{V}O_{2\max}$ (Taylor *et al.*, 1955; Mitchell *et al.*, 1958). The works of Wyndham *et al.* (1959), later confirmed by that of Sloninger *et al.* (1996) using multiple observations for each workload to account for biological and technical errors of measurement, has unequivocally shown that $\dot{V}O_2$ tends to an asymptote when sufficiently high exercise intensities are achieved. However, while Wyndham *et al.* (1959) found that $\dot{V}O_{2\max}$ seldom does indeed reach the values predicted by the asymptote, so that “no simple

criterion to define the level of maximum O_2 intake will suffice”, ger and colleagues (1996) found that the predicted asymptote actually underestimated the real $\dot{\text{V}}\text{O}_{2\text{max}}$.

In spite of the criticism and concerns over the adequacy of the verification phase test and the “plateau phenomenon” itself (Myers *et al.*, 1990; Noakes & St Clair Gibson, 2004), it is accepted by all that the $\dot{\text{V}}\text{O}_{2\text{max}}$ values measured at the end of incremental exercise tests are indeed representative of a “true” physiological upper ceiling, provided that a strict set of requirements are taken into account (Sloniger *et al.*, 1996; Levine, 2008).

$\dot{\text{V}}\text{O}_{2\text{max}}$ indicate true biological limitation during incremental exercise tests?

Since their introduction by Taylor *et al.* (1955) and Mitchell *et al.* (1958), the traditional test to determine $\dot{\text{V}}\text{O}_{2\text{max}}$, and no other protocol has consistently produced higher values of O_2 uptake, even though there is now a wide variety of incremental test protocols with different stage durations and load increments (Sloniger *et al.*, 1996; Yoon *et al.*, 2007). This reinforces the belief that values measured at the end of the traditional incremental exercise tests are indeed representative of the “true” physiological ceiling, specifically in the capacity to deliver O_2 to the exercising muscles. Thus it follows that as exercise increases in intensity, stroke volume (SV) levels off (Bassett, Jr. & Howley, 1997; Bassett, Jr. & Howley, 2000). Some even suggest that SV actually falls (Gonzalez-Alonso, 2008; Trinity *et al.*, 2012), causing O_2 delivery and $\dot{\text{V}}\text{O}_2$ to plateau (Levine, 2008).

In **Chapters 4-7** however it was repeatedly shown that a protocol based on decremental workloads (DEC) can produce higher $\dot{\text{V}}\text{O}_{2\text{max}}$ values than those achieved during a traditional incremental test (INC), even when accounting for the typical error of measurement, which encompasses both biological variability and technical errors in the measurement (**Chapter 4**) (Hopkins, 2012). This difference in $\dot{\text{V}}\text{O}_{2\text{max}}$ between the DEC and INC has been reproduced in well-trained (**Chapter 4**) and elite runners

(**Chapter 5**), who would theoretically have less “room” for acutely improving their $\dot{V}O_{2\max}$ due to their already elevated state of fitness (Shephard, 2009). It was also shown using cycling as the exercise modality (**Chapters 6 and 7**), although it appears that the magnitude of differences in $\dot{V}O_{2\max}$ between DEC and INC are somewhat more elevated in uphill running.

Nonetheless, if $\dot{V}O_{2\max}$ is indeed limited by cardiac output ($\dot{Q}$), as currently believed (Levine, 2008), then $\dot{Q}$ must have been higher during DEC to explain the higher in $\dot{V}O_{2\max}$. Although participants from the study performed in **Chapter 7** did show an increased $\dot{Q}$ during DEC compared to INC, which apparently corroborates the current belief, this did not result in higher O_2 delivery, because arterial saturation was lower in DEC and partly off-set the increase in $\dot{Q}$. Curiously, the difference in $\dot{Q}$ in **Chapter 7** resulted from higher heart rate (HR) with an unchanged SV, whereas in **Chapter 6**, also using cycling, HR was similar between tests. Although HR is expected to reach its peak during an incremental test, differences of 3-5 beats.min⁻¹ between exercise protocols are not uncommon when incremental cycling is the exercise modality (Miyamura & Honda, 1972; Roca *et al.*, 1989; Basset & Boulay, 2000; Peltonen *et al.*, 2001; Lepretre *et al.*, 2004). Furthermore, HR measured at $\dot{V}O_{2\max}$ was 87.6% and 90.2% of predicted (220-age) maximum values during INC₁ and DEC respectively, which are threshold values for maximal effort (Roca *et al.*, 1992; Barker *et al.*, 2011).

The $\dot{Q}$ -limited $\dot{V}O_{2\max}$ theory proposes that it is SV, not HR, which limits maximal $\dot{Q}$ during incremental exercise (Mortensen *et al.*, 2005; Levine, 2008; Larsen & Ekblom, 2012). According to this interpretation, SV is possibly limited at $\dot{V}O_{2\max}$ by vasoconstriction in the exercising muscles, which decreases venous return (Mortensen *et al.*, 2008), even though the heart itself is working sub-maximally at $\dot{V}O_{2\max}$ during incremental exercise tests (Brink-Elfegoun *et al.*, 2007b; Lundby *et al.*, 2008; Elliott *et al.*, 2013). In the present study venous return must have been higher during DEC to maintain a similar SV (and higher $\dot{Q}$) between tests in spite of different HR; otherwise SV would fall resulting in similar $\dot{Q}$ between tests, as shown when HR is artificially raised through atrial pacing (Bada *et al.*, 2012) or through administration of amphetamines (Chandler & Blair, 1980). It is also hypothesized that SV could be limited

by a physical restriction of the pericardium, which would limit diastolic filling and thus SV (Shabetai *et al.*, 1992; Spurway *et al.*, 2012). It has been demonstrated that pericardectomy increases $\dot{Q}$ during exercise in untrained dogs (Stray-Gundersen *et al.*, 1986) and pigs (Hammond *et al.*, 1992) associated with a proportional increase in $\dot{V}O_{2\max}$, in line with the theory that a maximal $\dot{Q}$ limits the $\dot{V}O_{2\max}$. Similarly, stroke volume (SV) – and thus $\dot{Q}$ – appears to be the main determinant of $\dot{V}O_{2\max}$ and exercise performance in rats selectively bred for running performance (Gonzalez *et al.*, 2006).

However it is not known if the pericardium poses any restriction to $\dot{Q}$ in trained humans exercising maximally in the upright position on two limbs, since the end diastolic volume is lower during upright than during supine exercise (Rowell, 1993). Furthermore, it has been demonstrated, at least in situ, that $\dot{V}O_{2\max}$ can be dissociated from O_2 delivery (Hogan *et al.*, 1989; Richardson *et al.*, 1998), suggesting a role for O_2 diffusion limitation in the regulation of $\dot{V}O_{2\max}$. Moreover, in humans ATP infusion during exercise successfully increases blood flow and oxygen delivery to the exercising muscles without altering the $\dot{V}O_{2\max}$ (Calbet *et al.*, 2006; Barden *et al.*, 2007). Alternatively, higher $\dot{V}O_{2\max}$ values can be achieved when breathing hyperoxic mixtures, without necessarily increasing blood flow to the exercising muscles (Knight *et al.*, 1993; Nielsen *et al.*, 1998; Peltonen *et al.*, 2001), but this is not a universal finding (Pedersen *et al.*, 1999; Esposito *et al.*, 2010). Similarly, treatment with recombinant human erythropoietin can increase $\dot{V}O_{2\max}$ through increased arterial concentration of O_2 (CaO_2) and O_2 extraction without augmenting skeletal muscle blood flow (Lundby *et al.*, 2008). Due to the divergent results from these studies, there is currently no consensus as to which factor(s) limit $\dot{V}O_{2\max}$ during whole-body exercise (Levine, 2008; Noakes, 2008a; Wagner, 2011). In fact, data from **Chapter 7** suggests that indeed there is no such “single” factor responsible for limiting the $\dot{V}O_{2\max}$, but instead the interplay between the multiple determinants of O_2 transport can all independently affect $\dot{V}O_{2\max}$ to a greater or smaller extent, as has been suggested previously (Wagner, 1993; Wagner, 1996). Because multiple variables are responsible for determining $\dot{V}O_{2\max}$ at any single time, it makes no biological sense to argue for a “limitation” in $\dot{V}O_{2\max}$, unless there is a reduction in PO_2 to a level at which there is not enough pressure

gradient to drive it into the mitochondria. But this would likely cause ischemic damage to muscle, which does not occur.

What is responsible for the difference in $\dot{V}O_{2\max}$ measured during DEC?

It has been suggested that during decremental exercise the “excess” $\dot{V}O_2$ measured could be attributed to the payment of the “ O_2 debt” from previously active fibers (Yano *et al.*, 2000; Yano *et al.*, 2007). On the other hand, it is proposed that during fatiguing trials the recruitment of less efficient type II fibers contribute to an increase in O_2 consumption (Vanhatalo *et al.*, 2011). In **Chapter 5** the lack of difference in electromyographic activity of the lower limbs muscles at $\dot{V}O_{2\max}$ between DEC and INC in spite of the lower power output provides some evidence for loss of efficiency of the exercising muscles (Vanhatalo *et al.*, 2011), but results were inconclusive. In addition, the difference in venous blood temperature found in Chapter 7 between DEC and INC also suggest an increase in mitochondrial cost of O_2 for ATP production, though this is likely of little importance due to the magnitude of differences (Willis & Jackman, 1994). An alternative hypothesis is that the additional pulmonary $\dot{V}O_2$ measured during hyperoxia, and possibly during DEC in the present study, is used by non-active organs such as the liver (Nielsen, 2003) or the brain (Rasmussen *et al.*, 2010). Our experimental methods do not allow us to distinguish between these possibilities. In Chapter 7 however there was likely an increase in leg $\dot{V}O_2$ and blood flow, which we believe were obscured by a type II error. If this is correct, a loss of efficiency in the active limbs seems a more attractive hypothesis to explain the difference in $\dot{V}O_2$ between DEC and INC.

Interestingly, in **Chapters 6 and 7** the difference in $\dot{V}O_2$ between DEC and INC was coupled with differences in pulmonary ventilation ($\dot{V}E$) and breathing rate (BR), which was not the case in the running studies (**Chapters 4 and 5**). This suggests a possible role for the respiratory muscles, at least during cycling, as responsible for at least part of the difference in $\dot{V}O_2$ measured between DEC and INC. However, it has been demonstrated that although unloading the respiratory muscles during high-intensity exercise decreases $\dot{V}O_2$ (Harms *et al.*, 1998; Romer *et al.*, 2007), the opposite

does not happen, so that higher $\dot{V}O_{2\max}$ values are not achieved by simply requiring the respiratory muscles to perform more work (Harms *et al.*, 1998; Harms *et al.*, 2000).

$\dot{V}O_{2\max}$, the “plateau phenomenon”, and beyond

This thesis was designed to test the common assumption that a plateau in the $\dot{V}O_2$ versus workload relationship at the end of incremental exercise tests represents a physiological limitation in the heart’s capacity to pump oxygen-enriched blood to the exercising muscles (Levine, 2008). We hypothesized that the “plateau phenomenon” was not an unambiguous proxy of a true physiological ceiling, as this would, in theory, imply that the heart would fail during maximal exercise in similar fashion as in pathological conditions (Clark-Kennedy & Owen, 1927).

The first approach used to test whether the “plateau phenomenon” could be safely assumed to indicate a maximal capacity to consume oxygen, restrained only by physiological limitations of the cardiovascular system, consisted in determining the accuracy of both the gas analyzers (**Chapter 2**) and the mathematical methods used to define the “plateau phenomenon” (**Chapter 3**). Lastly, a new protocol for exercise testing was designed to test whether participants could achieve values for $\dot{V}O_{2\max}$ beyond those defined during incremental exercise tests (**Chapters 4-7**), currently believed to be the optimal method to measure maximal oxygen uptake.

As already anticipated (Howley, 2007), even though modern gas analyzers can perform well when compared to measurements using the Douglas Bag Method, which is considered the gold-standard method for determining gas exchange parameters, their accuracy compromises researchers’ ability to detect small changes in $\dot{V}O_2$, as required by the mathematical definition of the “plateau phenomenon” (**Chapter 2**). A practical example of this possibility was provided in **Chapter 3**, in which it was demonstrated that even at sub-maximal stages during which oxygen uptake most certainly increased between the compared intervals, “false-positive” plateau phenomena were often detected by several established methods. The frequent occurrence of “false” plateau phenomena compromises the definition of “true”

phenomena, as the only difference between the two is the timing when they occur during the exercise test. It was further shown that even though secondary criteria might be of assistance in this situation, they are also known to be of limited value, as they are commonly achieved during sub-maximal exercise, or not at all (**Chapter 3**). As such, the first two studies in this thesis (**Chapters 2-3**) indicate that researchers should pay greater attention to the accuracy of their equipment, and consider that when designing exercise protocols in which deviations from the “normal” $\dot{V}O_2$ versus workload relationship are to be detected. Furthermore, it also suggests that exercise physiologists should move away from the differentiation between $\dot{V}O_{2peak}$ and $\dot{V}O_{2max}$, as without additional testing it is not possible to determine any biological difference between the two, especially with regards to test quality. If any doubts are left following an incremental test, supra-maximal verification tests should be performed (**Chapters 4-6**).

When the above-mentioned issues are taken into consideration and incremental exercise tests are performed with sufficiently large increments between stages and respiratory data are appropriately treated with regards to time-averaging, $\dot{V}O_{2max}$ becomes a reproducible phenomenon (**Chapter 4**). Even without the presence of the “plateau phenomenon”, it can be used for several scientific, athletic or clinical purposes. This does not mean, however, that because $\dot{V}O_{2max}$ is “maximal” and reproducible it represents the ceiling of cardiovascular capacity (**Chapter 4-7**). We have demonstrated that even when all recommendations are strictly followed to determine $\dot{V}O_{2max}$ in trained (**Chapter 4**) and elite athletes (**Chapter 5**) $\dot{V}O_{2max}$ is not the maximal rate at which their bodies are able to consume oxygen, a finding that also applies to cycling (**Chapters 6-7**). Even though it was already known that the body could combine cardiac output and O_2 extraction in different ways to reach $\dot{V}O_{2max}$ (Lepetre *et al.*, 2004; Lundby *et al.*, 2008), in **Chapter 7** it was demonstrated for the first time how these variables can combine to reach higher $\dot{V}O_{2max}$ values than those measured during incremental tests. This suggests the existence of cardiovascular “reserve” capacity even at maximal exercise.

Scientists have searched for years for the ultimate factor responsible for limiting $\dot{V}O_{2max}$ in humans (Noakes, 2006). Rather than determining which is the “ultimate”

factor within the O_2 transport chain that limits $\dot{V}O_{2max}$, the studies in **Chapters 4-6**, and especially from **Chapter 7**, show how the complex interaction between these factors can be altered depending on acute physiological conditions (**Chapter 7**) resulting in $\dot{V}O_{2max}$ values different from those expected, with regards to the “limiting” site where O_2 is being used during exercise (**Chapter 6-7**). For example, the difference in $\dot{V}O_{2max}$ between the decremental and incremental exercise tests is that the more acidic milieu in the exercising muscles seen during the decremental test developed in **Chapter 4** and adapted for cycling in **Chapter 6** facilitates the unloading of O_2 from hemoglobin during exercise, increasing O_2 extraction at very high cardiac outputs (**Chapter 7**).

In our understanding all these findings add up to confirm our hypothesis that $\dot{V}O_{2max}$ does not indicate the attainment of an absolute ceiling in cardiovascular capacity, even though it represents the highest value than one can typically achieve under certain conditions. Additionally, the data that we have reported for $\dot{V}O_{2max}$ to fitness and athletic parameters. It does however open new possibilities, as not all subjects responded to this new test in the same manner (for example, figure 2 in **Chapter 5**, it is not established whether the decremental test is better than the incremental test for $\dot{V}O_{2max}$ and such parameters. Also, it remains to be determined whether there is a better protocol for changing the load during the decremental tests in comparison to those used in **Chapters 4-7** in order to obtain $\dot{V}O_{2max}$ values. We strongly suggest researchers to further investigate these questions.”

CONCLUSION

$\dot{V}O_{2\max}$ is not an absolute phenomenon. For each fraction of inspired O_2 and exercise modality, an incremental test will end at a different $\dot{V}O_{2\max}$ value. Still, for any given set of conditions $\dot{V}O_{2\max}$ will be considered a fairly reproducible variable, and increasing the workload beyond that in which $\dot{V}O_{2\max}$ is first measured will not produce higher $\dot{V}O_{2\max}$ values. As a result, most tests, regardless of the presence of the “plateau phenomenon” during the continuous part will eventually result in a “plateau” if supra-maximal exercise is performed; either because $\dot{V}O_2$ fails to increase or because exhaustion occurs before $\dot{V}O_2$ is able to reach its truly maximal levels. Whether these two scenarios represent the same biological phenomenon remains debatable.

It has long been considered that this “plateau” indicated a limitation of the cardiovascular system to provide O_2 enriched blood to the exercising muscles. The results from **Chapters 2 and 3** indicate the care must be taken when mathematically detecting the occurrence of the “plateau phenomenon”, since there is an abundance of false-positive “plateau phenomena” during incremental exercise, partly due to poor precision of modern gas analyzers in detecting very small changes in $\dot{V}O_2$.

The results from the studies described in **Chapters 4-7** further question the biological implications often attributed to the “plateau phenomenon”, and propose a different explanation for the regulation of $\dot{V}O_{2\max}$. Even though the capacity for delivering O_2 to the muscles must be limited, this upper ceiling is not reached during traditional incremental exercise tests. In addition, more extreme physiological conditions can be achieved during severe exercise, facilitating the unloading of O_2 from the hemoglobin and increasing $\dot{V}O_2$ through increased extraction.

Speaking of what variable “limits” $\dot{V}O_{2\max}$ and thus is responsible for the “plateau phenomenon” does not seem appropriate. It is probably wiser to acknowledge that for each given set of conditions there will be a $\dot{V}O_{2\max}$, which is determined, rather than limited, by the interaction between the different components of the O_2 transport chain.

Proposed guidelines for best practice when testing for maximal oxygen uptake

Based on the collective findings from the different studies of the present thesis, we would like to propose the following guidelines for best practice to be used when testing for maximal oxygen uptake:

- All equipment (automated gas analyzers or Douglas Bag systems) should be evaluated for its validity and reliability against a system of known accuracy prior to its use in clinical or scientific settings;
- All testing should be preceded by the full calibration of all equipment used, including the ventilation, O₂ and CO₂ sensors;
- All participants should be allowed to warm-up prior to a maximal exercise test;
- Whenever possible, participants should be allowed at least one familiarization trial prior to a maximal test;
- Incremental tests should be designed with increments that result in $\dot{V}O_2$ changes higher than the error margin of the gas analyzer between the relevant analysis interval;
- Alternatively, when ramp tests are used instead of stage tests, the rate of increase in load should result in $\dot{V}O_2$ slopes that are larger than the error margin of the gas analyzer;
- $\dot{V}O_2$ data should be averaged over 30 s to 60 s intervals, as shorter periods can lead to the inappropriate use of "spikes" in data;
- The test for $\dot{V}O_{2max}$ should be designed in a way that participants exercise for at least 2 minutes before the interval where $\dot{V}O_{2max}$ is measured starts, to ensure a steady state of $\dot{V}O_2$ kinetics;
- A "plateau" $\dot{V}O_2$ should not be considered an unambiguous indicator of "maximal" or "physiological";
- A reference of the $\dot{V}O_2$ x workload relationship at high intensities should be performed taking into account the equipment precision and each participant's $\dot{V}O_2$ x workload relationship measured during sub-maximal,

steady-state conditions; it should never be based on absolute threshold values published in the literature, even if for a similar population;

- In order to determine the attainment or not of $\dot{V}O_{2max}$, performance of an additional bout of exercise after the incremental tests is encouraged (the so-called verification test);
- Verification tests should be performed at a intensity equivalent to one stage higher than the last completed stage during a stage test; alternatively intensity can be raised by 5-10% in the case of ramp tests (variation according to the maximal load achieved and the precision of the gas analysis system);
- Verification tests should be immediately preceded by 3-5 minutes of light exercise, in order to allow $\dot{V}O_2$ kinetics to fully develop during the main part of the test;
- While we encourage the scientific development of the decremental protocol devised in this thesis, we do not yet advise its use in clinical or commercial settings, since the test was developed purely to test the validity of an accepted physiological concept.

Directions for future research

The most intriguing finding from this thesis was that during a new exercise testing protocol, incorporating decremental workloads, most participants were able to reach higher $\dot{V}O_{2\max}$ values in comparison to the standard incremental exercise test. I choose to name this the decremental test paradox. This new paradox in exercise physiology poses some interesting questions:

- Is there an ideal load of rate decrement that results in the highest possible $\dot{V}O_{2\max}$ values?
- Is blood flow and $\dot{V}O_2$ in the lower limbs really increased during the decremental test?
- Is O_2 extraction by the exercising muscles during the decremental test increased to the same extent as reported in hypoxia studies? If so, are the mechanisms similar?
- Why did participants retain the higher $\dot{V}O_{2\max}$ values two day after performing the decremental test (**Chapters 4 and 5**) in running?
- Why was the above-mentioned effect not noticed during the cycling study (**Chapter 6**)?
- Why is pulmonary ventilation higher during the decremental test in comparison to the incremental test in the cycling studies but not in the running studies?
- Will sedentary participants also show the decremental test paradox?
- Will the $\dot{V}O_{2\max}$ values achieved during the decremental exercise test present a different correlation to health and athletic parameters than the standard $\dot{V}O_{2\max}$ values measured during traditional exercise tests?
- Since the physiological stress during the decremental test is higher than that during an incremental test, can it be used as a training tool for health and/or athletic purposes?

We encourage future researchers to tackle these issues, so that it is possible to better understand the physiological reasons and implications, as well as the practical applications, of the decremental test paradox.

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